Analgesics
From Chemistry and Pharmacology to Clinical Application

Edited by
Helmut Buschmann, Thomas Christoph, Elmar Friderichs,
Corinna Maul, Bernd Sundermann

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Preface

Pain has been a human problem since the beginning of time but the last decade has seen an explosion of information about the transmitters, receptors and channels involved in the transmission and modulation of noxious messages generated in peripheral tissues. This has lead to the identification of a number of potential new targets for analgesic therapy. We now have more experimental drugs available, which allows us to study the roles of transmitters and receptors in physiological events. There are now numerous animal models for clinical pain states such as inflammation and neuropathies, and these models have shown that several transmitter systems which have minor actions in acute pains can play important roles in more persistent pains.

This ability to test drugs in contexts other than acute pain models has arisen from good communication between bench scientists, clinicians and industry. Until recently investigations into the mechanisms of clinical pain syndromes all relied on animal studies using acute stimuli. The symptoms of pain arising from nerve injury, neuropathic pain, such as allodynia, spontaneous pain, hyperalgesia, sensory deficits and in some cases a sympathetic component are simply not seen in the older acute models. There are now several animal models which mimic peripheral and central neuropathic states. The same is true for inflammation.

A number of major discoveries over the last thirty years, such as the opioid receptors and the endogenous opioids, the spinal, supraspinal and peripheral sites of action of opioids, the key role of glutamate in signalling in the nervous system, the important actions of peptides such as substance P, the ability of descending controls, both noradrenergic and serotoninergic, to influence pain transmission may not yet have yielded many new drugs but has enormously aided the conceptual basis for understanding pain and analgesia. The Gate Theory of Pain, published in 1965, was the first study to make us think about the ways in which transmission of pain could alter yet the pharmacological details have only recently been eluidated. Plasticity, the capacity of the pain signalling and modulating systems to alter in different circumstances, has changed our ways of thinking about pain control. Signalling events are not fixed, not the same in all pain conditions, but subject to alteration. Types of pain, symptoms of pain, the intensity and area are all factors that alter the pharmacology.

High throughput screening should expedite the identification of useful agents, which, combined with improved combinatorial chemistry, should lead to fast and efficient production of novel agents with good affinity for particular targets. Genomics can be used to identify targets related to specific disease, and, in the field of pain and analgesia, to identify targets associated with particular pathological processes within this area. Here again, the validity of the models will be critical in screening molecules. Realistically many discoveries are still serendipitous, but with a better understanding of the neurobiology of pain their effects can be better verified. Molecular and genetic approaches have recently allowed the identification of channels or receptors that has lead to a far better understanding of the peripheral processes that lead to pain from thermal, chemical and other stimuli. At the level of the peripheral nerve, the roles of particular sodium channels in the generation of activity after tissue damage may provide local-anaesthetic-like drugs that target only pain-related activity. Agents acting on calcium channels that control both neuronal activity and transmitter release also have potential.

Within the last 10, years several new compounds were launched in the field of non-steroidal antiinflammatory drugs (NSAIDs) with a clear focus on cyclooxygenase type 2 selective compounds. In the field of opioids on the other hand no new drugs have passed phase III clinical trials. In this field innovation has been achieved through new pharmaceutical formulations of known drugs such as transdermal systems, e.g. buprenorphine patch, transmucosal systems, e.g. fentanyl lollipop, or rectal delivery systems containing e.g. morphine. These were developed in order to reduce opioid side effects, but also to overcome pharmacokinetical limitations, in particular to prolong compliance and duration of action.

This book deals with established analgesics as well as with new chemical entities for established and new targets. Compounds are classified with respect to their physiological target. In every case the structural formula is given as well as further information e.g. on the pharmacological profile, synthetic routes or major metabolites if important. In addition, structure-activity relationships are discussed if available. Consequently following this scheme, a huge number of compounds is evaluated and a clear picture especially of pain research in the industrial setting is constructed.

Based on the snapshot-like collection of information in this book the cyclooxygenase and the opioid systems are still the most attractive targets and ligands acting on this system are still not surpassed by ligands of other targets. It is also clear, however, that we are at the beginning of an era of promising new approaches to pain therapy. The future will show which targets will survive the race for the best therapy.

The book presented here will be highly valuable for advanced students in pharmaceutical and medicinal chemistry as well as for scientists in the field of chemistry, biochemistry and pharmacology in industrial and academic research.

August 2002

Anthony Dickenson, London

Ulrike Holzgrabe, Würzburg

A View from Grünenthal

Pain research is a traditional and well established field within the pharmaceutical industry. Beginning with the isolation of morphine in a small pharmacy by Adam Sertürner (1806), the next major breakthrough in pain treatment was achieved by the synthesis of acetylsalicylic acid by Felix Hoffmann in the Bayer Laboratories in Wuppertal (1897). Further outstanding contributions by the pharmaceutical industry were the first fully synthetic opioids pethidine (1939) and methadone (1946). Continued efforts up to now have resulted in many potent and clinically accepted analgesics with reasonable side effects and covering nearly all facets of pain treatment. However, pain treatment is far from being satisfactory in respect to more complex pain states, e.g. neuropathy, visceral pain or migraine.

Grünenthal's interest in pain research started in 1962, when Kurt Flick designed a simple molecule containing the essential structural elements of morphine to be a potent analgesic. This prediction was clinically confirmed and today this compound – tramadol – is one of the leading centrally acting analgesics.

Forty years after the discovery of tramadol, research at Grünenthal is still focused on the search for even better analgesics. Due to the intense accumulation of knowledge about pain the idea was born to collect this in a comprehensive overview with the intention to stimulate further scientific efforts in this area. It is our hope that this book will be a useful reference meeting the challenge to improve pain therapy.

Klaus-Dieter Langner and Eric-Paul Pâques

Aachen, August 2002

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Part I

Introduction

1 What is Pain?

Helmut Buschmann

Introduction

Pain - a common phenomenon

Pain is the most common symptomatic reason for seeking a medical consultation.

Everyone is affected by pain at some point in their lives, whether it is from headaches, cuts and bruises or more severe pain resulting from surgery, which would be pre-controlled in anticipation of the event.

Acute pain as a warning mechanism

Although chronic types of pain may generally appear to have no purpose, acute pain acts as an important warning mechanism to the person by instructing the brain to remove the individual from that particular pain stimulus. If for example a person lifts a hot object, pain signals to the brain to put the object down to avoid severe burns.

The treatment of pain, a major problem in medicine

The treatment of pain, a major problem in medicine, is complicated by many factors. Pain is not a uniform sensation, as illustrated by its many common descriptions, e.g. sharp, dull, aching, burning, shooting, cramping, stabbing and throbbing. There are several ways to classify pain, but the first distinction usually made is that between acute and chronic pain. Pain is a subjective sensation which cannot be measured objectively, and its intensity is not always a direct reflection of the nociceptive inputs provoking it. Nociceptive inputs which are easily ignored by an individual in one situation may be unbearable in another.

Definition of Pain

Various definitions of pain have been proposed but the most widely accepted is that of the International Association for the Study of Pain (IASP):

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A definition of pain (IASP)

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. It is unquestionably a sensation in part of the body, but it is also unpleasant and therefore also an emotional experience. Many people report pain in the absence of tissue damage or any likely pathophysiological state; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage, if we take this subjective report. If they regard their experience as pain and if they report it in the same way as pain caused by tissue damage, it should be accepted as pain (Merksey, 1979).

Before the 1960s, pain was considered an inevitable sensory response to tissue damage, but since that time the definition has broadened to encompass the affective dimension of pain with greater consideration of the effects of genetic differences, past experience, anxiety and expectation.

...pain remains grossly undertreated and is frequently mistreated...

Research in understanding the underlying mechanisms behind people's pain has progressed rapidly, but despite these critical advances pain remains grossly undertreated and is frequently mistreated. In the words of Jean-Marie Besson, President of the IASP at the 9th IASP Congress in Vienna, Austria, in August 1999: '*Some pain states can now be controlled, yet there are still others which are far from being treatable.*'

Side-effects of analgesics

Pain, as a whole, is currently a very active area for pharmaceutical R&D, largely because of its undertreatment and frequent mistreatment, but also because the older and still widely-used compounds can cause unacceptable side-effects.

Side-effects of NSAIDs

This is especially true for the traditional non-steroidal anti-inflammatory drugs (NSAIDs) which are associated with serious gastrointestinal complications such as bleeding, lesions and ulcers.

Side-effects of central acting analgesics

Conversely, the perceived dangerous side-effects and fears of addiction and tolerance associated with potent opioid analgesics (e.g. morphine) has led to restrictions and controversy regarding their use. Clinical studies, however, have demonstrated that these risks are low and potent opioid analgesics today are more widely accepted for treating severe cancer pain, but experts are still calling for broader use of opioids in non-malignant chronic pain.

Class	2000 sales ($ billion)	% of global sales	% growth (vs. 1999)
Anti-ulcer	17.4	5.5	+ 13
Cholesterol & triglyceride reducers	15.9	5.0	+ 21
Antidepressants	13.4	4.2	+ 18
Calcium antagonists (plain)	9.8	3.1	+ 2
NSAIDs	9.5	3.0	+ 26
ACE inhibitors	7.3	2.3	+ 3
Cephalosporins & combinations	6.9	2.2	- 5
Antipsychotics	6.0	1.9	+ 22
Non-narcotic analgesics	6.0	1.9	+ 3
Oral antidiabetics	5.9	1.9	+ 26

Table 1: Global pharma sales - the leading therapeutic classes.

Source: IMS Health Word Reviews 2001, SCRIP No. 2630, March 30th 2001, p. 18.

Physiology and Pathophysiology of Pain

Pain transmission

The physiological aspects of lasting pain become apparent when a mechanical, thermal, chemical or electrical stimulus strong enough to damage tissue or affect cellular metabolism stimulates the nociceptive free nerve endings of C-fibers, which are found all over the surface of the body and its organs. Several subtypes of A-fibers also carry afferent nociceptive impulses. The damaged tissue sends out nerve impulses through nerve tracts in the spinal cord to the brain (cerebral cortex) where the stimulus translates to a conscious pain sensation.

Contribution of endogenous inflammatory and/or pain-producing substances

In addition to nervous pain impulses, injured tissues produce inflammatory pain-producing substances, including bradykinin and other kinins, serotonin, histamine, acetylcholine, excesses of potassium ions, proteolytic enzymes and prostaglandins, which can act in synergy to increase pain levels. Many of these substances, especially the proteolytic enzymes, can cause direct damage to the pain nerve endings, but others, such as bradykinin and prostaglandins, can cause extreme direct stimulation of pain nerve fibers without actually damaging them. Local changes accompanying the injury, such as muscular spasm, ischeamia and inflammation, can also contribute to the intensity and character of the pain.

Severe and sustained pain can cause long-lasting reflexes in the spinal cord and sympathetic nervous system that can lead to changes in the secretion of hormones and other substances and to a chronic state of increased pain

sensitivity known as hyperalgesia. This, in turn, can give rise to pain sensations even in response to non-noxious stimuli that can be difficult to treat. Hyperalgesia characteristically develops during inflammation and research has shown that prostaglandins are the main inflammatory mediators responsible for the development of hyperalgesia. Futhermore, both prostaglandins and leukotrienes play a key role in the sensitization of pain mediating receptors. In fact, the basis for the analgesic actions of non-steroidal anti-inflammatory drugs (NSAIDs) is their ability to prevent the production of prostaglandins.

Peripheral pain mechanisms

Pain receptors in the skin (cutaneous) and other tissues (non-cutaneous) are all free nerve endings. They are widespread in the superficial layers of the skin and in certain internal tissues such as the arterial walls and joints. Most other deep tissues contain few free nerve endings and so tissue damage there is more likely to cause a slow, chronic, dull ache rather than acute pain in these areas.

Different groups of nerve endings

Different groups of nerve endings in the skin relay messages about four basic sensations:

- **Warmth:** very sensitive non-myelinated fibers
- **Cold:** thinly myelinated A-fibers
- **Touch:** mechano-receptive afferent fibers
- **Pain:** nociceptive fibers

Nociceptors

Nociceptors, or nociceptive fibers, are peripheral nerve endings. They are mostly found in the skin but can also be of non-cutaneous origin. Nociception itself is a response to the excitation of these nociceptors, which will only occur after a strong stimulus such as a pinch or knock that may hurt briefly or a more severe injury causing bruising and/or broken skin. The signals transmitted from the nociceptors following this stimulus reach the brain and are interpreted as pain. Although nociception may give rise to the experience of pain, pain may also arise in the absence of nociception. Conversely, nociception may also occur in the absence of pain. The complex relationship between pain and injury is highlighted by the fact that analgesics such as morphine cannot abolish pain due to nerve injury (neuropathic pain) as efficiently as they abolish pain due to tissue damage (nociceptive pain). Furthermore, the intensity of chronic pain frequently bears little relation to the extent of tissue damage, making the perception of pain itself an important issue.

Central pain mechanisms

The central nervous system (CNS) - the brain and spinal cord - is involved in the reception and interpretation of peripheral afferent nociceptive impulses. Reflexes mediated by spinal interneurons and the gating functions of the dorsal horn of the spinal cord are particularly crucial. However, our knowledge of brain mechanisms is still limited.

The spinal cord

If a spinal cord is cross-sectioned, the gray matter appears as a roughly H-shaped area in its middle which is, divided into dorsal (posterior), lateral, and ventral (anterior) horns. The horns are interconnected by a crossbar, the gray commissure. The rest of the spinal cord is the white matter, made up largely of tracts of myelinated nerve fibers (axons). Ascending tracts carry afferent sensory impulses towards the brain, descending tracts transmit motor impulses from the brain to the motor neurons in the ventral or lateral horns of the gray matter.

... spinal reflexes

Neurons in different regions of the gray matter can connect with each other, forming spinal reflex arcs between sensory nerves bringing together noxious stimuli and motor nerves controlling avoidance responses. The behavioral consequences of such spinal reflexes are familiar in everyday life: the eye blinks as an object approaches, the hand is withdrawn from a hot plate, both without conscious control.

... the dorsal horn

The dorsal horn of the spinal cord houses a type of pain-inhibitory complex where the pain signals can be blocked before they are relayed to the brain. The dorsal horn itself contains axons from sensory spinal neurons that pass into the dorsal ascending tracts and to higher levels of the spinal cord and the brain. The neurons in the dorsal horn are immediately involved in the processing of pain signals and the control of pain. Mechanisms by which they modulate pain messages transferred to the brain are referred to as segmental and supraspinal. Nerve tracts ascend from the dorsal horn to parts of the brain, including the periaqueductal gray matter, and on to the cerebral cortex, where the pain is localized to a particular body region. Conscious appreciation of pain occurs in the frontal lobes of the brain.

The dorsal horn of the spinal cord contains many transmitters and receptors. Some of these include: peptides, e.g. substance P, somatostatin and neuropeptide Y; excitatory amino acids, e.g. glutamate and aspartate; inhibitory amino acids, e.g. γ-aminobutyric acid (GABA); nitric oxide; endogenous opioids; adenosine; and the monoamines, e.g. serotonin and noradrenaline. There is, therefore, diverse therapeutic potential for

pharmacological control of the transmission of nociceptive information to the brain.

... dorsal horn neurons

Available evidence suggests that the neurons of the dorsal horn do not have fixed functions, that their functional characteristics are modifiable, and that pathological processes can have a potent influence on them. Wall (1989) summarizes three main processes that can affect the functioning of dorsal horn cells and thus, by implication, the experience of pain: gate control, sensitivity control and connectivity control.

... gate control

The widely accepted theory of gate control was put forward by Melzack and Wall (1965). Wall (1978) later restricted the term to describe the immediate reception and control of sensory inputs that lead to effector triggering and sensation. Descending impulses from the raphe nuclei, reticular formation and other regions of the brain affect - and, in particular, inhibit - the activity of neurons in the dorsal horn, where gating functions are thought to be localized. Only when the gate is open does pain information pass to the brain.

... sensitization

Unlike most receptors, nociceptors can become increasingly sensitive after injury (i.e. when the stimulus is very strong) or when the stimulus is continuing or repeated. This sensitization means that there can be a reduction in the threshold for activation (i.e. pain signals will be transmitted in response to even gentle stimuli), an increase in the response to a given stimulus, or even the appearance of spontaneous activity.

Sensitization results from the actions of second messenger systems activated by the release of inflammatory mediators such as the prostaglandins described above. These effects cause some of the features of hyperalgesia produced by pathological processes as opposed to physiological pain that occurs in response to a noxious stimulus. Much of the peripheral sensitization of nociceptors is caused by primary hyperalgesia occurring at the site of the damaged tissue, although some sensitization appears to be due to central mechanisms of hyperexcitability (Besson, 1999).

Pathological pain involves:

- **Allodynia**: a lowered pain threshold
- **Hyperalgesia**: increased responsiveness
- **Hyperpathia**: prolonged pain sensations after the initial stimulus

Psychology of Pain

Organic factors alone cannot explain why different patients report different levels of pain. There is an important psychological reaction to pain that must be assessed and physicians must adopt a comprehensive approach, encompassing psychosocial and behavioral factors as well as organic ones. Melzack and Wall's (1965) gate control theory explains this as the suppression of nociceptive afferent peripheral impulses in the dorsal horn by descending central messages relating to the individual's emotional, cognitive and attention state.

Everyday evidence exists to support the notion that physical pain is closely associated with emotion, cognition and attention. For example, people who have injured themselves whilst participating in a sporting event may not fully realize the extent of their injuries until they stop and their concentration is focused on the injury rather than the game.

Placebo effect

Even the placebo effect goes some way towards demonstrating that if patients believe that they are taking a strong drug this will improve their condition and they will start to feel better even if the drug is really an inactive imitation. Similarly, if during an examination patients are advised that nothing is wrong with their health, their mind's logic may be strong enough to convince them that the pain has no pathological cause and therefore does not really exist. The placebo response is of vital importance in pain management and pain research. In the clinical setting, one may seek to optimize the placebo response to achieve a greater success rate during treatment, but in research one aims to rule out the placebo response.

The role of emotions

Although in the majority of cases, a physician would find an underlying pathological cause to explain a patient's physical pain, it is possible for pain to be brought on by emotional disturbances such as a reaction to grief, anxiety (panic disorder), depression or anger (Ray and Yoham 1992). These emotions themselves can be consequences of pain, perhaps even reinforcing each other, or lead to pain. Painful conditions can be mimicked by neuroendocrine and autonomic changes brought on by emotional distress. Furthermore, a great problem for terminally-ill patients is the resulting distress or suffering from the belief that their pain cannot be cured or pain relief improved. This creates a negative outlook leaving the patient with feelings of despair, hopelessness, helplessness and pessimism.

On the other hand the body can also control pain by producing its own analgesic molecules (endorphins).

Individual perception and tolerance

The way a person relates to pain is in the domain of cognitive psychology. How sufferers cope depends on their own cognitive evaluation of the situation, and if their treatment or prognosis is incorrect the patient will respond inappropriately by over- or under-reacting to it.

Pharmaceutical Pain Management

Analgesics and anesthetics adequately cover pain management and are sometimes used with adjuvant therapies such as sedatives, antidepressants and anxiolytics. In the following sections, and for convenience, general pain therapy will refer to opioid and non-opioid analgesics that normally are given orally (or transdermally). Non-general pain therapy will refer to peripheral localized analgesia and anesthesia, such as that provided by topically applied agents and regional or central blockade methods (nerve blocks, spinal and epidural anesthesia/analgesia).

Principal treatment of pain

- NSAIDs (non-opioid analgesics)
- Opioids
- Adjuvants

At present, the principal treatment for pain are non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, but both of these classes suffer from drawbacks in clinical use. The third group of drugs which is currently used for the treatment of pain is represented by analgesic adjuvants, a class of compounds that include antidepressants and local anesthetics that are used empirically to treat chronic pain states. Some of the NSAIDs are associated with gastric damage as well as kidney and liver toxicity and an increase in blood clotting time, while the opioids can produce tolerance and dependence, along with constipation, nausea, respiratory depression and sedation. In certain pain states, both NSAID and opioid use are ineffective.

Non-opioid analgesics (NSAIDs)

Almost all non-opioid analgesics are non-steroidal anti-inflammatory drugs (NSAIDs) and have varying degrees of analgesic, anti-inflammatory and antipyretic activity. Acetylsalicylic acid (Aspirin®), used to relieve mild to moderate pain and certain types of severe pain, is the archetypal NSAID and is probably the best known and most used therapeutic drug worldwide.

The basis for the analgesic action of NSAIDs is their ability to prevent the production of prostaglandins. Prostaglandins are derived from the arachidonic acid cascade and are implicated in the production of inflammatory pain and in sensitizing nociceptors to the actions of other mediators.

Their antipyretic action means that NSAIDs do not reduce normal body temperature or elevated temperatures in heat

stroke, which is due to hypothalamic malfunction. During fever, interleukin-1 (IL-1) is released and acts directly on the thermoregulatory centre in the hypothalamus to increase body temperature. This is associated with an increase in brain prostaglandins. Aspirin prevents the temperature-raising effects of IL-1 and the rise in brain prostaglandin levels.

Older and still widely-used analgesic compounds can cause unacceptable side-effects. This is especially the case for the traditional non-steroidal anti-inflammatory drugs (NSAIDs) which are associated with serious gastrointestinal complications such as bleeding, lesions and ulcers. Every year it is estimated that 16,500 NSAID-related deaths occur in the US alone, with 75,000 patients hospitalized.

COX-2 selective inhibitors

The main advantage of the highly selective COX-2 inhibitors may be a significant improvement in the unacceptable gastrointestinal side-effects commonly caused by NSAIDs in patients with chronic pain of an inflammatory origin. Other adverse events of conventional NSAIDs include: nephrotoxicity, since prolonged analgesic use over several years is associated with papillary necrosis and chronic renal failure; bronchospasm; skin rash and other allergies.

Opioid analgesics

Opioid analgesics, some of which are the most powerful analgesics (narcotics) are used to relieve moderate to severe pain and can also be used as adjuncts to anesthesia.

Many myths surround the stronger opioids and their use can become restricted when the definitions of addiction, tolerance and physical dependence are confused. Some opioids, e.g. codeine, are less potent and are readily available in OTC products.

Morphine is the gold standard opioid against which all others are compared (McQuay, 1999) and it is the analgesic of choice for terminal pain.

Local legislation limits the availability and choice of opioids in many countries, but there can also be a reluctance to prescribe opioids for non-cancer pain due to the assumption that susceptible individuals may become addicted to these potent drugs through abuse. However, if opioids are prescribed on an individual basis with adequate support and education for the patients as well as their familes or carers, a great deal of unnecessary chronic pain and suffering can be prevented. But today there are still several unresolved clinical issues surrounding opioid use for which there is no data to construct a suitable policy.

Adjuvant therapy

Adjuvants are agents other than the primary opioid analgesics, which can be used to assist total pain management. They can directly diminish pain, counteract opioid side-effects, or help manage concurrent psychiatric symptoms. Adjuvants include agents such as non-opioid analgesics (e.g. NSAIDs), corticosteroids, anticonvulsants, antidepressants and muscle relaxants that can decrease pain directly. In addition, antinauseants, laxatives and psychostimulants can also be administered alongside opioids to help counteract the three most common problems of opioid therapy - nausea, constipation and sedation. This ensures that patients can tolerate higher doses of opioids if their pain so dictates. Furthermore, pain can be aggravated by feelings of depression and anxiety, so various antidepressants and anxiolytics can also be given.

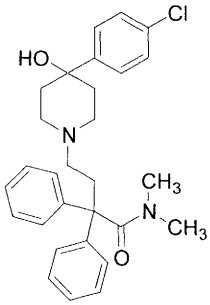
Outlook

Continuing advances in understanding the pharmacology of pain and analgesia that have resulted from the application of molecular biology techniques and the development of selective ligands for the various receptor classes involved in nociceptive transmission, have established that pain is an extremely complex and dynamic process involving multiple, interrelated neurotransmitter/neuromodulator systems in the spinal cord, in ascending and decending spinal pathways, and at supraspinal sites.

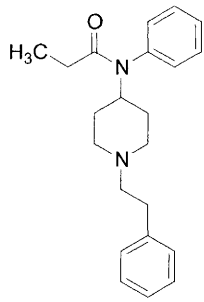
The identification of novel compounds which more effectively treat both acute and chronic pain states, and which lack side-effects associated with current therapies, remains a major challenge in biomedical and pharmaceutical research. Over the last two decades analgesia research has focused largely on identifying safer NSAIDs resulting in the current COX-2 inhibitors and safer opioids.

This situation was commented on in a review article as follows (Williams et al., 1999):

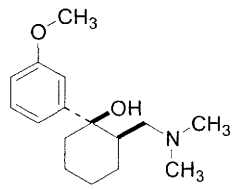
'Despite an intensive research effort over the past two decades involving many innovative approaches in the global academic community and by the pharmaceutical industry, the latter representing an aggregate investment in excess of $ 2.5 billion, the only new opioid-based pain medications either in clinical development or on the market are alternative dosage forms of the classical opioids, morphine, loperamide, and fentanyl, or compounds such as tramadol.'



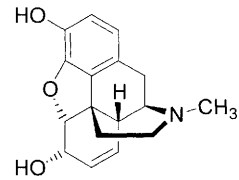
Loperamide



Fentanyl



Tramadol



Morphine

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Part II

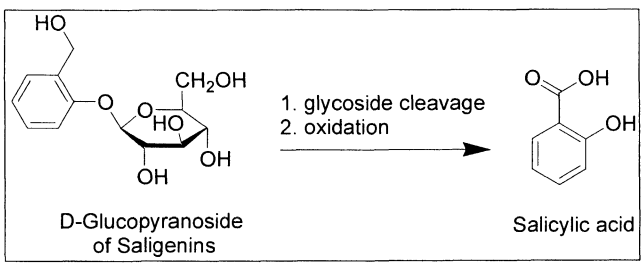
Pain Therapy Today

2 Cyclooxygenase Inhibiton: From NSAIDs to Selective COX-2 Inhibitors

Thomas Christoph and Helmut Buschmann

History of NSAIDs

For many decades the pain relieving properties of willow bark extracts have been used in folk medicine (Fig.1). Glycoside cleavage and oxidation is necessary for the biosynthesis of salicylic acid from the plant precursor β-D-glucopyranoside, the so-called saligenins.



Scheme 1: Biosynthesis of salicylic acid.

The active principle was shown to be salicin by Buchner in 1828 but the bitter taste and damage to the gastric mucosa limited its use. Piria isolated salicylic acid from salicin in 1838. In 1859, Kolbe discovered the structure and synthesis of salicylic acid and in 1897 acetylsalicylic acid was synthesized by Hoffmann. Two years later, in 1899, acetylsalecylic acid, the first nonsteroidal anti-inflammatory drug was registered under the name Aspirin (Fig. 2).

More than 100 years later, acetylsalicylic acid is still the best-known nonsteroidal anti-inflammatory drug and is used in almost every household for the treatment of mild to moderate pain states and fever. Despite the fact that there had already been a long period from the initial use of aspirin to ist introduction as a registered drug, it took

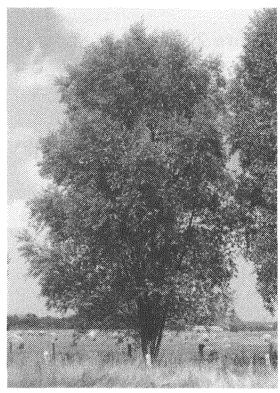


Figure 1: Willow (*Salix alba*).

NSAIDs in Plants?

Salicylic acid and related molecules are involved in endogenous defence mechanisms (Dong, 2001). Infection with tabac mosaic virus leads to an induction of salicylic acid in the infected leafs of a tabac plant (Hennig et al., 1993).

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another 70 years until the mechanism of action of acetylsalicylic acid could be elucidated. In 1971, it was shown that the analgesic action of nonsteroidal anti-inflammatory drugs is due to the inhibition of the enzymatic production of prostaglandins (Vane, 1971).

Cyclooxygenase (COX), one of the two activities of prostaglandin endoperoxide synthase (PGHS), is the key enzyme in the conversion of arachidonic acid derived from lipids of the cell membrane to prostaglandins and other eicosanoids (Fig. 3).

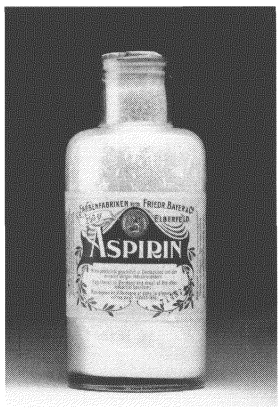


Figure 2: Historical bottle of Aspirin.

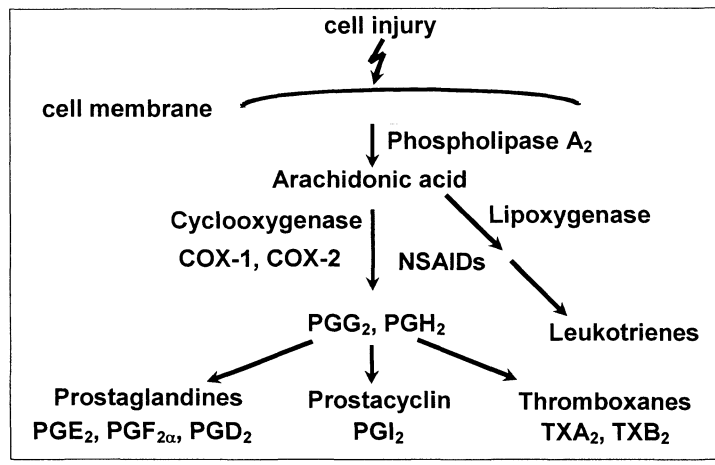


Figure 3: Simple schema of the biosynthesis of prostaglandins (PG), thromboxanes (TX), and leukotrienes from arachidonic acid consequent to cell injury (modified after Bonica, 1990).

Prostaglandin Receptors and Pain

EP1 receptor knock-out mice show reduced pain sensitivity, suggesting an important role for this receptor subtype in pain perception (Stock et al., 2001). Furthermore these mice show a significant reduction in systolic blood pressure and an increased renin angiotensin activity, suggesting a role also in cardiovascular homeostasis.

The eicosanoids (Fig. 4) are important mediators in pain and inflammation leading to hyperalgesia by sensitization of nerve fibers and fever. Furthermore they fulfill important roles in the protection of the gastric mucosa, in platelet aggregation and maintenance of normal kidney function (Vane et al., 1998). The diversity of physiological functions of the eicosanoids is reflected by a variety of different receptors. Five main receptor types have been described, designated DP, FP, IP, TP, and EP which show the greatest apparent affinity for PGD, PGF, PGI_2, TXA_2, and PGE, respectively (Pierce et al., 1995). PGE_2, the eicosanoid which plays a key role in pain perception, exerts this and other functions via the four subtypes of the EP receptors, EP1 to 4.

Isoforms of Cyclooxygenase

Two different isoforms, COX-1 and COX-2, encoded by two genes have been cloned and sequenced (Table 1; Funk et al., 1991; Kujubu et al., 1991; Hla and Neilson, 1992; O'Banion et al., 1992). The cloning of the two isoforms led to further characterization of the enzymes by means of a whole range of molecular biology tools from experiments using knock-out mice (Dinchuk et al., 1995; Langenbach et al., 1995; Morham et al., 1995) to protein structure determination (Picot et al., 1994; Kiefer et al., 2000).

COX-1 is constitutively expressed in many cells of the body and responsible mainly for the production of eicosanoids serving normal physiological functions. One important physiological role is the protection of the gastric mucosa. Inhibition of COX-1 therefore often brings pain relief together with gastrointestinal side-effects.

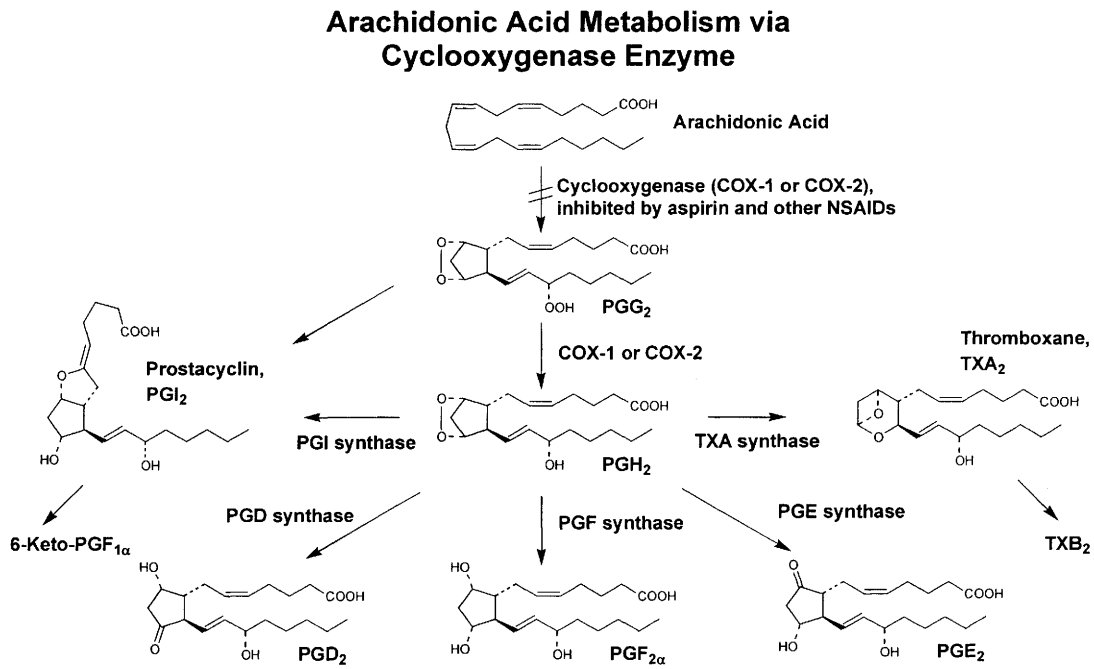


Figure 4: Chemical structure of eicosanoids.

COX-2 expression is induced during inflammation and is thought to be responsible for the production of eicosanoids

in inflammatory conditions related to fever and pain. Furthermore, COX-2 is expressed in the central nervous system and might play a more direct role in central pain processing.

The characteristic differences in expression of the two COX isoforms suggest a potential for new drugs addressing inflammatory and painful conditions specifically via inhibition of COX-2. This analgesic and anti-inflammatory potential should come without the well-known gastrointestinal side-effects of classical NSAIDs which target COX-1 in combination with COX-2 or alone. As usual, nature is not that simple. COX-1 expression is also subject to regulatory processes and can be increased in inflammatory conditions (Crofford et al., 1994). COX-2 expression on the other hand is increased in the gastric mucosa by inflammatory stimuli and *Helicobacter pylori* infection (Sawaoka et al., 1998, McCarthy et al., 1999). Finally, analysis of COX-1 and COX-2 knock-out mice indicate that both isoforms can contribute to an inflammatory response and have significant roles in the maintenance of physiological homeostasis and in carcinogenesis (Langenbach et al., 1999). These data suggest that the initial roles attributed to COX-1 and COX-2 under normal and inflammatory conditions, respectively, should be re-assessed.

Ultimately, comparison of clinical data from the COX-2 selective drugs with those of the classical NSAIDs will answer the question of whether or not COX-2-inhibitors will take a leading position within the NSAIDs.

Table 1: Comparison of COX-1 and COX-2 (Dannhardt and Laufer, 2000).

Parameter	COX-1	COX-2
Sequence identity	60 %	
Gene size	22 kb	8.3 kb
Exons	11	10
Molecular weight	67,000 Da	72,000 Da
Chromosome	9q32-q33,3	1q25,2-q25,3
MRNA	2.8 kb	4,1 kb
mRNA regulation	Constitutive	Inducible (>50 fold)
Inducers	-	Cytokine, LPS, Phorbolester
Amino acids	599	604
Localization	Nuclear membrane, ER	Nuclear membrane, ER

Table 1: continued

Parameter	COX-1	COX-2
Co-factors	1 Mol Heme	1 Mol Heme
Glycosylation	-N, 3 site	-N, 3 or 4 site
Acetylsalicylic acid (ASS)-acetylation site	Ser 529	Ser 516
Substrate specificity	Arachidonic acid (AA) γ-linolenic acid	Arachidonic acid (AA) γ- linolenic acid, eicosapentenoic acid
Activity	23 mmol AA/mg/min	11 mmol AA/mg/min
Role of COX-1 and COX-2	Homeostatic functions • Protection of normal gastric mucosa • Platelet function • Renal	Pathological functions a) Inflammation, pain, fever b) Dysregulated proliferation Physiological functions 1. Tissue repair 2. Vasculature 3. Reproduction 4. Renal 5. Bone 6. Islet cells 7. Lung 8. Brain

AA, arachidonic acid; LPS, endotoxin lipopolysaccharide; ER, endoplasmic reticulum.

Classification of NSAIDs According to their Chemical Structure

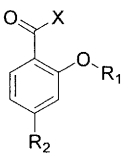
Many nonsteroidal anti-inflammatory drugs of different chemical structures (Fig. 5) have been introduced for the treatment of inflammatory and painful conditions. Many years of clinical experience with these drugs have shown that there is no induction of tolerance or dependence and no respiratory depression as seen with opioids. The major side-effects of these compounds with COX-1 selectivity or balanced COX-1 and COX-2 inhibition are damage to the gastric mucosa, prolongation of bleeding time and renal failure.

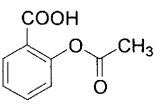
According to their chemical structure the following classification of the classical NSAIDs can be made:

- Salicylates
- Anthranilates
- Arylacetic acids
- Arylpropionic acids

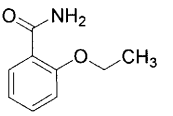
- Pyrazolinone derivatives
- Para-aminophenol derivatives
- Acidic enolic compounds (pyrazolidine-3,5-diones, oxicams)

Salicylates

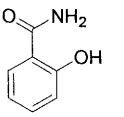




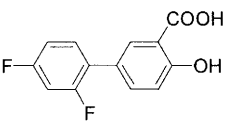
Acetylsalicylic acid



Ethenzamide

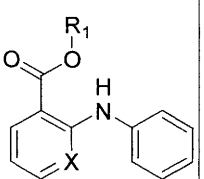


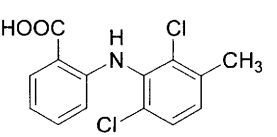
Salicylamide



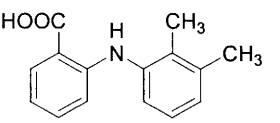
Diflunisal

Anthranilates

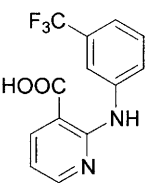




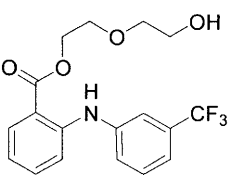
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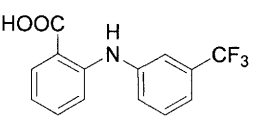
Mefenamic acid



Niflumic acid

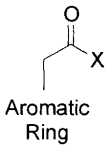


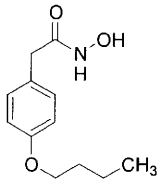
Etofenamate



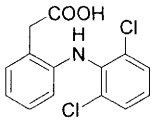
Flufenamic acid

Arylacetic Acids

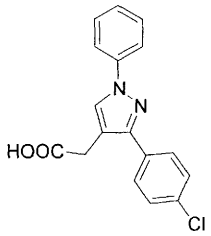




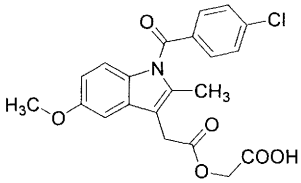
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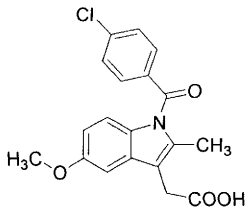
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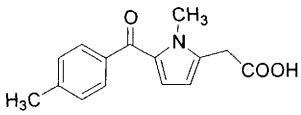
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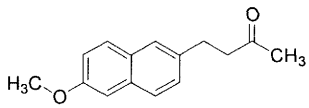
Acemetacin



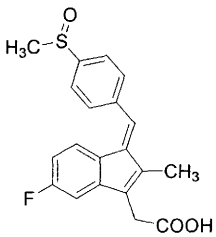
Indomethacin



Tolmetin

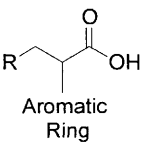


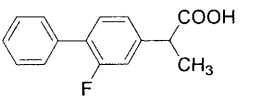
Nabumetone



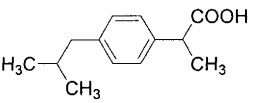
Sulindac

Arylpropionic Acids

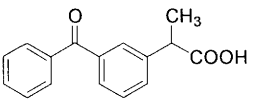




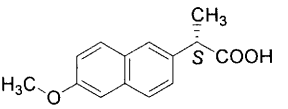
Flurbiprofen



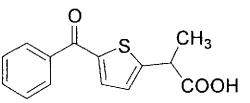
Ibuprofen



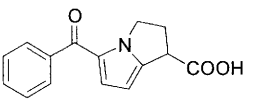
Ketoprofen



Naproxen

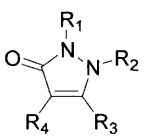


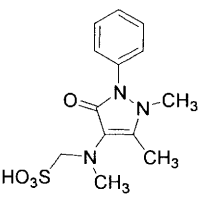
Tiaprofenic acid



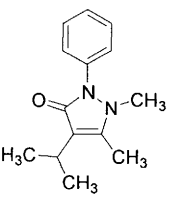
Ketorolac

Pyrazolinone Derivatives



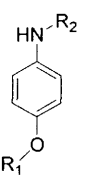


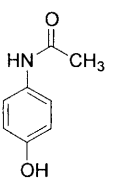
Metamizol



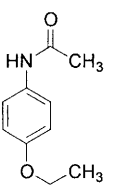
Propyphenazone

Para-Aminophenol Derivatives

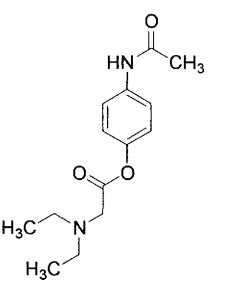




Paracetamol

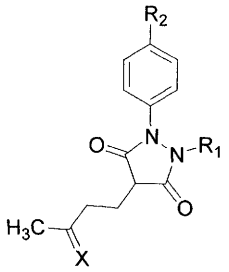


Phenidine

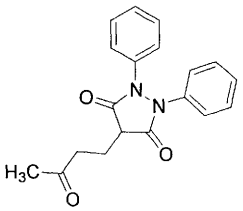


Propacetamol

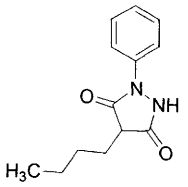
Acidic Enolic Compounds



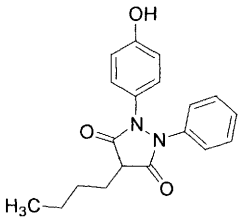
Pyrazolidine-3,5-diones



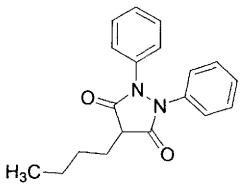
Kebuzone



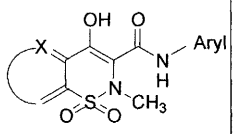
Mofebutazone



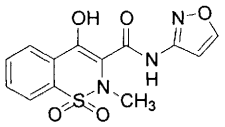
Oxyphenbutazone



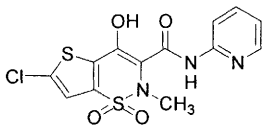
Phenylbutazone



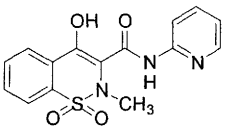
Aryl-sulfonamides (Oxicams)



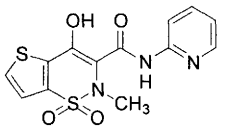
Isoxicam



Lornoxicam



Piroxicam



Tenoxicam

Figure 5: Chemical diversity of NSAIDs.

The Development of COX-2 Selective Inhibitors

Classification According to COX Isoenzyme Selectivity

The discovery of the inducible isoform led to the identification of drugs that show a stronger inhibition of COX-2 compared to COX-1 (Table 2).

Table 2: COX-2 selective inhibitors.

Group	Compound	COX-2 Selectivity (enzyme)	COX-2 Selectivity (whole blood)
first generation	etodolac	n.d.	10 [1]
	meloxicam	75 [2]	11.2 [1]
	nimesulide	n.d.	18 [1]
second generation	celecoxib	375 [3]	7.6 [4]
	rofecoxib	76 [5]	75 [6]
third generation	etoricoxib	>20 [4]	105 [4]
	valdecoxib	28000 [6]	30 [4]
	parecoxib	valdecoxib prodrug	valdecoxib prodrug

Selectivity of inhibition of COX-2 vs. COX-1 based on enzyme assays and whole blood cellular assays (IC50 COX-1/IC50 COX-2)

(1) Patrignani et al. (1997)
(2) Churchill et al. (1996)
(3) Penning et al. (1997)
(4) Riendeau et al. (2001)
(5) Chan et al. (1999)
(6) Warner et al. (1999)
(7) Talley et al. (2000a)

COX Inhibition Assays
Inhibition of COX can be quantified in recombinant or natural enzyme preparations, cellular systems, isolated human cell populations such as platelets (COX-1) and white blood cells (COX-2), or in *ex vivo* stimulated whole blood samples The closer the experimental system is to the physiological state, the lower the selectivity for most COX-2-inhibitors. The standard test for comparison is considered to be a whole blood assay which mimics *in vivo* conditions like plasma binding (e.g. Patrignani et al., 1996). It is commonly accepted that reasonable variations occur between different laboratories (see data for Piroxicam). Therefore, whenever possible, data of several compounds generated with a given test system should be compared with each other.

Table 2 shows the classification of COX-2 selective inhibitors based mainly on their historical development. The first generation of COX-2 inhibitors such as meloxicam still shows inhibition of COX-1 in physiological plasma concentrations and hence, still generate the same gastrointestinal side-effects as COX-1 inhibitors although to a lower degree. These compounds were developed before the discovery of the inducible isoform of COX. The second generation COX-2 inhibitors, which have been identified by screening their inhibitory potentials on both COX isoforms, reached the market in 1999 with celecoxib and rofecoxib and shows a clear selectivity for COX-2 compared to COX-1 in enzyme preparations and whole blood assays. The gastrointestinal side-effects of these drugs seem to be clearly lower than those of classical NSAIDs such as naproxen. Nevertheless, the FDA has so far rejected claims of reduced side-effect profile of celecoxib. The third generation of COX-2 inhibitors is under development showing an even greater selectivity for COX-2.

The fastest-growing product in 2000 was Pharmacia/Pfizer's COX-2 inhibitor, Celebrex (Celecoxib), for osteoarthritis, which was launched in early 1999.

Celecoxib generated $ 2.4 billion in sales, up by 65 % from previous year (Table 3).

Table 3: The top ten selling drugs in 2000.

Product	2000 sales ($ billion)	% of global sales	% growth (vs. 1999)
Losec / Prilosec	6.1	1.9	+ 9
Lipitor	5.4	1.7	+ 44
Zocor	4.4	1.4	+ 15
Norvasc	3.3	1.1	+ 15
Orgastro / Prevacid	3.1	1.0	+ 33
Prozac	2.9	0.9	- 1
Seroxat / Paxil	2.4	0.8	+ 20
Zyprexa	2.4	0.8	+ 30
Celebrex	**2.4**	**0.7**	**+ 65**
Zoloft	2.2	0.7	+ 12

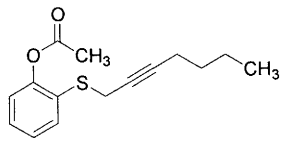
Source: IMS Health Word Reviews 2001, SCRIP No 2630 March 30th 2001 p. 18

Including the classical NSAIDs in a classification based on isoenzyme selectivity, it turns out that beside low dose aspirin there is no COX-1 selective inhibitor on the market. Instead, most of the classical NSAIDs belong to the group of nonselective COX inhibitors.

- **Selective COX-1 inhibitors** (low dose aspirin)
- **Nonselective COX inhibitors** (e.g. high dose aspirin, indomethacin)
- **Preferential COX-2 inhibitors** (e.g. meloxicam)
- **Highly selective COX-2 inhibitors** (e.g. celecoxib, second and third generation of COX-2 inhibitors, sometimes called coxibs)

Classification According to Drug-Protein Interactions

Another classification is based on the mode of interaction between drug and enzyme (Kurumbail et al., 1996).



APHS (2-**a**cetoxy-**p**henyl-**h**ept-2-ynyl **s**ulfide)

- **Irreversible inhibitors of COX-1** (aspirin) **or COX-2** (2-acetoxyphenylhept-2-ynyl sulfide, APHS; Kalgutkar et al., 1998). Aspirin and APHS acetylate the amino acid serine so that endogenic arachidonic acid is prevented from reaching the catalytic center of the enzymes.
- **Reversible, competitive inhibitors of COX-1 and COX-2.** Inhibitors such as ibuprofen, piroxicam or mefenamic acid compete against arachidonic acid to bind at the catalytic center.
- **Slow, time-dependent, reversible inhibitors of COX-1 and COX-2**. E.g. indomethacin and flurbiprofen seem to act by ionic interactions between their carboxylic acid function and the arginine residue of the enzyme. This effect seems to influence the helix D of the protein followed by a significant loss of flexibility in the enzyme protein.
- **Slow, time-dependent irreversible inhibitors of COX-2** (e.g. celecoxib and rofecoxib) The last group shows a weak competitive inhibition of COX-1 which is of minor clinical relevance compared to the slow, time-dependent COX-2 inhibition.

Chemical Classification of Selective COX-2 Inhibitors

The large number of newly developed COX-2 inhibitors demonstrates how promising this field of anti-inflammatory agents is expected to be. More than 1000 COX-2 inhibitors have been described over the past few years (Prous database, March 2002). The chemical structures of COX-2 inhibitors are heterogenic. Contrary to the classical NSAIDs, this new class of enzyme inhibitors lacks a carboxylic acid group, thus effecting COX-2 affinity by a different orientation within the enzyme without formation of a salt bridge in the hydrophobic channel of the enzyme.

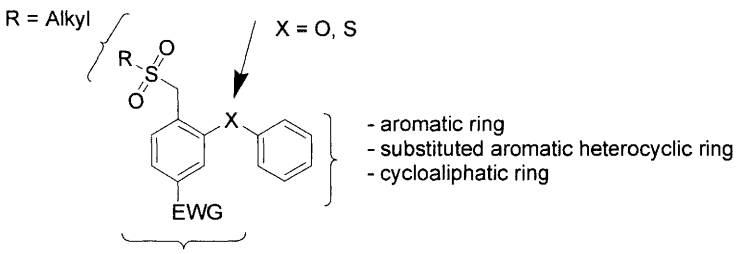
Most of the selective COX-2 inhibitors belong to one of the following different structural classes (Thalley et al., 1999; Dannhardt and Kiefer, 2001; Jiménez et al., 2000):

- **Diaryl- or aryl-heteroaryl ethers and thioethers** (sulfonanilide inhibitors) such as nimesulide or flosulide
- **Heterocycles or carbocycles with vicinal diaryl substitution**, e.g. celecoxib and rofecoxib
- **Modified, known NSAIDs to improve COX-2 selectivity**: L-748780, L-761066, meloxicam, etodolac
- **Antioxidative compounds**

- **1,2-Diarylethylene derivatives** (*cis*-stilbenes)
- **Miscellaneous structures**

Diaryl- and Aryl-Heteroaryl Ether and Thioether

Diaryl- and aryl-heteroaryl ether and thioether compounds belong to the first generation of selective COX-2 inhibitors (Fig. 6). One of the first COX-2 inhibitors was compound NS-398 wich has a completely different structure from classic NSAIDs.The compound showed inhibition of prostaglandin synthesis in inflammatory cells and was largely free of unwanted gastrointestinal effects in animal models. Moreover, NS-398 did not affect prostaglandin production in the stomach or kidney. On recognizing that NS-398 was a more or less preferential selective inhibitor of COX-2, new interest in this class of anti-inflammatory agents evolved. Nimesulide and flosulide are two other compounds with a diaryl ether and thioether structure, respectively, which bear a methansulfonanilide moiety. The sulfonamide structure with its NH-acidity in all these compounds seems to be obligatory.



Scheme 2: General structure of a sulfonanilide COX-2 inhibitor (first generation scaffold).

Carbocycles and Heterocycles with Vicinal Aryl Substitution (Second Generation of COX-2 Inhibitors)

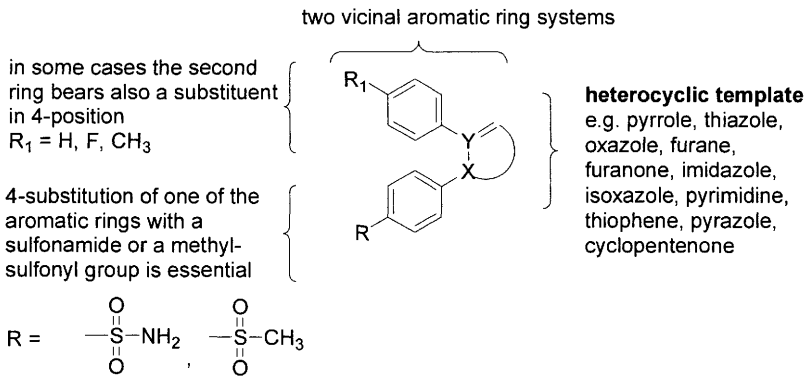
By far the greatest amount of research in the area of COX-2 inhibition has been carried out in the preparation and evaluation of this class of compounds. The compounds are characterized by a central carboxylic or heterocyclic ring system bearing two vicinal aryl moieties. These compounds represent the most important group of COX-2 inhibitors. During the last few years a large number of compounds has been developed as potential candidates.

Celecoxib, rofecoxib, valdecoxib, parecoxib sodium, and etoricoxib all belong to this chemical class.

The diaryl heterocycles described in the last 2 years can be classified into the following subcategories according to the nature of their linkages (Fig. 7; Jiménez et al., 2000):

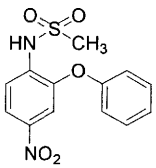
- Diaryl heterocyles linked via a 4-membered ring
- Diaryl heterocyles linked via a 5-membered ring
- Diaryl heterocyles linked via a 6-membered ring
- Diaryl heterocyles linked via a fused-ring system

It is assumed that the heterocyclic core structure is responsible for the appropriate orientation of the aromatic rings in space and finally for binding to the enzyme. A wide variety of heterocycles can serve as templates, i.e. pyrrole, thiazole, oxazole furane, furanone, imidazole, isoxazole, pyrimidine and thiophene, but at the moment pyrazole and cylopentenone seem to be the most appropriate for achieving COX-2 specificity. For optimal activity, one aromatic ring must be substituted with a methylsulfonyl or a sulfonamide substituent in the para position. Substitution at position 4 of one of the aromatic systems with a sulfonamide or a methylsulfonyl group is essential for COX inhibition. Replacement of the methylsulfonyl group by a sulfonamide group reduces COX-2 selectivity but improves oral bioavailability.

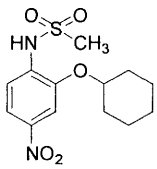


Scheme 3: General structure of carbocycles and heterocycles with vicinal aryl substituents. The structural pre-requisites shown are obligatory for enhanced activity towards COX-2.

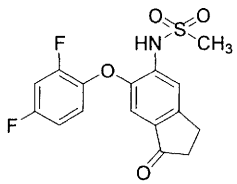
Diaryl- or aryl-heteroaryl-ethers and thioethers



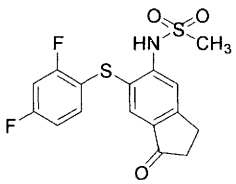
Nimesulid



NS-398



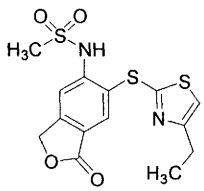
Flosulid



L-745337

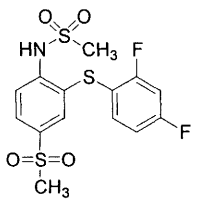
COX-1: IC_{50} > 30 μM

COX-2: IC_{50} = 9.67 μM



COX-2: IC_{50} = 0.005 μM

COX-1: IC_{50} > 100 μM

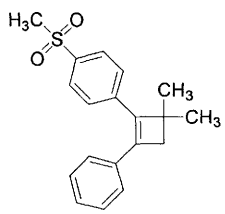


(Nycomed Austria)

Figure 6: Chemical structures of diaryl- or aryl-heteroaryl-ethers and thioethers

Carbocycles and heterocycles with vicinal diaryl substituents

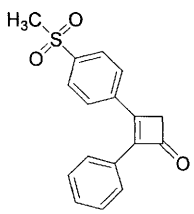
- Diaryl heterocycles linked via a 4-membered ring



COX-1: IC_{50}=0.12 μM

COX-2: IC_{50} = 0.002 μM

COX-2/COX-1 = 0.017

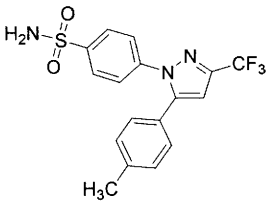


COX-1: IC_{50} > 5 μM

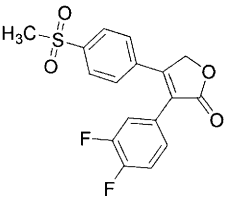
COX-2: IC_{50} = 0.11 μM

COX-2/COX-1 < 0.022

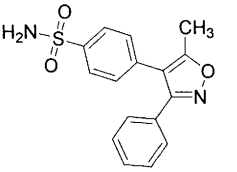
- Diaryl heterocycles linked via a 5-membered ring



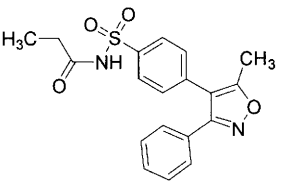
Celecoxib (Celebrex)



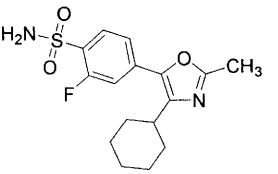
Rofecoxib (Vioxx)



Valdecoxib



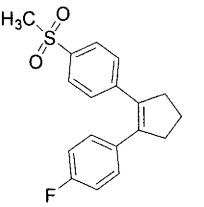
Parecoxib



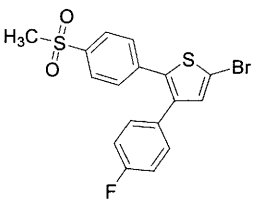
JTE-522

COX-2: IC_{50} = 0.085 μM

COX-1: IC_{50} = 0.64 μM



SC-57666

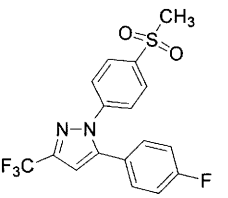


DuP-697

COX-1: IC_{50} = 1.18 μM

COX-2: IC_{50} = 0.06 μM

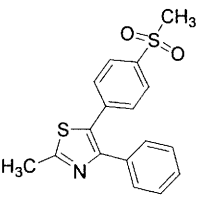
COX-2/COX-1 = 0.051



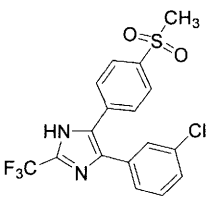
SC-5812 5

COX-1: IC_{50} > 30 μM

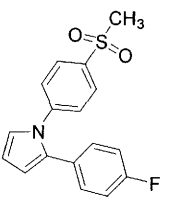
COX-2: IC_{50} = 2.25 μM



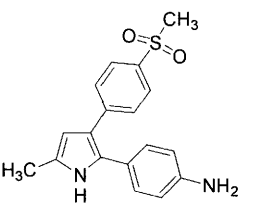
(GD Searle & Co)



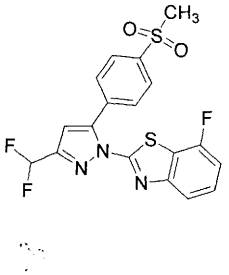
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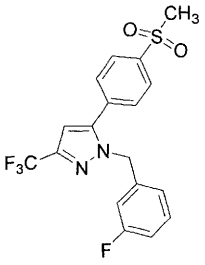
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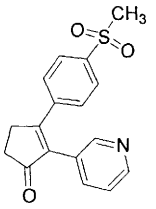
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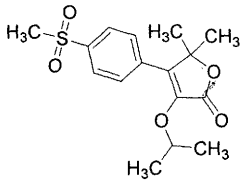
(Grelan Pharmaceutical)



(Fujisawa Pharm.)



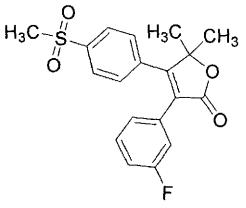
L-784506



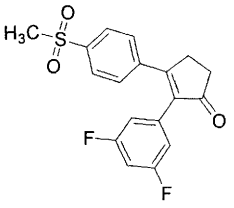
DFP

COX-2: IC_{50} = 0.3 μM

COX-1: IC_{50} = 3 μM



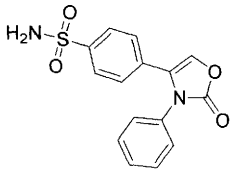
DFU



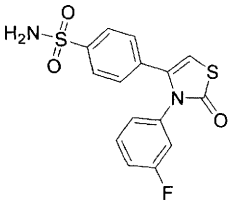
L-776967

COX-2: IC_{50} = 0.03 μM

COX-1: IC_{50} = 0.1 μM



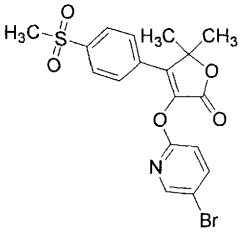
(Almirall Prodesfarma)



(Laboratoires UPSA)

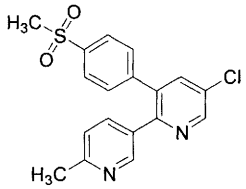
COX-2: IC_{50} = 0.06 μM

COX-1: IC_{50} = 328 μM

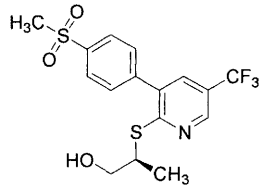


L-778736

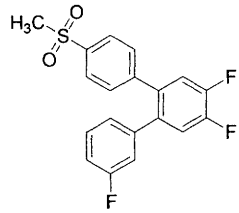
- Diaryl heterocycles linked via a 6-membered ring



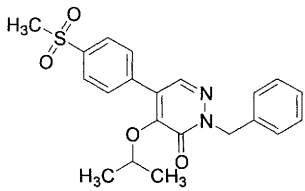
Etoricoxib



(Merck Frosst Canada)

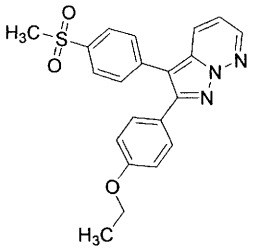


COX-1: IC_{50} = 5.5 μM
COX-2: IC_{50} = 0.002 μM
COX-2/COX-1 = 0.00036

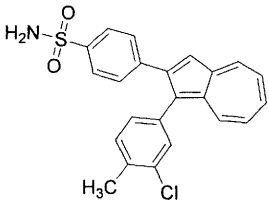


L-804600

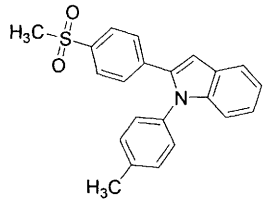
- Diaryl heterocycles linked via a fused ring system



(Glaxo Group)
COX-2: IC_{50} = 0.003 μM
COX-1: IC_{50} = 90 μM



(Kotobuki Seiyaku)
COX-2: IC_{50} = 0.0026 μM
COX-1: IC_{50} = 4.2 μM



(Laboratoires UPSA)

Figure 7: Chemical structures of heterocycles or carbocycles with vicinal diaryl substitution.

Metabolism Considerations in the Discovery and Selection of COX-2 Inhibitors

Compound DuP-697 with a bromo-substituted thiophene ring is a typical representative which fulfills these pre-requisites. However, the clinical data obtained from DuP-697 showed a very long plasma half-life of 242 h in humans as a result of its enterohepatic recirculation and rendered in unacceptable for further evaluation. Today this compound serves as a pharmacological tool.

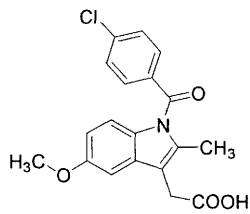
A continuous effort was made in chemical research to develop a second generation of COX-2 inhibitors, structurally different from existing ones, that would show very high COX-2 selectivity, a suitable pharmacokinetic profile and *in vivo* efficacy in animal models. Based on these properties, DFP (5,5-dimethyl-3-(2-isopropyl)-4-(4-methanesulfonyl-phenyl)-2-(5*H*)-furanone and DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5*H*)-furanone) were selected for human clinical trials (Chauret et al., 2001). However, during phase I clinical studies, both of these compounds showed poor pharmacokinetic characteristics in humans: DFP had a very long half-life (64 h) while DFU exhibited pharmacokinetics that varied significantly from individual to individual (12.2 h to > 72 h). In addition, pharmacokinetic studies in rats showed that the clearance of DFP was significantly increased upon multiple dosing. The basis of these pharmacokinetic behaviors was investigated through *in vitro* metabolic studies. It was found that DFP was poorly metabolized in human microsomes and hepatocytes, and a low rate of metabolism *in vivo* probably accounts for the very long half-life. *In vivo* studies in hepatocytes indicated that DFP induces its own metabolism in the rat, probably through the induction of CYP3A, and this phenomenon was related to the faster clearance of DFP upon multiple dosing. Regarding the metabolism of DFU, it was discovered that the compopund was metabolized by a single, polymorphic cytochrome P450 (CYP2C19), which explains the variable pharmacokinetics obtained *in vivo*.

In light of these observations, it was considered critical that the metabolic fate of any potential second generation COX-2 inhibitor should be carefully examined as early as possible.

Modification of Known NSAIDs and Compounds Without Common Structural Features

Modifying well-known NSAIDs into more selective COX-2 inhibitors represents an interesting strategy (Dannhardt and Laufer, 2000). However, the methodology utilized in NSAID modification does not follow a general scheme.

Modification of indomethacin



Indomethacin

COX-2: IC_{50} = 0.96 μM (human)

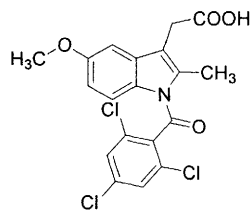
COX-1: IC_{50} = 0.08 μM (human)

Classic NSAIDs such as indomethacin possess both COX-1 and COX-2 inhibiting affinity. Various attempts have been made to shift the enzyme selectivity of indomethacin from COX-1 to COX-2 while keeping the potency at the same level and reducing the unwanted side-effects at the same time. In principle, the strategy consisted of introducing larger substituents to fit the active site volume of COX-2 resulting in compounds like L-748780. Introducing a larger trichlorobenzoyl analog instead of the chlorobenzoyl analog optimized COX-2 selectivity. Altering the side chain by introducing a beta-branched butyric acid and replacing the benzoyl group of indomethacin with a 4-bromo benzyl-substituent finally produced compound L-761066 which has a high potency and remarkable selectivity (Black et al., 1996; Leblanc et al., 1996) Transformation of the aryl acetic acid moiety of indomethacin to esters or amides produces molecules capable of binding tightly to COX-2 but not COX-1 (Kalgutkar, 2000a).

Abbott (Brooks et al. (Abbott), 1998a; Woods et al. (Abbott), 1998) published two patent applications in which the carboxyl moiety of indomethacin was substituted with an iminooxy or substituted thiazole. On the other hand, Kotobuki Seiyaku (Tomiyama et al. (Kotobuki Seiaku Co. LTD), 1998) used a novel approach, exchanging the indole nucleus for an azulene (Jiménez et al., 2000).

Conversion of indomethacin to selective COX-2 inhibitors

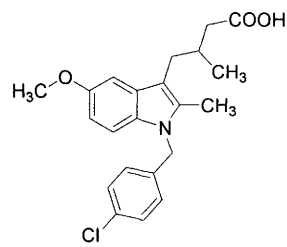
Some of the most potent and selective indomethacin derivatives are shown



L-748780

COX-2: IC_{50} = 0.06 μM

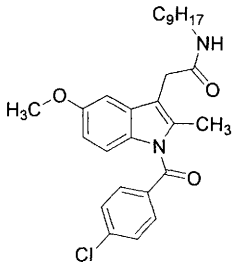
COX-1: IC_{50} > 10 μM

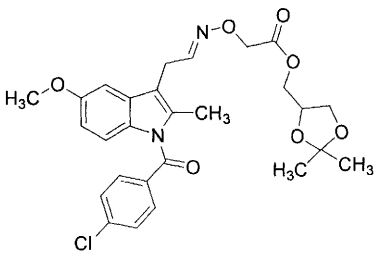


L-761066

COX-2: 0.04 μM (human)

COX-1: 66 μM (bovine)

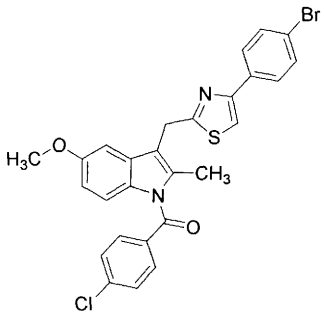


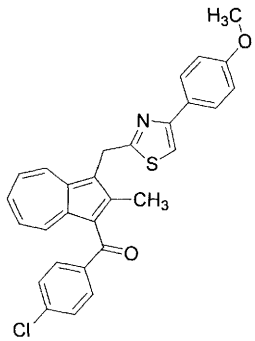


(Abbott Laboratories)

COX-2: IC_{50} = 0.0003 μM

COX-1: IC_{50} > 100 μM





(Abbott Laboratories)

COX-2: IC_{50} = 0.003 μM

COX-1: IC_{50} = 0.09 μM

(Kotobuki Seiyaku)

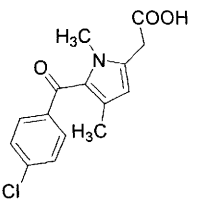
COX-2: IC_{50} = 0.0008 μM

COX-1: IC_{50} > 100 μM

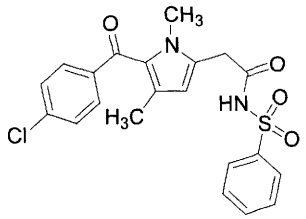
Figure 8: Modification of known NSAIDs.

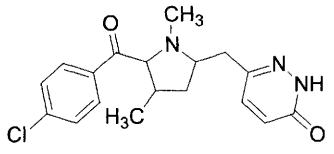
A similar strategy was used for the modification of zomepirac, basically a COX-1 selective drug. The desired COX-2 selectivity was achieved by replacing the acetic acid group by other moieties such as a pyridazinone ring or an N-acyl aminosulfonyl phenyl group to yield RS-57067 and RS-1048934, respectively (Barnett et al., 2000)

Modification of zomepirac



zomepirac





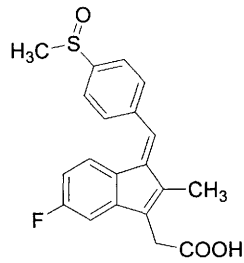
RS-1048934

RS-57067

Figure 9: Modification of zomepirac.

Modification of sulindac

American Home Products (Failli et al. (American Home Products), 1998a; 1999) has described analogs of sulindac that act as selective COX-2 inhibitors, where a tetronic acid moiety is used as an isosteric replacement for the carboxylic acid group (Jiménez et al., 2000).

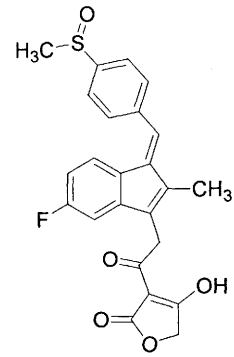


Sulindac

COX-2: IC_{50} = 10.43 μM

COX-1: IC_{50} = 1.02 μM

(sulfide derivative, active metabolite)



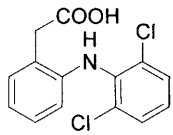
(American Home Products)

COX-2: IC_{50} = 0.027 μM

COX-1: IC_{50} = 10 μM

Figure 10: Modification of sulindac.

Modification of diclofenac

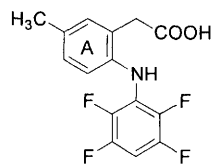


diclofenac

COX-2: IC_{50} = 0.05 μM

COX-1: IC_{50} = 0.14 μM

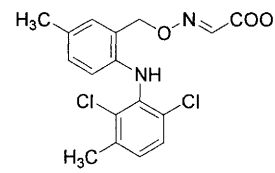
Two different strategies have been used in the modification of diclofenac. First Novartis (Fujimoto et al. (Novartis), 1999) described the substitution of ring A of diclofenac with an alkyl group resulting in a compound, which has very good COX-2 potency and selectivity, while Abbott Laboratories (Brooks et al. (Abbott), 1998b; 1999), using a modification of the acidic moiety, obtained moderately selective inhibitors (Jiménez et al., 2000).



(Novartis)

COX-2: IC_{50} = 0.007 μM

COX-1: IC_{50} = 30 μM



(Abbott Laboratories)

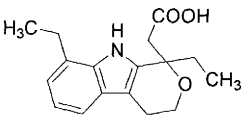
COX-2: IC_{50} = 0.022 μM

COX-1: IC_{50} = 0.85 μM

Figure 11: Modification of diclofenac.

Modification of etodolac

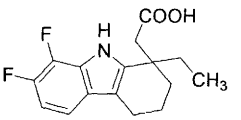
In 1995, etodolac (American Home Products) was shown to be a selective COX-2 inhibitor (Glaser et al., 1995). An increase in selectivity was achieved by replacing the oxygen with a methylene group at the 4-position of the pyran ring, by transformation of the ketone into an oxime or by complete reduction of the pyran ring (Failli et al. (American Home Products), 1998b; Kreft et al. (American Home Products), 1998a; 1998b; Jiménez et al., 2000).



etodolac

COX-2: $IC_{5\ 0}$= 0.041 μM

COX-1: IC_{50} = ~50 μM



(American Home Products)

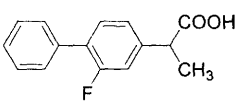
COX-2: IC_{50} = 0.7 μM

COX-1: IC_{50} = 23 μM

Figure 12: Modification of etodolac.

Modification of flurbiprofen

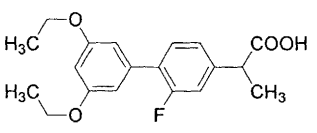
Flurbiprofen has been successfully modified by Merck Frosst using comparative computer modeling studies of the X-ray crystal structures of COX-1 and COX-2 (Bayly et al., 1999, (Merck Frosst), 1999). Optimal selectivity was conferred by a 3-atom lipophilic substitution at the 3′ position of the unsubstituted phenyl ring. The most effective analog was obtained by introducing two ethoxy groups at the 3′and 5′position of flurbiprofen, yielding a compound, which has 77-fold greater selectivity than the parent compound (Jiménez et al., 2000).



Flurbiprofen

COX-2: IC_{50} = 6.42 μM

COX-1: IC_{50} = 0.41 μM



(Merck Frosst)

(*S*)-Flurbiprofen (Dannhardt and Kiefer, 2001)

COX-2: IC_{50} = 2.56 μM

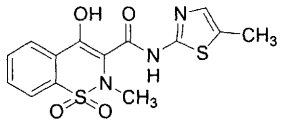
COX-1: IC_{50} = 0.29 μM

Figure 13: Modification of flurbiprofen.

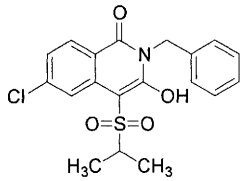
Modification of meloxicam

Meloxicam was discovered to be an anti-inflammatory agent with relatively few gastrointestinal side-effects, when compared with other NSAIDs (Ogino et al., 1997). New attempts to modify the modestly selective COX-2 inhibitor led to a new series of isoquinoline-1,3-diones, which are orally active COX-2-selective inhibitors (Lazer et al. (Boehringer Ingelheim), 1997; 1998). The most

representative example of this series is BIRL-790 (Boehringer Ingelheim).



melioxicam



BIRL-790

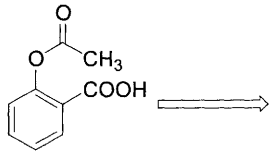
COX-2: IC_{50} = 0.06 μM

COX-1: IC_{50} = 1.44 μM

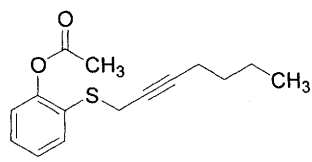
Figure 14: Modification of melioxicam.

APHS as an aspirin derivative

Aspirin is the only known NSAID that covalently bonds to serine and inhibits COX-1 more significantly than COX-2. Many systematic structural modifications have been carried out resulting in the development of APHS characterized by a 60-fold increase in activity and a 100-fold increase in selectivity for COX-2 than aspirin. Inhibition of COX-2 also occurs by acetylation of the same serine residue that is acetylated by aspirin, indicating that the mechanism of APHS inhibition is not identical to that of other selective COX-2 inhibitors (Kalgutkar et al., 1998a; 1998b).



aspirin



APHS
(Vanderbilt University)

COX-2: IC_{50} = 0.8 μM

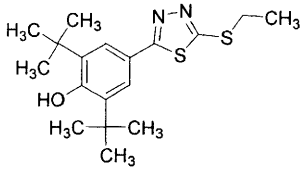
COX-1: IC_{50} = 17 μM

Figure 15: Modification of aspirin.

Compounds with Antioxidative Moieties

The mode of action of these compounds, which are under investigation, is via an antioxidative mechanism. Since COX enzyme catalysis involves radical intermediates, a radical scavenging moiety such as a di-*tert*-butylphenol interferes with the cyclooxygenase reaction. Linkage of phenolic substructure with a thiazolone, oxazolone, thiadiazole or oxadiazole derivative produces non-

ulcerogenic, orally active anti-inflammatory agents as a novel class of COX-2 inhibitors. The most potent and COX-2 selective compound of this class was the thiadiazole derivative (Song et al., 1999).



COX-1: IC_{50} > 100 μM

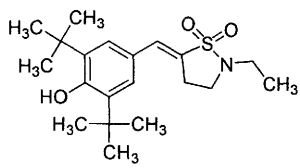
COX-2: IC_{50} = 0.14 μM

(purified enzymes)

Figure 16: COX-2 inhibitor with an antioxidative moiety.

Dual COX-2 and 5-LO Inhibitors

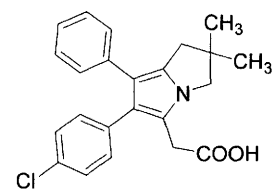
A new class of compounds is reported to have dual inhibitory properties. They have a γ-sultam skeleton and show potent inhibitory effects towards both COX-2 and 5-lipoxygenase as well as production of IL-1 in *in vitro* assays. These compounds have also proved to be effective in several animal arthritic models without any ulcerogenic activity. Among these compounds S-2474 ((*E*)-(5)-(3,5-di-*tert.*-butyl-4-hydroxy-benzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide) was selected as an anti-arthritic drug candidate and is now under clinical investigations (Inagaki et al., 2000).



S-2474

ML-3000 ((2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl)-acetic acid) is a non-antioxidant dual inhibitor of both cyclooxygenase and 5-lipoxygenase. ML-3000 has been compared to indomethacin in a number of experimental models of inflammation. The analgesic effects of ML-3000 have also been assessed in a number of animal models. Phase II studies have shown a wide range of activities, including anti-inflammatory, analgesic, antiplatelet and anti-asthmatic properties (Laufer et al., 1994; Chin and Wallace, 1999).

Non-antioxidant dual COX-2 and 5-LO inhibitors



ML-3000

COX: IC_{50} = 0.21 μM

5-LO: IC_{50} = 0.18 mM

Diarylethylene Derivatives (*cis*-Stilbene Compounds)

Reduction of the furanone ring led to active inhibitors with an open-ring diol structure. Ring opening and elimination of the heteroatom led to *cis*-stilbene derivatives which still contain the prerequisites for COX-2 inhibition: vicinal orientation of two aromatic rings, substitution pattern at the aryl moiety as seen in potent COX-2 inhibitors, i.e. a methylsulfonyl moiety in combination with a halogen. This group of compounds is presently undergoing biological testing (Dannhardt and Kiefer, 2001).

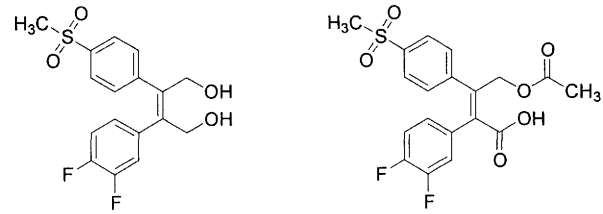
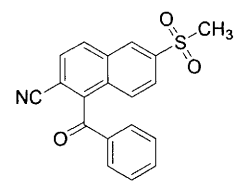


Figure 17: COX-2 inhibitors with a diarylethylene moiety.

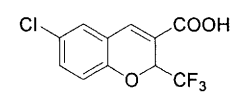
Miscellaneous Structures

Novel structural series that do not fit the above-mentioned categories have been included in the miscellaneous group. Hoffmann-La Roche (Rotstein and Sjorgen (Hoffmann-La Roche), 1998) has described aroylnaphthalene derivatives, which exhibit good *in vitro* activity and selectivity. Searle (Carter, 1998) has also disclosed a series of chromenes with good COX-2 selectivity and remarkable oral activity (57 % inhibition of paw edema at 30 mg/kg p.o.). Chugai Seiyaku claimed a series of indoles and diazaindoles, as well as substituted indenes. Interestingly, these compounds lack the alkanoic acid side chain typical of COX inhibitors (Matsuoka et al. (Chugai Seiyku Kabushiki Kaisha), 1998; 1999). Pfizer (Nakao et al. (Pfizer), 1999; Okumura et al. (Pfizer), 1999) has also reported indole derivatives as selective COX-2 inhibitors. Additionally, Pfizer modified the indole framework, yielding benzimidazole derivatives (Stevens et al. (Pfizer), 1999; Jiménez et al., 2000).

Computational and combinatorial chemistry methodology helped to create a highly selective phenothiazine derivative which can serve as a novel lead compound for further development in the field of COX-2 inhibitors (Dannhardt and Kiefer, 2001).



(Hoffmann La Roche)



(GD Searle & Co)

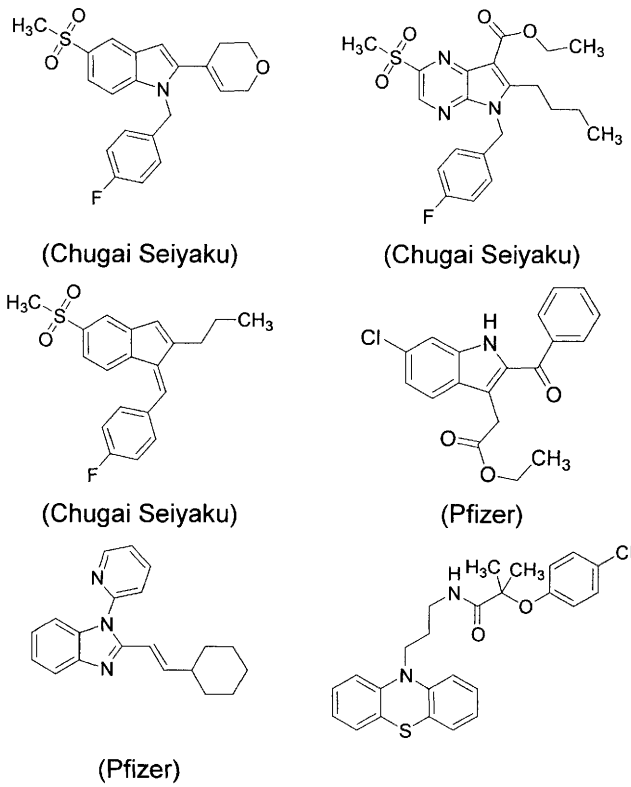


Figure 18: Miscellaneous structures.

Clinical Experiences with COX-2 Inhibitors

A variety of clinical studies have shown the efficacy of the new COX-2 inhibitors in several indications of pain and inflammation. Two large clinical studies have been performed in order to assess clinical efficacy and side-effects of rofecoxib (VIGOR trial) and celecoxib (CLASS trial), respectively (Fitzgerald and Patrono, 2001). Both studies show an increased gastrointestinal safety profile compared to the standard medication, although only rofecoxib shows a statistically significant improvement. In terms of cardioprotection, naproxen had a better outcome compared to rofecoxib. This may represent the negative aspects of the COX-1-sparing novel COX-2 inhibitors, since they do not inhibit platelet-derived thromboxanes, which are important in indications such as myocardial infarction. Likewise, the influence of COX-2 inhibition on renal function and hypertension has to be addressed in future studies, since COX-2 expression is essential for kidney development and function (Dinchuk et al., 1995; Komhoff et al., 2000).

Gastro-intestinal safety

The Vioxx Gastrointestinal Outcomes Research (VIGOR) trial (Bombardier et al., 2000) and the Celecoxib Long-Term Arthritis Safety Study (CLASS) trial (Silverstein et al., 2000) were designed to assess both efficacy and gastrointestinal side-effects. The VIGOR trial included 8076 patients with rheumatoid arthritis receiving either rofecoxib or naproxen. The CLASS trial comprises of a total of 8059 patients with rheumatoid arthritis or osteoarthritis in two studies, comparing celecoxib to diclofenac and ibuprofen, respectively.

Colon cancer

Celecoxib leads to a significant reduction in the number of colorectal polyps in patients with familial adenomatous polyposis in a 6-months study (Steinbach et al., 2000).

NSAIDs and COX-2 inhibitors show good potencies and efficacies in mild to moderate pain conditions and in inflammation. Many combinations of NSAIDs with other principles are on the market or under development. Misoprostol (on the market) and NO donors (under development) reduce the side-effects of NSAIDs on the gastric mucosa and opioid analgesics (standard WHO ladder of pain treatment) add to the analgesic potency of NSAIDs.

Alzheimer's disease

A prospective, population-based cohort study of 6989 patients aged 55 years or older, who were free of dementia at baseline, showed a beneficial effect of long-term treatment with NSAIDs (in't Veld et al., 2001).

In addition to the treatment of pain and inflammation, COX-2 inhibitors might be of benefit in other indications. Expression of COX-2 in colon cancer, intestinal adenomas, and other cancer cells as well as clinical studies with COX-2 inhibitors, suggest the use of COX-2 inhibitors in cancer (Masferrer et al., 1999). Expression of COX-2 in angiogenesis follows the same route since angiogenesis is important for blood supply and hence the growth of many tumors.

COX-2 expression was also found in brain areas related to memory (hippocampus, cortex) in patients with Alzheimer's disease (Ho et al., 1999, Yasojima et al., 1999). These findings together with clinical data suggest new options for the use of COX-2 inhibitors in this indication.

Aspirin has gained additional importance in the last few years due to its inhibition of platelet aggregation. Low dose treatment with aspirin leads to irreversible acetylation of COX-1 in platelets and is used in the acute treatment and chronic prevention of myocardial infarction and stroke (Higgs et al., 1987).

Additional Effects of COX-inhibitors

Some NSAIDs show anti-inflammatory and anti-proliferative effects independent of their COX activity. Modulation of intracellular signalling pathways might contribute to these activities (Tegeder et al., 2001). A key question with respect to those additional activities is whether or not concentrations of the respective compound at the presumed sites of actions is sufficient in order to add to the main mode of action, COX inhibition.

NSAIDs as Transcriptional Modulating Drugs

In addition to bioactive eicosanoids, the inflammatory response involves the sequential activation of various signaling pathways, including reactive oxygen intermediates, cytokines, growth factors, enzymes,

receptors, and adhesion molecules. Increased expression of most of these proteins is the result of enhanced gene transcription. Changes in gene transcription of proteins involved in inflammation are usually regulated by transcription factors such as modulators of activated T cells (NF-AT), nuclear factors (NF-κB), and activated protein (AP-1).

The role of glucocorticoids

Therefore, drugs able to inhibit transcription of genes involved in the inflammatory process could be potent anti-inflammatory agents. Thus among the most effective anti-inflammatory drugs are the glucocorticoids, whose actions occur by inhibition of the transcription of several genes which are responsible for the induction of cytokines, chemokines and COX-2 in response to inflammation. Inhibition of pro-inflammatory transcription factors such as AP-1, NF-AT or NF-κB is thought to be the major action of glucocorticoids

The role of other immunosuppressants such as cyclosporin A (CsA)

Another class of transcription-modulating drugs is the immunosuppressants such as cyclosporin A (CsA), which inhibit T cell activation and proliferation, events playing a central role in the immune response and therefore in the inflammatory process. CsA blocks transcriptional induction of cytokines by inhibiting the phosphatase calcineurin, and by the subsequent inhibition of the activation of NF-AT and NF-AT-dependent activation genes.

Cyclooxygenase-independent mechanisms of NSAIDs

Several studies have demonstrated that certain NSAIDs have anti-inflammatory and anti-proliferative effects that seem to be mediated through mechanisms independent of cyclooxygenase activity and prostaglandin production. These effects are generally mediated through inhibition of several transcription factors such NF-AT, NF-kB, or AP-1. It may be these properties of NSAIDs that add to the therapeutic benefits of these drugs in diseases such carcinogenesis and Alzheimer's disease. In most cases, the doses required to ameliorate chronic inflammatory diseases and tumor progression are much higher than those doses required to inhibit prostaglandin synthesis. In addition, NSAIDs which are poor inhibitors of COX-2 are able to reduce inflammation and hyperalgesia. The anitumor activity of NSAIDs such as inhibition of cell cycle progression, induction of apoptosis and inhibition of angiogenesis has become apparent at high concentrations of the drugs. This suggests that the effects of high doses of certain NSAIDs are mediated by cyclooxygenase-independent mechanisms. Some of these mechanisms decribed for NSAIDs are summarized below. The enzymatic inhibition of NSAIDs as well as the transcriptional regulation is shown in Fig. 19.

Summary of mechanisms of actions of NSAIDs as transcriptional regulators on the functional and molecular level

Functional level

- Inhibition of immune cell activation
- Inhibition of cytokine production
- Induction of apoptosis

Molecular level

- Inhibition of transcription factors NF-κB, NF-AT, and AP-1
- Alteration of MAP kinases cascade
- Modulation of the activity of nuclear receptors (PPARs)
- Induction of transcription factors and genes (STAG, NAG, NFGI-B)

PPAR (Peroxisome Proliferator-Activated Receptor)

PPAR is a nuclear receptor-transcription factor and is ligand-dependent and expressed in several tissues. It is initially involved in adipocyte differentiation and fatty acid synthesis. Fatty acid and eicosanoids bind to PPAR and regulate transcription. PPAR activation inhibits monocyte differentiation and expression of several pro-inflammatory genes such as iNOS, TNF, etc. PPAR activation inhibits tumor cell proliferation (epithelial, colon, prostate). PPAR is involved in angiogenesis.

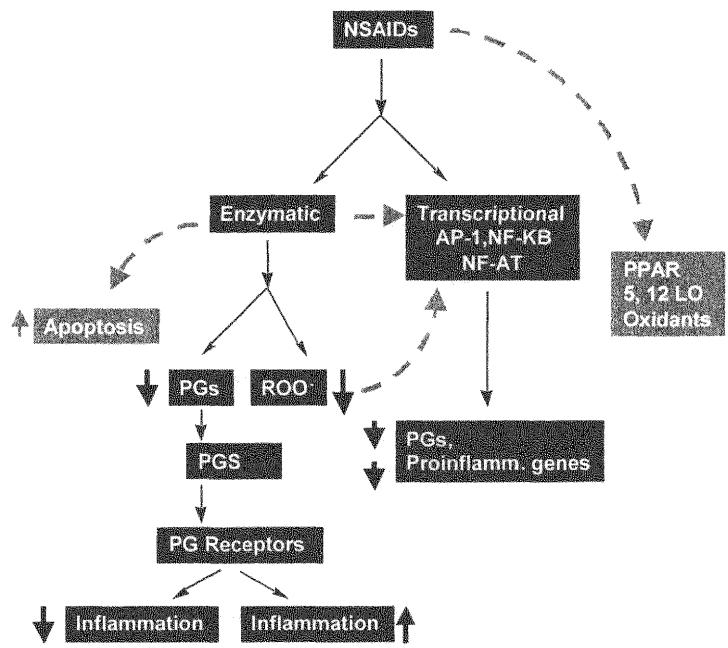
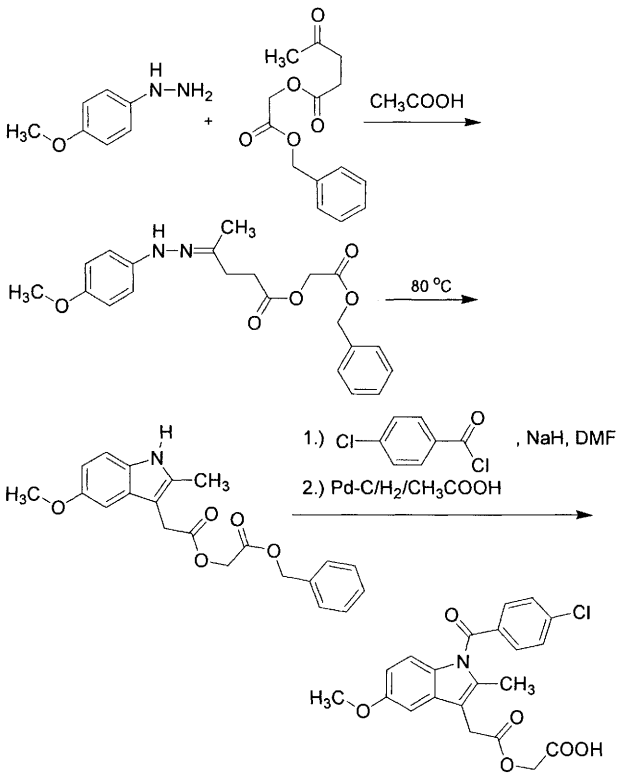


Figure 19: Mechanism of action of NSAIDs as enzyme inhibitors and/or transcriptional regulators.

COX-1 and COX-2 Inhibitors in Clinical Use

Acemetacin

Synthesis (Boltze and Kreisfeld, 1977; Kleemann et al., 1999):

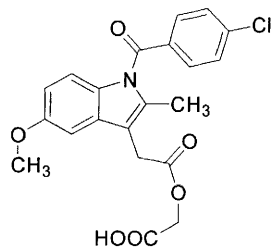


Scheme 4: Synthesis of acemetacin.

The reaction product of indomethacin (see below) with benzyl bromo acetate can also be hydrogenated to Acemetacin.

Clinical use: Acemetacin (Jacobi and Dell, 1980) is a nonsteroidal anti-inflammatory drug which acts directly via its major metabolite indomethacin. Acemetacin is used in chronic joint pain as well as in postoperative pain. The recommendation for oral dosing is between 120 and 360 mg daily.

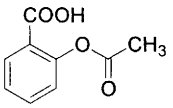
Acemetacin



[53164-05-9], [1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetic acid carboxymethyl ester, $C_{21}H_{18}ClNO_6$, M_r 415,82, *mp* 150-153 °C (fine pale yellow crystals)

Trade name: Rantudil (Ger), Emflex (UK)

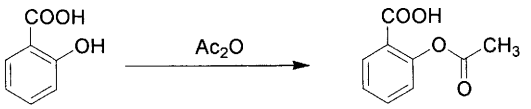
Acetylsalicylic Acid



[*50-78-2*], 2-Acetoxy-benzoic acid, $C_9H_8O_4$, M_r 180.04, *mp* 135 °C

Acetylsalicylic Acid

Synthesis (Kleemann et al., 1999; Kuhnert, 1999):



Scheme 5: Synthesis of acetylsalicylic acid.

Clinical use: Acetylsalicylic acid is the prototype of a nonsteroidal anti-inflammatory drug and is used in a large number of inflammatory and pain indications including musculoskeletal, soft tissue and joint disorders, headache, dysmenorrhoea and fever (Symposium on new perspectives on aspirin therapy 1983, various authors). Furthermore, acetylsalicylic acid is used as an antiplatelet drug in the acute treatment of myocardial infarction in combination with thrombolytics and for the prevention of myocardial infarction and stroke (Patrono, 1994).

Depending on the assay system, acetylsalicylic acid shows a balanced inhibition of COX-1 and COX-2 or a selectivity towards COX-1. When used in low doses as antiplatelet drug, the main target is COX-1. In addition to a COX inhibition, acetylsalicylic acid modulates the activities of several cellular kinases which may contribute to its anti-inflammatory effects (Tegeder et al., 2001).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	9.6	16.0	0.6
cell culture (2)	0.3	50	0.006
whole blood (3)	4.4	13.9	0.3

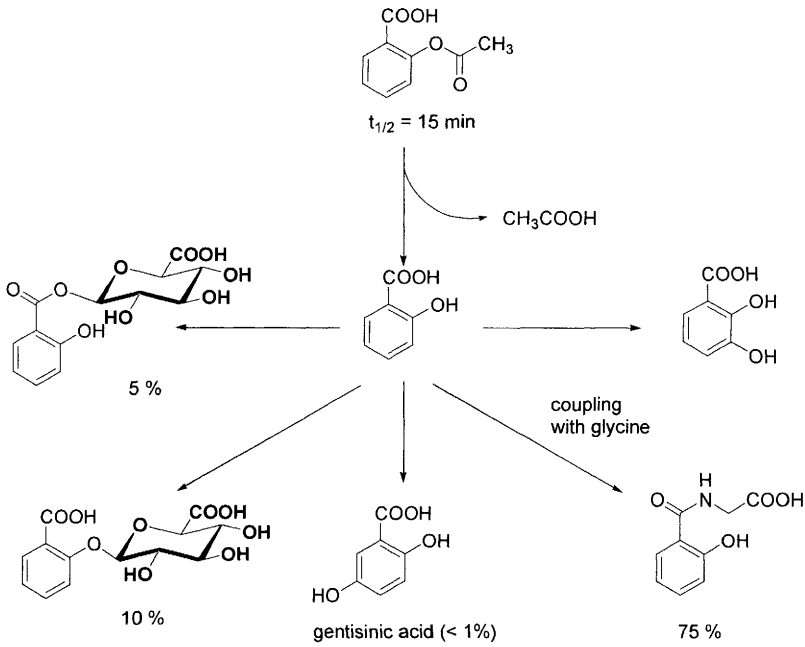
(1) Berg et al. (1997)

(2) Mitchell et al. (1993)

(3) Cryer and Feldman (1998)

The inhibition of COX-1 and COX-2 does not follow a competitive mechanism like other nonsteroidal anti-inflammatory drugs but rather is due to a covalent enzyme inhibition via acetylation. After absorption, acetylsalicylic acid is hydrolyzed to salicylate which itself still shows some COX inhibition. Both compounds are bound (80-90%) to plasma proteins. The plasma elimination half-life is about 15 min for acetylsalicylic acid and between 3 and 22 h for salicylate depending on the dose (Needs and Brooks, 1985).

The metabolic pathway of acetylsalicylic acid is shown in scheme 6.



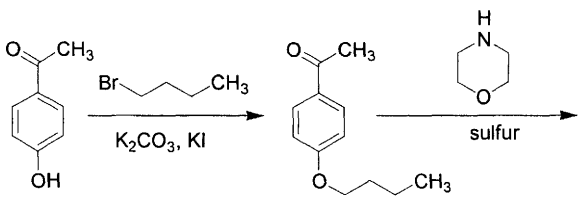
Scheme 6: Metabolic pathway of acetylsalicylic acid

Acetylsalicylic acid is usually given by oral administration (0.5-8 g/day) for pain and inflammation and for antiplatelet therapy (75-100 mg/day). It is also available in rectal and topical formulations and as a soluble lysine derivative for intravenous or intramuscular application. Acetylsalicylic acid is often used in multi-drug preparations. The main side-effects are gastrointestinal disorders. Use in children is limited due to the risk of Reye's syndrome (Waldmann et al., 1982). The lithium, magnesium, calcium, and aluminium salts of acetylsalicylic acid are used in some special preparations.

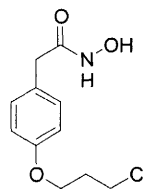
Trade name: Aspirine (F), Aspirin, Aspisol (Ger), Aspro (UK), Alka Seltzer (US)

Bufexamac

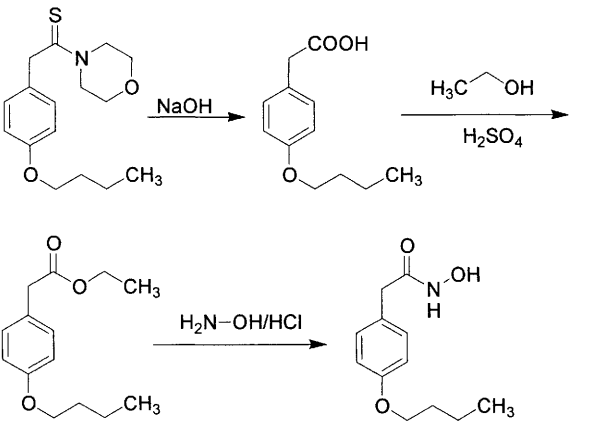
Synthesis (Buu-Hoi, 1965; Kleemann et al., 1999):



Bufexamac



[2438-72-4], 2-(4-Butoxyphenyl)-N-hydroxyacetamide, $C_{12}H_{17}NO_3$, M_r 223.12, *mp* 153-155 °C



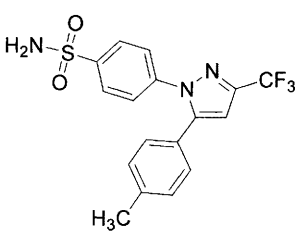
Scheme 7: Synthesis of bufexamac.

Trade name: Duradermal (Ger), Parfenac (France, Ger)

Clinical use: Bufexamac (Brogden et al., 1975) is a nonsteroidal anti-inflammatory drug used in topical formulations for mild skin disorders and as suppositories (250-500 mg/day) for haemorrhoids.

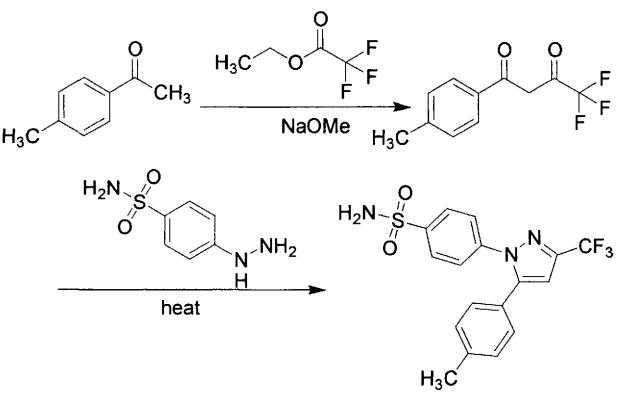
Celecoxib

Celecoxib



[169590-42-5], 4-(5-p-Tolyl-3-trifluoromethyl-pyrazol-1-yl)-benzenesulfonamide, $C_{17}H_{14}F_3N_3O_2S$, M_r 381.37; *mp* 157-159 °C (pale yellow solid)

Synthesis: The condensation of 4-methylacetophenone with ethyl acetate by means of NaOMe in refluxing methanol gives 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione, which cyclized with 4-hydrazinophenyl-sulfonamide in refluxing ethanol (Graul et al., 1977; Talley et al. (Searle & Co.), 1995.



Scheme 8: Synthesis of celecoxib.

Clinical use: Celecoxib (Graul et al., 1997; Wallace and Chin, 1999) is a second generation selective COX-2 inhibitor and the first drug of this group which reached the market. Its selectivity for COX-2 compared to COX-1 is about 375-fold greater in human recombinant enzyme preparations and about 8-fold in a whole blood assay. Celecoxib has been approved for rheumatoid arthritis, osteoarthritis, acute pain and primary dysmenorrhoea in the US and has been launched in an increasing number of countries since 1999.

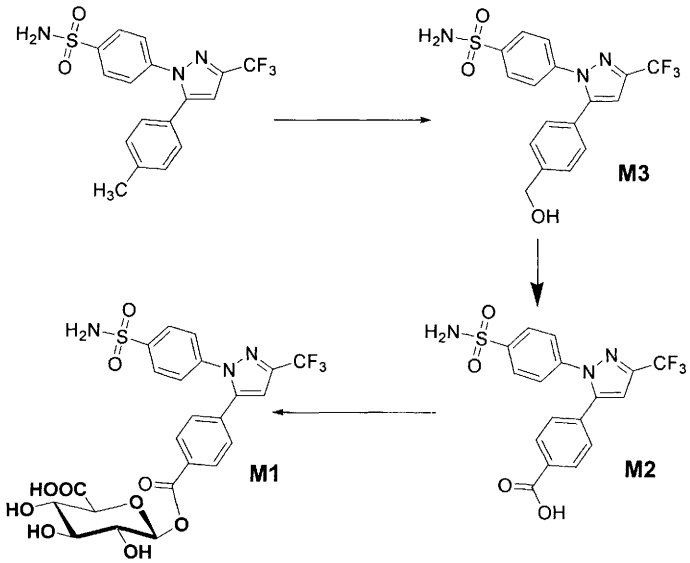
COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
recomb. enzyme (1)	15.0	0.040	375
whole blood (2)	6.7	0.87	7.6

(1) Penning et al. (1997)

(2) Riendeau et al. (2001)

Plasma peak concentrations are achieved within 2 h and the elimination half-life is about 12 h. Within the clinical dose range, there is high plasma protein binding (~97%). Celecoxib is metabolized primarily via cytochrome P450 2C9 to three inactive main metabolites. It is excreted in faeces (~57%) and urine (~27%) as determined by administration of a single oral dose of radiolabeled drug. Celecoxib is given orally (200-400 mg/day).



Proposed metabolic pathway for celecoxib in humans (Paulson et al., 1999, 2000)

Scheme 9: Metabolic pathway of celecoxib.

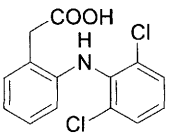
Use of celecoxib is associated with a reduced incidence of gastroduodenal ulcers in comparison to naproxen (Goldstein et al., 2001) ibuprofen, or diclofenac (Silverstein et al., 2000) in patients with arthritis.

Celecoxib is approved for the use in familial adenomatous polyposis in the US and leads to a reduction in the number of colorectal polyps in these patients (Steinbach et al., 2000).

Trade name: Celebrex (US, EC)

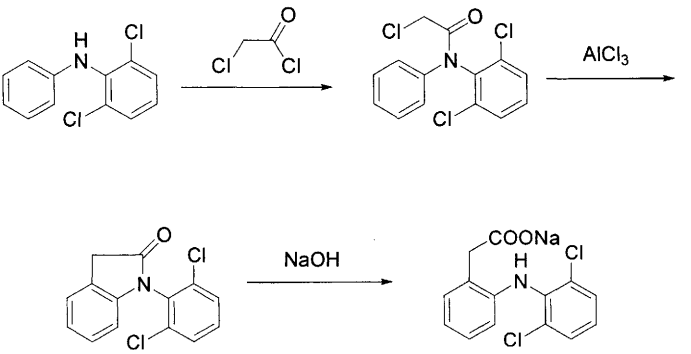
Diclofenac

Diclofenac



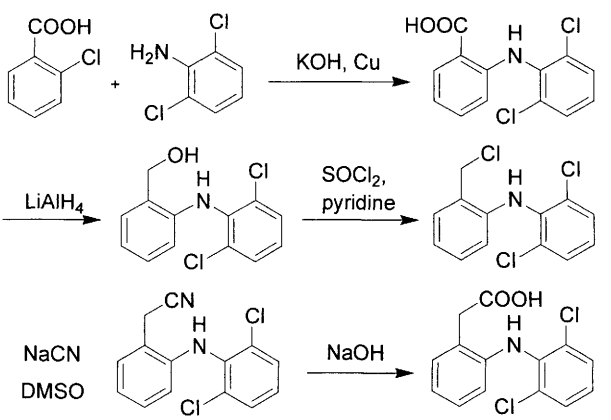
[*15307-86-5*], [2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid, $C_{14}H_{11}Cl_2NO_2$, M_r 296.15, *mp* 156-158 °C; sodium salt [*15307-79-6*], $C_{14}H_{10}Cl_2NNaO_2$, M_r 318.13, *mp* 283-285 °C

Synthesis (Moser et al., 1990): acylation of *N*-phenyl-2,6-dichloroaniline with chloroacetyl chloride gives the corresponding chloroacetanilide, which is fused with aluminum chloride to give 1-(2,6-dichlorophenyl)-2-indolinone. Hydrolysis of the indolinone with dilute aqueous-alcoholic sodium hydroxide affords the desired sodium salt directly.



Scheme 10: Synthesis of diclofenac.

Another synthesis using 2-chloro benzoic acid as starting material is shown below.:



Scheme 11: Synthesis of diclofenac.

Clinical use: Diclofenac (Todd and Sorkin, 1988) is a nonsteroidal anti-inflammatory drug with balanced COX-1 and COX-2 inhibition. It is commonly used for a variety of inflammatory and pain conditions such as musculoskeletal and joint disorders, periarticular disorders, soft tissue

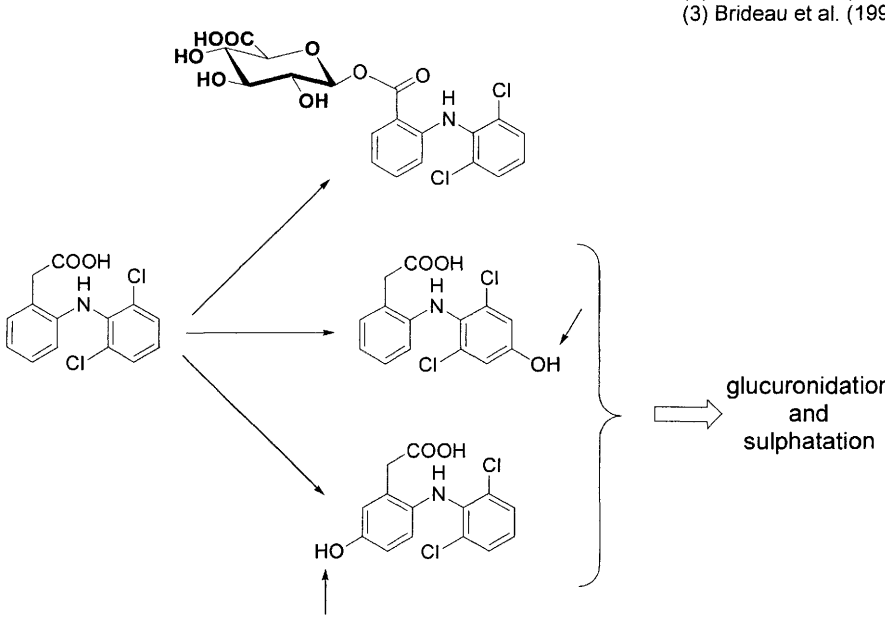
disorders, renal colic, acute gout, dysmenorrhoea as well as postoperative pain.

The plasma protein binding of diclofenac is greater than 99.5 % and the plasma elimination half-life is between 1 and 2 h. The metabolites of diclofenac, 4'-hydroxy-diclofenac, 5-hydroxydiclofenac, 3'-hydroxy-diclofenac, and 4',5-dihydroxydiclofenac are excreted as glucuronide and sulphate conjugates in the urine (~65%) and in the bile (~35%).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
recomb. Enzyme (1)	0.059	0.031	1.9
cell culture (2)	0.5	0.35	1.4
whole blood (3)	0.14	0.05	2.8

(1) Churchill et al. (1996)
(2) Mitchell et al. (1993)
(3) Brideau et al. (1996)



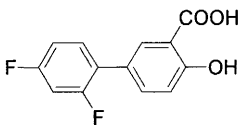
Scheme 12: Metabolic pathway of diclofenac.

Diclofenac is used mainly as the sodium salt orally or parenterally (75-150 mg/day) and as an ophthalmic solution. Topical formulations may contain the diethylammonium or epolamine salt. Diclofenac is combined with misoprostol to reduce gastrointestinal effects, which are the main side-effects.

Trade name: Voltaren (Ger, US), Voltarène (France), Voltarol (UK)

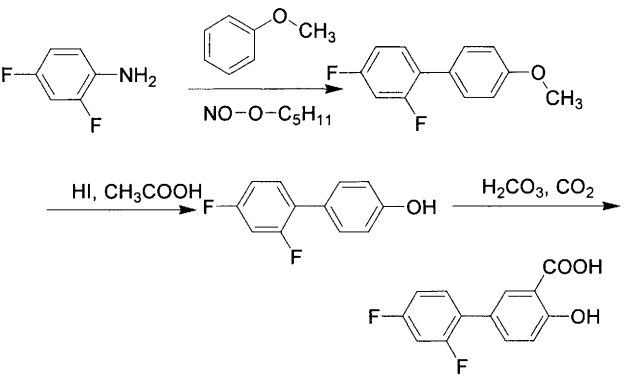
Diflunisal

Diflunisal



[*22494-42-4*], 2'4'-Difluoro-4-hydroxy-biphenyl-3-carboxylic acid, $C_{13}H_8F_2O_3$, M_r 250.20, *mp* 210-211 °C (also reported as 212-213 °C)

Synthesis (Arrigoni-Martelli, 1978a; Hannah et al., 1978; Jones and Hauser (Merck & Co.), 1980; Kleemann et al., 1999): The diazotation of 2,4-difluoroanaline with isoamyl nitrite and condensation with anisole gives 4-(2,4-difluorophenyl)anisole, which is hydrolyzed with HI in refluxing acetic acid yielding 4-(2,4-difluorophenyl)phenol. Finally this compound is carbonated with K_2CO_3 and CO_2 at 175 °C and 90 bar.



Scheme 13: Synthesis of diflunisal.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
whole blood (1)	232	52	4.5

(1) Young et al. (1996)

Clinical use: Diflunisal (Brogden et al., 1980) is a nonsteroidal anti-inflammatory drug used in the treatment of mild to moderate pain including osteoarthritis, rheumatoid arthritis and primary dysmenorrhoea. It is used as base or lysine- or arginine-salt for oral or parenteral application.

Diflunisal shows weak inhibition of both, COX-1 and COX-2 in a whole blood assay.

Peak plasma concentrations are reached within 2 to 3 h after oral dosing. Diflunisal is heavily bound to plasma protein (>99 %), has a long elimination half-life (8-12 h) and non-linear kinetics. Hence, it is used with an initial loading dose (1000 mg) and a lower maintenance dose (500-1000 mg/day). Diflusinal is excreted as glucuronide in the urine.

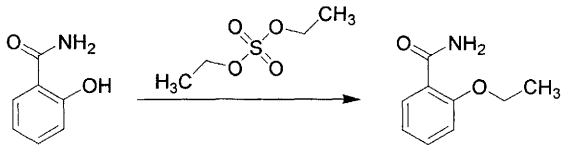
Trade names: Dolobis (France), Dolobid (UK, USA)

The main side-effects are gastrointestinal disturbances, headache and rash.

Analgesics. Edited by H. Buschmann, T. Christoph, E. Friderichs, C. Maul, B. Sundermann
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ISBN: 3-527-30403-7

Ethenzamide

Synthesis: Salicylamide (see below) is ethylated with diethyl sulfate (Lundbeck, 1948; Kleemann et al., 1999).

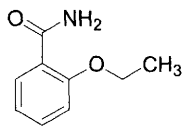


Ethenzamide

Scheme 14: Synthesis of ethenzamide.

Clinical use: Ethenzamide is a nonsteroidal anti-inflammatory drug used mainly in combination with other ingredients for the treatment of mild to moderate pain including musculoskeletal and joint disorders.

Ethenzamide

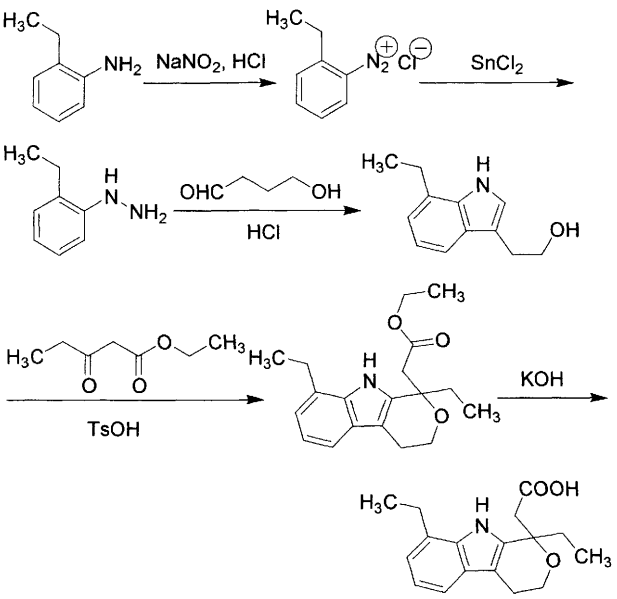


[*938-73-8*], 2-Ethoxybenzamide, $C_9H_{11}NO_2$, M_r 165.19, *mp* 132-134 °C

Trade name: Trancalgyl (France), Kolton, Antiföhnon-N (Ger)

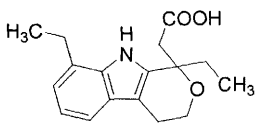
Etodolac

Synthesis (Demerson et al. (American Home Products), 1974; Demerson et al., 1975; 1976; Castaner and Arrigoni-Martelli, 1977a):



Scheme 15: Synthesis of etodolac.

Etodolac



[*41340-25-4*], (1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl)-acetic acid, $C_{17}H_{21}NO_3$, M_r 287.35, *mp* 145-148 °C

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	~50	0.041	~1000
whole blood (2)	34	3.4	10

(1) Riendeau et al. (1997)

(2) Patrignani et al. (1997)

Trade name: Lodine (USA, UK, France)

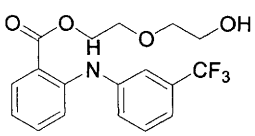
Clinical use: Etodolac (Bellamy, 1997) is a drug, invented before the discovery of the COX isoenzymes. Thus, there was clinical experience with the drug, before it was shown, that etodolac has a 10-fold selectivity for COX-2 compared to COX-1 in human whole blood. Etodolac belongs to the first generation of COX-2 inhibitors (Vane et al., 1998). Clinical data indicate fewer gastrointestinal side-effects in comparison to naproxen (Taha et al., 1989; Bianchi Porro et al., 1991). Etodolac is a racemate with an active (S)-enantiomer and an inactive (R)-enantiomer.

Etodolac shows efficacy in a wide variety of diverse pain states. It is used for the treatment of mild to moderate acute and chronic pain including rheumatoid arthritis and osteoarthritis. Etodolac is administered orally (400-1200 mg/day).

Peak plasma concentrations are reached within 2 h. Etodolac shows 99 % binding to plasma protein and an elimination half-life of about 7 h (Brooks and Jamali, 1994). Etodolac is metabolized almost completely to the main metabolites 6- or 7-hydroxy-etodolac, acyl-glucuronide, 8-(1'-hydroxyethyl)-etodolac and 4-ureido-etodolac. The metabolites and a small quantity of etodolac are excreted in the urine. A small amount of conjugated etodolac is excreted through the biliary tract.

Etofenamate

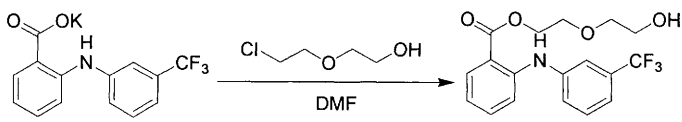
Etofenamate



[*30544-47-9*], 2-(3-Trifluoromethyl-phenylamino)-benzoic acid 2-(2-hydroxy-ethoxy)-ethyl ester, $C_{18}H_{18}F_3NO_4$, M_r 369.34, pale yellow viscous oil, thermolabile at 180 °C, bp 130-135 °C at 0.001 mm pressure

Trade name: Rheumon (Austria, Ger, Switz.)

Synthesis: The esterification of the potassium salt of flufenamic acid (see below) with 2-(2-chloroethoxy)ethanol in dimethyl formamide as solvent yields Etofenamate (Boltze and Kreisfeld, 1977; Kleemann et al., 1999).

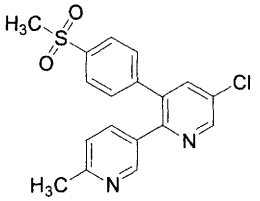


Scheme 16: Synthesis of etofenamate.

Clinical use: Etofenamate (Coletta et al., 1988) is a nonsteroidal anti-inflammatory drug which is used for the treatment of joint, musculoskeletal and soft tissue disorders. Etofenamate is used mainly as a topical formulation (500-1300 mg/day) and is also available for intramuscular injection (1 g/day).

Etoricoxib

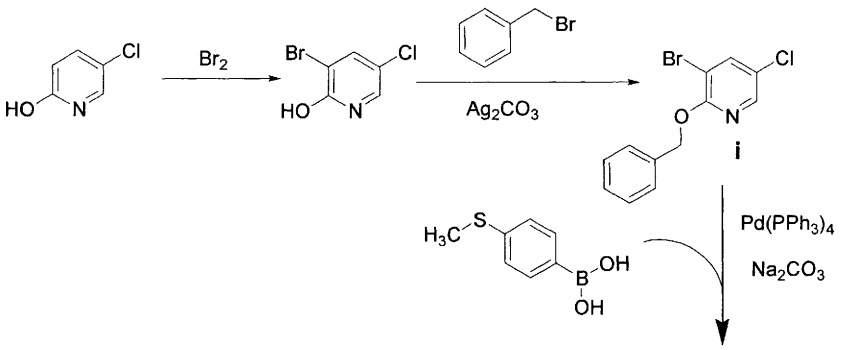
Etoricoxib

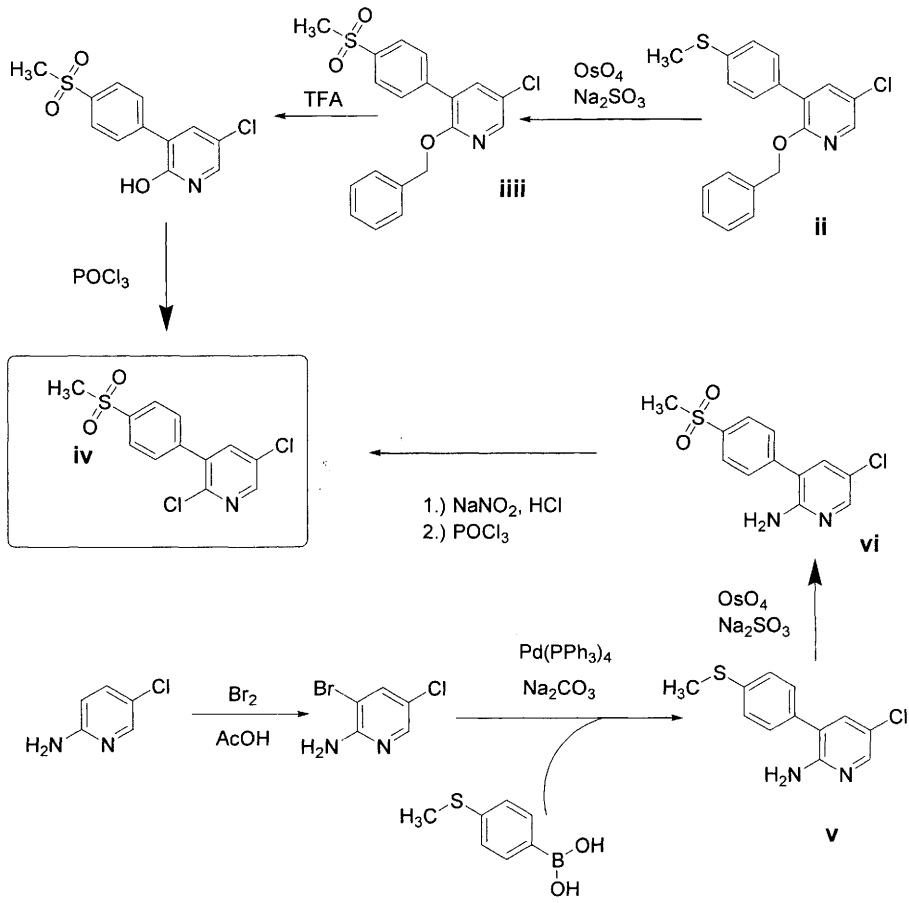


[*202409-33-4*], [*202409-40-3*] (mono HCl) , 5-Chloro-3-(4-(methylsulfonyl)phenyl)-2-(6-methylpyridin-3-yl)pyridine; 5-chloro-6′-methyl-3-(4-(methylsulfonyl)phenyl)-2,3′-bipyridine $C_{18}H_{15}ClN_2O_2S$, 358.848; crystals mp 271.5-138.1 °C (136.7 °C DSC onset)

Synthesis: A number of synthetic strategies to the COX-2 specific inhibitor etoricoxib have been described (Dube et al. (Merck Frosst), 1998; Davies et al., 2000; Sobera et al., 2001a). In schemes 17-20 the two major routes via a dichloropyridine derivative or a ketosulfone, respectively, as the key intermediates are discussed.

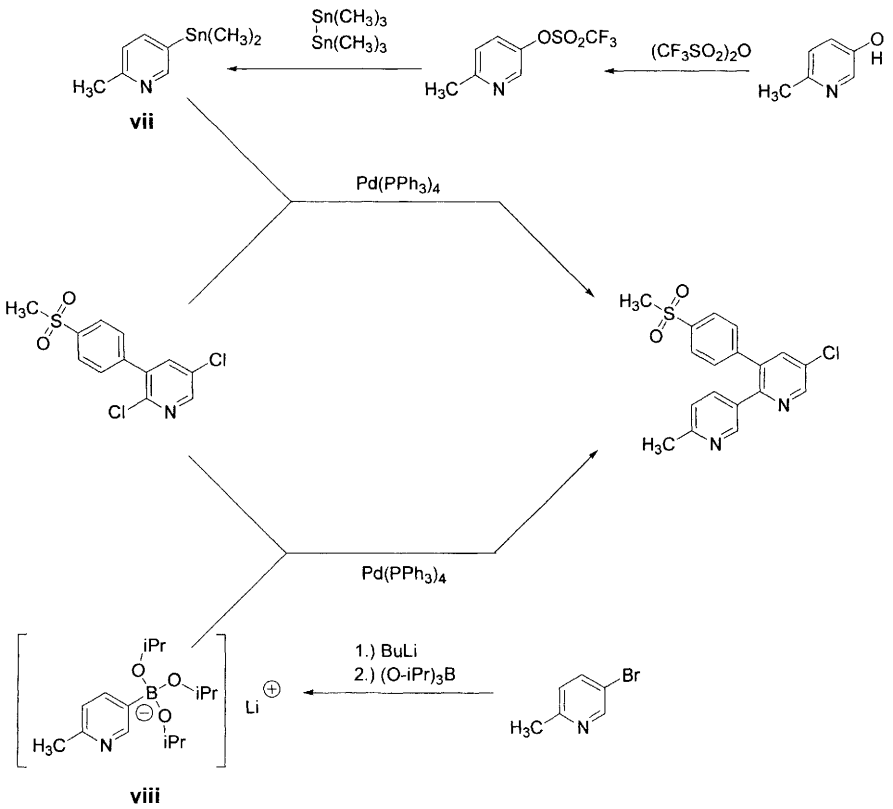
The bromination of 5-chloro-2-hydroxypyridine with bromine gives 3-bromo-5-chloro-2-hydroxypyridine, which is treated with benzyl bromide and silver carbonate yielding the benzyl ether-protected derivative (**i**). Condensation of (**i**) with 4-(methylsulfanyl)phenylboronic acid by means of $Pd(PPh_3)_4$ in refluxing ethanol(benzene affords 2-(benzyloxy)-5-chloro-3-(4-(methylsulfanyl) phenyl)pyridine (**ii**), which is treated with osmium tetroxide and sodium sulfite to furnish sulfone (**iii**). Treatment of the sulfone (**iii**) with TFA provides the 2-hydroxypyridine derivative, which is reacted with $POCl_3$ to yield the key intermediate 2,5-dichloro-3-(4-(methylsulfonyl)phenyl) pyridine (**iv**). An alternative pathway to (**vi**) starts with the bromination of 2-chloropyridine with bromine in acetic acid to yield 2-amino-3-bromo-5-chloropyridine, which is coupled with (methylsulfanyl)phenylboronic acid by means of $Pd(PPh_3)_4$ and sodium carbonate in refluxing ethanol/benzene to give 5-chloro-3-(4-methanesulfonylphenyl)-pyridin-2-ylamine (**v**). The subsequent oxidation of (**v**) with osmium tetroxide as before yield compound (**vi**), which is converted to the key intermediate (**iv**) by treatment first with $NaNO_2$ and HCl and then chlorination with $POCl_3$.





Scheme 17: Synthetic pathways to the key intermediate 2,5-dichloro-3-(4-(methylsulfonyl)phenyl) pyridine (**iv**).

Finally, compound (**iv**) is condensed with either trimethyl(6-methyl-3-pyridyl)tin or the boronate ester by means of $Pd(PPh_3)_4$ to afford etoricoxib. The metallated pyridine (**vii**) is obtained by esterification of 3-hydroxy-2-methylpyridine with triflic anhydride to give the corresponding triflate, which is treated with a tin reagent to yield the target tin intermediate. The boron lithium salt (**viii**) is prepared by treatment of 5-bromo-2-methylpyridine with butyllithium followed by addition of triisopropyl borate.

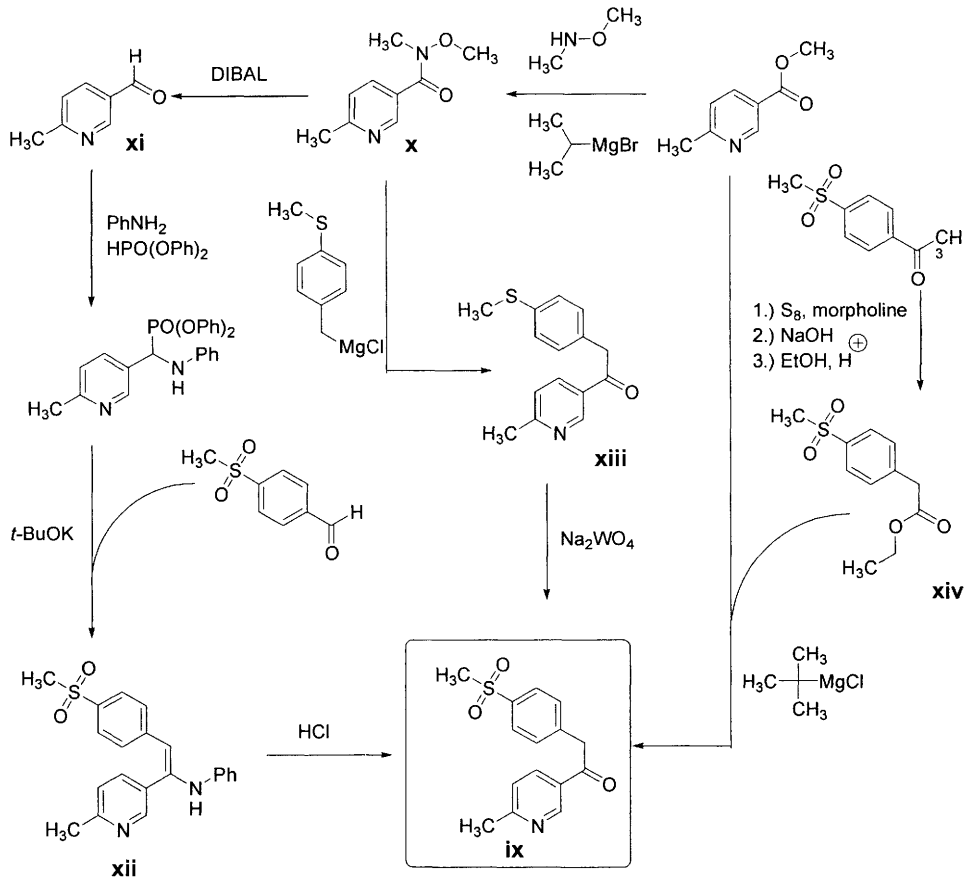


Scheme 18: Condensation pathways from the key intermediate 2,5-dichloro-3-(4-(methylsulfonyl)phenyl) pyridine (**iv**) with the tin or boronate derivative, respectively, to afford etoricoxib.

The reaction of 6-methylpyridine-3-carboxylic acid methyl ester with N,O-dimethylhydroxylamine and isopropylmagnesium chloride in toluene gives the N-methoxyamide derivative (**x**), which is reduced with diisobutyl aluminium hydride (DIBAL) to afford 6-methylpyridine-3-carbaldehyde (**xi**). The reaction of the aldehyde (**xi**) with a phosphite provides the diphenyl phosphonate derivative, which is condensed with 4-(methylsulfonyl)benzaldehyde in the presence of potassium *tert*-butoxide in HF to yield the enimine (**xii**). Finally, this compound is hydrolyzed with HCl to yield the ketosulfone (**ix**).

Another synthetic route starts with the condensation of N-methoxyamide (**x**) with 4-(methylsulfanyl) benzylmagnesium bromide to give 1-(6-methylpyridin-3-yl)-2-(4-(methylsulfanyl)phenyl)ethanone (**xiii**), which is finally oxidized with the wolframate to ketosulfone (**ix**).

Alternatively the oxidation of 4′-(methylsulfonyl) acetophenone with S_8 and morpholine produces the 2-(4-(methylsulfonyl)phenyl)acetic acid ethyl ester (**xiv**), which is condensed with 2-methylpyridine-3-carboxylic acid methyl ester by means of *tert*-butyl magnesium chloride in hot tetrahydrofurane to give the ketosulfone (**ix**).



Scheme 19: Synthetic pathways to the ketosulfone (**ix**) as the key intermediate for the etoricoxib synthesis.

Finally etoricoxib can be obtained by several related cylization reactions:

- Cyclization of the ketosulfone (**ix**) with 2-chloro-3-hydroxy-propenal (**xv**) in the absence of ammonium acetate
- Cyclization with the aniline derivative (**xvi**) in the absence of ammonium acetate

- Cyclization with aminoacrolein (**xvii**), which is prepared by treatment of with 2-chloro-3-hydroxy-propenal (**xv**) with isopropanol, yielding the ether derivative and followed by reaction with ammonium hydroxide
- Cyclization of the lithium enolate of the ketosulfone (**iv**) with 2,3-dichloroacrolein (**xviii**), obtained by treatment of 2-chloro-3-hydroxy-propenal (**xv**) with oxalyl chloride and DMF in toluene, followed by reaction with ammonium acetate or anhydrous ammonia
- Reaction of the ketosulfone (**ix**) with 2-chloro-1,3-bis(dimethylamino)trimethinium salt (**xix**) in the presence of an equimolar amount of *tert*-BuOK followed by treatment with acetic acid and TFA and reflux with an excess of ammonium hydroxide. 2-Chloro-1,3-bis(dimethylamino)trimethinium hexafluoro-phosphat (**xix**) is obtained by reaction of chloroacetic acid with hot dimethylformamide and $POCl_3$. Finally the reaction mixture is treated with NaOH and hexafluorophosphoric acid in water.

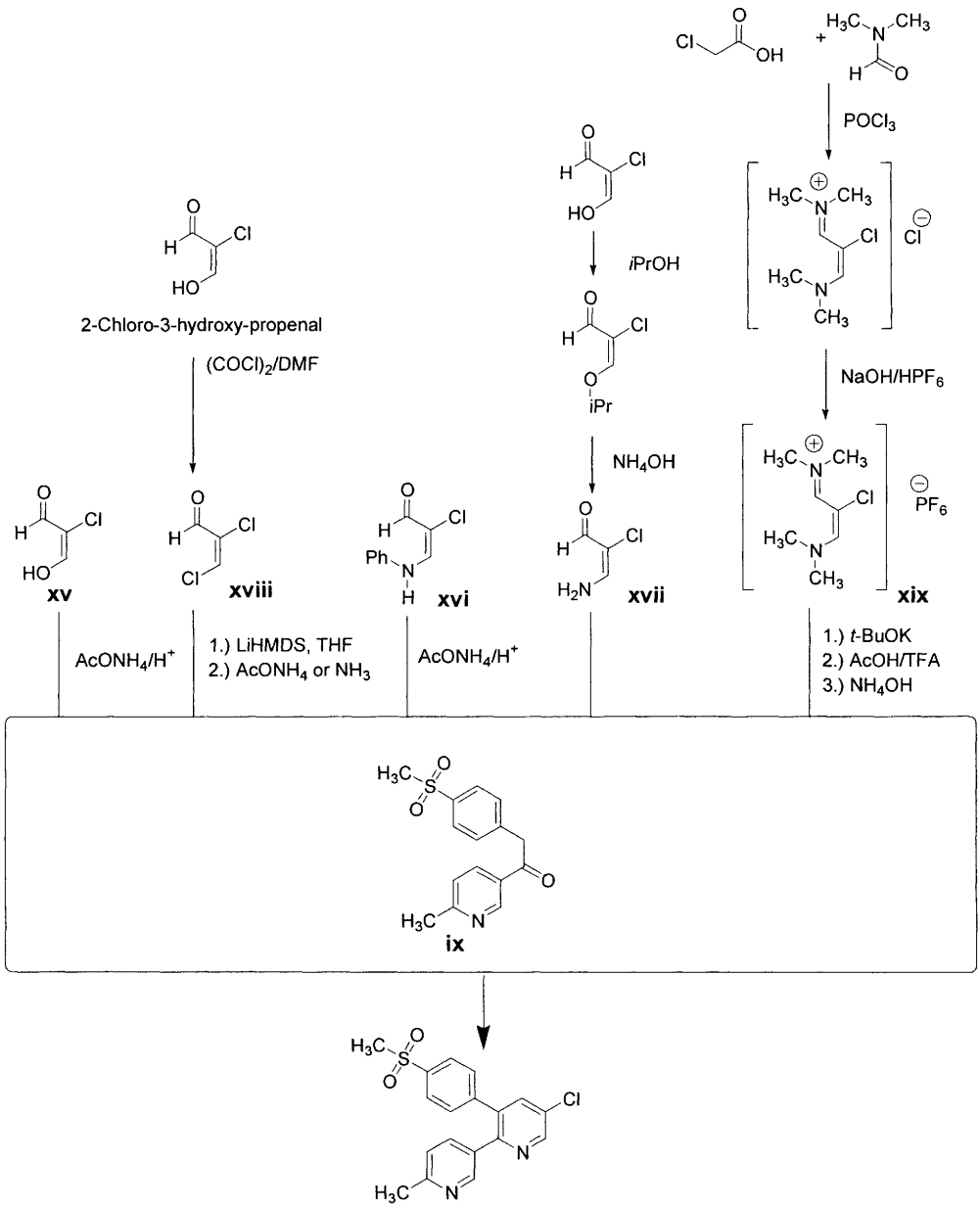
Clinical use: Etoricoxib (Sorbera et al., 2001a) is a third generation COX-2 inhibitor in clinical development. It shows a 100-fold selectivity for COX-2 in a whole blood assay. Classical recombinant enzyme preparations of COX-1 could not be blocked by etoricoxib. When lowering the substrate concentration (0.1μM arachidonic acid) in a microsomal enzyme preparation, thus generating an assay of high sensitivity, etoricoxib showed 6- and 240-fold lower IC_{50} values for COX-1 compared to rofecoxib and celecoxib, respectively (Riendeau et al., 2001). Etoricoxib shows efficacy in a variety of animal models of inflammation without affecting gastrointestinal permeability even at high doses (Riendeau et al., 2001).

Plasma peak concentrations are achieved within 1.0 to 1.5 h after administration in healthy volunteers and the elimination half-life is about 15 h. Etoricoxib is 60% metabolized via members of the cytochrome P450 3A family (Kassahun et al., 2001). *In vitro* studies provide no evidence for active metabolites with respect to COX-1 and COX-2 (Chauret et al., 2001).

COX selectivity

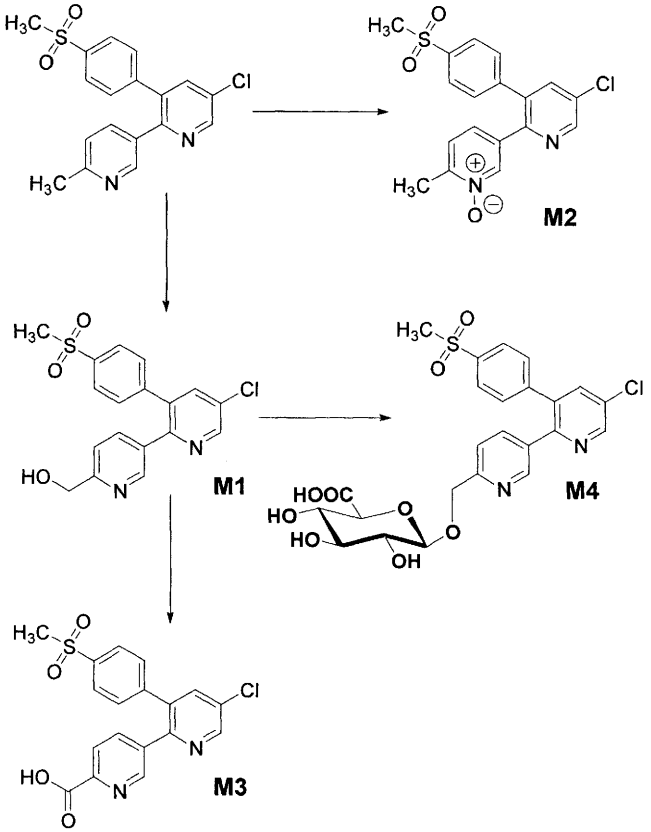
IC50 [μM]	COX-1	COX-2	ratio
recomb. enzyme (1)	>100	5.0	>20
cellular assay (1)	>50	0.079	>633
whole blood (1)	116	1.1	105

(1) Riendeau et al. (2001)



Scheme 20: Different cyclization pathways of of the ketosulfone to etoricoxib.

The metabolic profile of etoricoxib is shown below (Chauret et al., 2001):

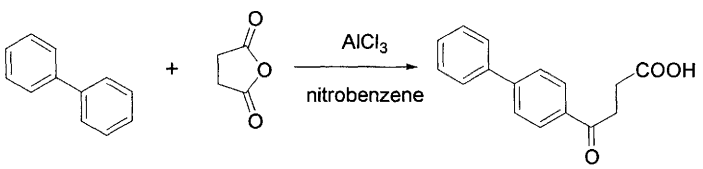


Scheme 21: *In vitro* metabolic pathways of etoricoxib.

Trade name: Arcoxia (US)

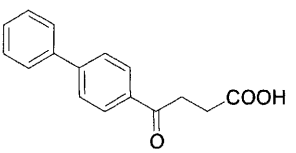
Fenbufen

Synthesis: Fenbufen is prepared by the Friedel-Crafts (aluminum chloride-nitrobenzene) acylation of biphenyl with succinic anhydride (Tomcufcik et al. (American Cyanamid Co.), 1972).



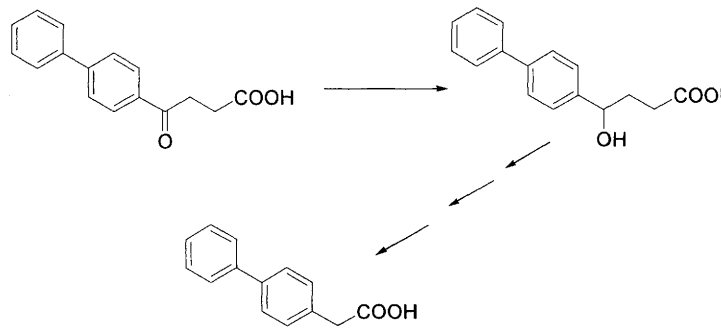
Scheme 22: Synthesis of fenbufen.

Fenbufen



[*36330-85-5*], γ-oxo(1,1′biphenyl)- 4-butanoic acid, 4-Biphenyl-4-yl-4-oxo-butyric acid, $C_{16}H_{14}O_3$, M_r 354.29 , *mp* 185–187 °C

Clinical use: Fenbufen (Brogden et al., 1981a) has been found to be an effective, well-tolerated drug for the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. The compound is metabolized in humans first to 4-hydroxy-4-biphenylbutyric acid (t_{max} 2.5 h) then to 4-biphenyl acetic acid (t_{max} 7.5 h). Both metabolites are more active than fenbufen itself (Kerwar, 1983) and circulate for several hours ($t_{1/2}$ 10 h). This slow conversion of fenbufen to active metabolites having relatively long plasma half-lives allows for once a day dosing (900 mg) with this agent.



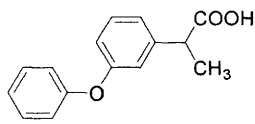
biphenyl acetic acid
(felbinac)

Trade names: Clincopal (Spain), Lederfen (UK), Napanol (Japan)

Scheme 23: Metabolic pathway of fenbufen.

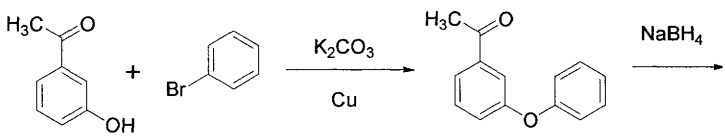
Fenoprofen

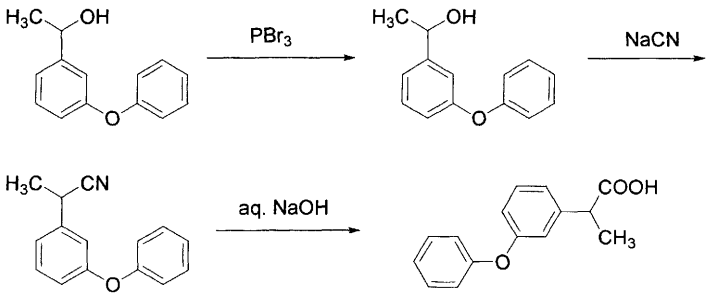
Fenoprofen



[*31879-05-7*], α-methyl-3-phenoxybenzeneacetic acid, 2-(3-Phenoxy-phenyl)-propionic acid, $C_{15}H_{14}O_3$, M_r 242.28, *bp* 168-171 °C (0.015 kPa)

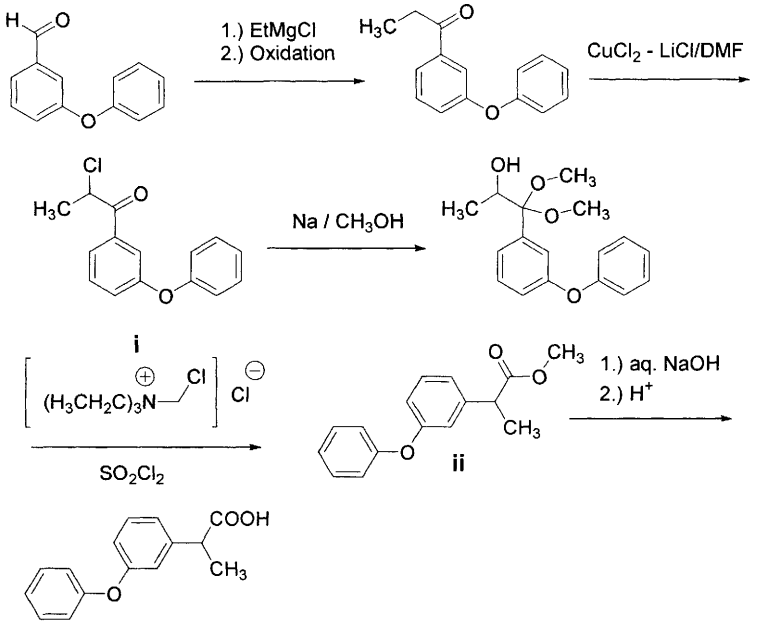
Synthesis (Marshall (Eli Lilly & Co.), 1971): Sodium borohydride reduction of 3-phenoxyacetophenone followed by bromination of the resulting alcohol with PBr_3 gives α-methyl-3-phenoxybenzyl bromide. Reaction of this bromide with sodium cyanide in dimethyl sulfoxide gives the corresponding nitrile, which is hydrolyzed using sodium hydroxide. Acidification affords fenoprofen.





Scheme 24: Synthesis of fenoprofen.

Sonawane et al. (Sonawane et al., 1994) described a practical and efficient synthesis of fenoprofen using commercially available *m*-phenoxybenzaldehyde as the starting material. The key step in the synthesis is the transformation of the α-hydroxyacetal (**i**) into its chlorosulfonyl ester *in situ* and its concomitant rearrangement to the methyl ester (**ii**) in high yields. The required α-hydroxyacetal (**i**) can be readily prepared from *m*-methoxybenzaldehyde by the routine sequence of reactions: Grignard reaction with ethyl bromide or chloride, oxidation and finally α-chlorination with $CuCl_2$-LiCl/DMF.



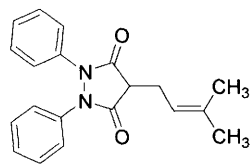
Scheme 25: Synthesis of fenoprofen.

Trade names: Fenopron (South Africa, UK), Fepron (Italy), Nalfon (US, Austria, Canada), Nalgesic (France), Progesic (UK)

Clinical use: Fenoprofen (Gruber, 1976; Brogden et al., 1977) is used as its calcium salt dihydrate in the treatment of rheumatoid arthritis and osteoarthritis at a daily dose of 1.2-3.0 g. The drug is rapidly absorbed and excreted with a plasma half-life of about 3 h despite being extensively bound (99%) to plasma protein. Fenoprofen is well tolerated, with dyspepsia being the main adverse effect.

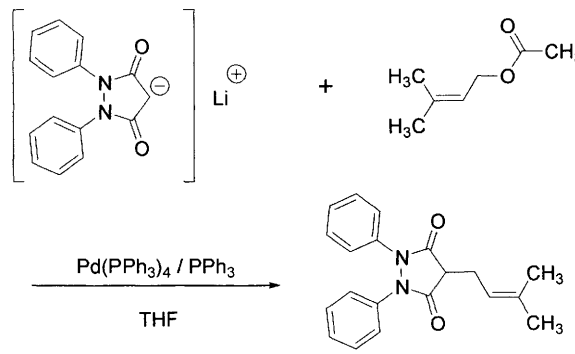
Feprazone

Feprazone



4-(3-Methyl-but-2-enyl)-1,2-diphenyl-pyrazolidine-3,5-dione

Synthesis: Feprazone is prepared by the condensation of acetic acid 3-methyl-but-2-enyl ester with the lithium salt of 1,2-diphenyl-pyrazolidine-3,5-dione in the presence of tetrakis(triphenylphosphin)palladium in anhydrous tetrahydrofuran.



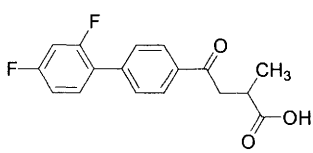
Scheme 26: Synthesis of feprazone.

Trade names: Zepelin (Austria, Italy), Brotazona (Spain)

Clinical use: Feprazone (Fletcher et al., 1975) is used in conditions of mild to moderate pain associated with musculoskeletal and joint disorders in daily oral doses of 400-600 mg. Peak plasma concentrations are seen 4-6 h after oral administration. The plasma half-life is in the range of 24 h.

Flobufen

Flobufen



[*112344-52-2*], 2′,4′-Difluoro-α-methyl-γ-oxo-(1,1′-biphenyl)-4-butanoic acid, 4-(2′,4′-Difluoro-biphenyl-4-yl)-2-methyl-4-oxo-butyric acid $C_{17}H_{14}F_2O_3$, M_r 304.29

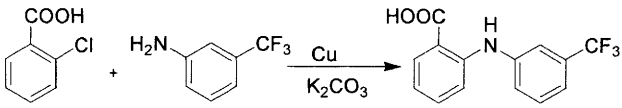
Synthesis (Rejholec, 1989; Fujimoto, 1999): Flobufen is prepared by Friedel-Crafts acylation of 2,4-difluorobiphenyl with methylsuccinic anhydride. The biphenyl is prepared by the Gomberg reaction of benzene with the diazonium salt derived from 2,4-difluoroaniline. Methylsuccinic anhydride is prepared by condensation of ethyl 2-bromopropionate with ethyl cyanoacetate followed by hydrolysis of the nitrile, decarboxylation of the resultant β-ketoacid, and dehydration.

Clinical use: Flobufen (Fujimoto, 1999) an inhibitor of both, cyclooxygenase and lipoxygenase, is in late clinical

development for the treatment of symptoms associated with rheumatoid arthritis and osteoarthritis.

Flufenamic Acid

Synthesis: 2-Chlorobenzoic acid is reacted with 3-trifluoromethylaniline in the presence of copper and potassium carbonate (Moffett and Aspergen, 1960; Parke Davis, 1961; Kleemann et al., 1999).



Scheme 27: Synthesis of flufenamic acid.

Clinical use: Flufenamate is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain of musculoskeletal, joint or soft tissue origin.

Flufenamate shows a preference for COX-1 in enzyme preparations of recombinant human enzymes.

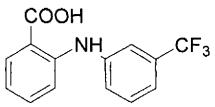
It is marketed in a variety of topical formulations alone or in combination with other ingredients.

Flufenamate is not recommended in patients with acute porphyria and was associated with a case of acute proctocolitis (Ravi et al., 1986).

The elimination half-life of flufenamate is about 2 h.

In addition to its action on prostaglandin synthesis, fenamates have been shown to modify several ion channel functions, e.g. inhibition of non-selective cation conductance (Gögelein et al., 1990), calcium-activated chloride channels (White and Aylwin, 1990), voltage-gated calcium channels, voltage-gated and ATP-sensitive potassium channels (Grover et al., 1994; Lee and Wang, 1999), as well as blocking gap junctions (Harks et al., 2001). The clinical relevance of these activities for the analgesic and anti-inflammatory potential of flufenamate is unknown.

Flufenamic Acid



[*530-78-9*], 2-(3-Trifluoromethyl-phenylamino)-benzoic acid, $C_{14}H_{10}F_3NO_2$, M_r 281.23, mp 124-125 °C (also reported as 134-136 °C); aluminium salt (3 : 1) [*16449-54-0*], $C_{42}H_{27}AlF_9N_3O_6$, M_r 867.66

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	2	29.5	0.07
whole blood (2)	30.6	n.d.	n.d.

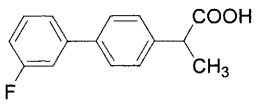
(1) Gierse et al. (1995)

(2) Young et al. (1996)

Trade name: Dignodolin (Ger)

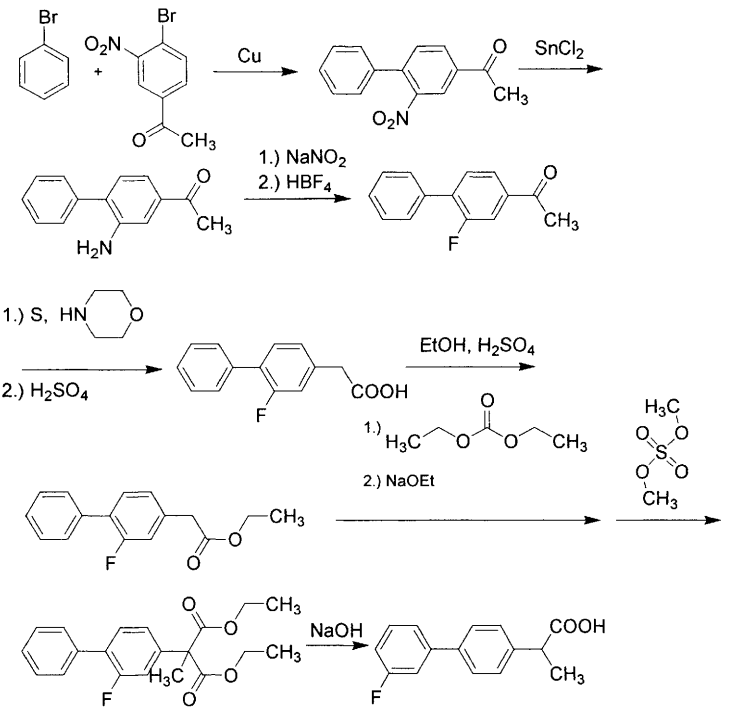
Flurbiprofen

Flurbiprofen



[*5104-49-4*], 2-(3'-Fluoro-biphenyl-4-yl)-propionic acid, 2-fluoro-a-methyl[1,1´-biphenyl]-4-acetic acid, $C_{15}H_{13}FO_2$, M_r 244.26, *mp* 110-111 °C; sodium salt [*56767-76-1*], $C_{15}H_{12}FNaO_2$, M_r 266.25

Synthesis (Thiele and v. Bebenburg (Degussa), 1966; 1970; v. Bebenburg et al., 1979; 1981; 1983; Kleemann et al., 1999):

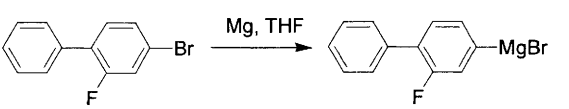


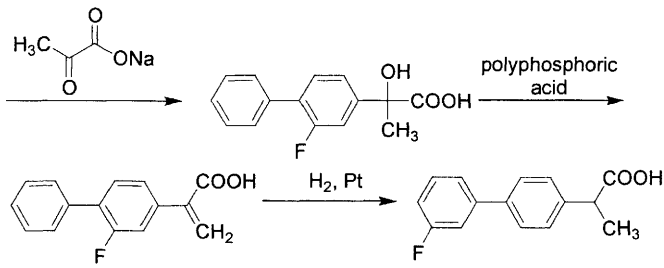
Scheme 28: Synthesis of flurbiprofen.

Clinical use: Flurbiprofen is a nonsteroidal anti-inflammatory drug used for the treatment of pain and inflammation associated with musculoskeletal and joint disorders as well as neuralgias, dysmenorrhoea and postoperative pain.

Flurbiprofen is a racemic mixture with the COX inhibitory activity in the S-enantiomer. Flurbiprofen shows a preference for COX-1 compared to COX-2.

Alternative synthesis:





Scheme 29: Synthesis of flurbiprofen.

Flurbiprofen is given orally or rectally (150-200 mg/day, max. 300 mg/day) and as ophthalmic solutions. Peak plasma concentration appears 1 to 2 hours after oral administration. The plasma protein binding is about 99.5% and the elimination half-life is in the range of 3-5 h.

Flurbiprofen is metabolized mainly by hydroxylation and conjugation in the liver and excreted in the urine.

Flurbiprofen has been proposed as an anti-platelet agent following myocardial infarction (Brochier, 1993).

In addition to COX inhibition, flurbiprofen shows weak inhibition of the transcription factors NF-κB and AP-1. This activity resides in the R-enantiomer which has no COX-inhibiting properties (Tegeder et al., 2001). The clinical relevance of this activity is unknown. Furthermore, the anti-proliferative potential of R-flurbiprofen has been investigated in the treatment of cancer (Wechter et al., 2000).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
Cell culture (1)	1.8	4.0	0.45
Whole blood (2)	0.44	6.42	0.07

(1) Riendeau et al. (1997)

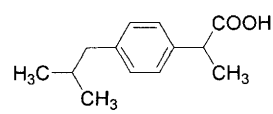
(2) Brideau et al. (1996)

Trade name: Cebutid (France), Froben (Ger, UK), Ocufen (Ger, UK, US)

Ibuprofen

- 1960 patented by the Boots Pure Drugs Co.
- (*R*)- and (*S*)-isomers have similar *in vivo* potency
- Only the (*S*)-isomer inhibits prostaglandin synthetase *in vitro*
- Chiral inversion of the (*R*)-isomer to the active (*S*)-isomer occurs *in vivo*
- Production and sale of ibuprofen as a racemic mixture
- In 1985 the US patent on ibuprofen expired opening the generic prescription market.
- 1989 a US patent was filed: analgesia was more rapidly attained and enhanced in effect, when (*S*)- (+)-ibuprofen was used.

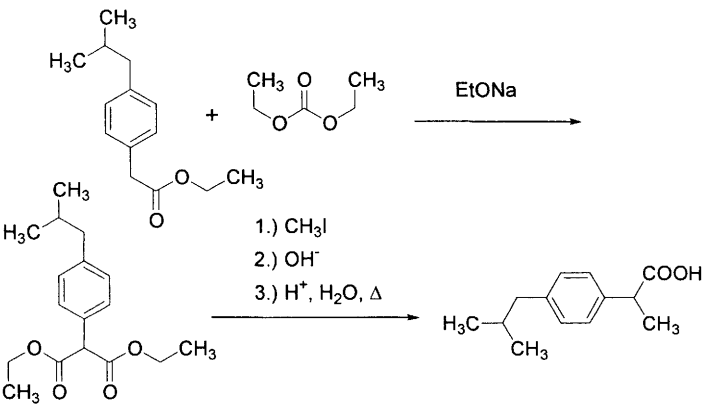
Ibuprofen



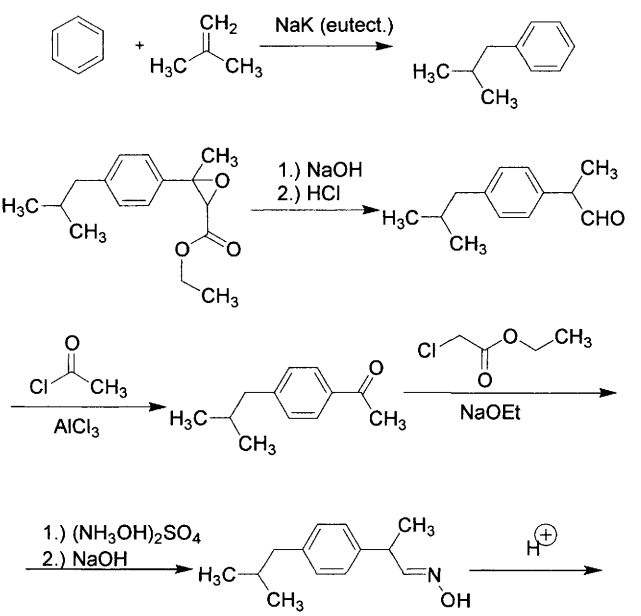
[*15687-27-1*], 2-(4-Isobutyl-phenyl)-propionic acid, $C_{18}H_{18}O_2$, M_r 206.28, *mp* 75-77 °C

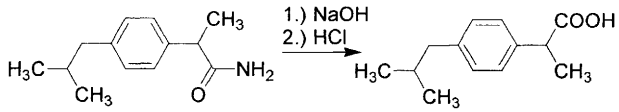
- The S(+)-enantiomer is available in some countries (dexibuprofen)

Synthesis (Mayer and Testa, 1997; Cleij et al., 1999; Kleemann et al., 1999): *a)* Treatment of ethyl 4-isobutylphenylacetate and diethyl carbonate with sodium ethoxide gives diethyl 4-isobutylphenylmalonate, which is methylated using methyl iodide and sodium ethoxide. Saponification followed by decarboxylation of the resulting malonic acid derivative affords ibuprofen.



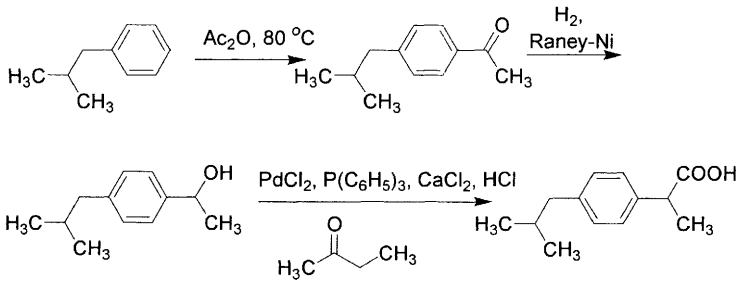
Scheme 30: Synthesis of ibuprofen.





Scheme 31: Boots process (industrial process).

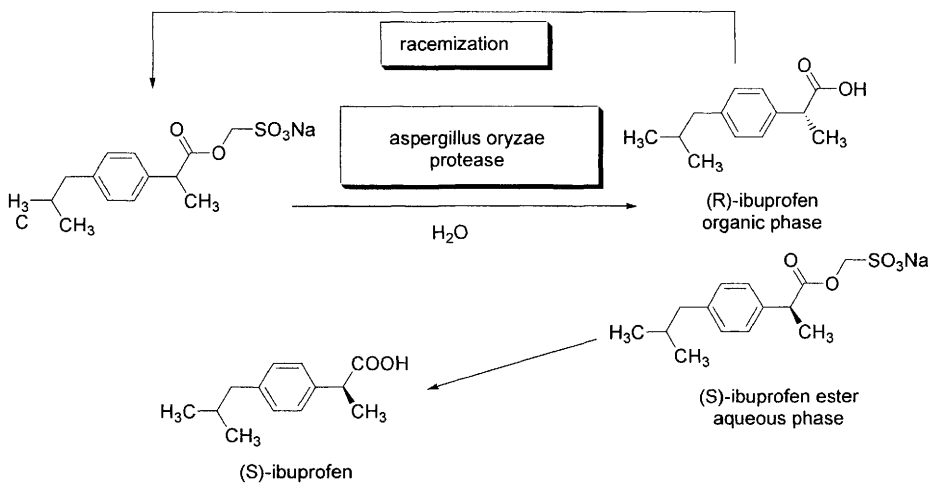
Boots-Hoechst-Celanese process: More recently, a shorter three-step catalytic route has been developed and is illustrated in the following scheme. Here, a Pd catalyzed carbonylation reaction is employed in the final step to introduce the carboxyl group.



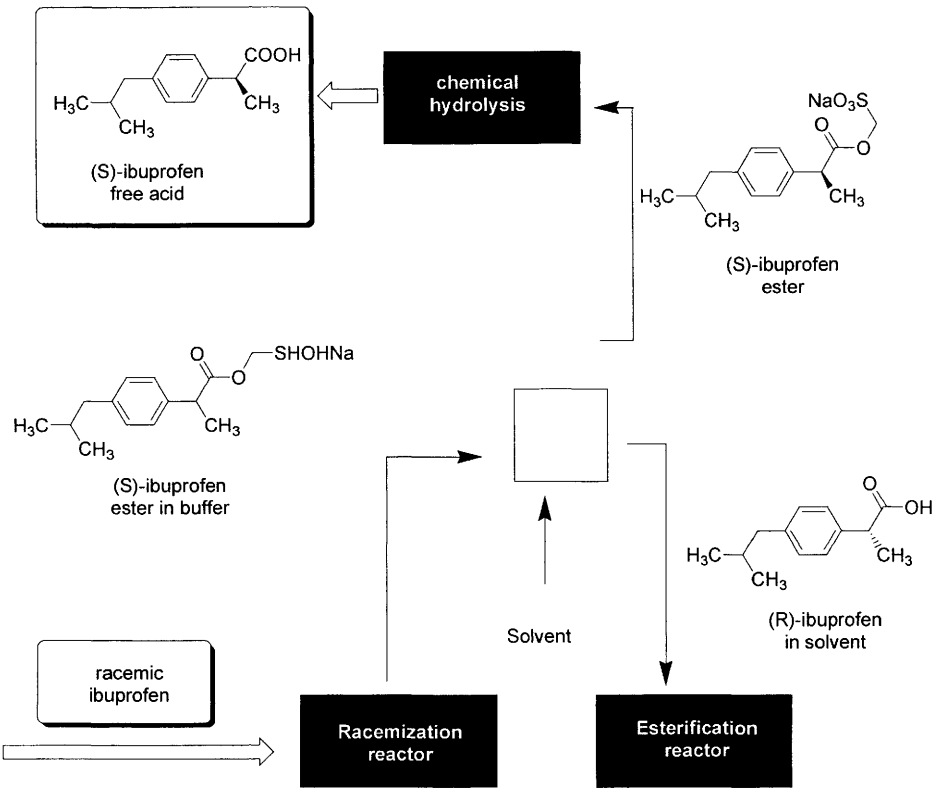
Scheme 32: Boots-Hoechst-Celanese process.

Several alternative processes are described in the literature.

For the preparation of the (*S*)-enantiomer an enzymatic racemic resolution process using A*spergillus oryzae* protease was developed by Sepracor:



Scheme 33: Racemic resolution of ibuprofen with hydrolase (Sepracor Process).



Scheme 34: Flow scheme for the enzymatic resolution process of ibuprofen.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	1 μg/ml	46 μg/ml	0.02
cell culture (2)	1.07	1.12	0.95
whole blood (3)	4.75	>30	<0.1

(1) Mitchell et al. (1993)

(2) Berg et al. (1999)

(3) Brideau et al. (1996)

Clinical use: Ibuprofen (Busson, 1986) is a nonsteroidal anti-inflammatory drug, commonly used for the treatment of mild to moderate pain. It is used in conditions like rheumatoid arthritis, osteoarthritis, joint and soft tissue pain, dental pain, postoperative pain, dysmenorrhoea and headache, including acute migraine attacks.

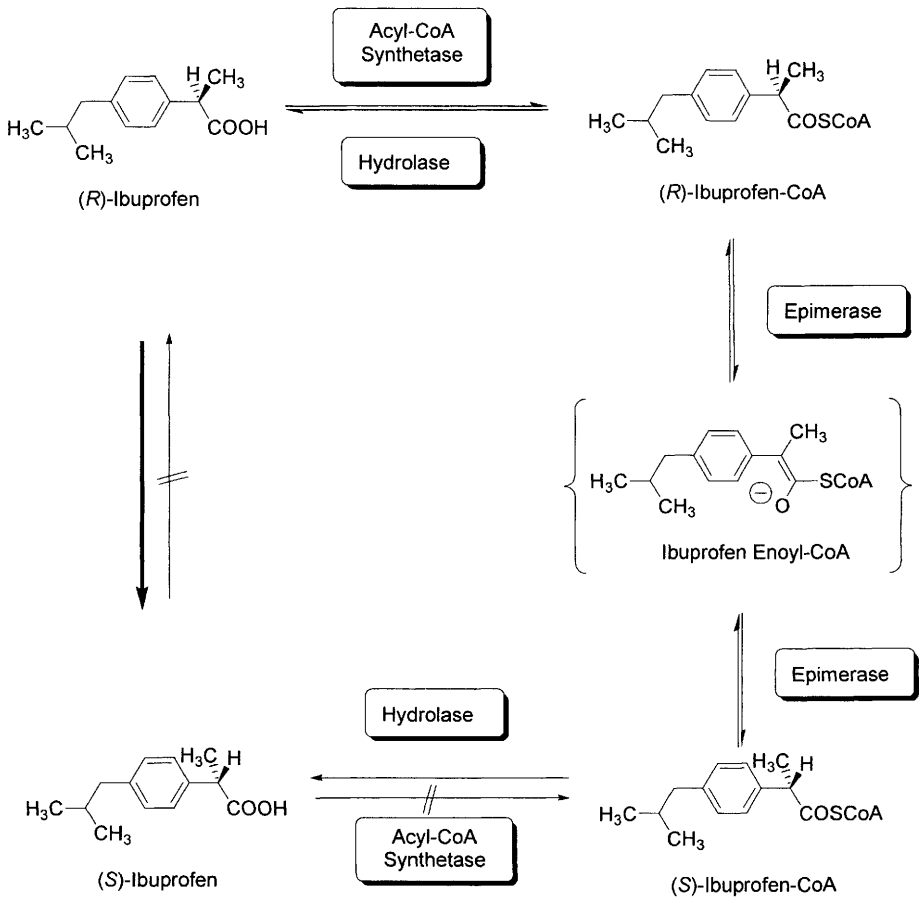
Ibuprofen inhibits both COX-1 and COX-2 with some preference for COX-1, depending on the assay system.

Ibuprofen is given by oral, rectal or topical application (800-2400 mg/day) and in a lower dose (40 mg/kg day) for the treatment of fever in children. Ibuprofen is given as free base or a variety of salts, esters and other derivatives.

Peak plasma concentrations of ibuprofen occur 1 to 2 h after oral administration. It is heavily bound to plasma proteins (90-99%) and has a plasma half-life of 2 h. Ibuprofen is excreted as metabolites and conjugates in the urine (Davies, 1998a).

Ibuprofen is a racemate, the active enantiomer being the S(+)-enantiomer which is commercially available in some

countries (dexibuprofen). (*R*)-ibuprofen is converted to the (*S*)-enantiomer via a metabolic chiral inversion process shown in Scheme 35.



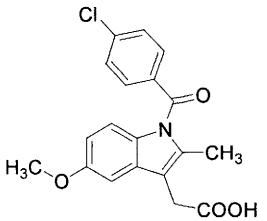
Scheme 35: Metabolic chiral inversion of (*R*)-ibuprofen to the (*S*)-enantiomer.

Ibuprofen shows the typical side-effects of nonsteroidal anti-inflammatory drugs but seems to be better tolerated than other such drugs. This may be due to its inhibition of COX-2.

Trade name: Anco (Ger), Imbim (Ger), Motrin (US)

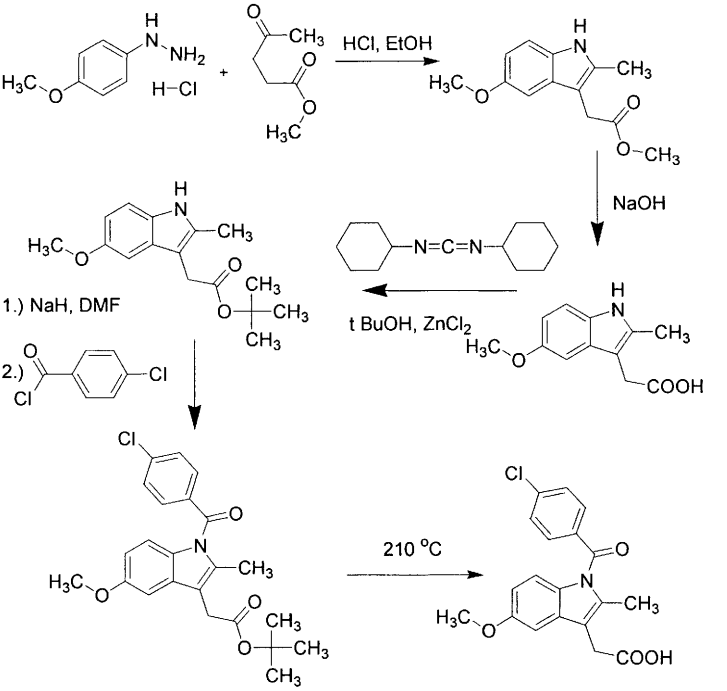
Indomethacin

Indomethacin

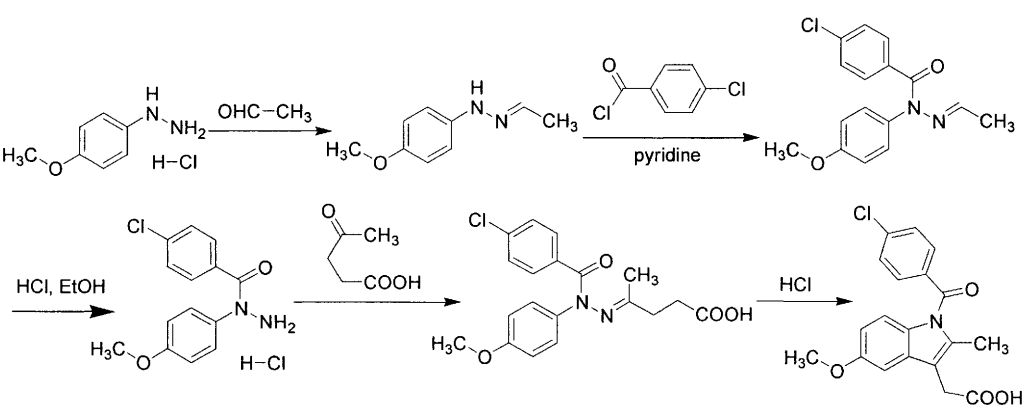


[*53-86-1*], [1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetic acid, $C_{19}H_{16}ClNO_4$, M_r 357.79, *mp* 153-154 °C (crystals exhibiting polymorphism, *mp* for another form is 162 °C); sodium trihydrate [*74252-25-8*], $C_{19}H_{15}ClNNaO_4 \cdot 3H_2O$, M_r 433.82, pale yellow crystaline powder

Synthesis (Shen et al., 1963; Kleemann et al., 1999):



Scheme 36: Synthesis of indomethacin: Merck & Co. process (Shen (Merck & Co.), 1962; 1964).



Scheme 37: Synthesis of indomethacin: Sumitomo process (Yamamoto et al., 1968).

Clinical use: Indomethacin is a nonsteroidal anti-inflammatory drug commonly used for the treatment of mild to moderate pain. It is also used in acute and chronic pain states such as rheumatoid arthritis, osteoarthritis,

joint and soft tissue pain, dental pain, postoperative pain and dysmenorrhoea.

Indomethacin is a COX-1 selective inhibitor with up to 10-fold selectivity compared to COX-2 depending on the assay.

It is given by oral, rectal or topical administration (50-150 mg/day, maximal daily dose 200 mg) as well as an ophthalmic solution.

Indomethacin is absorbed after oral administration reaching peak plasma concentrations after 2 h. About 99% binds to plasma proteins and it has a variable terminal half-life from 2.6 to 11.2 h in adults and up to 30 h in neonates. The metabolites generated in the liver are desmethyl-indomethacin, desbenzoyl-indomethacin, desmethyl-desbenzoyl-indomethacin and glucuronides. Indomethacin and its conjugates undergo enterohepatic circulation and are excreted mainly in the urine.

The major side-effects of indomethacin are gastrointestinal and central nervous system disturbances such as depression, drowsiness, tinnitus and convulsions.

Indomethacin is available as the sodium, meglumine, or *L*-arginine salt or as the prodrug (proglumetacin maleate).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	0.1	0.35	0.3
cell culture (2)	0.0045	0.045	0.1
whole blood (3)	0.16	0.46	0.3

(1) Churchill et al. (1996)

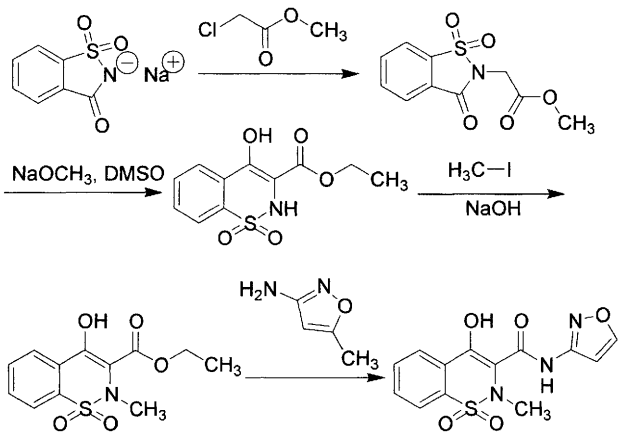
(2) Berg et al. (1999)

(3) Brideau et al. (1996)

Trade name: Indocid (F, UK), Amuno (Ger), Indocin (US), Protaxon (Proglumetacin, Ger)

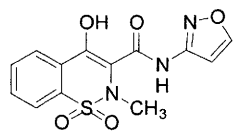
Isoxicam

Synthesis (Lombardino and Wiseman, 1971; Zinnes et al. (Warner-Lambert), 1972; Zinnes et al., 1982; Kleemann et al., 1999):



Scheme 38: Synthesis of isoxicam.

Isoxicam

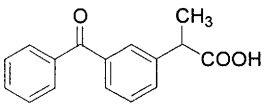


[*34552-84-6*], 4-Hydroxy-2-methyl-1,1dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid isoxazo-3-ylamide, 4-hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1 dioxide, $C_{14}H_{13}N_3O_5S$, M_r 335.34, *mp* 265-271 °C (decomp.); sodium salt, $C_{14}H_{12}N_3NaO_5S$, M_r 343.29, *mp* 270-272 °C

Clinical use: Isoxicam (Downie et al., 1984) is a nonsteroidal anti-inflammatory drug withdrawn from the market following reports of fatal skin reaction.

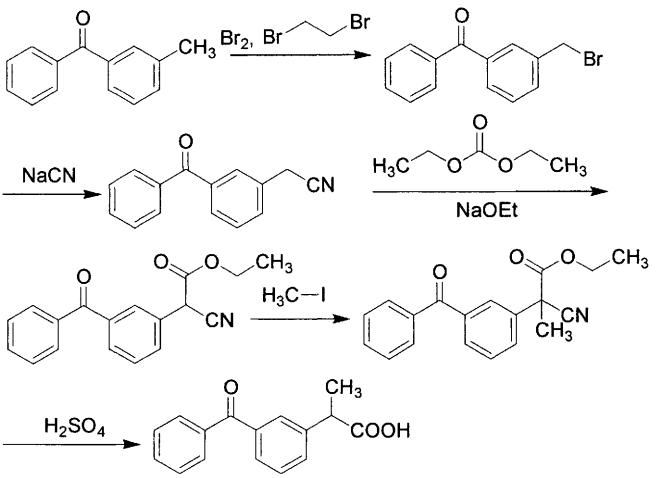
Ketoprofen

Ketoprofen



[*22071-15-4*], 2-(3-Benzoyl-phenyl)-propionic acid, 3-benzoyl-α-methylbenzene-acetic acid, $C_{16}H_{14}O_3$, M_r 254.28, *mp* 94 °C; lysine salt (1 : 1) [*57469-78-0*], $C_{16}H_{14}O_3 \cdot C_6H_{14}N_2O_2$, M_r 400.47; sodium salt [*57495-14-4*], $C_{16}H_{13}NaO_3$, M_r 276.27

Synthesis (Farge et al. (Rhône-Poulenc), 1972; Kleemann et al., 1999):



Scheme 39: Synthesis of ketoprofen.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	0.0061	0.12	0.05
whole blood (2)	0.02	1.08	0.02
whole blood (3)	0.11	0.18	0.6

(1) Riendeau et al. (1997)

(2) Brideau et al. (1996)

(3) S-Ketoprofen, Patrignani et al. (1997)

Clinical use: Ketoprofen (Hommeril et al., 1994) is a nonsteroidal anti-inflammatory drug used for the treatment of a variety of acute and chronic pain and inflammatory conditions including rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, postoperative pain and dysmenorrhoea. It is given by oral, rectal, topical or intramuscular application (100-200 mg/day, maximal dose 300 mg/day) as the sodium or lysine salt.

Ketoprofen is usually given as the racemate, the pharmacological action being mainly carried out by the S(+)-enantiomer. The active enantiomer is available in some countries since 1997 as the trometamol salt, which is said to be absorbed more quickly thus leading to an earlier onset of action.

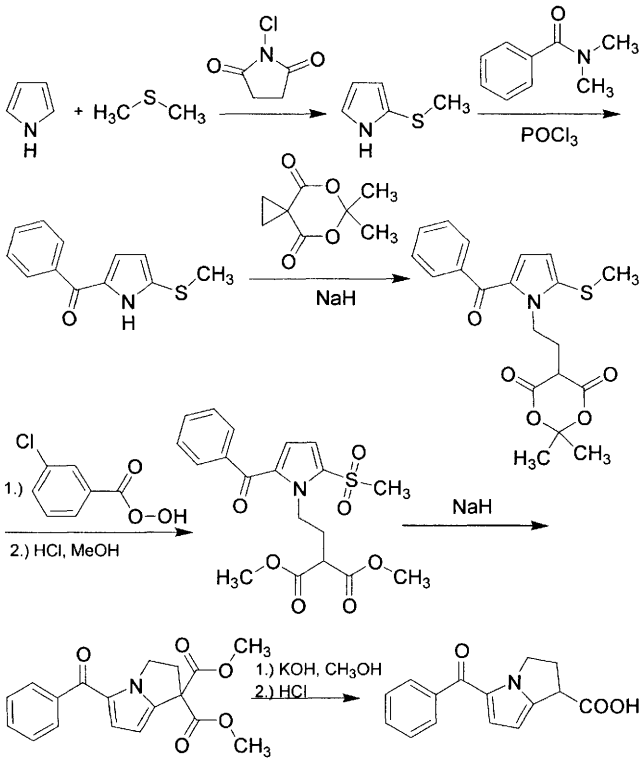
Ketoprofen shows selectivity for COX-1 compared to COX-2.

Trade name: Profénid (F), Orudis (Ger, UK, US), S(+)-Ketoprofen: Keral (UK)

Peak plasma concentration after oral application occurs within 2 h. Ketoprofen is bound to plasma protein up to 99% and shows a plasma elimination half-life of 1.5 to 4 h. It is metabolized mainly by glucuronidation and excreted mainly in the urine (Jamali and Brooks, 1990).

Ketorolac

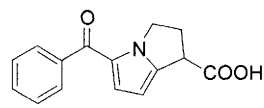
Synthesis: The benzoylation of 2-methylthiopyrrole with *N,N*-dimethylbenzamide by means of $POCl_3$ in refluxing CH_2Cl_2 gives 5-benzoyl-2-methylthiopyrrole, which is condensed with spiro[2,5]-5,7-dioxa-6,6-dimethyloctane-4,8-dione by means of NaH in DMF. The oxidation of this product with m-chloroperbenzoic acid in CH_2Cl_2 affords the sulfone, which is submitted to methanolysis with methanol and HCl giving 1-(3,3-dimethoxycarbonylpropyl)-2-methanesulfonyl-5-benzoylpyrrole. Cyclization with NaH in DMF yields dimethyl-5-benzoyl1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1,1-dicarboxylate, which is finally hydrolyzed and decarboxylated with KOH in refluxing methanol (Franco et al., 1982; Synthex 1982; 1984; 1989); Arrigoni-Martelli, 1983; Muchowski et al., 1985; Guzman et al., 1986; Kleemann et al., 1999).



Scheme 40: Synthesis of ketorolac.

Clinical use: Ketorolac (Gillis and Brogden, 1997) is a nonsteroidal anti-inflammatory drug mainly used for the treatment of moderate to severe postoperative pain.

Ketorolac



[*74103-06-3*], 5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, $C_{15}H_{13}NO_3$, M_r 255.27, *mp* 160-161 °C; tromethamine salt (1 : 1) [*74103-07-4*], $C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$, M_r 376.41, (+)-form *mp* 174 °C, $[\alpha]_D$ +173° (*c* = 1, CH_3OH), (-)-form *mp* 169-170 °C, $[\alpha]_D$ -176° (*c* = 1, CH_3OH); monosodium salt [*110618-38-7*], $C_{15}H_{12}NNaO_3$, M_r 277.26

Ketorolac shows a balanced inhibition of both COX-isoenzymes in a variety of assay systems and is a racemate with an active (S)-enantiomer.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
recomb. enzyme (1)	1.23	3.50	0.35
cell culture (2)	0.025	0.039	0.6
whole blood (3)	0.11	0.06	1.8

(1) Jett et al. (1999)

(2) Berg et al. (1999)

Ketorolac is given as the trometamol salt intramuscularly, intravenously, or orally. The maximal daily dose is 90 mg for the parenteral and the oral route. The duration of treatment is restricted due to side-effects. Ketorolac is also used as a 0.5% ophthalmic solution.

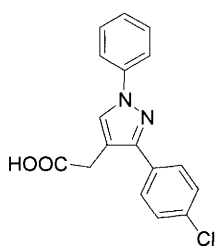
The peak plasma concentration of oral ketorolac is reached within 30 to 60 min and may be slower after intramuscular administration. It is bound to plasma proteins by more than 99%. The terminal plasma half-life is about 4 to 6 h and is prolonged in elderly and in patients with renal dysfunction. Ketorolac is metabolized mainly by glucuronidation and to a minor extent by para-hydroxylation and is excreted in the urine (~90%) and faeces (~10%) (Buckley and Brogden, 1990).

Trade name: Tora-Dol (F, I), Acular (Ger), Toradol (UK, US)

Due to a number of severe side-effects including gastrointestinal disturbances, liver function changes, renal failure, skin and other hypersensitivity reactions ketorolac has been withdrawn in many countries.

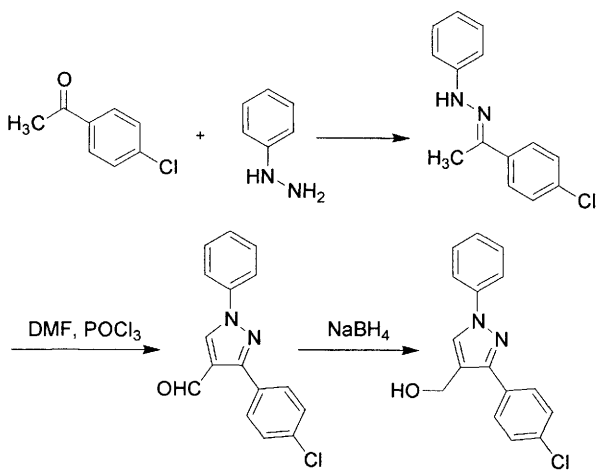
Lonazolac

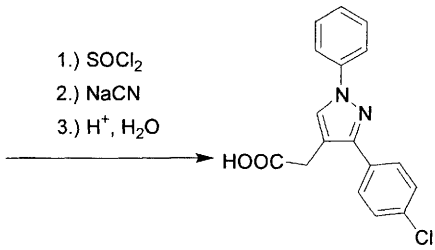
Lonazolac



[*53808-88-1*], [3-(4-Chloro-phenyl-1*H*-pyrazol-4-yl]-acetic acid, $C_{17}H_{13}ClN_2O_2$, M_r 312.75, *mp* 150-151 °C; calcium salt (2 : 1) [*75821-71-5*], , $C_{34}H_{24}CaCl_2N_4O_4$, M_r 663.57, *mp* 270-290 °C (decomp.)

Synthesis: The pyrazole-4-carbaldehyde synthesized according to Vilsmeier is reduced to the alcohol, which is chlorinated. The chloro derivative is reacted with sodium cyanide to give the nitrile, which is hydrolyzed to Lonazolac. The calcium salt, slightly soluble in water, is formed by adding calcium chloride to the free acid (Rainer et al., 1981; Unterhalt, 1982; Rainer et al. (Byk Gulden), 1969; 1982; Kleemann et al., 1999).





Scheme 41: Synthesis of lonazolac.

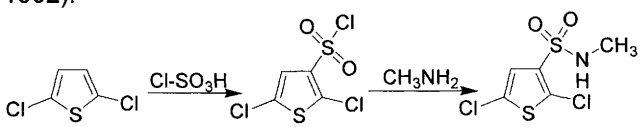
Clinical use: Lonazolac (Riedel et al., 1981) is a nonsteroidal anti-inflammatory drug used for the treatment of acute inflammatory pain conditions of joint and soft-tissue disorders as well as posttraumatic and postoperative pain. It is used as its calcium salt and is given by oral (600 mg/day, initial dose up to 900 mg/day) or rectal administration (800 mg/day).

Trade name: Argun (Ger), Irritren (Austria, Belg., Switz.)

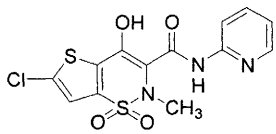
Lonazolac has a terminal half-life of about 6 h in young volunteers. The terminal half-life is prolonged to about 12 h in elderly patients (Huber et al., 1990).

Lornoxicam

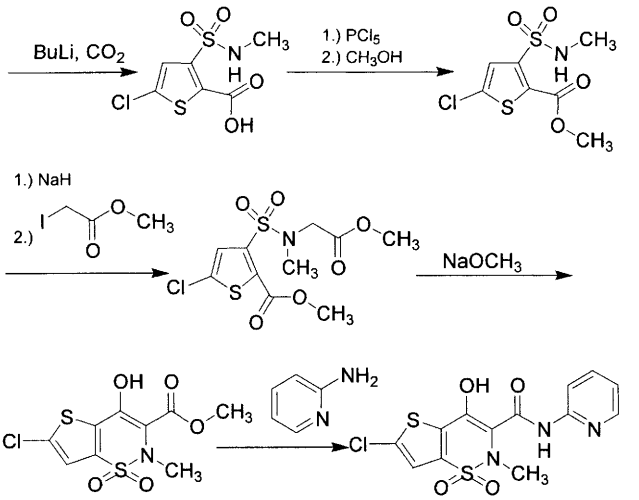
Synthesis: The sulfonation of 2,5-dichlorothiophen with $ClSO_3H/SOCl_2$ gives 2,5-dichlorothiophene-3-sulfonic acid chloride, which by reaction with methylamine in $CHCl_3$ yields the corresponding methylamide. Carboxylation with butyl lithium and CO_2 in ether affords 5-chloro-3-(*N*-methylsulfamoyl)thiophene-2-carboxylic acid, which is esterified with PCl_5 and methanol to the methyl ester. The condensation with methyl iodoacetate by means of NaH in DMF gives 5-chloro-3-[*N*-(methoxycarbonylmethyl)-N-methylsulfamoyl]thiophene-2-carboxylic acid methyl ester, which is cyclized with sodium methoxide in methanol yielding 6-chloro-4-hydroxy-2-methyl-2*H*-thieno[2,3-*e*]-1,2-thiazine-3-carboxylic acid methyl ester-1,1-dioxide. Finally this compound is treated with 2-aminopyridine in refluxing xylene (Drugs Fut., 1992).



Lornoxicam



[*70374-39-9*], 6-Chloro-4-hydroxy-2-methyl-1,1-dioxo-1,2-dihydro-1l^6-thieno[2,3-*e*][1,2]thiazine-3-carboxylic acid pyridin-2-ylamide, 6-chloro-4-hydroxy-2-methyl-*N*-(2-pyridyl)-2H-thieno[2,3-*e*]-1,2-thiazine-3-carboxamide-1,1-dioxide, $C_{13}H_{10}ClN_3O_4S_2$, M_r 371.81, *mp* 225-230 °C (decomp.)



Scheme 42: Synthesis of lornoxicam.

COX selectivity

IC50 [µM]	COX-1	COX-2	ratio
cell culture (1)	0.003	0.008	0.4
whole blood (1)	0.13	0.13	1

(1) Berg et al. (1999)

Clinical use: Lornoxicam (Pruss et al., 1990) is a nonsteroidal anti-inflammatory drug with a strong and balanced inhibition of both COX isoenzymes.

It is used orally (8-24 mg/day) for the treatment of mild to moderate pain including postoperative pain, rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Balfour et al., 1996).

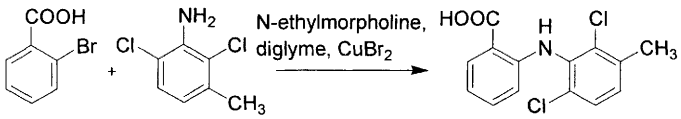
Lornoxicam reaches peak plasma concentrations within 2 to 6 h and shows high degree of binding to plasma protein (99.7%). In contrast to other oxicams, lornoxicam has a short plasma elimination half-life of about 4 h (Olkkola et al., 1994) and is metabolised mainly to the inactive compound 5'-hydroxy-lornoxicam (Dittrich et al., 1990) and excreted in the urine (~33%) and faeces (~66%) (Hitzenberger et al., 1990).

Trade name: Xefo (Austria, I, D, Switz, UK)

In addition to the inhibition of COX, lornoxicam shows weak inhibition of LPS-induced inducible nitric oxide synthase (iNOS; IC_{50} 65 µM) and LPS-induced interleukin-6 (IC_{50} 54 µM), both of which could contribute to its potent anti-inflammatory and analgesic action (Berg et al., 1999).

Meclofenamic Acid

Synthesis: By condensation of 2-bromobenzoic acid with 2,6-dichloro-3-methylaniline by means of $CuBr_2$ in diethyleneglycol dimethyl ether containing *N*-ethylmorpholine, and heating at 145-155 °C (Scherrer and Short (Parke Davis), 1961; 1967); Juby et al., 1968; Arrigoni-Martelli, 1978b; Kleemann et al., 1999).



Scheme 43: Synthesis of meclofenamic acid.

Clinical use: Meclofenamate (McLean and Geuckman, 1983) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain, musculoskeletal and joint disorders such as rheumatoid arthritis and osteoarthritis as well as dysmenorrhoea.

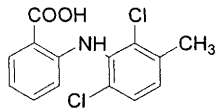
Meclofenamate shows balanced inhibition of both COX-1 and COX-2 based on data with purified enzymes.

Meclofenamate is given by oral administration (300-400 mg/day).

Peak plasma concentration of meclofenamate occur 0.5 to 1 h after oral administration. The binding to plasma proteins is over 99% and the plasma elimination half-life is about 2 to 4 h. Meclofenamate is metabolized by oxidation, hydroxylation, dehalogenation, and glucuronidation. Metabolites are excreted mainly in the urine with about 20 to 30% being excreted in the faeces (Koup et al., 1990). A 3-hydroxymethyl metabolite of meclofenamate has been reported to be active.

In addition to its action on prostaglandin synthesis, fenamates have been shown to modify several ion channel functions, e.g. inhibition of non-selective cation conductance (Gögelein et al., 1990), calcium-activated chloride channels (White and Aylwin, 1990), voltage-gated calcium channels, voltage-gated and ATP-sensitive potassium channels (Grover et al., 1994; Lee and Wang, 1999), as well as blocking gap junctions (Harks et al., 2001). The clinical relevance of these activities for the analgesic and anti-inflammatory potential of meclofenamate is unknown.

Meclofenamic Acid



[*644-62-2*], 2-(2,6-Dichloro-3-methyl-phenylamino)-benzoic acid, $C_{14}H_{11}Cl_2NO_2$, M_r 296.15, *mp* 257-259 °C; monosodium salt monohydrate [*6385-02-0*], $C_{14}H_{10}Cl_2NNaO_2 \cdot H_2O$ M_r 336.15, *mp* 289-291 °C

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	0.040	0.050	0.8
whole blood (2)	2.3	n.d.	n.d.

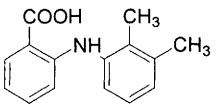
(1) Kalgutkar et al. (2000a)

(2) Young et al. (1996)

Trade name: Meclomen (Austria, I, Spain, Switz., US)

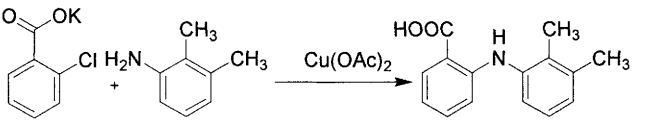
Mefenamic Acid

Mefenamic Acid



[*61-68-7*], 2-(2,3-Dimethylphenylamino)-benzoic acid, $C_{15}H_{15}NO_2$, M_r 241.29, *mp* 230-231 °C; monosodium salt $C_{15}H_{14}NNaO_2$, M_r 263.27

Synthesis (Scherrer (Parke Davis), 1961; 1967); Kleemann et al., 1999):



Scheme 44: Synthesis of mefenamic acid.

Clinical use: Mefenamic acid is a nonsteroidal anti-inflammatory drug which is used for the treatment of mild to moderate pain conditions, musculoskeletal and joint disorders such as rheumatoid arthritis and osteoarthritis, and dysmenorrhoea.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	0.04	3	0.01
cell culture (2)	0.21	6.32	0.03
whole blood (3)	1.9	0.16	12

(1) Gierse et al. (1995)

(2) Lora et al. (1998)

(3) Cryer and Feldman (1998)

Mefenamate inhibits both COX isoforms with some preference for COX-2 in a whole blood assay and for COX-1 in an enzyme preparation of recombinant human enzymes and in a cellular assay. The COX-1 preference in the cellular assay shows a time dependency as pre-incubation of the cells decreases the ratio of COX isoform selectivity from 0.03 to 0.005 (Lora et al., 1998).

Mefenamic acid is given orally (1500 mg/day maximal dose).

After oral administration, mefenamic acid reaches peak plasma concentrations after 2 to 4 h. Mefenamic acid is heavily bound to plasma proteins and plasma elimination half-life is about 2 to 4 h.

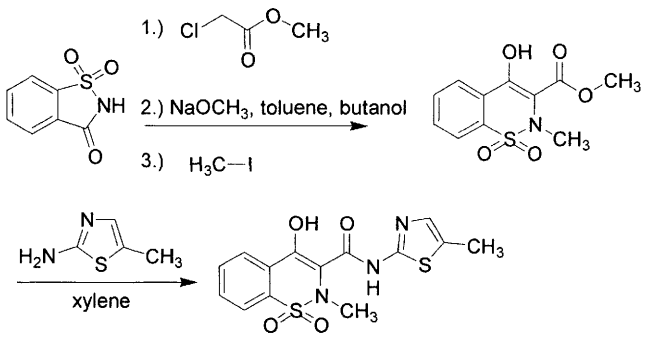
The main side effects concern the gastrointestinal system and include diarrhea (Marks and Gleeson, 1975).

Trade name: Ponstyl (F), Parkemed (Ger), Ponstan (UK), Ponstel (US)

In addition to its action on prostaglandin synthesis, fenamates have been shown to modify several ion channel functions, e.g. inhibition of non-selective cation conductance (Gögelein et al., 1990), calcium-activated chloride channels (White and Aylwin, 1990), voltage-gated calcium channels, voltage-gated and ATP-sensitive potassium channels (Grover et al., 1994; Lee and Wang, 1999), as well as blocking gap junctions (Harks et al., 2001). The clinical relevance of these activities for the analgesic and anti-inflammatory potential of mefenamate is unknown.

Meloxicam

Synthesis: The reaction of benzothiazolo-3(*2H*)-one-1,1-dioxide with methyl chloroacetate gives the methyl 2(*3H*)-acetate derivative, which is isomerized with sodium methoxide in toluene/*tert*-butanol yielding methyl 4-hydroxy-*2H*-1,2-benzothiazine-3-carboxylate-1,1-dioxide. The subsequent methylation with methyl iodide in methanol yields the 2-methyl compound. Finally this compound is treated with 2-amino-5-methylthiazole in xylene (Trummlitz et al. (Thomae GmbH), 1979; Trummlitz et al., 1989; Kleemann et al., 1999).



Scheme 45: Synthesis of meloxicam.

Clinical use: Meloxicam (Engelhardt et al., 1995) is used for the acute and chronic treatment of mild to moderate pain including arthritis and ankylosing spondylitis.

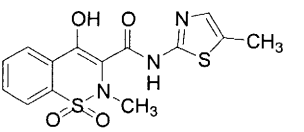
Meloxicam is 10-times more selective for COX-2 compared to COX-1 in human whole blood and belongs to the first generation of COX-2 selective drugs (Vane et al., 1998).

Meloxicam is given by oral administration (7.5-15 mg/day).

Meloxicam reaches peak plasma concentrations about 8 h after oral dosing. More than 99.5% binds to plasma proteins and it has an elimination half-life of 20 h. Meloxicam is metabolized to four inactive metabolites and excreted in the urine and faeces.

Meloxicam shows central antinociceptive effects in rats which seem to be independent of the COX-inhibitory activity (Lopez-Garcia and Laird, 1998).

Meloxicam



[71125-38-7], 4-Hydroxy-2-methyl-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2]thiazine-3-carboxylic acid (5-methyl-thiazol-2-yl)-amide, 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazoyl)-*2H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide, $C_{14}H_{13}N_3O_4S_2$, M_r 351.40, *mp* 264 °C (decomp.)

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
recomb. enzyme (1)	36.6	0.49	75
cell culture (2)	1.8	0.006	300
whole blood (3)	4.8	0.43	11.2

(1) Churchill et al. (1996)

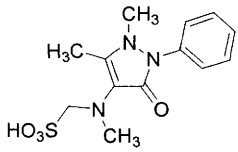
(2) Riendeau et al. (1997)

(3) Patrignani et al. (1997)

Trade name: Mobic (US, EC, J)

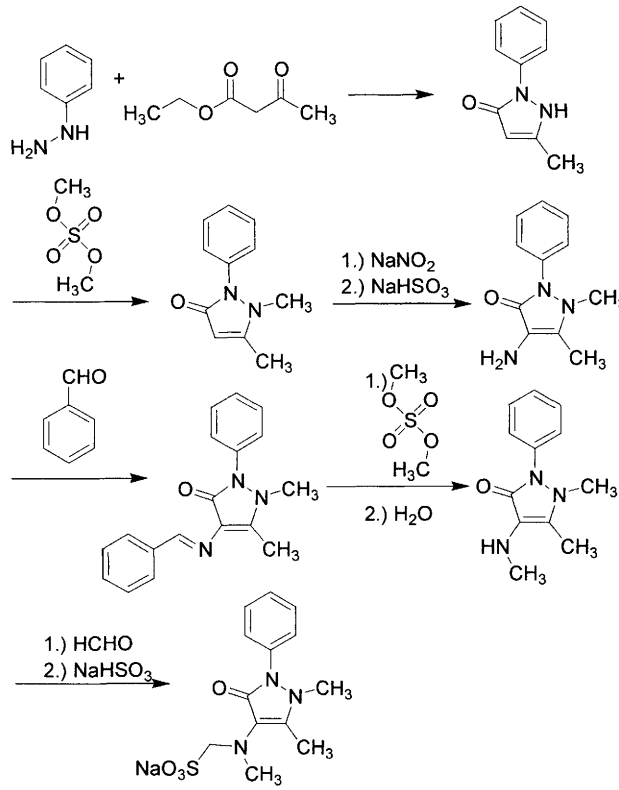
Metamizol (Dipyrone)

Metamizol (Dipyrone)



[*50567-35-6*] [(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-methyl-amino]-methanesulfonic acid, $C_{13}H_{17}N_3O_4S$, M_r 311.36; sodium salt [68-89-3], $C_{13}H_{16}N_3NaO_4S$, M_r 333.34; sodium salt monohydrate [*50567-35-6*], $C_{13}H_{16}N_3NaO_4S \cdot H_2O$, M_r 351.36

Synthesis (Boskmühl and Schwarz (I.G. Farben), 1922; Erhart and Ruschig, 1972; Kleemann et al., 1999):



Scheme 46: Synthesis of metamizole.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	420	420	1
whole blood (1)	4855	1364	3.6

(1) Campos et al. (1999)

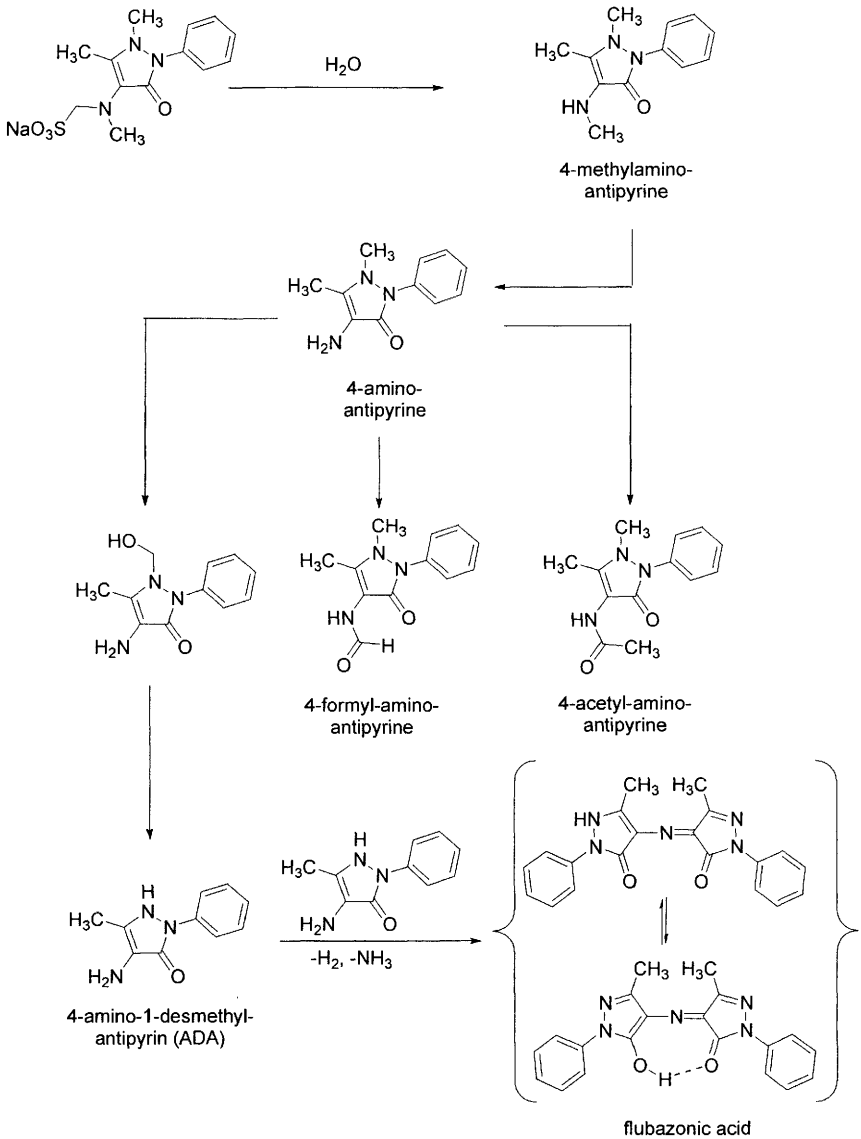
Clinical use: Metamizol is the water-soluble sodium sulfonate of amidopyrine. After oral administration it is rapidly hydrolyzed to the active 4-methyl-amino-antipyrine and metabolized to various metabolites (Levy et al., 1995; scheme 47). Metamizol has strong analgesic, spasmolytic and antipyretic action, but no anti-inflammatory properties. The exact mechanism of action is unknown but may include inhibition of prostaglandin synthesis. Inhibition of both COX isoenzymes has been demonstrated, although only in extremely high concentrations, thus questioning the relevance of this activity.

Metamizol is used for the treatment of medium to severe pain, often in combination with opioids, for fever reduction and for the treatment of colic pain. It is given by mouth in doses of 500 mg up to 4 g daily, and by intravenous or rectal routes.

The active metabolite 4-methyl-amino-antipyrine reaches peak plasma concentrations 1.2 to 2 h after oral administration and is further metabolised with a mean elimination half-life of 2.6 to 3.5 h. Of the four main metabolites about 60% are excreted in the urine. Protein binding of these metabolites is less than 60% (Levy et al. 1995).

Metabolites of metamizol:

4-methyl-amino-antipyrine, 4-formyl-amino-antipyrine, 4-amino-antipyrine, 4-acetyl-amino-antipyrine



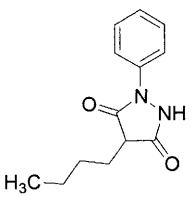
Scheme 47: Metabolic pathway of metamizol (Weithmann and Alpermann, 1983)

Trade name: Baralgin (Ger), Novalgin (Ger)

Metamizol is relatively free of acute side-effects but in rare cases may induce severe and life-threatening allergic reactions such as agranulocytosis, allergic skin reactions and allergic shock. Therefore, the compound is not used in the UK, US or Scandinavian countries.

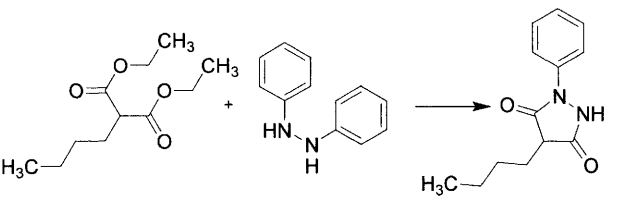
Mofebutazone

Mofebutazone



[*2210-63-1*], 4-Butyl-1-phenyl-pyrazolidine-3,5-dione, $C_{13}H_{16}N_2O_2$, M_r 232.28, *mp* 102-103 °C; sodium salt [*41468-34-2*], $C_{13}H_{15}N_2NaO_2$, M_r 254.27

Synthesis (Büchi et al., 1953; Comm. Farmaceutica Milanese, 1957; Kleemann et al., 1999):



Scheme 48: Synthesis of mofebutazone.

Clinical use: Mofebutazone is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain including inflammatory and degenerative rheumatic disorders and musculoskeletal pain.

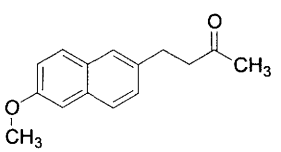
Mofebutazone is given as oral, rectal (900-1200 mg/day), or intramuscular preparations (650 mg/day).

Mofebutazone reaches peak plasma concentrations within 1.4 h and is extensively bound to plasma proteins (~99%). The plasma elimination half-life of mofebutazone is 1.9 h. Mofebutazone is excreted mainly as the glucuronides of its metabolites in the urine (Loew et al., 1985).

Trade name:Clinit (Austria), Diadin, Mofesal (Ger)

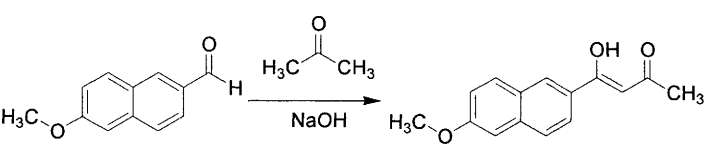
Nabumetone

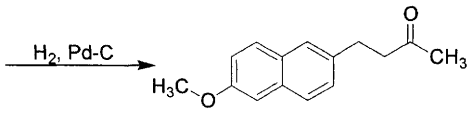
Nabumetone



[*42924-53-8*], 4-(6-Methoxy-naphthalen-2-yl)-butan-2-one, $C_{15}H_{16}O_2$, M_r 228.29, *mp* 80 °C

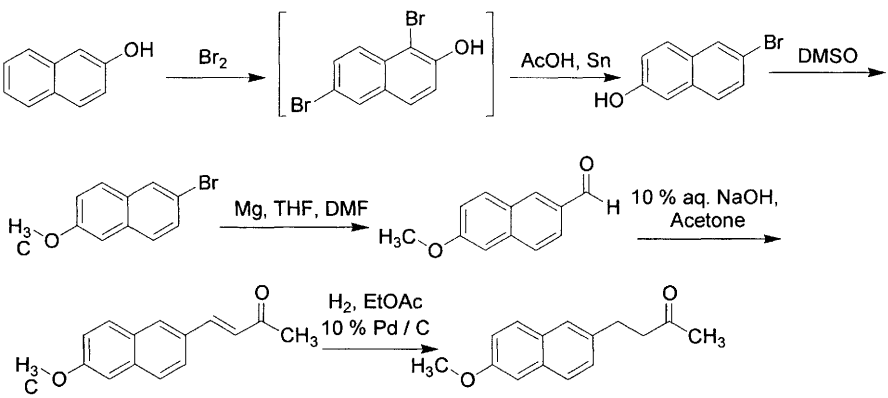
Synthesis: The condensation of 6-methoxy-2-naphthaldehyde with acetone by means of NaOH in water gives 4-(6-methoxy-2-naphthyl)-3-buten-2-one, which is reduced with H_2 over Pd-C in ethyl acetate (Goudie et al., 1978; Neumann, 1981; Lake and Rose (Beecham), 1974; 1983; Kleemann et al., 1999; Prabhakar et al., 1999).





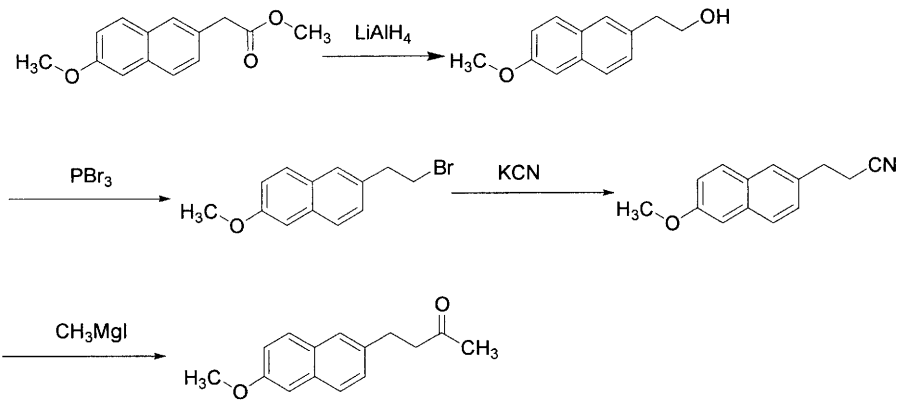
Scheme 49: Synthesis of nabumetone.

The following route starts with the bromination of β-naphthol, using molecular bromine and resulting in the formation of 2,5-dibromo-6-naphthol, which on monodebromination with tin metal affords the 6-bromo-2-naphthol in high yields. The bromophenol thus obtained is O-methylated using dimethyl sulfate to afford 2-bromonaroline in quantitative yields, which in turn is converted to 6-methoxy-2-naphthaldehyde via 6-methoxynaphthylmagnesium bromide using a Mg/DMF/THF protocol. The aldol condensation of the aldehyde with acetone resulted in the formation of the enone. The final reduction of the enone, using Pd/C or Raney-Ni, proceeded smoothly to produce nabumetone.



Scheme 50: Synthesis of nabumetone.

The reduction of methyl 6-methoxy-2-naphthyl acetate with lithium aluminium hydride in refluxing ether gives 2-(6-methoxy-2-naphthyl)ethanol, which by treatment with PBr_3 in refluxing benzene is converted into 2-(6-methoxy-2-naphthyl)ethyl bromide. Further reaction with KCN in refluxing ethanol-water affords 3-(6-methoxy-2-naphthyl) propionitrile, which is finally treated with methylmagnesium iodide in refluxing ethanol.



Scheme 51: Synthesis of nabumetone.

Several further synthetic methods have been described.

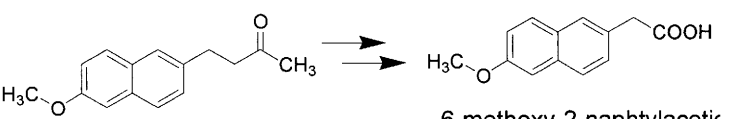
Clinical use: Nabumetone (Friedel et al., 1993) undergoes rapid first-pass metabolism to the active metabolite 6-MNA (6-methoxy-2-naphthyl acetic acid). 6-MNA shows balanced inhibition of both COX-1 and COX-2 in cell culture and a whole blood assay.

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	2.3	~5	~0.5
whole blood (2)	278	187	1.5

(1) Riendeau et al. (1997)

(2) Patrignani et al. (1997)



6-methoxy-2-naphtylacetic acid (6-MNA)

Scheme 52: 6-Methoxy-2-naphtylacetic acid (6-MNA), the active metabolite of nabumetone.

Nabumetone is used for the treatment of pain and inflammation associated with osteoarthritis and rheumatoid arthritis. The drug is is administered orally (500-2000 mg/day).

Trade name: Relafen (US), Relifex (UK)

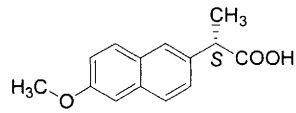
Nabumetone is well absorbed from the gastrointestinal tract following oral administration and undergoes rapid and extensive first-pass metabolism in the liver to 6-MNA and other inactive metabolites. There is 99% binding of 6-MNA to plasma protein and it has an elimination half-life of up to 22 h with marked individual differences. 6-MNA is metabolized by O-methylation and conjugation and is excreted to about 80% in the urine as inactive or conjugated metabolites (Davies, 1997).

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Naproxen

The 2-arylpropionic acid derivatives (profens) are important classes of NSAIDs that have been in clinical use for over 20 years. The profens have been used clinically as racemic agents with the exception of (*S*)-(+)-naproxen, which has been developed and used only as a single enantiomeric drug.

Naproxen



[*22204-53-1*], (+)-(*S*)-2-(6-Methoxy-naphthalen-2-yl)-propionic acid, $C_{14}H_{14}O_3$, M_r 230.26, *mp* 155.3 °C (also reported as 152-154 °C),), $[\alpha]_D$ +66° (*c* = 1, $CHCl_3$); sodium salt [*26159-34-2*], $C_{14}H_{13}NaO_3$, M_r 252.25, *mp* 244-246 °C, $[\alpha]_D$ -11° (*c* = 1, $CHCl_3$)

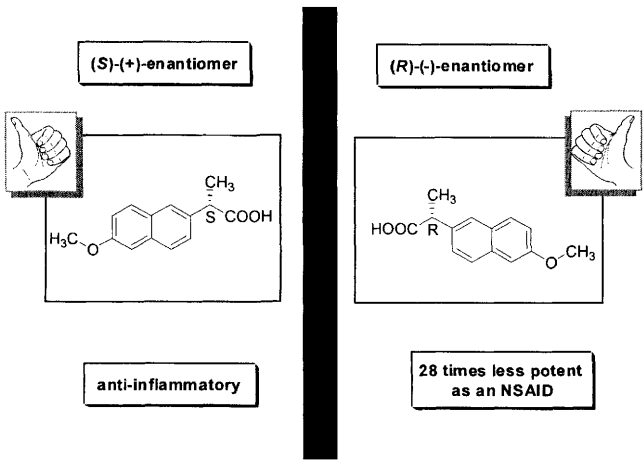


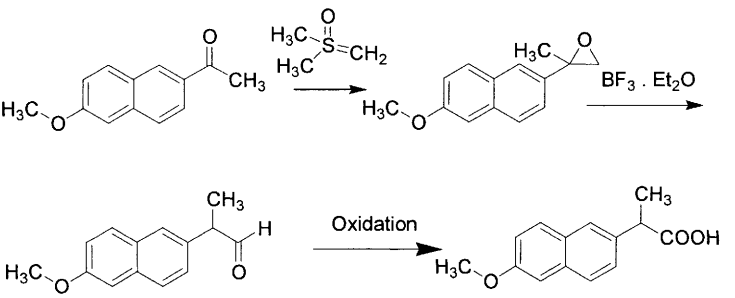
Figure 20: Enantiomers of naproxen.

Drug history

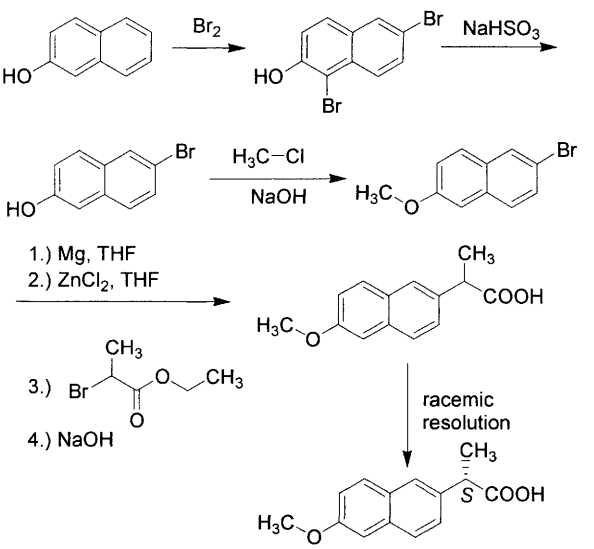
- 1967 first publication (SYNTEX)
- 1972 intrudoction to the pharmaceutical market as an anti-inflammatory, analgesic and antipyretic drug in the form of the free acid, later as the sodium salt (naproxen sodium)
- 1988 expiry of the patent protection (development of the generic market in many countries)
- 1993 expiry of the patent protection in the US
- 2-Arylpropanoic acid derivative with one chiral center
- (*S*) enantiomer is 28 times more active as an anti-inflammatory agent than the (*R*) enantiomer
- Naproxen is the only NSAID drug currently on the market in an enantiomerically pure form
- Current production of naproxen and naproxen sodium: 1000 tons per annum
- Bulk price (1990) US $ 140-150 per kg.

Synthesis (Shen, 1972; Dorfman, 1975; Sonawane et al., 1992; Kleemann et al., 1999)

Synthesis of racemic naproxene: Friedel-Crafts acylation (aluminum chloride - nitrobenzene) of β-naphthol methyl ether affords 2-acetyl-6-methoxy naphthalene, which, when treated with either dimethyl sulfonium or dimethylsulfoxonium methylide, gives 2-(6-methoxynaphthalen-2-yl)propylene oxide. Treatment of the latter with boron trifluoride etherate in tetrahydrofuran gives 2-(6-methoxynaphthalen-2-yl)propionaldehyde, which is oxidized using Jones reagent (4 M chromic acid) to yield the racemic 2-(6-methoxynaphthalen-2-yl)propionic acid.



Scheme 53: Synthesis of racemic naproxen.



Scheme 54: First large-scale manufacturing process of Syntex (Alvarez, 1972):

For racemic resolution of naproxen the use of cinchonidine, *N*-alkyl-*D*-glucamine, dehydroabietylamine or (*S*)-α-phenylethylamine has been described.

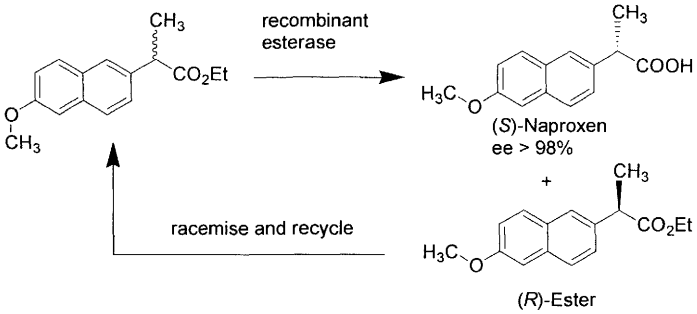
For the enzymatic cleavage of esters of racemic naproxen, cloned esterases, which are cheap and easy to produce, have been developed (a 100 tons per annum process is planned).

Advantages of the enzymatic process

Cloned esterase (isolated from *Bacillus subtilis* and cloned in *E. coli*), cheap and easy to produce

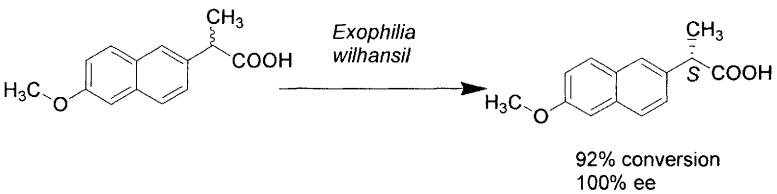
Utilizes insoluble non-toxic ester, 150 g/l, conversion 39%

Very simple processing to recover product, 100 tons per annum (SHASUN Process)

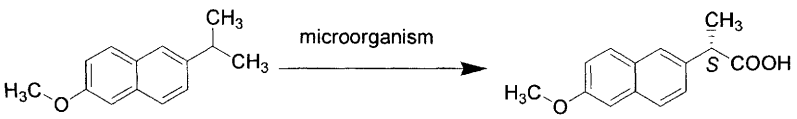


Scheme 55: Enzymatic cleavage of esters of racemic naproxen.

Other biocatalytic processes for (*S*)-naproxen production from the academic area are shown below:

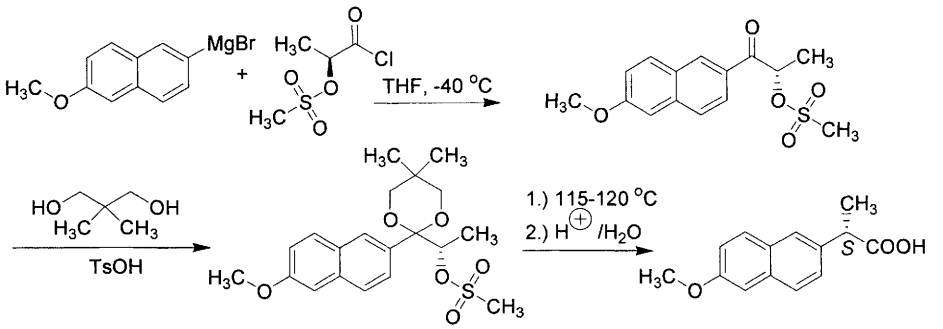


Scheme 56: Direct isomerization of racemic naproxen using the biocatalyst *Exophialia wilhansil.*

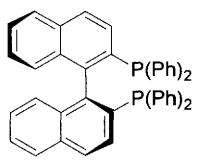


Scheme 57: One-step synthesis by microbial oxidation (IBIS).

Several other synthetic routes also exist. The stereospecific Syntex process is an example using chiral technology to produce enantiomerically pure naproxen:

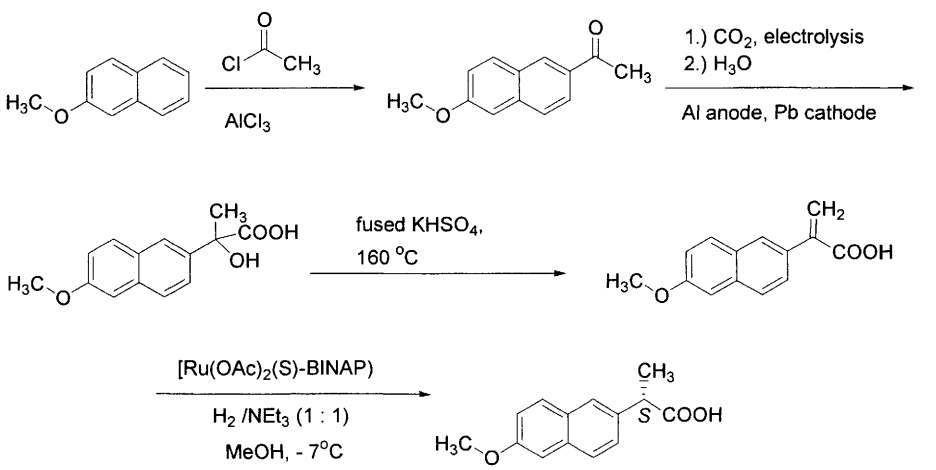


Scheme 58: Stereospecific Syntex process starting with ethyl-(*S*)-lactate (Schloemer (Syntex), 1986):



(*S*)-BINAP

The asymmetric hydrogenation of 2-(6-methoxy-2-naphthyl)acrylic acid using ruthenium-BINAP complexes also yields enantiomerically pure naproxen.



Scheme 59: Synthesis of naproxen by asymmetric hydrogenation.

Clinical use: Naproxen (Todd and Clissold, 1990) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain and inflammatory pain conditions such as rheumatoid arthritis, osteoarthritis, soft tissue disorders, postoperative pain and dysmenorrhoea. It is also used to treat migraine. Naproxen shows balanced inhibition of both COX isoenzymes in a cellular assay and a preference for COX-1 in a whole blood assay and in an enzyme assay using recombinant human enzymes.

Naproxen has fewer cardiovascular side-effects in comparison with the COX-2 selective inhibitor rofecoxib (Mukherjee et al., 2001).

Naproxen is the (+)-enantiomer. It is given orally or rectally with a common initial dose of 500 mg (up to 1250 mg/day). The major side-effects are gastrointestinal disturbances.

Naproxen is available as a free base, as sodium salt and in combination with misoprostol for the reduction of the gastrointestinal side-effects.

The plasma elimination half life of naproxen is about 13 h. Naproxen is heavily bound to plasma proteins (>99%) at therapeutic concentrations. Approximately 95% of the compound is excreted as naproxen and its 6-*O*-desmethyl metabolite (Davies and Andersson, 1997).

The synthesis and *in vitro* evaluation of novel morpholinyl- and methylpiperazinylacyloxyalkyl prodrugs of naproxen for topical drug delivery has been described recently (Rautio et al., 2000).

COX selectivity

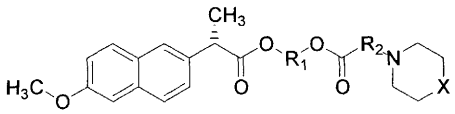
IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	1.1	36	0.03
cell culture (2)	2.2	1.3	1.7
whole blood (3)	7.8	73.7	0.1

(1) Gierse et al. (1995)

(2) Mitchell et al. (1993)

(3) Brideau et al. (1996)

Trade name: Proxen (Ger), Apranax (F, Ger), Naprosyn (UK, US)



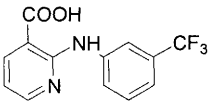
$R_1 = (CH_2)_2, (CH_2)_4$
$R_2 = CH_2, (CH_2)_2$
$X = O, N\text{-}CH_3$

Figure 21: Novel morpholinyl- and methylpiperazinylacyloxy alkyl prodrugs of naproxen for topical drug delivery.

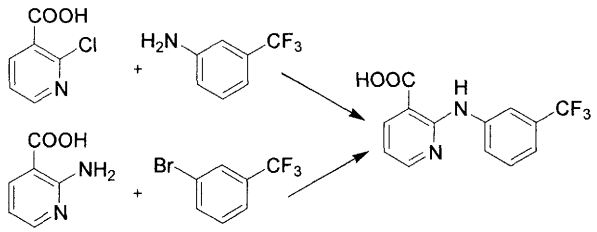
Niflumic Acid

Synthesis: Condensation of 2-chloronicotinic acid with 3-trifluoromethylaniline or reaction of 2-aminonicotinic acid with 1-bromo-3-trifluoromethylbenzene yields niflumic acid (Sherlock and Sperber (Schering Corp.), 1967; Faure and Hoffman (Labs. U.P.S.A.), 1968; Kleemann et al., 1999).

Niflumic Acid



[*4394-00-7*], 2-(3-Trifluoromethyl-phenyl-amino)-nicotinic acid, 2-[[3-(trifluoromethyl)]amino]-3-pyrodinecarboxylic acid, $C_{13}H_9F_3N_2O_2$, M_r 282.22, *mp* 204 °C



Scheme 60: Synthesis of niflumic acid.

Clinical use: Niflumic acid (Auclair et al., 1989) is a nonsteroidal anti-inflammatory drug used for the treatment of inflammation and pain in musculoskeletal and joint disorders such as rheumatoid arthritis as well as traumatic and postoperative pain. Niflumic acid is used in oral, rectal or topical preparations (up to 750 mg/day).

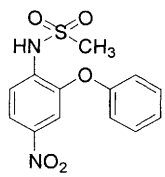
The morpholinoethyl ester morniflumate, which is used in topical formulations was shown to inhibit both cyclooxygenase and 5-lipoxygenase, thus suggesting an additional potential in anti-inflammatory therapy (Civelli et al., 1991).

In addition to its action on prostaglandin synthesis, fenamates have been shown to modify several ion channel functions, e.g. inhibition of non-selective cation conductance (Gögelein et al., 1990), calcium-activated chloride channels (White and Aylwin, 1990), voltage-gated calcium channels, voltage-gated and ATP-sensitive potassium channels (Grover et al., 1994; Lee and Wang, 1999), as well as blocking gap junctions (Harks et al., 2001). The clinical relevance of these activities for the analgesic and anti-inflammatory potential of niflumic acid is unknown.

Trade name: Niflurid, Niflugel (B, F, Switz.), Actol (Austria, S)

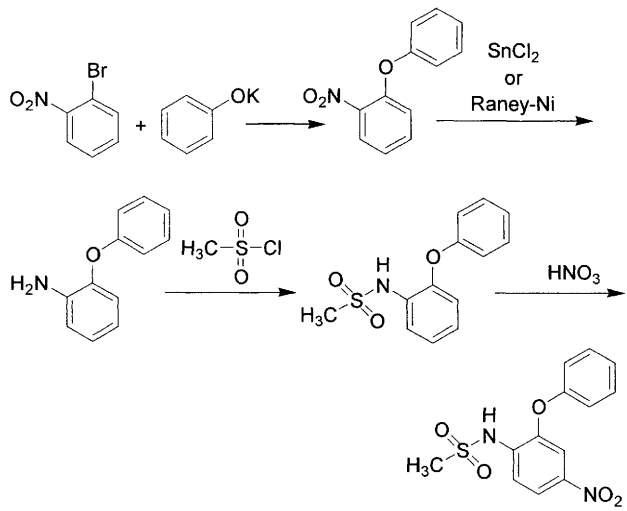
Nimesulide

Nimesulide



[*51803-78-2*], N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide, $C_{13}H_{12}N_2O_5S$, M_r 308.05, *mp* 143-144.5 °C

Synthesis (Riker, 1973; 1974; Kleemann et al., 1999):



Scheme 61: Synthesis of nimesulide.

Clinical use: Nimesulide (Davis and Brogden, 1994) is a first-generation COX-2 inhibitor with up to 100-fold

selectivity for COX-2 compared to COX-1, depending on the assay system.

It is used for the short-term treatment of inflammatory conditions, fever and pain, including musculoskeletal and joint disorders. Nimesulide is used as an oral or rectal formulation (up to 400 mg/day).

Peak plasma concentrations are reached within about 2 to 3 h after oral administration. The terminal plasma elimination half-life is between 2 and 5 h. Nimesulide is subject to extensive metabolism. The principal active metabolite is 4-hydroxy-nimesulide. Nimesulide and its metabolites are excreted in the urine (~70%) and the faeces (~20%).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	0.78	0.009	87
whole blood (2)	9.2	0.52	18

(1) Riendeau et al. (1997)

(2) Patrignani et al. (1997)

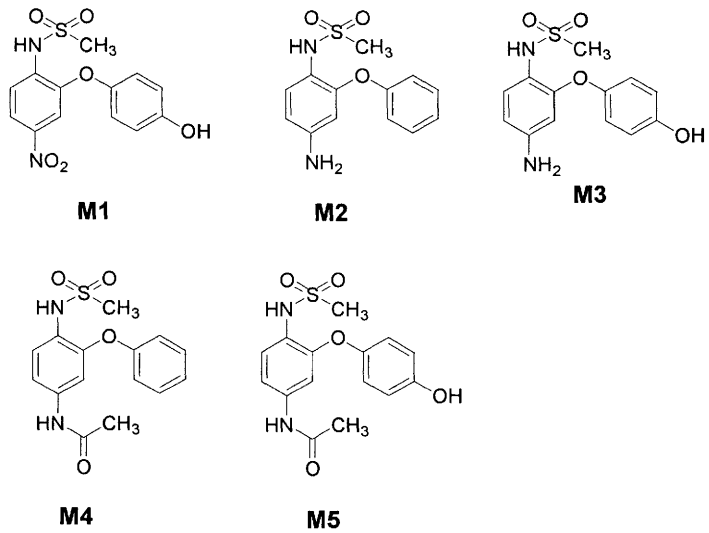


Figure 22: Urinary metabolites of nimesulide in man (Singla et al., 2000) including M1, 4-hydroxy-nimesulide, an active metabolite of nimesulide

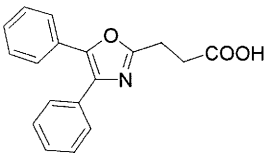
Nimesulide has been reported to induce hepatic failure in some cases (McCormick et al., 1999; Ferreiro et al., 2000).

In addition to the inhibition of COX, nimesulide has been shown to inhibit the production of the pro-inflammatory cytokine TNF-α under inflammatory conditions (Azab et al., 1988).

Trade name: Nexen (France), Aulin (Italy, CH)

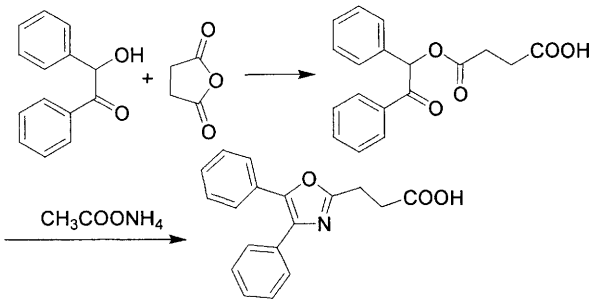
Oxaprozin

Oxaprozin



[*21256-18-8*], 3-(4,5-Diphenyl-oxazol-2-yl)-propionic acid, $C_{18}H_{15}NO_3$, M_r 293.32, *mp* 160.5-161.5 °C

Synthesis: Brown (Wyeth), 1971; Arrigoni-Martelli, 1978c; Kleemann et al., 1999):



Scheme 62: Synthesis of oxaprozin.

Clinical use: Oxaprozin (Miller, 1992) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain including rheumatoid arthritis and osteoarthritis.

COX selectivity

IC50 [µM]	COX-1	COX-2	ratio
cell culture (1)	2.2	36	0.06
whole blood (2)	15	37	0.4

(1) Kawai et al. (1998)

(2) Cryer and Feldman (1998)

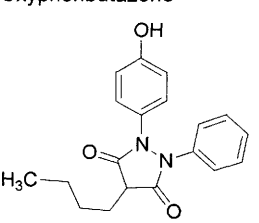
Oxaprozin shows selectivity for COX-1 in human cellular assays and a more balanced inhibition of both COX-1 and COX-2 in a whole blood assay. It is given orally (600-1200 mg/day, maximum dose 1800 mg/day).

Oxaprozin reaches peak plasma concentrations 2 to 6 h after oral administration (Davies, 1998b). It shows slow kinetics with an elimination half-life of about 24 h. Oxaprozin is metabolized and glucuronized and excreted in the urine and the bile. Two hydroxlated metabolites have been shown to have anti-inflammatory activity.

Trade names: Deflam (S. Afr.), Daypro (US)

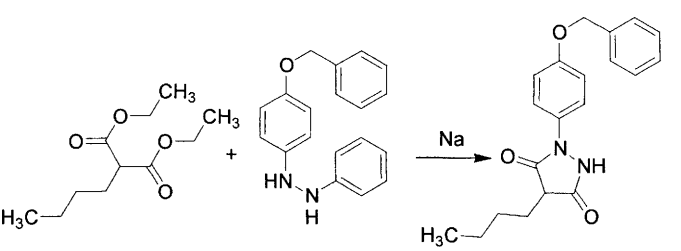
Oxyphenbutazone

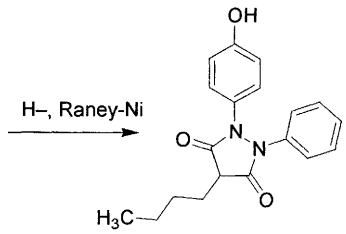
Oxyphenbutazone



[*129-20-4*], 4-Butyl-1-(4-hydroxy-phenyl)-2-phenyl-pyrazolidine-3,5-dione, $C_{19}H_{20}N_2O_3$, M_r 324.37, *mp* 124-125 °C; monohydrate [*7081-38-1*], $C_{19}H_{20}N_2O_3 \cdot H_2O$, M_r 342.40, *mp* 96 °C

Synthesis (Häflinger (Geigy), 1956; Kleemann et al., 1999):





Scheme 63: Synthesis of oxyphenbutazone.

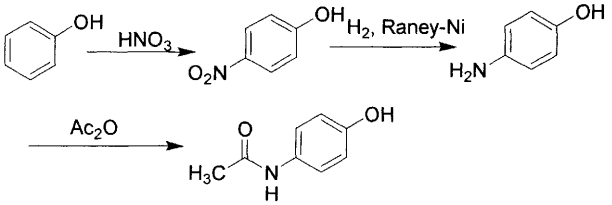
Clinical use: Oxyphenbutazone is a nonsteroidal anti-inflammatory drug used for the acute treatment of ankylosing spondylitis, chronic polyarthritis and gout. Oxyphenbutazone is a metabolite of phenylbutazone and is limited in use because of a high incidence of hematopoietic side-effects such as fatal agranulocytosis and aplastic anemia (Bottiger and Westerholm, 1973). Therefore, oxyphenbutazone should only be used if other nonsteroidal anti-inflammatory drugs do not show sufficient efficacy.

Oxyphenbutazone can be administered as oral, rectal or topical preparations (400-600 mg/day).

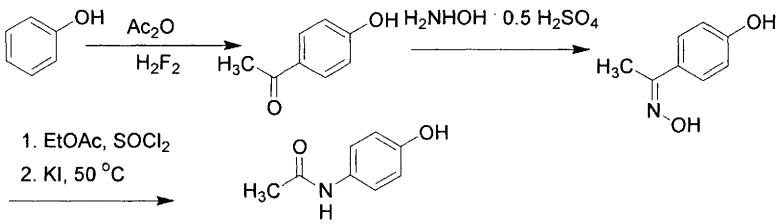
Trade name: Tanderil (Austria, Eire, Switz.), Phlogont (Ger)

Paracetamol (Acetaminophen)

Synthesis (Wilbert and DeAngelis (Warner-Lambert), 1961; Kleemann et al., 1999):

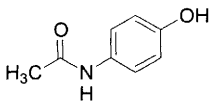


Scheme 64: Synthesis of paracetamol, classical route.



Scheme 65: Synthesis of paracetamol, Hoechst-Celanese process.

Paracetamol



[*103-90-2*], *N*-(4-Hydroxyphenyl)-acetamide, $C_8H_9NO_2$, M_r 151.16, *mp* 168-169 °C

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cellular assay (1)	2.7	20	0.1
whole blood (2)	>100	49	<2
whole blood (3)	42	11	4

(1) Mitchell et al. (1993) (IC_{30} values are given, because 50% inhibition of COX-2 was not reached at concentrations up to 1 mg/kg)

(2) Warner et al. (1999)

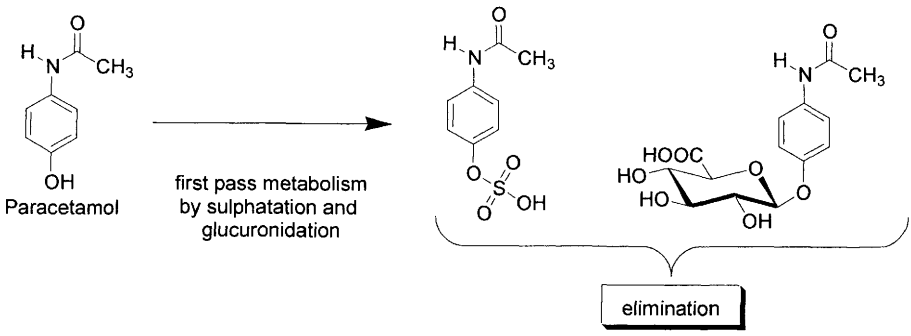
(3) Cryer and Feldman (1998)

Clinical use: Paracetamol (Ameer and Greenblatt, 1977; Clissold, 1986) has analgesic and antipyretic properties, but no relevant anti-inflammatory action. It is used for the treatment of various mild to moderate pain conditions and to reduce fever. Paracetamol is one of the most popular analgesics as a single drug or in multi-ingredient preparations, often in combination with NSAIDs or weak opioids.

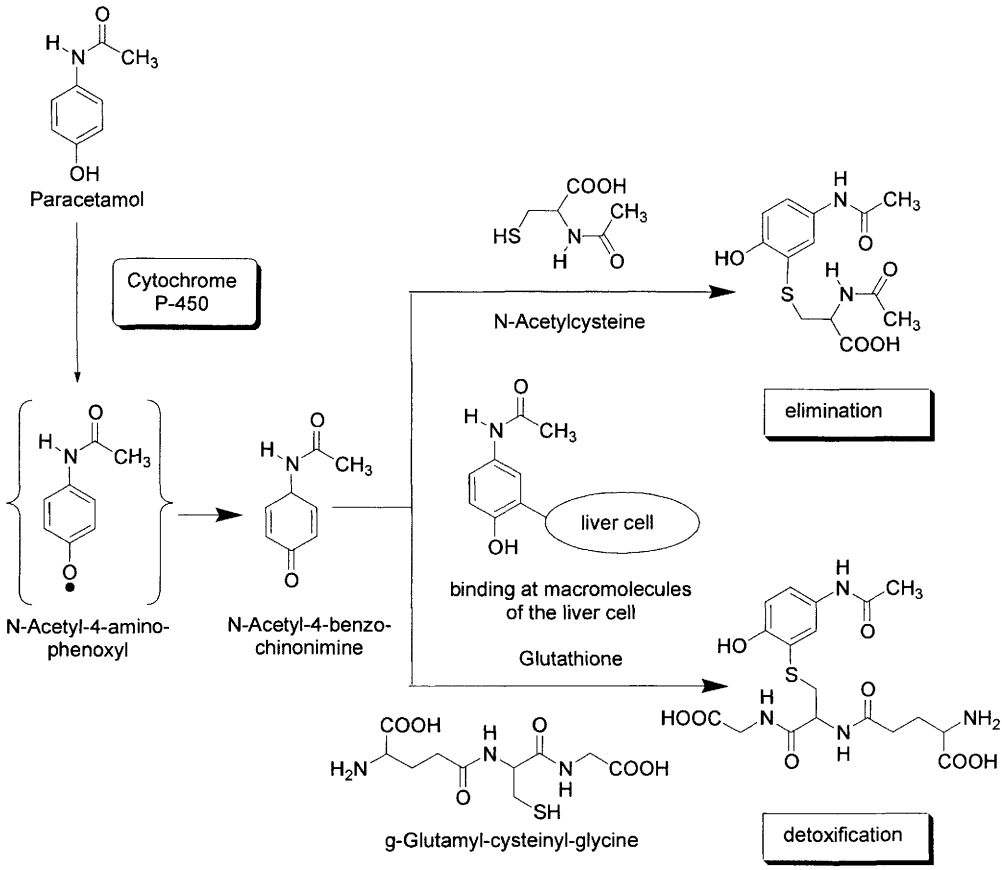
Despite its long clinical history after its discovery in 1893 (von Mering, 1893), the mechanism of action of paracetamol is not fully understood. It shows some weak inhibition of the COX isoenzymes and there is speculation on a third COX isoenzyme, COX-3, induced during the resolution phase of an inflammatory response, that might be specifically targeted by paracetamol (Willoughby et al., 2000). Furthermore, there is evidence for a possible central analgesic effect mediated indirectly by 5-HT (Courade et al., 2001).

Paracetamol is used orally or rectally as suppositories, the oral dose range is 500-1000 mg every 4-5 h up to 4 g daily.

Paracetamol reaches peak plasma concentrations within the first hour after oral administration and shows only a low tendency for plasma protein binding at therapeutic concentrations. The elimination half-life is between 1 and 3 h. Paracetamol is metabolized mainly in the liver and excreted in the urine as glucuronide and sulphate conjugates. The metabolic pathway of paracetamol is shown in Schemes 66 and 67:



Scheme 66: Formation of the glucuronide and sulphate conjugates of paracetamol.



Scheme 67: Formation of the liver toxic metabolite *N*-acetyl-4-benzochinonimine and its elimination and detoxification with gluthathione or (*N*-acetyl)cysteine.

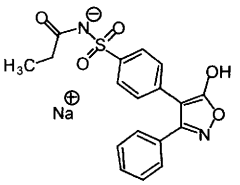
Side-effects are rare and may include hematological reactions, leucopenia, agranulocytosis and other hypersensitivity reactions. Paracetamol has a narrow therapeutic dose range and overdosage induces severe liver and renal damage (Lewis and Paloucek, 1991) via accumulation of a toxic metabolite, *N*-acetyl-benzoquinoneimine (NABQI). Acetylcysteine or methionine, which increase glutathione conjugation of the metabolite, are used as the antidote.

Paracetamol is not soluble in aqueous solutions and cannot be given parenterally. A soluble glycine prodrug derivative of paracetamol is on the market as parenteral form (propacetamol).

Trade name: Dafalgan (Fr), Benuron (Ger, Switz), Tylenol (Austr, Austral, Canad, US, Irl, Sp), Alvedon (Norw, Swed, UK)

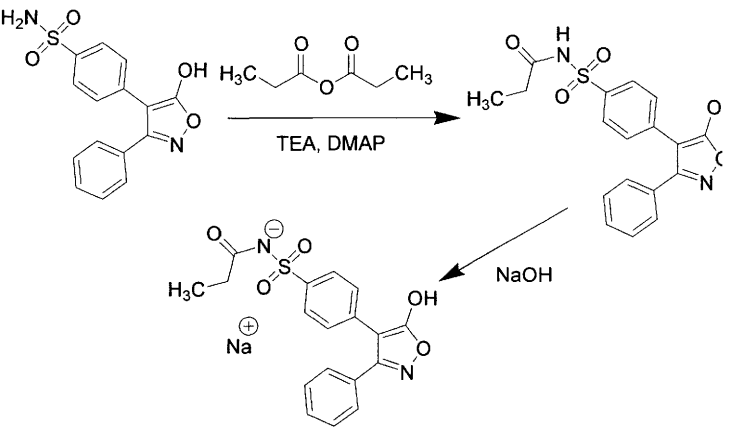
Parecoxib sodium

Parecoxib sodium



[*198470-85-8*], [*198470-84-7*] (free acid) , N-(4-(5-methyl-3-phenylisoxazol-4-yl)phenylsulfonyl)propion-amide sodium salt $C_{19}H_{17}N_2O_4SNa$, 392.409; crystals mp 271.5-272.7 °C

Synthesis: The acylation of 4-(5-methyl-3-phenylisoxazol-4-yl)benzenesulfonamide (valdecoxib), with propionic anhydride in a solution of TEA and DMAP in tetrahydrofurane gives *N*-(4-(5-methyl-3-phenylisoxazol-4-yl)phenylsulfonyl)propionamide, which is treated with NaOH in ethanol to give parecoxib sodium salt (Talley (Parmacia Corp.), 1996; 2000b; Sorbera, 2001b).



Scheme 68: Synthesis of parecoxib sodium.

Clinical use: Parecoxib (Cheer and Goa, 2001; Gotta, 2001; Sorbera et al., 2001b) is a third generation COX-2 inhibitor. Parecoxib is a prodrug of valdecoxib with aqueous solubility sufficient for the use of the substance in parenteral formulations. Valdecoxib shows about 30-fold selectivity for COX-2 in whole blood assays (see Valdecoxib; Talley et al., 2000a; Riendeau et al., 2001). Parecoxib shows efficacy in a variety of animal models of inflammation (Talley et al., 2000b).

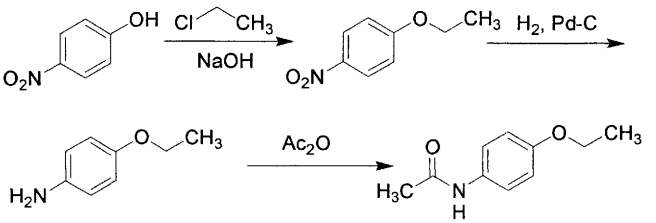
Parecoxib is rapidly hydrolyzed by the liver to its active metabolite valdecoxib. Plasma peak concentrations for valdecoxib are achieved within 1.1 to 3.5 and 0.27 to 2 h after i.m. and i.v. administration, respectively, in healthy volunteers. The elimination half-life for parecoxib is 15 to 35 min and 5 min for i.m. and i.v. administration respectively, in healthy volunteers. Metabolism of parecoxib follows the metabolism of the active metabolite valdecoxib which is a substrate for cytochrome P450 3A4 and 2C9.

Trade name: Xapit, Dynastat, Rayzon (EC)

Parecoxib is used in doses of 20 and 40 mg of its sodium salt for the short-term treatment of postoperative pain.

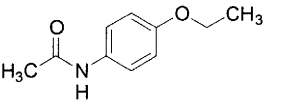
Phenidine (Phenacetin)

Synthesis (Kleemann et al., 1999):



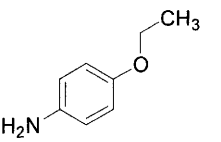
Scheme 69: Synthesis of phenidine

Phenidine



[*62-44-2*], *N*-(4-Ethoxy-phenyl)-acetamide, $C_{10}H_{13}NO_2$, M_r 179.22, *mp* 134-135 °C

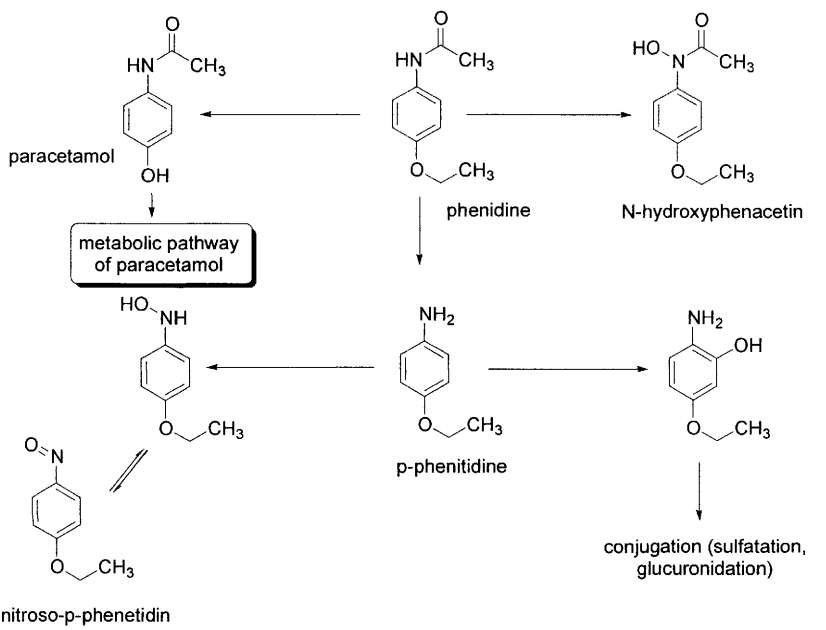
Clinical use: Phenidine (Clissold, 1986) is a weak analgesic and antipyretic compound without anti-inflammatory action. It has been used in combination with other compounds like aspirin, caffeine or codeine, but due to hematological and nephrotoxic side-effects (Dubach et al., 1983) has been withdrawn from many markets.



p-phenetidine, the toxic metabolite of phenidine

Phenidine is rapidly metabolized to a great extent to its metabolite paracetamol which seems to be responsible for the therapeutic action of phenidine. Another metabolite, *p*-phenetidine is responsible for the toxic side-effects.

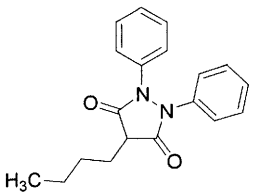
Trade name: Gripponyl (F), Cratodin (Spain)



Scheme 70: Metabolic pathway of phenidine with the formation of *p*-phenetidine, the toxic metabolite of phenidine.

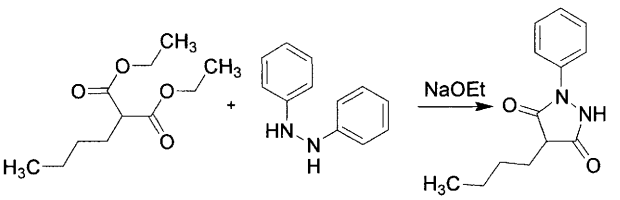
Phenylbutazone

Phenylbutazone



[*50-33-9*], 4-Butyl-1,2-diphenyl-pyrazolidine-3,5-dione, $C_{19}H_{20}N_2O_2$, M_r 308.37, *mp* 105 °C; sodium salt [*129-18-0*], , $C_{19}H_{19}N_2NaO_2$, M_r 331.36; calcium salt (2 : 1) [*36298-23-4*], , $C_{38}H_{36}CaN_4O_4$, M_r 656.83; piperazine salt (1 : 1) [*4985-25-5*], , $C_{19}H_{20}N_2O_2 . C_4H_{10}N_2$, M_r 395.51, *mp* 140-141 °C

Synthesis (Stenzl (Geigy), 1951; Kleemann et al., 1999):



Scheme 71: Synthesis of phenylbutazone.

Clinical use: Phenylbutazone (Brogden, 1986) is a nonsteroidal anti-inflammatory drug used for the acute treatment of ankylosing spondylitis, chronic polyarthritis and gout. Phenylbutazone on its own shows only weak inhibition of COX-1 and COX-2 with IC50s >30 µM (Brideau et al., 1996) and active metabolites are mainly responsible for its actions.

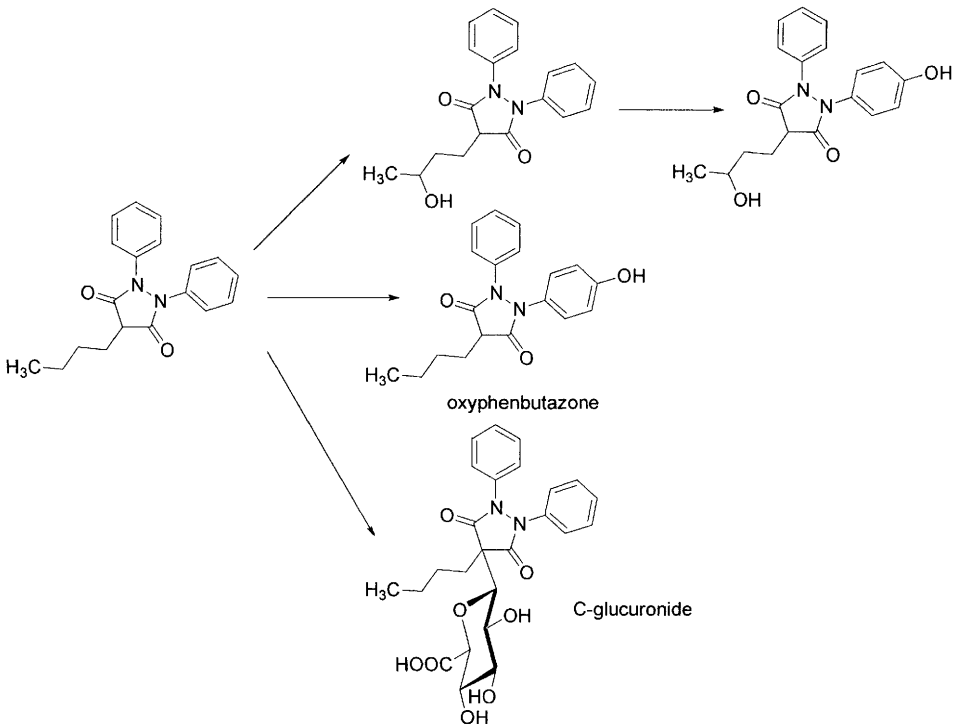
Side-effects include disturbances of the hematopoietic system such as agranulocytosis and aplastic anaemia (Faich, 1987) and limit its use to the treatment of conditions in which other nonsteroidal anti-inflammatory drugs do not show sufficient efficacy.

Phenylbutazone is given as oral, rectal, intramuscular or topical formulation (up to 600 mg/day initial dose, up to 400 mg/day maintenance dose).

The peak plasma concentration is reached 2 h after oral administration. The degree of binding of phenylbutazone to plasma proteins is 98%. The long elimination half-life of phenylbutazone (mean ~70 h) exhibits large interindividual and intraindividual variation. It is metabolized in the liver by oxidation and glucuronidation and excreted in the urine and to a lower degree (~25%) in the faeces (Aarbakke, 1978). Oxyphenbutazone is an active metabolite of phenylbutazone. The metabolic pathway of phenylbutazone is shown in Scheme 72.

Trade name: Butazolidin (F, Ger, UK, USA), Ambene (Ger)

Phenylbutazone is also used in veterinarian medicine in many species including camels (Wasfi et al., 1997).



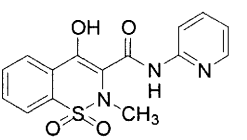
Scheme 72: Metabolic pathway of phenylbutazone.

Piroxicam

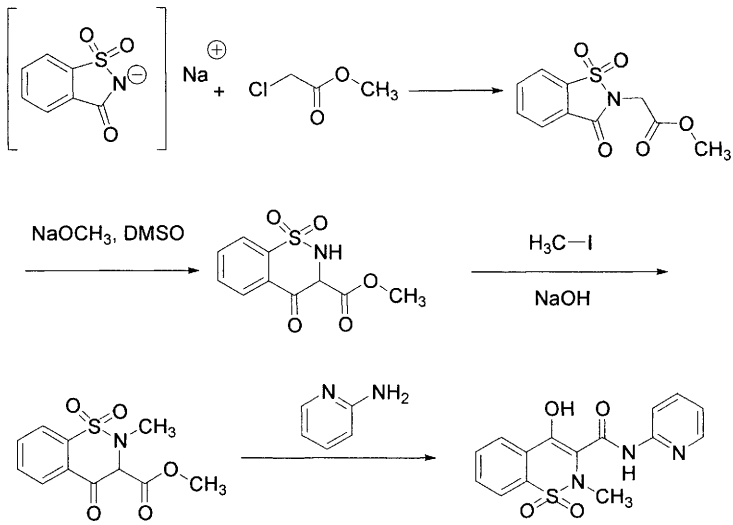
Synthesis (Lombardino (Pfizer), 1971; 1984); Lombardino et al., 1973; Hromatka et al. (Hoffmann-La Roche), 1975; Wiseman et al., 1976; Castaner and Arrigoni-Martelli, 1977b; Guzmann, 1986):

An improved procedure using 2-methoxyethyl 2-chloroacetate in place of methyl 2-chloroacetate for the alkylation of sodium saccharin has been described. The resulting 2-methoxyethyl saccharin-2-acetate is treated with sodium 2-methoxyethoxide in dimethyl sulfoxide, then acidified to give 2-methoxyethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide, which is *N*-alkylated with methyl iodide in acetone-aqueous sodium hydroxide. The resulting 2-methoxyethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide is heated with 2-aminopyridine in xylene to give piroxicam.

Piroxicam

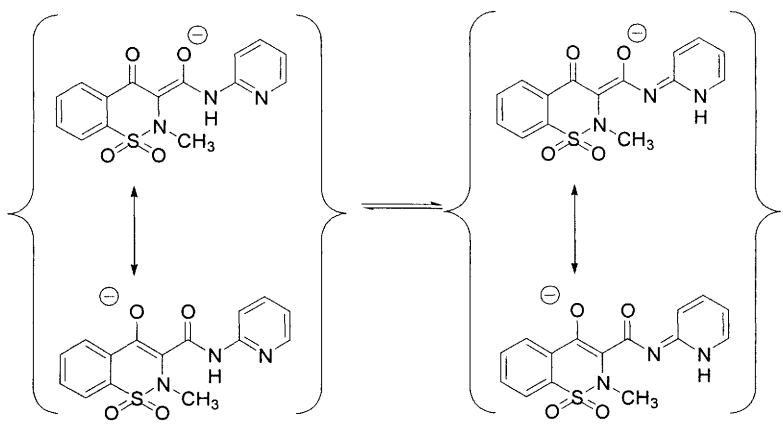


[*36322-90-4*], 4-Hydroxy-2-methyl-1,1-dioxo-1,2-dihydro-1λ^6-benzo[*e*][1,2] thiazine-3-carboxylic acid pyridin-2-ylamide, 4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide, $C_{15}H_{13}N_3O_4S$, M_r 331.35, *mp* 198-200 °C.



Scheme 73: Synthesis of piroxicam.

With a pK_A of 5.5 piroxicam is a weak acidic enol. The resonance stabilization of the anionic form is shown below.



Scheme 74: Resonance stabilization of the anionic form of piroxicam.

Clinical use: Piroxicam (Brogden et al., 1981b) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate acute and chronic pain and inflammation including musculoskeletal, soft tissue and joint disorders such as ankylosing spondylitis, chronic polyarthritis and gout (Brogden et al., 1984).

Piroxicam shows up to 12-fold selectivity for COX-1 compared to COX-2 in several assay systems. The variations of the results in whole blood assays from different laboratories (see also Warner et al., 1999; ratio COX-1/COX-2: 0.3 and Young et al., 1996; ratio COX-1/COX-2: 0.4) stress the importance of careful interpretation of COX selectivity ratios.

Piroxicam is given by oral, rectal, intramuscular or topical administration (10-30 mg/day, maximal initial dose 40 mg/day) as the free base, as a complex with beta-cyclodextrin and as the cinnamate or pivalate.

After oral administration, piroxicam reaches peak plasma concentration after 3 to 5 h, shows 99% binding to plasma protein and a long half-life of about 50 h. Piroxicam is metabolized in the liver by hydroxylation and glucuronidation and excreted mainly in the urine.

The metabolic pathway of piroxicam is shown in Scheme 75.

COX selectivity

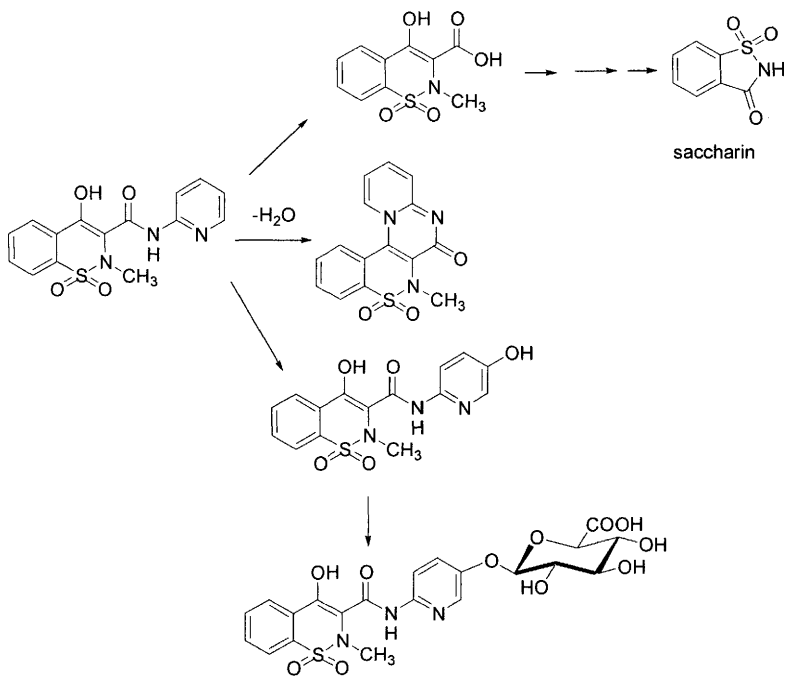
IC50 [μM]	COX-1	COX-2	ratio
purified enzyme (1)	13	>100	<0.1
cell culture (2)	0.45	0.77	0.6
whole blood (3)	2.9	0.93	3
whole blood (4)	0.76	8.9	0.08

(1) Gierse et al. (1995)

(2) Berg et al. (1997)

(3) Patrignani et al. (1997)

(4) Brideau et al. (1996)



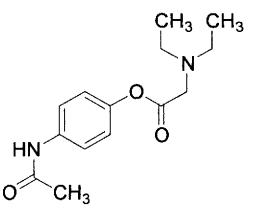
Scheme 75: Metabolic pathway of piroxicam.

Trade name: Feldéne (F), Felden (Ger), Feldene (UK, US)

Beside COX inhibition, piroxicam weakly inhibits the accumulation of nitric oxide generated by inducible nitric oxide synthase (iNOS, IC_{50} 240 µM) and LPS-induced interleukin-6 formation (IC_{50} 470 µM; Berg et al., 1999). These activities were measured not in whole blood, but in LPS-stimulated cell lines and occur only at high concentrations. Another COX-independent activity of piroxicam is a neuroprotective role against hypoxia and reperfusion by modulation of molecules from the intercellular signaling cascade (Vartiainen et al., 2001). Since all these activities are seen in the high concentration range, the anti-inflammatory potential of piroxicam, which is evident at a mean serum concentration (i.e. not the target compartment of local inflammation) of 16.6 µM after a standard dose of 20 mg/day (Cryer and Feldman, 1998), is questionable.

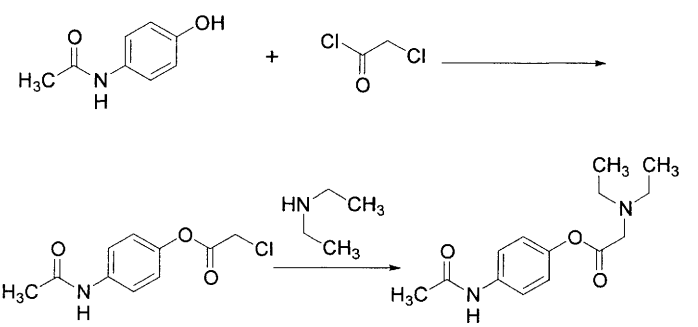
Propacetamol

Propacetamol



([*66532-85-2*], *N,N*-diethylglycine-4-(acetylamino)phenyl-ester, $C_{14}H_{20}N_2O_3$, M_r 264.33). It is used as hydrochloride ([*66532-86-3*] , $C_{14}H_{20}N_2O_3 \cdot HCl$, M_r 300.79)

Synthesis (Dittert et al., 1968; Kleemann et al., 1999): 4-Hydroxyacetaniline is coupled with chloroacetylchloride to yield *p*-acetamidophenyl-chloroacetate, which is finally reacted with diethylamine to produce propacetamol.



Scheme 76: Synthesis of propacetamol.

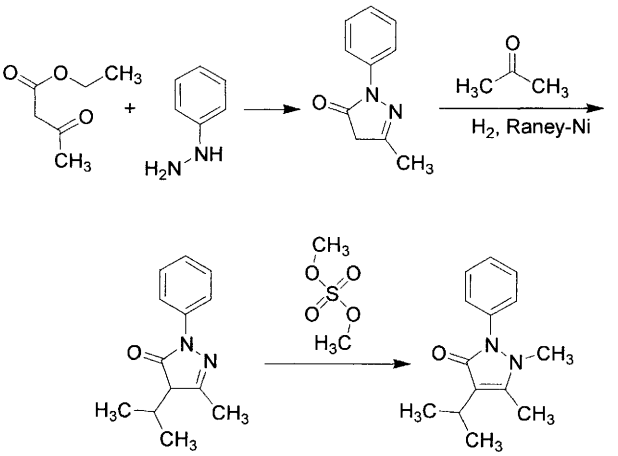
Trade name: Pro-Dafalgan (Belg, Fr, Switz), Pro-Efferalgan (Italy)

Propacetamol is a soluble glycine prodrug derivative of paracetamol. It is administered by the intramuscular or intravenous route and is rapidly metabolized to free paracetamol (Depre et al., 1992).

Propacetamol is given in doses of 1 to 2 g per day.

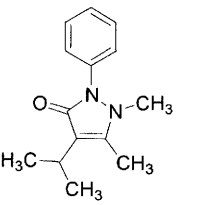
Propyphenazone

Synthesis (Hoffmann-La Roche, 1931; Volk (Riedel-de Haen), 1954; Kleemann et al., 1999):



Scheme 77: Synthesis of propyphenazone.

Propyphenazone



[479-92-5], 1,2-Dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl-3*H*-pyrazol-3-one, $C_{14}H_{18}N_2O_3$, M_r 230.31, *mp* 103 °C

Clinical use: Propyphenazone is a derivative of phenazone and has similar analgesic and antipyretic properties.

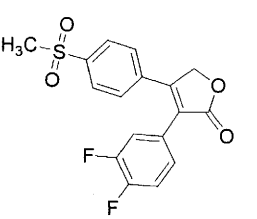
Propyphenazone is also used in multi-ingredient preparations.

Trade name: Demex (Ger)

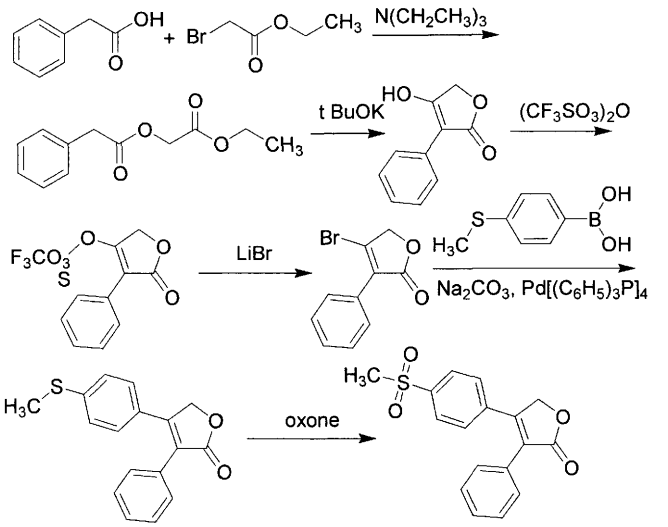
Rofecoxib

Synthesis: The condensation of phenylacetic acid with ethyl bromoacetate by means of triethylamine in THF yields 2-(phenylacetoxy)acetic ethyl ester, which is cyclized to the hydroxyfuranone by means of potassium *tert*-butoxide in *tert*-butanol. The reaction with triflic anhydride and diisopropylethylamine in CH_2Cl_2 affords the corresponding triflate, which by reaction with LiBr in hot acetone yields the bromofuranone. Condensation with 4-(methylsulfanyl)phenylboronic acid by means of Na_2CO_3 and $Pd[(C_6H_5)_3P]_4$ in hot toluene gives 4-[4-(methylsulfanyl)-phenyl]-3-phenylfuran-2(*5H*)-one, which is finally oxidized with $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$ (oxone) (Drugs Fut., 1998).

Rofecoxib

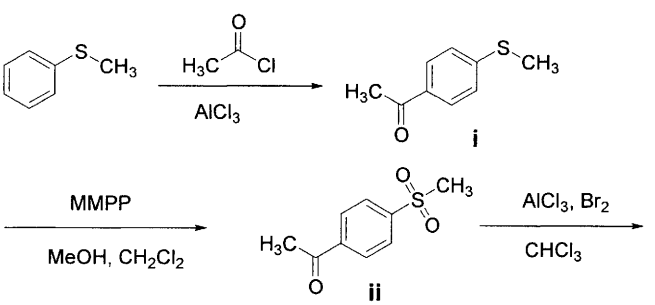


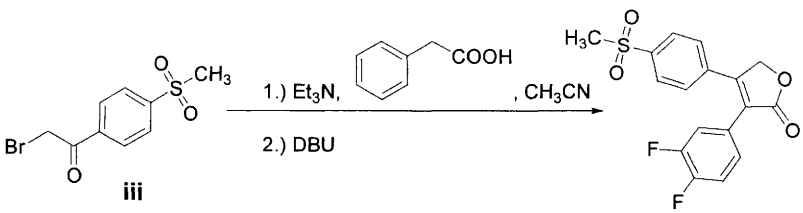
[162011-90-7], 4-[4-(Methylsulfonyl)phenyl]-3-phenylfuran-2(*5H*)-one, $C_{17}H_{14}O_4S$, M_r 314.36



Scheme 78: Synthesis of rofecoxib.

The synthesis of rofecoxib can be achieved by several different routes (Drugs Fut., 1998). A highly efficient synthesis for rofecoxib was recently described (Thérien et al., 2001). As illustrated in Scheme 79, acetophenon (**i**) is prepared according to the literature, by Friedel-Crafts acylation with thioanisole. Oxidation with MMPP (magnesium monoperoxyphthalate hexahydrate) affords the sulfone (**ii**), which is reacted with bromine in chloroform in the presence of a trace amount of $AlCl_3$, to give (**iii**). Bromoketone (**iii**) is than coupled and cyclized in a second step, one-pot procedure with phenylacetic acid. Firstly, the mixture of bromoacetophenone (**iii**) and phenylacetic acid in acetonitrile is treated with triethylamine at room temperature, to provide the ester intermediate, subsequent cooling and addition of DBU effected the cyclization to provide rofecoxib as the final product.





Scheme 79: Alternative synthesis of rofecoxib.

Clinical use: Rofecoxib (Sorbera et al., 1998) is a second generation COX-2 selective inhibitor. It was the second COX-2 selective drug to reach the market in 1999. Rofecoxib is used for the treatment of rheumatoid arthritis, osteoarthritis and pain.

The selectivity for COX-2 compared to COX-1 is more than 800-fold in cellular assays and more than 10-fold in whole blood assays.

Rofecoxib is used for a once-daily treatment of rheumatoid arthritis, osteoarthritis (12.5-25 mg/day) and pain (50 mg/day).

Rofecoxib reaches peak plasma concentrations between 2 to 9 h after oral administration. It is bound ~87% to plasma protein and has an elimination half-life of about 17 h. Its main metabolites in the liver are the *cis*-dihydro and *trans*-dihydro derivatives which are excreted mainly in the urine (72%) with some unchanged drug excreted in the faeces (14%).

Rofecoxib shows significantly less gastrointestinal toxicity compared to ibuprofen in studies with osteoarthritis patients (Laine et al., 1999) and compared to naproxen in patients with rheumatoid arthritis (Bombardier et al., 2000).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
recomb. enzyme (1)	26	0.34	76
cell culture (1)	>15	0.018	>833
whole blood (2)	63	0.84	75

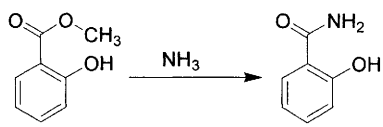
(1) Chan et al. (1999)

(2) Warner et al. (1999)

Trade name: Vioxx (EC, US)

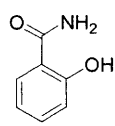
Salicylamide

Synthesis (Hoffenberg und Hauser, 1955; Kleemann et al., 1999):



Scheme 80: Synthesis of salicylamide.

Salicylamide



[*65-45-2*], 2-Hydroxybenzamide, $C_7H_7NO_2$, M_r 137.14, *mp* 140 °C

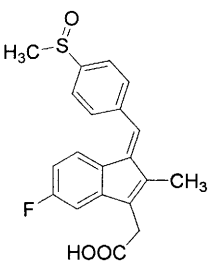
Clinical use: Salicylamide has analgesic and antipyretic effects and is used in multidrug combinations for the treatment of a variety of mild pain conditions including musculoskeletal, soft tissue and joint disorders.

Trade name: Percutalgine (F), Glutisal (Ger), Intralgin (UK), Anabar (US)

Salicylamide is given orally in daily doses of 1 to 2.5 g or applied topically in concentrations of about 5%. It is metabolized to inactive metabolites during absorption or in the liver.

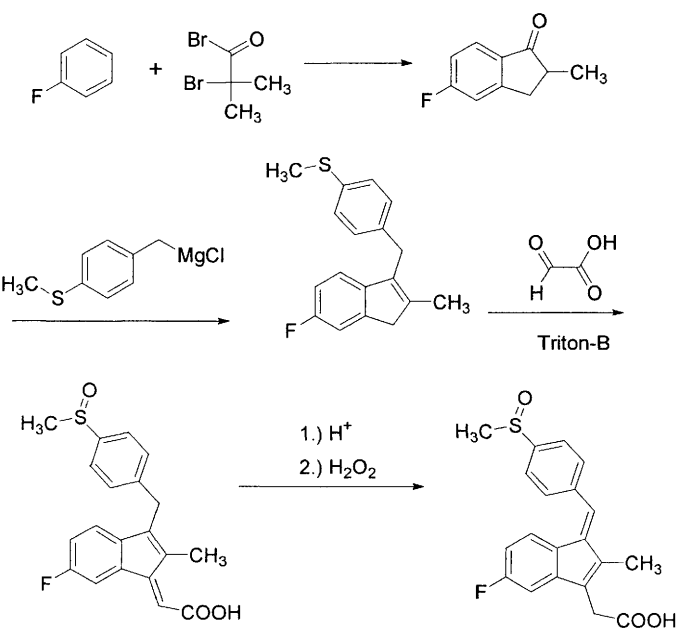
Sulindac

Sulindac



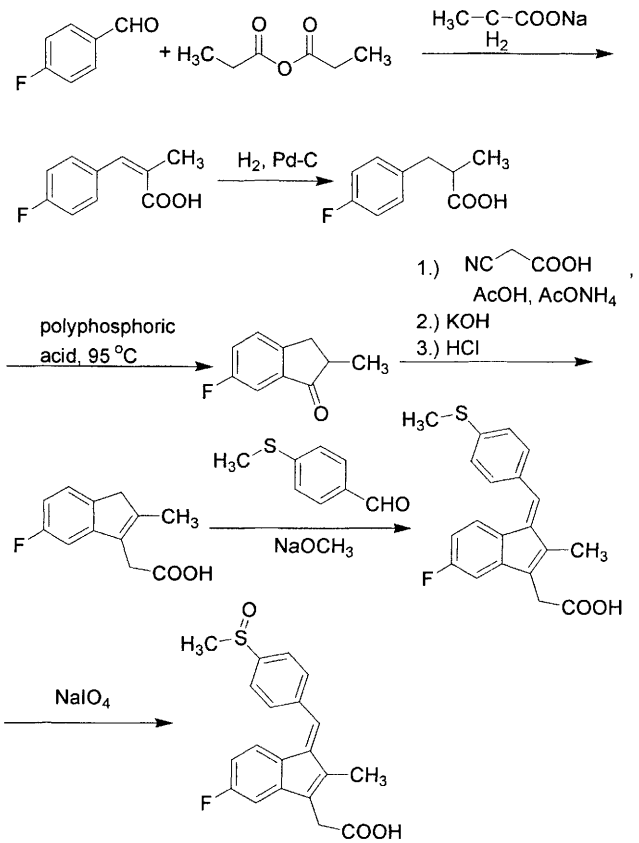
[*38194-50-2*], (*Z*)-[6-Fluoro-3-(4-methanesulfinyl-benzylidene)-2-methyl-3*H*-inden-1-yl]-acetic acid, $C_{20}H_{17}FO_3S$, 356.41, *mp* 182-185 °C (decomp.)

Synthesis (Tull et al. (Merck & Co.), 1975; 1976) Friedel-Crafts reaction of fluorobenzene and α-bromoisobutyryl bromide gives 5-fluoro-2-methylindan-1-one, which is treated with 4-methylthiobenzylmagnesium chloride to yield 5-fluoro-2-methyl-1-(4-methylthiobenzyl)indene. Condensation with glyoxylic acid in the presence of *N*-benzyltrimethyl ammonium hydroxide (Triton B) gives 3-carboxy methylene-5-fluoro-2-methyl-1-(4-methylthio-benzyl) indene, which is isomerized in acid to 5-fluoro-2-methyl-1-(4-methylthiobenzylidene)indene-3-acetic acid. Oxidation with hydrogen peroxide affords sulindac.



Scheme 81: Synthesis of sulindac.

Another synthesis starting from *p*-fluorobenzaldehyde is shown:



Scheme 82: Synthesis of sulindac.

Clinical use: Sulindac (Brogden, 1978a) is a nonsteroidal anti-inflammatory drug used in the treatment of mild to moderate pain including musculoskeletal and joint disorders such as rheumatoid arthritis, osteoarthritis and gout.

Sulindac shows no relevant inhibition of cyclooxygenase (Warner et al., 1999), whereas the active metabolite sulindac sulfide shows inhibition of both isoenzymes with a preference for COX-1 in a whole blood assay (see also Brideau et al., 1996; ratio COX-1/COX-2 = 0.1). Sulindac is one of the NSAIDs, extensively studied in cancer reseach (Haanen, 2001). The metabolite sulindac sulfone induces apoptosis in tumor cells.

Sulindac is absorbed from the gastrointestinal tract and reversibly metabolised to sulindac sulfide and irreversibly metabolised to sulindac sulfone. Peak plasma

COX selectivity of the active metabolite sulindac sulfide

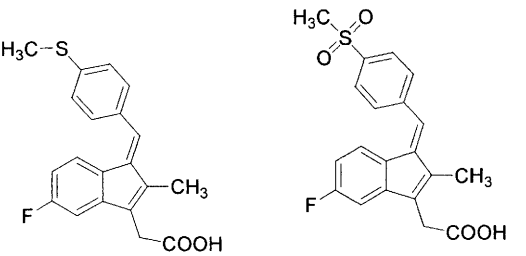
IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	0.028	0.004	7
whole blood (2)	1.9	55	0.03

(1) Riendeau et al. (1997)

(2) Warner et al. (1999)

concentrations of sulindac sulfone are reached within 2 h. Sulindac as well as the sulfone and sulfide metabolites show extensive plasma protein binding. The plasma elimination half-life of sulindac and sulindac sulfide is 7 to 8 h and 16 to 18 h, respectively. Sulindac and its sulfone metabolite as well as the respective glucuronates are excreted mainly in the urine, whereas only a small amount of sulindac sulphide is excreted in the urine.

Trade name: Arthrocine (F), Clinoril (UK, US)



sulindac sulfide sulindac sulfone

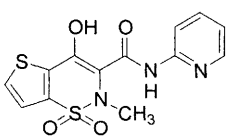
Structures of the metabolites sulindac sulfide and sulindac sulfone

Figure 23: Metabolites of sulindac.

The main side effects are gastro-intestinal disturbances and renal stones (Whelton et al., 1983).

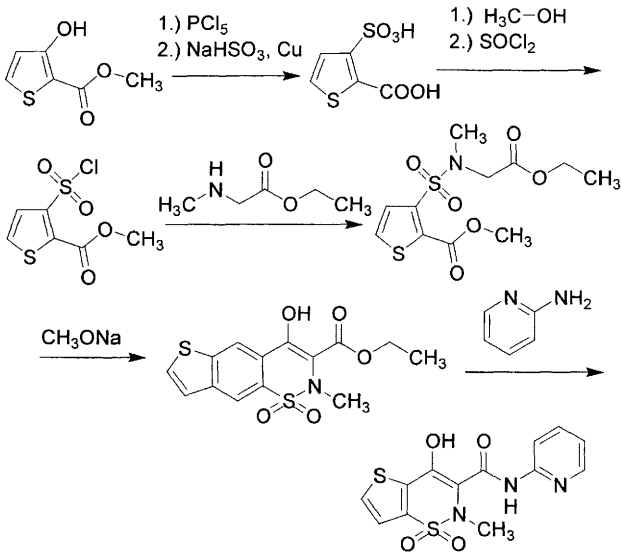
Tenoxicam

Tenoxicam



[*59804-37-4*], 4-Hydroxy-2-methyl-1,1-dioxo-1,2-dihydro-1λ^{6}thieno[2,3-*e*][1,2]thiazine-3-carboxylic acid pyridin-2-ylamide, 4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-thieno[2,3-*e*]-thiazine-3-carboxamide-1,1-dioxide, $C_{13}H_{11}N_3O_4S_2$, M_r 337.38, *mp* 209-213 °C (decomp.)

Synthesis: The reaction of methyl 3-hydroxythiophen-2-carboxylate with PCl_5 in refluxing CCl_4 gives 3-chlorothiophene-2-carboxylic acid, which by treatment with $NaHSO_3$ and Cu in basic water at 143 °C in a pressure vessel is converted into 3-sulfothiophene-2-carboxylic acid. The first esterification with refluxing methanol affords methyl-3-sulfothiophene-2-carboxylate, which by reaction with refluxing $SOCl_2$ yields methyl-3-chlorosulfonyl thiophene-2-carboxylate. The following condensation with sarcosine ethyl ester in hot $CHCl_3$ gives 3-(*N*-ethoxycarbonylmethyl-*N*-methylsulfamoyl)thiophene-2-carboxylate, which is cylized by treatment with sodium methoxide in refluxing methanol affording 3-ethoxycarbonyl-4-hydroxy-2-methyl-2*H*-thieno-[2,3-*e*]-1,2-thiazine 1,1-dioxide. Finally this compound is condensed with 2-aminopyridine in refluxing toluene (Hromatka et al. (Hoffmann-La Roche), 1975; Arrigoni-Martelli, 1982; Kleemann et al., 1999).



Scheme 83: Synthesis of tenoxicam.

Clinical use: Tenoxicam (Todd and Clissold, 1991) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain states in musculoskeletal, soft tissue and joint disorders such as rheumatoid arthritis, osteoarthritis and gout.

Tenoxicam shows a balanced inhibition of both, COX-1 and COX-2.

Tenoxicam is given by oral, rectal or intramuscular routes (20 mg/day, maximal dose 40 mg/day).

Peak plasma concentration appears within 1 to 6 h depending on fasted or fed status. There is 99% binding of tenoxicam to plasma proteins and a long plasma elimination half-life of 49 to 81 h. Tenoxicam is eliminated by liver metabolism. The main metabolites are 5'-hydroxy-tenoxicam and the 6-O-glucuronidate which are excreted in urine and bile, respectively (Nilsen, 1994). The hydroxylated metabolites of tenoxicam are shown in Scheme 84:

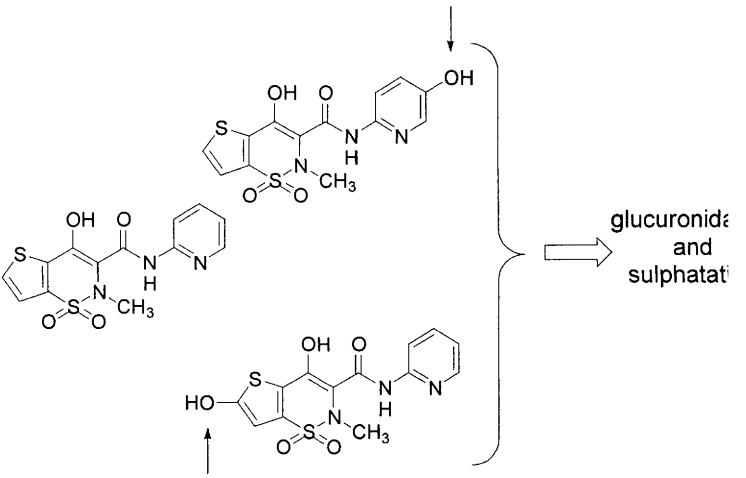
COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
cell culture (1)	0.32	0.13	2.5
whole blood (2)	2.3	14.2	0.2

(1) Berg et al. (1999)

(2) Brideau et al. (1996)

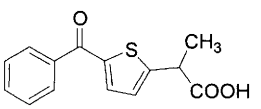
Trade name: Tilcotil (Aust, Austral, Belg, F, Ger, Ital, Neth, Spain), Mobiflex (Canad, Irl, UK)



Scheme 84: Hydroxylated metabolites of tenoxicam.

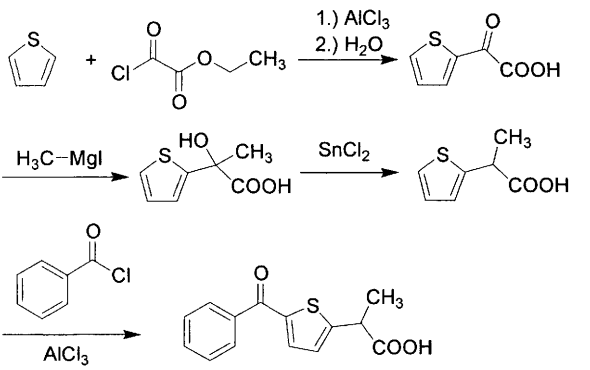
Tiaprofenic Acid

Tiaprofenic Acid



[*33005-95-7*], 2-(5-Benzoyl-thiophen-2-yl)-propionic acid, $C_{14}H_{12}O_3S$, M_r 260.31, *mp* 96 °C

Synthesis (Clemence (Roussel-Uclaf), 1970; Kleemann et al., 1999):



Scheme 85: Synthesis of tiaprofenic acid.

Clinical use: Tiaprofenic acid (Plosker and Wagstaff, 1995) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain states in musculoskeletal, soft tissue and joint disorders as well as for postoperative pain.

Tiaprofenic acid is a racemate and given as oral or rectal preparations (600 mg/day) and as an intramuscular injection of the trometamol salt.

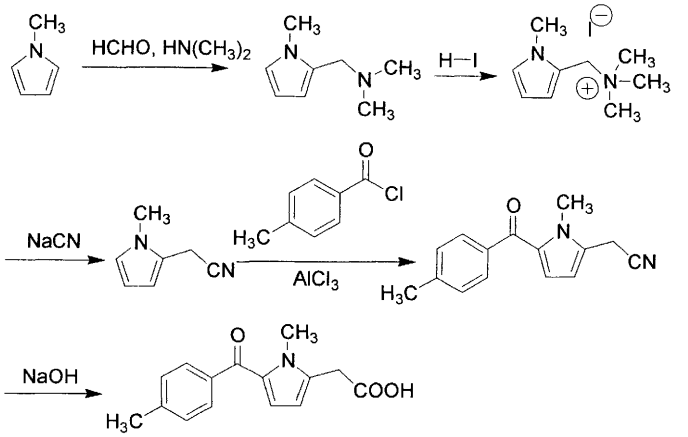
After oral administration, tiaprofenic acid reaches peak plasma concentrations after 1.5 h. Tiaprofenic acid binds efficiently to plasma proteins (~98%) and has a short elimination half-life of about 2 h. Tiaprofenic acid and its metabolites are excreted mainly in urine (Davies, 1996).

Trade name: Surgam (Austr, Belg, F, Ger, Irl, Neth, UK, Switz), Suralgan (Ital), Surgamic (Spain)

The main side-effects are urinary tract symptoms such as cystitis and bladder irritation (Mayall et al., 1994).

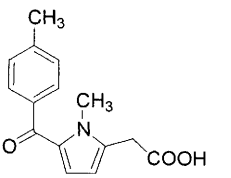
Tolmetin

Synthesis (Carson et al., 1971; Carson (McNeil), 1973; Kleemann et al., 1999):



Scheme 86: Synthesis of tolmetin.

Tolmetin



[*26171-23-3*], [1-Methyl-5-(4-methyl-benzoyl)-1*H*-pyrrol-2-yl-acetic acid, $C_{15}H_{15}NO_3$, M_r 257.28, *mp* 155-157 °C; sodium salt [35711-34-3], $C_{15}H_{14}NNaO_3$, M_r 279.27; sodium salt dihydrate [64490-92-2], $C_{15}H_{14}NNaO_3 \cdot 2\ H_2O$, M_r 315.30

Clinical use: Tolmetin (Brogden et al., 1978b) is a nonsteroidal anti-inflammatory drug used for the treatment of mild to moderate pain states in musculoskeletal, soft tissue and joint disorders such as rheumatoid arthritis, osteoarthritis and gout as well as juvenile rheumatoid arthritis.

Tolmetin inhibits both isoforms of cyclooxygenase with a preference for COX-1 in whole blood assays (see also COX-1/COX-2 ratios of 0.2, 0.4 and 0.5 in Brideau et al., 1996; Young et al., 1996 and Cryer and Feldman, 1998, respectively).

COX selectivity

IC50 [μM]	COX-1	COX-2	ratio
whole blood	0.35	0.82	0.4

Warner et al. (1999)

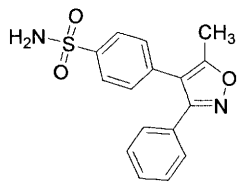
Tolmetin is given as oral, rectal (600-1800 mg/day) or topical preparation (5% topical gel).

The peak plasma concentrations are reached within 30 to 60 min after oral administration. Tolmetin shows a high plasma protein binding of 99% and a biphasic plasma half-life of 1 to 2 and 5 h, respectively. Tolmetin and its metabolites and conjugates are excreted in the urine (Grindel, 1981).

Trade name: Tolectin (Austria, Switz., UK, US)

Valdecoxib

Valdecoxib



[*181695-72-7*], 4-(5-Methyl-3-phenylisoxazol-4-yl)benzenesulfonamide $C_{16}H_{14}N_2O_3S$, 314.366; mp 172-173 °C

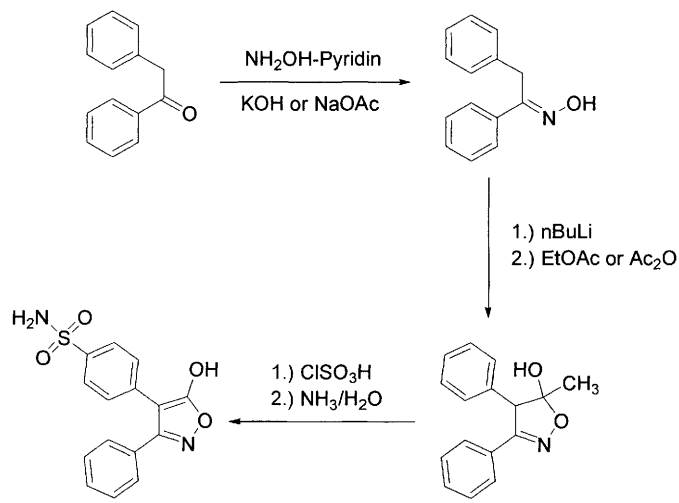
Synthesis: Deoxybenzoin is converted to the corresponding oxime by treatment with hydroxylamine under basic conditions with sodium acetate in aqueous ethanol or in toluene in the presence of potassium hydroxide in absolute ethanol. Treatment of the oxime under nitrogen with two equivalents of butyllithium in tetrahydrofurane is followed by cyclization in ethyl acetate or acetic anhydride to the isoxazoline derivative. Finally, treatment of the isoxazoline with cold chlorosulfonic acid followed by reaction of the intermediate with aqueous ammonia affords the desired product. (Talley, 2000a; Sorbera, 2001b).

COX selectivity

IC50 [µM]	COX-1	COX-2	ratio
recomb. enzyme (1)	140 1120 (2)	0.005 0.18 (2)	28000 6222 (2)
whole blood (1)	25.4 >50 (2)	0.89 0.329 (2)	28.5 >152 (2)

Talley et al. (2000a)

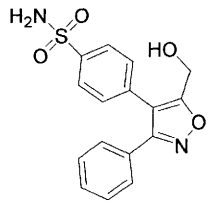
activity of the active metabolite of valdecoxib



Scheme 87: Synthesis of valdecoxib.

Clinical use: Valdecoxib (Sorbera et al., 2001b) is a third generation COX-2 inhibitor in clinical development. It shows about 30-fold selectivity for COX-2 in a whole blood assay (see also Riendeau et al., 2001; COX-1/COX-2 = 30). Valdecoxib shows efficacy in a variety of animal models of inflammation (Talley et al., 2000a).

Valdecoxib is converted in rodents and dogs, and in a low abundance in humans, by hydroxylation of the methyl group to an active metabolite (4-(5-Hydroxymethyl-3-phenyl-isoxazol-4-yl)-benzenesulfonamide) (Talley et al., 2000a). Pharmacological evaluation of the independently synthesized metabolite showed that it possessed oral activity in the acute anti-inflammatory assay (carrageenan paw edema, ED_{50}=1.06 mg/kg). Chronic inflammatory activity was achieved with the metabolite in the rat adjuvant arthritis model (ED_{50}=1.49 mg/kg/day). *In vitro* the metabolie showed an IC_{50} of 1120 μM against COX-1 and an IC_{50} of 0.18 μM against COX-2.



Structure of the active metabolite of valdecoxib

Trade name: Bextra (US)

Valdecoxib is a substrate for cytochrome P450 3A4 and 2C9 (Cheer and Goa, 2001; Gotta, 2001).

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3 Opioids

3.1 Introduction

Elmar Friderichs

Definition

Opioids is the common name for all compounds which have the same mode of action as the constituents of opium, the dried milky liquid of the poppy seed, *Papaver somniferum* (Brownstein, 1993). All opioids interact in biological systems with the same type of receptor, the so-called opioid receptor.

Natural and synthetic opioids, opioid peptides

With respect to structural features (Casy and Parfitt, 1986) opioids can be divided into three groups:

- The first group contains the natural products morphine, codeine and thebaine, which have been isolated from the natural product opium. In addition, the group contains various semi-synthetic derivatives of morphine, codeine and thebaine, which are prepared by chemical modifications of these natural products
- The second group comprises fully synthetic compounds which often have a totally different chemical structure as compared to the semi-synthetic analogs, but interact with the same opioid receptors and show the same spectrum of analgesia and side-effects as the natural compounds. The older name 'opiates' is still in use to describe both groups

Opiates is the older name for non-peptidic opioids

- The third group consists of naturally occurring and synthetic peptides with opioid-like properties. The opioid peptides were discovered during the search for endogenous ligands of the opioid receptors and share the same action and side-effect profile as the non-peptidic compounds

Functions of the endogenous opioid system:

1. Central and (peripheral) pain processing
2. Regulation of autonomous functions
3. Regulation of immune and inflammatory processes

The endogenous opioid system (Akil et al., 1984) is widely distributed within the body, it is phylogenetically very old and is expressed in all vertebrates. A high density of opioid receptors is found in the brain (Mansour et al., 1995) and spinal cord, where these receptors are involved in pain inhibition and additionally in many other central regulatory processes. In addition to localisation in the CNS, opioid receptors are expressed in many peripheral organs (Herz, 1983). Of great importance are the opioid receptors of the gastrointestinal system, which regulate stomach emptying, gut motility and intestinal fluid secretion. Opioid receptors are found in cells of the immune system and peripheral opioids seem to be involved in the regulation of inflammatory and immunological processes (Stefano et al., 1996). In addition to the outstanding role in central pain inhibition,

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action at peripheral opioid receptors, which are expressed in high density during inflammation and immune stimulation, may add to central pain inhibition (Stein et al., 1999).

Opioid Receptor Types

The action profile of synthetic opioids reinforced speculation, that more than one type of opioid receptor exists and is involved in the analgesic activity of these compounds. Martin and co-workers in 1960 investigated these differences in a specially developed test model, the chronic spinal dog (Martin et al., 1976). According to the analgesia and side-effect profile they postulated three types of opioid receptors, the μ-receptor (ligand = morphine), the κ-receptor (ligand = ketazocine) and the σ-receptor (ligand = SKF 10081).

Table 1: Differentiation of opioid receptors in the chronic spinal dog by Martin et al. (1984).

Prototypic compound	Action profile	Receptor type
Morphine	Analgesia, miosis, respiratory depression, bradycardia, hypothermia, inattention to external stimuli	μ-receptor
Ketazocine	Miosis, strong sedation, inhibition of flexor reflex	κ-receptor
N-Allylnormetazocine (SKF-10047)	Mydriasis, respiratory stimulation, tachycardia, delirium	σ-receptor

Opioid receptor subtypes: μ, κ, and δ

This was later confirmed by binding experiments with radioactively labeled ligands and by the different binding and action profiles of the endogenous opioid peptides, the enkephalins and endorphines (Fowler and Fraser, 1994), which led to the identification of the δ-opioid receptor. Since the σ-receptor today is no longer considered to be an opioid receptor, three opioid receptor subtypes, the μ-, κ-, and δ-opioid receptor, had been confirmed (Martin, 1983; Dhavan et al., 1996).

ORL1- receptor

More recently, a fourth opioid receptor type, named ORL1-receptor has been added (Meunier et al., 1995). It was

detected as an cDNA, which coded for a protein with opioid receptor-like properties. Within a short time, the endogenous ligand, a peptide named nociceptin, was isolated, which depending on the place and route of administration, induced pro-nociceptive or anti-nociceptive actions. The full spectrum of biological activity of nociceptin and the physiological role of the ORL-1 opioid receptor in pain processing is still under evaluation.

Subtypes of the Different Opioid Receptors

Functional significance of subtypes of opioid receptors still contoversial

Corresponding to other receptor systems, binding studies as well as functional investigations indicate that subtypes of opioid receptors exist (Wood, 1982; Pasternak and Wood, 1986). Within the μ- and δ-receptor type 2 subtypes, the μ-1 and μ-2 and δ-1 and δ-2 have been described. The κ-receptor contains an additional κ-3 subtype. It was postulated by some investigators, that analgesia and opioid-side effects occur at different receptor subtypes, this would therefore make it possible to separate analgesia from the unwanted opioid side effects. According to Pasternak and Wood (1986) analgesia should be mediated by the μ-1 receptor site, whereas respiratory depression and addiction is mediated via the μ-2 receptor subtype. But in contrast to binding experiments, the functional separation of the μ-1 and μ-2 and other subtypes is more equivocal and a clear separation of analgesia from respiratory depression and addiction potential has never been found among the μ-opioids. Therefore, an attempt to differentiate μ-opioid analgesics according to subtype specificity is no longer maintained.

It is interesting, that the subtypes of opioid receptors could not be confirmed in cloning experiments (Gaveriaux-Ruff and Kieffer, 1999), since for each receptor only one individual gene with an homogenous transcript was detected. Therefore, possible heterogeneity of opioid receptor subtypes must result from a later modification which is independent from the gene level. Possible variations could include splice variants, receptor association, posttranslational modifications (e.g. glycosidation) or coupling with different transduction mechanisms.

Molecular Pharmacology of Opioid Receptors

The development of the polymerase chain reaction (PCR) technique made it possible to amplify isolated opioid receptor cDNA and to synthesize small amounts of the receptor protein necessary to identify their amino acid

sequence. The first successfully cloned opioid receptor was the δ-opioid receptor of the mouse, which was described in parallel by Kieffer et al. (1992) and Evans et al. (1992). Both used neuroblastoma-glioma hybridoma cells which express a high density of δ-receptors in their membranes. The isolated receptor protein consisted of 372 amino acids and had a molecular mass of 40.644. The amino acid sequence showed a partial overlap with the receptors of somatostatin (37%), angiotensin (31%) and interleukin-8 (22%). Similar to the somatostatin receptor, seven hydrophobic domains were identified by which the receptor is inserted into the lipid bilayer of the cell membrane. Shortly thereafter the rat and human δ-receptors were cloned and they showed nearly total homology with the rat receptor (mouse - rat homology: 97%; rat - human homology: 99%).

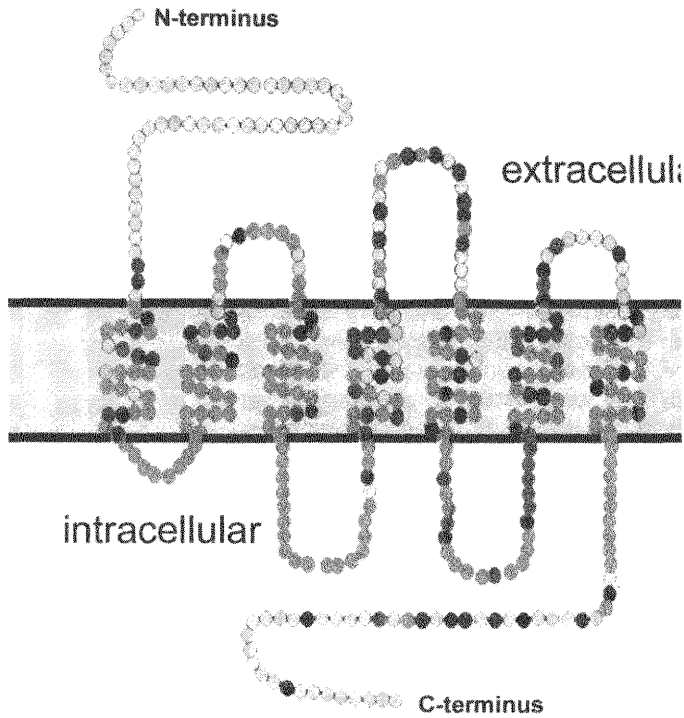


Figure 1: Common model for the μ-, κ- and δ-opioid receptor (modified from Gaveriaux-Ruff and Kieffer, 1999).

Using the same technique a novel opioid-like cDNA was isolated (Reinscheid et al., 1995), which coded for an unknown opioid receptor. The new receptor had many similarities with the classical opioid receptors (Calo et al., 2000) and was added as the fourth member to the opioid

receptor family under the name ORL-1 ('opioid receptor–like' protein). In addition to rat and mouse receptors, the human types of all four opioid receptors have now been identified. They show a high degree of structural identity, which corresponds to their widely overlapping biological functions (Knapp et al., 1995; Gaveriaux-Ruff and Kieffer, 1999).

Molecular structure of opioid receptors and receptor homology

All opioid receptors belong to the group of pertussis toxin-sensitive G protein coupled receptors of the rhodopsine family with seven transmembrane spanning hydrophobic domains. The N-terminal is oriented to the outer side of the cell membrane and is involved in the selection and binding of the receptor-specific ligands. Studies with chimeric or point-mutated receptors indicate that predominantly the second and third extracellular loop determine receptor selectivity. The N-terminal sequence contains several free amino groups which can be conjugated with sugar residues. The carboxy terminal is directed towards the interior of the cell and is involved in the signal transduction cascade. The carboxy terminal contains groups which can be phosphorylated and which are involved in receptor internalization and inactivation. The seven transmembrane regions are connected by extracellular and intracellular loop regions of different length. Comparing the sequences of the μ-, δ- and κ-receptor reveals that the highest degree of similarity is located in the transmembrane regions and in the intracellular loop, whereas the external loops and both terminal regions are more heterogeneous. The external loops and the terminal moiety are involved in the selection and binding of ligands and contain the structural elements which determine the receptor selectivity.

Structure-Activity Relationship of Opioid Receptor Interaction

Structural elements for opioid receptor binding

The structural and conformational prerequisites for opioid receptor binding have been most extensively studied for the μ-opioid receptor. These investigations, which were aimed at finding clinically improved opioid analgesics were initiated long before the receptor binding technique was elaborated. The basic concept dates back to the middle of the last century when Beckett and Casy (1954) developed a receptor model with three binding sites. This model was refined by Janssen and Jageneau (1957) to include the binding characteristic of the phenylpiperidine opioids. As essential elements the receptor ligand must contain an aromatic ring system, a central quarternary carbon atom and a side chain of two carbon atoms with a terminal basic

nitrogen group which should be substituted with at least one methyl group (Janssen and van der Eycken, 1968). An hydroxyl group in the meta-position of the aromatic ring increases receptor binding and analgesic potency. In the morphine molecule, the side chain is incorporated into ring D and corresponds to atoms C15 - C16. Synthetic opioids of the fentanyl and oripavine type indicate, that a second aromatic ring system is advantageous, provided that both rings are at an optimal distance to each other and to the basic nitrogen group.

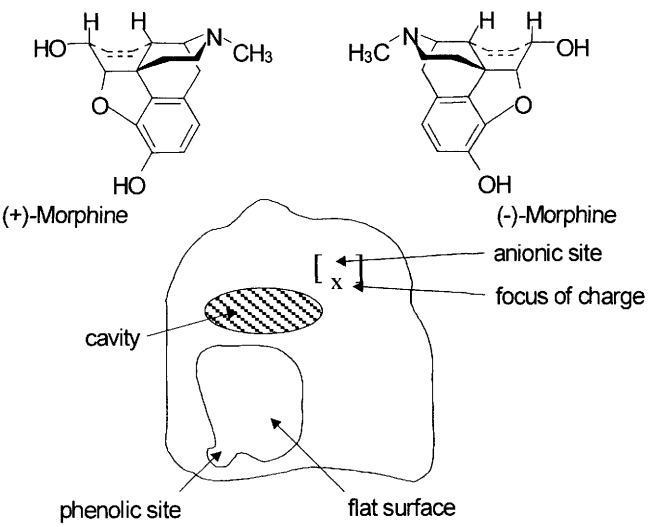


Figure 2: The classical opioid receptor model developed by Casy (modified from Casy and Parfitt, 1986).

A more recent modeling (Brandt et al., 1993) of the three-dimensional structure of the μ-opioid ligands resulted in a binding model with seven essential binding areas. This model fits peptidic and non-peptidic μ-opioid structures. Area A represents the anionic center of the binding pocket which interacts with the protonated nitrogen of the opioid ligand. Area B contains a hydrophilic binding area which interacts with the phenolic hydroxyl or analogous groups via hydrogen bridges. Binding area C is a lipophilic site which interacts with the aromatic ring system. Area D is a further lipophilic site, where the second aromatic ring of molecules with more than one aromatic group is bound. Areas E, F and G are hydrophilic regions of possible interactions with corresponding polar groups of the ligand. Essential for a high receptor binding affinity is the

appropriate distance between the aromatic rings and the basic nitrogen. It was shown in modeling experiments, that the optimal distance between the two aromatic rings is in the range of 10 Å. An optimal receptor interaction results when all seven areas are occupied by corresponding structural or electrically charged counterparts.

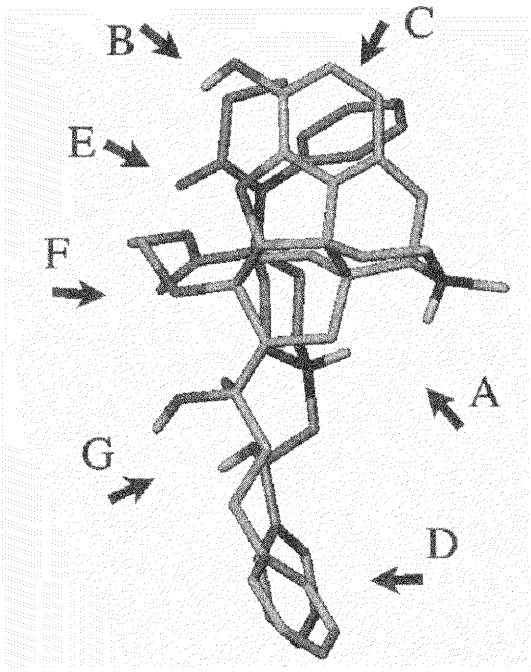


Figure 3: The refined model according to Portoghese et al. (1981) and Brandt et al. (1993, 2002).

Transduction Mechanisms of Opioid Receptor Interaction

Cellular effects of opioid receptor activation (see Fig. 4)

Functional studies of the opioid receptors (Childers, 1991) revealed that all four receptor types belong to the group of G protein coupled receptors (GPCRs). Agonistic binding at the receptor induces association of the α-, β- and γ-subunit of the G protein which triggers several biochemical reactions within the cell (McFadzean, 1988; Simonds, 1988; Blake et al., 1997; Law and Loh, 1999). The pharmacological properties of the opioids depend mostly on the following three mechanisms:

- Activation of a hyperpolarizing K^+ channel (inward rectifying K^+ channel)
- Inactivation of voltage dependent Ca^{++} channels (N-, P- and R-type)

- Inhibition of adenylate cyclase

A further relevant mechanism involved in the inhibition of synaptic transmission by opioids is a direct impairment of the exocytotic release of neurotransmitters, induced by stabilization of the presynaptic membrane.

Additional actions, with only partially understood relevance, are activation of phospholipases (PLH2, PLC7), activation of MAP-kinases and activation of some voltage dependent Ca^{2+} channels (L-type and T-type).

As a result of these actions opioids inhibit neurotransmission at the presynaptic and postsynaptic sites. Presynaptic inhibition depends mostly on the direct inhibitory effect on transmitter exocytosis from membrane-associated storage vessels. This direct effect is increased by the inhibition of Ca^{2+} channels, since Ca^{2+} ions trigger the transmitter release. Activation of K^+ ions induces membrane hyperpolarization which is the most important action component of postsynaptic inhibition.

Activation of K^+ channels, inactivation of Ca^{2+} channels and direct inhibition of neurotransmitter release are powerful mechanisms by which opioids inhibit the neuronal transmission of the pain signal.

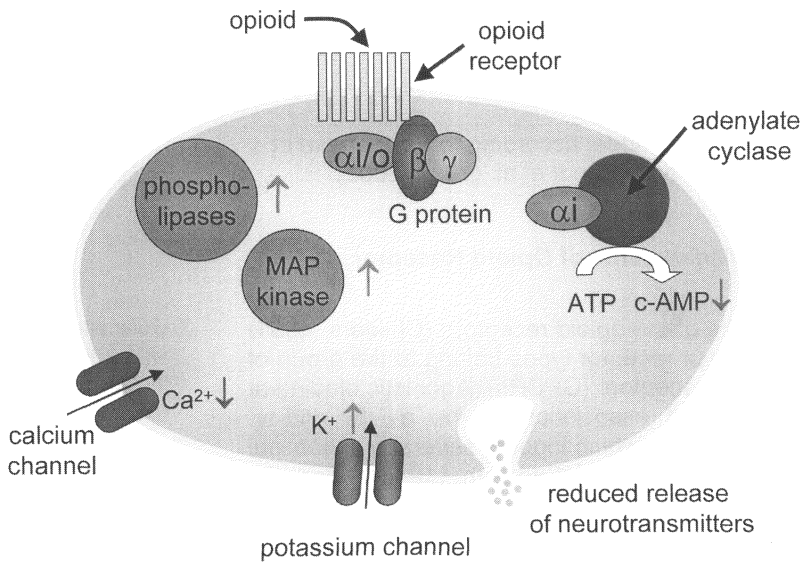


Figure 4: Cellular effector system, activated by opioid receptor binding.

Inhibitory and excitatory mechanisms of opioids

Opioid receptors are not only located at excitatory synapses, but are also expressed at inhibitory neurons. At these synapses, opioids inhibit the transmission of the

Table 3: Pharmacological actions of opioids at different receptors.

μ-receptor	κ-receptor	δ-receptor	ORL1-receptor
Analgesia	Analgesia	Analgesia?	Analgesia?
Respiratory depression	Diuresis	Anxiolysis?	Pro-nociception
Constipation	Dysphoria		Anxiolysis?
Euphoria	Hallucinations		

μ-Opioid Receptors

Morphine-like analgesics are μ-selective

Binding experiments and investigations in pain models have shown that the μ-receptor and to a lesser degree the κ-and δ-receptors predominates in the mediation of pain inhibition.

Table 4: Binding affinities of μ-selective opioid analgesics at recombinant human opioid receptor membranes expressed in CHO-K1 or HEK293 cells (Grünenthal, Dep. of Molecular Pharmacology).

Compound	Opioid Receptor Affinity (Ki / nM)			
	μ-OR (^{3}H-Naloxone)	κ-OR (^{3}H-CI-977)	δ-OR (^{3}H-Deltorphin II)	ORL1 (^{3}H-Nociceptin)
Sufentanil	0.00085	0.34	0.047	0.12
Fentanyl	0.0079	3.71	1.1	2.45
Buprenorphine	0.00032	0.0004	0.0017	0.034
Hydromorphine	0.004	0.35	0.13	>10
Morphine	0.025	1.7	0.95	>> 10
Levomethadone	0.0070	12.9	0.83	>> 10
Ketobemidone	0.023	1.15	0.27	>> 10
Pethidine	2.0	>> 10	27	>> 10
Meptazinol	0.23	~10	~3	24.7
Detropropoxyphene	0.34	19.7	2.4	>> 10
Codeine*	1.78	1.0	3.2	>> 10
Tilidine*	2.42	> 10	>> 10	>> 10
Tramadol*	32.6	>> 10	>> 10	>> 10

Most of the opioid analgesics in clinical use (Cherny, 1996) have a prevalence for the μ-opioid receptor and this

confirms that μ-receptor activation is the common mechanism of these compounds. Since morphine is the prototype, they are called 'morphine-like' analgesics.

Their common side-effect profile includes respiratory depression, constipation and addiction and dependence. All these side-effects, corresponding to analgesia, are mediated via μ-opioid receptor activation. This is the reason why the primary goal of the development of synthetic opioids, i.e. the separation of analgesia from the addiction and dependence potential, has never been achieved. The involvement of the same receptor population in the analgesia and side-effect profile of morphine and morphine-like opioids was impressively confirmed in μ-knock-out mice (Matthes et al., 1996; Kieffer, 1999). In these animals, morphine had no analgesic effect and no side effects. There was no respiratory depression and no inhibition of gastrointestinal motility and secretion. In behavioral models in μ-knock-outs, morphine did not induce liking or any other signs of addiction and repeated treatment with morphine did not produce signs of tolerance or physical dependence.

κ-Opioid Receptors

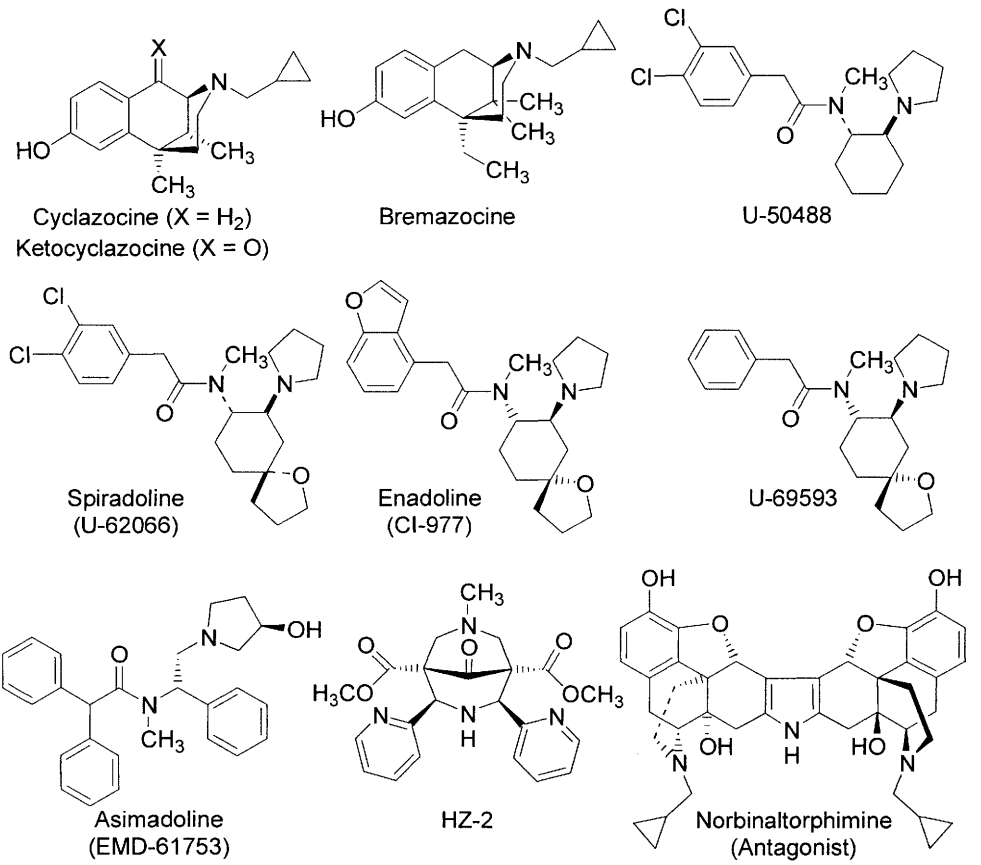
Clinical failure of κ-agonists because of psychotomimetic side-effects

Activation of κ-receptors induces clinically-relevant pain inhibition (Scopes, 1993; Barber and Gottschlich, 1997), which seem to be less efficacious than μ-receptor-mediated analgesia. The most prominent side-effects of κ-activation are sedation and diuresis. In contrast to μ-agonists, which induce well-being and euphoria, activation of κ-receptors in the limbic system induces dysphoria and other unpleasant psychic effects such as e.g. hallucinations and spatial disorientation. The dose range of analgesia and psychic side-effects partly overlap and this causes problems in the potential medical use of κ-receptor agonists. This may explain why despite an intensive search for κ-selective analgesics, no selective κ-agonist has been successfully developed up to clinical use.

κ-component induces analgesia in mixed agonists-antagonists

κ-Receptor interaction is a common action component of the so called partial opioid agonists or agonists-antagonists like e.g. pentazocine, nalbuphine, butorphanol and buprenorphine (Hoskin and Hanks, 1991; Jacobs and Youngblood, 1992; Archer, 1992). In contrast to buprenorphine, which has κ-antagonistic activity, the other compounds have an agonistic or partial agonistic κ-action, which is responsible for analgesia and the often unpleasant side-effects of these compounds. Most of them

have an additional μ-partial agonistic or μ-antagonistic action. The combination of both components results in a moderate to marked analgesia, weak respiratory depression, and a weak abuse and dependence potential.



Scheme 1: Selective κ-opioid agonists and norbinaltorphimine (antagonist).

In compounds with stronger μ-agonistic activity (buprenorphine and pentazocine), the abuse potential is marked and in contrast to earlier estimations, both compounds had to be subjected to narcotic control. In compounds with a marked κ-agonistic component (cyclazocine, nalorphine) the dysphoric side effects are so prominent that the compounds could not be used for therapeutic purposes.

Pain-relevant peripheral opioid receptors – even now an unverified therapeutic option

The investigations of Stein (1991) and Stein et al. (1999) have shown that pain-relevant opioid receptors are not only situated in the central nervous system but also in peripheral organs and tissues. Investigations in pain models with peripherally-acting opioids indicate that κ-

receptors may predominantly mediate peripheral pain inhibition.

Table 5: Receptor binding profile of mixed agonists-antagonists.

Compounds	κ-receptor	μ-receptor
Cyclazocine, Nalorphine, Nalbuphine	+	-
Butorphanole, Pentazocine, Dezocine	+	(+)
Buprenorphine	-	(+)
Meptazinole, Propiram		(+)

+ = Agonist; (+) = partial Agonist; - = Antagonist

Expression of peripheral opioid receptors and peptides seems to be triggered by immunological and inflammatory processes, and it is speculated that these opioid receptors represent a second peripheral pain inhibitory system. This has triggered an intensive search for peripherally-acting κ-agonists, which are devoid of the centrally-mediated psychogenic side-effects and which are believed to be attractive new compounds for the treatment of traumatic, inflammatory and burn-induced pain.

δ-Opioid Receptors

In contrast to μ-, and κ-receptors, indications for a prominent role of δ-receptors in the pain process are less obvious (Dondio et al., 1997; Scheideler, 2000).There is an on-going broad but still controversial discussion concerning a genuine analgesic effect mediated by δ-1 or δ-2 receptors, since analgesia is scarcely observed in compounds having purely δ-agonistic activity. Many δ-agonists have an additional μ-action component or they are metabolized to a μ-agonistic metabolite (SNC-80), which is responsible or at least involved in the analgesic effect (Thomas et al., 2001)

Cross-talk of μ- and δ-receptors may increase analgesic effect

Other investigations show that δ-active compounds increase the analgesic effect of μ-opioids, which is explained by 'cross-talk' between μ- and δ-receptors (Vaught et al., 1982). By this cross-talk δ-agonists inhibit the spinal release of pronociceptive cholecystokinin (CCK-

8), which is induced by μ-receptor activation (Noble et al., 1994). The μ-, δ-receptor interaction was confirmed in δ-knock-out mice (Gomes et al., 2000) and although no δ-selective analgesic has been developed so far, mixed μ-, δ-agonists may become clinically-relevant pain inhibitors.

ORL-1 Receptors

Pain relevance of ORL1-receptors

The ORL-1 receptor (Calo et al., 2000; Mollereau and Mouledous, 2000) is more diverse in its peptide sequence compared to the classical opioid receptors. In contrast to these, ORL-1 receptor activation by the endogenous ligand nociceptin, at least at the supraspinal level, has a pro-nociceptive effect and induces pain. Release of CCK-8, NMDA or PGE-2 is discussed as the mechanism of pronociceptive activity. Spinal administration, in contrast, induces antinociception. With the systemically not bioavailable peptidic ligand nociceptin the question whether peripheral administration of an ORL-1 agonist would induce analgesia or a pain reaction can not be answered. To circumvent the restrictions of peptidic compounds, non-peptidic ORL-1 agonists and antagonists are in development which will elucidate whether ORL-1 agonists or antagonists are the better compound for pain inhibition. In addition to interaction with pain processing, ORL-1 agonists have a broad spectrum of somatic and vegetative effects, e.g. sedation, anxiolysis, anticonvulsant activity, memory impairment, inhibition of food consumption and cardiovascular stimulation. With the aid of the non-peptidic agonists and antagonists, which are currently under development, it will be possible to elucidate the contribution of the ORL-1 opioid system to pain processing and to other physiological processes (Bertorelli et al., 2000).

Location of Opioid Receptors in the Pain Pathway

The endogenous opioid system (Mansour et al., 1995) is the most important component of the pain inhibitory system of the body. Opioids act at different levels in the pain pathway and their action effects different aspects of pain processing (Lipp, 1991; Yaksh, 1997). This results in:

- Inhibition of the transmission of the pain signal
- Inhibition of the emotional aspect of pain
- Inhibition of pain realization

Pain pathway

Pain processing is inhibited at the spinal and supraspinal level. Spinal opioid receptors are located pre- and post

synyptically at interneurons of the substantia gelatinosa of the dorsal horn. The opioid interneurons inhibit the release of excitatory transmitters and reduce the transmission of the pain signal from the primary afferents to the secondary neurons of the ascending spinal pain pathway. Supraspinally, opioids are located in different regions of the brainstem, in the periaquaeductal gray matter, in the limbic system, in the thalamic nuclei, in the basal ganglia and in the cortex. Cortical and thalamic localization is involved in the perception of the pain stimulus; the cortical regions are also involved in identifying the source of the pain.

Opioids in the different parts of the limbic system suppress the emotional component of pain and the pain suffering. Opioids in the formatio reticularis inhibit the pain-induced activation of autonomous functions, e.g. increase in respiration, increase in blood pressure and sweating. The inhibitory actions at the formatio reticularis are responsible for the major side-effects of the opioids such as respiratory depression, bradycardia and the central component of gastrointestinal inhibition.

In addition to the inhibitory effect at the ascending pain transmission, opioids activate a descending pain inhibitory system, which originates from different centers of the pons and medulla, e.g. nucleus coeruleus, areas of the periaquaeductal gray matter and areas of the raphe nuclei. The descending nerve fibers terminate at the spinal interneurones in noradrenergic and serotoninergic inhibitory synapses, which suppress the ascending pain signal. Thus, opioids inhibit the spinal pain processing by two mechanisms, one is a direct pre- and postsynaptic inhhibition of the ascending pain pathway and the other is a centrally-mediated activation of the descending pain inhibitory system.

Use of Opioids in Pain Treatment

Opioids are potent therapeutic options for almost all acute and chronic pain states

Opioids are used for the treatment of moderate to severe or very severe pain of acute or chronic type (Stein, 1999). Nearly all forms of pain are sensitive to opioid treatment and in contrast to traditional opinions even neuropathic pain is reasonably sensitive to higher doses of opioids. This was clearly shown in well-controlled clinical studies (Watson, 2000). The most important use of opioids in acute pain treatment is postoperative pain, whereas treatment of cancer pain, often accompanied by a neuropathic pain component, is the classical domain of chronic opioid treatment.

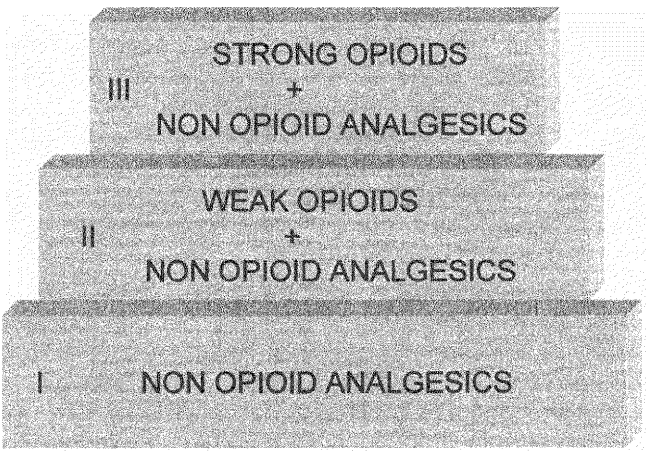


Figure 5: The WHO analgesic ladder.

WHO Guidelines for cancer pain treatment

(WHO, 1986)

To improve the world-wide under-treatment of chronic cancer pain, especially in underdeveloped countries, a guideline for chronic cancer pain treatment was published by the WHO in 1986. Meanwhile these guidelines have become the standard not only for malignant pain but also for benign chronic and acute pain states. The WHO proposal is often described as treatment 'by mouth, by the clock and by the ladder' (Jadad and Browman, 1995). This means that for repeated administration of the analgesic, the oral route should be used. The dosing interval should be regular and selection of the compounds should follow an increase in potency and efficacy as indicated in the three-step analgesic ladder. The first step starts with the single use of a non-opioid analgesic. If pain control is not sufficient, a weak opioid may be added. If a further increase in analgesic efficacy is needed, the weak opioid should be replaced by a strong opioid and the non-opioid may be omitted. Each stage of the treatment may be supplemented by the use of co-analgesics and other pharmacological and non-pharmacological interventions to improve pain control and to reduce side-effects.

Opioid Side-effects

Table 6: Side-effect profile of μ-opioids (Martin, 1983).

1. Respiratory system
- respiratory depression
- chest wall rigidity

2. Gastrointestinal system
- inhibition of gastric emptying
- inhibition of gut motility
- inhibition of intestinal fluid secretion
- constipation
- nausea and emesis

3. Cardiovascular system
- bradycardia
- impairment of cardiac conduction

4. CNS effects
- sedation and tranquilization
- deepening of anestesia
- euphoria
- addiction

5. Systemic adaption
- induction of tolerance and physical dependence

Respiratory Depression

Respiratory depression - the most dangerous side effect of postoperative opioid use

Opioids induce respiratory depression via inhibition of the respiratory center of the medulla oblongata, which respond to the pCO_2 content of the blood (Etches et al., 1989; Shook et al., 1990). The inhibitory effect is more prominent with respect to the respiratory frequency than to the volume of respiration. At higher opioid dosages, respiration becomes irregular and gasping occurs. Opioid-induced respiratory depression is augmented by other CNS-depressant compounds, like e.g. sedatives and hypnotics. In patients who are awake, respiratory depression by opioids can be voluntarily compensated over a broad dose range. Respiratory impairment is not a prominent feature in pain patients who are awake, since pain itself is a strong stimulus for respiration.

Respiratory depression becomes an important side-effect when opioids are used for postoperative pain treatment, since the anesthetic agent and most adjuncts of anesthesia induce a long-lasting depressant effect on respiration, which can increase the opioid effects up to respiratory arrest. Therefore careful supervision of respiration during the postoperative period is mandatory (Mulroy, 1996). Opioid-induced respiratory depression can be interrupted by the opioid antagonist naloxone. Naloxone has a short duration of action and repeated administration may be necessary to successfully counteract the effect of longer-acting opioids. The degree of respiratory depression corresponds to opioid receptor affinity and highly potent opioids induce severe respiratory

depression in the higher dose range. In addition, they induce a stiffness of the chest musculature (Jackson, 1994), called chest rigidity or 'wooden chest', which is mediated via stimulation of dopamine release in the nucleus caudatus. Chest rigidity further increases the respiration impairment caused by these compounds. Therefore use of higher doses of potent opioids such as fentanyl and analogs, as used for anesthesia, must be accompanied by artificial or at least assisted ventilation.

Respiratory depression is most prominent with μ-type opioid compounds and less so with κ-agonists and δ-agonists. δ-Agonists seem to have a compensatory stimulant action component, which counteracts respiratory depression.

Cardiovascular Effects

Nearly all opioids induce bradycardia (Bowdle, 1998), most likely mediated via central stimulation of the vagus nerve. Cardiovascular depression associated with most opioids is moderate and only the stronger opioids of the fentanyl group induce a more severe effect. Morphine and some of its analogs induce a non-opioid receptor-mediated release of histamine, which can result in a decrease in blood pressure and compensatory tachycardia.

Gastrointestinal Effects

Gastrointestinal side-effects of opioids are used for treatment of diarrhea

Opioids induce an inhibitory effect on gastrointestinal motility and fluid secretion (Kromer, 1990). The effect is peripherally and centrally mediated. The peripheral component is related to μ- and κ-receptors in intestinal organs, which are densely equipped with opioid receptors. They are located at parasympathic ganglia and inhibit the release of acetylcholine, which stimulates the contraction of smooth muscles. Inhibition of the intestinal fluid secretion is mediated via inhibition of adenylate cyclase. The intestinal effects of opioids extend to all parts of the gut and results in inhibition of stomach emptying and inhibition of secretion and motility of duodenum, jejunum, colon and rectum.

Reduced motility and secretion can lead to constipation, which is the most common side-effect of chronic opioid treatment (Mancini and Bruera, 1998). Opioid-induced constipation can increase to the stage of megacolon or paralytic ileus. Therefore chronic opioid treatment should be accompanied by concomitant use of laxatives. Besides their peripheral actions, opioids are involved in the central

regulation of intestinal functions which are located in the formatio reticularis. This explains why the intestinal side-effects of opioids are not restricted to the more hydrophilic compounds like morphine, but are also seen with the use of more centrally active lipophilic analogs. During chronic opioid treatment a varying degree of tolerance towards the intestinal side-effects may occur.

The intestinal inhibitory action of opioids can be used for treatment of diarrhea (De Luca and Coupar, 1996). The clinically most important anti-diarrheal opioid is loperamide (Heel et al., 1978). After oral administration, loperamide acts locally within the gastro-intestinal tract. After parenteral administration, the compound is rapidly inactivated and does not reach the CNS. Therefore loperamide does not show the typical central opioid side-effects, has no analgesic action and has no abuse potential.

Emetic Activity

Opioids in the circulating blood induce nausea and emesis and an anti-emetic effects after penetration of the blood brain barrier

Nausea and emesis are common unpleasant side-effects of opioids (Campora et al., 1991; Aparasu et al., 1999). They are most intensively experienced at the beginning of the treatment. During chronic administration, tolerance may occur, which reduces the emetic sequelae. Nausea and emesis are induced via activation of chemoreceptors which are located in the trigger zone of the area postema of the formatio reticularis. The receptors are at the tissue surface and in contact with the circulating blood. Thus the emetic effect of opioids is not mediated centrally, i.e. after penetration of the blood-brain barrier, but rather peripherally via the amount of the compound, which is distributed in the circulating blood.

After passage through the blood brain barrier, opioids have an anti-emetic effect (Blancquaert et al., 1986). Emesis inhibition is induced via blockade of an emesis centre located in a more central area of the formatio reticularis. This explains why the emetic effect of opioids is most apparent immediately after anministration, especially after rapid intravenous administration and is reduced or terminated when the compound has reached the CNS. The more hydrophilic opioids like morphine have stronger emetic side-effects than lipophilic compounds like methadone or fentanyl (Barnes et al., 1991), which are rapidly transported into the CNS.

Tolerance and Dependence

μ-Opioid compounds induce a feeling of well-being and euphoria, which is mediated by the release of dopamine within the limbic system. κ-Opioids induce an opposite effect with dysphoria, disorientation and hallucinations (Pfeifer et al., 1986). Repeated activation of the μ-opioid rewarding system may induce a psychological dependence, which leads to addiction and compulsive drug seeking behavior (Brown and Lo, 2000). In addition, higher opioid dosages, as used for non-medicinal purposes, induce tolerance and as a consequence a further increase of the dose is needed to achieve the intended effect. In the course of tolerance development opioid users becomes physically dependent on a supply of the compound (Taylor and Fleming, 2001) and suspension of the treatment or blocking of the opioid receptors with an antagonist induces withdrawal reactions, characterized by strong dysphoria, restlessness, pain and various symptoms of autonomic dysregulation such as diarrhea, shivering, chills and cardiovascular collapse.

Opioid kick

The euphorigenic effect of opioids, the 'opioid kick', is more intensely induced by lipophilic compounds such as diacetylmorphine (heroine), which rapidly penetrates the CNS. The feeling of euphoria at one site and the absence of well-being at another site is increased by rapidly changing brain concentrations of the opioid and this intensifies drug seeking behavior and psychological as well as physical dependence.

Regular use and adequate dosing of opioids minimizes tolerance and dependence development in the treatment of chronic pain

In contrast to recreational use, the treatment of chronic pain with opioids has only a limited risk of inducing psychological dependence and drug addiction (Heit, 2001). Regular dosing can often postpone tolerance development for longer time periods. The most important precaution for avoiding tolerance and dependence development is to ensure constant plasma levels of the opioid, which should be high enough to give complete pain relief. This can be reasonably achieved by oral administration of retarded formulations or by using a patch (Reder, 2001). Breakthrough pain, which is often induced by a fluctuation in pain intensity, should be rapidly addressed by the administration of additional treatment with an immediate-release formulation of the same or a similar opioid.

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3.2 Opioid Peptides

Bernd Sundermann and Corinna Maul

Introduction

Opioids, the most important analgesics for the treatment of moderate to severe pain, can be divided into three groups (Buschmann et al., 2002):

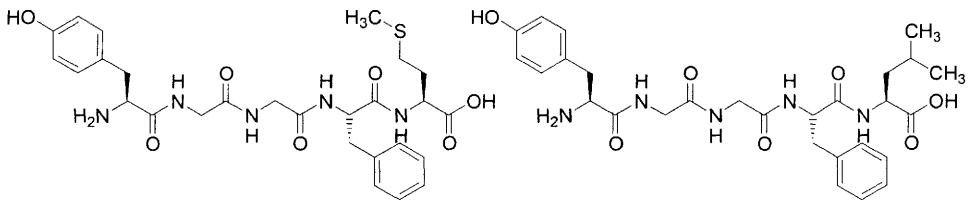
Classification of μ-opioids

- Morphine and codeine as well as their natural and synthetic derivatives ultimately derived from opium ('opiates')
- Purely synthetic non-peptidic compounds with opioid properties but non-morphinan structure
- Naturally-occurring and synthetic opioid peptides

Discovery of Endogenous Opioids

Discovery of Met- and Leu-enkephalin

While morphine as a component of opium has been in use for centuries and the first synthetic opioid, pethidin, was prepared as early as 1939, opioid peptides, the endogenous pentapeptides Met- and Leu-enkephalin (YGGFM and YGGFL), were identified in brain extracts only in 1975 by Kosterlitz and Waterfield (Hughes et al., 1975; also see: Cox et al., 1975; Hughes, 1975; Lord et al., 1977).



Met- (X = Met) & Leu-enkephalin (X = Leu)
(YGGFX = H-Tyr-Gly-Gly-Phe-X-OH)

Simple linear peptides consisting of natural L-amino acids are rapidly broken down *in vivo* (and in many *in vitro* systems) by ubiquitously occurring aminopeptidases

These peptides were characterized *in vitro* and found to be powerful opioid agonists in the mouse vas deferens (MVD) and guinea pig ileum (GPI) assay. In vivo (rat tail-flick) they are only active when administered directly to the brain – a general limitation of simple linear peptides consisting of natural L-amino acids – but with less potency and shorter duration of action than morphine (Casy and Parfitt, 1986).

Discovery of further endogenous opioid peptides

This discovery was the starting point for further investigations that led to the identification of more endogenous opioids. Remarkably, not all endogenous opioids have been isolated from animal brains: the

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Amino acid sequences of prominent endogenous opioid peptides are given in Table 1

dermorphins and deltorphins (see below) have been identified in skin extracts of the South African frogs *Phyllomedusa sauvagei* and *Phyllomedusa bicolor*, respectively (Montecucchi et al., 1981; Erspamer et al., 1989). The opioid peptides β-casomorphine-5 (YPFPG) and morphiceptin (YPFP-amid) are fragments of bovine β-casein (Brandt et al., 1979; Holzgrabe et al., 1997).

Table 1: Selected human opioid peptide sequences.
(with modifications from Straßburger and Friderichs, 2002)

Peptide	Amino acid sequence	Selectivity
Endomorphin-1[(1)]	**Y** *P* W **F**-amid	μ
Endomorphin-2[(1)]	**Y** *P* F **F**-amid	μ
β-Endorphin[(2)]	**Y G G F** *M* T S E **K S** Q T P **L** V T L F K N A I I K N A Y K K G E	μ ≈ δ
Met-Enkephalin[(3)]	**Y G G F** *M*	δ > μ
Leu-Enkephalin[(3)]	**Y G G F L**	δ > μ
Methorphamid[(3)]	**Y G G F** *M* **R R** V-amid	μ >> δ > κ
Dynorphin A[(4)]	**Y G G F L R R** I R P K L **K** W D N **Q**	κ >> μ, δ
Dynorphin B[(4)]	**Y G G F L R R** Q F *K* V V T	κ >> μ, δ
α-Neoendorphin[(4)]	**Y G G F L R** K Y P *K*	κ >> μ, δ
Nociceptin[(5)]	F **G G F** T G A R **K S** A R **K L** A N **Q**	ORL1

Corresponding amino acids are shown in bold or italic.

Precursor peptides: [(1)]pro-endomorphin*, [(2)]pro-opiomelanocortin, [(3)]pro-enkephalin, [(4)]pro-dynorphin, [(5)]pro-nociceptin (* presumed to exist).

Amino acid one letter code:

A: alanine	F: phenylalanine	K: lysine	P: proline	T: threonine
C: cysteine	G: glycine	L: leucine	Q: glutamine	V: valine
D: asparagic acid	H: histidine	M: methionine	R: arginine	W: tryptophane
E: glutamic acid	I: isoleucine	N: asparagine	S: serine	Y: tyrosine

Biological role of endogenous opioids

Endogenous opioids influence a variety of biological functions (reviewed yearly, see Vaccarino and Kastin, 2001), but particularly pain perception: while acute pain in itself has a protective function for the organism, in a 'fight or flight' situation - as an extreme example - endogenous opioids may render even excruciating pain tolerable and thus assure survival. Furthermore, endogenous opioids are released in stressful situations and are assumed to be at least partly responsible for the euphoria sometimes experienced by athletes. The biological functions of the heptadecapeptide nociceptin (Meunier et al., 1995; Reinscheid et al., 1995), the endogenous agonist of ORL1, the latest member of the opioid receptor family to be identified, are still a topic of intensive investigation.

Subtype selectivity of endogenous opioids

Opioid peptides with the common N-terminal sequence YGGF, the so-called 'opioid message area', do not show a very pronounced selectivity for μ, δ or κ receptors. β-Endorphin for example is non-selective with respect to μ and δ receptors while the enkephalins bind an order of magnitude more potently to δ receptors. The highly selective endomorphins and nociceptin on the other hand differ specifically in the classical 'message area'. In this respect today the N-terminal tetrapeptide sequence YGGF is considered to be necessary and sufficient for μ and δ receptor affinity, Y (Tyr^1) and F (Phe^4) being essential, while additional C-terminal amino acids are necessary for κ receptor affinity.

Endomorphins and nociceptin – subtype selective endogenous opioids

The μ selectivity of the endomorphins (and morphiceptin) is thought to arise from the substitution of Gly^2 by the cyclic amino acid proline which significantly increases rigidity of the peptide backbone. With an N-terminal F instead of Y, nociceptin (NC) lacks the aromatic hydroxy function common among natural as well as many synthetic opioids. Although NC has some structural similarity to dynorphin A and β-endorphin, this major difference sets NC apart from all other known endogenous opioids and thus is believed to give rise to its selectivity.

Synthetic Peptidic Tool Compounds

...derived from enkephalin; deltorphins and dermorphin

Endogenous opioid peptides and structurally related tool compounds have played an important role in the differentiation and characterization of opioid receptor subtypes as well as in the elucidation of opioid receptor function. A selection of prominent opioid peptides is given below (review: Hruby and Gehrig, 1989):

- **DALAMID**: [D-Ala^2]Leu-enkephalin — H-Tyr-D-Ala-Gly-Phe-Leu-OH
- **DADL(E)**: [D-Ala^2,D-Leu^5]enkephalin — H-Tyr-D-Ala-Gly-Phe-D-Leu-OH
- **DSL(E)T**: [D-Ser^2,Thr^6]enkephalin — H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH
- **DTLET**: [D-Thr^2,Leu^5,Thr^6]enkephalin — H-Tyr-D-Thr-Gly-Phe-Leu-Thr-OH
- **DA(M)GO**: [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin — H-Tyr-D-Ala-Gly-N(CH_3)Phe-Gly-ol
- **DALCE**: [D-Ala^2,D-Leu^5,Cys^6]enkephalin — H-Tyr-D-Ala-Gly-Phe-D-Leu-Cys-OH
- **DAMME**: [D-Ala^2,MePhe^4,Met(O)-ol^5]enkephalin — H-Tyr-D-Ala-Gly-N(CH_3)Phe-Met(O)-ol
- **[D-Met^2,Pro^5]enkephalinamide** — H-Tyr-D-Met-Gly-Phe-Pro-NH_2
- **[D-Ala^2]deltorphin I** — H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2
- **[D-Ala^2]deltorphin II** — H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2
- **[D-Ala^2]dermorphin** — H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2
- **DALDA**: — H-Tyr-D-Arg-Phe-Lys-NH_2

Unfavorable pharmacokinetic properties of opioid peptides (very short half-life; inability to cross the blood-brain barrier) prohibit their therapeutic use as analgesics.

While opioid peptides have been very useful for investigating the pharmacology of different opioid receptor subtypes, pharmacological investigations have established that no pharmacodynamic advantage is to be expected from opioid peptides with respect to analgesic activity or side-effects. Furthermore, they have their own shortcomings with respect to potential clinical applications. Most importantly their peptidic structure usually prohibits administration by the oral or transdermal route, which are the routes of choice for pain treatment.

Strategies for the design and synthesis of more stable opioid peptides

Therefore, until the present time not a single opioid peptide has become a marketed drug, although many attempts have been made to overcome their serious disadvantages in comparison to non-peptidic opioids. Among others, the following strategies have been followed:

- Incorporation of 'unnatural' D-amino acids (e.g. D-Ala instead of Gly^2)
- Incorporation of derivatized L-amino acids (e.g. N-methylated Phe^4 or Met^5 with an oxidized sulfide side chain)
- Conversion of the terminal COOH to an amide (e.g. $CONH_2$) or reduction to CH_2OH
- Formation of cyclic peptides (e.g. DPDPE) less prone to enzymatic degradation by aminopeptidases
- Formation of peptide dimers or oligomers (e.g. biphalin)
- Incorporation of β-amino acids

Parenterally-active Opioid Peptides

FK 33-824 (DAMME)

The first significant advances towards parenterally-active opioid peptides were made by a Sandoz group (Roemer et al., 1977; Pless et al., 1979). Within the pentapeptide [D-Ala^2,$MePhe^4$,Met(O)-ol^5]enkephalin (FK 33-824, DAMME) several approaches towards a stable enkephalin analog have been combined. FK 33-824 has been reported to be several orders of magnitude more potent than morphine when applied i.c.v. and to be orally active.

[D-Met^2,Pro^5]enkephalin-amide

[D-Met^2,Pro^5]enkephalinamide has been shown to be nearly equipotent to FK 33-824 when administered i.v. (Casy and Parfitt, 1986, p. 352).

Biphalin

(H-Tyr-D-Ala-Gly-Phe-$NH)_2$

The dimeric tetrapeptide hydrazide biphalin, essentially an abbreviated enkephalin dimer, is a potent μ and δ receptor agonist preclinically evaluated as a potential analgesic (Horan et al., 1993). When administered via the i.c.v. route in mice it was found to be two orders of magnitude more potent than morphine. Although biphalin crosses the

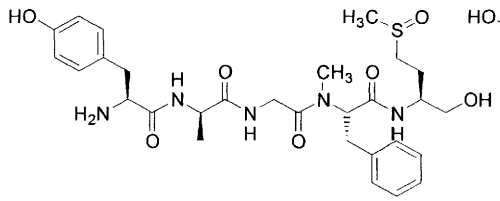
blood-brain barrier, its clinical development was halted due to low plasma stability.

The agonistic δ_1 selective enkephaline analog DPDPE is conformationally constrained through the formation of a disulfide bond between Pen^2 and Pen^5 (Mosberg et al., 1983) and is reported to have an enhanced stability in blood ($t_{1/2}$ > 500 min; Weber et al., 1991). While DPDPE - as well as its even more potent [p-Cl-Phe^4] derivative - crosses the blood-brain barrier in mice after systemic and oral administration, DPDPE is also a substrate for the P-glycoprotein efflux mechanism (Witt et al., 2000) and is rapidly excreted biliarily (Weber et al., 1992; Chen and Pollack, 1997).

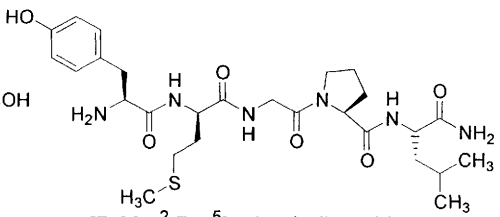
DPDPE

[D-Pen^2,D-Pen^5]enkephalin

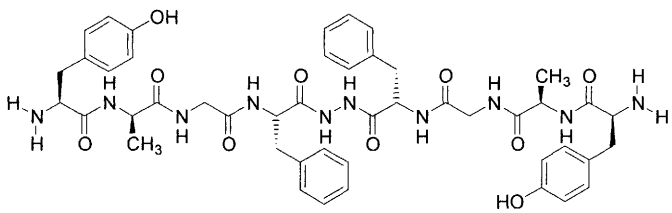
(H-Tyr-D-Pen-Gly-Phe-D-Pen-OH)



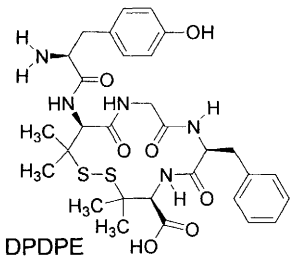
FK 33-824 (DAMME)



[D-Met^2,Pro^5]enkephalinamide



Biphalin



DPDPE

Important peptidic tool compounds for opioid receptors

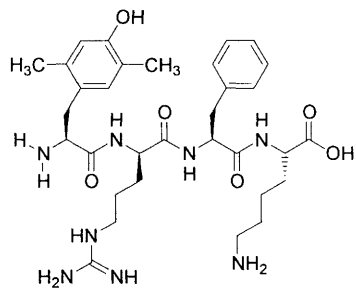
While no further development of FK 33-824, Biphalin, DPDPE or other prominent opioid peptides has been reported, opioid peptides are still among the most important tool compounds in opioid receptor pharmacology. An overview is given in Table 2 (Corbett et al., 2002):

Table 2: Peptidic opioid receptor tool compounds.

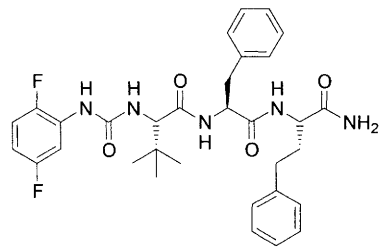
	μ	δ	κ	ORL1
agonists	DA(M)GO Endomorphin I Endomorphin II	δ_1: DPDPE (DADL(E)) δ_2: [D-Ala2]deltorphin I [D-Ala2]deltorphin II (DSLET)	-	nociceptin
antagonists	-	δ_1: (DALCE)	-	-

Recent patent activity

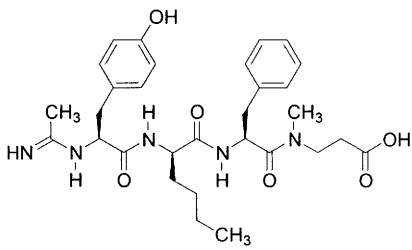
Patent applications disclosed in the last several years reveal further activities directed towards the discovery and potential development of opioid peptides as analgesics (e.g. Kim et al. (Biomeasure Inc.), 1994; Dooley and Houghten (Torrey Pines Institute for Molecular Studies), 1996-1999; Grandy et al. (Oregon Health Sciences University), 1998; Kahn et al. (Molecumetics Ltd.), 1998; Moreau et al. (Biomeasure Inc.), 1997; Wang (Astra Aktiebolag), 1997); Junien et al. (Ferring B.V.), 1999; Persons et al. (Sepracor Inc.), 1999; Sakurada et al. (Daiichi Pharmaceutical Co.), 1999; Szeto (Cornell Research Foundation), 2002). Selected examples include:



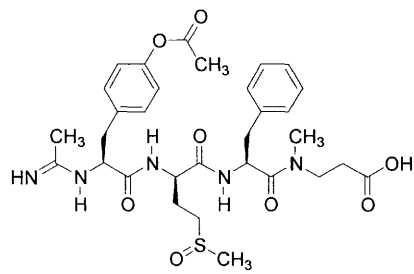
cpd. 1 - [Dmt[1]]DALDA (Szeto, 2002)



cpd. 2 (Persons et al., 1999)



cpd. 3 (Sakurada et al., 1999)



cpd. 4 (Sakurada et al., 1999)

Cpds. 3 and 4 have been described to be orally active in a tail pressure-stress assay in mice with ED_{50} values of 7.9 and 1.2 mg/kg, respectively, compared to 22 mg/kg for morphine.

Summary

Opioid peptides have an action and side-effect profile identical to non-peptidic opioids, but in general have significantly less favorable physicochemical and pharmacokinetic properties. Considering the numerous attempts to overcome these drawbacks undertaken since the mid-1970s that have not resulted in a single opioid

peptide being successfully developed for clinical use, it seems unlikely to expect opioid peptides to ever reach the market. Nevertheless, recent patent activity discloses opioid peptides still to be considered as potential analgesics (see above).

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3.3 Synthetic Opioids

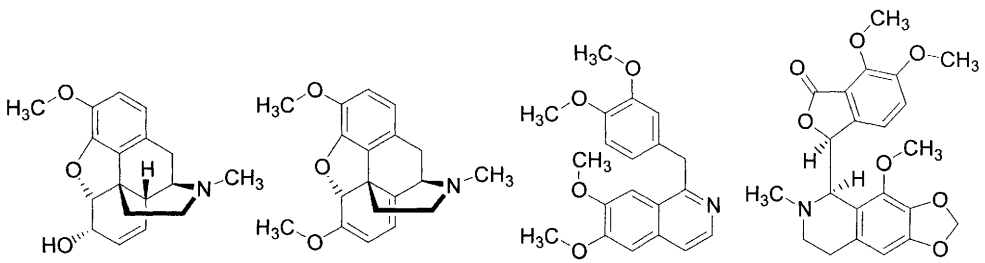
Corinna Maul, Helmut Buschmann, and Bernd Sundermann

Introduction

Morphine has always been an accepted standard analgesic, the medicament without which, until recently, no one could practice medicine effectively. Its use, however, bears some risks of side-effects. If the dose is only a little too high, breathing may be depressed to a life-threatening degree. Nausea, vomiting, sweating, dizziness, and sluggishness occur frequently. The heart rate is slowed and the blood pressure may fall. With repeated use of morphine, the analgesic effects wane and the dose has to be increased. Furthermore, morphine can cause addiction, an accommodation of the cells of the body to its presence so that its use must be continued or a withdrawal syndrome appears. Thus the search for a better analgesic is a search for a better morphine, a substance with morphine's beneficial properties and with attenuated or no harmful side-effects including tolerance and dependence (Eddy and May, 1973).

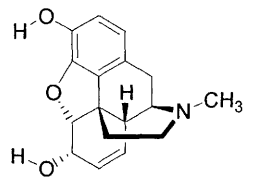
1805	Morphine
1874	Heroin
1925	Oxycodone
1939	**Pethidine**
1946	**Methadone**
1961	**Fentanyl**
1962	**Tramadol**
1965	**Tilidine**

Following the isolation of morphine by Sertürner in 1805 further alkaloids were isolated from opium, for example narcotine in 1817, codeine in 1832, thebaine in 1835 and papaverine in 1848.



Scheme 1: Opium alkaloids isolated in the 19th century.

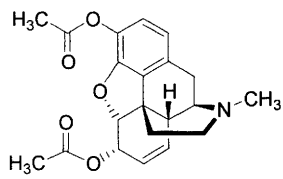
The earliest attempts to develop a non-dependence-inducing morphine derivative resulted in the preparation of heroin (3,6-diacetylmorphine) by acetylation of morphine (Wright, 1874, Dreser, 1898). The potency of heroin was soon recognized. It underwent more investigation than any other product of the time, and was introduced into clinical medicine in 1898. Reports of its reduced respiratory depression and dependence liability were soon shown to be unfounded, but its analgesic effects in animals and man (twice morphine) were confirmed. Pharmacological examination of acyl derivatives of morphine showed that



Morphine

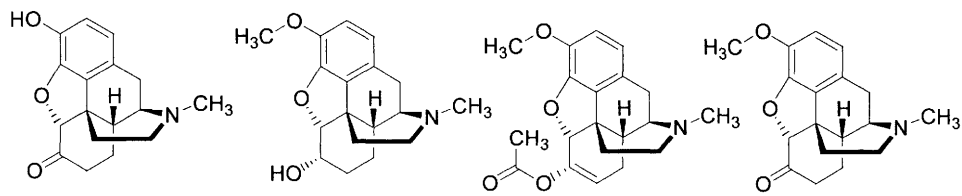
Analgesics. Edited by H. Buschmann, T. Christoph, E. Friderichs, C. Maul, B. Sundermann
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heroin and its higher and lower acyl homologs have similar analgesic potencies in rodents and have high physical dependence liability (May and Jacobson, 1977).



Heroin

The introduction of heroin, although based on inaccurate observations and interpretation, undoubtedly influenced the trend and objectives of morphine research and marked the beginning of the search for an improved analgesic. During the 25 years after the introduction of heroin, other morphine derivatives were incorporated into medical practice some of which are still being used today. These include dihydrocodeine, differing from codeine only in the saturation of one double bond, hydrocodone (dihydrocodeinone) which is very similar in activity, having in addition one hydroxyl replaced by a keto group and an effective dose one-sixth that of codeine, and thebacon (acetyldihydrocodeinone) an acetylation product of hydrocodone and similar to it in activity. All of these are analgesics, but mainly used as antitussives. Also introduced in that period was hydromorphone (dihydromorphinone) which is very similar to heroin in its action.



Hydromorphone Dihydrocodeine Thebacon Hydrocodone

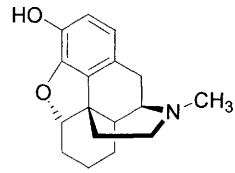
Scheme 2: Opioids introduced into clinical practice at the beginning of the 20th century.

The Search for Opioids with Reduced Side-Effects

In the 1920s a most significant change in analgesic research came about: the beginning of the first systematic study of structure-action relationships which endeavored to separate analgesic effectiveness from side-effects and addiction liability.

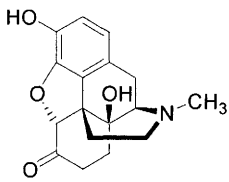
In the USA, this plan was directed from 1929-1939 by the Committee on Drug Addiction of the National Research Council (NRC) with financial support from the Rockefeller Foundation. The program consisted of modification of the morphine molecule at all accessible points and also targeted (modified) partial structures of the morphine molecule, such as phenanthrene, hydrogenated phenanthrene, isoquinoline, dibenzofuran, and carbazole. More than 150 derivatives of morphine and more than 300

synthetic products were tested for analgesic, respiratory, gastrointestinal, sedative, and other central nervous system effects. The significance of the phenolic and alcoholic hydroxyls for intensity of analgesic action was established. Removal of the latter, as in desomorphine, resulted in the most rapidly acting and potent analgesic known at that time (Eddy and May, 1973).

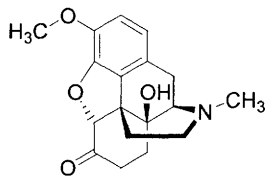


Desomorphine

After 10 years of intensive research, no significant dissociation of potent analgesia and dependence liability was accomplished. As an indirect result of the systematic program the identification of the 17-hydroxy-7,8-dihydro compounds oxycodone (patented in 1925 by E. Merck AG, Germany) and oxymorphone, derived from thebaine, are of particular note.



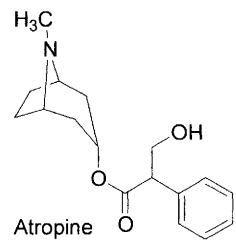
Oxymorphone



Oxycodone

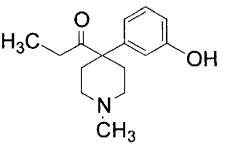
Scheme 3: Structures of oxymorphone and oxycodone.

During the late 1930s some 4-phenylpiperidine derivatives were examined as potential spasmolytics on the basis of their chemical relationship to atropine. The antinociceptive properties of one member, ethyl 1-methyl-4-phenyl-piperidine-4-carboxylate, was detected in screening tests and the compound was subsequently introduced into clinical use by Eisleb and Schaumann in 1939. The compound, well known as pethidine in Europe and meperidine in North America (proprietary names include Demerol, Dolantin, and Dolosal), was soon in widespread use for the relief of pain, and it is remarkable how pethidine, the original non-opioid-derived opioid analgesic, has retained its popularity for many years in the face of competition from other synthetic analgesics introduced since 1939.



Atropine

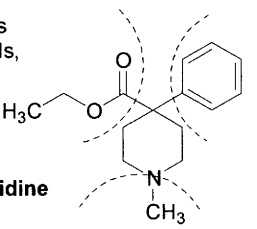
Thousands of phenylpiperidines, related to pethidine, were synthesized during the following years. Some of these variations and other drugs which were developed are shown in scheme 4. But again there has been no significant progress in relating specific structural features with analgesia or side-effects and dependence.



Ketobemidone

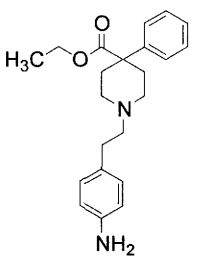
structural variation:
- other esters (bulky esters usually reduce activity, except adamantyl
- inversed esters (usually leads to an increase in potency)
- ether or keto oxygen functions (leads to analgesic compounds, e.g. ketobemidone)

replacement by other aromatic groups:
- gross increase in size leads to inactive compounds
- replacement of phenyl by heteroaromatic groups is usually disadvantageous in analgesics (*exception: 2-furyl, 2-thienyl*)
- substitution generally leads to fall in activity (*exception: the presence of a meta phenolic hydroxyl in ketobemidone elevates activity 13-14 times*)

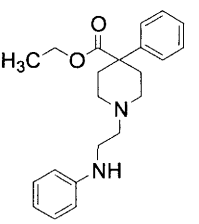


Pethidine

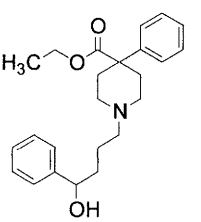
structural variation:
alkyl, phenalkyl, N- or O-containing alkyl substituents lead to various new analgesics; some of them have been used clinically but have only a historical role
examples: anileridine, piminodine, phenoperidine, furethidine



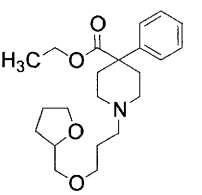
Anileridine



Piminodine

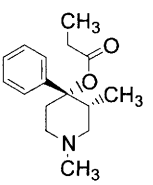


Phenoperidine

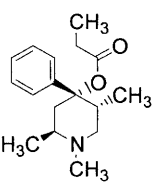


Furethidine

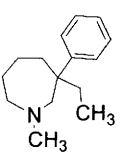
further variations:
- alkyl substitution in the piperidine ring (examples: α-prodin (nisentil), trimeperidine (γ-promedol))
- ring size (examples: profadol, meptazinol)



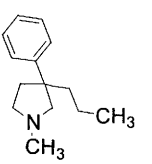
α-Prodin



Trimeperidine



Meptazinol



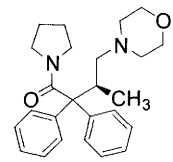
Profadol

Scheme 4: Variations of the pethidine structure. Ketobemidone and pethidine itself are the only compounds which are still in frequent clinical use.

During World War II, chemists working in the Hoechst Laboratories of I.G. Farbenindustrie discovered that

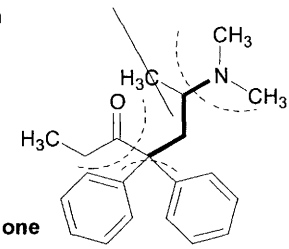
certain derivatives of 3,3-diphenyl-N,N-dimethylpropylamine have analgesic properties. The best known member of this group, 6-dimethylamino-4,4-diphenylheptan-3-one (methadone), was introduced into clinical practice in 1946. The path from pethidine to the methadone structure was never clearly revealed, but both contain several features in common with morphine and with each other. Methadone matched the pharmacological profile of morphine qualitatively, but there are significant differences in the time-courses of action. It is as effective as morphine as an analgesic but longer acting when administered orally. Compared with morphine and heroin, the methadone abstinence syndrome is slower in onset, longer in duration, and much less intense.

NCCCPh$_2$-chain
- methyl substituent adjacent (α) to nitrogen is optimal usually
- in the case of pyrrolidin-substitution at the oxygen function, β-methyl derivatives show higher potencies (*dextromoramide*)



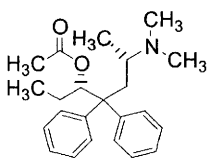
Dextromoramide

variants of the ethyl ketone function
ester, sulphone, secondary alcohols (also acylated, see *levomethadyl acetate*), tertiary amides (*dextromoramide*)



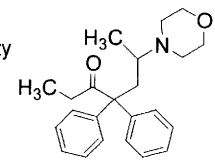
Methadone

variation of the basic group
In general, dimethylamino gives optimum activity, but 5- and 6-membered alicyclic basic units also yield strong analgesic compounds (e.g. *phenadoxone, dipipanone, dextromoramide*)

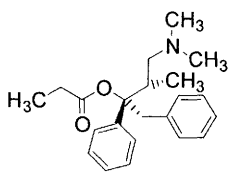


Levomethadyl acetate

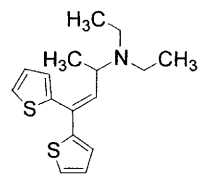
variation of the diphenyl unit
- removal of one phenyl abolishes activity
- replacement by 2-thienyl leads to loss of activity (exception: related derivatives like *themalon*)
- substitution of one phenyl by benzyl leads to analgesics with moderate potency (*dextropropoxyphene*)



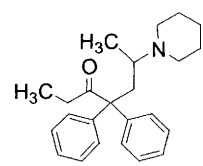
Phenadoxone



Dextropropoxyphene



Themalon

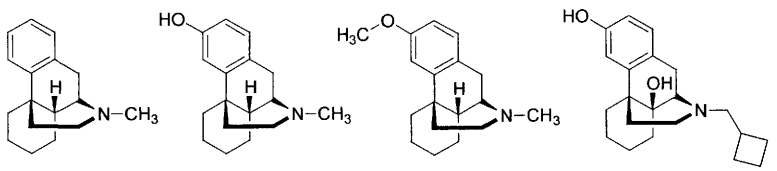


Dipipanone

Scheme 5: Variations of methadone. Levomethadyl acetate, levomethadone, dextropropoxyphene and dextromoramide are still in clinical use

Many methadone derivatives have been prepared with varying degrees of analgesic potency and duration of action. Variations of the methadone structure and some derivatives are shown in scheme 5.

The attempts to synthesize morphine led to the synthesis of its basic skeleton by Grewe, published in 1946. This work, continued by Schnider et al. (1950; 1951), yielded the significant discovery that the complete morphine structure is not essential for potent analgesic activity. N-Methylmorphinan is analgesic, and (-)-3-hydroxy-N-methylmorphinan (levorphanol) is an effective therapeutic agent, more potent than morphine.

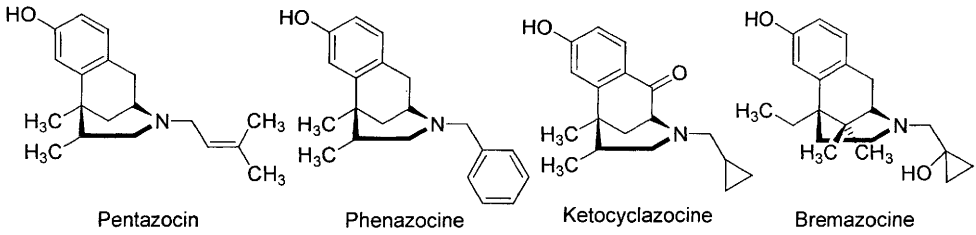


N-Methylmorphinan Levorphanol Dextromethorphan Butorphanol

Scheme 6: Selected morphinanes

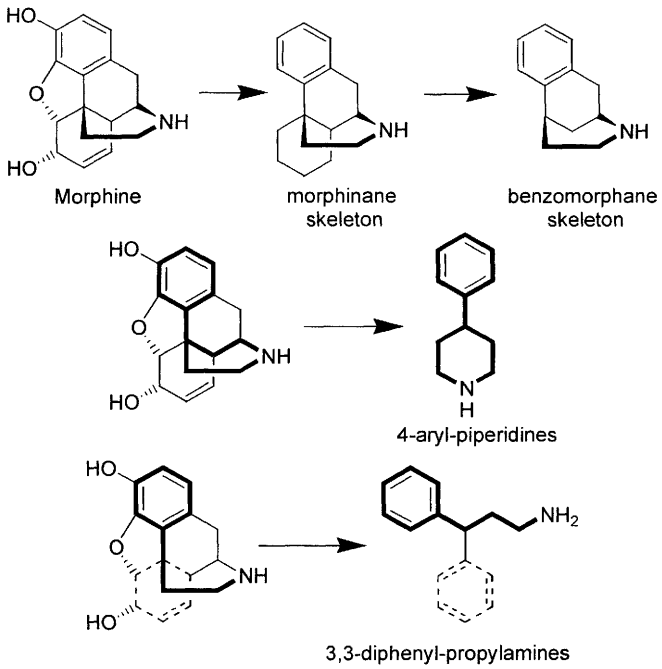
The activity of levorphanol prompted the synthesis of two even simpler modifications, phenylmorphans and 6,7-benzomorphans starting in 1953. Levorphanol, a morphinan, has one ring less than morphine, and benzomorphan has one ring less than levorphanol. The first of the benzomorphans to be brought to general attention was phenazocine. When administered orally, it is an effective analgesic with a significantly lower dependence capacity in monkeys and a somewhat lower dependence liability in man. The attachment of a phenethyl group to the nitrogen atom led to a systematic study of the role of the tertiary amine in opioid action, which showed that although activity was reduced by N-ethyl substitution, it began to be restored and increased with increasing size of the N-alkyl group (from propyl up to phenacyl).

Ketocyclazocine, an analog with an oxidized C-1 methylene, although active in antinociceptive tests, differs from most other opioids in failing to elicit a 'Straub tail' reaction and mydriasis in mice. It was found that the main activity of the compound is κ-agonism (Casy and Parfitt, 1986). Another derivative, bremazocine, carrying an hydroxy substituent in its alkyl side chain, is a potent, long-acting κ-agonist with activity at μ-sites as well. However, it was found to possess strong psychomimetic side effects, a problem which frequently occurs with κ-agonists.



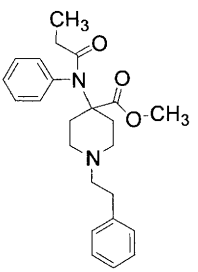
Scheme 7: Selected benzomorphanes

The morphinanes and benzomorphanes are structurally derived from morphine as it is shown in figure 8. Furthermore, the opioids known by the end of the 1950s from the pethidine group and the methadone group retrospectively incorporate substructures of the morphine skeleton. All active compounds possess at least one aromatic ring. The most common aromatic entity is the phenyl ring, where substituents are generally disadvantageous with the exception of a correctly placed hydroxy group. The other important substructure is the basic nitrogen atom, where a methyl substituent is most commonly associated with agonism.

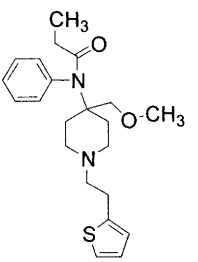


Scheme 8: From morphine to synthetic opioids

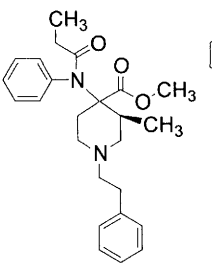
An important step for the treatment of severe pain was made in the early 1960s: Paul Janssen's exploitation of 4-piperidone chemistry proved remarkably successful in that it led to the clinical use of both a major tranquilizer (haloperidol) and a potent narcotic analgesic, fentanyl. Fentanyl is related to pethidine and also to basic anilides with analgesic properties and is characterized by high potency and short duration of action. Again, a series of derivatives was synthesized over the following decades which led to several products for clinical use, however fentanyl is still very important for the treatment of severe pain.



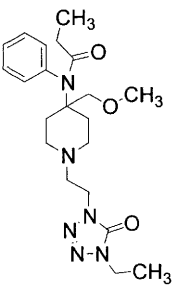
Carfentanil



Sufentanil

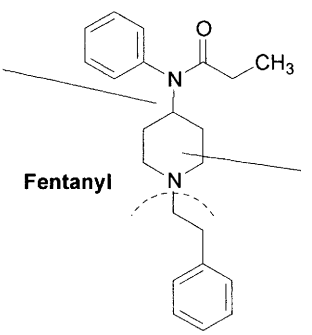


Lofentanil



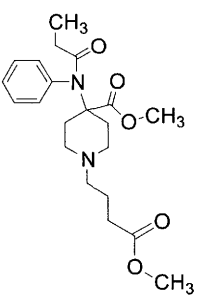
Alfentanil

substitution at C-4
- carboalkoxy (*carfentanil, lofentanil*)
- alkoxymethyl (*alfentanil, sufentanil*)
- acyl



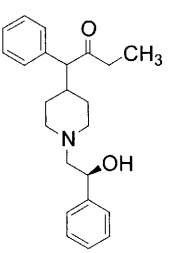
Fentanyl

variations of the piperidine ring
- one-carbon expansion or contraction diminishes potency
- the cis-3-methyl analog possesses increased activity (e.g. *lofentanil*)



Remifentanil

variations at the nitrogen of the piperidine ring
- N-ethyl is clearly the optimal unbranched spacer between the piperidine ring and the phenyl ring
- insertion of a methyl group in α-position to the piperidine nitrogen elevates potency
- insertion of hydroxyl in β-position to the piperidine nitrogen can elevate potency (e.g. *ohmefentanyl*)
- substitution of phenyl can lead to strong analgesics (e.g. *sufentanil, remifentanil, alfentanil*)

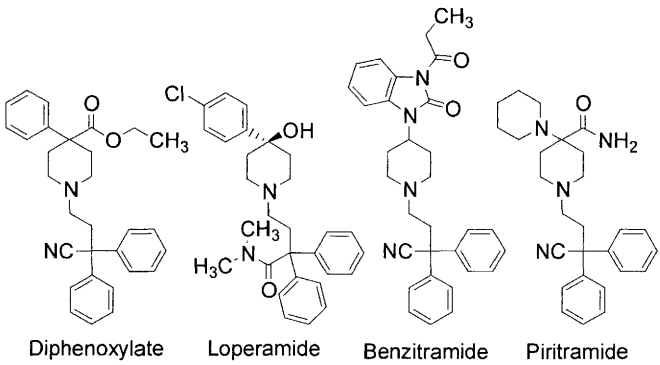


Ohmefentanyl

Scheme 9: Variations of the fentanyl structure.

Several compounds have been prepared with the probable aim of combining the most attractive features of both

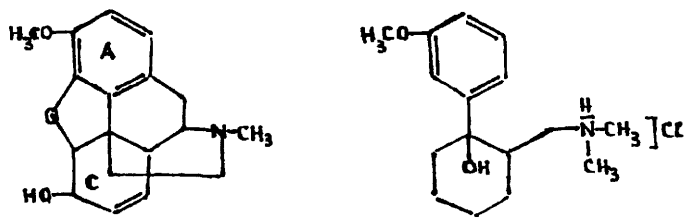
diphenylpropylamine and 4-phenylpiperidine analgesics. The derivatives diphenoxylate and loperamide have limited access to the brain and are used for the prevention of diarrhea, whereas benzitramide and piritramide represent potent analgesics.



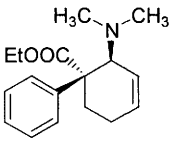
Scheme 10: Combined diphenylpropylamine/4-phenylpiperidine opioids.

From a structural point of view, cyclohexane forms the common element of a variety of opioid analgesics that are otherwise difficult to classify.

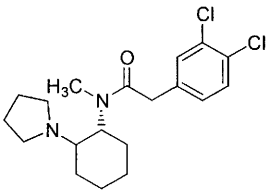
In 1962, Flick (Grünenthal, Germany) aimed to synthesize a new antitussive compound. He took codeine as a model and simplified the complex structure as shown in figure 11, which can be regarded as an early rational design (Flick et al., 1978). The resulting compound, tramadol, did have antitussive properties, but due to its outstanding combination of analgesic properties and low potential for abuse or dependence (Scott and Perry, 2000) became one of the most important drugs for moderate to severe pain by the late 1990s.



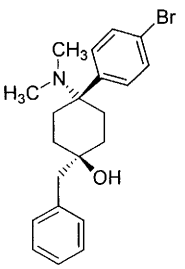
Scheme 11: Reaction scheme taken from the original notes of Kurt Flick (1962): early rational design of a new opioid (left: codeine; right: tramadol).



Tilidine



U-50488



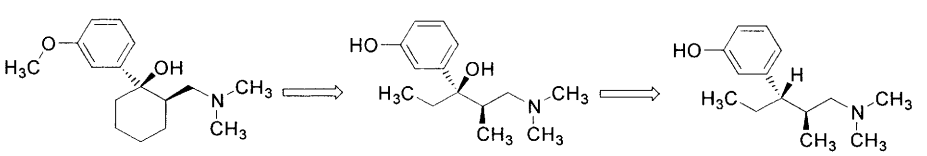
1-Benzyl-4-(4-bromo-phenyl)-4-dimethylamino-cyclohexanol

A number of cyclohexane derivatives with tertiary amino substituents, especially dimethylamine, have proven to be opioid analgesics. Tilidine, which was synthesized at the beginning of the 1960s, is one of these compounds. Its trans NMe_2/CO_2Et configuration is important for activity since the corresponding cis-isomer is less potent.

Another aminocyclohexane with relevant analgesic properties is the 1,2-diaminocyclohexane derivative U-50488 and its analogs. Biological evaluation suggests that they are κ- rather than μ-agonists. The 3,4-dichlorophenyl unit is also present in other cyclohexane derivatives showing κ-agonistic activity (Holzgrabe et al., 1997)

The discovery of 1-aryl-1-dimethylamino-cyclohexanes resulted from a surrey of compounds in which aromatic and basic features, both critical structural requirements of opioid analgesics, but usually separated by two or three carbon atoms, are linked to the same quaternary carbon. The synthesis of these compounds yielded a series of highly potent opioids (e.g. 1-Benzyl-4-(4-bromo-phenyl)-4-dimethylamino-cyclohexanol), however none of them are in clinical use (Lednicer et al., 1981).

Reduction of the morphine structure (via tramadol) to the known essential substructures of efficient opioids (basic nitrogen atom plus a m-phenol) led to the synthesis of a series of open-chained potent analgesics. Moreover, the removal of the tertiary hydroxy group further increased analgesic potency. The (+)-enantiomer of the resulting derivative is, to the best of our knowledge, the smallest μ-opioid agonist at least equipotent to morphine ever described. The (-)-enantiomer, having a dual mechanism of action like tramadol, is being investigated in clinical trials (Buschmann et al., 2002).



Scheme 12: Small but potent new μ-opioid agonists.

Starting from morphine, the search for an ideal opioid analgesic has resulted in a huge number of μ-opioids - many of them are in clinical use today. Over recent decades, efforts to find further μ-opioids with attenuated side-effects were clearly reduced, but with respect to the fact that μ-opioids are still the only drugs for the treatment

of severe pain there may be a renewed interest in novel μ-opioids in the near future.

References

For compound-specific and mechanism-directed literature see corresponding chapters.

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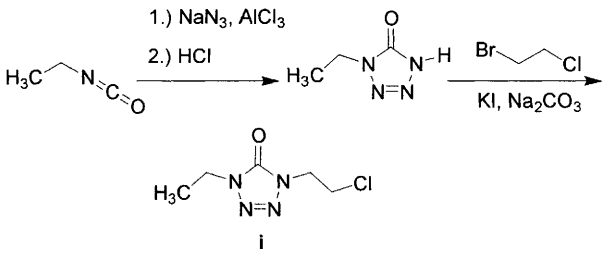
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3.4 Opioids with Clinical Relevance

Elmar Friderichs and Helmut Buschmann

Alfentanil

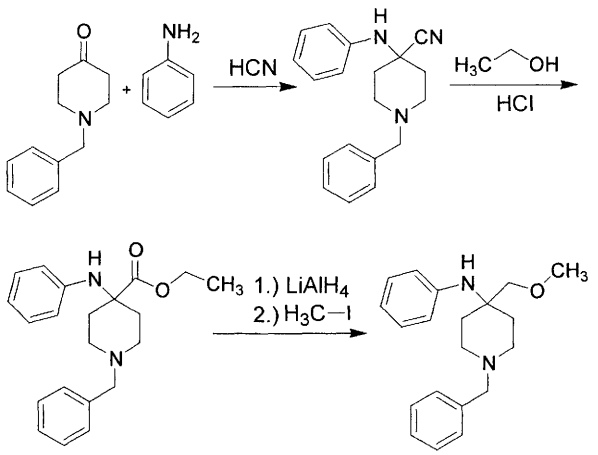
Synthesis: The cyclization of ethyl isocyanate with sodium azide by means of $AlCl_3$ in refluxing THF gives 1-ethyl-1,4-dihydro-5*H*-tetrazol-5-one, which is alkylated with 1-chloro-2-bromo-ethane in the presence of Na_2CO_3 and KI in refluxing 4-methyl-2-pentanone to afford 1-ethyl-4-(2-chloroethyl)1,4-dihydro-5*H*-tetrazol-5-one **i** (Janssen (Janssen), 1978; Janssens et al., 1986; Hopkins, 1981; Kleemann et al., 1999).



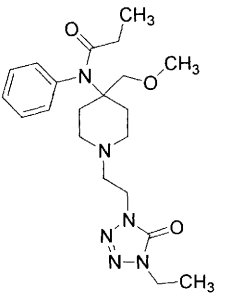
i

Scheme 1: Synthesis of 1-ethyl-4-(2-chloroethyl)-1,4-dihydro-5*H*-tetrazol-5-one.

N-(4-methoxymethyl-4-piperidinyl)-*N*-phenyl-propionamide **ii** is synthesized according the following scheme starting from 1-benzyl-4-piperidone:

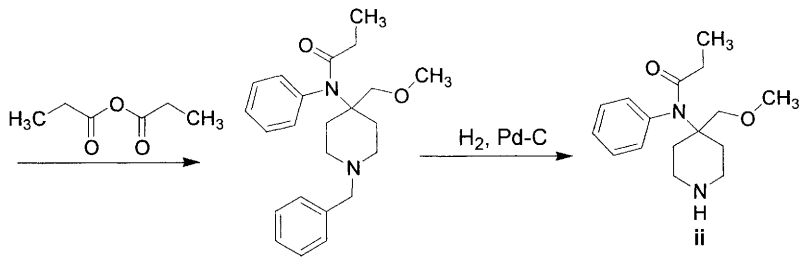


Alfentanil



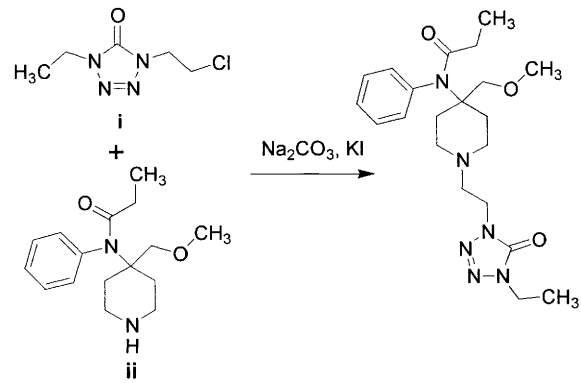
[71195-58-9], *N*-{1-[2-(4-Ethyl-5-oxo-4,5-dihydro-tetrazol-1-yl)-ethyl]-4-methoxymethyl-piperidin-4-yl}-*N*-phenyl-propion-amide, $C_{21}H_{32}N_6O_3$, M_r 416,25; hydrochloride monohydrate [*70879-28-6*], $C_{21}H_{32}N_6O_3 \cdot HCl \cdot H_2O$, M_r 470.99, *mp* 138.4-140.8 °C

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Scheme 2: Synthesis of *N*-(4-methoxymethyl-4-piperidinyl)-*N*-phenyl-propionamide

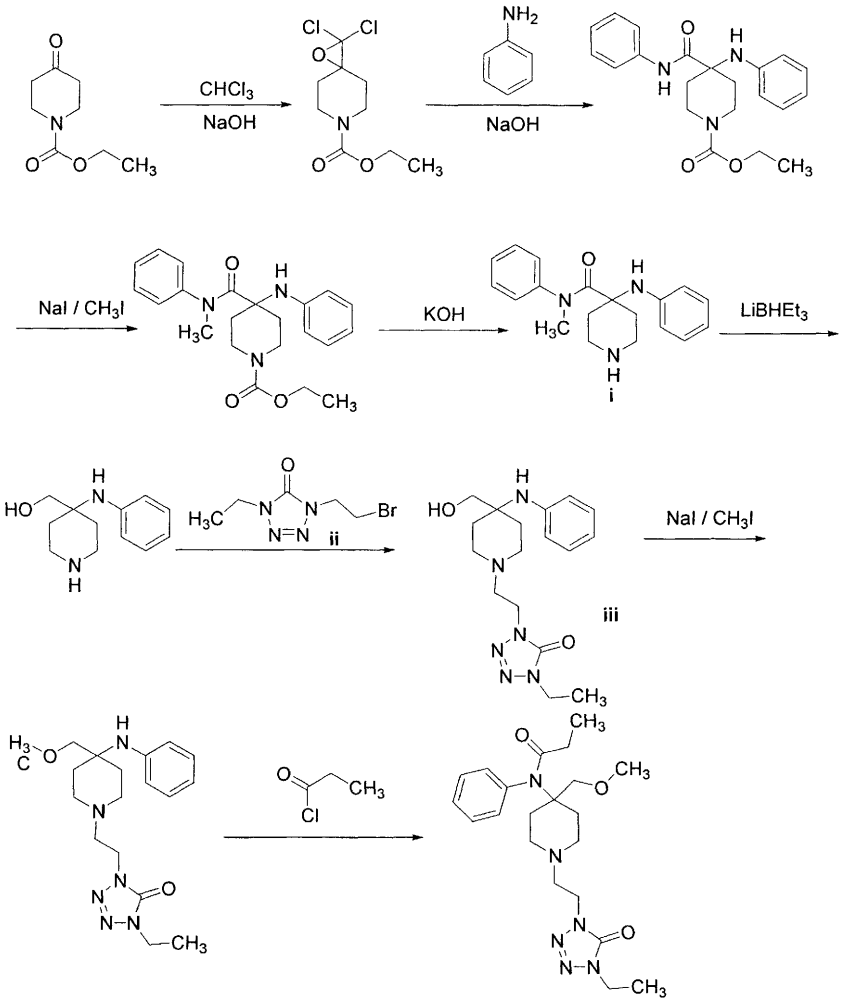
Finally the tetrazole derivative **i** is condensed with the propionamide **ii** by means of Na_2CO_3 and KI in refluxing 4-methyl-2-pentanon.



Scheme 3: Synthesis of alfentanil.

An alternative synthetic route is described in the literature (Kleemann et al., 1999): the reaction of 4-oxopiperidine 1-carboxylic acid ethyl ether with chloroform, benzyltriethylammonium chloride and NaOH in THF/water gives the corresponding spirooxetane, which is treated with aniline and NaOH to yield the anilide. The methylation of the amide nitrogene by means of sodium hydide and methyliodide in THF affords the methylated anilide. The following reaction with KOH in refluxing isopropanol causes elimination of its ethoxycarbonyl group, providing compound **i**, which is reduced with lithium triethyl-borohydride in THF to yield 4-(hydroxymethyl)-4-(phenyl-amino)piperidine. Condensation with the tetrazolone derivative **ii** by means of KI in refluxing acetonitrile yields the adduct **iii**, which is methylated with NaH and methyliodide in THF to afford the methoxy derivative. Finally, this compound is acylated with propionyl chloride in chloroform to provide the target compound. The

intermediate tetrazolone derivative **ii** has been obtained by reaction of 1-ethyl-4,5-dihydro-1H-tetrazol-5-one with 1,2-dibromoethane by means of TEA in acetonitrile.



Scheme 4: Synthesis of alfentanil.

Opioid receptor binding: Alfentanil is a μ-selective opioid (Cookson et al., 1983) with a receptor affinity in the range of morphine and fentanyl.

Analgesic efficacy and clinical use: Alfentanil is a short-acting potent opioid with analgesic and anesthetic properties (Larijani and Goldberg, 1987). It is less potent than fentanyl but administration can be better controlled. It is mostly used as a supplement to general anesthesia or as a primary anesthetic e.g. in cardiac surgery. Intra-

venous or epidural bolus or on-demand administration can be used for postoperative pain treatment.

Dosages and routes of administration: Alfentanil is only used parenterally. Because of strong respiratory depression administration under spontaneous respiration has to be confined to a dose range up to 200 µg/h. Higher doses as used in anesthesia need assisted ventilation.

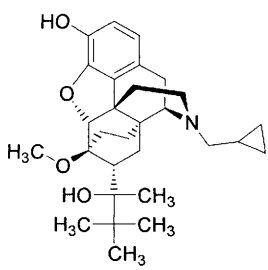
Pharmacokinetic properties: Intravenous alfentanil (Hull, 1983) has a rapid onset and a short duration of action. It has a shorter elimination time (terminal half-life 1-2 h) than fentanyl. It is less lipid-soluble and the short duration of action is more dependent on metabolic inactivation than on redistribution. Alfentanil has a high (90%) plasma protein binding. Metabolic inactivation is effected by oxidative N- and O-demethylation.

Trade name: Rapifen (Ger, Fr, UK); Alfenta (USA)

Side-effects: Alfentanil has a strong respiratory depressant action and high doses induce chest wall rigidity. The compound has a µ-type addiction and dependence potential.

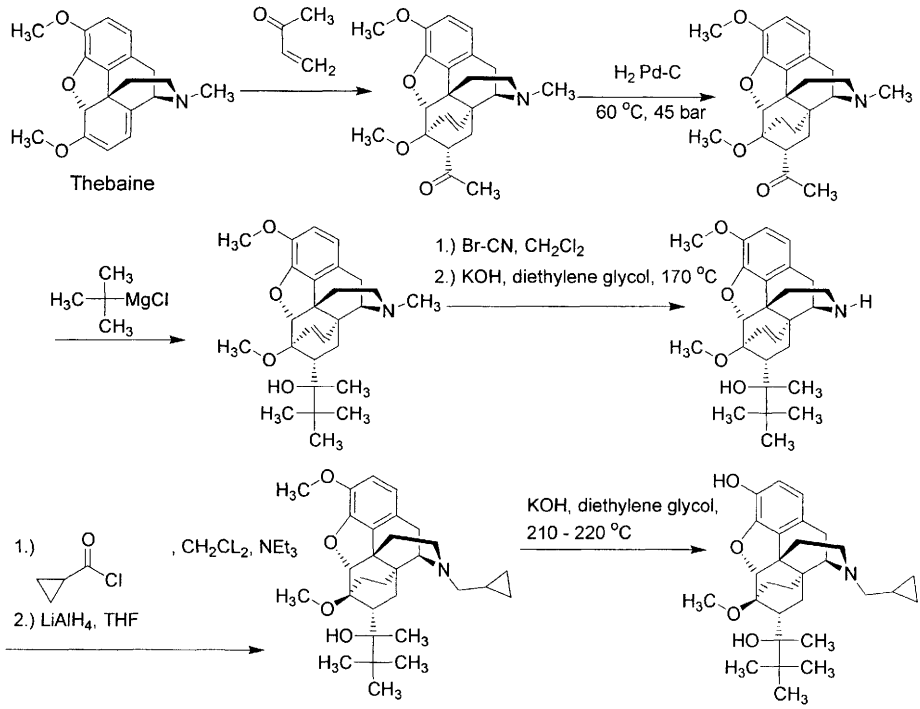
Buprenorphine

Buprenorphine



[52485-79-7], (αS,5α,7α)-17-(Cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol, $C_{29}H_{41}NO_4$, M_r 467.30, *mp* 209 °C; hydrochloride *[53152-21-9]*, $C_{29}H_{41}NO_4 \cdot HCl$, M_r 504.10

Synthesis (Bentley (Reckitt & Sons), 1963; 1966; 1969; Dorner, 1986, Kleemann et al., 1999; Husbands and Lewis, 2000; Christoph, 2002): condensation of thebaine and but-3-en-2-one yields 7-acetyl-6,14-endoetheno-6,7,8,14-tetrahydrothebaine, which is hydrogenated to the corresponding *endo*-ethano derivative. The reaction of the *endo*-ethano-derivative with tertbutyl-magnesium chloride in ether-benzene yields 7-α-(2-hydroxy-3,3-dimethyl-2-butyl)-6,14-*endo*-ethano-6,7,8,14-tetrahydrothebaine. The following reaction with BrCN in methylene chloride affords 7-α-(2-hydroxy-3,3-dimethyl-2-butyl)-6,14-*endo*-ethano-N-cyano-6,7,8,14-tetrahydrothebaine, which is treated with potassium hydroxide in ethylene glycol to give 7-α-(2-hydroxy-3,3-dimethyl-2-butyl)-6,14-*endo*-ethano-N-cyano-6,7,8,14-tetrahydronorthebaine. This compound is treated with cyclopropylcarbonyl chloride in methylene chloride containing triethylamine, followed by reduction with $LiAlH_4$ in refluxing THF yielding 7-α-(2-hydroxy-3,3-dimethyl-2-butyl)-6,14-*endo*-ethano-6,7,8,14-tetrahydronorthebaine.
In the final step this compound is demethylated with KOH in diethylene glycol at 210-220°C.



Scheme 5: Synthesis of buprenorphine.

Opioid receptor binding: Buprenorphine has a mixed agonistic-antagonistic action profile with a high affinity for the μ-, κ-, and δ-opioid receptors (Huang et al., 2001). An approximately 100-fold lower affinity was observed for the ORL1-receptor. The compound dissociates slowly from the receptor which may explain some peculiarities in its pharmacological actions.

Analgesic efficacy and clinical use: Buprenorphine (Heel et al.,1979) is a potent mixed agonistic-antagonistic opioid analgesic, which is used for the treatment of moderate to severe pain. The potency is about 20-30 times higher than that of morphine. No ceiling of analgesia is observed in clinical dosages (Zenz et al.,1985). Buprenorphine may be used for premedication or as adjunct to anesthesia. The compound has a long duration and a slow offset of action and is used in the treatment of opioid addiction as well. Due to its partial agonistic properties it can act in combination with full agonists as an antagonist, reducing their effect and precipitating a withdrawal reaction in opioid agonist-dependent persons. Antagonistic properties are seen in doses much higher than the analgesic dose range.Therefore no precautions are necessary when

changing the treatment from a standard opioid agonist to buprenorphine or vice-versa (Atkinson et al.1990).

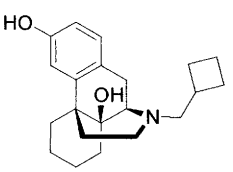
Dosages and routes of administration: Buprenorphine is given parenterally, orally (sublingual) or by the transcutanous route as a patch. The doses for slow intravenous or intramuscular administration are 300-600 µg, the sublingual doses 200-400 µg, both given every 6-8 h. A transdermal formulation of buprenorphine is available as an advanced matrix patch with release rates of 35; 52.5 and 70 µg/h, corresponding to daily dosages of about 0.8, 1.2 and 1.6 mg, respectively and providing 3 days pain control.

Pharmacokinetic properties: Buprenorphine (Kuhlman et al., 1996) is subject to considerable first-pass metabolism after oral application, but sublingual administration results in a high rate of transmucosal absorption and good bioavailabilty. Buprenorphine is highly lipophilic and about 96% is bound to plasma proteins. Plasma elimination half-lives are between 1 and 7 h. There is only a weak correlation between plasma levels and analgesic effect. The compound is metabolized by N-dealkylation to norbuprenorphine, which has a reasonable µ-opioid receptor affinity and may be involved in the analgesic action (Huang et al., 2001). An essential part of the drug is excreted unchanged in the faeces.

Side-effects: Buprenorphine induces µ-opioid-type side effects including respiratory depression, drowsiness, nausea and vomiting. In the clinical literature, however, there are only few cases of significant respiratory depression. Reversal of respiratory depression may need higher doses of naloxone (Gal, 1989). Buprenorphine has a limited abuse potential and withdrawal reactions, due to slow receptor dissociation, are mild and delayed.

Trade name: Temgesic (Ger, Fr, UK); Bupenex (USA); Transtec

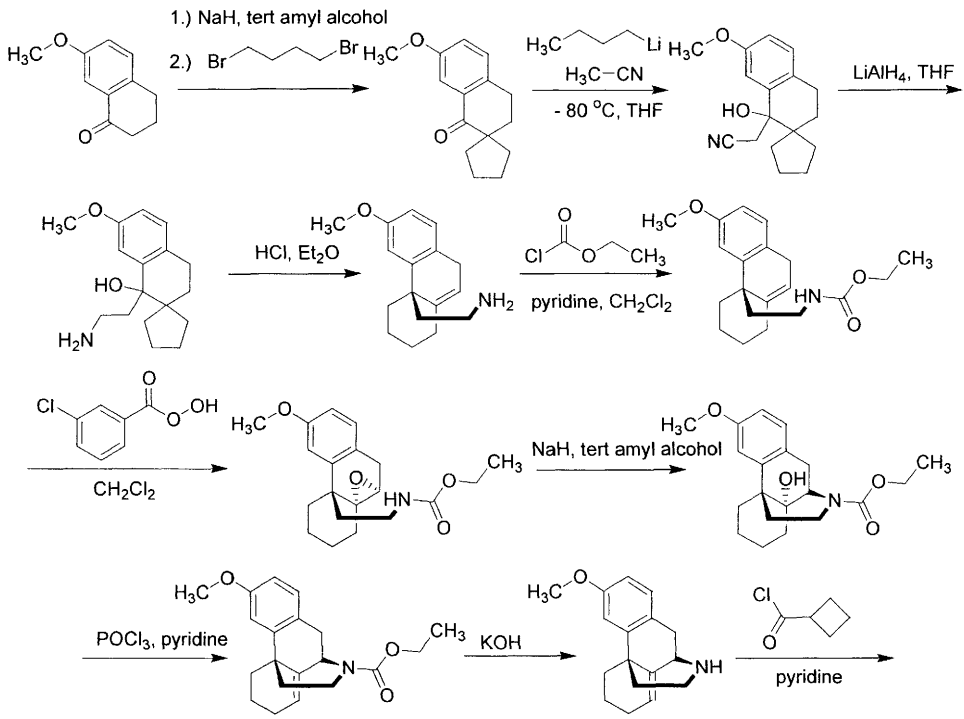
Butorphanol

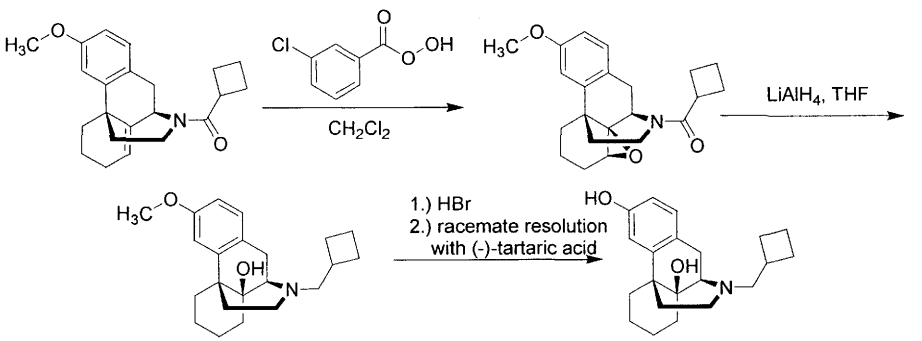


Synthesis (Monkovic and Conway (Bristol-Myers), 1973; Monkovic, 1973; 1987; Kleemann et al., 1999): Condensation of 7-methoxy-3,4-dihydro-1(2H)-naphthalenone with tetramethylene dibromide by means of NaH in benzene or *tert* amyl alcohol gives 3,4-dihydro-7-methoxy-2,2-tetramethylene-1(2H)-naphthalene (bp (0,05 mbar) 120-123 °C), which is treated with acetonitrile and butyllithium in THF yielding 1-hydroxy-7-methoxy-1,2,3,4-tetrahydro-2,2-tetramethylene-1-naphthalene-acetonitrile (mp 140-142 °C). This compound is reduced with $LiAlH_4$ in THF to afford hydro-2,2-tetramethylene-1-naphthol (mp 178-180 °C), and isomerized to 4a-(2-aminoethyl)-1,2,3,4,4a,9-hexahydro-6-methoxy-phenantrene **i** (mp 187 °C).

This amine **i** is cyclized by reaction with bromine in $CHCl_3$ giving 3-methoxy-9a-bromonorhasybanan hydrobromide (mp 207.0-208.5 °C (decomp.)), and isomerized with dehydrobromination by treatment with $NaHCO_3$ in DMF affording 3-methoxy-δ(8,14)-morphinan (mp 180-184 °C). The acetylation of this compound with trifluoroacetic anhydride yields 3-methoxy-N-trifluoroacetyl-δ(8,14)-morphinan (mp 94-96 °C) which is epoxidized with m-chloroperbenzoic acid in methylene chloride giving 8,14-epoxy-3-methoxy-N-trifluoroacetylmorphinan (mp 102-105 °C). The deacetylation of this intermediate with $NaSH_4$ in ethanol gives 8,14-epoxy-3-methoxymorphinan, an oily product that is treated with $LiAlH_4$ in THF to open the epoxide ring and yield 14-hydroxy-3-methoxymorphinan (HCl salt, mp 243-244 °C (decomp.)). The condensation of this derivative with cyclobutylcarbonyl chloride by means of pyridine in CH_2Cl_2 affords N-cyclobutylcarbonyl-14-hydroxy-3-methoxymorphinan (mp 183-185 °C), which is reduced with $LiAlH_4$ in refluxing THF giving N-cyclobutylmethyl-14-hydroxy-3-methoxymorphinan (HCl salt, mp 248- 250 °C (decomp.)). Finally the methoxy ether is demethylated by treatment with refluxing 48% HBr. Resolution of the racemic mixture is achieved by crystallization of the diastereomeric tartaric acid salt.

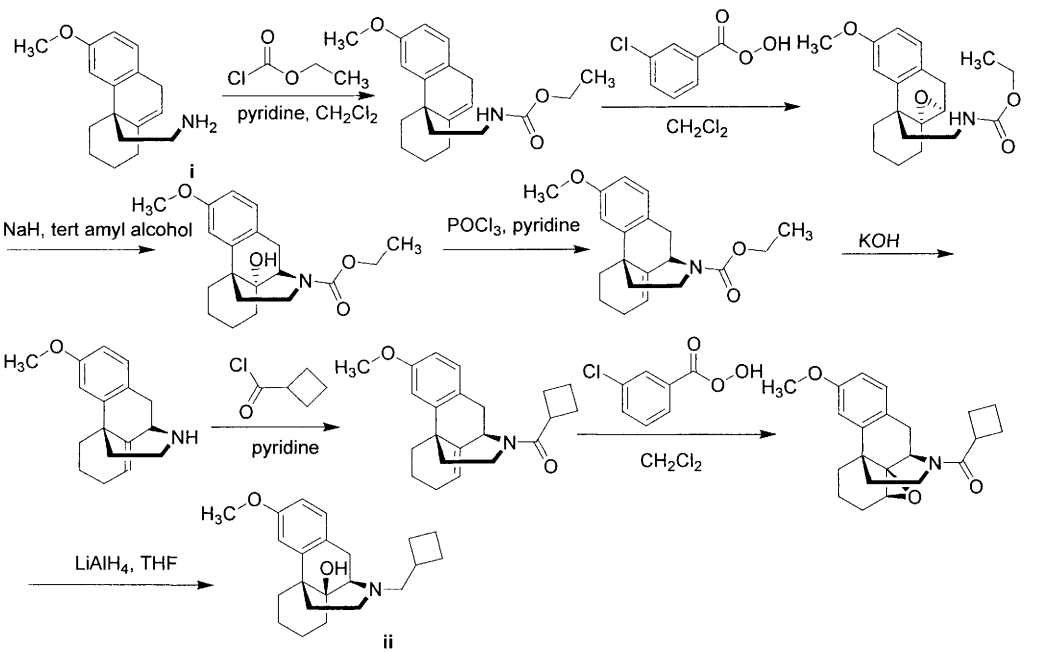
[42408-82-2], 11-Cyclobutylmethyl-1,2,3,4,9,10-hexahydro-4a,10-propanophenanthrene-6,10a-diol, $C_{21}H_{29}NO_2$, M_r 327.22, *mp* 215-217 °C, $[\alpha]_D$ -70° (*c* = 0.1, CH_3OH); tartrate[*58786-99-5*], $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$, M_r 477.55, *mp* 217-219 °C, $[\alpha]_D$ -64.0° (*c* = 0.4, CH_3OH)





Scheme 6: Synthesis of butorphanol.

Alternatively the cyclization of the amine **i** to the methoxy ether derivative **ii** can be performed by the following reaction sequence.



Scheme 7: Synthesis of butorphanol.

Opioid receptor binding: Butorphanol (Rosow, 1988) is a mixed agonist-antagonist opioid with full agonistic activity at the κ-receptor and partial agonistic-antagonistic effect at the μ-receptor. The compound has a high μ- and κ-receptor affinity.

Analgesic efficacy and clinical use: Butorphanol (Heel et al., 1978; Ameer and Salter, 1979) is a fairly potent opioid

analgesic, the analgesic properties are effected by activation of μ- and κ-opioid receptors. It is used for the treatment of moderate to severe pain, for migraine and headache (Homan, 1994) and as an adjunct to anesthesia.

Dosages and routes of administration: Butorphanol is orally inactive but can be given by the nasal route (Homan 1994). The usual administration is via the intramuscular or intravenous route. The intramuscular doses are 1-4 mg every 3-4 h, the intravenous doses are 0.5-2 mg. Nasal doses are ~ 1mg/spray in each nostril.

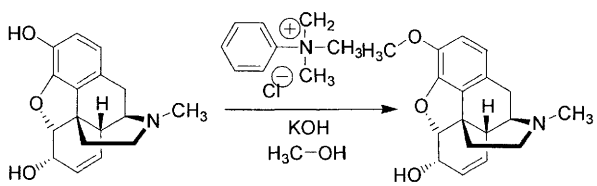
Pharmacokinetic properties: Butorphanol (Vachharajani et al., 1997) is rapidly inactivated by first pass metabolism in the gut. Intramuscular and nasal administration induces a peak effect between 0.5–1hr and a duration of action of about 3 h, corresponding to the plasma half-life time of the compound. Butorphanol has a plasma protein binding of about 80%, metabolic inactivation includes hydroxylation, N-dealkylation and glucuronidation and only about 5% remain unchanged.

Side-effects: Butorphanol (Rosow, 1988) has a side-effect profile combining morphine- and pentazocine-like symptoms. They include drowsiness, weakness, sweating, feelings of floating, and nausea. It has respiratory depressant properties similar to morphine but with a ceiling effect. Naloxone can be used as an antidote. Overt hallucinations or other psychotic effects are rare and less often reported than with pentazocine. The compound has a very low abuse potential and has not been submitted to narcotic control.

Trade name: Stadol (USA)

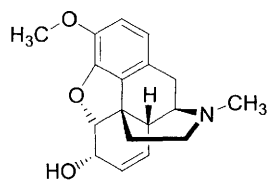
Codeine

Preparation: Codeine is extracted from opium (present in opium from 0.7 to 2.5 % depending on the source), but mostly prepared by methylation of morphine in a phase transfer reaction (Boehringer, 1912; Ehrhart and Ruschig, 1972; Casy and Parfitt, 1986).



Scheme 8: Synthesis of codeine.

Codeine



[76-57-3], $C_{18}H_{21}NO_3$, M_r 299.36, *mp* 154-156 °C (monohydrate from water or diluted alcohol), sublimes when anhydrated at 140-145 °C under 1.5 mm pressure, $[\alpha]_D^{25}$ -136° (*c* = 2, CH_3CH_2OH); monohydrate *[6059-47-8]*, $C_{18}H_{21}NO_3 \cdot H_2O$, M_r 317.38, *mp* 154-156 °C; hydrochloride dihydrate $C_{18}H_{21}NO_3 \cdot HCl \cdot 2H_2O$, M_r 371.86, *mp* 280 °C (decomp.), $[\alpha]_D$ -108°; hydrobromide dihydrate *[125-25-7]*, $C_{18}H_{21}NO_3 \cdot HBr \cdot 2H_2O$, M_r 416.31, *mp* 190-192 °C (anhydrous), $[\alpha]_D$ -96.6°; phosphate *[52-28-8]*

Opioid receptor binding : Codeine has a low affinity at μ-, δ-, and κ-opioid receptors and the *in vivo* effects are predominantly induced by morphine, formed by metabolic O-demethylation.

Analgesic efficacy and clinical use: Codeine (Honig and Murray, 1984) has a morphine-like action profile with analgesic and antitussive properties. As compared to morphine the analgesic potency is 5–10fold lower. The compound is used for the treatment of mild to moderate pain and for cough inhibition (Eccles,1996).

Dosages and routes of administration: Codeine is used orally in single doses of 30 to 60 mg up to a total dose of 240 mg per day for pain relief. Codeine is used in the form of different salts such as hydrochloride, phosphate and sulfate. To increase the duration of action, slow-release preparations have been developed. Codeine is often combined with other analgesics e.g. acetyl salicylic acid or paracetamol. For cough inhibition lower doses are sufficient.

The following scheme shows the codeine consumption in different European countries and the United States.

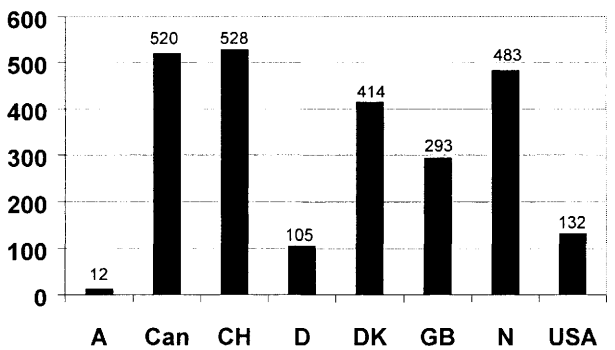
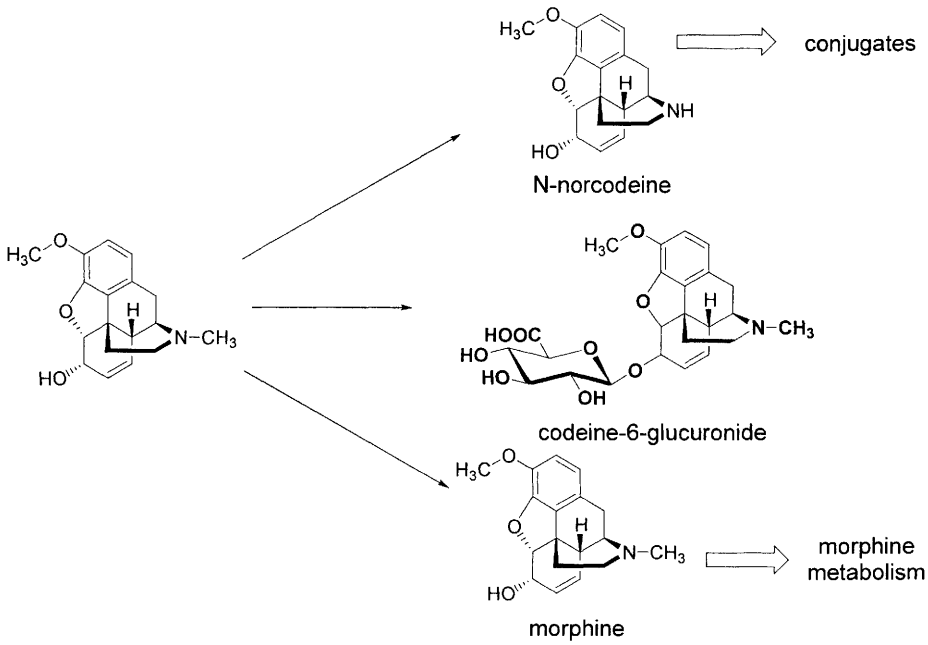


Figure 1: Codeine consumption (1995) in different countries in kg compound /Mio inhabitants (Sohn and Zenz, 1998).

Pharmacokinetic properties: Codeine (Sindrup and Brosen, 1995) has a good oral bioavailability. The compound is extensively metabolized by O- and N-demethylation followed by glucuronidation. The main metabolites are norcodeine, morphine and hydrocodeine and their glucuronides. There are indications (Yue et al., 1997), that the analgesic effect is reduced in persons with low CYP2D6 activity (poor metabolizers).



Scheme 9: Metabolic pathway of codeine.

Side-effects: Codeine has a similar spectrum of side-effects as morphine including nausea, vertigo and somnolence, but with a lower intensity. Most prominent are constipation, excitement and convulsions in the higher dose range. Abuse and dependence are less prevalent as compared to morphine, which can be explained by the fact that the opioid principle is only available after metabolic activation.

Trade name:
Codipront (Gerri);
Codicaps (Ger), Codimal (USA)

Dextromoramide

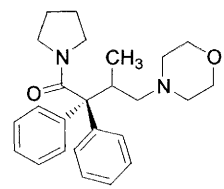
Synthesis (Janssen and Karel (Janssen), 1956; Kleemann et al., 1999).

Opioid receptor binding: Dextromoramide is a μ-selective opioid with a higher receptor affinity than morphine.

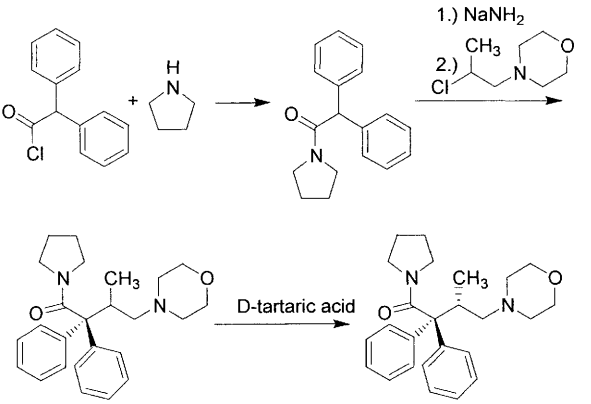
Analgesic efficacy and clinical use: Dextromoramide tartrate (Kay, 1973) is a strong opioid related to methadone and is used in the treatment of severe pain (Judd et al., 1981).

Dosages and routes of administration: Dextromoramide is administered orally and rectally. The parenteral potency is in the range of morphine, but the duration of action is shorter.

Dextromoramide



[*357-56-2*], (+)-(*S*)-3-Methyl-4-morpholin-4-yl-2,2-diphenyl-1-pyrrolidin-1-yl-butan-1-one, $C_{25}H_{32}N_2O_2$, M_r 392.55, *mp* 183-184 °C, $[\alpha]_D^{25}$ +25.5° (*c* = 5, benzene), $[\alpha]_D^{25}$ +16° (*c* = 5, ethanol), D-tartrate [*2922-44-3*], $C_{25}H_{32}N_2O_2.C_4H_6O_6$ M_r 542.64, *mp* 189-192 °C, $[\alpha]_D^{25}$ +25.5° (*c* = 5, water), $[\alpha]_D^{25}$ +30.5° (*c* = 5, CH_3OH)



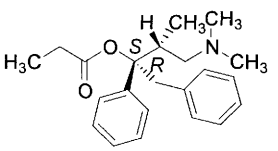
Scheme 10: Synthesis of dextromoramide

Side-effect profile: The compound has a morphine-type abuse and dependence potential.

Trade name: Palfium (Fr, UK, NL)

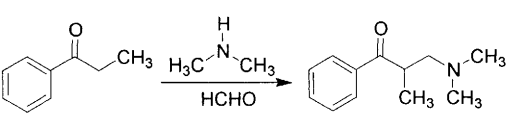
Dextropropoxyphene

Dextropropoxyphene



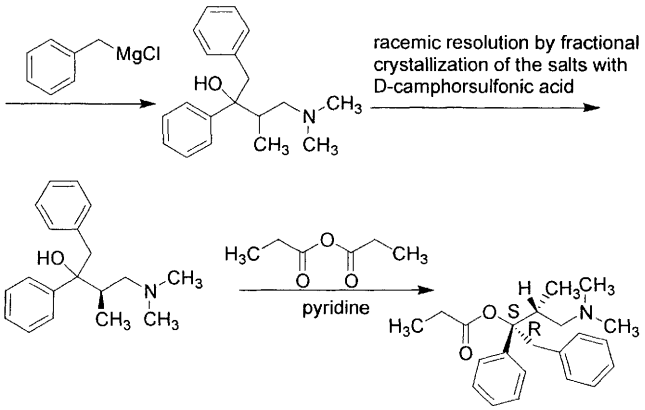
[*469-62-5*], Propionic acid 1-benzyl-3-dimethylamino-2-methyl-1-phenyl-propyl ester, [*S*-(*R**,*S**)]-α-[2-(dimethylamino)-1-methylethyl]-α-phenylbenzeneethanol propanoate (ester), $C_{22}H_{29}NO_2$, M_r 339.47, *mp* 75-76 °C, $[\alpha]_D^{25}$ +67.3° (*c* = 0.6, $CHCl_3$); hydrochloride [1639-60-7], $C_{22}H_{29}NO_2$ HCl, M_r 375.93, *mp* 163-168.5 °C, $[\alpha]_D^{25}$ +59.8° (*c* = 0.6, water)

Synthesis (Pohland, 1953; 1955; 1963; janssen and Karel (Janssen)1956; Sullivan et al., 1963): In the Grignard reaction of 3-dimethylamino-2-methyl-1-phenyl-propan-1-one with benzylmagnesium chloride 4-dimethylamino-3-methyl-1,2-diphenyl-butan-2-ol is formed. The preferred product is the a-diastereomer(75 % α-form, 15 % β-form). The α-form crystallizes and the diastereomeric β-form remains in solution, because of its better solubility. Racemic resolution to obtain the analgetically (+) enantiomer can be achieved on the pure α-Grignard product via fractional crystallization of the salts with *D*-camphorsulfonic acid. Alternatively the resolution can be achieved by treating the racemic mannich product 3-dimethylamino-2-methyl-1-phenyl-propan-1-one with (-)-dibenzoyltartaric acid in acetone as solvent.



Mannich reaction

Opioid receptor binding: Dextropropoxyphene has a lower μ-opioid receptor binding capacity than morphine. Binding at other opioid receptors is even weaker.



Scheme 11: Synthesis of dextropropoxyphene.

Analgesic efficacy and clinical use: Dextropropoxyphene (Grover,1988) is a moderately potent opioid analgesic often combined with paracetamol or acetylsalicylic acid or other NSAIDs (Collins et al., 2000). As the hydrochloride or napsylate it is used orally for the treatment of mild, moderate, or severe pain (Beaver, 1984).

Dosages and routes of administration: Dextropropoxyphene is mostly administered by the oral route. Parenteral injection and rectal administration is painful and induces tissue damage. The ordinary oral doses are 65 mg of the hydrochloride and 100 mg of the napsylate.

The following scheme shows the dextropropoxyphene consumption in different European countries and the United States.

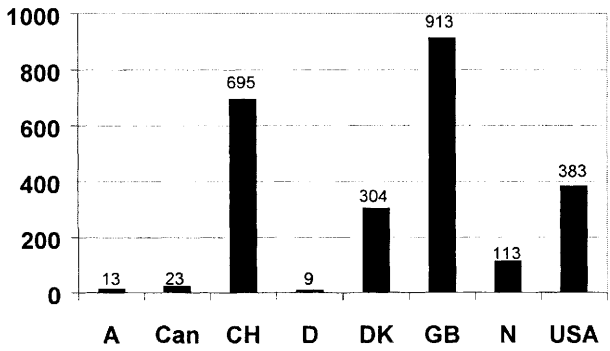


Figure 2: Dextropropoxyphene consumption (1995) in different countries in kg compound /Mio inhabitants (Sohn and Zenz, 1998).

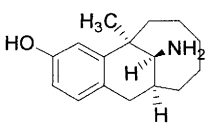
Pharmacokinetic properties: Dextropropoxyphene (Pearson, 1984) has a reasonable oral bioavailability and is readily absorbed from the gastrointestinal tract. Peak plasma concentrations are reached within 1–2 hrs. It is rapidly distributed and about 80% are bound by plasma proteins. The compound is metabolized by N-demethylation to the active metabolite nordextropropoxyphene and other inactive metabolites which are excreted in the urine. Accumulation of the compound and the metabolites may occur with chronic use and the nor-metabolite may contribute to toxicity (Inturrisi et al., 1982).

Trade name: Develin (Ger), Darvon (USA), Doloxene (UK), Antalvic (Fr)

Side-effects: Adverse reactions in the therapeutic range are mild and include drowsiness, dizziness, sedation and nausea. Overdosage can induce serious adverse reactions including profound sedation, respiratory depression, cardiovascular disturbances, convulsions and psychotic reactions, often with fatal outcome (Lawson and Northridge, 1987). Oral dextropropoxyphene has a relatively low abuse liability. Abuse by injection is impeded by severe irritation at the injection side.

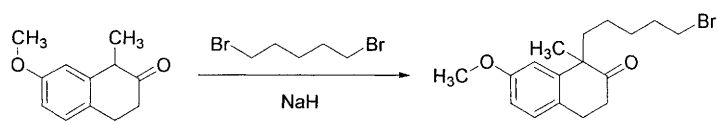
Dezocine

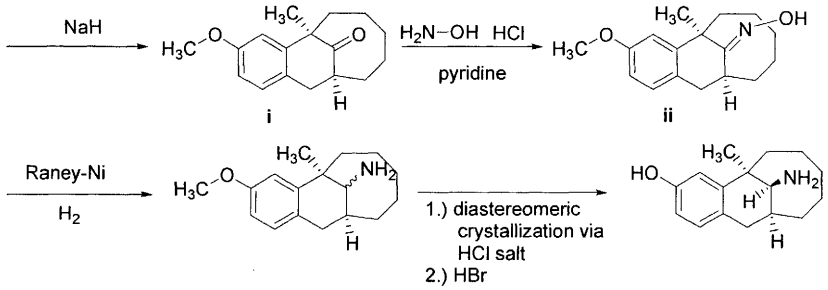
Dezocine



[53648-55-8], 15-Amino-1-methyl-tricyclo[7.5.1.0^{127,255}] pentadeca-2-4-6-trien-4-ol, [5*R*-(5α,11α,13*S**)]-13-amino-5,6,8,9,10,11,12-octahydro-5-methyl-5,11-methanobenzocyclodecen-3-ol, $C_{16}H_{23}NO$, M_r 245.36; hydrobromide *[57236-36-9]*, $C_{16}H_{23}NO \cdot HBr$, M_r 326.27, *mp* 269-270 °C

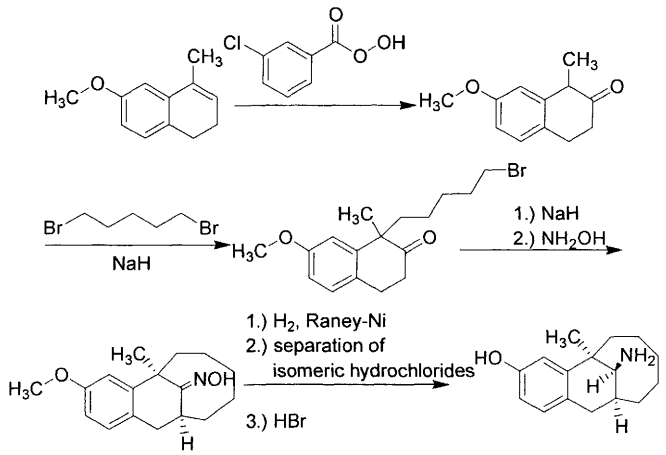
Synthesis (Freed and Potoski (American Home), 1971; Freed, 1973; Kleemann et al., 1999,): Dezocine is prepared through the following sequence: The condensation of 1-methyl-7-methoxy-2-tetralone with 1,5-dibromopentane by means of NaH or potassium tertbutylate affords 1-(5-bromopentyl)-1-methyl-7-methoxy-2-tetralone; this product is cyclized with NaH to give 5-methyl-3-methoxy-5,6,7,8,9,10,11,12-octahydro-5,11-methanobenzocyclodecen-13-one **i**. The ketone **i**, by reaction with hydroxylamine hydrochloride in pyridine, is converted into its oxime **ii**, which is reduced with H_2 over Raney Ni to a mixture of isomeric amines which were separated by crystallization of the HCl salts giving 5-α-methyl-3-methoxy-5,6,7,8,9,11α,12-octahydro-5,11-methanobenzocyclodecen-13β-amine, which is finally cleaved with concentrated HBr.





Scheme 12: Synthesis of dezocine.

Another synthetic pathway to dezocine is shown in the following scheme:



Scheme 13: Synthesis of dezocine.

Opioid receptor binding: Dezocine (Chen et al., 1993) is a mixed agonist-antagonist with binding affinity to the μ-receptor in the range of morphine. The δ- and κ-affinity is 10-100-fold lower (O'Brien and Benfield, 1989).

Analgesic efficacy and clinical use: Dezozine has medium opioid analgesic potency and is used for treatment of moderate to moderately severe pain.

Dosages and routes of administration : The compound is only used parenterally in single dosed of 5-20 mg.

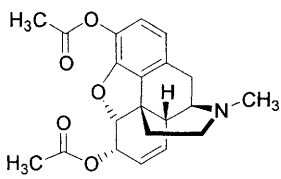
Pharmacokinetic properties: The compound is subject to an intensive first-pass metabolism via glucuronidation of the free phenolic hydroxyl group (Wilson et al.,1995; Strain et al.,1996). This strongly reduces oral bioavailability and induces a short duration of action.

Trade name: Dalgan (USA)

Side-effects: Dezocin induces μ-opioid-type side-effects with nausea, vomiting and drowsiness. Overdoses may be treated with naloxone. The compound has a low abuse potential and is not under narcotic control. Because of its partial antagonistic properties dezocine can precipitate withdrawal in opioid-dependent subjects (Strain et al., 1996).

Diamorphine (Diacetylmorphine, Heroin)

Diamorphine (Heroin)



[*561-27-2*], $C_{21}H_{23}NO_5$, M_r 369.41, *mp* 173 °C, $[\alpha]_D^{25}$ -166° (*c* = 1.49, CH_3OH); hydrochloride monohydrate [*561-27-2*], $C_{21}H_{23}NO_5 \cdot HCl \cdot H_2O$, M_r 423.89, *mp* 243-244 °C, $[\alpha]_D^{25}$ -156° (*c* = 1.044)

Synthesis: morphine is acetylated with acetic anhydride (Ehrhart and Ruschig, 1972).

Opioid receptor binding: Diamorphine (Inturrisi et al.,1983) has a 10-100fold lower μ-opioid receptor binding affinity than morphine. The relevant opioid properties originate from the high μ-receptor affinity of the metabolites 6-acetylmorphine and morphine (Umans and Inturrisi, 1981).

Analgesic efficacy and clinical use: Diamorphine is a strong opioid analgesic used for the treatment of severe pain, especially in terminally ill cancer patients (Sawynok, 1986). In addition it can be used for the treatment of cough associated with terminal lung cancer.

Dosages and routes of administration: Diamorphine is given by the oral as well as by parenteral (i.m., s.c) or intrathecal routes. Diamorphine is about twice as potent as morphine. The parenteral doses are 5-10 mg every 4 h, oral doses are up to two-fold higher.

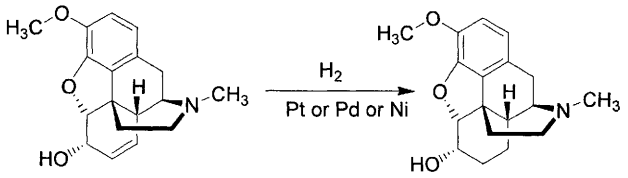
Pharmacokinetic properties: Diamorphine is a lipophilic morphine derivative, which is well absorbed from the intestinal tract and rapidly penetrates into the CNS. It is already metabolized during the transport to the CNS yielding the active metabolites 6-acetylmorphine and morphine. The rapid brain access induces a quick onset of action and seems to be the reason for its high abuse potential (Inturrisi et al., 1984).

Trade name: Diagesil (UK)

Side-effects: Diamorphine has in principle the same side-effect profile as morphine. High doses as used by addicts may cause fatal pulmonary edema (Darke and Zadol, 1996). Because of its high abuse potential therapeutic administration is prohibited in many countries including Germany and the USA, in other countries like the UK it is used for severe pain mostly among terminally ill patients.

Dihydrocodeine

Synthesis (Stein, 1955, Ehrhart and Ruschig, 1972; Kleemann et al., 1999): Hydrogenation of codeine yields Dihydrocodeine (Kleemann et al., 1999).



Scheme 14: Synthesis of dihydrocodeine.

Opioid receptor binding: Dihydrocodeine has a low μ-opioid receptor binding and its opioid properties are mostly due to metabolic activation to dihydromorphine.

Analgesic efficacy: Dihydrocodeine has codeine-like analgesic and antitussive properties and is used for the treatment of moderate to severe pain (Edwards et al., 2000) and as antitussive (Matthys et al., 1985).

Dosages and routes of administration: Dihydrocodeine is mostly used in the form of immediate or sustained release oral formulations (Lloyd et al., 1992). For pain treatment the dose range is 30-80 mg, for cough inhibition doses are in the range of 10 mg.

The following scheme shows the dihydrocodeine consumption in different European countries and the United States.

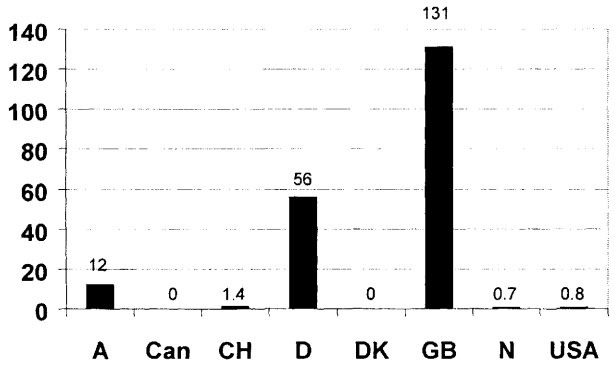
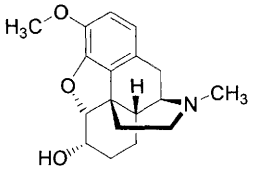


Figure 3: Dihydrocodeine consumption (1995) in different countries in kg compound /Mio inhabitants (Sohn and Zenz, 1998)

Pharmacokinetic properties: Like codeine, dihydrocodeine is metabolized by CYP2D6 yielding the active metabolite dihydromorphine (Ammon et al., 1999). N-Demethylation

Dihydrocodeine



[*125-28-0*], (5α,6α)-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol, $C_{18}H_{23}NO_3$, M_r 301.38, *mp* 112-113 °C; tartrate (1 : 1) [*5965-13-9*], $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$, M_r 451.47, *mp* 192-193 °C (commercial medicinal grade usually melts at 186-190 °C), $[\alpha]_D^{25}$ -72° to -75° (*c* = 1.0, water)

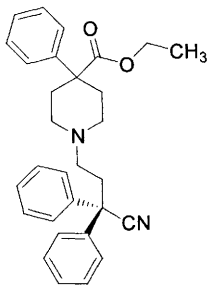
to nordihydrocodeine and nordihydromorphine takes place to a lesser extent.

Trade name: Paracodin (Ger), Dicodin (Fr), DHC Continus (UK), Synalgos (USA)

Side-effects: Dihydrocodeine induces morphine-type side-effects with a lower intensity than morphine. Chronic treatment may produce dependence and abuse has been reported. On the other hand, the compound has been used as substitution therapy for morphine dependence (Banbery et al., 2000)

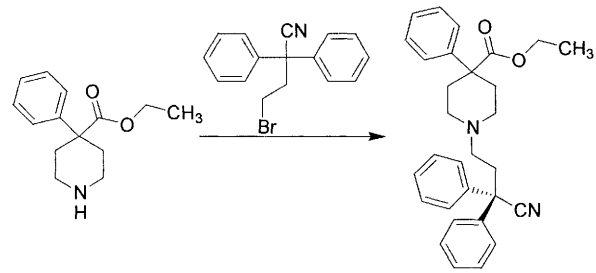
Diphenoxylate

Diphenoxylate



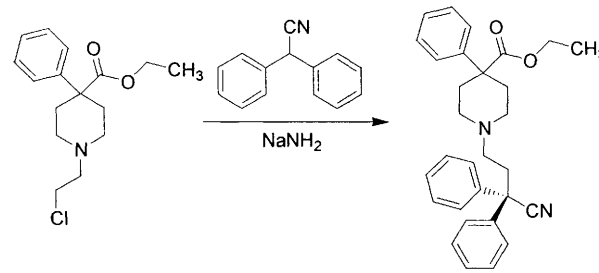
[*915-30-0*], 1-(3-Cyano-3,3-diphenyl-propyl)-4-phenyl-piperidine-4-carboxylic acid ethyl ester, $C_{30}H_{32}N_2O_2$, M_r 452.59; hydrochloride [*3810-80-8*], $C_{30}H_{32}N_2O_2 \cdot HCl$, M_r 489.06, *mp* 220.5-222.0 °C

Synthesis (Janssen, 1959; Dryden and Erickson (Searle), 1978, Kleemann et al., 1999): The reaction of 4-phenyl-piperidine-4-carboxylic acid ethyl ester with 4-bromo-2,2-diphenyl-butyronitrile yields diphenoxylate (Kleemann et al., 1999, p. 250).



Scheme 15: Synthesis of diphenoxylate.

Alternatively the condensation of diphenylacetonitrile with 1-(2-chloro-ethyl)-4-phenyl-piperidine-4-carboxylic acid ethyl ester by means of sodium amide can be carried out.



Scheme 16: Synthesis of diphenoxylate.

Opioid receptor binding: Diphenoxylate and its active metabolite difenoxine (Niemegeers et al., 1972) has a high affinity and selectivity for the μ-type of opioid receptor.

Analgesic efficacy and clinical use: Diphenoxylate is a synthetic pethidine analog with a limited access to the brain and minimal analgesic activity. It has mainly peripheral opioid activity and oral administration induces inhibition of gastrointestinal motility and secretion. The compound is used for the treatment of acute and chronic diarrhea (Shee and Pounder, 1980; Lustman et al., 1987).

Dosages and routes of administration: Diphenoxylate is used orally at initial doses of 10 mg, followed by 5 mg every 5 h. The standard formulation contains 1% atropine to inhibit parenteral misuse.

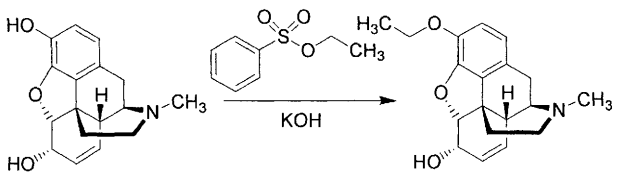
Pharmacokinetic properties: The compound is readily absorbed from the gastrointestinal tract, but rapidly and extensively metabolized in the liver (Karim et al.,1972), which strongly reduces systemic and CNS availability. The main metabolite is the free diphenoxylic acid, which still has anti-diarrheal properties. Other inactive metabolites and their glucuronides are excreted in faeces.

Side-effects: The compound induces mainly peripheral side-effects (Ginsburg, 1973) such as anorexia, nausea and vomiting, and abdominal distension. After higher doses and chronic treatment paralytic ileus and toxic megacolon can occur. Despite restricted access to the CNS, centrally mediated symptoms such as headache, drowsiness, dizziness, euphoria or depression can occur. Diphenoxylate potentiates the effect of CNS depressants and may provoke toxic interactions with MAO inhibitors. Prolonged use of high dosages may induce morphine like addiction and dependence. Illicit use is discouraged by addition of atropine.

Trade name: Reasec (Ger, Ital), Diaserd (Fr), Tropergen (UK), Lomotil (USA)

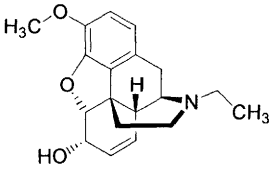
Ethylmorphine

Synthesis: Ethylation of morphine with ethyl benzenesulfonate (E. Merck, 1902; Ehrhart and Ruschig, 1972; Kleemann et al., 1999).



Scheme 17: Synthesis of ethylmorphine

Ethylmorphine



[*76-58-4*], (5α,6α)-7,8-Didehydro-4,5-epoxy-3-ethoxy-17-methylmorphinan-6-ol, $C_{19}H_{23}NO_3$, M_r 313.40, *mp* 199-201 °C; hydrochloride dihydrate [6746-59-4], $C_{19}H_{23}NO_3 \cdot HCl \cdot H_2O$, M_r 385.89, *mp* 123 °C (decomp.), anhydrous form melts at 170 °C (decomp.)

Opioid receptor binding: Ethylmorphine is an ethyl congener of codeine and has a low opioid receptor affinity (Chen et al., 1991). Like codeine, it is metabolized to the active principle morphine.

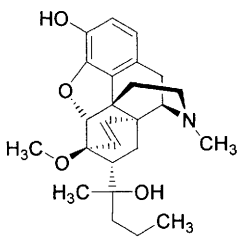
Analgesic efficacy and medical use: Ethylmorphine has an action profile similar to codeine with analgesic, antitussive and antidiarrheal properties. It has been used in similar circumstances to codeine as a cough suppressant and analgesic, but today it is mostly out of use.

Pharmacokinetic properties: Ethylmorphine (Aasmundstad et al., 1995) has a reasonable oral bioavailability. Like codeine, it is metabolized by O- and N-desalkylation, leading to nor-ethylmorphine, morphine, nor-morphine, and the respective glucuronides.

Trade name: Codethyline (B), Trachyl (Fr), Collins Elixir (UK)

Side-effects: Ethylmorphine has a side-effect profile comparable to codeine (Klinger, 1976) and a low to limited abuse and dependence potential (Jonasson et al., 1999).

Etorphine



[*14521-96-1*], [5α,7α(*R*)]-4,5-Epoxy-3-hydroxy-6-methoxy-α,17-dimethyl-α-propyl-6,14-ethenomorphinan-7-methanol, $C_{25}H_{33}NO_4$, M_r 411.53, *mp* 214-217 °C; hydrochloride [*13764-49-3*], $C_{25}H_{33}NO_4 \cdot HCl$, M_r 447.99, *mp* 266-267 °C

Etorphine

Synthesis (Bentley (Reckitt & Colman), 1963; 1966, Bentley (Reckitt & Sons) 1969, Dorner, 1986; Kleemann et al., 1999): Starting from thebaine etorphine can be synthesized in a similiar way to buprenorphine (see buprenorphine) (Boehringer, 1912; Ehrhart and Ruschig, 1972; Trauner et al., 1983; Mulzer and Trauner, 1999).

Opioid receptor binding: Etorphine (Lee et al., 1999) has a high affinity and selectivity for the μ-opioid receptor.

Analgesic efficacy and medical use: Etorphine (Wallach, 1969) is one of the most potent synthetic opioids with a potency 400-1000-fold higher than morphine. In addition to ist analgesic properties etorphine induces potent CNS depression and is mostly used in veterinary practice for anesthesia, immobilization and pain treatment of large animals (Alford et al.,1974).

Routes of administration: Etorphine is only used parenterally.

Pharmacokinetic properties: Etorphine has an immediate onset of action and an intermediate duration of action (1-1.5 h), indicating rapid absorption and metabolization (Friedrich et al., 1991).

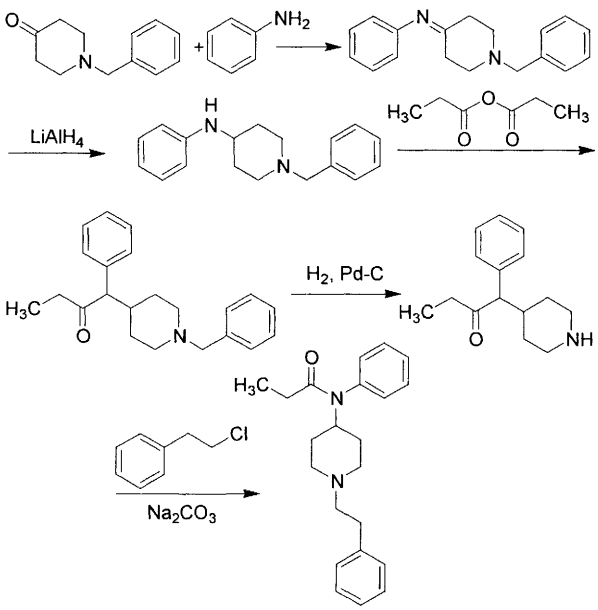
Trade name: Immobilon (UK)

Side-effects: Etorphine induces potent CNS depression with impairment of respiration leading to coma and death in higher doses. Because of its rapid penetration through skin and mucosa and its outstanding potency, special precautions are necessary to avoid contamination during

medical use. As antidote or to terminate anesthesia or immobilization, naloxone or the mixed κ-agonist/μ-antagonist diprenorphine (Alford et al., 1974) can be used. Etorphine has a morphine-type abuse and dependence potential.

Fentanyl

Synthesis (Janssen, P. (Janssen), 1964; 1965; Kleemann et al., 1999):

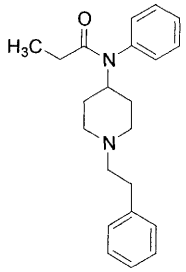


Scheme 18: Synthesis of fentanyl.

Opioid receptor binding: Fentanyl is a μ-selective potent opioid with a similar receptor binding affinity to morphine. The higher *in vivo* potency results from its greater lipophilicity (Subramanian, 2000).

Analgesic efficacy and clinical use: Fentanyl (Clotz and Nahata, 1991) is a potent analgesic and anesthetic compound. It is used for the treatment of severe acute and chronic pain, as a pre-medication or adjunct to anesthesia and as a primary anesthetic for the induction or maintenance of anesthesia. In combinations with neuroleptics e.g. droperidole, it induces a pain free and calm state known as neuroleptanalgesia (Foldes, 1973). In this condition, surgery can be performed in an awake patient, who is able to cooperate with the surgeon.

Fentanyl



[*437-38-7*], *N*-(1-Phenethyl-piperidin-4-yl)-*N*-phenyl-propionamide, $C_{22}H_{28}N_2O$, M_r 336.47, *mp* 83-85 °C; citrate (1 : 1) [*990-73-8*], $C_{22}H_{28}N_2O \cdot C_6H_8O_7$, M_r 528.60, *mp* 149-151 °C

Dosages and routes of administration: For acute (postoperative) pain and for anesthesia, fentanyl is given by the intravenous route. For pre-medication in anesthesia and for break-through pain the compound can also been given as an oral-transmucosal formulation (Ashburn and Streisand, 1994). A transdermal patch has been developed for chronic pain treatment (Jeal and Benfield, 1997; O'Siordin, 1998). The intravenous doses for premedication are 50-100 µg, oral-transmucosal systems contain 200-400 µg and patch formulations have a delivery rate of 25-100 µg/h.

Pharmacokinetic properties: Fentanyl (Scholz et al., 1996) is a highly lipophilic compound and about 80% binds to plasma proteins. After parenteral administration it has a rapid onset and a short duration of action. The compound is rapidly transported into the CNS and lipid tissues. The short duration of action is due to redistribution rather than metabolic inactivation or excretion. It is released from tissue depots with a half-life of about 4 h and the terminal half-life is up to 7 h. The main metabolites, excreted in urine are 4-N-(N-propionylanilino)-piperidine and the N-hydroxypropionyl derivative.

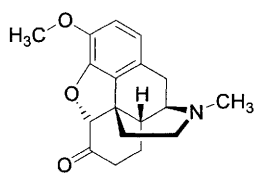
Trade name: Durogensic (Ger, UK), Fentanyl Janssen (Ger), Sublimase (UK; USA), Duragesic (USA), Thalamonal (combination with Droperidol)

Side-effects: The side-effect profile (Poklis, 1995) is typical of potent µ-opioids with respiratory depression, increased muscle tone (chest wall rigidity during fentanyl anesthesia), strong sedation and emesis being most prominent. Adverse reactions can be antagonized with naloxone.

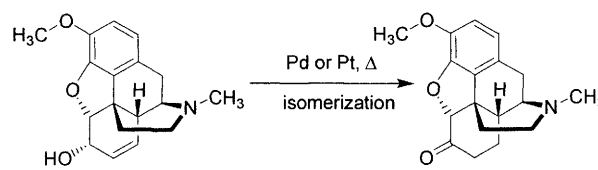
Fentanyl and fentanyl derivatives (so-called designer drugs) have a fundamental abuse potential (Buchanan and Brown, 1988)) and induce morphine-type physical dependence.

Hydrocodone

Hydrocodone



Synthesis (Knoll AG, 1935; Erhart, 1972, Kleemann et al., 1999): Palladium or platin catalyzed isomerization of codeine yields hydrocodone (Kleemann et al., 1999).



Scheme 19: Synthesis of hydrocodone.

Opioid receptor binding: Hydrocodone has a µ-opioid receptor binding that is 10-100 fold higher than codeine. In

contrast to codeine, the compound itself in addition to the active metabolite hydromorphone is responsible for the opioid properties.

Analgesic efficacy and clinical use: Hydrocodone has an action profile similar to codeine, but with higher analgesic and antitussive potency. It is used for the treatment of moderate to moderately severe pain (Palangio et al., 2002) and for cough inhibition (Homsi et al., 2000). Combinations with paracetamol, acetylsalicylic acid or other weak analgesics are common for pain treatment and hydrocodone is added to multi-ingredient cough preparations.

Dosages and routes of administration: Hydrocodone is used orally in doses of 5-10 mg.

Pharmacokinetic properties: Hydrocodone is metabolized by CYP2D6 to the O-desmethyl derivative hydromorphone (Otton et al., 1993). Further steps of metabolization include N-demethylation and glucuronidation.

Side-effects: Hydrocodone induces side-effects similar to codeine and morphine and has an appreciable abuse and dependence potential (Morrison, 1979).

[*76-42-6*], (5α)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-one, $C_{18}H_{21}NO_3$, M_r 299.36, *mp* 198 °C; hydrochloride [*25968-91-6*], $C_{18}H_{21}NO_3 \cdot HCl$, M_r 335.83; hydrochloride monohydrate [*124-90-3*], $C_{18}H_{21}NO_3 \cdot HCl \cdot H_2O$, M_r 353.85, *mp* 185-186 °C (decomp.), $[\alpha]_D^{27}$ -130° (*c* = 2.877); bitartrate hemipentahydrate [*34195-34-1*], $C_{18}H_{21}NO_3 \cdot C_4H_6O_6$ 5/2 H_2O, M_r 988.99, *mp* 118-128 °C

Trade name: Dicodid (Ger, UK), Vicodin (USA)

Hydromorphone

Synthesis: Morphine is hydrogenated over a palladium catalyst, and the resulting dihydromorphine is oxidized with benzophenone and potassium *tert*-butoxide. Alternative oxidants are cyclohexanone with aluminium tri(*tert*-butoxide) or aluminium triphenoxide (Rapoport, 1950; Pfister and Tishler (Merck & Co), 1955; Kleemann et al., 1999).

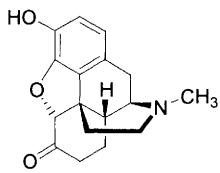
Opioid receptor binding: Hydromorphone has a high affinity and selectivity for the μ-opioid receptor, the μ-affinity is about 10-fold higher than that of morphine.

Analgesic efficacy and clinical use: Hydromorphone is a strong morphine-type analgesic and is used for the treatment of moderate to severe pain and for cough inhibition (Sarhill et al., 2001; Quigley, 2002).

Dosages and routes of administration: Hydromorphone is used in doses of 1-2 mg by subcutaneous, intramuscular, slow intravenous or rectal administration, and in oral doses between 2-4 mg. The doses for cough inhibition are 1 mg, given as a syrup.

Pharmacokinetic properties: Hydromorphone (Hagen et al.,1995) is rapidly but incompletely absorbed from the gastrointestinal tract. It is metabolized in the gut and liver

Hydromorphone



[*466-99-9*], (5α)-4,5-Epoxy-3-hydroxy-17-methylmorphinan-6-one, $C_{17}H_{19}NO_3$, M_r 285.34, *mp* 266-267 °C; monohydrochloride [*71-68-1*], $C_{17}H_{19}NO_3 \cdot HCl$, M_r 321.80

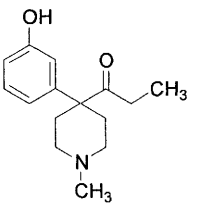
by glucuronidation and N-demethylation, and the conjugates are excreted in urine.

Trade name: Dilaudid (Ger, UK, USA)

Side-effects: Hydromorphone shows the typical morphine-like side-effects, and has a relatively high potential for addiction and dependence (Hill and Zacny, 2000).

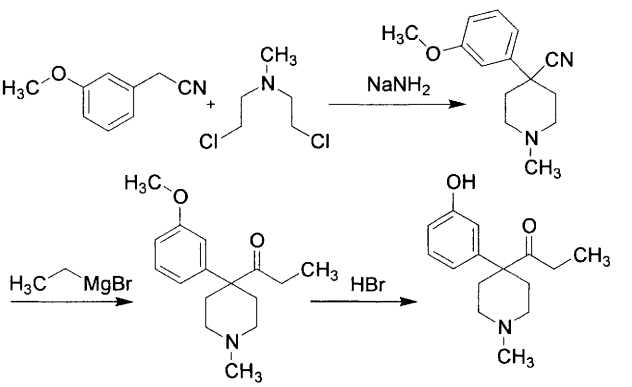
Ketobemidone

Ketobemidone



[*469-79-4*], *N*-(3-Hydroxyphenyl)-*N*-(1-methylpiperidin-4-yl)-propionamide, $C_{15}H_{21}NO_2$, M_r 247.34, *mp* 150-151 °C; hydrochloride [*5965-49-1*], $C_{15}H_{21}NO_2$. HCl, M_r 283.80, *mp* 201-202 °C

Synthesis: 4-Cyano-4-(3-methoxyphenyl)-1-methyl-piperidine is converted into a ketoethyl group by reaction with ethylmagnesium bromide. Subsequent ether cleavage by means of HBr yields ketobemidone (Eisleb (I. G. Farben, 1941; 1942; Kägi, 1949, Kleemann et al.,1999):



Scheme 20: Synthesis of ketobemidone.

Opioid receptor affinity: Ketobemidone is a μ-selective synthetic opioid with a receptor affinity similar to morphine (Christensen, 1993).

Analgesic efficacy and medical use: Ketobemidone is a potent analgesic and is used for the treatment of moderate to severe pain (Ohqvist et al., 1991).

Dosages and routes of administration: Doses of 5-10 mg are given by mouth, intravenous injection or rectally.

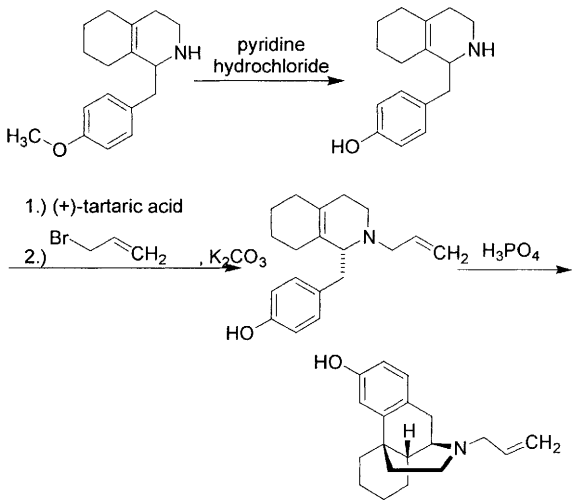
Pharmacokinetic properties: The compound has an acceptable oral bioavailability (Bondesson, 1980). Metabolic inactivation occurs via N-demethylation and glucuronidation at the phenolic hydroxyl.

Trade name: Cliradon (Ger, out of use), Ketogan (Sweden, Norway)

Side-effects: Ketobemidone has morphine-like side-effect and a similar abuse and dependence potential.

Levallorphan

Synthesis: The starting material for the synthesis of levallorphan is 1-(4-Methoxy-benzyl)-1,2,3,4,5,6,7,8-octa-hydro-isoquinoline **6** (*preparation see levorphanol*) (Schnider and Grüssner, 1951; Hellerbach, 1956; Ehrhart and Ruschig, 1972; Patron, 1979; Kleemann et al., 1999):



Scheme 21: Synthesis of levallorphan.

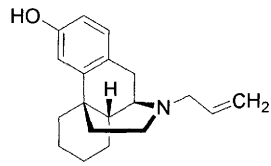
Opioid receptor binding: Levallorphan has a high binding affinity for the μ-opioid receptor.

Analgesic efficacy and clinical use: Levallorphan (Leimgruber et al., 1973) is an opioid antagonist with a minor agonistic component and practically no analgesic action. It has been used as one of the first relative pure antagonists for the treatment of opioid overdose, to reverse opioid central depression and to antagonize opioid-induced respiratory impairment (Foldes et al.,1969). The compound has now been widely replaced by naloxone (Evans et al., 1974).

Pharmacokinetic properties: The compound has low oral bioavailability because of strong first-pass glucuronidation in the gut and liver.

Side-effects: Levallorphan has virtually no opioid agonistic actions and does not induce analgesia (Blane and Boura, 1968).

Levallorphan

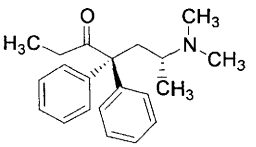


[*152-02-3*], 17-(2-Propenyl)morphinan-3-ol, 1,*N*-allyl-3-hydroxy-morphinan, $C_{19}H_{25}NO$, M_r 283.42, *mp* 180-182 °C, $[\alpha]_D^{25}$ -88.9° (CH_3OH); hydrogen tartrate (1 : 1) [71-82-9], $C_{19}H_{25}NO \cdot C_4H_6O_6$, M_r 433.50, *mp* 176-177 °C, $[\alpha]_D^{25}$ -39° (water)

Trade name: Lorfan

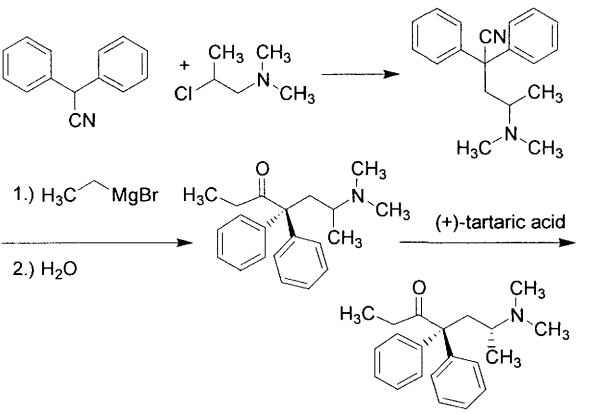
Levomethadone

Levomethadone



[*125-58-6*], (*R*)-6-Dimethylamino-4,4-diphenyl-heptan-3-one, $C_{21}H_{27}NO$, M_r 309.45, *mp* 98-100 °C (*mp* racemic form [*57-42-1*] 78-79 °C), $[\alpha]_D^{20}$ -32° (c = 1.8, CH_3CH_2OH), hydrochloride [*5967-73-7*], $C_{21}H_{27}NO \cdot HCl$, M_r 345.91, *mp* 240-241 °C (*mp* racemic form [*1095-90-5*] 231 °C), $[\alpha]_D^{20}$ -169° (c = 2.0, water)

Synthesis (Bockmühl and Ehrhart (Farbw. Hoechst), 1941; 1948; Schultz et al.,1947; Howe and Sletzinger, 1949; Solmssen and Wenis,1948; Larsen et al.,1948, Howe et al. (Merck & Co), 1953; Zaugg (Abott), 1961):



Scheme 22: Synthesis of levomethadone.

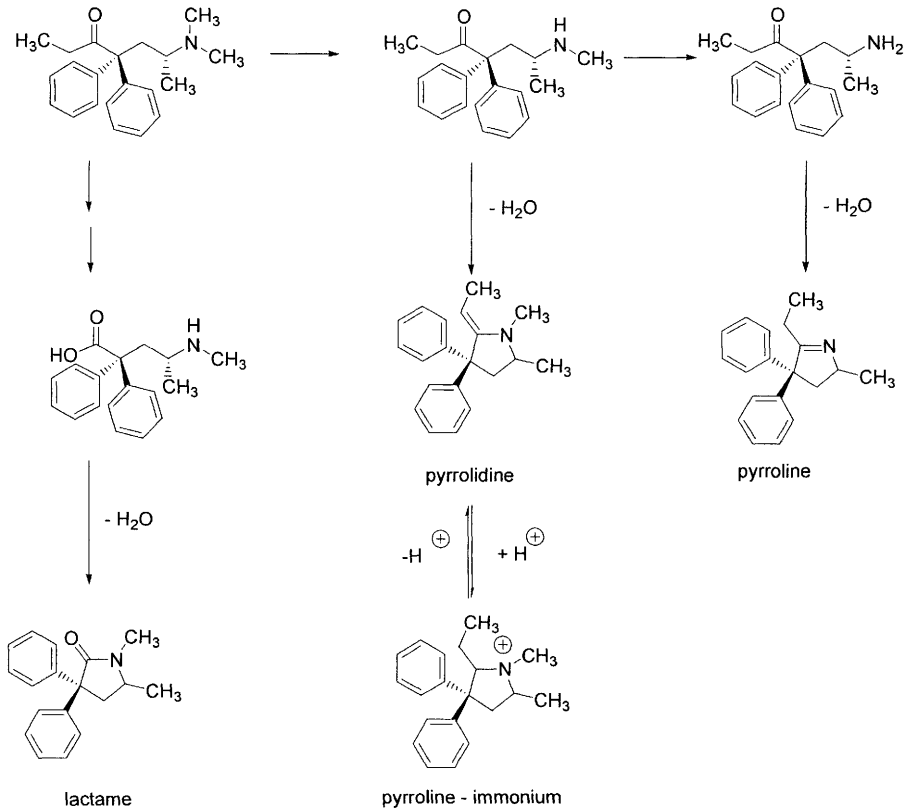
Opioid receptor binding: Levomethadone is the more potent and μ-selective levo-enantiomer of racemic methadone (Sim, 1973). It has an opioid receptor affinity in the range of morphine.

Analgesic efficacy and clinical use: Levomethadone like racemic methadone is a potent and long-acting opioid analgesic and can be used for the treatment of moderate to severe pain (Jamison, 2000; Davis and Walsh, 2001). It has an action profile similar to morphine and has significant antitussive properties, for which it is used in terminal lung cancer. The long duration of action makes the compound suitable for substitution treatment of opioid addiction (Joseph et al., 2000; Pallenbach, 2002). For practical and economic reasons the racemate instead of the levo-enantiomer is used in addicts.

Dosages and routes of administration: Levomethadone can be given by mouth or by intravenous, intramuscular, subcutaneous or intraspinal injection. For pain treatment, intravenous doses are between 2.5 and 5 mg and oral doses between 5 and 10 mg. For maintenance treatment in addicts, much higher oral doses up to more than 100 mg are used.

Pharmacokinetic properties: Levomethadone (Olsen et al., 1977) is readily absorbed from the intestinal tract and has high oral bioavailability. The compound is much more lipophilic than morphine and binds to plasma protein in the

range of 60-90 %. It undergoes considerable tissue distribution and has a long elimination half-life of around 18 h. Accumulation can occur after repeated administration. Levomethadone is metabolized by N-demethylation to the cyclic derivatives 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine and 2-ethyl-3,3-diphenyl-5-methyl-pyrrolidine, which are both inactive as analgesics (Moody et al., 1997). Together with unmetabolized levomethadone they are excreted in faeces and urine. The metabolic pathway of methadone is shown in the following scheme:



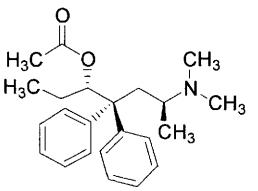
Scheme 23: Metabolic pathway of methadone.

Side-effects: Levomethadone has a morphine-like side-effect profile with stronger respiratory depression and less sedation than morphine (Kreek, 1973). The compound has a morphine-type abuse and dependence potential. Because of its slow elimination withdrawal reactions are more protracted and less severe than with morphine (Lowinson et al., 1978).

Trade name: l-Polamidon (Ger), Dolophine (USA), Physeptone (UK)

Levomethadyl-Acetate (l-α-Acetylmethadol (LAAM))

Levomethadyl-Acetate



[*34433-66-4*], Acetic acid 4-dimethylamino-1-ethyl-2,2-diphenyl-pentyl ester, [*S*-(*R*,R**)]-β-[2-(Dimethyl-amino)propyl]-α-ethyl-β-phenylbenzeneethanol acetate, $C_{23}H_{31}NO_2$, M_r 353.50; hydrochloride $C_{23}H_{31}NO_2$. HCl, M_r 389.96, *mp* 215 °C, $[\alpha]_D^{25}$ -60° (*c* = 0.2, water)

Synthesis: By reduction of dextromethadon and subsequent acylation (Pallenbach 2002, Carroll et al., 1976, Drugs Fut. 1979).

Opioid receptor binding: Levomethadyl acetate has a moderate affinity for opioid receptors with selectivity for the μ-type. Higher binding affinity is induced by the active metabolites *l*-alpha-nor-acetylmethadol, nor-methadol and methadol (Walczak et al., 1981; Carroll, 1976).

Analgesic efficacy and clinical use: Levomethadyl acetate, a methadone derivative, is a long-acting opioid analgesic, which is mostly used for the treatment of opioid dependence (Blaine et al., 1978; Ling, 1978)

Dosages and routes of administration: The compound is used orally. The initial doses range from 20 to 40 mg every 2-3 days and can be increased up to 140 mg. Due to the slow onset of action, supplementation with other shorter acting opioids is necessary during the first 3 days.

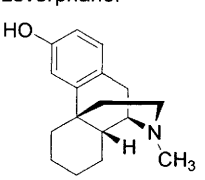
Pharmacokinetic properties: Levomethadyl acetate is well absorbed from the intestinal tract and is extensively metabolized to various active metabolites, which contribute to the long duration of action. The metabolites nor-acytylmethadol, di-nor-acetylmethadol, nor-methadol, and methadol are formed by N-demethylation and hydrolytic cleavage of the ester bond (Moody et al., 1997)

Trade name: Orlaam (USA)

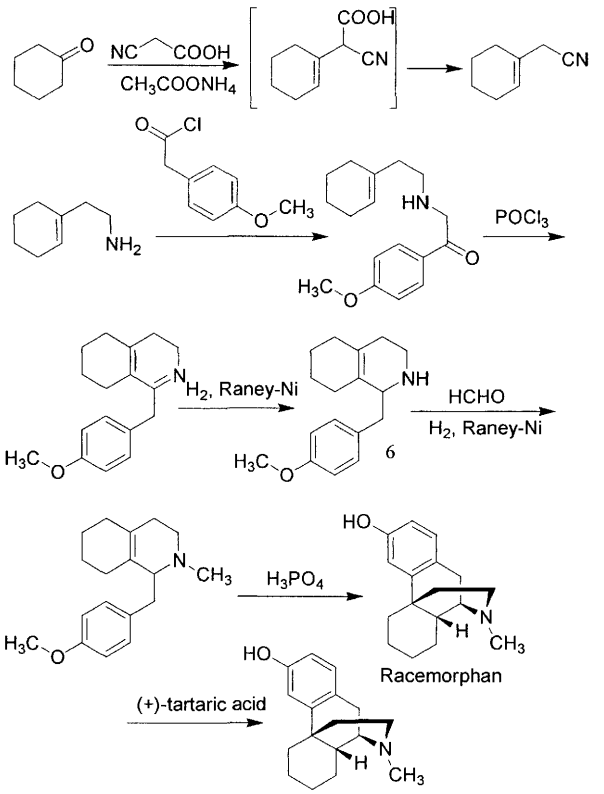
Side-effects: Levomethadyl acetate induces opioid-type side-effects with respiratory depression, bradycardia and impairment of cardiac contractility (Wolven and Archer, 1976). The compound increases the QT-interval and may induce Torsade de pointes (Deamer et al., 2001). Because of the cardiovascular side effects (Q/T interval prolongation) Levomethadyl acetate was recently withdrawn from the market in most of the european countries.

Levorphanol

Levorphanol



Synthesis: The analgesic activity of racemorphan is due to the (-) isomer, levorphanol, which is obtained by resolving the racemate with (+)-*D*-tartaric acid. Resolution can also be carried out on the intermediate 1-(4-Methoxy-benzyl)-1,2,3,4,5,6,7,8-octahydro-isoquinoline **6** prior to *N*-methylation (Grewe 1946, Schnider and Hellerbach, 1950, Schnider and Grüssner, 1951, Ehrhart and Ruschig 1972, Kleemann et al. 1999).



Scheme 24: Synthesis of levorphanol.

Opioid receptor binding: Levorphanol is a μ-selective synthetic opioid with a higher receptor affinity than morphine (Childers et al., 1979).

Analgesic efficacy and clinical use: Levorphanol is a potent and long-acting analgesic used for the treatment of moderate to severe pain (Dixon et al., 1983).

Dosages and routes of administration: The compound is used as the tartrate in doses of 2-4 mg by mouth or 2-3 mg by subcutaneous or slow intravenous injection. In addition, levorphanol can be used as ananesthetic supplement.

Pharmacokinetic properties: After oral administration levorphanol has a relatively slow onset, but a long (up to 8 h) duration of action. Metabolic inactivation occurs via glucuronidation of the phenolic hydroxyl and via N-demethylation (Dixon et al., 1983).

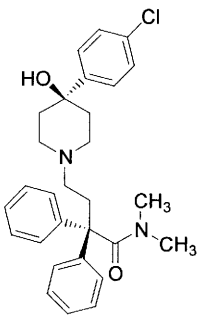
[*77-07-6*], 17-Methylmorphinan-3-ol, (-)-3-hydroxy-*N*-methyl-morphinan, $C_{17}H_{23}NO$, M_r 257.37, *mp* 198-199 °C, $[\alpha]_D^{20}$ -56° (c = 3, CH_3CH_2OH); tartrate dihydrate [*5985-38-6*], $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2\ H_2O$, M_r 257.37, *mp* 113-115 °C (anhydrous *mp* 206-208 °C), $[\alpha]_D^{20}$ -14° (c = 3,water)

Trade name: Levo-Dromoran (USA)

Side-effects: Levorphanol has morphine-type side-effects with significant respiratory depression in the high dose range. It induces morphine type addiction and dependence (Coniam, 1991).

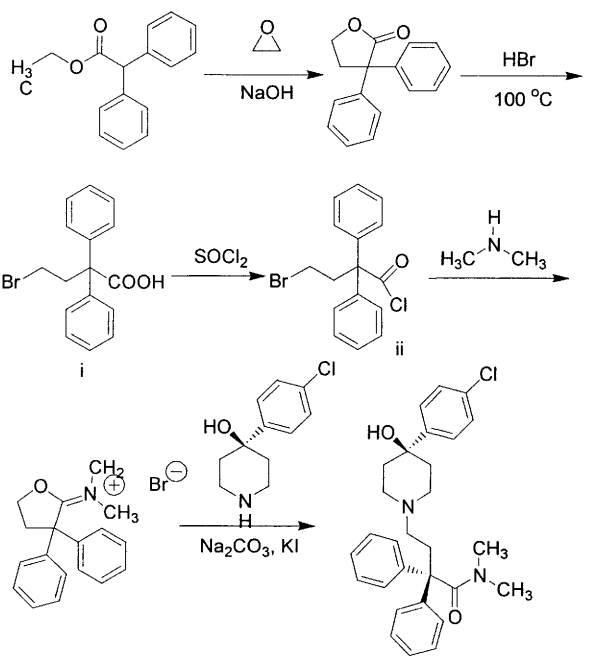
Loperamide

Loperamide



[*53179-11-6*], 4-[4-(4-Chloro-phenyl)-4-hydroxy-piperidin-1-yl]-*N,N*-dimethyl-2,2-diphenyl-butyramide, 4-(4-chlorophenyl)-4-hydroxy-*N,N*-diemethyl-α,α-diphenyl-1-piperidinebutanamide, $C_{29}H_{33}ClN_2O_2$, M_r 477.04; monohydrochloride [*34552-83-5*], $C_{29}H_{33}ClN_2O_2 \cdot HCl$, M_r 513.51, *mp* 222-223 °C (decomp.)

Synthesis (Kleemann et al. 1999, Janssen (Janssen), 1973; Janssen et al. (Janssen), 1973, Stokbroekx et al., 1973, Niemegeers et al., 1974):): Treatment of 2-oxo-3,3-diphenyl-tetrahydrofuran, synthesized by treatment of diphenyl-acetic acid ethyl ester with ethylene oxide, with HBr(gas) yields bromo derivative **i**, which is then converted into butyryl chloride derivative **ii** by means of thionyl chloride in refluxing chloroform. Reaction of derivative **ii** with dimethylamine in toluene affords dimethyl (tetrahydro-3,3-diphenyl-2-furylidene)ammonium bromide, which is then condensed with 4-(4-chlorophenyl)-4-piperidinol by means of Na_2CO_3 and KI in refluxing 4-methyl-2-pentanone to provide loperamide.



Scheme 25: Synthesis of loperamide.

Opioid receptor binding: Loperamide has a morphine-like affinity and selectivity for the μ-opioid receptor (Dashwood et al., 1990).

Analgesic efficacy and clinical use: Systemic loperamide does not reach the CNS and the compound has no analgesic action. Oral loperamide acts locally in the gut to inhibit intestinal motility and secretion (Awouters et al., 1993). In addition to the strong μ-opioid action, calcium and calmodulin antagonism are involved in the anti-diarrheal activity. The compound is used for the treatment of acute and chronic diarrhea and for the management of colostomies and ileostomies (Heel et al., 1978b, Wheeler 2000).

Dosages and routes of administration: Loperamide is used in various oral formulations with single doses of 4-8 mg and daily doses up to 16 mg.

Pharmacokinetic properties: About 40% of the drug is absorbed from the intestinal tract but nearly completely inactivated by first pass metabolism in the liver (Killinger et al., 1979). The main metabolites are N-desmethyl-loperamide and the di-desmethyl derivative (Yoshida et al., 1979). The elimination half life is about 10 h.

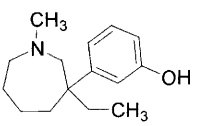
Side-effects: Adverse effects include nausea, dry mouth and dizziness. High doses can induce toxic megacolon and paralytic ileus. The compound has no abuse or dependence potential (Ericsson and Johnson, 1990).

Trade name: Imodium (Ger, Belg., USA, F), Arret (UK)

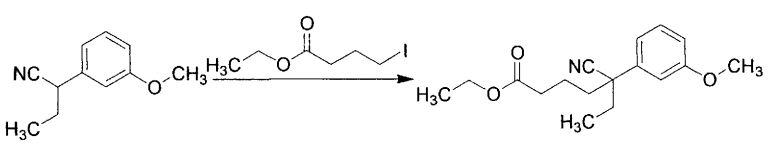
Meptazinol

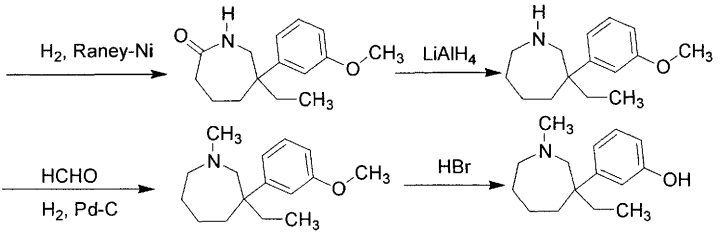
Synthesis (Cavalla and White (Wyeth), 1969; 1969; Bradley 1980; Kleemann et al. 1999): By condensation of 2-(m-methoxyphenyl)butyronitrile with ethyl 4-iodobutyrate by means of $NaNH_2$ in liquid NH_3 to give ethyl 5-cyano-5-(m-methoxyphenyl)heptanoate, which is cyclized by hydrogenation with H_2 over Raney Ni in cyclohexane to yield 6-ethyl-6-(m-methoxyphenyl)hexahydro-2H-azepin-2-one; this ketone is reduced with $LiAlH_4$ in THF to 3-ethyl-3-(m-methoxyphenyl)hexahydro-1H-azepine, which in turn, is reductively methylated with HCHO, H_2 and Pd/C in ethanol to give 1-methyl-3-ethyl-3-(m-methoxyphenyl)-hexahydro-1H-azepine, and finally demethylated by refluxing with 80% HBr to yield a racemic mixture of the final product.

Meptazinol

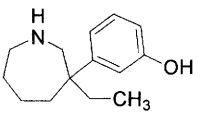


[54340-58-8], 3-(3-Ethyl-1-methyl-azepan-3-yl)-phenol, 3-(3-Ethylhexahydro-1-methyl-1*H*-azepin-3-yl-phenol, $C_{15}H_{23}NO$, M_r 233.35, *mp* 127.5-133 °C; hydrochloride *[59263-76-2]*, $C_{15}H_{23}NO \cdot HCl$, M_r 269.81





Scheme 26: Synthesis of meptazinol.



i

The enantiomers are obtained by diastereomeric crystallization of the demethylated methoxyazepine derivative **i** with (*D*)-(+)-tartaric acid followed by methylation with HCHO, H_2 and Pd/C in ethanol.

Opioid receptor binding: Meptazinol (Holmes and Ward, 1985) is a partial μ-agonist with a high μ-receptor affinity; the selective action at a special μ1-subtype is controversial (Pasternak et al., 1985).

Analgesic efficacy and clinical use: Meptazinol is a medium potent opioid analgesic with an additional cholinergic component. It is used for the treatment of moderate to moderately severe pain. The compound has a shorter duration of action than morphine (Holmes and Ward, 1985; Kay, 1985).

Dosages and routes of administration: Meptazinol is used in parenteral doses of 50-100 mg, given every 2-4 hrs by intramuscular or slow intravenous injection. For short-term treatment of moderate pain, the compound can be given by oral administration in doses of 200 mg every 3-6 h.

Pharmacokinetic properties: Meptazinol has poor oral bio-availability due to extensive first-pass metabolism. Systemic availability is improved after rectal administration. Peak plasma concentrations are reached 30 min after rectal or intramuscular administration and plasma half-life is about 2 h. Plasma protein binding is low (~ 30%). Meptazinole is extensively metabolized in the gut and liver mainly to the glucuronide derivative. Only about 10% is excreted unmetabolised in the faeces (Franklin, 1988).

Side-Effects: The most common side-effects are nausea, vomiting and dizziness. Other vegetative side-effects include sweating, hypotension and drowsiness. The compound is reported to be relatively free of respiratory depressant activity, which was attributed to selective binding to a subtype (μ-1) of the opioid receptor

(Pasternak et al., 1985) , an alternative explanation may be the cholinergic action component (Holmes and Ward, 1985) In accordance with a low κ-receptor affinity, the incidence of psychotomimetic actions and hallucinations is low.

Meptazinole may precipitate withdrawal in persons under long-term opioid treatment. The compound has a low abuse potential (Johnson and Jasinski, 1987) and is not under narcotic control.

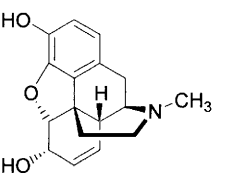
Trade name: Meptid (Ger, UK)

Morphine

Preparation: By extraction of poppy capsules or opium (opium contains 9-14 % morphine depending on the source) with water, precipitation with aqueous Na_2CO_3 solution, washing of the precipitate with ethanol and dissolution in dilute acetic acid (Hörner et al. (Knoll), 1977, Trauner et al.,1983, Hudlicky et al.,1996).

The total synthesis of (-)-morphine has been a challenging target for organic chemists for many decades (Casy and Parfitt, 1986; Frackenpohl, 2000). Although a number of successful synthesis have been developed to date (Hudlicky et al., 1996, Casy and Parfitt, 1986, Novak et al., 2000, Bentley 2000) since the first accomplishment by Gates in 1952 (Gates 1952), only a few could produce the alkaloid in an enantio- and diastereocontrolled manner (e.g.: Meuzelaar et al.,1999; Mulzer and Trauner,1999, Davies et al., 2001; Nagata et al., 2001)

Morphine



[*57-27-2*], (5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol, $C_{17}H_{19}NO_3$, M_r 285.34, *mp* 254 °C (decomp., 197 °C is reported for a metastable phase), $[\alpha]_D^{25}$ -132° (*c* = 1, CH_3OH); hydrochloride [*52-26-6*], $C_{17}H_{19}NO_3 \cdot HCl$, M_r 321.80, *mp* 200°C (trihydrate), $[\alpha]_D^{25}$ -113.5° (*c* = 2.2, water); sulfate (2 : 1) [*64-31-3*], $C_{17}H_{19}NO_3 \cdot 1/2\ H_2SO_4$, M_r 668.76; sulfate pentahydrate [6211-15-0], $C_{17}H_{19}NO_3 \cdot 1/2\ H_2SO_4 \cdot 5H_2O$, M_r 758.85, *mp* 250 °C (decomp.), $[\alpha]_D^{25}$ -108.7° (*c* = 4, water)

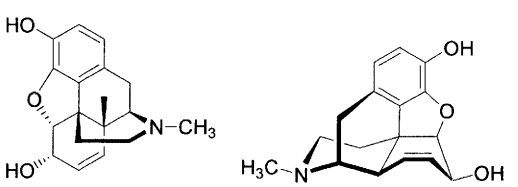
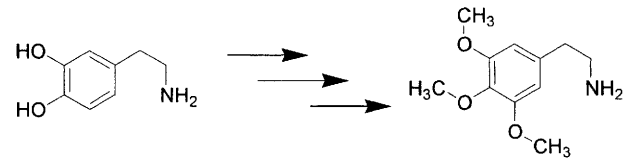


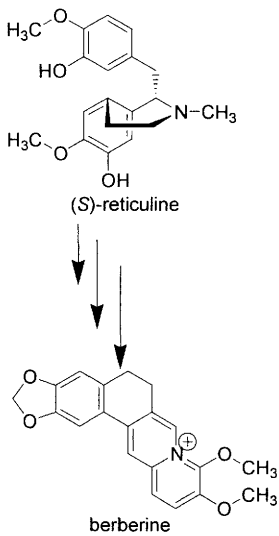
Figure 4: Morphine in the conventional presentation (left) and in a stereoformula according to IUPAC rules (right).

Morphine and codeine biosynthesis (Samuelsson, 1999; Herbert et al., 2000; Novak et al., 2000): *S*tudies on the biosynthesis of morphine have been carried out mainly on cell cultures mainly of *Coptis japonica* and species of *Thalictrum*. Two enzymes (*tyrosine decarboxylase* and *phenolase*) catalyze the formation of dopamine from one molecule tyrosine. Dopamine is also the key intermediate in the biosynthesis of mescaline.



dopamine mescaline

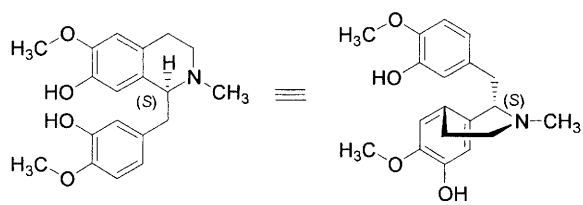
Scheme 27: Biosynthesis of morphine: conversion of dopamine to mescaline.



Scheme 28: Conversion of (S)-reticuline to berberine.

(L)-tyrosine transaminase catalyses the reaction between a second molecule of tyrosine and α-ketoglutaric acid yielding 4-hydroxyphenylpyruvic acid which is decarboxylated by *4-hydroxyphenylpyruvate carboxylase* resulting in the formation of 4-hydroxyphenylacetaldehyde. *(S)-narcoclaurine synthase* catalyzes a stereoselective condensation of dopamine and 4-hydroxy-phenyllacetaldehyde to (S)-norcolaurine. The enzyme has an apparent molecular weight of around 15,000. Methylation of the 6-OH of norcolaurine is catalyzed by *(R,S)-norcolaurine-6-O-methyl-transferase* (6-OMT) yielding (*S*)-coclaurine. N-methylation of coclaurine is mediated by *(S)-coclaurine-N-methyltransferase* resulting in the formation of (*S*)-N-methylcoclaurine which is oxidized by a phenolase to yield (*S*)-3´-hydroxy-N-methylcoclaurine. Methylation of the 4´-OH in this compound, catalyzed by *(S)-3´-hydroxy-N-methyl-(S)-coclaurine-4´-O-methyltransferase* (4-OMT) yields (*S*)-reticuline. This alkaloid is a key compound in the biosynthesis of other benzylisoquinoline alkaloids such as berberine.

In order to understand the continuation of the biosynthesis of codeine and morphine from reticuline, the structure for (*S*)-reticuline can be written as follows:

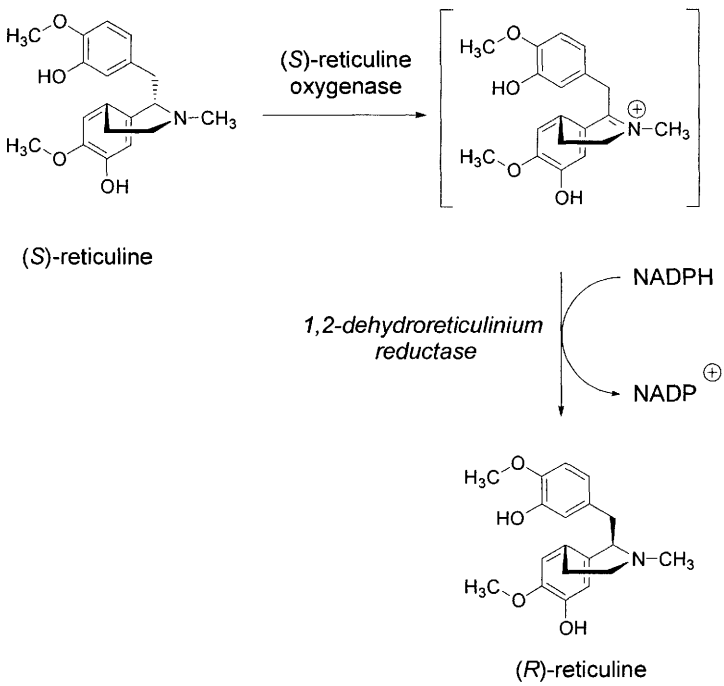


(*S*)-reticuline

Figure 5: Two different formulas showing the stereochemistry of (*S*)-reticuline

Reticuline occurs in the two enantiomeric forms: (*S*)-(+)- and (*R*)-(-)reticuline. Curiously this compound has the

opposite configuration to that found in the morphine family of alkaloids. The two isomers are interconvertable via the 1,2-dehydroreticulinium ion, an intermediate which has been shown to be naturally occuring in *Papaver somniferum*. An enzyme, *1,2-dehydroreticline reductase* (EC 1.5.1.27) has been isolated from seedlings of *Papaver somniferum*. This enzyme stereospecifically reduces 1,2-dehydroreticuline to (*R*)-reticuline. The isolated enzyme is cytosolic, NADPH dependent and constitutes a single polypeptide with a molecular weight of 30,000. It is highly substrate-specific and has been found only in morphinan alkaloid-containing plants. Unlike many other oxireductases, the NADPH-dependent enzyme does not catalyze the physiologically reverse reaction *in vitro*. The 1,2-dihydroreticulinium ion is produced by oxidation of the (*S*)-enantiomer catalyzed by (S)-reticuline-oxidase.



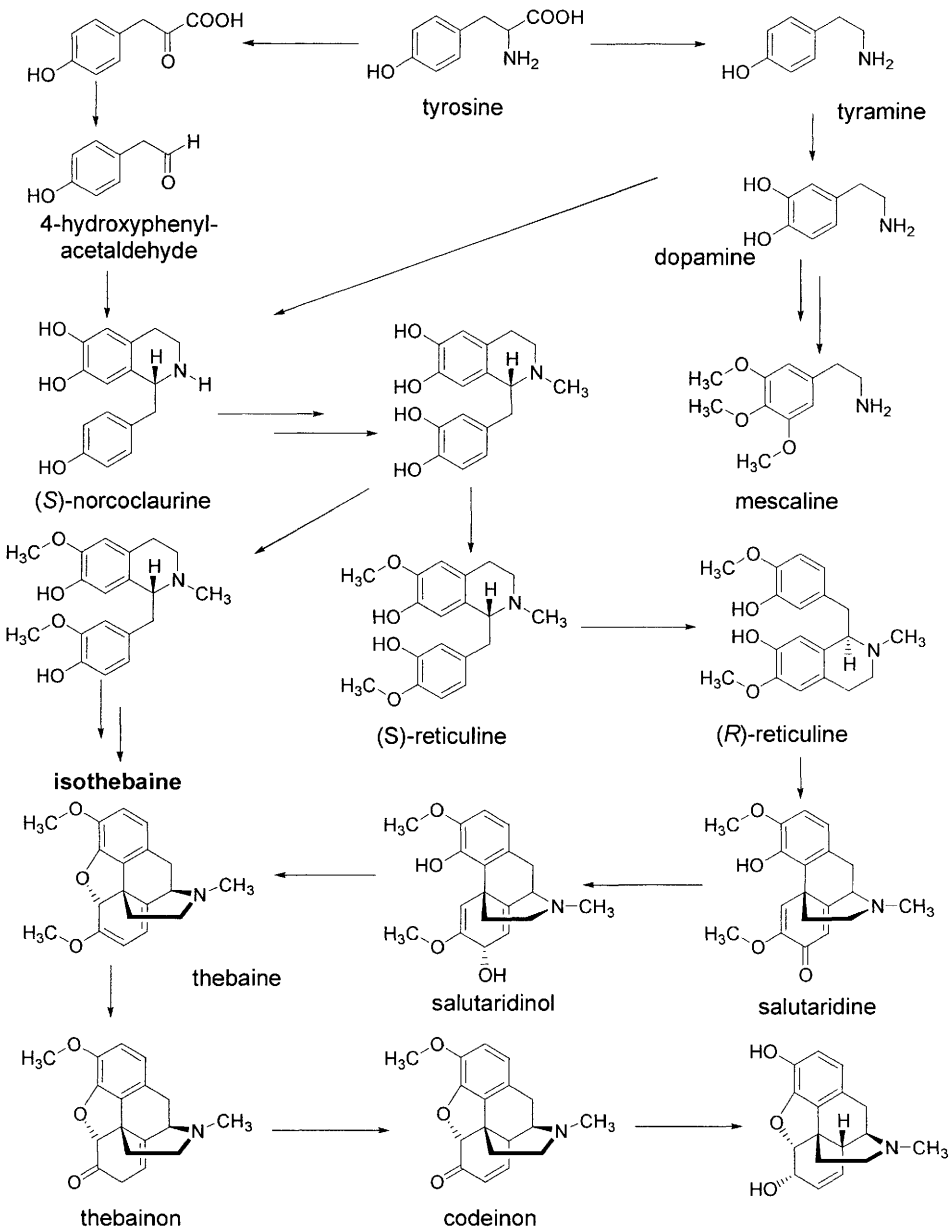
Scheme 29: The stereochemical inversion of (*S*)-(+)- to (*R*)-(-) reticuline via the enzymes *(S)-reticulinium oxygenase* and *1,2-dehydroreticuline reductase.* The 1,2-dehydro-reticulinium ion is proposed as an intermediate.

In the next step (*R*)-reticuline is first transformed to the dienone salutaridine by regioselective *para-ortho* oxidative coupling, catalyzed by a stereo- and regioselective

cytochrome P-450-linked microsomal enzyme, which has not yet been isolated. Salutaridine is reduced to salutaridinol by *salutaridine-NADPH 7-oxireductase.* This reaction proceeds at pH 6.0-6.5. The enzyme can also catalyze the reverse reaction - salutaridinol to salutaridine - but the pH optimum for that reaction is much higher, 9.0-9.5. The enzyme is a single polypeptide of molecular mass 52 kDa which is absolutely dependent on NADPH/NADP as pyridine nucleotide cofactors. It was originally isolated from cell cultures of *Papaverum somniferum* but has also been shown to be present in capsules and seedlings of the plant. This enzyme has not been found in any other Papaver species except the thebaine-producing *P. bracteatum.* It is thus highly specific for plants producing the morphinandione skeleton.

The oxide bridge in codeine and morphine is closed in a reaction catalyzed by the enzyme *acetyl-coenzyme A salutaridinol-7O-acetyltransferase* which transfers an acetyl group from acetyl-CoA to the hydroxy group at C-7 of salutaridinol. The product taridinol-7-(O)-acetate undergoes a spontanenous allylic elimination yielding the alkaloid thebaine. The spontaneous elimination reaction has been demonstrated to occur in vitro at pH 8-9 and no enzyme catalyzing this reaction has been found. It is therefore assumed that the elimination reaction also occurs spontaneously *in vivo. Acetyl-coenzyme A salutaridinol-7O-acetyltransferase* was isolated from a cell culture of *Papaver somniferum* and has a molecular mass of 50 kDa. The enzyme is highly substrate specific. The epimer of salutaridinol 7-epi-salutaridinol is not acetylated by this enzyme, thus confirming the previous finding that this compound is not a precursor for thebaine. Thebaine is demethylated to yield neopinone which spontaneously rearranges to codeinone. Codeinone is reduced to codeine by a highly substrate specific and stereoselective enzyme known as *codeine/NADP oxireductase.* Finally, demethylation of codeine yields morphine. The two demethylation reactions are unusual in a biosynthetic pathway. It has been suggested that the methyl groups serve as protecting groups during the biosynthesis, thereby avoiding other possible reactions in which the hydroxy groups in question could participate.

The overall pathway from tyrosine to morphine is illustrated in the following scheme.



Scheme 30: The overall biosynthetic pathway from tyrosine to morphine

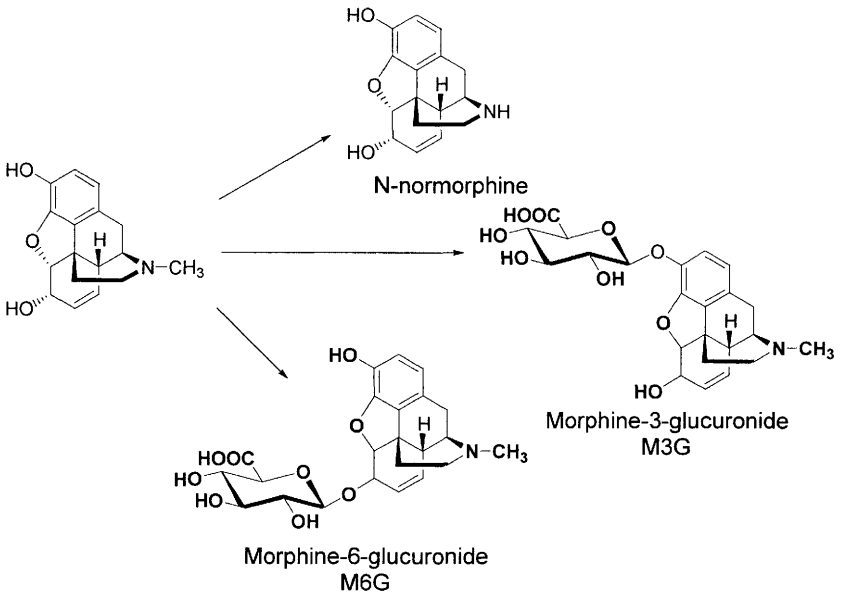
Opioid receptor binding: Morphine has a high (nanomolar) binding affinity for the opioid receptor. The affinity for the δ- and κ- receptor is at least 10-fold lower.

Analgesic efficacy and clinical use: Morphine (Benyhe, 1994) is a very potent analgesic and is used for the treatment of moderate to severe acute and chronic pain of various origins. It is more active in nociceptive and in inflammatory as compared to neuropathic pain and is regarded as the gold standard for pain treatment (Coluzzi,1998).

Dosages and routes of administration: Morphine is available in different salt forms but the hydrochloride and sulfate (Vermeire and Remon, 1999) are used preferentially. The compound can be administered by the oral, parenteral or intraspinal route. Oral application is preferred for chronic pain treatment and various slow release forms have been developed to reduce the administration frequency to 2-3 times per day (Bourke et al., 2000). Parenteral morphine is used in intravenous or intramuscular doses of 10 mg, mostly for postoperative pain and self-administration devices are available for patient-controlled analgesia (PCA). Morphine is additionally used for intraspinal (epidural or intrathecal) administration. Morphine is absorbed reasonably well in the lower gastrointestinal tract and can be given as suppositories.

Pharmacokinetic properties: Morphine is extensively metabolized by glucuronidation at both hydroxyl groups at position 3 and 6 and by N-demethylation (Osborne et al.,1990; Christrup, 1997). The 3-glucuronide has minimal opioid receptor binding affinity and is devoid of analgesic action, whereas the morphine-6-glucuronide (Schwarzinger et al., 2001) has a binding affinity similar to morphine, is analgesically active and seems to be involved in pain inhibition during chronic oral treatment (Lotsch and Geisslinger, 2001). Despite the polar sugar residue the glucuronide can cross the blood-brain barrier. The N-demethyl derivative normorphine is analgesically active, but has a lower μ-receptor affinity than morphine. The main metabolites of morphine are shown in Scheme 31.

Side-effects: Morphine induces a variety of centrally- and peripherally-mediated side-effects. The most important of which is respiratory depression following parenteral administration, especially in the postoperative situation. Chronic oral application induces constipation and chronic treatment with oral morphine must be supplemented with laxatives. Other frequent side-effects are nausea, vomiting, dizziness and sedation.



Scheme 31: Metabolic pathway of morphine.

Morphine is a controlled substance since it has a high euphorigenic potential and is liable to abuse and dependence. The euphorigenic effect is less expressed in the context of pain treatment and tolerance and dependence can largely be avoided by appropriate dosing and administration intervals, ensuring constant and pain appropriate plasma levels of the compound.

The following scheme shows the morphine consumption in different European countries and the United States.

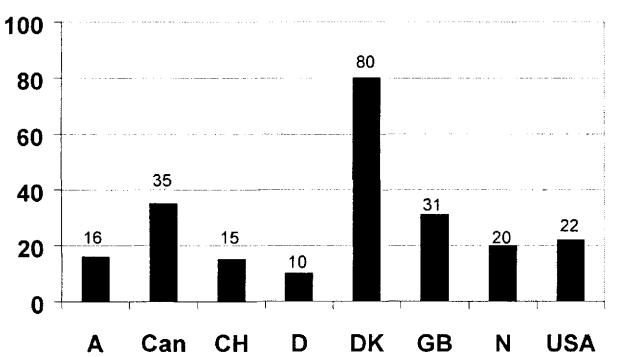
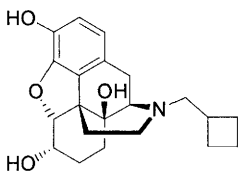


Figure 6: Morphine consumption (1995) in different countries in kg compound /Mio inhabitants (Sohn and Zenz, 1998)

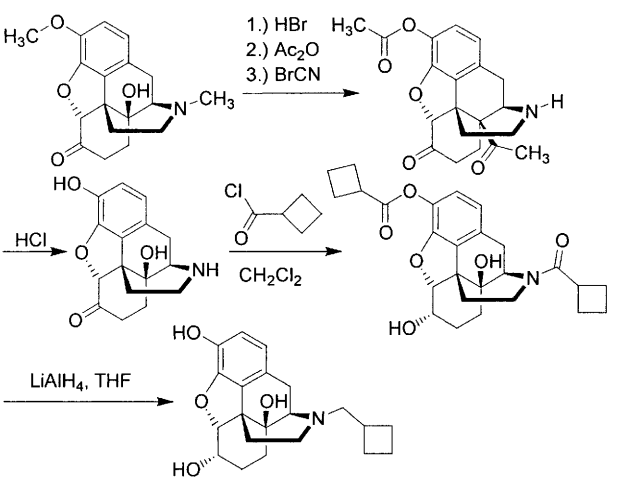
Nalbuphine

Nalbuphine



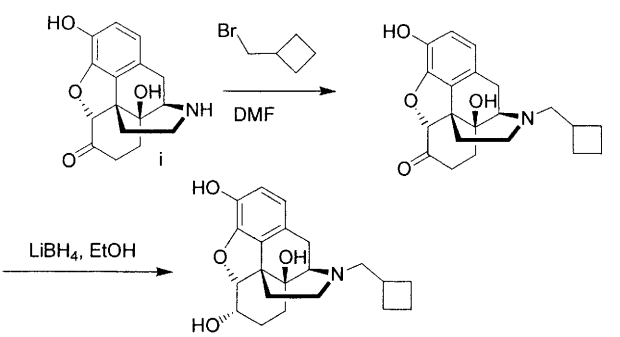
[*20594-83-6*], (5a,6a)-17-(Cyclobutylmethyl)-4,5-epoxymorphinan-3,6,14-triol, $C_{21}H_{27}NO_4$, M_r 357.44, *mp* 230.5 °C; hydrochloride [*23277-43-2*], $C_{21}H_{27}NO_4 \cdot HCl$, M_r 393.91, *mp* 291-292 °C (decomp.)

Synthesis: The starting material for the nalbuphine synthesis is oxycodone (*see oxycodone*). After ether cleavage to oxymorphone the product is acylated and the N-methyl group is removed by treatment with cyanogen bromide. The acetyl groups are hydrolyzed with dilute hydrochloric acid. The resulting 14-hydroxydihydro-normorphinon **i** can be *N*-alkylated with cyclobutylmethyl bromide, and the carbonyl group at C-6 is reduced (Blumberg et al. (Endo Laboratories), 1967; 1970; Castaner and Roberts, 1977, Kleemann et al. 1999).



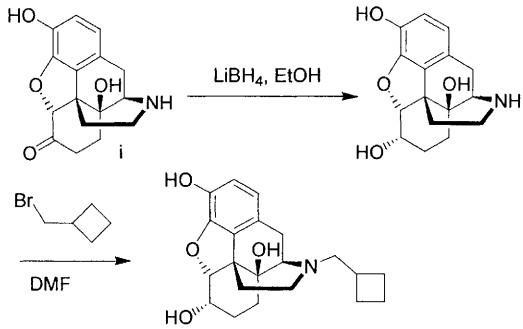
Scheme 32: Synthesis of nalbuphine.

Alternatively the condensation of **i** with cyclobutylmethyl bromide in DMF gives the N-cyclobutylmethyl derivative, which is then reduced with $NaBH_4$ in ethanol.



Scheme 33: Synthesis of nalbuphine.

The reduction of **i** with $NaBH_4$ also gives 14-hydroxy-dihydronormophine, which is then alkylated with cyclobutylmethyl bromide in DMF.



Scheme 34: Synthesis of nalbuphine.

Opioid receptor binding: Nalbuphine is a mixed agonist-antagonist opioid with a high affinity for the μ- and κ-opioid receptor. At the κ-receptor the compound is an agonist, at the μ-receptor a partial agonist with a very low intrisic activity, thus acting more as a μ-antagonist (De Souza et al., 1988).

Analgesic efficacy and clinical use: Nalbuphine (Errick and Heel, 1983; Schmidt et al., 1985) is a medium potent opioid analgesic used for the treatment of moderate to severe pain and inter-operatively as adjunct to anesthesia. Because of its antagonistic action component, analgesia may be subject to a ceiling level.

Dosages and routes of administration: Nalbuphine is used only parenterally by the subcutaneous, intramuscular or intravenous route. Single doses are 10-20 mg and for the treatment of myocardial infarction 30 mg.

Pharmacokinetic properties: Due to intensive first-pass metabolism, nalbuphine has a low oral bioavailability of less than 10%. After intramuscular administration peak plasma concentrations are reached after 30 min, half-life time is about 5 h. The compound is metabolized by glucuronidation and to a minor extent by N-dealkylation, and less than 10% is excreted unmetabolized (Lo et al., 1987).

Side-effects: The most frequent side-effect is drowsiness, others are nausea, vomiting, sweating, dizziness, vertigo, dry mouth and headache. Hallucinations and psychotomimetic reactions are less frequent than with pentazocine, reflecting a relative weak kappa component of the compound. Nalbuphine-induced respiratory depression is

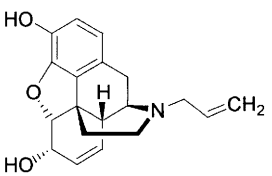
less severe than with morphine and is limited by a ceiling level (Pugh et al., 1989).

Trade name: Nubain (Ger, F, UK, USA)

Nalbuphine has a low abuse potential and is not subject to narcotic control.

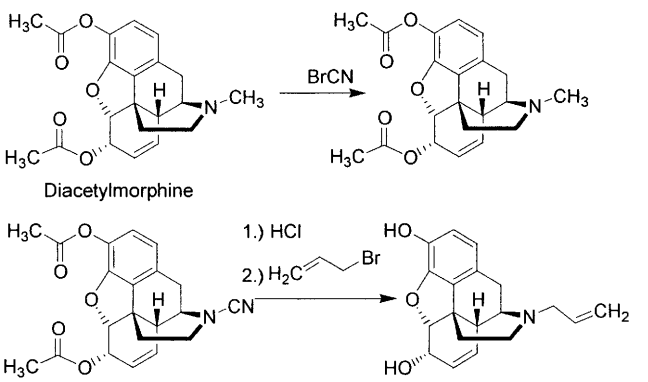
Nalorphine

Nalorphine



[62-67-9], (5a,6a)-7,8-Didehydro-4,5-epoxy-17-(2-propenyl)morphinan-3,6-diol, $C_{19}H_{21}NO_3$, M_r 311.38, *mp* 208-209 °C, $[\alpha]_D^{25}$ -155.3° (*c* = 3, CH_3OH); hydrochloride [*57-29-4*], $C_{19}H_{21}NO_3 \cdot HCl$, M_r 347.84, *mp* 260-263 °C; hydrobromide [*1041-90-3*], $C_{19}H_{21}NO_3 \cdot HBr$, M_r 392.29, *mp* 258-259 °C (decomp.)

Synthesis: Diacetylmorphine (Heroin) is demethylated with cyanogene bromide and hydrolyzed to normorphine, which is alkylated to allyl bromide (, Weijlard and Erickson (Merck & Co.), 1944; Weijlard (Merck & Co), 1959; Kleemann et al. 1999).



Scheme 35: Synthesis of nalorphine.

Opioid receptor binding: Nalorphine is a mixed agonist-antagonist with high affinity and low intrinsic action at the μ- and κ-opioid receptors.

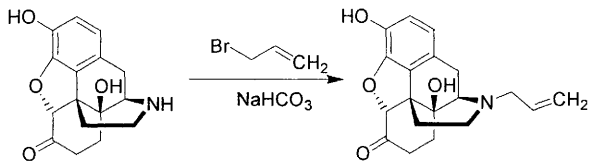
Analgesic efficacy and clinical use: Nalorphine has substantial analgesic activity and was the first opioid in which a combination of analgesia with antagonistic properties was detected (Eckenhoff et al., 1951; Takemori et al., 1969).

Trade name: Lethidrone (Ger, UK), Nalline (USA), Nalorphine Serb (F)

Side-effects: The compound has prominent κ-type adverse reactions like anxiety, hallucinations and dysphoric mood alterations and is no longer used clinically (Lattin, 1976).

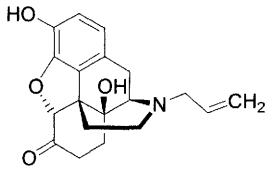
Naloxone

Synthesis: 14-Hydroxydihydronormorphinon (*see nalbuphine*) is *N*-alkylated with allyl bromide (Lewenstein and Fishman, 1962, Olofson et al., 1977, Kleemann et al. 1999, Moser et al., 1990):



Scheme 36: Synthesis of naloxone.

Naloxone



Opioid receptor binding: Naloxone (Simantow and Snyder, 1977) is a pure opioid antagonist with a high affinity and a limited selectivity for the μ-receptor.

Efficacy and clinical use: Naloxone (Handal et al., 1983; Goodrich, 1990) has no analgesic activity. This compound is the standard antidote for treating opioid adverse reactions, opioid overdoses or to terminate a therapeutic use of an opioid compound. Typical indications are inhibition of opioid-induced respiratory depression, termination of opioid anesthesia or protection of neonates following opioid treatment during labor. Naloxone has a short duration of action and repeated administration may be necessary to antagonize longer-acting compounds. To avoid parenteral misuse of non-scheduled oral opioid formulations (tilidine, pentazocine) a small amount of naloxone is added which is inactivated by the oral route, but is fully active after parenteral administration.

Dosages and routes of administration: Naloxone is only used parenterally, mostly as an intravenous bolus or by infusion. The compound is used in single or repetitive doses of 0.4-2 mg up to a total dose of 10 mg.

Pharmacokinetic properties: Oral naloxone is extensively metabolized in the gut and liver, predominantly by glucuronidation of the phenol function (Berkowitz, 1976). The parenteral half-life is about 1 h.

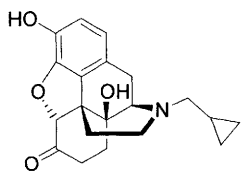
Side-effects: Naloxone induces mainly mild and non-specific side-effects. Higher doses may induce nausea and vomiting and in rare cases a reduction of the seizure threshold.

Trade name: Narcanti (Ger, Austral.), Narcan (F, UK, USA)

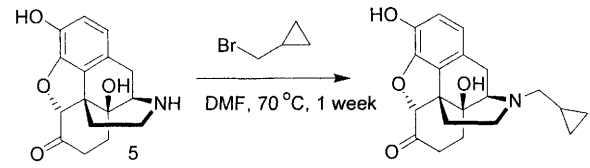
Naltrexone

Synthesis: (a) Its preparation is the same as that for naloxone starting from oxycodone via the intermediate **i** except that the nitrogen atom is alkylated with cyclopropyl bromide (Blumberg et al. (Endo Laboratories), 1967; 1973; Drugs Fut. 1977; 2000; Kleemann et al. 1999):

Naltrexone

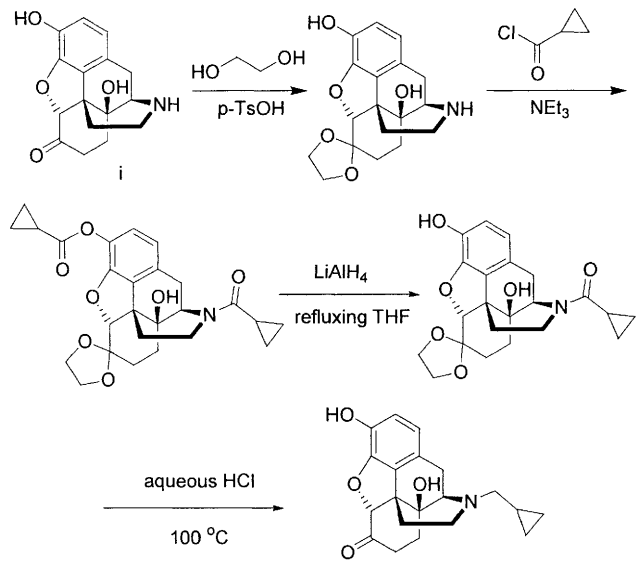


[*16590-41-3*], (5α)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-morphinan-6-one, $C_{20}H_{23}NO_4$, M_r 341.40, *mp* 168-170 °C; hydrochloride [*16676-29-2*], $C_{20}H_{23}NO_4 \cdot HCl$, M_r 377.87, *mp* 274-276 °C



Scheme 37: Synthesis of naltrexone

(b) *Alternative route*: Product **i** is ketalized with ethylene glycol by means of p-toluenesulfonic acid giving the cyclic ketal (mp 311-313 °C); this in turn, is treated with cyclopropyl carbonyl chloride in a mixture of methylene chloride and triethylamine yielding the N,O-dicyclopropyl-carbonyl derivative (mp 219-220 °C). The N,O-dicyclopropylcarbonyl derivative is reduced with $LiAlH_4$, in refluxing THF yielding the ethylene ketal of naltrexone (mp 221-222 °C) which is finally hydrolyzed with aqueous HCl at 100 °C.



Scheme 38: Synthesis of naltrexone.

Opioid receptor binding: Naltrexone has a high affinity and selectivity for the μ-opioid receptor (Höllt and Herz, 1978).

Efficacy and clinical use: Naltrexone (Crabtree, 1984; Gonzalez and Brogden, 1988) is a pure opioid antagonist and has no analgesic activity. It is used for the treatment of opioid adverse effects, for opioid detoxification and as maintenance treatment for former addicts to avoid a relapse. In chronic opioid users, naltrexone may precipitate an acute withdrawal reaction.

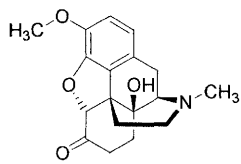
Dosages and routes of administration: Naltrexone (O'Brien et al., 1978) is given orally and parenterally. It is given intravenously as an acute opioid antidote and as a challenge when dependency is suspected. Oral application us used for long-term treatment of former addicts. Naltrexone challenge is started with 200 µg and gradually increased to 1.6 mg, which is repeated until acute abstinence symptoms are no longer seen. Long-term treatment starts with 25 mg and is increased to 50 mg daily. The weekly doses of 350 mg can be given as 100 mg every second day or 150 mg given every third day.

Pharmacokinetic properties: Naltrexone (Misra, 1981) is absorbed from the gastrointestinal tract, but is subject to considerable first-pass metabolism in the liver, yielding the active metabolite 6-beta-naltrexole. Naltrexone has low plasma binding of about 20%. The half-life of naltrexone is ~3 h and of 6-beta-naltrexole is ~13 h.

Side-effects: Naltrexone itself has only weak and uncharacteristic side-effects, stronger effects, as seen in opioid users, are mostly the result of an acute withdrawal reaction.

Trade name: Nemexin (Ger), Nalorex (F, UK), Revia (USA)

Oxycodone



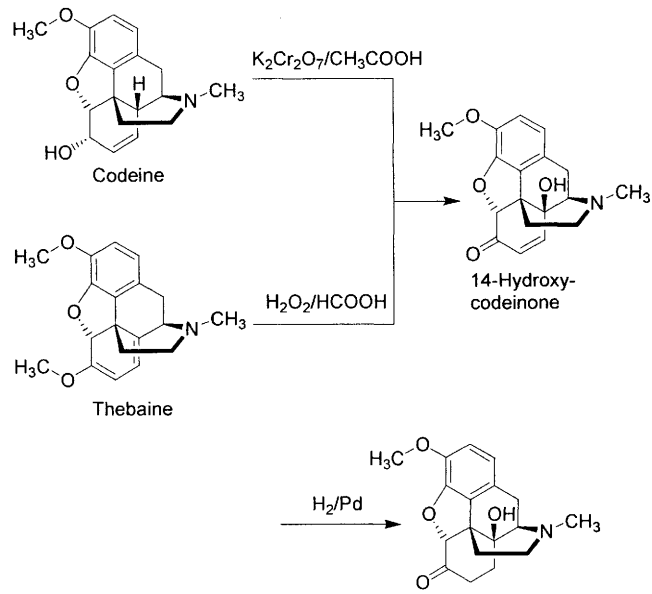
Synthesis (Krauß (E. Merck), 1925, Juby et al., 1968, Ehrhart and Ruschig 1972): Thebaine is oxidized with hydrogene peroxide to 14-hydroxycodeinone (Bentley 1954, Hauser 1974), which is hydrogenated directly or via its oxime, or its bromination products to oxycodone. The reduction of 14-hydroxycodeinone can also be carried out with sodium hydrosulfite. Alternatively 14-hydroxy-codeinone is prepared by oxidation of codeine.

Opioid receptor binding: Oxycodone (Chen et al., 1991) is a µ-selective opioid with a 10-fold higher receptor affinity than codeine. Both the parent compound and the high affinity metabolite oxymorphone mediate the opioid effects of the compound (Cleary et al., 1994).

Analgesic efficacy and clinical use: Oxycodone is a potent opioid and is used for the treatment of moderate to severe pain (Poyhia et al,. 1993).

Dosages and routes of administration: Oxycodone is given by mouth in single doses of 5-10 mg or as controlled release preparations with doses of 40 mg (Cairns, 2001). Rectal administration is also possible. Oral formulations often contain combinations with paracetamol or acetylsalicylic acid.

[*76-42-6*], (5α)-4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one, 14-hydroxydihydrocodeinone, dihydrone, dihydrohydroxycodeinone, $C_{18}H_{21}NO_4$, M_r 315.36, *mp* 218-220 °C; hydrochloride [*124-90-3*], $C_{18}H_{21}NO_4 \cdot HCl$, M_r 351.81, *mp* 270-272 °C (decomp.), $[\alpha]_D^{20}$ -125° (*c* = 2.5, water)



Scheme 39: Synthesis of oxycodon.

Pharmacokinetic properties: Oxycodone (Kaiko et al., 1996) is well absorbed from the gastrointestinal tract. It is metabolized to nor-oxycodone and to a lesser extent to the active metabolite oxymorphone (Poyhia et al., 1993). Metabolites and unchanged parent drug are excreted in the urine.

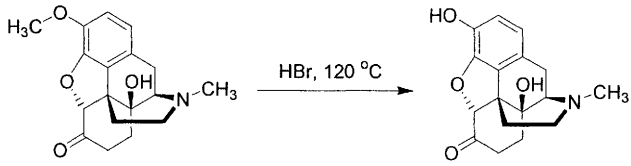
Trade name: Roxicodone, OxyContine (USA), Oxygesic, Eukodal (Ger), Eubine (F), Proladone (UK)

Side-effects: Oxycodone has a morphine-like side-effect profile. Respiratory depression has been found in children. The compound has a relevant abuse and dependence potential and illicit use of the retarded preparations has been reported.

Oxymorphone

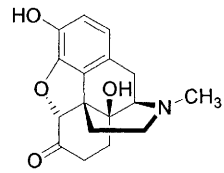
Synthesis (Lewenstein and Weiss, 1955; Weiss 1955): oxycodone is hydrolyzed with boiling concentrated hydrobromic acid.

Opioid receptor binding: Oxymorphone is a μ-selective opioid with a binding affinity in the range of morphine.



Scheme 40: Synthesis of oxymorphone

Oxymorphone



[*76-41-5*], (5α)-4,5-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one, dihydrohydroxymorphinone, dihydro-14-hydroxymorphinone, 14-hydroxydihydromorphinone, $C_{17}H_{19}NO_4$, M_r 301.34, *mp* 248-249 °C (decomp.); hydrochloride [357-07-3], $C_{17}H_{19}NO_4 \cdot HCl$, M_r 337.80

Analgesic efficacy and medical use: Oxymorphone is a potent analgesic. It is used for the treatment of moderate to severe pain, including labor pain, and as an adjunct to anesthesia. Oxymorphone has no cough suppressant activity.

Dosages and routes of administration: Oxymorphone is used parenterally by intramuscular or subcutaneous doses of 1-1.5 mg and as suppositories with a content of 5 mg. For patient controlled analgesia (PCA) i.v. bolus doses up to 300 µg are used (Sinatra and Harrison, 1989).

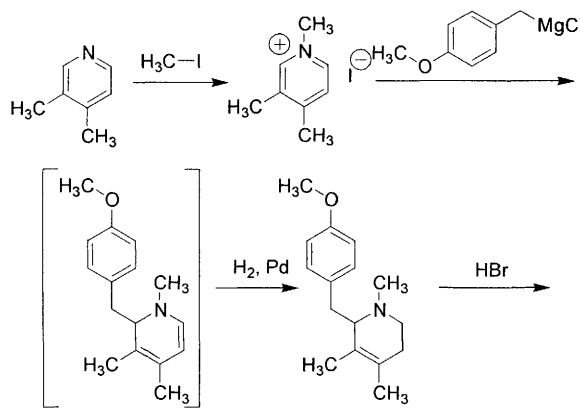
Pharmacokinetic properties: Oxymorphone is excreted into the urine as the unmetabolized drug and to a greater extent as the O-glucuronide (Cone et al., 1983).

Side-effects: Oxymorphone has a morphine-type side-effect profile and can induce addiction and dependence (Copland et al., 1987).

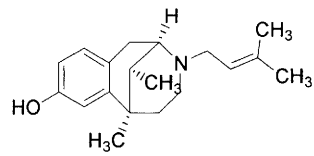
Trade name: Numorphan (USA, Canad.)

Pentazocine

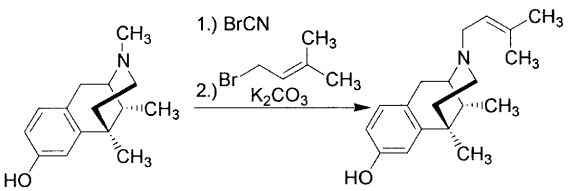
Synthesis (Archer et al., 1964; Kleemann et al. 1999):



Pentazocine



[359-83-1], 6,11-Dimethyl-3-(3-methyl-but-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-benzo[*d*]azocin-8-ol, (2α,6α,11*R**)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-mmethyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol, $C_{19}H_{27}NO$, M_r 285.42, *mp* 145-148 °C; hydrochloride [*2276-52-0*], $C_{19}H_{27}NO \cdot HCl$, M_r 321.89; lactate (1 : 1) [*17146-95-1*], $C_{19}H_{27}NO \cdot C_3H_6O_3$, M_r 375.51



Scheme 41: Synthesis of pentazocine.

Opioid receptor binding: Pentazocine (Brogden et al., 1973) is a mixed opioid agonist-antagonist with agonistic effects at the kappa and partial antagonistic effects at the μ-type of opioid receptor.

Analgesic efficacy and clinical application: Pentazocine is a fairly potent analgesic and is used for the treatment of moderate to severe pain (Goldstein, 1985).

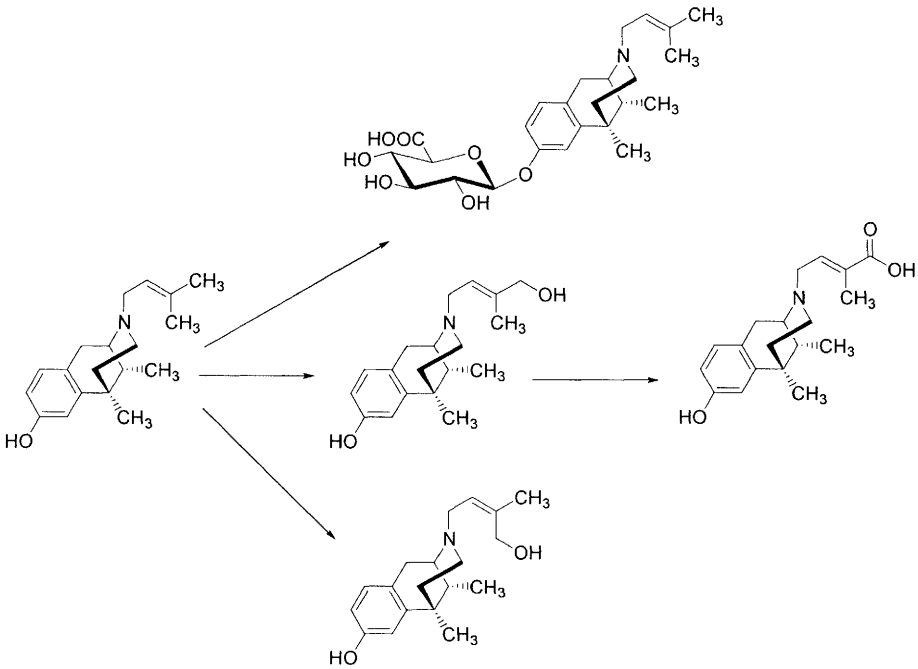
Dosages and routes of administration: Pentazocine is used as the hydrochloride or lactate in oral, parenteral and rectal formulations. Doses for the treatment of moderate to severe pain are in the range of 25-100 mg.

Pharmacokinetic properties: Pentazocine is orally bioavailable but there is a high degree of fluctuation in resorption. The compound is rapidly and extensively metabolized. Metabolites are formed by oxidative demethylation of the methyl residues of the dimethylallyl group and by glucuronidation (Berkowitz, 1973). The metabolic pathway of pentazocine is shown in scheme 42.

Side-effects: Pentazocine induces morphine-type side-effects such as dizziness, nausea, vomiting, sedation and sweating. In high doses seizures may occur (Challoner et al., 1990). In addition, it can cause kappa-agonist type psychotomimetic effects including hallucinations, nightmares and thought disturbances (Kane and Pokorny, 1975). Respiratory depression is weaker than with morphine and is subject to a 'ceiling' effect (Nagle and Pilcher, 1972).

Trade name: Fortral (Ger, F, UK), Talwin (USA)

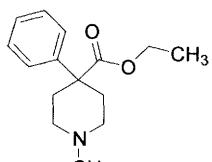
The compound has a relevant abuse potential (Reed and Scholl, 1986) and high doses may produce dependence of the morphine type. Pentazocine effects can be antagonized with naloxone and combinations with naloxone are available to discourage parenteral misuse (Baum et al., 1987).



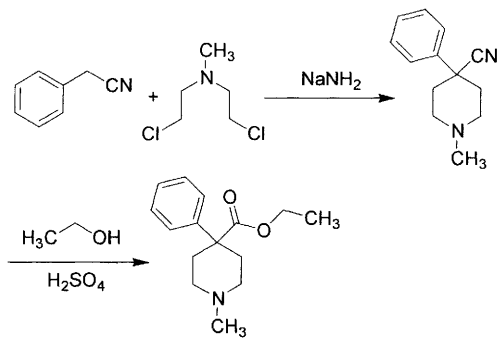
Scheme 42: Metabolic pathway of pentazocine.

Pethidine (Meperidine)

Synthesis (Eisleb (I.G. Farben), 1937, Eisleb (Winthrop), 1939, Smissman and Hite, 1959; Kleemann et al. 1999): the original synthesis involved condensation of benzyl cyanide with *N,N*-bis(2-chloro-ethyl)-*N*-methyl-amine, which is a skin irritant and a carcinogen.

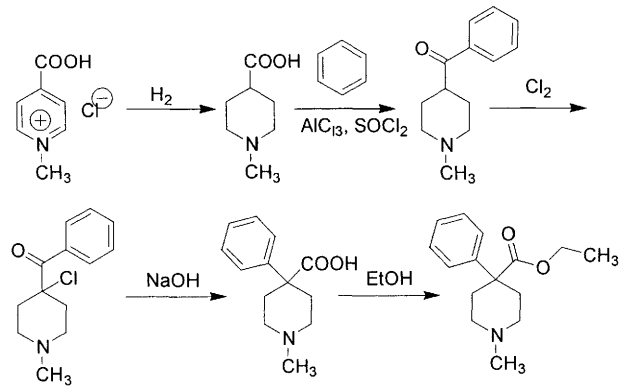


[*57-42-1*], 1-Methyl-4-phenyl-piperidine-4-carboxylic acid ethyl ester, $C_{15}H_{21}NO_2$, M_r 247.33; hydrochloride [*50-13-5*], $C_{15}H_{21}NO_2 \cdot HCl$, M_r 283.80, *mp* 186-189 °C.



Scheme 43: Synthesis of pethidine.

Another synthesis starts with pyridine-4-carboxylic acid:



Scheme 44: Synthesis of pethidine.

Pethidine, introduced in 1939, was the first fully synthetic analgesic with morphine-like activity.

Opioid receptor binding: Pethidine is a μ-selective synthetic opioid with an intermediate receptor affinity (Pert and Snyder, 1976).

Analgesic efficacy and clinical use: Pethidine (Clark et al.,1995; Latta et al., 2002) is used for the treatment of moderate to severe pain including labor pain. It is also used as preoperative medication and as an adjunct to anesthesia. Due to its anti-muscarinic properties, it has a weaker muscle stimulant activity than other opioids and does not increase biliary pressure, which makes it suitable for the treatment of pain associated with pancreatitis or biliary colic.

Dosages and routes of administration: For pain inhibition pethidine is used in oral doses of 50-150 mg every 4 hrs, or in subcutaneous, intramuscular or slow intravenous doses of 25-100 mg.

Figure 7 shows the pethidine consumption in different european countries and the United States.

Pharmacokinetic properties: Pethidine (Mather and Meffin, 1978) has a faster onset and a shorter duration of action than morphine. After oral administration about 50% of the drug is eliminated by first-pass metabolism. N-demethylation yields the active metabolite nor-pethidine, and hydrolytic cleavage the inactive metabolites pethidinic and nor-pethidinic acid. The half-life of pethidine is about 3- 6 h. Nor-pethidine has a much slower elimination with a half life of up to 20 h.

Trade name: Dolantin (Ger), Dolosal (F), Demerol (USA)

Side-effects: Pethidine induces morphine-type side-effects with a lower incidence of constipation. Higher doses induce central stimulation and antimuscarinic effects

accompanied with pupil dilatation. In even larger doses toxic symptoms including muscular twitching, tremor, mental confusion and convulsions occur, which are partly attributed to the more toxic metabolite nor-pethidine (Jiraki, 1992). Severe side-effects including coma and cyanosis have been observed in combination with MAO inhibitors (Meyer and Halfin, 1981). Pethidine induces morphine type tolerance and dependence and addicts using high doses of pethidine have an increased risk of excitatory side-effects.

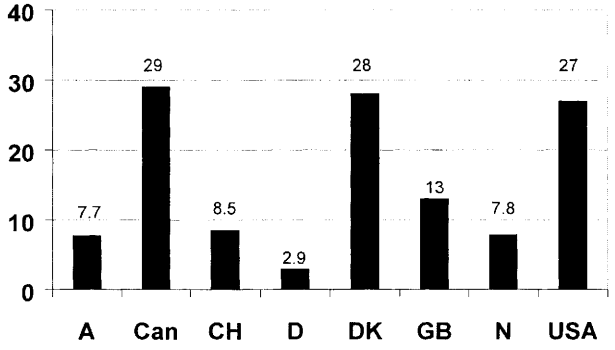
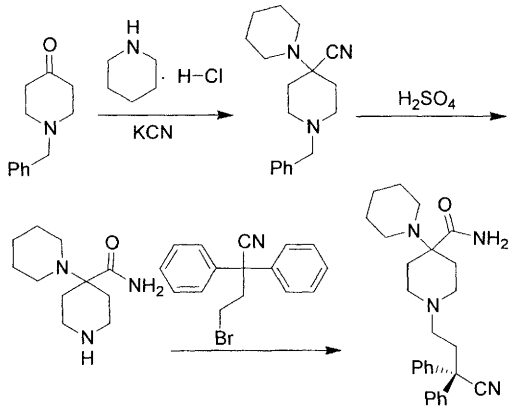


Figure 7: Pethidine consumption (1995) in different countries in kg compound /Mio inhabitants (Sohn and Zenz, 1998)

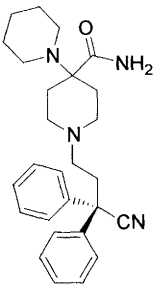
Piritramide

Synthesis: By condensation of 4-piperidinopiperidine-4-carboxamide with 3,3-diphenyl-3-cyano propyl bromide (Janssen, P. (Janssen), 1961; Kleemann et al. 1999).



Scheme 45: Synthesis of piritramide.

Piritramide



[*302-41-0*], 1'-(3-Cyano-3,3-diphenyl-propyl)-[1,4']bipiperidinyl-4'-carboxylic acid amide, $C_{27}H_{34}N_4O$, M_r 430.59, 149-150 °C

Opioid receptor binding: Piritramide is a synthetic μ-opioid with morphine-like affinity and receptor selectivity.

Analgesic efficacy and clinical use: Piritramide (Gibb and Pikler, 1973; Kumar and Rowbotham, 1999) is used for the treatment of acute, preferentially postoperative pain (Lehmann et al., 1986) and as an adjunct to anesthesia. It is less potent than morphine.

Dosages and routes of administration: Piritramide is used only parenterally by the intravenous, intramuscular or subcutaneous route. The standard dose is 15 mg.

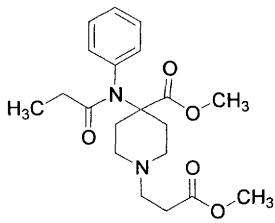
Pharmacokinetic properties: Piritramide is extensively metabolized by first-pass metabolism in the liver and gut (Bouillon et al., 1999). After parenteral administration it is rapidly distributed. The compound is eliminated slowly and has a half-life of about 10 h.

Trade name: Dipidolor (Ger, B, NL), Piridolan (S)

Side-effects: Piritramide has a morphine-like side-effect profile and may induce tolerance and dependence.

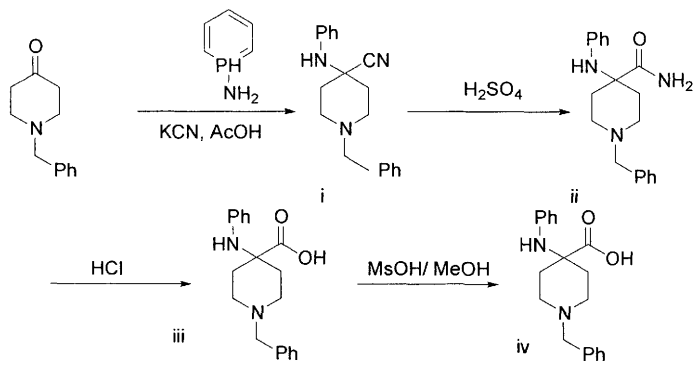
Remifentanil

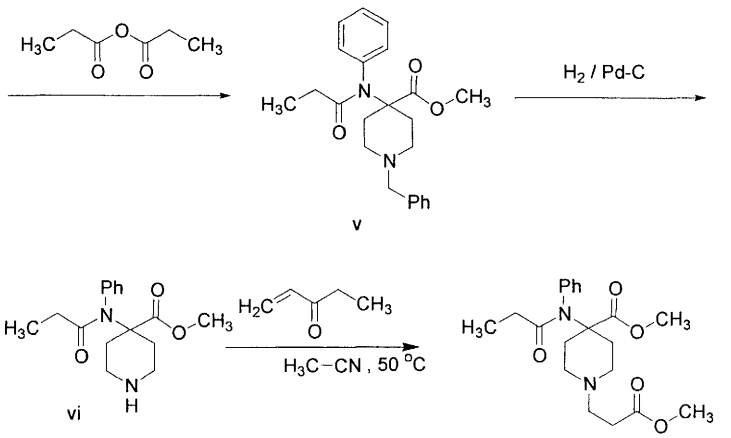
Remifentanil



[*132875-61-7*], 1-(2-Methoxycarbonyl-ethyl)-4-(phenyl-propionyl-amino)-piperidine-4-carboxylic acid methyl ester, 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid methyl ester, $C_{20}H_{28}N_2O_5$, M_r 376.45; monohydrochloride [132539-07-2], $C_{20}H_{28}N_2O_5 \cdot HCl$, M_r 412.91; oxalate (1 : 1) [*132875-62-8*], $C_{20}H_{28}N_2O_5 \cdot C_2H_2O_4$, M_r 466.49

Synthesis: The reaction of 1-benzyl-4-piperidone with aniline and KCN in acetic acid gives the aminonitrile **i**, which is treated with H_2SO_4 at room temperature to yield the carboxamide **ii**. The hydrolysis of **ii** with refluxing aqueous HCl affords the carboxylic acid **iii**, which is esterified with MsOH and methanol to provide the methyl ester **iv**. Acylation of the NH group of **iv** with propionic anhydride gives the propionamide **v**, which is debenzylated with H_2 over Pd/C to yield the piperidine **vi**. Finally condensation of 4-(phenyl-propionyl-amino)-piperidine-4-carboxylic acid methyl ester with acrylic acid methyl ester in hot acetonitrile affords the target compound (Feldman et al. (Glaxo) 1990; Feldmann et al., 1991; Drugs Fut. 1994, Drugs Fut. 1995, Drugs Fut. 1997, Coleman 1999; Kleemann et al. 1999).





Scheme 46: Synthesis of remifentanil.

Opioid receptor binding: Remifentanil (Patel and Spencer, 1996) is a μ-opioid with a higher receptor affinity than fentanyl.

Analgesic efficacy and clinical use: Remifentanil (Glass,1995) has short-acting strong analgesic and anesthetic properties. It has been developed as an ultra-short anesthetic and can be used for ambulatory surgery (Servin, 1997; Haigh, 2000), general surgery and intensive care. As primary anesthetic or adjunct to other anesthetics it affords potent intra-operative analgesia and has a sparing effect on sedatives and hypnotics (Cohen and Royston, 2001).

Dosages and routes of administration: Remifentanil is given as intravenous short infusion in doses of 0.5-1 μg/kg or as continuous infusions in the range of 0.0025-2 μg/kg/min.

Pharmacokinetic properties: Remifentanil is an ultra-short acting compound (Michelsen and Hug, 1996), rapidly inactivated by plasma and tissue esterases. The terminal elimination half-life is 10-20 min.

Side-effect profile: Remifentanil has a μ-opioid-type side-effect profile with strong CNS and respiratory depressant properties. It has a morphine-like addiction and dependence potential.

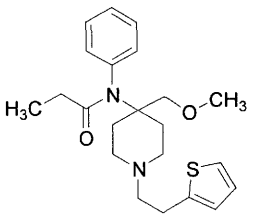
Trade name: Ultiva (Ger, UK)

Sufentanil

Synthesis: In the literature several synthetic pathways to sufentanil have been described (Janssen and van Daele

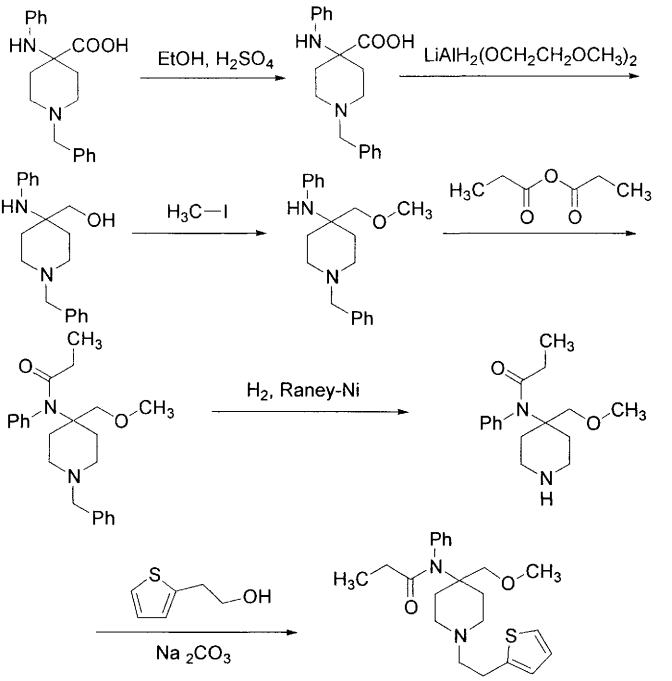
(Janssen), 1976, van Daele et al., 1976; Castaner and Roberts, 1977; Bever et al., 1976; Kleemann et al. 1999).

Sufentanil



The esterification of 1-benzyl-4-(phenylamino) piperidine-4-carboxylic acid with ethanol and H_2SO_4 gives the corresponding ethyl ester, which is reduced with $LiAlH_2(OCH_2CH_2OCH_3)_2$ in benzene affording 1-benzyl-4-(phenylamino)piperidine-4-methanol. The methylation of this compound with methyl iodide and NaH in HMPA yields 1-benzyl-4-methoxymethyl-4-(phenylamino)-piperidine, which is refluxed with propionic anhydride to give N-[4-(methoxymethyl)-1-benzyl-4-piperidyl]propionanilide . The following hydrogenolysis of with H_2 over Raney Ni in methanol yields N-[4-(methoxymethyl)-4-piperidyl]propionanilide, which is finally condensed with 2-thiopheneethanol methanesulfonate by means of Na_2CO_3 in methyl isobutyl ketone.

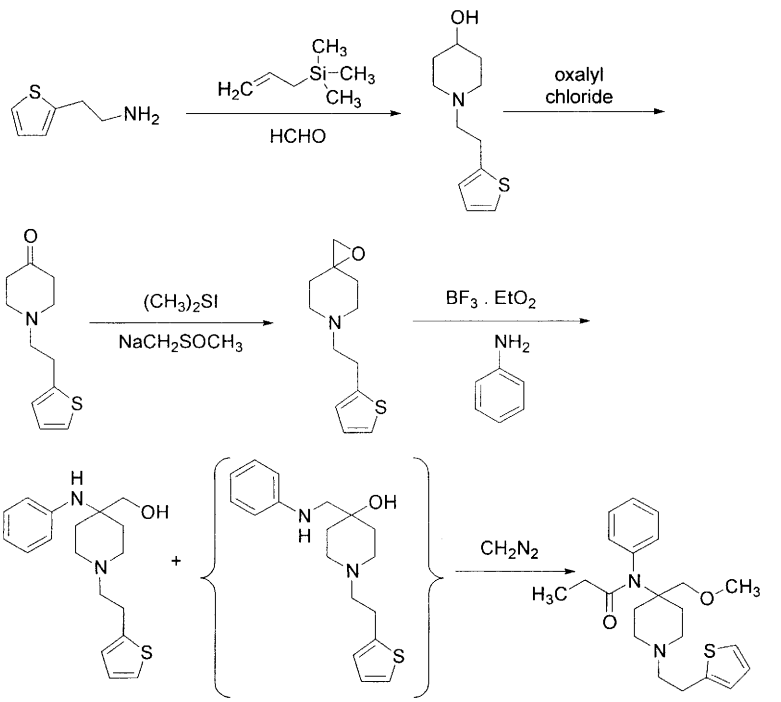
[*56030-54-7*], N-[4-(Methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide, *N*-[4-Methoxymethyl-1-(2-thiophen-2-yl-ethyl)-piperidin-4-yl]-*N*-phenyl-propionamide, $C_{22}H_{30}N_2O_3S$, M_r 386.56, *mp* 96.6 °C; citrate (1 : 1) [*60561-17-3*], $C_{22}H_{30}N_2O_3S \cdot C_6H_8O_7$, M_r 578.68



Scheme 47: Synthesis of sufentanil.

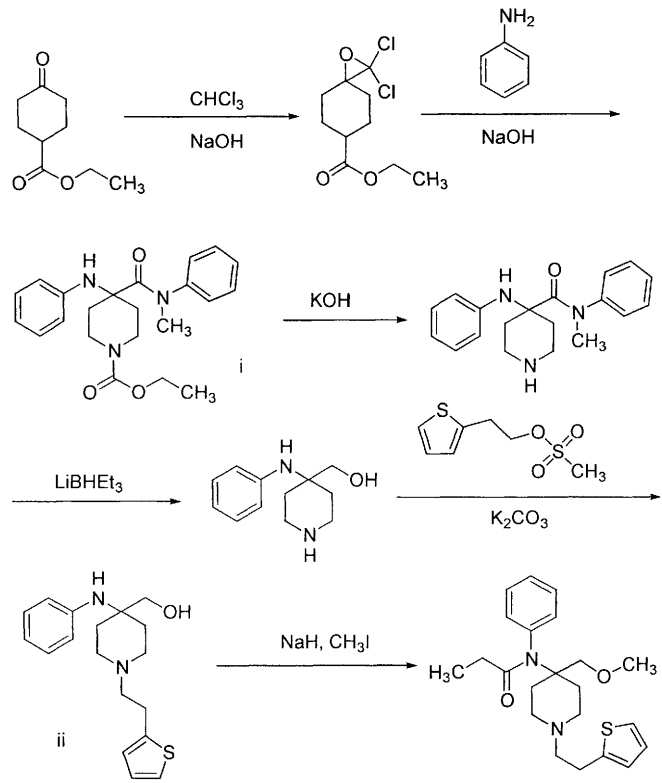
Another total synthesis of sufentanil has been described: the cyclization of 2-(2-thienyl)ethylamine with allyl-trimethylsilane and formaldehyde gives 4-hydroxy-1-[2-(2-thienyl)ethyl]piperidine, which is oxidized with oxalyl chloride in DMSO/dichloromethane to 1-[2-(2-thienyl) ethyl]piperidin-4-one. The epoxidation of this compound by means of trimethylsulfonium iodide and the sodium salt of DMSO yields the spiro-epoxide, which is opened with

aniline and boron trifluoride ethearate giving a 1.8 : 1 mixture of 4-(hydroxymethyl)-4-(phenylamino) piperidine and 4-hydroxy-4-(phenylamino) piperidine which can be conveniently separated. The methylation of the OH group with diazomethane and SiO_2 affords the methoxymethyl compound, which is finally acylated with propionic anhydride to provide sufentanil.



Scheme 48: Synthesis of sufentanil.

The reaction of 4-oxopiperidine-1-carboxylic acid ethyl ester with $CHCl_3$, benzyl triethylammonium chloride and NaOH in THF/water gives the spirooxirane derivative, which is treated with aniline and NaOH to yield the corresponding anilide. The methylation of the amide nitrogen by means of NaH and CH_3I in THF affords the methylated anilide. The reaction of methylated anilide with KOH in refluxing isopropanol causes elimination of its ethoxycarbonyl group, providing compound **i**, which is reduced with lithium triethylborohydride in THF to give 4-(hydroxymethyl)-4-(phenylamino)piperidine. The condensation of *N*-(4-methoxymethyl-piperidin-4-yl)-*N*-phenyl-propionamide with 2-(2-thienyl)ethyl mesylate by means of K_2CO_3 in refluxing acetonitrile yields the adduct **ii**, which is methylated with NaH and CH_3I in THF to afford the methoxy derivative sufentanil.



Scheme 49: Synthesis of sufentanil.

Opioid receptor binding: Sufentanil is a μ-selective opioid with an about 10-fold higher receptor affinity than fentanyl (Leysen et al., 1983).

Analgesic efficacy and clinical use: Sufentanil (Rosow, 1984; Monk et al., 1988) is a very potent fentanyl analog with analgesic and anesthetic properties and a more rapid onset and a shorter duration of action. It is used for perioperative analgesia, short duration anesthesia and as an adjunct to various anesthetic procedures including neuroleptanalgesia (Isaacson, 1992).

Dosages and routes of administration: Sufentanil is mostly given parenterally as an intravenous bolus or as brief injection or infusion during anesthesia. For pain treatment, intravenous or epidural on-demand procedures are in use. Doses up to 8 μg/kg are adequate for pain treatment and higher doses up to 30 μg/kg for surgery (Grass, 1992).

Pharmacokinetic properties: Following parenteral administration sufentanil has a rapid onset and a short duration of action. The compound is very lipophilic and is subject to high plasma protein binding of ~ 90%. The short

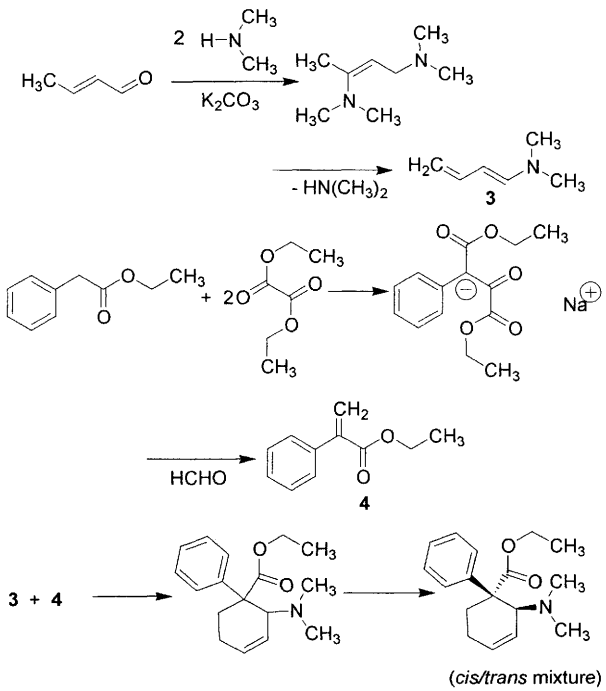
duration of action is more dependent on redistribution than on metabolic inactivation. Redistribution half-life is about 20 min as compared to a elimination half-life of 2-3 h. The compound is metabolized by oxidative N- and O-demethylation to polar metabolites which are excreted in the urine.

Side-effects: Sufentanil has a morphine-type side-effect profile and induces severe respiratory depression and chest wall rigidity in anesthetic dosages (Goldberg et al., 1985). High dose levels have been associated with seizures. Prolonged use may induce tolerance and dependence.

Trade name: Sufenta (B, Ger, F, N, USA)

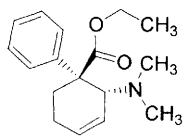
Tilidine

Synthesis: The reaction of buta-1,3-dienyl-dimethylamine **3** with ethyl atropate **4** yields tilidine as a *cis/trans* mixture (trans : cis = 2 : 3). Most of the analgesically inactive *cis* isomer is separated as a zinc complex and the trans isomer is isolated as the hydrochloride. The *cis* isomer can be epimerized to the trans form by treating the epimeric mixture with acid (Satzinger (Gödecke), 1965; Sallay (Warner-Lambert), 1969, Satzinger, 1972; Satzinger et al., 1978; Overman et al., 1979; Kleemann et al. 1999).



Scheme 50: Synthesis of tilidine.

Tilidine



[*51931-66-9*], *trans*-2-Dimethylamino-1-phenyl-cyclohex-3-enecarboxylic acid ethyl ester, $C_{17}H_{23}NO_2$, M_r 273.17, *mp* 34 °C, *bp* 95.5-96 °C at 0.01 mm pressure; hydrochloride [*27107-79-5*], $C_{17}H_{23}NO_2 \cdot HCl$, M_r 309.84, *mp* 159 °C; hydrochloride hemihydrate $C_{17}H_{23}NO_2 \cdot HCl \cdot 1/2\ H_2O$, M_r 318.80, *mp* 125 °C

Opioid receptor binding: Tilidine itself has a low opioid receptor binding affinity, whereas the active metabolites nor-tilidine and bisnor-tilidine have a high affinity and selectivity for the μ-type of opioid receptor (Schulz et al.,1978).

Analgesic efficacy and clinical use: The compound is used for the treatment of acute and chronic moderate to severe pain.

Dosages and routes of administration: Tilidine is most commonly used in oral formulations containing about 10% naloxone to avoid parenteral misuse (Worz and Worz, 1995). Tilidine without naloxone is given rectally and by various parenteral routes including intravenous, intramuscular and subcutaneous routes. Parenteral doses of up to 400 mg/day can be administered; the oral and rectal single doses are 50 and 75 mg, respectively (Martin et al., 1999).

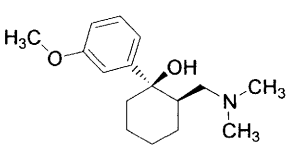
Pharmacokinetic properties: Tilidine is a pro-drug (Vollmer et al., 1989) which is rapidly metabolised in the gut and liver to the analgesically active N-demethyl derivatives nor-tilidine and bisnor-tilidine.

Trade name: Valoron N (Ger), Tilidate (Spain)

Side-effects: Tilidine has a morphine-like side-effect and abuse potential (Trojan and Beil, 1978; Jasinski and Preston, 1986). To inhibit parenteral abuse of oral formulations, a small amount of naloxone is added. Naloxone is completely inactivated after oral administration and does not impede analgesia, whereas after parenteral administration the naloxone component is active and blocks the tilidine effect and can induce withdrawal reactions (Vollmer, 1988). The combination with naloxone, in contrast to pure tildine, is available under normal prescription.

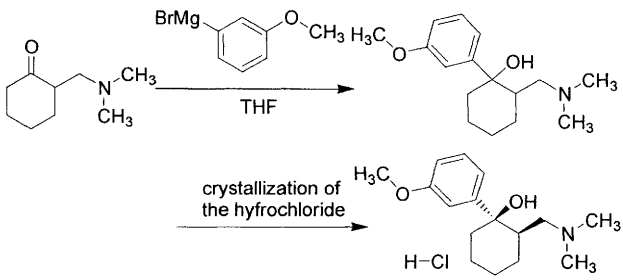
Tramadol

Tramadol



[*27203-92-5*], *cis*-2-Dimethylaminomethyl-1-(3-methoxy-phenyl)-cyclohexanol, $C_{16}H_{25}NO_2$, M_r 263.38; hydrochloride [*36282-47-0*], $C_{16}H_{25}NO_2$. HCl, M_r 299.84, *mp* 180-181°C

Synthesis: The Grignard reaction of 2-(dimethyl-aminomethyl)cyclohexanone (obtained by Mannich reaction of cyclohexanone, formaldehyde, and dimethyl-amine hydrochloride) and the Grignard reagent of 3-bromoanisole yields tramadol as a *cis/trans* mixture (*cis* : *trans* = 85 : 15). Tramadol (*cis* isomer) is separated from the reaction mixture via crystallization of the hydrochloride salt. A crystallization of the hydrate or hydrobromide salts has also been described. (Frankus and Flick (Grünenthal) 1964; 1971; Flick et al. 1978; Kleemann et al. 1999).



Scheme 51: Synthesis of tramadol.

Opioid receptor affinity: Tramadol (Frink et al., 1996) itself has a weak opioid receptor affinity, the active metabolite O-desmethyl-tramadol has μ-selectivity and μ-affinity about 10 times lower than that of morphine.

Analgesic efficacy and clinical use: Tramadol HCl (Friderichs et al., 1978; Raffa and Friderichs, 1996) is a centrally-acting analgesic with a μ-opioid and non-opioid component of action (Raffa et al., 1992). The non-opioid component induces inhibition of spinal pain transmission via inhibition of noradrenaline (NA) and serotonin (5HT) re-uptake (Driessen and Reimann, 1992; Driessen et al. 1993) Tramadol is a racemate and uptake inhibition and opioid properties are differentially distributed between the enantiomers (Raffa et al., 1993) and the active O-desmethyl metabolite. μ-Opioid receptor binding, NA uptake inhibition and 5HT uptake inhibition are regarded as the relevant (Desmeules et al., 1996; Raffa and Friderichs, 1996) components of the complex analgesic action profile of the compound; (+)-O-desmethyl-tramadol mainly contributes to μ-activity, (-)-tramadol to NA uptake inhibition and (+)-tramadol to 5HT uptake inhibition, respectively.

Tramadol is a potent analgesic (Lee et al., 1993). The compound is marketed worldwide and has become one of the most important centrally-acting analgesics (Bamigbade, 1998; Scott and Perry, 2000) for the treatment of acute and chronic moderate to severe pain.

Dosages and routes of administration: Tramadol is used in single doses of 50-100 mg up to daily doses of 300-400 mg. The compound has high oral bio-availability and can be given by mouth, rectally or by intramuscular, subcutaneous or slow intravenous injection or infusion (Lintz et al. 1981).

Pharmacokinetic properties: Tramadol is extensively metabolized by O- and N-demethylation yielding the active metabolite O-desmethyl-tramadol (M1) and several

inactive derivatives like N-desmethyl-tramadol (M2), N-bis-desmethyl-tramadol (M3), O-desmethyl-N-desmethyl-tramadol (M5) and O-desmethyl-N-bis-desmethyl-tramadol (M4).

Trade name: Tramal (Ger, Switzerland, Austr.,Fin.), Zydol (UK), Ultram (USA), Adolonta (Spain), Contramal (F,I, Belg.), Nobligan (N)

Side-effects: Typical side-effects of tramadol are nausea, sweating and dizziness. In rare cases seizures after high i.v. doses are reported, mostly in combination with other proconvulsant componds or in patients with reduced seizure theshold (Gardner et al., 2000). Tramadol shows a reduced level of opioid side-effects, especially respiratory depression and constipation are less frequent and severe than with standard opioids such as morphine. Tramadol has a very limited abuse potential and is not subject to narcotic control (Cossmann et al., 1997).

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3.5 Drug Delivery Systems for Opioids

Johannes Bartholomäus

Introduction

Natural opioids have been used as medicines since ancient times (Seefelder, 1986), when Hippocrates and Galen, for example, obtained them from the juice of the poppy. Throughout the Middle Ages from Avicenna to Paracelsus, the number of extraction forms increased, culminating in Sydenham' entry of opium tincture in the pharmacopoeias. The isolation of the active principles, the synthesis of clearly defined new morphine derivatives, analog active principles, and progress in formulation technology paved the way for classical formulations such as injections, oral solutions, tablets, capsules and suppositories. With regard to 'non-therapeutic' use, in which it is not so much a question of an indication-adequate dosage, alternative administration by means of pulmonary or nasal inhalation (opium pipe or alkaline sniffing) came on the scene at an early stage

The development of modern formulations for opioids was and is still determined by the following trends:

- prolongation of action
- reduction of side-effects
- individual therapy
- patient convenience
- leading to an increase in patient compliance

The trends in parenteral, oral, rectal, transdermal, pulmonary and transmucosal administration will be described with the aid of products that are already on the market or are under development.

PCA

Medico-technical instruments such as infusion pumps can be used in PCA (*patient-controlled analgesia*, Fig. 1) to provide patient-orientated and therapy as required, e.g. with morphine injection solutions. Depending on the patients' perception of pain, they may add small doses of analgesics to the basic infusion by means of an electrically controlled infusion pump. The physician specifies the basic dose, which is infused independent of patient demands, the boluses that can be demanded, an hourly maximum dose and a refractory time that cannot be reduced between two doses. The infusion may be given intravenously, subcutaneously, epidurally or intraspinally.



Figure 1: PCA system.

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Such technologies focus on acute pain, for example after surgery (Lehmann, 2001).

Oral Prolonged-release Formulations

In (sub)chronic treatment long-acting medicines are mainly used. The breakthrough was the development of oral prolonged-release morphine formulations. The relatively short half-life of morphine (1.7 - 4.5 h) requires a high dosing frequency of 4 - 6 times a day of tablets, capsules or solutions. With the introduction of prolonged-release tablets the frequency of administration was first reduced to twice daily, which was particularly suitable for '"dosing by the clock' in chronic pain instead of symptomatic dosing on the recurrence of pain.

From a technical point of view the challenge of oral morphine prolonged-release was solved by the first preparation MST® Retard/ MS Contin comprising tablets with a matrix of hydrophobic (cetostearyl alcohol) and hydrophilic (hypromellose) elements that release the active substance over a period of 8 - 10 hours (Fig. 2). Various dosage strengths (10, 30, 60, 100 mg and more recently 200 mg) are available to allow for variations in pain intensity and dose adjustment due to the development of tolerance. The different colored tablets of 10 - 100 mg are about 7 mm in diameter and the 10 - 60 mg formulations have virtually the same *in vitro* profile rate (the percentage of the total dose against time, Fig. 2), and the release of the higher 100 mg dose is somewhat more prolonged in order to achieve optimal therapeutic results. This is the aim with the larger 200 mg tablet (approx. 8 mm in diameter), which is bioequivalent despite its somewhat slower *in vitro* release.

Another technical solution for twice-daily morphine treatment are capsules with prolonged-release pellets such as M-long® or Capros®. Release is prolonged by means of diffusion coatings, usually on an ethylcellulose base. The various doses of pellet preparations are obtained by adjusting the number of identical pellets per capsule, and therefore the release profiles of the various 'strengths' are identical. Due to the low dose per pellet and the high solubility of morphine, resulting in the total dissolution of the active substance within the pellets, a first-order release kinetic is achieved after a relatively short time. First-order kinetics and root-t kinetics are quite similar, and therefore M-long® and the above-mentioned matrix tablets are bioequivalent. Using the pellet formulation, in which the dose is distributed over small

subunits, the substance can be administered via a stomach tube in patients who have to be fed artificially.

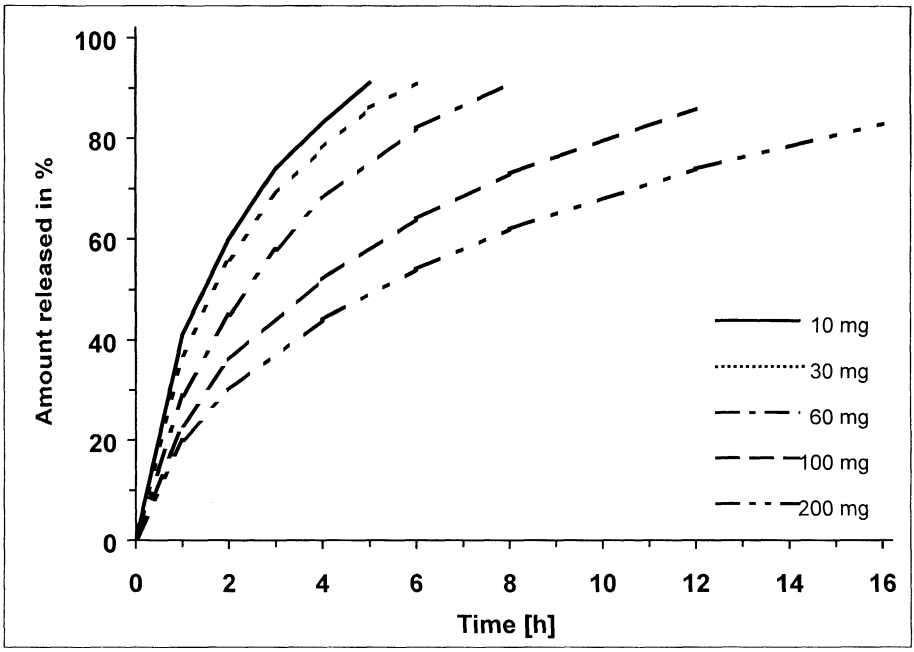


Figure 2: In-vitro release of MST 10 / 30 / 60 / 100 / 200 Mundipharma/MS Contin prolonged-release tablets.

The success of prolonged-release morphine prompted the development of prolonged-release formulations for other opioids, for example the matrix made of hydrophobic and hydrophilic matrix formers, for example on hydrocodeine (DHC retard with cetostearyl alcohol and hydroxyethyl-cellulose), oxycodone (oxygesic with stearyl alcohol and polyacrylate) and tramadol (tramundin with cetostearyl alcohol and ethylcellulose). By virtue of the oblong shape of hydrocodeine and tramadol tablets the prolonged-release tablets can be divided, whereby compared with whole tablets release from the divided tablets is slightly accelerated. The difference with these forms is that with increasing dose the release slows down.

A purely hydrophilic matrix of hypromellose prolongs the release of tramadol from Tramal® long (Fig. 3) developed by Gruenenthal. The tablets have the same dimensions, resulting in an identical release profile for all dosages (100, 150, 200 mg, see Fig. 4). For a titrated effect linear pharmacokinetics on increasing doses produce dose-proportional blood levels at any time. External influences, such as pH value, mechanical stress, surface-active

substances and food, have no effect on release (Anonymus, 1995).

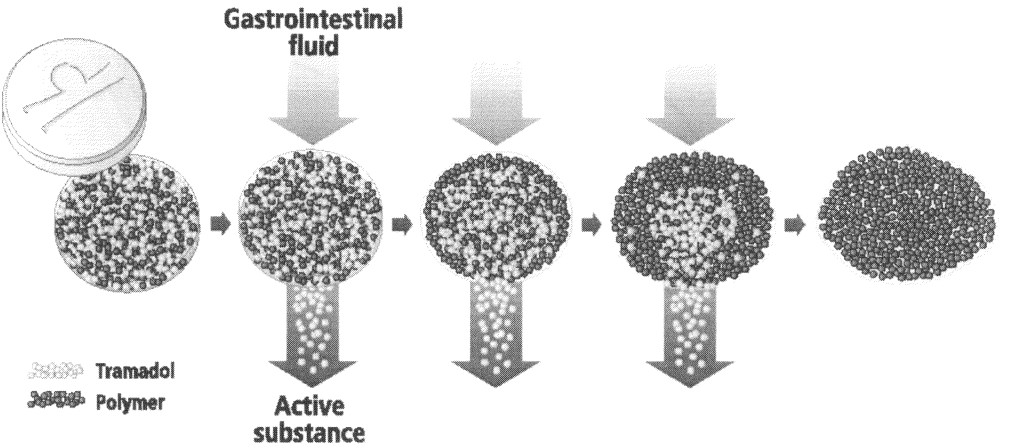


Figure 3: Release from hydrophilic matrices with Tramal®/Contramal®/Adolonta® long of Gruenenthal as an example.

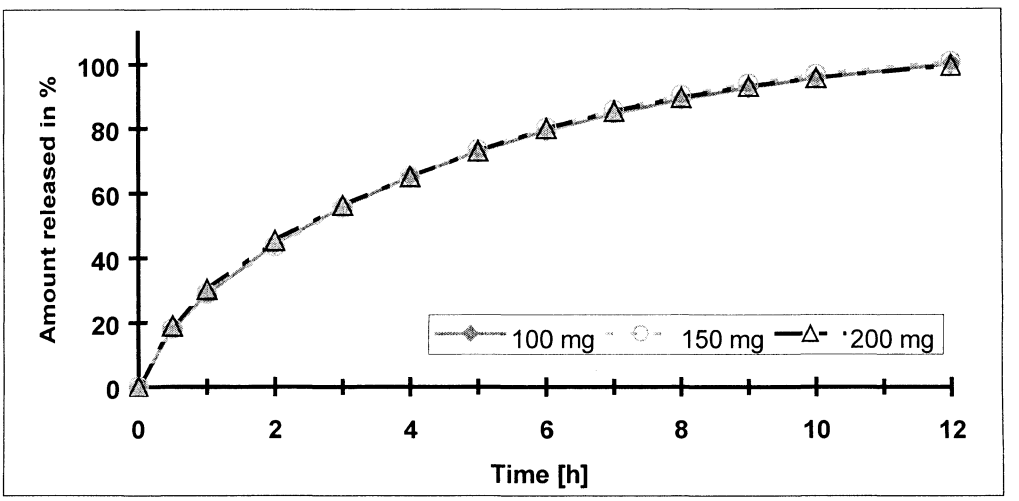


Figure 4: Dose-independent in-vitro release of tramadol hydrochloride from Tramal®/ Contramal®/Adolonta® long 100, 150 and 200 mg (medium up to 0.5 h artificial gastric juice pH 1.2; up to 2 h pH 2.3; up to 3 h pH 6.8; up to 12 h pH 7.4).

Tramadol (Tramadolor® long) is also available as sustained-release pellets with an identical release profile.

Long-acting Oral Formulations

In a number of indications once-daily dosages have proved to be beneficial, and therefore attempts have been made to develop once-daily formulations of opioids. In the case of morphine, a step in this direction was achieved with MST® continus long, which has been approved for once or twice-daily use, by incorporating the active substance in a purely fatty matrix to produce granules with a particle size of about 1 mm, which are then filled into single-dose sachets. Other morphine solutions have been incorporated in prolonged-release pellets with a release rate of about 24 h (e.g. Morphelan developed by Elan).

In Great Britain tramadol is available as once-daily tablets made up of granules in a purely fatty matrix (Zydol® XL), as described above for morphine, which again reduces the release rate compared with the granules.

In other indications such as hypertension, the OROS technology (oral osmotic system developed by ALZA) has already proven its value, and therefore it seemed appropriate to use this technology with its typical, virtually constant, release rate (zero-order kinetics) for once-daily opioid tablets. Such a morphine tablet is already available, but is not yet on the market, and a hydromorphone OROS is currently being clinically developed by Knoll.

Long-acting Enteral Forms

All enteral formulations have one thing in common (at least to date), namely, in view of the gastrointestinal passage times, the dosing frequency must be at least once daily. The only exceptions would be active substances with very long half-lives and having a duration of action of several days. However, these do not include analgesics. Alternative formulations such as Moranex®, which has recently been approved in Great Britain (Fig. 5) and consists of morphine delivered by a rectal xerogel system (Hycore™ Technology developed by CeNeS), still have to gain therapeutic acceptance. The hydrogel formed in the rectum releases the active substance by virtue of the special formulation of the cross-linked gel body via zero-order kinetics, thus producing almost constant blood levels (Fig. 6). However, a new system has to be administered once daily on account of defaecation.

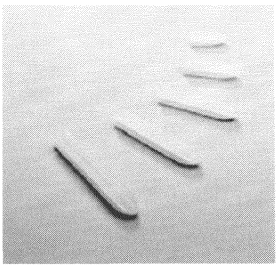


Figure 5: Moraxen™ rectal delivery systems with 35, 50, 75, 100 and 125 mg based on the Hycore™–R technology.

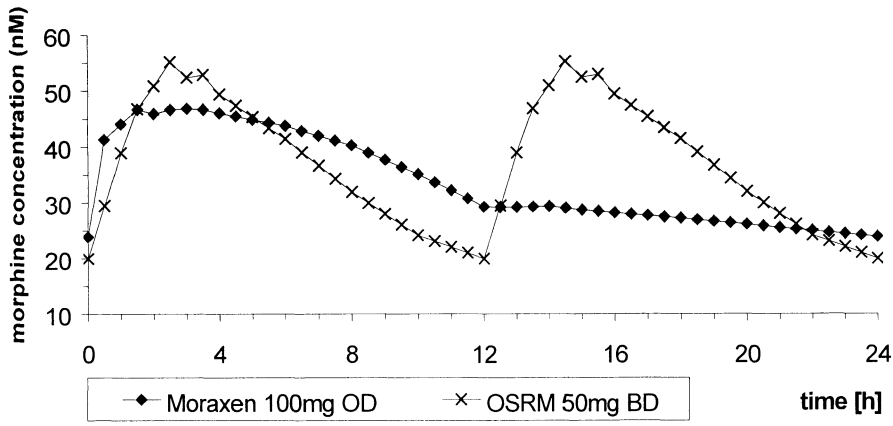


Figure 6: Moraxen™ rectal delivery system. Comparative steady-state simulation of morphine plasma levels after single daily rectal administration of Moraxen™ with 100 mg morphine compared with twice-daily 50 mg morphine (OSRM) in an oral prolonged-release formulation. The simulation is based on the plasma levels from a single-dose study.

Transdermal Patches

A quantum leap in pain therapy with distinct advantages in the form of reduced side-effects and application frequency was achieved with transdermal opioid administration. Transdermal application requires a number of characteristics on the part of the active substance (Fig. 7), the most restrictive being the daily dose, and only very potent opioids which are effective in very low doses, such as fentanyl and buprenorphine, are an option (Sittl and Likar, 2001).

A technical breakthrough was reached by the Drug Delivery System (DDS) developer ALZA with a TTS (Transdermal Therapeutic System) of fentanyl on the basis of a reservoir patch about 0.5 mm thick (Fig. 8). In order to release therapeutically effective amounts of fentanyl from the TTS through the poly(ethylene, vinylacetate) membrane controlling the release of the active substance release and skin penetration, ethanol was used as the solvent and enhancer. The solution in the TTS reservoir is gelated with hydroxyethylcellulose. The 3-day TTS with doses of 25, 50, 75 and 100 µg/h have been clinically developed, approved and marketed by Janssen as Durogesic®. The various dosages that correlate linearly with the corresponding fentanyl blood levels are obtained by proportionally increasing the contents of the reservoir (active substances and excipients) and the contact area

(25 µg/h with 2.5 mg fentanyl citrate and 10 cm^2 area up to 100 µg/h with 10 mg fentanyl citrate and 40 cm^2 area).

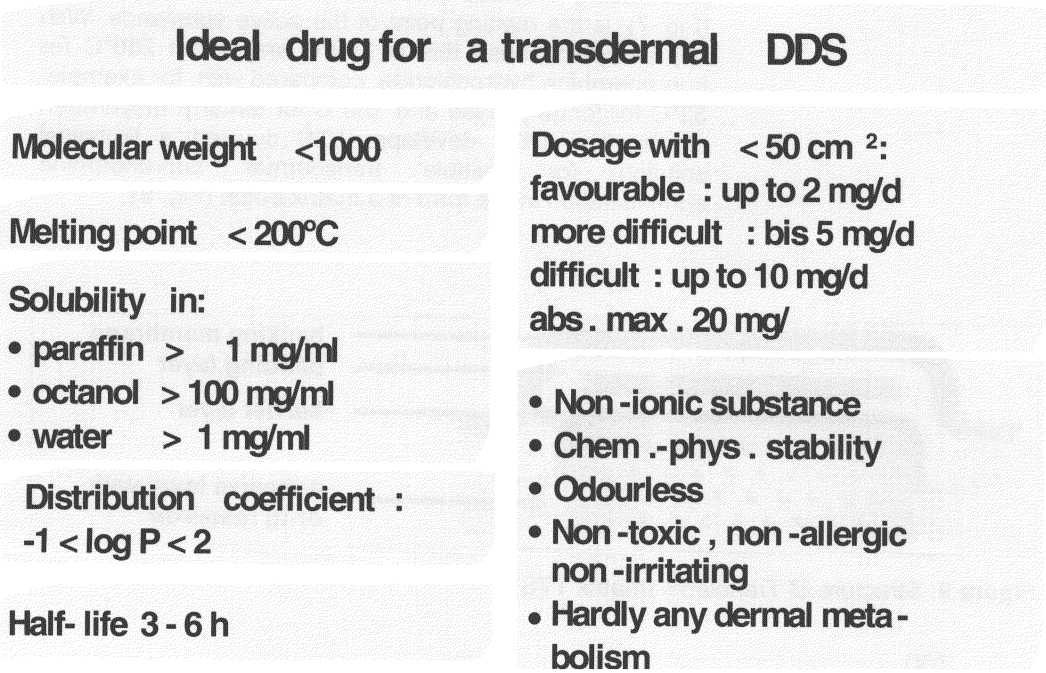


Figure 7: Requirements for an ideal substance for transdermal administration.

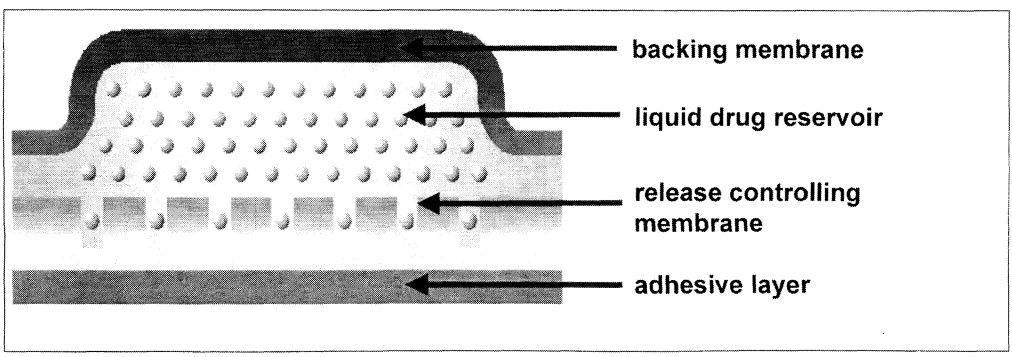


Figure 8: Structure of a reservoir TTS (Durogesic®).

Buprenorphine, the other option for transdermal application on account of its potency, is more difficult to apply dermally in therapeutic doses (Roy et al., 1994; Grond et al., 2000). A surrogate parameter for skin penetration, provided the other prerequisites are fulfilled (Fig. 7), is the melting point of the active substance. With buprenorphine base this is 209°C and about 260°C for buprenorphine hydrochloride, compared with, for example, 83°C for fentanyl base and 150°C for fentanyl dihydrogen citrate. The DDS developer LTS devised a technical solution for reliable transdermal buprenorphine administration in the form of a matrix patch (Fig. 9).

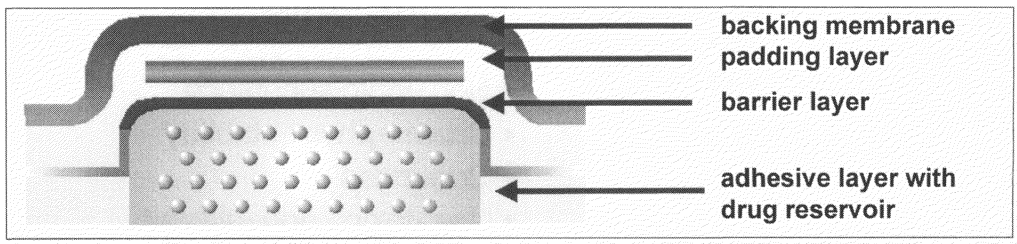


Figure 9: Structure of Transtec® (matrix TTS).

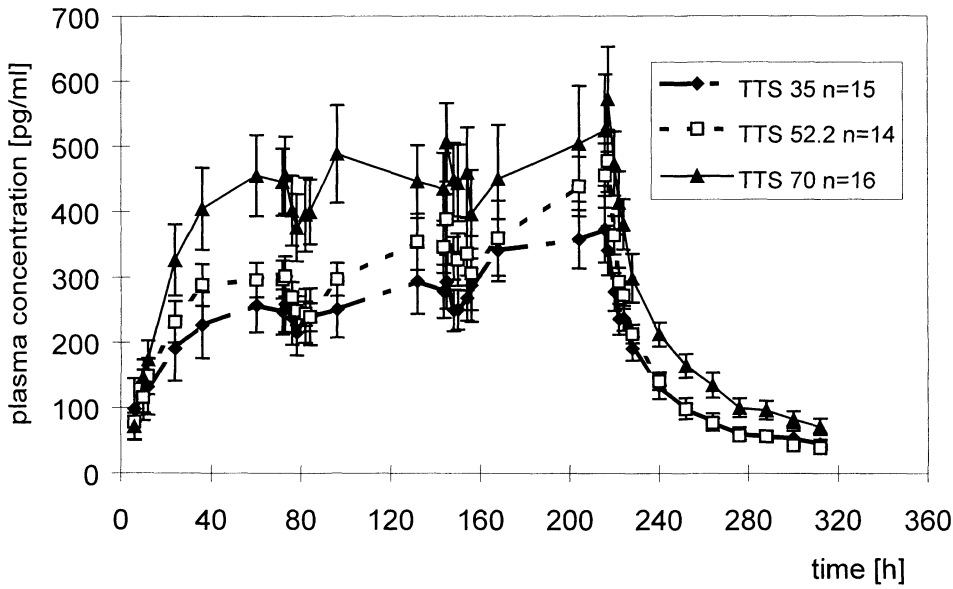


Figure 10: Plasma levels after multiple application of Gruenenthal's Transtec® 35, 52.5 and 70 µg/h for 9 days (three consecutive applications of 3 days each)

In contrast to reservoir patches, there is no risk of dose-dumping a with matrix patch, which releases buprenorphine from a prolonged-release matrix in direct contact with the skin for at least 3 days. The improved solubility necessary to achieve an adequate concentration of dissolved buprenorphine base is obtained by means of well tolerated organic acids that do not form practically insoluble salts with buprenorphine in the undercooled mass in the matrix of the TTS. In Europe transdermal buprenorphine was developed by Grünenthal as Transtec®, which was launched in Germany in 2001 and recently approved in major Europoean markets with dosages of 35, 52.5 and 70 µg/h containing 20, 30 and 40 mg buprenorphine per TDS respectively (Fig. 10; Terlinden et al., 2000).

A typical pharmacokinetic feature of transdermal therapy is a lag-time before measurable concentrations of the active substance circulate in the blood with the first TDS (Grond et al. 2000) from a depot of the active substance in the upper layers of the skin. Therefore when assessing efficacy at least 48 h should elapse before deciding whether the selected patch strength is sufficient or whether a switch should be made to a higher dosage. This depot is also the reason why after removal of the TDS the concentration of the active concentration in the blood only decreases gradually. Mechanical stimulation on removal of the patch also increases perfusion of the skin, producing a small plasma level peak. Steady-state plasma levels are usually reached after a few days and are determined more by the flip-flop kinetics of the delayed absorption of the active substance from the TDS through the skin and the completion of the distribution phase of the relatively lipophilic substances in the various compartments, than by the elimination half-life of the active substance.

In line with the trend towards prolonged application intervals, 3M and Purdue have started developing a fentanyl patch that is effective for 7 days. It will be interesting to see how well long-term application and occlusion at a particular site will be tolerated in comparison with the relatively good dermal tolerance of three days' treatment.

Alternative Transdermal Drug Delivery Systems

An alternative approach to transdermal drug application that differs from patches and classic gels led to the foundation of Acrux, an Australian drug delivery company. The key finding was that certain widely used sunscreens, including C_8- to C_{18}-alkyl substituted-cinnamate,

-methoxycinnamate, or -salicylate, caused a significant enhancement of drug absorption through the human skin.

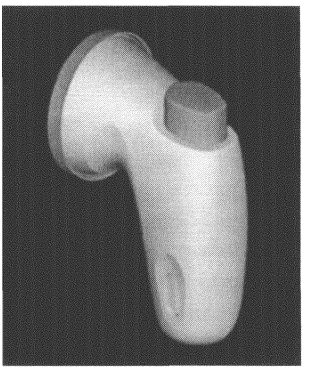


Figure 11: The Acrux MDTS® (Metered Dose Transdermal System) Applicator.

These so-called Across® enhancers are dissolved together with the drug in a volatile solvent for administration. The solution is dosed by means of a volumetric valve combined with a spray nozzle resembling a metered dose inhaler system. Due to its use for transdermal application, the device is called the Metered Dose Transdermal System (MDTS, Fig. 11). The patient positions the unit against the skin and pushes the actuator button to spray a small accurate volume of liquid onto a defined area of skin. The liquid rapidly evaporates leaving an invisible, water resistant depot from which drug is slowly absorbed into the body. This process essentially forms an 'invisible patch' within the upper layer of the skin. One expected advantage of such delivery systems is improved skin tolerability when compared to classical patches. The dosing frequency of these systems would usually be once daily. In addition to formulations containing sex hormones, preliminary results in pigs with fentanyl are available (Klose et al., 2002), exhibiting practically the same absorption rate through human skin as the Duragesic patches (Fig. 12).

Transdermal systems with electrophoretic release of the active substance are under development, e.g. ETRANS® by ALZA with fentanyl for postoperative pain in clinical phase III. By virtue of the electrical field administration of the active substance is faster and on demand, e.g. similar to PCA therapy.

Extremely Long-acting Parenteral Systems

With patches a dosing interval of 1 week may become a possibility in the treatment of pain. Even longer dosing intervals may be achieved by employing implant techniques and formulations that have already become available in the field of hormon treatment including LHRH antagonist formulations. For safety reasons and to remain within the therapeutic window opioids need to be dosed without the 'initial burst' that very often occurs with parenteral microsphere depot formulations based on biodegradable polymers. Very reliable and exact dosing over a long time period has now become available in the form of the DUROS DDS originally developed by ALZA e.g. the once per year Viadur system with leuprolide. This has now been applied to the field of pain control with sufentanyl 3 months depot by DURECT under the brand Chronogesic™, now reaching phase III of clinical development.

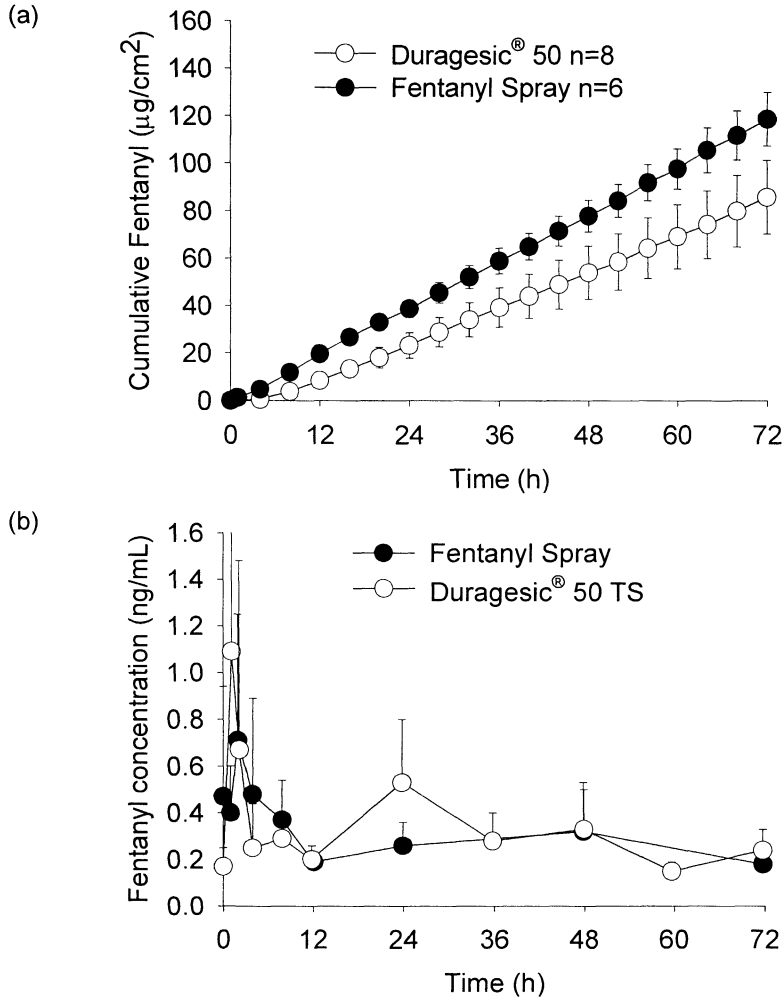


Figure 12: *In vitro* permeation through human skin and *in vivo* animal data after dermal application of fentanyl MDTS. (a) Absorption of fentanyl across human epidermis. Comparison of a fentanyl MDTS® and Duragesic® 50 TS. (b) Mean plasma concentration of fentanyl after a fentanyl MDTS® and Duragesic® 50 TS in male pigs (n = 7).

The DUROS consists of a cylindrical titanium chamber (Fig. 13). The original version was approximately 4 mm in diameter and 4 cm in length; other sizes can be produced (Fisher, 2002). One end of the cylinder is capped with a semipermeable membrane; the other is capped with an orifice designed to permit unidirectional release of the drug. Inside the cylinder, at the membrane end, is a salt tablet, adjacent to which is a piston. The remainder of the cylinder is the reservoir in which the drug is stored.

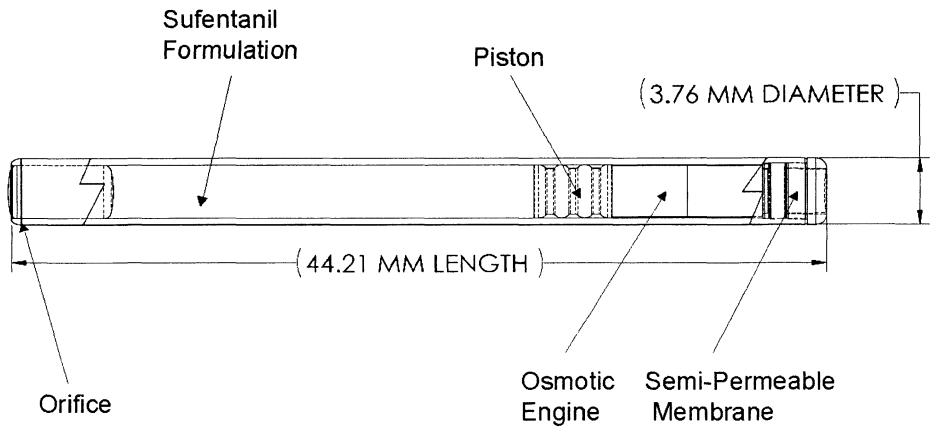


Figure 13: The Durect Chronogesic™ implantable osmotic pump.

During storage in a dry environment, there is no osmotic activity. When the implant is placed in the subcutaneous space or some other body space, there is sufficient relative humidity or water around the implant system to create an osmotic gradient across the membrane. Water is absorbed across the membrane at a zero-order rate that can be controlled to be as low as 0.4 µl (or lower) per day (thereby permitting delivery for 12 months). Water crossing the membrane expands the salt tablet, pushing the piston, and forcing the drug through the orifice into the subcutaneous space from which it is then absorbed.

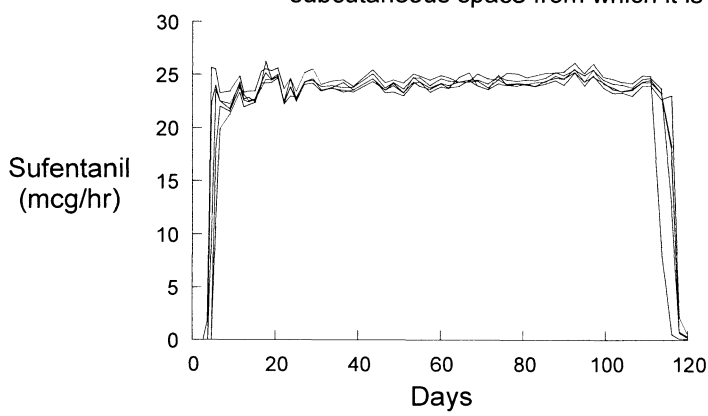


Figure 14: In-vitro release of Sufentanyl from Chronogesic™ DUROS.

Sufentanyl was the selected opioid because it is a very powerful analgesic and could be formulated in a tailor-made high-concentration solution enabling the release of effective doses of drug throughout a 3 months period despite the small capacity of approximately 155 µl filling

volume of the Chronogesic™ system. The zero-order *in vitro* release of sufentanyl from the system is shown in Fig. 14.

The Duros implant requires implantation. To date, clinical trials have examined only one implant site, the mesial surface of the upper arm, ~ 10 cm above the antecubital crease. The procedure is performed on an outpatient basis using local anesthesia and requires less than 10 min tocarry out. The polished titanium surface minimizes any adhesions, permitting rapid explant and reimplant (at the same site) once the device is nearly expended.

Fast-acting Transmucosal Formulations

During treatment with long-acting opioid formulations, breakthrough pain (pain peaks) may occur for which basic therapy does not suffice and which requires rapid treatment. In addition to classical formulations such as drops (e.g. Tramal® or morphine solutions), sublingual buprenorphine formulations (Temgesic® sublingual tablets) and FlashTab® dosage forms that rapidly disintegrate in the saliva and are then swallowed, Actiq®, a new transmucosal form in the shape of a fentanyl lozenge on a stick, (Fig. 15), is already on the market in the USA and Europe. The active substance is incorporated into a matrix of sucrose and glucose syrup on a stick in doses of 200, 400, 600, 800, 1200 and 1600 µg. When pain peaks occur the lozenge is moved around in the mouth, especially along the cheeks, twirled often, and actively sucked in about 15 min. About 25% of the active substance is absorbed through the mucous membranes of the mouth, and one-third of the remaining 75% swallowed is said to be absorbed enterally, giving a total bioavailability of about 50% of the fentanyl dose. Plasma level curves are shown in Fig. 16. Any unused remains of the formulation are destroyed as soon as possible by dissolving under running hot water in a wash-basin. Naturally the system must be child-proof. In the USA a 'welcome kit' is also available, with a child-resistant lock, a portable locking pouch, and a child-proof temporary storage bottle for used lozenges.

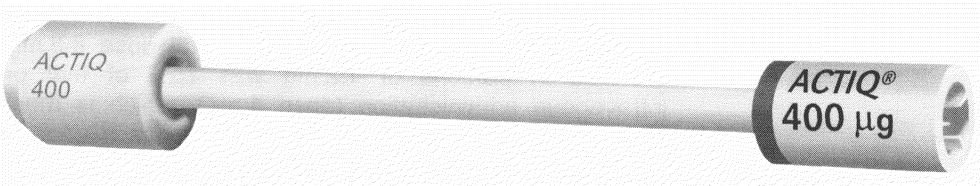


Figure 15: Transmucosal fentanyl drug delivery system Actiq™.

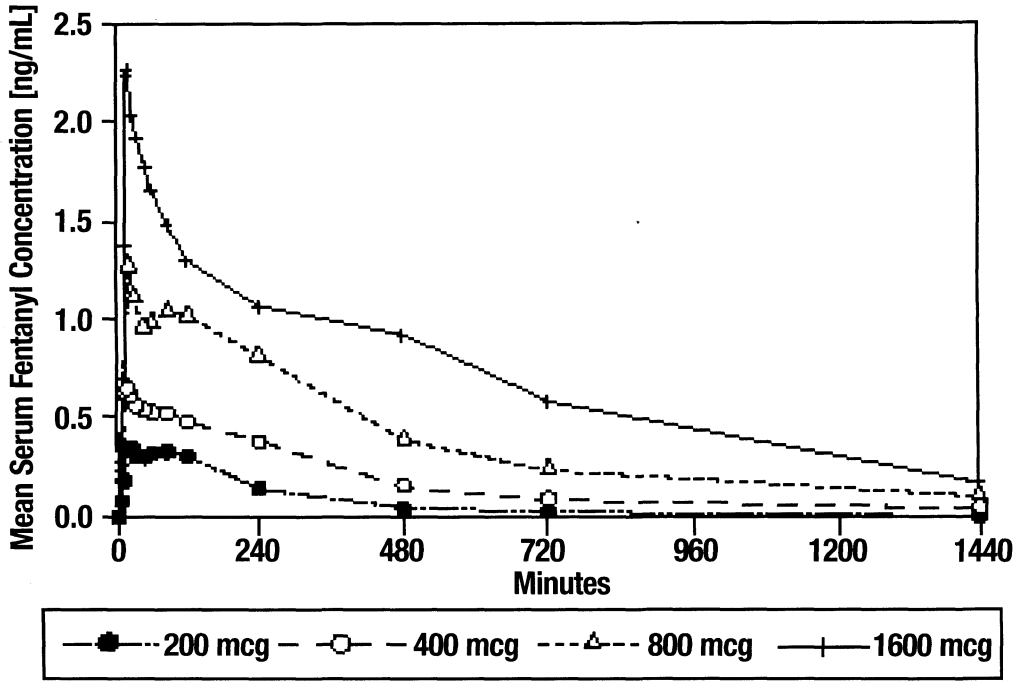


Figure 16: Fentanyl serum levels after sucking Actiq™ at doses of 200, 400, 800 and 1600 µg.

Rapidly Acting Aerosols

In addition to injections, pulmonary administration also allows rapid absorption of analgesics into the blood stream. The AerX® pain management system, which is currently being developed jointly by Aradigm and Glaxo Smith Kline for morphine and fentanyl, produces an aerosol from an active substance solution (Fig. 17).

The active substance is mechanically ejected from a blister through laser-drilled nozzles with a diameter of a few µm and integrated into the blisters by means of punch, to produce a mist consisting of drops measuring 1 - 6 µm. The blister is inserted into an electronically controlled dosing unit that measures the airstream and induces aerosolization in synchrony with inspiration. The bioavailability of morphine sulphate (total dose 8.8 mg) blistered in portions of 1.2 mg was 75% of that on intravenous administration (dose 4 mg), which is much higher than conventional aerosol formulations (Gonda et al., 1999; Otulana et al., 1999).

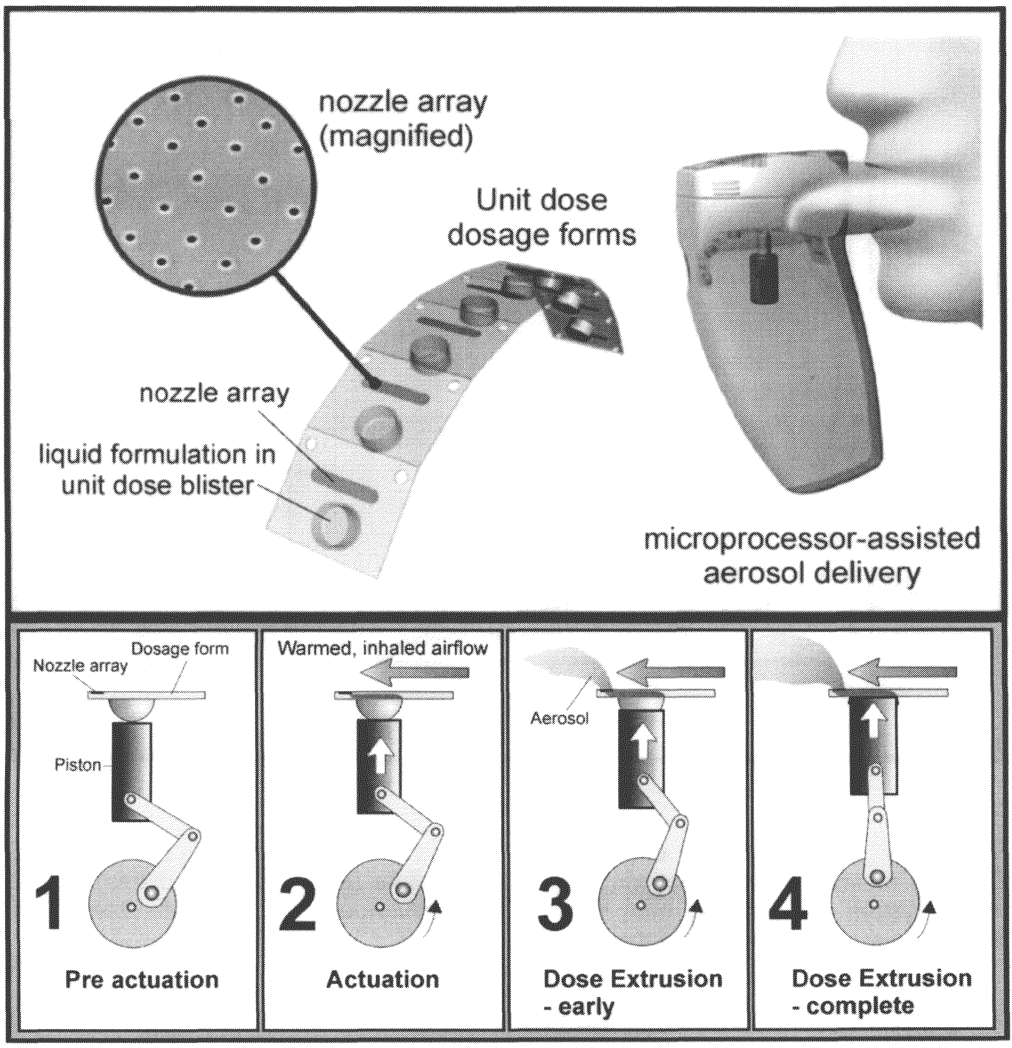


Figure 17: AerX pain management system: the single-dosed active substance solution in the blister is aerosolized by means of a punch through the laser bored nozzles. The blister is inserted into a dosing unit controlled by a microprocessor. Aerosolization is induced by the punch (actuation), when the dosing unit measures a sufficient inspiratory stream and the mist is synchronized with inspiration.

Plasma levels on pulmonary administration corresponded to those resulting from i.v. administration, T_{max} being reached in 2 min (Fig. 18). The electronic control of the AerX system offers interesting opportunities, for example with regard to narcotic safety, such as user identification or lock-out times after a certain number of applications within a certain period to prevent overdosing.

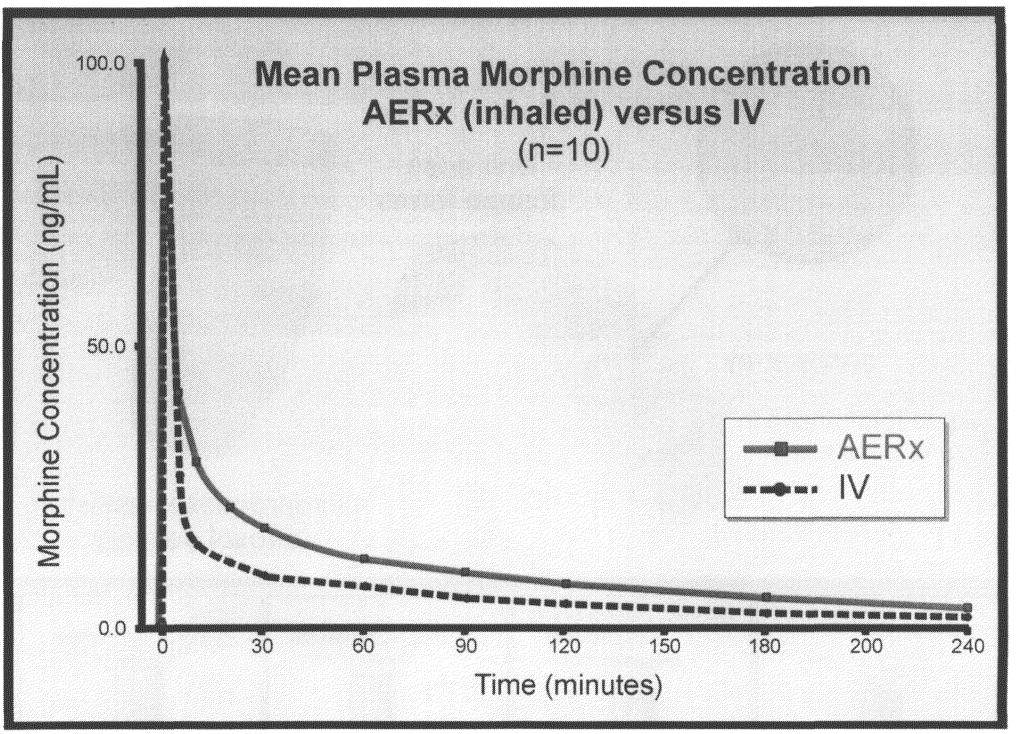


Figure 18: Plasma profiles of inhaled ('AERx') versus intravenous ('IV') morphine. Subjects received four inhalations of morphine sulfate (8.8 mg loaded doses) via the AERx system and 4.0 mg morphine sulfate intravenously on separate days. Both dosages were delivered over a 4 min period.

Conclusions

The development of modern formulations for opioids in the last few years has contributed significantly to pain therapy. New developments will probably optimize pain treatment and thus improve the quality of life of sufferers.

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4 NA and 5-HT Reuptake Inhibitors and α_2 agonists

Thomas M. Tzschentke

The discovery that drugs elevating extracellular levels of noradrenaline and/or serotonin have analgesic potential was circumstantial. In 1960, Paoli et al. reported that during an attempt to treat reactive depression in chronic pain patients with the tricyclic antidepressant imipramine they observed an improvement of the patients´ neuralgic pain. Subsequently, it became well established that antidepressant drugs can improve both depression and chronic pain states.

Use of antidepressants against chronic pain

Over the years, antidepressant drugs have become an important treatment option in chronic pain states, in their own right and as adjuncts to opiate treatment. In fact, tricyclic antidepressants are the mainstay of treatment of neuropathic pain conditions such as polyneuropathy, diabetic neuropathy, postherpetic neuralgia and peripheral nerve injury (Sindrup, 1997; Sindrup and Jensen, 1999). Other chronic pain states responsive to antidepressants include osteo- and rheumatoid arthritis, fibromyalgia, and chronic tension headache.

In preclinical tests, antidepressants can also be effective in acute pain models, but in humans the acute analgesic effects are rather small and of no therapeutic relevance. When used as adjunctive treatment, usually doses lower than those required for the treatment of depression are sufficient for improvement of pain, reducing the side-effects arising from this adjunctive treatment. However, antidepressant treatment has clear beneficial effects only in a proportion of patients (McQuay and Moore, 1997) and may not be equally effective in all chronic pain states (Onghena and van Houdenhove, 1992).

It is interesting to note that despite the very widespread use and the generally accepted efficacy of antidepressants in pain therapy, at present not all antidepressant drugs are formally approved for the treatment of pain. Thus, the widespread use of these drugs is, to some extent, off-label, and there does not seem to be much effort in the pharmaceutical industry to market antidepressants explicitley as analgesic drugs.

One important question to bear in mind when considering the analgesic effectiveness of antidepressant drugs in chronic pain is to what extent this effectiveness is related to a genuine analgesic effect, and to what extent the psychotropic effects of the drugs might contribute to their analgesic effects. It is well known that depressed mood can exacerbate the perception of pain and that chronic pain is often accompanied by depressed mood (´reactive´ depression), thus it appears feasible that drug-induced mood improvement could contribute to a reduction in perceived pain or to an increase in pain tolerance. In the past, a number of possible mechanisms have been proposed to be responsible (at least in part) for the analgesia observed with antidepressants: alleviation of a masked depression, alleviation of a manifest depression, a

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Antidepressant-induced analgesia seems to be largely independent of their mood-altering effects

general sedative effect, or a placebo-like effect (see Onghena and van Houdenhove, 1992). There are several reasons, however, to suggest that antidepressant-induced analgesia is largely independent of their mood-altering effects. Doses of antidepressants needed to produce pain relief are often considerably lower than those that produce antidepressant effects. The onset of analgesia clearly precedes the onset of antidepressant effects; in chronic pain, maximal analgesia is usually achieved within a few days, and in experimental acute pain, antidepressants show an acute analgesic effect, while the antidepressant effects do not become apparent until 2-3 weeks of chronic treatment. Antidepressants that clearly lack sedative effects (e.g. desipramine) show analgesic efficacy, while clearly sedative, benzodiazepines have very little, if any, effect, arguing against the hypothesis that sedative effects contribute to the apparent analgesia (see Onghena and van Houdenhove (1992) for references). This issue is still under discussion, but it seems safe to assume that the main effect is indeed due to a mechanistical pain reduction, although the possibility that in some chronic pain states or in some patients an antidepressant action contributes to the improvement of general well-being and possibly greater pain tolerance cannot be discounted.

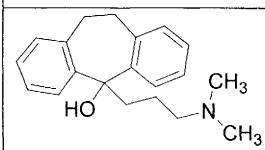
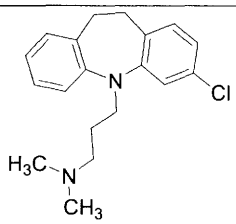
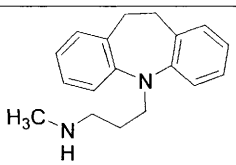
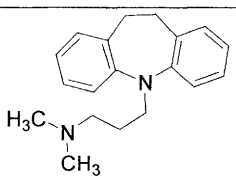
Pharmacological Effects of Antidepressants

Note that there may be a discrepancy between the amount of published data on a particular drug and the actual frequency of clinical use of this drug, since clinical trials are not always well documented in the literature, especially when the trial was unsuccessful

The analgesic action of antidepressant drugs is thought to arise mainly from their ability to block the reuptake of the monoamines noradrenaline and/or serotonin, thus increasing the extracellular levels of these transmitters (see Table 1), although other possible mechanisms of action, such as direct interaction with opiate receptors (Sierralta et al., 1995) or histamine receptors (Rumore and Schlichting, 1986), stimulation of adenosine release or blockade of adenosine uptake (Phillips and Wu, 1982; Sawynok et al., 1999), blockade of sodium channels (Song et al., 2000; Sawynok et al., 2001), blockade of calcium channels (Peroutko et al., 1984), blockade of NMDA receptors (Eisenach and Gebhart, 1995), or blockade of substance P (NK1) receptors (Iwashita and Shimizu, 1992) have also been discussed, in particular with regard to tricyclic antidepressants. The latter effect may be important for the antinociceptive effects of antidepressants in pain states involving central sensitization (Eisenach and Gebhart, 1995). The focus of this chapter, however, will be on the monoamine reuptake inhibition produced by these drugs.

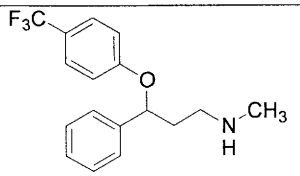
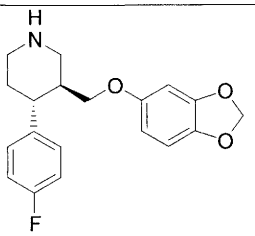
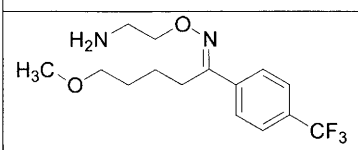
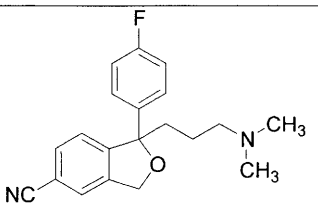
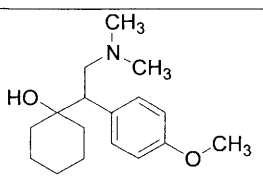
Table 1: Clinical properties of serotonin and noradrenaline reuptake inhibitors (only those drugs are included for which a resonable number of reports on controlled clinical trials are available). For more detailed information see Onghena and van Houdenhove (1992), Philipp and Fickinger (1993), McQuay et al. (1996), and Ansari (2000).

a) TCAs

drug	name/MF/MW/RN	treated symptoms and dosages (examples)	references (examples)
 amitriptyline	5-(3-Dimethylamino-propyl)-10,11-dihydro-5H dibenzo[a,d]cyclohepten-5-ol, $C_{20}H_{23}N$, MW 277.41, [50-48-6]	fibromyalgia (20-40 mg/day for 4-32 weeks); diabetic neuropathy (12.5-150 mg/day for 15 weeks)	Goldenberg et al., 1996; Max et al., 1992
 clomipramine	[3-(3-Chloro-10,11-dihydro-dibenzo[b,f]azepin-5-yl)-propyl]-dimethyl-amine, $C_{19}H_{23}ClN_2$, MW 314.86. [303-49-1]	diabetic neuropathy (75-125 mg/day for several weeks)	Sindrup et al., 1990a
 desipramine	[3-(10,11-Dihydro-dibenzo[b,f]azepin-5-yl)-propyl]-methyl-amine, $C_{18}H_{22}N_2$, MW 266.39, [50-47-5]	diabetic neuropathy (12.5-150 mg/day for 15 weeks)	Max et al., 1992; Sindrup et al., 1990a
 imipramine	[3-(10,11-Dihydro-dibenzo[b,f]azepin-5-yl)-propyl]-dimethyl-amine, $C_{19}H_{24}N_2$, MW 280.42, [50-49-7]	diabetic neuropathy (25-350 mg/day for 7 weeks)	Sindrup et al., 1990b

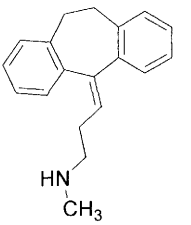
Prominent side-effects common to the whole drug class (magnitude may vary between drugs): weight gain, drowsiness, dry mouth, constipation, orthostatic hypotension, blurred vision, urinary retention, sedation, dizziness, confusion (especially in the elderly)

Table 1: continued. b) SSRIs, SNRIs

drug	name/MF/MW/RN	treated symptoms and dosages (examples)	references (examples)
 fluoxetine	Methyl-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-amine, $C_{17}H_{18}F_3NO$, MW 309.33, [54910-89-3]	headache, migraine (10-80 mg/day for 6-16 weeks); fibromyalgia (20-40 mg/day for 4-32 weeks); *not effective* against diabetic neuropathy (20-40 mg/day for 17 weeks)	Adly et al., 1992; Goldenberg et al., 1996; Max et al., 1992
 paroxetine	3-(Benzo[1,3]dioxol-5-yloxymethyl)-4-(4-fluoro-phenyl)-piperidine, $C_{19}H_{20}FNO_3$, MW 329.37, [61869-08-7]	headache, migraine (10-50 mg/day for 3-9 months); diabetic neuropathy (40 mg/day for 7 weeks)	Black and Sheline, 1995; Sindrup et al., 1990b
 fluvoxamine	5-Methoxy-1-(4-trifluoromethyl-phenyl)-pentan-1-one O-(2-amino-ethyl)-oxime, $C_{15}H_{21}F_3N_2O_2$, MW 318.34, [54739-18-3]	headache, migraine (50-100 mg/day for 8-12 weeks)	Manna et al., 1994
 citalopram	1-(3-Dimethylamino-propyl)-1-(4-fluoro-phenyl)-1,3-dihydro-isobenzofuran-5-carbonitrile, $C_{20}H_{21}FN_2O$, MW 324.40, [59729-33-8]	diabetic neuropathy (40 mg/day for 8 weeks); *not effective* against headache and fibromyalgia (20-40 mg/day for 8 -32 weeks)	Sindrup et al., 1992; Norregard et al., 1995
 venlafaxine	1-[2-Dimethylamino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanol, $C_{17}H_{27}NO_2$, MW 277.41, [93413-69-5]	various forms of chronic pain (headache, migraine, neuropathies) (37.5-300 mg/day for 4-12 weeks)	Taylor and Rowbotham, 1996

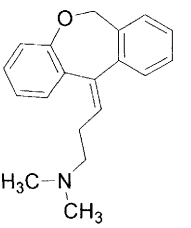
Prominent side-effects common to the whole drug class (magnitude may vary between drugs) agitation: akathisia, insomnia, sexual dysfunction, nausea, gastrointestinal distress/diarrhea, headache, withdrawal effects.

Tricyclic antidepressants (TCAs)



Nortriptyline

Drugs from this class have multiple pharmacological effects. The most prominent and most important action (for both, antidepressant and analgesic effects) is serotonin and noradrenaline reuptake inhibition. In addition, these drugs act as M1-muscarinic antagonists, α1-adrenergic antagonists, and H1-histaminic antagonists. These actions do not contribute to the therapeutic effectiveness of tricyclic antidepressants but are rather responsible for a number of side-effects. All TCAs block serotonin as well as noradrenaline reuptake, albeit with different potencies. Whereas desipramine and nortriptyline show relative selectivity for noradrenaline, clomipramine shows relative selectivity for serotonin, and imipramine and amitriptyline show about equal affinities for both transporters.



Doxepine

By far the most data on the use of TCAs in the treatment of pain is available on amitriptyline. Because much less data are available on the other drugs, it is difficult to say whether the dominance of this drug in clinical studies reflects a superiority of amitriptyline over other drugs. Results from meta-analyses, however, would suggest that other drugs such as imipramine, doxepine or clomipramine may be equally effective but used less often.

The high efficacy of amitriptyline in chronic pain may be in part related to an antagonistic action at NMDA receptors (Eisenach and Gebhart, 1995) (see Table 2).

In preclinical studies, a number of TCAs (imipramine, amitriptyline, nortriptyline, desipramine) were shown to inhibit pain behavior in the formalin test after systemic as well as after i.t. administration, and this effect did not seem to be related to an antiinflammatory effect of these drugs (Sawynok and Reid, 2001). The effects of TCAs in preclinical acute pain models (involving acute thermal or mechanical stimuli) have been reviewed by Eschalier et al. (1999). TCAs were also active in models of chronic inflammation (Butler et al., 1985) and in models of neuropathic pain involving nerve injury (e.g. Ardid and Guilbaud, 1992; Abdi et al., 1998).

There have been numerous clinical studies examining the analgesic effects of TCAs in chronic pain, and the review of these is beyond the scope of this chapter. There are a number of reviews covering these studies (e.g. Onghena and van Houdenhove, 1992; McQuay et al., 1996; Feuerstein, 1997). In contrast, the effects of TCAs in acute pain have not received much attention in clinical research. There are only a few controlled studies with mixed results, reporting no effect of desipramine or amitryptiline on postoperative dental pain when given alone but enhanced

morphine analgesia when combined with desipramine (Levine et al., 1986). In another study, amitriptyline was more effective against acute low back pain than paracetamol (Stein et al., 1996).

Table 2. Pharmacological properties of serotonin and noradrenaline reuptake inhibitors (K_i values [nM]).

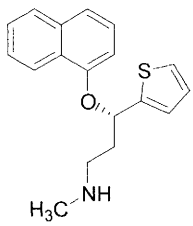
	drug	5-HT transporter	NA transporter	5-HT1A receptor	5-HT2A receptor	H1 receptor	M1 receptor	α1 receptor
TCAs	amitriptyline	70	45	>1000	12	2	32	88
	clomipramine	4	48	>1000	64	41	67	88
	desipramine	400	4	>1000	300	200	220	500
	doxepine	230	35	>1000	54	1	110	33
	imipramine	71	31	>1000	130	19	150	190
SSRIs, SNRIs	fluoxetine	11	340	>1000	770	>1000	>1000	>1000
	paroxetine	1	220	>1000	>1000	>1000	280	>1000
	fluvoxamine	7	620	>1000	>1000	>1000	>1000	>1000
	citalopram	2	>1000	>1000	>1000	>1000		
	venlafaxine	77	538					

SSRIs, NSRIs

After a almost 30-year dominance of TCAs in the treatment of depression, selective serotonin reuptake inhibitors (SSRIs) were introduced in the 1980s. Since then, these drugs have also been used in the treatment of chronic pain (Ansari, 2000). SSRIs appear to be less effective in most patients than TCAs. Meta-analyses have shown that, in general, TCAs seem to be more effective

than heterocyclic antidepressants or newer generation serotonin- or noradrenaline-selective drugs (Onghena and van Houdenhove, 1992; McQuay et al., 1996; Aigner and Bach, 2000, and references therein) (see Table 2). The same pattern of efficacy also seems to apply to acute pain animal models (Ardid et al., 1992). This pattern may be related to the fact that both serotonin and noradrenaline are involved in pain modulation (see below) and that only a delicately balanced elevation of both transmitters yields the most effective analgesia. On the other hand, SSRIs have an undisputedly better side-effect profile than TCAs. At present, SSRIs are generally viewed as second-choice drugs for patients who do not tolerate TCAs well.

More recently, the selective serotonin and noradrenaline reuptake blocker venlafaxine has been introduced. This drug has a dual action on monoamine reuptake like classical TCAs, yet lacks the side effect-critical affinities for histaminergic, adrenergic and muscarinergic receptors. Venlafaxine has also been used in the treatment of chronic pain, with some success (see Ansari, 2000). It is currently being developed for neuropathic pain. Duloxetine is in clinical development as an antidepressant drug. It has the same mechanisms of action as venlafaxine, but with 10-15-fold higher affinitiy for the NA- and 5-HT transporters. Whether this drug is more effective against chronic pain than venlafaxine remains to be seen.



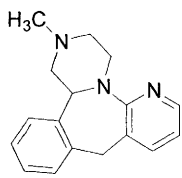
Duloxetine

Others

Due to their diverse pharmacological actions, and for want of a better categorization, the drugs mentioned below are often referred to as ´atypical antidepressants´, although eventually all of them also act via enhancement of noradrenergic and/or serotonergic neurotransmission.

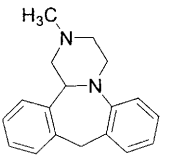
Mirtazapine, an analog of mianserine (see below), is a drug that combines α2-antagonistic properties (predominantly at inhibitory autoreceptors, thus increasing activity of noradrenergic and serotonergic neurons) with 5-HT2, 5-HT3 and histamine H1 antagonistic properties. 5-HT2 and 5-HT3 antagonism is thought to reduce sexual dysfunction and gastrointestinal problems, respectively, side-effects often observed with SSRIs. We are unaware of any published studies on the use of mitazepine in the treatment of pain.

Mianserine is an α2-antagonist with additional α1, 5-HT2, H1 antagonistic properties. It has been studied extensively for the treatment of chronic pain syndromes, with mixed results (see Ansari, 2000, for references).



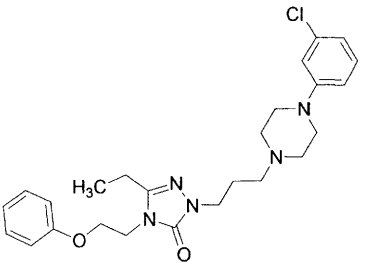
Mirtazapine

2-Methyl-1,2,3,4,9,13b-hexahydro-2,4a,5-triaza-tribenzo[a,c,e]cycloheptene, $C_{17}H_{19}N_3$, MW 265.36, [61337-67-5]



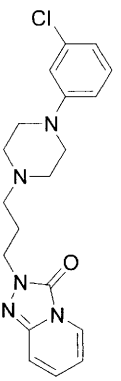
Mianserine

2-Methyl-1,2,3,4,9,13b-hexahydro-2,4a-diaza-tribenzo[a,c,e]cycloheptene, $C_{18}H_{20}N_2$, MW 264.37, [24219-97-4]



Nefazodone

2-{3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl}-5-ethyl-4-(2-phenoxy-ethyl)-2,4-dihydro-[1,2,4]triazol-3-one, $C_{25}H_{32}ClN_5O_2$, MW 506.48, [82752-99-6]



Trazodone

2-{3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl}-2*H*-[1,2,4] triazolo[4,3-*a*]pyridin-3-one, $C_{19}H_{22}ClN_5O$, MW 371.87, [19794-93-5]

Nefazodone blocks the reuptake of serotonin and noradrenalin and is a 5-HT2 antagonist. This drug is also launched as an analgesic and is being developed for migraine. We are unaware of any published studies in the literature on the use of mitazepine in the treatment of pain.

Trazodone blocks serotonin reuptake and is a 5-HT2, α1, and H1 antagonist. Thus far, the findings on the analgesic efficacy of trazodone are few and inconsistent (Goodkin et al., 1990; Wilson, 1999; see Ansari (2000) for further references).

The Descending Inhibitory Pain System as a Major Target for Noradrenaline and Serotonin Reuptake Inhibitors

The first convincing evidence for a descending inhibitory system came from the discovery of stimulation-induced analgesia: electrical stimulation of the midbrain periaqueductal grey (PAG) produced a highly specific suppression of behavioral responses to noxious stimuli (Reynolds, 1969). Later on, it was discovered that the PAG is part of a CNS circuit that controls the transmission of nociceptive information at the level of the spinal cord. It is in a position to integrate descending information from limbic cortical and diencephalic areas and ascending information from the spinal dorsal horn (Bandler and Keay, 1996). The PAG receives inputs from a number of monoaminergic brainstem nuclei (Herbert and Saper, 1992); in turn, its major descending afferents project to the the rostral ventrolateral medulla (RVM), including the serotonergic nucleus raphe magnus, and to the dorsolateral pons (DLP), including the noradrenergic cells groups A5 (locus coeruleus) and A7 (Cameron et al.,

1995). The RVM and DLP are also reciprocally interconnected (Clark and Proudfit, 1991a), and electrical stimulation of the RVM, like stimulation of the PAG, produces analgesia and inhibits dorsal horn responses to noxious stimuli (Basbaum and Fields, 1984). The analgesic effects of PAG stimulation at the spinal level appear to be relayed in large part via the RVM, since inactivation or inhibition of the RVM largely reduces the spinal effects of PAG stimulation (Behbehani and Fields, 1979). The PAG also projects rostrally to the orbitofrontal cortex and the medial thalamus (Coffield et al., 1992). These projections may underlie a possible ascending control of nociception.

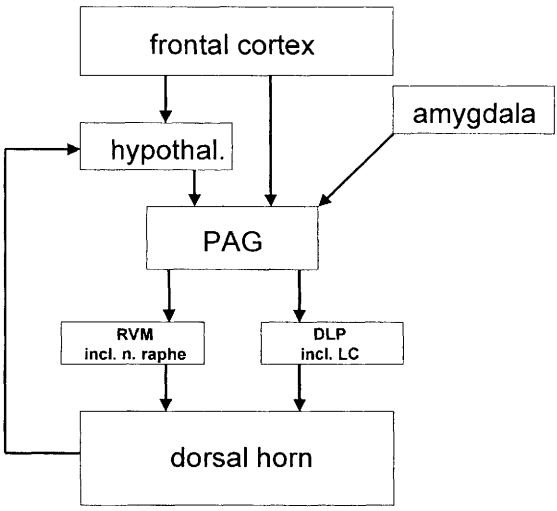


Figure 1: Descending pain modulary pathways: a simplyfied diagram.

Serotonergic and noradrenergic fibers travel from the brain stem through the dorsal lateral funiculus to the spinal cord and terminate in the dorsal horn where they modulate pain signals coming from the periphery (see Fig. 1). These projections have classically been described in terms of descending inhibitory pathways. More recently, evidence has been accumulating that these descending pathways may not only be of inhibitory nature but may modulate spinal pain transmission in a rather complex manner, including facilitatory mechanisms. However, a more detailed consideration of this issue is beyond the scope of this chapter (see Mason (1999) and references therein for details).

Serotonergic and non-serotonergic fibers originating in the RVM terminate predominantly in dorsal horn laminae I, II (substantia gelatinosa), and V, which are the main targets

of nociceptive primary afferents (Basbaum et al., 1978). Laminae I and II both contain excitatory and inhibitory interneurons, and both types of interneurons receive input from RVM projections.

The A5 and A7 cell groups are the main source of noradrenergic fibers projecting to the dorsal dorn (Clark and Proudfit, 1991b), and as in the case of PAG and RVM, electrical stimulation of these cell groups inhibits spinal withdrawal reflexes in response to noxious stimulation and the activity of dorsal horn nociceptive neurons (Carstens et al., 1980). In general, the inhibition of nociceptive transmission produced by noradrenaline at the spinal level is mediated via the α2-adrenoceptor (West et al., 1993; Willis and Westlund, 1997). This is consistent with the fact that spinally administered α2-agonists can produce potent analgesia (Curatolo et al., 1997; see below).

Serotonin and noradrenaline probably act both presynaptically on the terminals of nociceptive afferent fibers to reduce the release of excitatory transmitters such as glutamate, CGRP, or substance P (Fürst, 1999) and to increase the release of inhibitory transmitters such as enkephalins and GABA (Feuerstein, 1997; van Schayck et al., 1998), and postsynaptically to activate inhibitory (enkephalinergic or GABAergic) interneurons within the dorsal horn (Alhaider et al., 1991), or to directly inhibit ascending spinothalamic tract neurons (Giesler et al., 1981) (see Fig. 2).

Serotonin and noradrenaline probably act both presynaptically on the terminals of nociceptive afferents and postsynaptically

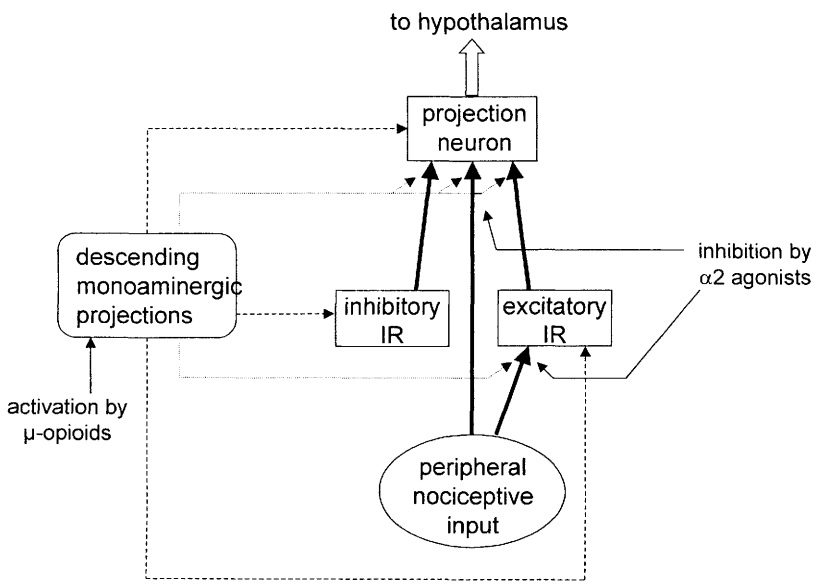


Figure 2: Neuronal circuitry in the dorsal horn of the spinal cord -simplified diagram.

From this anatomical organization it becomes clear that elevation of serotonin and noradrenaline can dampen transmission of nociceptive information at the spinal level via multiple mechanisms.

Possible Supraspinal Sites of Action and Interaction with Endogenous and Exogenous Opioids

Opioid-induced antinociception depends, to some degree, on monoaminergic signaling in the spinal dorsal horn. While opioids can act directly on dorsal horn terminals of primary afferent nociceptive fibers or on excitatory interneurons in lamina II of the dorsal horn to reduce the release of excitatory transmitters (Glaum et al., 1994), the supraspinally mediated analgesic effects of opioids, at least in part, involve interactions with central and spinal serotonergic and noradrenergic transmission.

By acting on monoaminergic brainstem nuclei, either directly or via action in the PAG (Bowker and Dilts, 1988; Budai et al., 1998; see Mason, 1999), opioids may activate descending monoaminergic inhibitory pathways that contribute to the analgesia produced by the activation of non-monoaminergic inhibitory mechanisms (Fitzgerald, 1986; Proudfit, 1988; Borszcz et al., 1996; but see Matos et al., 1992; Gao et al., 1997, 1998) (see Fig. 2). Although the extent of monoamine involvement in opioid action appears to depend on the type of painful stimulus and the test used to assess antinociception, the analgesic effects of intracerebral morphine are attenuated by blockade of serotonergic and α2-adrenergic receptors at the spinal level (Yaksh, 1979; Proudfit, 1988).

Serotonin is also involved in opioid effects on the transmission and processing of nociceptive information at a level rostral to the PAG. Borszcz (1999) and Borszcz and Streltsov (2000) have demonstrated that the antinociceptive effect of morphine administered into the PAG can be attenuated be serotonin antagonism in the central nucleus of the amygdala and the parafascicular nucleus of the thalamus, suggesting that serotonin is also involved in pain processing in higher brain centers.

This discussion shows that the well-documented enhancement of opioid effects by antidepressants (´opioid-sparing effect´) is likely to be related to a synergistic effect on monoaminergic transmission, possibly both at the spinal and at the supraspinal level. This synergistic interaction is based on the blockade by antidepressants of the reuptake of serotonin and/or noradrenaline released by the action of an opioid.

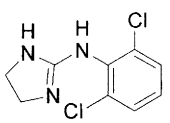
Analgesia Produced by α2-Agonists

In contrast to antidepressant drugs, directly-acting α2-agonists such as clonidine or demedetomidine are effective not only in chronic pain states, but also in acute pain. Unfortunately, these agents produce side effects such as cardiovascular depression and sedation when administered systemically; thus, they are most commonly administered by the epidural or intrathecal route, because side-effects are less pronounced following application via these routes. α2-agonists, like antidepressants, besides having analgesic effects, also have opiate-sparing (Motsch et al., 1990), anesthetic-sparing (Bonnet et al., 1990) and anxiolytic effects, making them useful adjuvants in clinical practice.

α2-Agonists Used Clinically

Clonidine has been launched for the treatment of cancer pain (Boehringer Ingelheim). It is effective against malignant and non-malignant pain after spinal administration (Glynn et al., 1988; Eisenach et al., 1995).

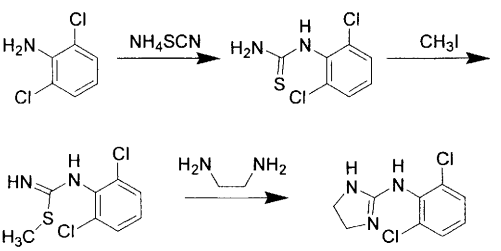
Clonidine



(2,6-Dichloro-phenyl)-(4,5-dihydro-1H-imidazol-2-yl)-amine, $C_9H_9Cl_2N_3$, MW 230.10, [4205-90-7]

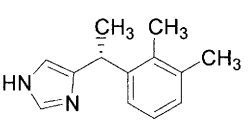
Clonidine can also be effective and tolerable when administered by the oral or transdermal route (Zeigler et al., 1992).

Synthesis (Zeile et al. (Boehringer Ingelheim), 1965; Kleemann et al. 1999):



Scheme 1: Synthesis of clonidine.

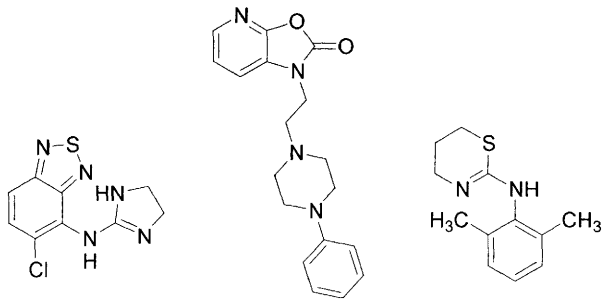
Dexmedetomidine



4-[1-(2,3-Dimethyl-phenyl)-ethyl]-1H-imidazole, $C_{13}H_{16}N_2$, MW 200.28, [113775-47-6]

Dexmedetomidine was launched for anesthesia and postoperative pain in the US in 2000 and in Australia in 2001. According to Pharmaprojects, dexmedetomidine alone is effective as an analgesic in 41-44% of patients. Treated patients who require additional analgesics achieve pain relief with half the dose required by those treated with placebo. Dexmedetomidine is generally well tolerated, possible side-effects in the high dose range are nausea, hypotension, decreased heart rate, atrial fibrillation and hypoxia,. The anxiolytic, sedative, analgesic, hemodynamic, stabilizing and anesthetic

potentiating effects have been demonstrated in several clinical trials (Orion Pharma; Abbott).



Tizanidine
launched as a muscle relaxant

S-12813-4

Xylazine

Scheme 2: α2-agonists with preclinical effectiveness.

Anatomy and Neuropharmacology of α2-Agonist-Mediated Analgesia

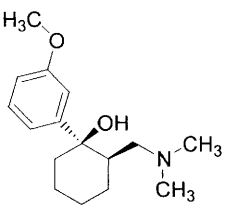
In the spinal cord, α2-agonists act on receptors located on the terminals of primary afferent fibers in the dorsal horn substantia gelatinosa to reduce nociceptive transmission by inhibiting the release of glutamate and substance P (Collin et al., 1994; Hamalainen and Pertovaara, 1995) (see Fig. 2). These receptors appear to be primarily of the α2A subtype which is negatively coupled to adenylate cyclase (Lakhlani et al., 1997; see Millan, 1999; but see Sawamura et al., 2000, and references therein for a discussion of the possible involvement of other α2-receptor subtypes in antinociception). Like activation of μ-opioid receptors, the activation of α2-receptors increases the potassium conductance of the cells bearing these receptors, thus reducing cellular excitability.

In mice with mutations in the α2A-receptor gene it was established that the α2A subtype mediates the analgesic and anesthesia-sparing effects of clonidine and dexmedetomidine, but unfortunately also the sedative and vasodepressor effects of these drugs (Lakhlani et al., 1997). Thus, it seems unlikely that new subtype-selective compounds will lack the major side-effects of the existing drugs.

Chronic constriction injury (CCI) models (Bennett and Xie, 1988; Kim and Chung, 1992; Mosconi and Kruger, 1996) have been used to examine chronic pain states in experimental animals. In these models, intrathecally administered α2-agonists reduced mechanical allodynia and thermal hyperalgesia that developed following nerve constriction (Levy et al., 1994; Yaksh et al., 1995). This

action may be partly due to the fact that α2-agonists inhibit the abnormally prolonged response to mechanical stimulation of some mechanoreceptors that develops after nerve ligation (Na et al., 1993). Interestingly, i.t. clonidine can also restore the reduced antinociceptive potency of i.t. morphine in the tail-flick test in nerve-ligated animals (Ossipov et al., 1995), and morphine-tolerant animals remain normally responsive to i.t. serotonin or noradrenaline (Reddy et al., 1980). This is consistent with the observation that in nerve-ligated rats, repeated treatment with desipramine does not result in tolerance to its antihyperalgesic effects (T. Christoph, Grünenthal GmbH, unpublished results). Dexmedetomidine showed antinociceptive actions against carrageenan-induced inflammatory pain and nerve injury pain in the rat (Idanpaan et al., 1994; Poree et al., 1998). It appears to be much more potent than clonidine and does not show an analgesic ceiling effect as is sometimes seen with clonidine (Sullivan et al., 1992).

Tramadol



(see chapter 3.4)

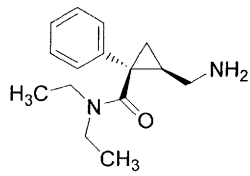
The ´atypical´ opioid drug tramadol has a dual mechanism of action. An important part of its analgesic effects is mediated by the agonistic activity of (+)-tramadol and its O-desmethyl-metabolite at μ-receptors. However, another part of its effects is mediated by noradrenaline and serotonin reuptake inhibition. Noradrenaline reuptake is inhibited primarily by the (-)-enantiomer of tramadol, while the reuptake of serotonin is primarily inhibited by the (+)-enantiomer (Frink et al., 1996), and it has been shown in preclinical models that reuptake inhibition by tramadol contributes to its overall analgesic effects, and that these different mechanisms can yield supra-additive analgesic effects (Kayser et al., 1992; Raffa et al., 1992; Miranda et al., 1999). This augmentation of the opioid effect by monoaminergic mechanisms may be the reason why tramadol can produce very satisfactory analgesia despite its relatively low μ-receptor affinity.

Recent Developments

Antidepressants as analgesics are almost a ´closed book´ as far as preclinical and clinical development is concerned. TCAs are an old drug class, and because of the rather problematic side-effect profile, interest in developing new drugs from this class is small. BL-1834 (Bioglan Lab.) is an intranasal formulation of doxepine that is in clinical development (phase II) for the treatment of severe pain. In patents on novel monoamine reuptake inhibitors, pain is usually claimed as a possible indication, but depression and anxiety are mentioned as the primary indications in most cases, and we are not aware of novel

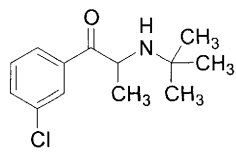
monoamine reuptake inhibitors that are exclusively developed for pain.

Among the established non-TCA drugs, nefazodone is currently being developed for migraine, and venlafaxine and dexmedetomidine are being developed for neuropathic pain.



Milnacipran

Milnacipran (Pierre Fabre), an equipotent serotonin and noradrenaline reuptake inhibitor, was launched in 1997 as an antidepressant and was licensed for development for the treatment of fibromyalgia and related chronic pain disorders in 2001. It is currently in late preclinical development.



Bupropion

The noradrenaline and dopamine reuptake inhibitor bupropion (GlaxoSmithKline) is currently in clinical development (phase II) for neuropathic pain.

Other novel antidepressants in development act on various targets other than noradrenaline or serotonin transporters; thus, the role of these drugs as analgesics is speculative, and its consideration is beyond the scope of this chapter.

VAN-H36 (Vita-Invest) is a serotonin and noradrenaline reuptake inhibitor and μ-receptor agonist (i.e. has a pharmacological profile of action similar to tramadol). It is in early clinical development as an analgesic.

RWJ-37210 (Johnson & Johnson) is the lead structure of a series of α2-receptor agonists in preclinical development for the treatment of pain.

Outlook

One point for future consideration is that the effects of antidepressants (and drugs like tramadol) may be limited by the activation of somatodendritic 5-HT1A autoreceptors in the raphe nucleus, resulting from the increased extracellular levels of serotonin produced by these drugs. This suggestion is supported by the findings that 5-HT1A-receptor blockade by pindolol accelerates the antidepressant effects of SSRIs (Artigas et al., 1996) and enhances the analgesic potency of tramadol, while the 5-HT1A-receptor agonist 8-OH-DPAT reduces the effects of tramadol (Rojas-Corrales et al., 2000). To our knowledge, it has not been determined whether 5-HT1A-receptor blockade would also enhance the antinociceptive effects of antidepressants, although this strategy would seem promising given the findings outlined above.

Thus, although major breakthroughs in the development of serotonin and noradrenaline reuptake inhibitors as analgesics are unlikely, a refinement of treatment and co-treatment methods may still hold substantial potential in yielding improved therapeutic effectiveness of this drug class.

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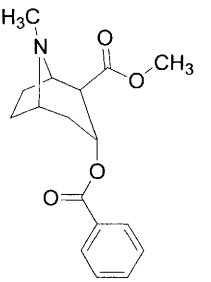
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6 Voltage-gated Ion Channels

6.1 Sodium Channels

Petra Bloms-Funke

Voltage-dependent sodium channels have long been recognized as potential drug targets for the treatment of pain. In fact, even before the mechanism of sodium channel blockade had been elucidated, Carl Koller had introduced in 1884, the use of the naturally-occurring alkaloid cocaine in clinical surgery, taking advantage of its local anesthetic properties (Vandam, 1987). Today sodium channel blockers, including local anesthetic, anticonvulsant and antiarrhythmic agents, are clinically used for local anesthesia and chronic pain management. As sodium channels are ubiquitously responsible for rapid excitatory transmission, the therapeutic window for the use of systemic sodium channel blockers is limited due to toxic side-effects especially on the cardiovascular and the central nervous system. The application of molecular biological techniques has led to the identification of sodium channel subtypes preferentially expressed in primary sensory neurons, thus opening promising approaches to find effective and well-tolerated sodium channel blockers in particular for their use in chronic pain (for review see Waxman et al., 2000; Anger et al., 2001; Wood et al., 2002).



The natural alkaloid **cocaine** was introduced as the first local anesthetic drug in clinical surgery

Voltage-gated Sodium Channels: Structure and Function

Voltage-gated sodium channels are large glycoproteins that form voltage-dependent, Na^+-selective pores through the plasma membrane of electrically excitable cells (i.e. neurons, cardiac cells, muscle cells). Their primary function is to generate the rapid regenerative upstroke of action potentials as first described by Hodgkin and Huxley (1952). They play a central role in modulation of the firing activity of excitable cells and in the transmission of depolarizing impulses through the neuronal network including pain pathways.

Voltage-gated sodium channels generate the rapid regenerative upstroke of action potentials and hence play a central role in modulation of the firing activity of excitable cells. They form heterotrimeric transmembrane Na^+-conducting pores

Sodium channels are densely expressed in axons, somata and dendrites of neurons. In the rat brain and spinal sensory neurons, sodium channels are heterotrimeric integral membrane proteins consisting of one major pore-forming α-subunit which associates with two smaller auxiliary β-subunits (one $\beta1$ or $\beta3$ assembled with one $\beta2$ subunit; Black et al., 1996; Morgan et al., 2000; for review see Catterall, 1992; Denac et al., 2000). The deduced

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primary structure of the α-subunit indicates four homologous domains (DI - DIV) each of which built up with six potential α-helical transmembrane segments (S1 - S6). There are several phosphorylation and glycosylation consensus sequences allowing differential modulation of channel function (Bennett et al., 1997; Fitzgerald et al., 1999; Tyrrell et al., 2001). Although the α-subunit alone is capable of establishing functional channels, the β1-subunit is crucial for rapid rates of channel activation and inactivation (Patton et al., 1994). Recently a β3-subunit, a β1-like member of the family, was cloned which shows largely a complemantary distribution to β1 and inactivates the sodium current more slowly when expressed in oocytes of the South African clawed frog, *Xenopus laevis* (Morgan et al., 2000). The β2-subunit is proposed to be an important regulator of channel expression and localization in neurons (Isom et al., 1995).

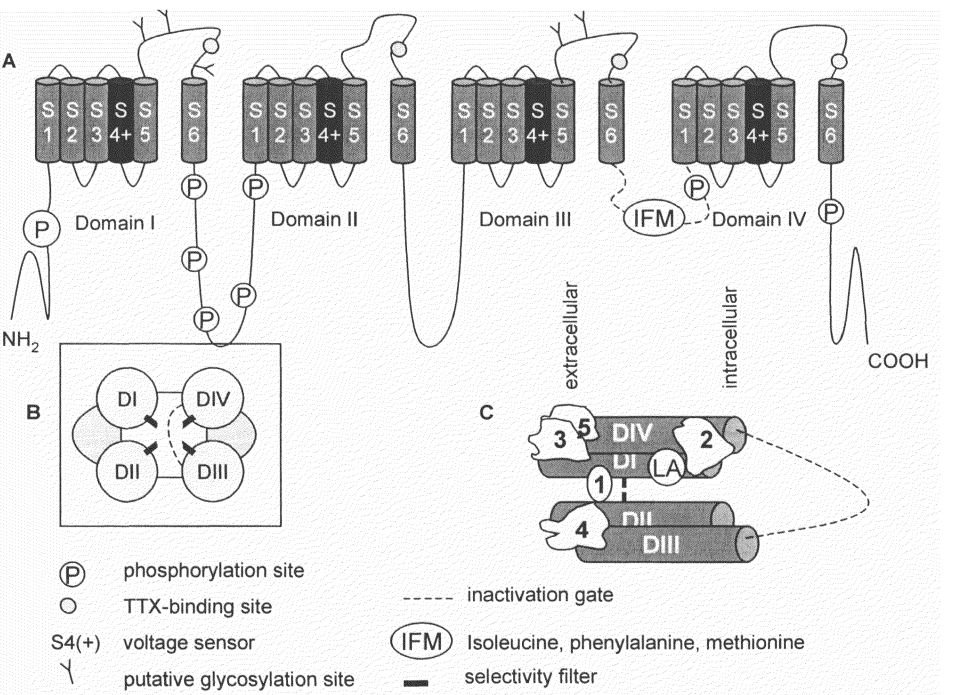


Figure 1: Topology of the α-subunit of voltage-gated sodium channels. (**A**) The α-subunit is built up of four homologous transmembrane domains (DI - DIV) each containing six transmembrane segments (S1 - S6). The extracellular and intracellular loops contain putative glycosylation and phosphorylation sites, as indicated. The IFM-motif is probalby crucial for channel inactivation. (**B**) Transmembrane arrangement of DI - DIV domains around the channel pore. (**C**). Binding sites (1 - 5) of the α-subunit. LA: putative local anesthetic binding site. TTX, tetrodotoxin. (Adapted from Denac et al., 2000).

Sodium channel blockers including local anesthetic, anticonvulsant and antiarrhythmic drugs exhibit voltage- and frequency-dependent blockade which makes them useful drugs for blocking hyperexcitable neurons

On depolarization of the plasma membrane, sodium channels are rapidly activated leading to a dramatic increase in Na^+ permeability and thus to an inward current of Na^+ ions into the cell. The fourth transmembrane segment, S4, of the α-subunit is believed to be the voltage sensor: According to the sliding helix model (Caterall, 1986), depolarization induces a spiral-shaped motion of the positively charged transmembrane α-helix with an outward displacement by 5 Å finally leading to channel activation. The open channel closes rapidly within a range of 0.5 to several milliseconds transforming to an 'inactivated', refractory state. The isoleucine, phenylalanine, methionine (IFM) motif within the intracellular loop between domains DIII and DIV is proposed to function as an inactivation gate (for review see Denac et al. (2000)). In addition to fast inactivation, sodium channels show a slow component of inactivation which lasts for several seconds. Repriming or reactivation of sodium channels requires repolarization of the plasma membrane leading to the so-called 'resting' state, which is non-conducting, but activatable.

Taking into account the fast gating properties of voltage-gated sodium channels, a simplified three-state model has been developed to understand voltage- and frequency-dependent properties which are characteristic for many sodium channel blockers including local anesthetic, anticonvulsant, and antiarrhythmic drugs (Caterall, 1987; Ragsdale et al., 1996; Marban et al., 1998). Voltage-dependency is attributed to a high binding affinity of the drug to the channel in the inactivated state ($k_3/k_{-3} \gg k_1/k_{-1}$, k_2/k_{-2}; Fig. 2). Thereby, the channel is stabilized in this state and the inactivation curve is shifted to more negative potentials, thus the extent of channels in the inactivated state is increased at the resting membrane potential . For example, the anticonvulsant drugs carbamazepine, phenytoin and lamotrigine have been shown to shift the voltage of half-maximal inactivation from -67 mV under control conditions to -74, -79 and -82 mV, respectively, at drug concentrations of 100 µM each as shown in a comprehensive study by Lang et al. (1993). Frequency dependency is explained by a faster rate of drug binding to the activated state ($k_2 \gg k_1, k_3$). If the drug detaches after a repeated stimulus, the amount of bound drug and thus channel block increases with repeated stimuli until the rates of association and dissociation are equal. This accumulated block during trains of stimulation is called use-dependency or also phasic block. The antagonistic potencies of the piperidine-derived local anesthetic drugs mepivacaine, ropivacaine, and bupivacaine, for instance, were increased 2-5 - fold by the 10^{th} pulse of a stimulus

train at a frequency of 2 Hz as compared to the block by the first pulse (i.e. tonic block; Bräu et al., 2000). Voltage- and frequency-dependent blocking mechanisms give rise to a preferred block of highly active cells and comparatively less interference with normal physiological sensory and motor function. With regard to chronic pain conditions these properties of many sodium channel blockers make them useful for blocking pain pathways with hyperexcitable neurons.

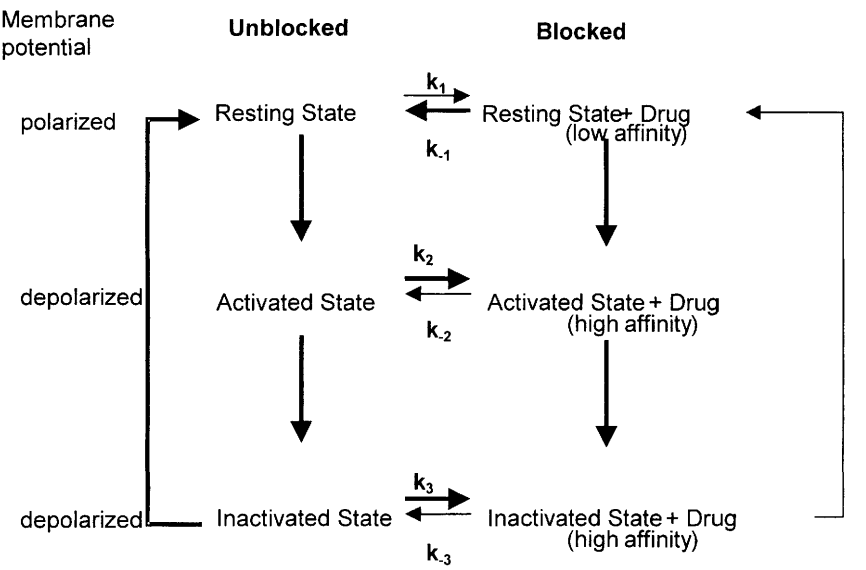


Figure 2: A simplified three-state model of sodium channel gating.

A variety of toxins that modulate voltage-gated sodium channels have been used to probe channel function. They can be classified on the basis of five discrete binding sites (Table 1). These binding sites are commonly found on all α-subunits and are being characterized at the molecular level. The sensitivity to the puffer fish poison tetrodotoxin (TTX) has been used to subdivide voltage-gated sodium channels (Table 3).

The puffer fish poison tetrodotoxin (TTX).

(4*R*,4a*R*,5*R*,7*S*,9*S*,10*S*,10a*R*,11*S*,12*S*)-Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol, $C_{11}H_{17}N_3O_8$, MW 319.27, [4368-28-9]

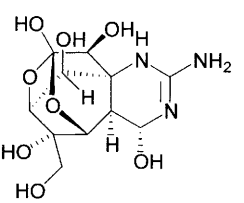


Table 1: Toxin and drug binding sites on α-subunits of voltage-gated sodium channels (Catterall, 1992).

Site	Modulators	Effect
1	Tetrodotoxin Saxitoxin	Inhibition of ion conductance
2	Batrachotoxin Veratridine	Persistent activation
3	α-Scorpion toxin Sea anemone toxins	Delay of inactivation, enhancement of persistent activation
4	β-Scorpion toxin	Shift of voltage-dependency of activation
5	Brevetoxin	Repetitive firing; enhancement of activation and block of inactivation
	Local anesthetics, antiarrhythmic, anticonvulsant drugs	Voltage- and use-dependent block

It has been found that six distinct α-subunits are expressed and often co-expressed in the primary sensory neurons, which play an important role in pain transmission. They are activated by nociceptive stimuli on the body surface, skeletal muscle and viscera and encode this information in the form of a series of action potentials which are conveyed to the spinal cord and brain (Black et al., 1996; Goldin et al., 2000; for review see Waxman et al., 2000; Wood et al., 2002). Among the heterogeneous sodium channel subtypes in the primary sensory neurons, $Na_v1.8$ and $Na_v1.9$ are characterized by their resistance to tetrodotoxin (TTX) with IC_{50} values in a micromolar range compared to low nanomolar ranges for TTX-sensitive (TTX-S) subtypes (Table 2).

Channel	Previous name	Gene symbol	Primary tissue
$Na_v1.1$	Type I	SCN1A	CNS, PNS
$Na_v1.2$	Type II	SCN2A	CNS, PNS
$Na_v1.3$	Type III	SCN3A	CNS, (PNS after axotomy)
$Na_v1.4$	SkM1	SCN4A	Skeletal muscle
$Na_v1.5$	SkM2	SCN5A	Heart
$Na_v1.6$	NaCh6	SCNA8A	CNS, PNS
$Na_v1.7$	PN1 / NaS	SCN9A	CNS, PNS
$Na_v1.8$	PN3 / SNS / NaNG	SCN10A	PNS
$Na_v1.9$	NaN / SNS II	SCN11A	PNS
Na_x	$Na_v2.1$ / NaG	SCN6A / SCN7A	heart, uterus, CNS, PNS (glia-specific)

CNS / PNS: central / peripheral nervous system.

Table 2: Subtypes of voltage-gated sodium channel α-subunits: nomenclature and distribution (Goldin et al., 2000)

In neuropathic pain conditions, TTX-resistant channels are downregulated and hyperexcitability of the injured primary sensory neurons is predominantly due to TTX-sensitive channels

Following nerve injury or inflammation of innervated peripheral tissue, primary sensory neurons become hyperexcitable showing spontaneous firing or abnormal high-frequency activity characteristic for chronic pain situations (Nordin et al., 1984; Zhang et al., 1997; Amir et al., 1999). At the same time, the sodium channel subtypes $Na_v1.3$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are distinguished by preferential distribution in primary sensory neurons and by temporally- and regionally-specific regulation of gene expression, respectively, which qualifies them as potential drug targets for selective treatment of chronic pain. The mRNA of $Na_v1.3$, which is expressed in embryonic, but not adult spinal sensory neurons, is re-expressed in animal models following injury of peripheral nerves either by axotomy or by chronic constriction injury (Waxman et al., 1994; Dib-Hajj et al., 1999; Kim et al., 2001). Since $Na_v1.3$ sodium channels are rapidly repriming, their upregulation might give rise to abnormal high-frequency activity which is recorded in dorsal root ganglions (DRGs) after nerve injury (Amir et al., 1999; Cummins et al., 2001). The $Na_v1.7$ channel, which is highly expressed in DRGs under normal conditions, shows a unique ability to activate upon slow membrane depolarization and has the ability to amplify small excitatory inputs like sensory generator potentials close to resting potential (Cummins et al., 1998). Therefore, the $Na_v1.7$ channel might play a crucial role in modulation of spontaneous activity of DRG neurons. Additionally, two distinct TTX-resistant (TTX-R) sodium channels $Na_v1.8$ and $Na_v1.9$ are densely expressed within a subpopulation of small- and medium-diameter, unmyelinated DRG neurons referred to as nociceptors (Akopian et al., 1996; Tate et al., 1998). Strong evidence for the important role of $Na_v1.8$ in chronic pain situations was revealed by Porreca et al. (1999) showing that intrathecal application of antisense oligo-deoxynucleotide (ODN) to $Na_v1.8$ prevented tactile allodynia as well as thermal hyperalgesia in rat models for neuropathic pain (spinal nerve injury model) and for chronic inflammatory pain (Complete Freund's adjuvant injection). Administration of antisense ODN to $Na_v1.9$, however, did not change pain behavior in neuropathic rats. On the other hand, mRNA for both TTX-R sodium channels are downregulated in the ipsilateral DRG, but are aggregated at the distal tip of injured neurons and upregulated in adjacent, uninjured DRG neurons after axotomy and chronic constriction injury (Dib-Hajj et al., 1996, 1998, 1999; Tzoumaka et al., 1997; Novakovic et al., 1998; Wood et al., 2002). As in animal models, $Na_v1.8$ and $Na_v1.9$ are also highly expressed in human DRGs and, several days after peripheral sensory nerve injury,

they are downregulated in the DRG and accumulate at the site of injury (Coward et al., 2000). The nerve damage-induced regulation of gene expression indicates that hyperexcitability in the respective DRG is predominantly due to TTX-S, and not to TTX-R channels. In fact, in animal models for neuropathic pain, increased firing rates of sensory neurons as well as the pain behavior is sensitive to low doses of TTX (Amir et al., 1999; Lyu et al., 2000). The role of TTX-R sodium channels upregulated at the site of injury and in the uninjured adjacent DRG remains to be elucidated (Waxman et al., 2000).

In inflammatory pain conditions, TTX-resistant channels are upregulated and play an improtant role in generation of hyperexcitability of primary sensory neurons

In contrast to neuropathic pain conditions, the mRNAs for $Na_v1.8$ and $Na_v1.9$ are upregulated in DRGs in chronic inflammatory pain models (Tanaka et al., 1998; Tate et al., 1998). Furthermore, a potentiation of sodium currents through $Na_v1.8$ channels by inflammatory mediators (e.g. prostaglandins, serotonin) probably via PKA-dependent phosphorylation has been shown (England et al., 1996; Gold et al., 1996; Fitzgerald et al., 1999). The inactivation kinetics of TTX-R sodium currents is about 10-fold slower than for TTX-S (about 5 ms versus 0.5 ms; Cummins and Waxman, 1997) thus giving rise to persistent TTX-R sodium currents. At the resting membrane potential of DRG neurons which is usually in the range of -55 to -60 mV (Zhang et al., 1997), more TTX-R Na_v 1.8 channels than TTX-S channels are in the resting state and can be activated by depolarizing stimuli as indicated by their relatively high voltage of half-maximal inactivation (Table 3). As a result of their functional properties together with their upregulation and potentiation by inflammatory mediators, TTX-R channels induce increased, persistent sodium conductance and hence might play an important role in generation of hyperexcitability in inflammatory pain conditions.

Table 3: Subtypes of voltage-gated sodium channel α-subunits: biophysical properties and TTX-sensitivity. V½: potential of 50 % activation or inactivation, respectively.

Channel	Activation V½	Inactivation V½	TTX-Block, IC_{50}
$Na_v1.3$	-26 mV [1]	-64.9 mV [1]	0.0018 µM [2]
$Na_v1.7$	-31 mV [3]	-78 mV [3]	0.0043 µM [3]
$Na_v1.8$	+ 13 mV [4]	-54 mV [4]	>100 µM [5]
$Na_v1.9$	-45 mV [6]	-44 mV [7]	1 µM [6]

[1]Cummins et al., 2001; [2] Joho et al., 1990, [3]Sangameswaran et al., 1997; [4]Koch et al., 1997; [5]Sangameswaran, 1996; [6] Tate et al., 1998; [7] Cummins et al., 1999.

Despite considerable efforts to develop subtype-specific sodium channel blockers, there are no specific compounds available as yet

The diversity of sodium channels indicates a complex interaction in modulation of the sensory neuronal activity which might be severely disturbed after dysregulation in neuropathic and inflammatory pain conditions. TTX-R and TTX-S sodium channels in primary sensory neurons can be blocked by several drugs with anti-neuropathic properties such as lidocaine, carbamazepine, lamotrigine and mexilitine in animal models and in patients (Roy and Narahashi, 1992; Xie et al., 1996; Bräu et al., 2001). However, these compounds are not selective for pain-relevant subtypes of sodium channels and despite considerable chemical efforts, there are no specific compounds available as yet. The development of new technologies for functional high-throughput screens (e.g. fluorescence resonance energy transfer (FRET); Gonzalez et al., 1999) should facilitate selective sodium channel drug discovery.

Sodium Channel Blockers in Clinical Use

Local Anesthetic drugs (LAs)

Local anesthetic drugs block sodium channels in a voltage- and frequency-dependent manner. They are not selective for pain-relevant subtypes of sodium channels

LAs block nerve conduction when applied locally to nervous tissue by a voltage- and frequency-dependent inhibition of sodium currents (see 'Voltage-gated Sodium Channels: Structure and Function'). Due to this mechanism, they preferentially block hyperexcitable cells and interfere comparatively less with normal physiological sensory and motor function. However, they are not selective for pain-relevant sodium channel subtypes so that they have a relatively high risk of adverse effects associated with the central nervous and cardiovascular systems when administered systemically. Known LAs are not active when administered orally.

Local anesthetic drugs probably bind to sodium channels in a charged form after they have penetrated the lipid plasma membrane in an uncharged form which explains why physico-chemical porperties largely determine their clinical properties including potency, onset and duration of action, and toxicitiy

LAs are reported to bind in a charged form within the pore of sodium channels near the cytoplasmic surface of the plasma membrane (for review see Butterworth and Strichartz, 1990; Tetzlaff, 2000). Before binding LAs have to cross the lipid layer of the plasma membrane probably in an uncharged form. The common structure of LAs is a hydrophilic moiety (usually a tertiary amine) linked by a short alkyl chain and an ester or amide group to a hydrophobic moiety (usually an aromatic residue). The linkage is enzymatically degraded in the plasma.

The pharmacological activity of LAs is determined by several physicochemical properties including lipophilicity, protein binding, and pKa which can be explained by their mechanism of action. A general structure - activity relationship was described by Courtney and Strichartz (1987), according to which an increase in the hydrophobicity leads to a parallel increase in anesthetic

potency and duration of action, but also an increase in toxicity which results from an improved access to the binding site within the membrane, leading to reduced degradation by plasma enzymes. These effects are further enhanced by high plasma protein binding. The pKa determines the onset of action: the lower the pKa value, the higher the percentage of the neutral form which can penetrate the plasma membrane.

Following plasma uptake by the vasculature of the tissue or after unintentional intravascular injection, LAs interfere with the function of all organs in which transmission of impulses occur due to their non-selective block of sodium currents, thus giving rise to adverse effects especially in the central nervous, cardiac and vascular systems. In fact, with high plasma concentrations LAs can induce tonic-clonic convulsions and cardiovascular depression possibly leading to severe ventricular arrhythmias. Therefore, it is essential that the main dose of LAs is injected incrementally until satisfactory anesthesia is achieved and with sufficient pauses between each bolus to allow observation of any systemic consequences. Furthermore, with application of a LA with a relatively high risk of systemic side-effects or for treatment in children or pregnant patients, the upper drug concentration of the preparation is limited. Allergic reactions to local anaesthetics are rare and are usually related to the presence of an ester function.

Table 4. Local anesthetic drugs: Physicochemical properties and plasma protein binding.

Drug	LogP	Protein binding	pKa
Benzocaine	1.86 [4]	-	3.5 [2]
Bupivacaine	3.41 [3]	95.5 % [1]	8.2 [1]
Cocaine	2.45 [4]	91 % [4]	8.8 [4]
Etidocaine	3.69 [3]	94 % [5]	7.7 [2]
Lidocaine	2.48 [3]	64 % [1]	7.8 [1]
Levobupivacaine	*	93.4 % [1]	8.2 [1]
Mepivacaine	1.95 [3]	77 % [1]	7.8 [1]
Prilocaine	8.0 [5]	55 % [1]	7.8 [1]
Procaine	1.91 [3]	6 % [5]	8.9 [2]
Ropivacaine	2.90 [3]	94 % [1]	8.2 [1]
Tetracaine	3.56 [3]	76 % [5]	8.6 [2]

[1]Whiteside and Wildsmith, 2001; [2]Tetzlaff, 2000; [3]Strichartz et al., 1990; [4] Dr. K. Fuchte, Grünenthal GmbH, personal communication; [5] Büch and Rummel, 1996, * (*S*)-enantiomer of bupivacaine.

When tolerability of the compound is sufficient to allow systemic administration, local anesthetic drugs can be employed for relief of neuropathic pain and acute treatment of migraine headache in addition to the broad application for local anesthesia as proven for lidocaine

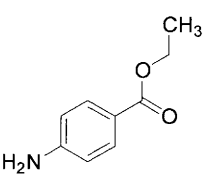
In clinical practice, LAs are applied for topical anesthesia using solutions, creams or patches, for infiltration anesthesia (injection directly into the wound), for field block anesthesia (parallel margin infiltration), for nerve block anesthesia (injection into or around a peripheral nerve), for intravenous regional anesthesia and for spinal anesthesia with intrathecal and epidural injection. Aiming at a prolongation of action and a reduction in plasma uptake, clinical preparations of LAs often contain a vasoconstrictor like adrenaline (1 : 200,000) to reduce local tissue perfusion. However, care must be taken to avoid tissue ischemia in areas without collateral blood supply such as nose, penis, fingers, or ears. In addition to these traditional applications of LAs to provide surgical anesthesia and analgesia for acute pain, LAs are also found to be effective in chronic pain conditions including neuropathic pain syndromes and acute migraine headache when administered systemically (for review see Backonja, 1994).

The chemical search for synthetic substitutes started in 1892 and gave rise to several compounds with improved properties which largely replaced the naturally occurring cocaine.

The natural substance cocaine was already beeing employed for local anesthesia in ophthalmological surgery in 1884 (Vandam, 1987). However, the clinical use of cocaine is limited because of its abuse potential, its intense vasoconstriction and eventual arrhythmias due to its reuptake-inhibition of catecholamines, and instability upon sterilization. The chemical search for synthetic substitutes started in 1892 and gave rise to several compounds without abuse potential and with improved onset and duration of action, tolerability and stability of the preparation.

Benzocaine

Benzocaine



4-Amino-benzoic acid ethyl ester, $C_9H_{11}NO_2$, MW 165.19, [94-07-7}

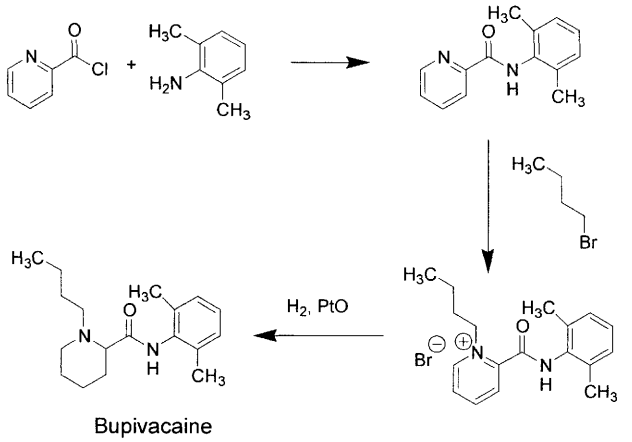
Anaesthesine® (Germany), Auralgan® , Tympagesic® (USA)

Benzocaine is an ester local anesthetic with a moderate onset of action and short duration. It is minimally absorbed and therfore relatively free from systemic adverse effects (toxic range of total dose: 200 to 300 mg; Tetzlaff, 2000).

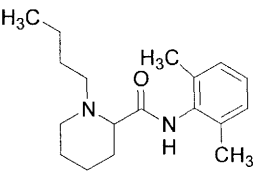
Clinical use: Due to the low lipophilicity and therefore the low ability to penetrate neuronal membranes, the clinical use of benzocaine is limited to topical anesthesia such as mucous membrane anesthesia prior to endoscopic examination or for temporary relief of oral or dental pain. With higher doses, oxidation of the ferric form of hemoglobin to the ferrous form can occur; the resulting methemoglobinemia is usually benign and can be reversed with methylene blue. Benzocaine is more likely to cause contact sensitization than amide-type LAs.

Bupivacaine

Synthesis (Kleemann et al., 1999):



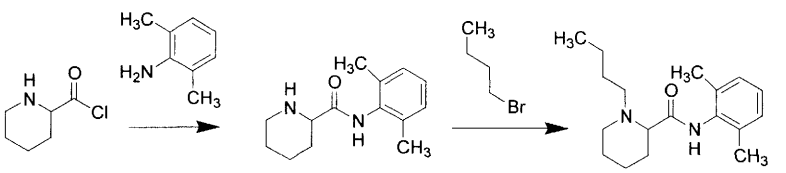
Bupivacaine



1-Butyl-piperidine-2-carboxylic acid (2,6-dimethyl-phenyl)-amide, $C_{18}H_{28}N_2O$, MW 288.44, [2180-92-9]

Trade names: Marcaine® (F, GB), Sensorcaine® (USA), Bupivacain® (D)

Alternative synthesis (Ekenstam and Egner, 1957; Kleemann et al., 1999):



Scheme 1: Synthesis of bupivacaine.

The racemic compound bupivacaine, which was first synthesized by Ekenstam et al. in 1957, is an amide-type LA with a high lipophilicity, protein binding and pKa giving rise to an intermediate onset and a long duration of action. At the same time, bupivacaine has a high toxicity potential relatively often associated with convulsions and life-threatening cardivascular collapse (Moore et al., 1978). Levobupivacaine, the (*S*)-enantiomer of bupivacaine, has recently been developed for clinical use addressing the enantioselectivity of side-effects of bupivacaine (see below).

Bupivacaine is a long-acting local anesthetic drug and has a high risk of side-effects which are enantio-selective and mainly associated with the (*R*)-enantiomer

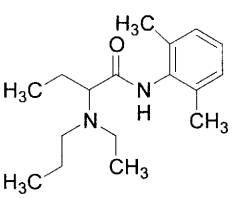
Clinical use: Because of its long duration of action, bupivacaine is indicated for long surgical anesthesia where a considerable amount of postoperative pain is expected such as dental and oral surgeries. Infiltration using a 0.25 % solution of bupivacaine produces sensory anesthesia with an onset of 2 to 5 min and a duration of 2 to 4 h or greater (Tetzlaff, 2000). A nerve conduction block with a duration of between 4 to 8 h and occasionally up to 24 h is achieved with injection of 0.5 to 0.75 %

bupivacaine often combined with adrenaline as an vasoconstrictor to decrease plasma uptake. For epidural analgesia employed in perioperative settings, bupivacaine is infused at a concentration of 0.1 or 0.25 % to an upper dose of 150 mg for surgery and 60 mg for labour (Singh and Erwin, 1998; Mandabach, 1999). Careful monitoring of the patient is necessary to avoid motor block and hypotension. A combination with opioids, e.g. 0.1 % bupivacaine with 10 µg/ml fentanyl, is infused epidurally for postoperative analgesia with satisfactory results. Bupivacaine with dextrose is used for spinal analgesia.

With local infiltration, toxic side-effects like convulsions and cardiovascular collaps occur in the dose range of 2.5 to 3 mg/kg body weight. Because of its systemic toxicity, bupivacaine is contraindicated for intravenous regional anesthesia.

Etidocaine

Etidocaine

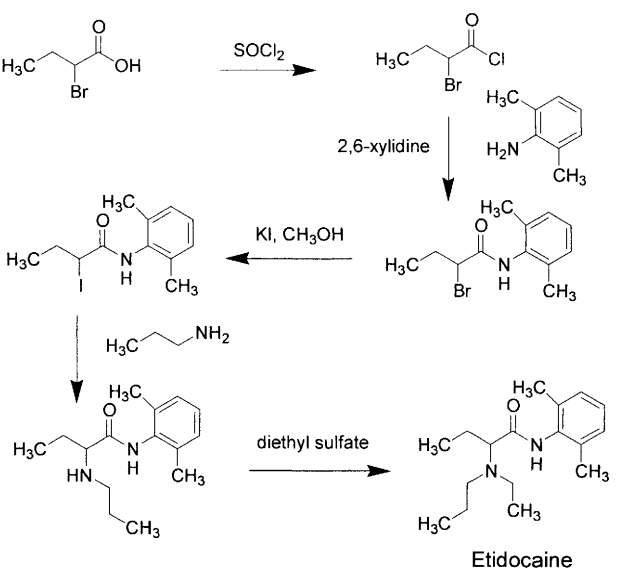


N-(2,6-Dimethyl-phenyl)-2-(ethyl-propyl-amino)-butyramide, $C_{17}H_{28}N_2O$, MW 276.43, [36637-18-0]

Duranest®

Etidocaine is a long-acting amide LA with a physicochemical and clinical profile similar to bupivacaine. Toxic side-effects occur at total doses of 300 to 400 mg. It is clinically available in combination with the vasoconstrictor adrenaline.

Synthesis (Adams et al., 1974; Kleemann et al., 1999):

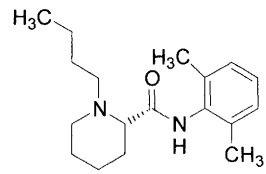


Scheme 2: Synthesis of etidocaine.

Clinical use: Etidocaine in combination with adrenaline is employed for infiltration anesthesia using solutions of 0.5% and peripheral nerve block at 0.5 and 1.0 % with a duration of 3 to 12 h (Tetzlaff, 2000). Epidural anesthesia is achieved with 1.0 to 1.5 % solutions with a duration of 3 to 5 h. Due to a profound motor block sometimes associated with unsatisfactory sensory block etidocaine is disadvantegous compared to bupivacaine.

Levobupivacaine

Levobupivacaine



(S)-(-)-Bupivacaine

(S)-1-Butyl-piperidine-2-carboxylic acid (2,6-dimethyl-phenyl)-amide, $C_{18}H_{28}N_2O$, MW 288.44, [27262-47-1]

Chirocaine®

Synthesis: cp. Bupivacaine

When the enantioselectivity of the cardiotoxicity of bupivacaine became apparent in preclinical models and in healthy human volunteers (Aberg, 1972; Bardsley et al., 1998), its (*S*)-enantiomer levobupivacaine was developed and finally approved as an alternative long-acting LA in 1999. The majority of preclinical and clinical studies indicate a similar potency, but lower risk of cardiovascular and, at least in preclinical investigations, fewer central side-effects. Levobupivacaine is vasoconstrictive in lower doses (up to 0.1 ml of 0.125 % solutions; Aps and Reynolds, 1978), which explains its longer duration of conduction block than that of bupivacaine, and vasodilatory at higher doses upon intradermal infiltration in healthy human volunteers. In adults, the recommended maximum single dose for surgical anesthesia is 150 mg. As for bupivacaine, the onset of action of levobupivacaine is slow (up to 15 min) with different methods of administration (for review see Foster and Markham, 2000; Whiteside and Wildsmith, 2001).

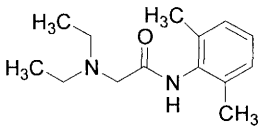
Levobupivacaine, the (S)-enantiomer of bupivacaine, was developed as an alternative long-lasting local anestethic compound with a similar potency, but improved tolerability.

Clinical use: The indications for levobupivacaine include wound infiltration (0.25 % solution), nerve conduction block (0.25 - 0.5 %), spinal analgesia (0.5 %) and epidural anesthesia (0.5 to 0.75 %). For labour analgesia, lower concentrations of levobupivacaine are recommended when administered as epidural injection (0.125 to 0.25 % up to 25 mg) or infusion (0.25 %). The maximum dose for ilioinguinal or iliohypogastric block in children is 1.25 mg/kg/side (0.25 to 0.5 % solutions). For postoperative pain management, levobupivacaine can be applied epidurally in combination with the opioids fentanyl or morphine or with the α_2-agonist clonidine.

The most common side-effect of levobupivacaine is hypotension. Therefore, levobupivacaine is contra-indicated for in intravenous regional block and patients with severe hypotension.

Lidocaine (Lignocaine)

Lidocaine



2-Diethylamino-N-(2,6-dimethyl-phenyl)-acetamide, $C_{14}H_{22}N_2O$, MW 234.34, [137-58-6]

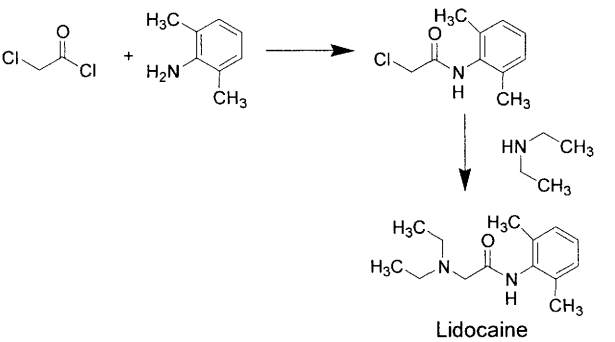
Xylocaine®, Gelicain®, Lidocaine®

Emla®

Lidoderm®

Lidocaine (synonyme: lignocaine) was introduced as the first amide in 1944 and is the most commonly used LA today. It has a rapid onset of action with intermediate duration and an intermediate toxicity. The maximum tolerated dose with infiltration or injection is 200 mg (500 mg when combined with adrenaline). Lidocaine is dealkylated in the liver to monoethylglycine xylidide and glycine xylidide which retain local anesthetic activity. It is available in a variety of preparations including creams, gels, patches and solutions, often in combination with adrenaline.

Synthesis (Löfgren and Lundquist, 1948; Kleemann et al., 1999):



Scheme 3: Synthesis of lidocaine.

Lidocaine is the most commonly used local anesthetic drug. In addition to its effectiveness for local anesthesia, it provides relief of neuropathic pain and acute migraine headache

Clinical use: Topical anesthesia is easiliy performed using 1 to 2 g/10 cm^2 skin of an eutectic mixture of the LAs (EMLA) lidocaine and prilocaine. Onset of action is achieved between 15 to 120 min depending on the tissue vascularization. Since EMLA is as effective as infiltration anesthesia, but avoids puncture pain and is usually well tolerated due to low risk of systemic absorption, it is widley used for procedures such as superficial skin surgery or lumbar puncture, especially in children. Effective relief of neuropathic pain was achieved with topical administration of lidocaine using a 5% gel or patch formulation (Rowbotham et al., 1995; Devers and Galer, 2000).

When used for infiltration anesthesia, a 1% solution is used which produces anesthesia within 2-3 min lasting for 2 to 4 h. On injection of 1.0 to 1.5 % lidocaine a peripheral nerve conduction block can be achieved with an onset of action of 4 to 10 min and a duration of 1 to 3 h, which can be prolonged with co-administration of adrenaline (Tetzlaff, 2000). Lidocaine is used for intravenous regional

anesthesia at a concentration of 0.5 % with a duration of 45 to 60 min. For epidural anesthesia, lidocaine is administered at a concentration of 1.5 to 2.0 % in combination with adrenaline and also with an opioid, e.g. fentanyl (Mandabach, 1999). Injection of lidocaine in a concentration of 5 % in a hyperbaric solution containing 7.5 % glucose is used for spinal anesthesia with a duration of 45 to 90 min.

Systemic injection of lidocaine was also shown to be effective and well tolerated in patients suffering from several neuropathic pain syndromes. Significant relief was reported in patients with neuropathic pain after peripheral nerve damage (Wallace et al., 1996) and after spinal cord injuries and stroke (Attal et al., 2000) and in patients with diabetic neuropathic pain (Kastrup et al., 1987), with postherpetic neuralgia (Rowbotham et al., 1991) and with fibromyalgia (Ellemann et al., 1989). Clinical data concerning cancer-related pain are inconsitent. While Brose and Cousins (1991) found an improvement after systemic lidocaine, there are other clinical trials showing no difference between lidocaine and placebo (Ellemann et al., 1989; Bruera et al., 1992).

Preclinical and clinical data indicate that lidocaine administered by intravenous injection or intranasally is effective in acute treatment of migraine (Bell et al., 1990; Kaube et al., 1994; Maizels, et al., 1996).

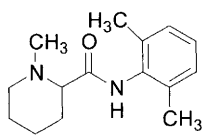
In addition to its use for anesthesia and analgesia, lidocaine is also used for acute treatment of ventricular arrhythmias.

Mepivacaine

Mepivacaine is an amide-type LA with a rapid onset, intermediate duration and intermediate toxicity. It has a slight vasoconstrictor action and produces less vasodilatation compared with lidocaine. The daily dose of mepivacaine should not exeed 400 mg.

Clinical use: Mepivacaine has been employed for all types of infiltration and conduction nerve block anesthesia using solutions of 1.0 and 1.5 % lasting for 1.5 to 3 h. Epidural anesthesia with 2.0 % mepivacaine has a rapid onset with a dense motor block. Hyperbaric solutions of mepivacaine have also been used for spinal anesthesia (Tetzlaff, 2000). Mepivacaine has been used for topical applications, but other LA such as lidocaine are more effective.

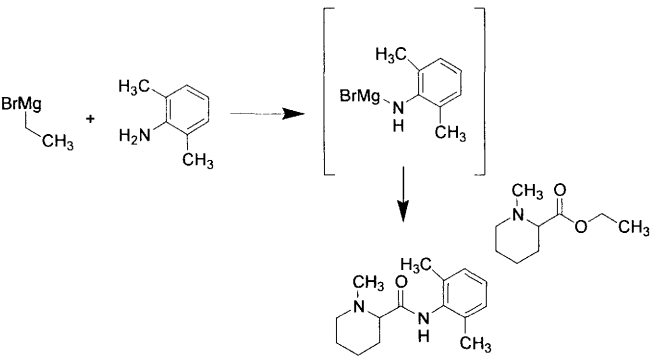
Mepivacaine



1-Methyl-piperidine-2-carboxylic acid (2,6-dimethyl-phenyl)-amide, $C_{15}H_{22}N_2O$, MW 246.35, [22801-44-1]

Carbocaine®, Polocaine®, Isocaine® , Scandicaine®

Synthesis (Ekenstam and Egner, 1957; Kleemann et al., 1999)

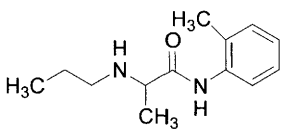


Mepivacaine

Scheme 4: Synthesis of mepivacaine.

Prilocaine

Prilocaine



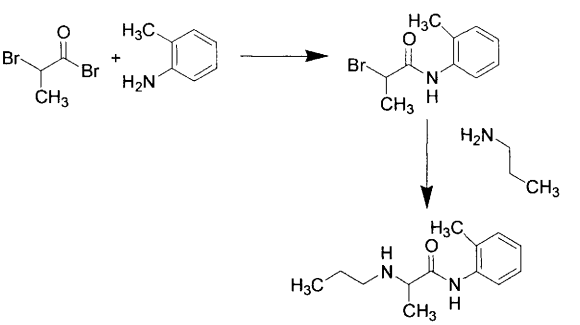
2-Propylamino-N-o-tolyl-propionamide, $C_{13}H_{20}N_2O$, MW 220.32, [721-50-6]

Citanest®, Xylonest® (G)

EMLA Creme® (G), Emlapatch® (GB), Emla® (USA)

Prilocaine is an amide-type LA with a rapid onset and an intermediate duration of action associated with a low toxicity. However, metabolism to ortho-toluidine can cause oxidation of the ferric form of hemoglobin to the ferrous form, creating methemoglobin. In most cases the methemoglobuniemia is benign, but sometimes tissue hypoxia is observed (Eriksson, 1966).

Synthesis (Löfgren et al., 1958; Kleemann et al., 1999):



Prilocaine

Scheme 5: Synthesis of prilocaine.

Clinical use: Topical anesthesia is easily achieved using an eutectic mixture of the LAs prilocaine and lidocaine (EMLA, see Lidocaine).

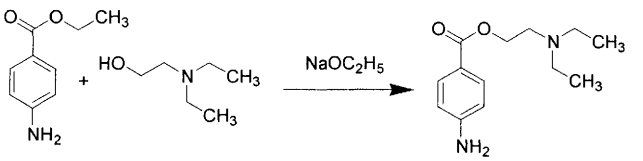
With infiltration of 0.5 to 1.0% prilocaine local anesthesia with a duration of 1 to 2 h is established. Peripheral nerve block is achieved with 1.5 to 2.0 % with a duration of 2 to 3

h. Upon peripheral application, the toxic dose is in the range of 600 mg (Teztlaff, 2000). Because of its low toxicity, prilocaine is also used for intravenous regional anesthesia. Epidural anesthesia with 2 to 3 % prilocaine gives satisfactory results for 1 to 3 h.

Procaine

Procaine, a para-aminobenzoic acid ester, was the first synthetic LA synthesized in 1904. It has a slow onset of action and a short duration and hence low toxicity. Due to its extremely low binding to plasma proteins (only 6 %) procaine is rapidly hydrolyzed by plasma cholinesterases to diethylaminoethanol and para-aminobenzoic acid, which inhibit the actions of salicylates and sulfonamides (Tetzlaff, 2000).

Synthesis (Kleemann and al., 1999):

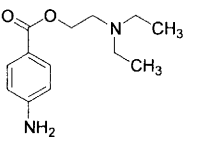


Procaine

Scheme 6: Synthesis of procaine.

Clinical use: Because of its poor penetration of intact mucous membranes, procaine is largely ineffective for topical applications and has been mainly used in injection in combination with adrenaline, although in general it has been replaced by other LAs such as lidocaine. For infiltration anesthesia, 0.25 to 0.5 % solutions of procaine have been used in doses up to 600 mg. For peripheral nerve block, a common dose of 500 mg of procaine has been given as a 0.5 to 2.0 % solution.

Procaine

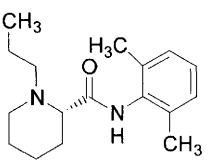


4-Amino-benzoic acid 2-diethylamino-ethyl ester, $C_{13}H_{20}N_2O_2$, MW 236.32, [59-46-1]

Novocaine®

Ropivacaine

In addition to levobupivacaine, ropivacaine is a new long-lasting amide-type LA that has been produced in order to address the enantioselectivity of the cardiotoxicity of bupivacaine. Ropivacaine, which is an (*S*)-enantiomer containing an n-propyl instead of the butyl moiety of bupivacaine, was launched in 2000. Clinical data indicate a late onset and long duration of action and the anesthetic potency of ropivacaine is comparable to that of bupivacaine (for review see McClellan and Faulds, 2000; Whiteside and Wildsmith, 2001). In animal models, the

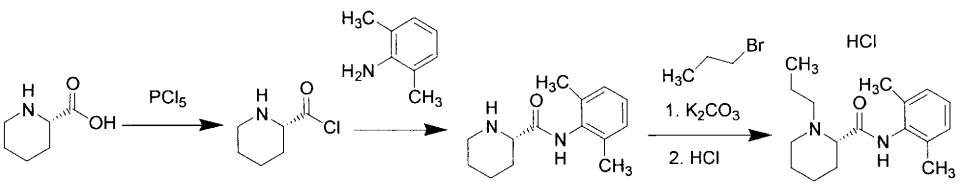


1-Propyl-piperidine-2-carboxylic acid (2,6-dimethyl-phenyl)-amide, $C_{17}H_{26}N_2O$, MW 310.87, [98717-15-8]

cardiotoxic side-effects of ropivacaine are intermediate between that of mepivacaine and bupivacaine. The maximum recommended single dose of ropivacaine is 300 mg. Ropivacaine has slight vasoconstrictor effects in lower doses in healthy human volunteers (Cederholm et al., 1994).

Naropin®, Naropeine®

Synthesis (Thuresson et al., 1985; Kleemann et al., 1999):



Ropivacaine hydrochloride

Scheme 7: Synthesis of ropivacaine..

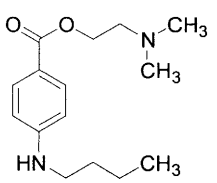
Ropivacaine, which is an (*S*)-enantiomer, is a new long-lasting amide local anesthetic drug that has been produced in order to address the enantioselectivity of the cardiotoxicity of bupivacaine.

Clinical use: Ropivacaine is used for local infiltrations such as field block (0.75 % solution) and for nerve block (0.75 %) up to 300 mg and for epidural anesthesia (0.75 and 1.0 %) up to 200 mg. When used for labour analgesia, epidural doses up to 40 mg are recommended. A combination of opioids is often administered via the epidural route for postoperative analgesia.

Hypotension was the most commomly reported side-effect. Co-administration with opioids may raise the incidence of hypotension, nausea, and pruritus (Scott et al., 1999).

Tetracaine

Tetracaine



4-Butylamino-benzoic acid 2-dimethylamino-ethyl ester, $C_{15}H_{24}N_2O_2$, MW 264.37, [94-24-6]

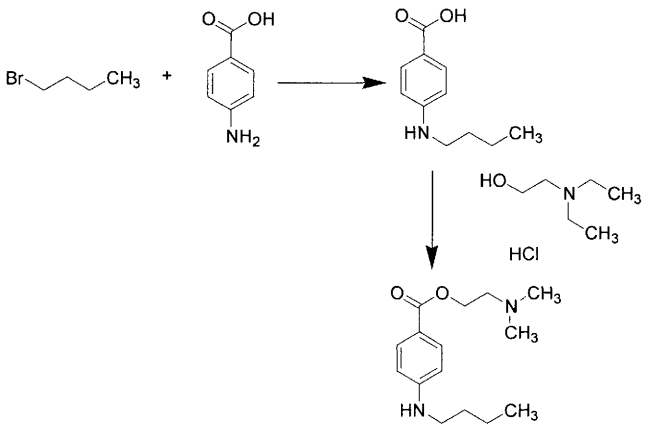
Pontocaine®

Tetracaine, an ester of para-aminobenzoic acid, has a slow onset, very short plasma half-life of 2.5 to 4 min and a long duration of action. Toxic effects are rare and only in the case of vascular absorbance from mucous membranes.

Clinical use: Tetracaine is employed by ophthalmologists for surface anesthesia as a 0.5 % solution and by endoscopists for anesthesia of mucous membranes including airways as a 2.0 % solution. For topical anesthesia, a 4.0 % cream of tetracaine can also be used, which is, however, less effective than a lidocaine/prilocaine cream in preventing venipuncture-induced pain in children (van Kan et al., 1997). A combination of tetracaine with adrenaline and cocaine (TAC) is widely used for repair of

lacerations to the face or scalp in children (Singh and Erwin, 1998). Tetracaine is commonly used for spinal analgesia at a total dose of 5 to 20 mg often in combination with other LAs with shorter duration in order to prolong anesthesia (Tetzlaff, 2000).

Synthesis (Kleemann et al., 1999; Eisleb, 1932):



Tetracaine

Scheme 8: Synthesis of tetracaine.

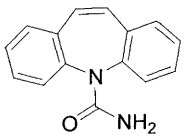
Anticonvulsant Drugs

Over the past few years, it has become increasingly apparent that anticonvulsant drugs are effective in neuropathic pain conditions in animal models and in patients (for review see McQuay, et al., 1995; Tremont-Lukats et al., 2000). Among the large number of anticonvulsant drugs this review will focus on carbamazepine, oxcarbazepine, lamotrigine, and phenytoin whose main mechanism is a voltage- and frequency-dependent block of sodium channels (see 'Voltage-gated Sodium Channels: Structure and Function'). As a result, they preferentially block highly active cells in pain pathways. However, they are not selective for pain-relevant sodium channel subtypes so that adverse effects associated with the central nervous and the cardiovascular systems occur. One of the advantages of the anticonvulsant sodium channel blockers over the local anesthetic lidocaine is that they are orally active.

Anticonvulsant drugs, which block voltage-gated sodium channels in a voltage- and frequency-dependent manner, are used to relief neuropathic pain and for migraine prophylaxis. They appear to have particular utility when there is a paroxysmal, lancinating component as in trigeminal neuralgia. The anticonvulsant drugs are not selective for pain-relevant sodium channel subtypes.

Carbamazepine

Carbamazepine



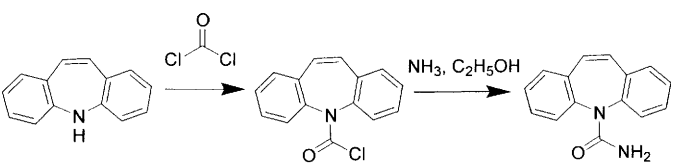
Dibenzo[b,f]azepine-5-carboxylic acid amide, $C_{15}H_{12}N_2O$, MW 236.27, [298-46-4]

Tegretal®, Tegretol®

Carbamazepine is recommended as the drug of first choice in the treatment of trigeminal neuralgia

In 1962, Blom was the first to report the analgesic properties of carbamazepine in neuropathic pain conditions. In clinical studies in patients suffering from trigeminal neuralgia, oral doses of up to 2400 mg/day for 5 to 14 days produced moderate to excellent results (Campbell et al., 1966; Killian and Fromm, 1968; Nicol, 1969). In fact, carbamazepine is recommended as the drug of first choice in the treatment of trigeminal neuralgia. Results from clinical trials have been positive in the treatment of painful diabetic neuropathy (Rull et al., 1969; Wilton, 1974; Gomez-Perez et al., 1996). After 4 weeks treatment with carbamazepine, central pain after stroke was improved only in five out of 14 patients (Leijon and Boivie, 1989). Carbamazepine was found to be effective for migraine prophylaxis (Rompel and Bauermeister, 1970).

Synthesis (Kleemann et al., 1999):



Carbamazepine

Scheme 9: Synthesis of carbamazepine.

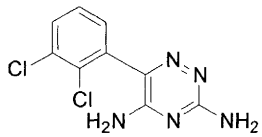
The most common side-effects in clinical trials among pain patients were moderate and largely associated with the central nervous system (i.e. sedation, tremor and incoordination). From its broad application as an anticonvulsant since the early 1960s, however, additional rare, but severe adverse effects are known including hematological changes such as aplastic anemia, hepatotoxicity and teratogenicity (for review see Perucca et al., 2000). The 10,11- double bond of carbamazepine is oxidized to an active epoxide metabolite and due to a remarkable induction of drug-metabolizing enzymes in the liver, carbamazepine is associated with intense drug interactions.

In addition to its use for the management of epilepsy and neuropathic pain, carbamazepine is employed for the treatment of manic depression (for review see Elphick,1989).

Lamotrigine

In patients with trigeminal neuralgia, some of whom may be resistant to carbamazepine, lamotrigine in oral doses up to 400 mg/day affords considerable and in some cases complete pain relief. Thus, lamotrigine offers an alternative approach for the management of trigeminal neuralgia (Canavero and Bonicalzi, 1997; Lunardi et al., 1997; Zakrzewska et al., 1997). Lamotrigine in daily doses up to 400 mg is effective in a dose-dependent manner in modulating and controlling diabetic neuropathic pain, as shown in one open and one randomized, placebo-controlled clinical trial (Eisenberg et al., 1988; Luria et al., 2000). In the treatment of painful human immunodeficiency virus (HIV) -associated peripheral neuropathy, lamotrigine in doses up to 300 mg/day produced effective pain relief versus placebo (Simpson et al., 2000). A clinical study in patients with central post-stroke pain indicates significant improvements with lamotrigine treatment for 8 weeks (200 mg/day).

Lamotrigine

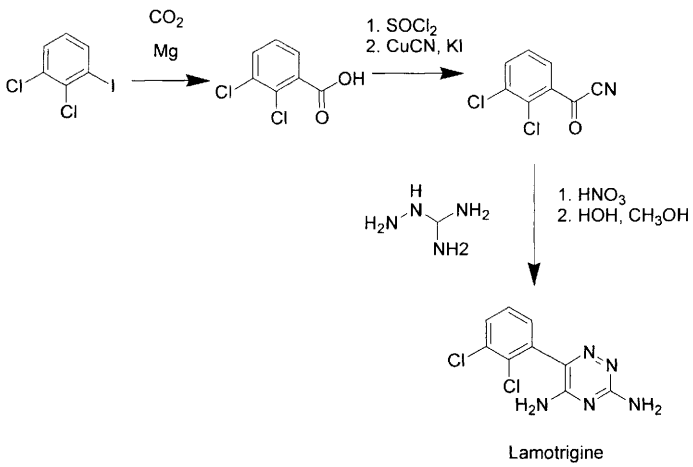


6-(2,3-Dichloro-phenyl)-[1,2,4]triazine-3,5-diamine, $C_9H_7Cl_2N_5$, MW 256.10, [84057-84-1]

Lamictal®

Lamotrigine, which has been shown to be effective in several neuropathic pain conditions, offers an alternative approach to carbamazepine for the management of trigeminal neuralgia

Synthesis (Kleemann et al., 1999):



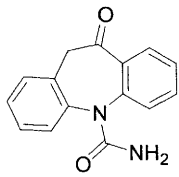
Scheme 10: Synthesis of lamotrigine.

Lamotrigine in doses up to 100 mg/kg/day was successful in migraine prophylaxis as shown in two clinical studies (D'Andrea et al., 1999; Lampl et al., 1999); however, a third study did not show significant effects versus placebo (Steiner et al., 1997). Further controlled trials are warranted to further validate the effectiveness of lamotrigine in prophylactic migraine treatment.

Side-effects are rare and include gastrointestinal adverse effects, skin rashes and headache.

Oxcarbazepine

Oxcarbazepine

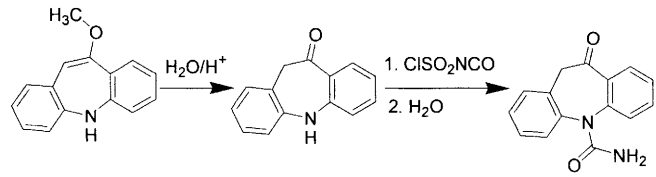


10-Oxo-10,11-dihydro-dibenzo[b,f]azepine-5-carboxylic acid amide, $C_{15}H_{12}N_2O_2$, MW 252.27, [28721-07-5]

Trileptal®

Oxcarbazepine is a sodium channel blocker which is structurally related to carbamazepine (McLean et al., 1994), but which has the advantage of a lower risk of drug interactions: In humans, it is rapidly and completely degraded to the active 10-monohydroxy metabolite and does not induce drug-metabolizing enzymes in the liver, so that comparatively few drug interactions are associated with this therapy (Feldmann et al., 1978). Administered orally, oxcarbazepine has been shown to be clinically as effective as carbamazepine against partial and generalized tonic-clonic seizures and has better tolerability (Reinikainen et al., 1987; Dam et al., 1989).

Synthesis:



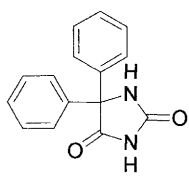
Oxcarbazepine

Scheme 11: Synthesis of oxcarbazepine.

In a clinical trial in trigeminal neuralgia patients, Lindström (1987) reported significant pain relief after treatment with 900-2100 mg/day oxcarbazepine which was comparable to the effects of carbamazepine (400-1200 mg/day).

Phenytoin

Phenytoin



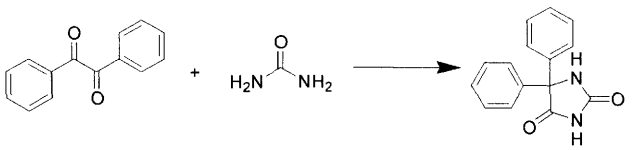
5,5-Diphenyl-imidazolidine-2,4-dione, $C_{15}H_{12}N_2O_2$, MW 252.27, [57-41-0]

Trade names: Dilantin® (USA), Epanutin® (G, GB), Aleviatin® (J)

Phenytoin was the first anticonvulsant to be tested in neuropathic pain patients (Bergouignan, 1942). In two following clinical studies with phenytoin in daily doses of up to 300 mg p.o. or 15 mg/kg i.v. clear reductions of diabetic neuropathic pain were observed (Chadda and Mathur, 1978; McCleane, 1999); however no effect was revealed in a third study using 300 mg/day p.o. (Saudek et al., 1977). Yajnik et al. (1992) found mild to moderate relief of cancer-related pain by phenytoin on its own and a significant enhancement of buprenorphine-induced analgesia.

The side-effect profile of phenytoin is quite similar to that of carbamazepine (see above).

Synthesis (Kleemann et al., 1999):



Phenytoin

Scheme 12: Synthesis of phenytoin.

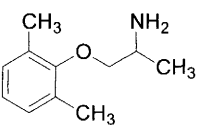
Antiarrhythmic Drugs

As in the previous section, only drugs with sodium channel blocking activities are considered.

Mexilitine

The class Ib antiarrhythmic drug mexilitine is structurally related to the local anesthetic agent lidocaine and also shows a voltage- and frequency-dependent block of sodium channels. Mexilitine is not selective for any pain-relevant subtype of sodium channel. As an advantage over lidocaine, mexilitine can be given orally.

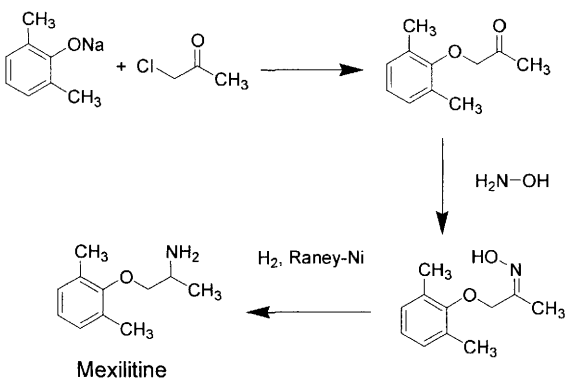
Mexilitine



2-(2,6-Dimethyl-phenoxy)-1-methyl-ethylamine, $C_{11}H_{17}NO$, MW 179.26, [31828-71-4]

Mexitil ®

Synthesis (Köppe et al., 1972; Kleemann et al., 1999):



Mexilitine

Scheme 13: Synthesis of mexilitine.

Several clinical trials indicate that mexilitine has analgesic properties in several neuropathic pain syndromes. Mexilitine was first shown to be effective for the treatment of diabetic neuopathic pain at oral doses of 10 mg/kg/day by Dejgard et al. (1988). However, in a large multi-center study by Stracke et al. (1992), the improvement in diabetic neuropathic pain resulting from the administration of mexilitine in daily doses up to 675 mg, was confined to certain subgroups of patients suffering from burning,

Mexilitine has analgesic properties in several neuropathic pain syndromes and is an alternative agent for treatment of patients who fail to respond to tricyclic antidepressants or who cannot tolerate them

stabbing, and heat sensation. In a double-blind placebo-controlled study, Chabal et al. (1992) found a significant improvement in neuropathic pain after peripheral nerve injury following oral administration of mexilitine in daily doses up to 750 mg. Furthermore, mexilitine at a dose of 10 mg/kg/day was used for treatment of a centrally-mediated pain syndrome and led to an improvement of neuropathic pain in eight out of nine patients (Awerbuch, 1990). Sloan et al. (1999) reported three cases of neuropathic cancer pain in which mexilitine was used as an adjuvant to opioids led to further relief of pain.

The most common side-effects in patients receiving mexilitine are gastrointestinal complaints, especially nausea (Jarvis and Coukell, 1998). Neurological side-effects such as tremor, headache, dizziness, and sleep disorders are rare. Although serious cardiac arrhythmias were not reported in neuropathic pain patients, transient tachicardia and palpitations occurred.

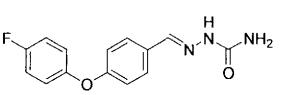
Mexilitine is an alternative for treatment of neuropathic pain in patients who fail to respond to tricyclic antidepressants or who cannot tolerate them (Chapter 4 this book; Jarvis and Coukell, 1998).

Sodium channel blockers in development

At present, a multitude of novel sodium channel blockers are in preclinical and clinical development. However, most compounds have been applied to many indications, especially epilepsy and stroke. This review includes only those substances for which activities in pain models have been reported.

In spite of remarkable efforts to develop sodium channel blockers which are selective for subtypes preferentially expressed in primary sensory neurons, no such pain-selective compound has as yet been found.

Co102862



Co102862, a semicarbazone, is a novel chemical entity structurally unrelated to other sodium channel blockers.

(Purdue Pharma)

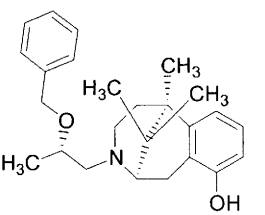
The semicarbazone Co102862 is a novel chemical entity structurally unrelated to other sodium channel blockers and is currently being investigated preclinically for epilepsy and pain. When studied in an HEK-293 cell line, stably expressing $Na_v1.4$ channels, Co102862 induced a concentration-dependent shift of the voltage of half-maximal inactivation from -77.6 to -95.4 mV at a concentration of 6 µM, thus indicating a preferential block of the sodium channel in the inactivated state (Illyin, 1999). Furthermore, channel repriming was significantly delayed. At steady-

state, Co102862 was more potent than the local anesthetic drugs lidocaine and bupivacaine. In addition to its anticonvulsant activities (Carter et al., 1997), Co102862 reduced pain behavior with ED_{50} values in the range of 5 to 10 mg/kg i.p. and p.o. in the formalin test in mice, a model for chronic, peristent pain (Tran et al., 1997). After spinal nerve injury in rats, reduction of tactile allodynia and mechanical hyperalgesia were marked and only moderate, respectively, in a dose range of 1.25 to 20 mg/kg p.o. (Carter et al., 1999).

In recent preclinical saftey studies, formation of bone lesions in the rat, probably mediated by the semicarbazide metabolite, have been reported (Srinivasan et al., 2001).

Crobenetine (BIII 890 CL)

Crobenetine, in phase II clinical studies for stroke, is characterized as a voltage- and frequency-dependent sodium channel blocker (Carter et al., 2000). Crobenetine shifted the half-maximal voltage of inactivation of $Na_v1.2$ channels by up to -27 mV in concentrations up to 10 µM. With trains of stimuli at 5 Hz, crobenetine at a concentration of 1.85 µM induces a pronounced use-dependent inhibition. The analgesic potential of the compound was investigated in a model for chronic inflammatory pain after induction of monoarthritis with intraatricular injection of Complete's Freund adjuvant into the right ankle (Laird et al., 2001). Following treatment for 5 days, crobenetine as well as mexilitine dose-dependently reduced mechanical joint hyperalgesia and impaired mobility with ED_{50} values of 15.5 and 18.1 mg/kg/day s.c., respectively. No effects on the edema and stiffness of the ankle were observed.



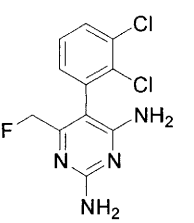
Crobenetine, a neuroprotectant compound, induces anti-hyperalgesia in an animal model for chronic inflammatory pain

(Boehringer Inglheim Pharma KG)

GW4030W92

Since lamotrigine has been shown to inhibit TTX-R channels (Xie et al., 1996), a systematic search for more potent analgesics from the lamotrigine series has led to the new compound GW4030W92. Due to steric loading and the restricted rotation around the two aromatic ring systems, GW4030W92 possesses a stereocenter with the R-(–)-confirmation being preferred (Nobbs and Rodgers, 1996).

GW4030W92



Derived from lamotrigine (Glaxo Wellcome), The compound is a potent voltage- and frequency-dependent blocker of TTX-resitant and TTX-sensitive sodium channels. The compound induces anti-hyperalgesic and anti-allodynic effects in animal models of chronic neuropathic and inflammatory pain

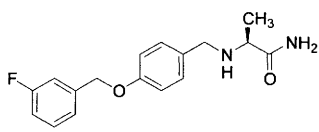
GW4030W92 has been shown to block TTX-R as well as TTX-S sodium channels in a voltage- and use-dependent manner (Trezise et al., 1998). TTX-R currents, induced by test pulses from – 60 to 0 mV, were reduced with an IC_{50} value of 22 µM. GW4030W92 leads to a shift of the voltage of half-maximal inactivation only with long-lasting prepulses (i.e. 4 s), but not with pulse width in the range of milliseconds. (see above: 'Voltage-gated Sodium Channels: Structure and Function'). Furthermore, use-dependency is obvious using trains of relatively long-lasting pulses (20 ms), but absent with application of short pulses (3.5 ms). These properties indicate a unique preferential effect of GW4030W92 on TTX-R channels in the slow inactivation state. TTX-S, currents induced from - 70 to 0 mV, are inhibited with an IC_{50} value of 5 µM and the steady-state inactivation curve is shifted to more negative potentials.

In a chronic constriction injury model for neuropathic pain in rats, GW4030W92 induces total relief of mechanical hyperalgesia and a significant reduction of mechanical allodynia after subchronic treatment with an oral dose of 10 mg/kg b.i.d. (Collins et al., 1998). In animal models for chronic, persistent and inflammatory hyperalgesia (Formalin test, Carrageenan and Complete Freund's adjuvant tests), GW4030W92 showed significant anti-hyperalgesic effects with ED_{50} values between 4 and 19 mg/kg p.o. (Clayton et al., 1998.). In addition, an anti-edemic property was found to be associated with GW4030W92 .

Development of GW4030W92 has been discontinued in phase II clinical trials.

Safinamide

Safinamide (NW1015, PNU 151774E)

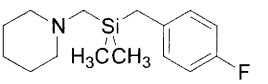


(Newron Pharmaceuticals)

Safinamide is a mixed Na^+ and Ca^{2+} channel blocker with anticonvulsant, neuroprotective and anti-parkinsonian properties and is currently in phase II clincical trials for the indications epilepsy and Morbus parkinson (for review see Chazot, 2001). Additionally, analgesic activity has been shown in acute pain models (hot plate, tail flick) and more pronounced in a chronic, persistent pain model (formalin test) in mice in a dose range of 7.5 to 120 mg/kg p.o. (Salvati et al., 1999).

RGH-5002

RGH-5002 (Silperisone)



(Gedeon Richter Ltd.)

RGH-5002 is a tolperisone-type centrally acting muscle relaxant. In isolated DRG neurons, RGH-5002 inhibited both TTX-R and TTX-S sodium currents. RGH-5002 shifted the voltage of half-maximal inactivation towards the hyperpolarizing direction for both channel types, thus indicating voltage-dependent channel block (Bielik et al., 1997). There was also a moderate fequency-dependency with trains of stimuli of 1 to 33 Hz. When investigated in hemisected spinal cord preparations excised form 6-day-old rats, RG-5002 inhibited ventral root potentials evoked by supramaximal stimulation of the dorsal root with a higher potency than the local anesthetic drug lidocaine (Farkas et al., 1997).

Development of RG-5002 has been discontinued in phase I clinical trials.

Conclusion and prospects

The therapeutic potential of voltage-gated sodium channels for pain management has been established by the efficacy of several sodium channel blockers including local anesthetic, anticonvulsant and antiarrhythmic drugs. In several preclinical and clinical studies, these drugs have been found to be useful for the treatment of various types of neuropathic pain. Further clinical trials are necessary to assess the risk - benefit ratio and hence the place of sodium channel blockers in the management of neuropathic pain especially in comparison with tricyclic antidepressant drugs (for review see McQuay et al., 1995, Chapter 4 this book).

The common mechanism of voltage- and frequency-dependent block of sodium channels gives rise to a preferred block of hyperexcitable neurons within pain pathways and comparatively less interference with normal physiological sensory and motor function. In clinical studies, adverse effects are usually reported as mild or moderate; however, there are also rare, but life-threatening incidents associated with the central nervous and cardiovascular systems.

The discovery of several subtypes of sodium channel with an outstanding relevance for pain has provided a rational basis to develop more selective and disease-specific compounds with an improved side-effect profile. Thus, there is a challenge to develop compounds combining the properties of voltage- and use-dependent block of sodium channels with subtype selectivity. However, despite considerable efforts, there are no such compounds available

as yet. Developments of new technologies for functional high-throughput should facilitate selective sodium channel drug discovery.

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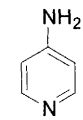
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6.2 Potassium Channels

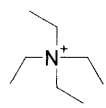
Wolfgang Schröder

Structure, Function and Distribution of K^+ Channels

Based on their functional properties as well as the degree of amino acid sequence homology, potassium (K^+) channels are grouped into several families and subfamilies with voltage-gated K^+ channels (Kv), Ca^{2+}-activated K^+ channels, inward rectifier K^+ (Kir) channels (including the ATP-dependent K_{ATP} channels) and two-pore-domain K^+ channels constituting the main classes. Over 80 mammalian genes for K^+ channel subunits have been cloned to date. In the case of Kv channels each subunit consists of six transmembrane (TM) segments. The fourth segment (S4) contains the voltage sensor. A pore-forming loop is located between S5 and S6. The subunits of Kir channels only possess two TM domains connected by the pore-forming loop. Unlike K_v channels they lack an intrinsic voltage sensor. Both functional Kv and Kir channels form homo- or heteromeric tetramers composed of four identical or related subunits, often with associated auxiliary β–subunits. K_{ATP} channels form octameric complexes of Kir6.x and regulatory sulphonylurea receptor (SUR) subunits with a 4:4 stochiometry. Three different SUR subunits have been identified so far (SUR1, SUR2A, SUR2B). They are expressed in a cell type-specific manner and confer different pharmacological sensitivities to the functional channel complexes. Subunits of the two-pore-domain K^+ channels may be regarded as composed of two connected Kir subunits. Hence, they possess 4 TM domains and two pore-forming loops. They probably dimerize to yield the functional channel.



4-aminopyridine



tetraethylammonium

Different K^+ channels can be distinguished by their characteristic electrophysiological properties as well as differential sensitivities to pharmacological modulation. For example, Kv channels are blocked by 4-aminopyridine (4-AP), tetraethylammonium (TEA) and millimolar concentrations of Ba^{2+}. In contrast, Kir channels are also blocked by TEA but show selective sensitivity to sub-millimolar concentrations of Ba^{2+}. Furthermore, certain peptide toxins (e.g. dendrotoxins, tertiapin) can sometimes discriminate between K^+ channels belonging to the same family. The K^+ channel openers diazoxide, pinacidil and cromakalim (see below) act on the three SUR subunits with a differential order of potency thereby affecting K_{ATP} channels in a cell type-specific manner (Yokoshiki et al., 1998).

Glx-Pro-Arg-Arg-Lys-Leu-Cys-Ile-Leu-His-Arg-Asn-Pro-Gly-Arg-Cys-Tyr-Asp-Lys-Ile-Pro-Ala-Phe-Tyr-Tyr-Asn-Gln-Lys-Lys-Lys-Gln-Cys-Glu-Arg-Phe-Asp-Trp-Ser-Gly-Cys-Gly-Gly-Asn-Ser-Asn-Arg-Phe-Lys-Thr-Ile-Glu-Glu-Cys-Arg-Arg-Thr-Cys-Ile-Gly

α-Dendrotoxin

The activation of K^+ channels leads to membrane hyperpolarization which exerts an inhibitory influence on

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H-Ala-Leu-Cys-Asn-Cys-Asn-Arg-Ile-Ile-Ile-Pro-His-Met-Cys-Trp-Lys-Lys-Cys-Gly-Lys-Lys-NH_2

Tertiapin

cell excitability. According to their electrophysiological properties the different types of potassium channels fulfill specialized functions in that they shape individual action potentials, determine the frequency of action potential firing, contribute to the afterhyperpolarization following an action potential or stabilize the membrane potential near the K^+ equilibrium potential. Common to all these actions is that they dampen excitability. An excellent introductory overview of the field of K^+ channel biology is provided by Hille (2001).

Activated K^+ channels in the neuronal circuitry of pain processing areas may either directly or indirectly interfere with the transmission of nociceptive signals

Depending on the location of a specific type of K^+ channel in the neuronal circuitry of pain processing areas they may either directly or indirectly interfere with the transmission of nociceptive signals when activated. For example, opening of K_{ATP} channels seems to mediate peripheral morphine analgesia by directly hyperpolarizing peripheral terminals of primary afferent fibers preventing action potential discharge and hence leading to antinociception. The activation of a K^+ conductance can also indirectly modulate pain processing via disinhibitory processes as will be discussed in detail for the supraspinal action of morphine (see below).

However, due to the ubiquitous expression of K^+ channels in virtually all excitable cells the general question arises how can they be exploited as targets for drug therapy of painful states? To put it in other words, what is required to ascertain a specific antinociceptive action when modulating K^+ channel functioning?

First, a considerable body of evidence for the involvement of K^+ channels in antinociceptive signaling that has been accumulated from both *in vitro* and *in vivo* studies of acute pain will be reviewed.

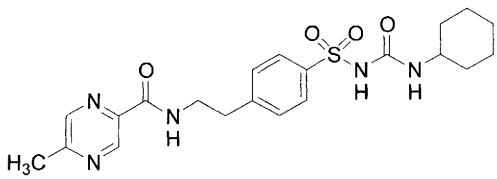
Second, the mechanisms by which altered K^+ channels contribute to the development of chronic pain states and what needs must be met by the channels in order to constitute attractive drug targets with respect to the problem of ensuring a specific antinociceptive action will be discussed. Corresponding examples for this will be given.

K^+ channels and supraspinal analgesia

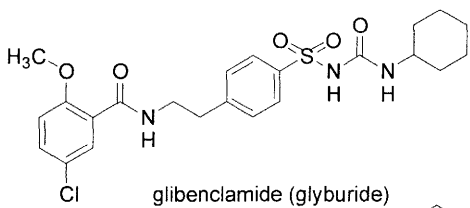
In 1956 it was recognized that insulin pretreatment enhanced morphine-induced analgesia (Davis et al., 1956). It was further suggested that this potentiation was due to a reduced level of intracellular ATP (Singh et al., 1983).

K_{ATP} channels

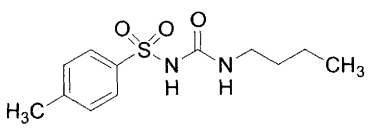
The identification of ATP-sensitive K^+ (K_{ATP}) channels in brain tissue (Bernardi et al., 1988) provoked a series of *in vivo* studies which demonstrated that these channels are involved in morphine-induced supraspinal analgesia (Table 1). Intracerebroventricular (i.c.v.) injection of K_{ATP} channel blockers antagonized morphine-induced antinociception in a dose-dependent manner (Ocana et al., 1990; Narita et al., 1992; Ocana et al., 1993). Interestingly, the order of potency of these blockers responsible for antagonizing the effect of morphine was the same as for blocking K_{ATP} channels in neurones, i.e. gliquidone > glipizide > glibenclamide > tolbutamide (Ocana et al., 1993). On the other hand, intracerebroventricularly administered K_{ATP} channel openers (KCOs) such as pinacidil (Vergoni et al., 1992), cromakalim (Ocana et al., 1996) as well as diazoxide and levcromakalim (Lohmann and Welch, 1999b) potentiated morphine-induced analgesia in a dose-dependent manner.



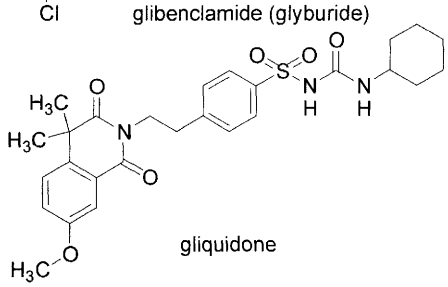
glipizide



glibenclamide (glyburide)

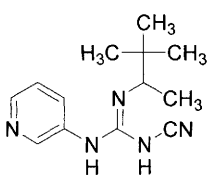


tolbutamide

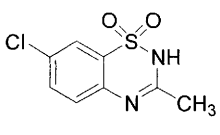


gliquidone

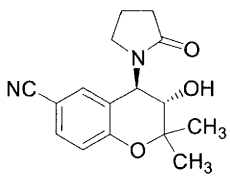
Scheme 1: K_{ATP} channel blockers.



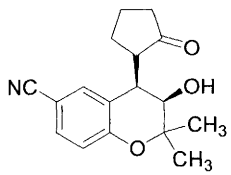
pinacidil



diazoxide



levcromakalim



L-cromakalim

Scheme 2: K_{ATP} channel openers.

Table 1: Involvement of K^+ channels in supraspinal analgesia.

Analgesics	Supraspinal K^+ channels		
	K_{ATP} channels	TEA, 4- AP sensitive K^+ channels	Ca^{2+} - activated K^+ channels
μ-opioid receptor agonists	+ (1, 2, 3, 4, 5, 6)	- (1, 7) + (8) (Kv1.1)	n.d.
κ-opioid receptor agonists	- (2, 5, 9)	- (9)	n.d.
$GABA_B$ receptor agonists	- (5, 9)	+ (8, 9) (Kv1.1)	n.d.
α_2 adrenoceptor agonists	+ (5, 9, 10, 11)	+ (11) (Kv1.1)	- (11)
Tricyclic antidepressants	+ (12)	+ (12, 13) (Kv1.1)	+ (12)
5-HT_{1A} receptor agonists	+ (14)	- (14)	n.d.
H1 histamine receptor antagonists	+ (15)	- (15) (Kv1.1)	+ (15)
A1 adenosine receptor agonists	+ (16)	- (16)	n.d.

(1) Ocana et al., 1990; (2) Narita et al., 1992; (3) Vergoni et al., 1992; (4) Ocana et al., 1993; (5) Ocana et al., 1996; (6) Lohmann and Welch, 1999a, b; (7) Ocana et al., 1995; (8) Galeotti et al., 1997a; (9) Ocana and Baeyens, 1993; (10) Raffa and Martinez, 1995; (11) Galeotti et al., 1999a; (12) Galeotti et al., 2001; (13) Galeotti et al., 1997b; (14) Robles et al., 1996; (15) Galeotti et al., 1999b; (16) Ocana and Baeyens, 1994

n.d. = not determined

The disinhibition of PAG output neurones mediates the supraspinal analgesia of morphine and serotonin

Morphine exerts its supraspinal analgesia by presynaptically blocking GABA release from tonically active inhibitory interneurones projecting to output neurones of the caudal ventrolateral periaqueductal grey matter (PAG) (Yaksh et al., 1976; Vaughan et al., 1997). This disinhibition of PAG output neurones stimulates serotoninergic and noradrenergic descending analgesic pathways which originate in the rostro-ventral medulla and terminate in the spinal cord where they modulate spinal nociceptive transmission (A detailed description of the PAG's functional anatomy can be found in Bandler and Shipley; 1994 and in Beitz, 1995).

Opioids including morphine acting on metabotrobic μ–opioid receptors produce their inhibitory effect on cellular excitability in part by activation of $G_{i/o}$-protein-coupled K^+ channels thereby hyperpolarizing the membrane potential (Williams et al., 1982; Han et al.,

1999; Ikeda et al., 2000; Narita et al., 2000). K_{ATP} channels have been shown to be opened by $G_{i/o}$-proteins (Edwards and Weston, 1993) and could thus mediate the inhibitory effect of morphine at the cellular level. Furthermore, such an interaction would also explain the antagonism and potentiation of morphine analgesia by K_{ATP} channel blockers and openers, respectively (see above). However, a recent electrophysiological study demonstrated that K_{ATP} channels do not mediate the effect of morphine in ventrolateral PAG neurones (Chiou and How, 2001).

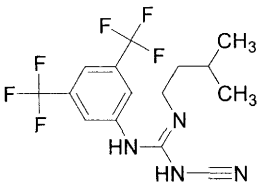
The disinhibition of PAG output neurones described above presumably also mediates supraspinal analgesia produced by serotonin which by acting on 5-HT_{1A} receptors stimulates a G-protein-gated inward rectifier K^+ current (Jeong et al., 2001). As with morphine, this leads to presynaptic inhibition of GABA release from inhibitory interneurones. Indeed, stimulation of 5-HT_{1A} and μ–opioid receptors synergistically modulate GABA release in PAG neurones since combining subthreshold concentrations of agonists of both receptors produces significant inhibitory effects (Kishimoto et al., 2001).

Currently, an alternative view of K_{ATP} channel involvement in supraspinal morphine analgesia is gradually emerging. Based on experiments which show that KCO-mediated antinociception is attenuated both by antisense oligonucleotides to opioid receptors and opioid antagonists, Lohmann and Welch (1999a, b) hypothesize that K_{ATP} channel openers stimulate the release of endogenous opioids which in turn may act on opioid receptors in the PAG. Most interestingly, when combining inactive doses of both KCOs and opioid receptor agonists a significant antinociception could be achieved. One important practical implication of this might be that morphine doses could be decreased when KCOs are concomitantly applied thereby reducing the side-effects of opioid therapy such as constipation, respiratory depression and abuse liability.

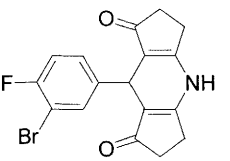
K_{ATP} channel openers seem to stimulate the release of endogenous opioids

However, currently available KCOs do not cross the blood-brain barrier and therefore had to be administered intracerebroventricularly for experimental purposes.

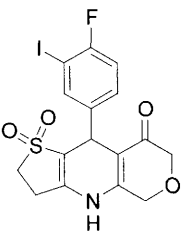
Novel classes of K_{ATP} channel openers have been claimed to be useful in the management of pain and migraine (Mogensen et al. (Novo Nordisk), 1999; Carroll et al., (Abbott), 2000-2001).



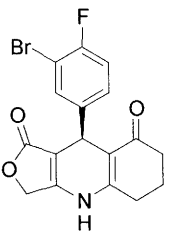
Mogensen et al. (Novo Nordisk), WO9958497



Carroll et al. (Abbott) WO0051986



Carroll et al. (Abbott), WO0183484



Carroll et al. (Abbott), WO0183480

Scheme 3: K_{ATP} channel openers.

Morphine interacts with the endogenous opioid system

The systemic administration of morphine has been shown to elevate endogenous opioid peptide levels in plasma and cerebrospinal fluid (Höllt et al., 1978; Natsuki and Dewey, 1993). Hence, K_{ATP} channel openers and blockers could interfere with this positive feedback loop to modulate supraspinal morphine analgesia by altering an additional antinociceptive component which is mediated by the morphine-induced release of endogenous opioids. Two findings may support this assumption. First, K_{ATP} channel blockers alone do not produce any effect on nociceptive thresholds (see below). Second, the antagonism of morphine-induced supraspinal analgesia by K_{ATP} channel blockers is not complete even with the highest doses tested. It is noteworthy that no cross-tolerance develops between KCOs and morphine, hence KCOs produce antinociception in morphine tolerant mice (Welch and Dunlow, 1993) possibly indicating that K_{ATP} channels interact with other transmitter systems involved in nociceptive processing.

Indeed, using the same experimental approach (i.e. i.c.v. administration of K_{ATP} channel openers and blockers) it was shown that supraspinal K_{ATP} channels are also involved in producing antinociceptive effects of several other classes of analgesic substances (Table 1). The K_{ATP} channels involved in supraspinal antinociception are not

tonically activated since none of the K_{ATP} channel blockers exerted any effect when given alone.

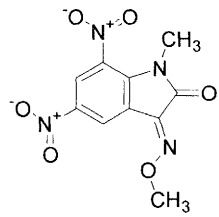
Kv1.1 channel

Likewise, by intracerebroventricular administration of antisense oligonucleotides to the voltage-gated K^+ channel Kv1.1 Galeotti and her colleagues (1997a) demonstrated that this K^+ channel is also associated with morphine-induced supraspinal analgesia. This finding is supported by electrophysiological experiments performed in the PAG demonstrating opioid-induced inhibition of inhibitory postsynaptic currents (due to reduced GABA release) by activating a 4-aminopyridine- and dendrotoxin-sensitive K^+ conductance (Vaughan et al., 1997). Again, supraspinal Kv1.1 channels have been implicated in antinociception produced by other classes of analgesic substances (Table 1). The fact that Kv1.1 knockout mice exhibit pronounced hyperalgesia and reduced responsiveness to morphine further underlines the important role played by this channel in nociceptive signaling (Clark and Tempel, 1998).

Ca^{2+}-activated K^+ channels

The i.c.v. injection of apamin or charybdotoxin, specific blockers of the SK and BK type of Ca^{2+}-activated K^+ channels, respectively, prevented the antinociception mediated by tricyclic antidepressants and H1 histamine receptor antagonists whereas α_2 adrenoceptor-mediated supraspinal analgesia did not depend on the activation of these K^+ channels (Table 1).

Immunoreactivities of the SK1 and IK1 type of Ca^{2+}-activated K^+ channels were demonstrated to be down-regulated by chronic nerve injury thereby probably contributing to increased excitability in neuropathic states (Boettger et al., 2002). SK, IK (Jensen et al. (Neurosearch), 2000) and BK channel opening compounds (Sit and Meanwell (BMS), 1998; Hewawasam et al. (BMS), 1999; Hewawasam and Starrett (BMS), 2000) were claimed to be suitable for the treatment of migraine.



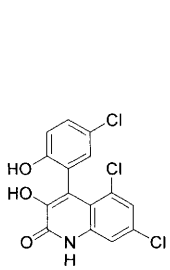
Jensen et al. (Neurosearch), WO0033834

Cys-Asn-Cys-Lys-Ala-Pro-Glu-Thr-Ala-Leu-Cys-Ala-Arg-Arg-Cys-Gln-Gln-His-NH_2

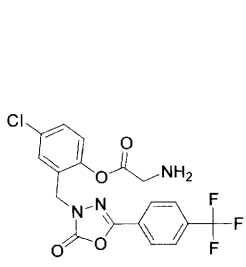
Apamin

pGlu-Phe-Thr-Asn-Val-Ser-Cys-Thr-Thr-Ser-Lys-Glu-Cys-Trp-Ser-Val-Cys-Gln-Arg-Leu-His-Asn-Thr-Ser-Arg-Gly-Lys-Cys-Met-Asn-Lys-Lys-Cys-Arg-Cys-Tyr-Ser

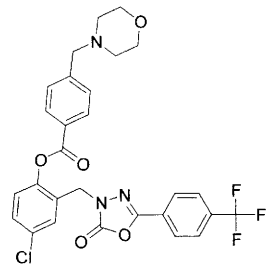
Charybdotoxin



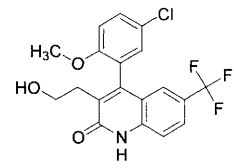
Sit and Meanwell (BMS), WO9823273



Hewawasam et al. (BMS), WO9938853



Hewawasam et al. (BMS), WO9938854



Hewawasam and Starrett (BMS), WO0034244

Scheme 4: BK channel openers.

K^+ channels and spinal analgesia

Spinal K_{ATP} channels were shown to be involved in the antinociception produced by intrathecally (i.t.) administered morphine, norepinephrine, apomorphine and carbachol as deduced from the dose-dependent inhibition by i.t. glibenclamide.

Table 2: Involvement of K^+ channels in spinal and peripheral analgesia

Analgesics	Spinal K^+ channels		
	K_{ATP} channels	TEA, 4- AP sensitive K^+ channels	Ca^{2+} -activated K^+ channels
μ-opioid receptor agonists	+ (1, 2, 3, 4, 5, 6)	n.d.	+ [1)]
α_2 adrenoceptor agonists	+ [(2, 3, 5)]	n.d.	n.d.
5-HT receptor agonists	- [(3)]	n.d.	n.d.
adenosine receptor agonists	- [(2, 3)]	n.d.	n.d.
dopamine D1/D2 receptor agonists	+ [(4)]	n.d.	n.d.
muscarinic receptor agonists	+ [(7)]	n.d.	n.d.
	Peripheral K^+ channels		
μ-opioid receptor agonists	+ [(8)]	- [(8)]	- [(8)]

(1) Welch and Dunlow, 1993; (2) Yang et al., 1998; (3) Kang et al., 1998a; (4) Kang et al., 1998b; (5) Asano et al., 2000; (6) Campbell and Welch, 2001; (7) Kang et al., 1997; (8) Rodrigues and Duarte, 2000

Interestingly, apart from their glibenclamide-sensitivity all these antinociceptive effects were also inhibited by i.t. naloxone suggesting a requirement for spinal opioid receptor activation. Kang et al. (1998b) proposed that norepinephrine, apomorphine and carbachol exert their spinal antinociception by releasing endogenous opioids which in turn act on opioid receptors that possibly coexist with K_{ATP} channels in neuronal membranes postsynaptic to opioidergic local interneurones. Morphine analgesia in the spinal cord is partly mediated by the release of adenosine from central terminals of capsaicin-sensitive primary afferent fibers (Sawynok et al., 1989). Accordingly, analgesia produced by i.t. norepinephrine could be blocked by both i.t. naloxone and the adenosine receptor antagonist aminophylline (Zhang et al., 1994). However, K_{ATP} channels do not participate in the analgesic effects elicited by adenosine agonists (Table 2).

Activation of spinal opioid receptors appears to be central for antinociception elicited by several classes of analgesics

Again, as is the case for K_{ATP} channels involved in supraspinal analgesia those participating in spinal antinociception are not tonically activated since none of the K_{ATP} channel blockers exerted any effect per se.

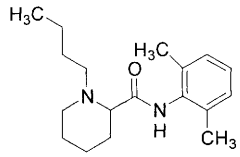
K^+ channels and peripheral analgesia

Peripheral endogenous opioid analgesia occurring under inflammatory conditions has been of much interest in the last few years (reviewed in Stein et al., 2001). Opioid receptors were shown to be expressed on the peripheral terminals of primary afferent fibers. Inflammation not only upregulates these receptors but also attracts immunocytes to the inflamed tissue to secrete endogenous opioid peptides thereby eliciting local analgesia. Rodrigues and Duarte (2000) showed that activation of K_{ATP} channels is involved in this process since peripherally applied blockers of those channels were able to reverse the antinociceptive effects of peripherally administered morphine. The hyperpolarization of primary afferent fibers due to K^+ channel activation leads to diminished action potential discharge and is thus thought to enhance the threshold for pain perception.

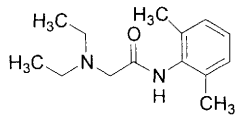
K^+ channels are affected by anesthetics

The action of several anesthetics has also been associated with a modulation of K^+ channels. In addition to blocking Na^+ currents in spinal neurones of the superficial dorsal horn the local anesthetics bupivacaine, lidocaine and mepivacaine reduce transient, A-type K^+ currents in these cells whereas delayed rectifier K^+ currents proved to be resistant (Olschewski et al., 1998). Since the A-type K^+ current determines the frequency pattern of repetitively firing neurones (Hille, 2001) their suppression in dorsal

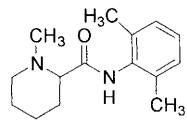
horn neurones might contribute to the modulation of pain perception. However, a reduced A-type K^+ current would increase the firing frequency of the respective neurone. How this might be related to the analgesic action is at present unknown. Bupivacaine also inhibits G-protein gated inward rectifier K^+ channels (Zhou et al., 2001).



Bupivacaine

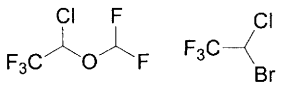


Lidocaine

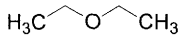


Mepivacaine

Scheme 5: Anesthetics modulating K^+ channels.



Isoflurane Halothane



Diethylether

$CHCl_3$

Chloroform

In contrast, currents through the two-pore-domain K^+ channels TREK-1, TASK-1 and TASK-2 are activated by the volatile general anesthetics halothane, isoflurane chloroform and diethyl ether (Patel et al., 1999; Sirois et al., 2000; Gray et al., 2000). The resulting hyperpolarization in cortical, hippocampal and cerebellar neurones where these channels are abundantly expressed may explain how anesthesia is produced by volatile anesthetics. Moreover, halothane also enhanced background currents through heteromeric Kir3.1/3.4 and homomeric Kir3.1 G-protein gated inward rectifier K^+ channels whereas homomeric Kir3.2-mediated currents were inhibited (Weigl and Schreibmayer, 2001; Yamakura et al., 2001). In addition, volatile anesthetics reduce neuronal excitability by stimulating $GABA_A$ and glycine receptors (Mihic et al., 1997) as well as by reducing AMPA and NMDA receptor functioning (Cheng and Kendig, 2000). The mechanisms of action of general anesthetics have been reviewed comprehensively by Thompson and Wafford (2001).

K^+ channels and chronic pain

So far, a role in mediating acute antinociceptive responses has been ascribed to different types of K^+ channels using *in vivo* pain models such as the tail-flick or the hot-plate test.

However, finding new drugs to combat chronic pain with negligible deleterious side-effects is a major challenge for pharmaceutical research. Side-effects generally arise when the drug's cellular target shows a broad tissue distribution and also becomes activated outside the area of interest.

For instance, cardiac expression of TASK and TREK (both in the myocardium and conductive tissue) is compatible with bradycardia and the negative inotropic effect observed with volatile anesthetics (Terrenoire et al., 2001).

In the CNS, K_{ATP} channels show an ubiquitous neuronal expression (Dunn-Meynell et al., 1998). Moreover, they have been detected in astroglial cells of the cortex, hippocampus and cerebellum (Zawar et al., 1999; Thomzig et al., 2001). Outside the nervous system K_{ATP} channels are well known to occur in heart (Kir6.2/SUR2A) pancreas (Kir6.2/SUR1) and vascular smooth muscle (Kir6.1/SUR2B) cells where they are involved in the phenomenon of ischemic cardiac preconditioning as well as in regulating insulin release and blood pressure (reviewed in Yokoshiki et al., 1998). Hence, interfering with K_{ATP} channel function may potentially provoke a series of effects apart from the intended modulation of pain perception mentioned above. To ensure a rather specific antinociceptive action an expression pattern of the target largely confined to areas involved in pain processing such as the PAG, dorsal root ganglion (DRG) cells or the spinal cord dorsal horn would be highly desirable.

Among K^+ channels, two candidates that meet these requirements (i.e. involvement in chronic pain as well as restricted expression) are now emerging: the KCNQ2/3 heteromere and the Kv1.4 homomere.

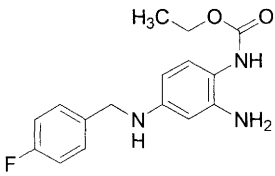
KCNQ2/3 K^+ channel

Activating KCNQ2/3 channels produces both anticonvulsant and analgesic effects

The heteromeric KCNQ2/3 K^+ channel is exclusively expressed in the nervous system with high abundance in neurones of the cortex, hippocampus, sympathetic ganglia and the DRG. This channel underlies a current referred to as the M-current since it is blocked by muscarinic agonists as was initially shown in sympathetic ganglion neurones (Brown and Adams, 1980; Wang et al., 1998). Due to its activity in the subthreshold voltage range the M-current critically determines cellular excitability and responsiveness to synaptic inputs. Most recently, the KCNQ2 protein was shown to be expressed in regions particularly prone to seizure generation namely in thalamo-cortical and septo-hippocampal neuronal circuits that are crucial determinants of synchronized activity in the brain (Cooper et al., 2001). Indeed, mutations in the channel protein resulting in a reduced current density have been associated with a special form of epilepsy (reviewed by Rogwaski, 2000). Hence, the potent and selective KCNQ2/3 agonist retigabine which is bioavailable after

systemic administration proved to be effective as an anticonvulsant both *in vitro* and *in vivo* (Hetka et al., 1999; De Sarro et al., 2001; Straub et al., 2001).

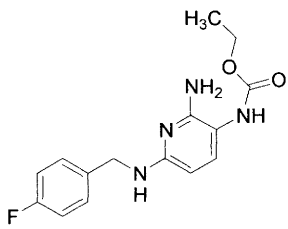
Retigabine



[2-Amino-4-(4-fluoro-benzylamino)-phenyl]-carbamic acid ethyl ester, $C_{16}H_{18}FN_3O_2$, MW 303.33, [150812-12-7]

Retigabine opens KCNQ2/3 channels by shifting the voltage of activation to more hyperpolarized potentials as well as by accelerating the rate of activation and slowing deactivation (Main et al., 2000; Rundfeldt and Netzer, 2000; Wickenden et al., 2000; Tatulian et al., 2001). An overview of pathophysiological conditions associated with KCNQ channels is given by Robbins (2001).

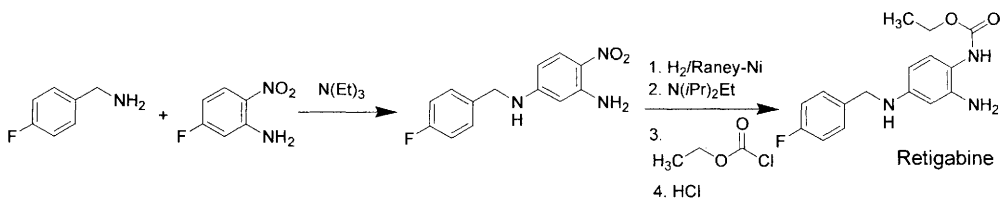
Retigabine which is in phase II clinical trials for epilepsy, is a derivative of the analgesic flupirtine which also has anticonvulsant activity. The anticonvulsant action of retigabine is greater than that of flupirtine, however retigabine is not effective in models of acute pain.

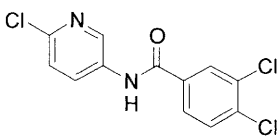


Flupirtine

Therefore, it was surprising to find that retigabine had remarkable antihyperalgesic properties (as demonstrated by the dose-dependent inhibition of the second phase of the formalin test). Furthermore, it exerted a robust anti-allodynic effect in the chronic sciatic nerve constriction model of neuropathic pain (Rostock et al., 2000; Rundfeldt et al., 2001). The usefulness of retigabine and KCNQ2/3 channel openers in general for the treatment or prevention of chronic inflammatory and neuropathic pain has also been stressed by Burbidge et al. (2001).

Synthesis (Dieter et al. (Asta Medica), 1993):





McNaughton-Smith et al. (Icagen), KCNQ2/3 channel opener WO0110380

The newly discovered KCNQ2/3 channel openers of the benzanilide class including those with a previously undescribed 2-substituted-5-aminopyridine substructure have been also demonstrated to be effective against inflammatory pain after systemic administration (McNaughton-Smith et al. (Icagen), 2001; Wickenden et al. (Icagen), 2001).

Kv1.4

Sciatic nerve injury causes a reduction of transient and delayed rectifier Kv currents in large-diameter DRG

neurones that leads to hyperexcitability (Everill and Kocsis, 2000). Recently, Rasband et al. (2001) showed a dramatic down-regulation of Kv1.1, Kv1.2 and Kv1.4 channels in DRG neurones in the same model of neuropathic pain. Most interestingly, homomeric Kv1.4 channels appear to critically determine C fiber excitability since no other Kv subunits are expressed by small-diameter DRG neurones. Consistent with this a dendrotoxin-insensitive transient A-type K^+ current with properties corresponding to homotetrameric Kv1.4 channels is found in these cells (Pearce and Duchen, 1994). Moreover, chronic bladder inflammation is also characterized by a suppression of a Kv1.4-like A-type K^+ current in C fiber bladder afferents resulting in enhanced excitability (Yoshimura and de Groat, 1999).

In most neurones Kv1.4 forms part of the heteromultimeric channels. Hence, the development of openers specific for homotetrameric Kv1.4 channels may prove an attractive alternative to Na^+ channel antagonists in selectively reducing excitability of nociceptive C fibers as was suggested by Rasband and colleagues.

Summary

In vivo studies employing tests of acute pain unequivocally showed the involvement of K_{ATP}, Kv1.1 and Ca^{2+}-activated K^+ channels in supraspinal, spinal and peripheral analgesia produced by different classes of analgesics. Furthermore, two-pore-domain and G-protein gated inward rectifier (Kir3.x) K^+ channels are affected by volatile anesthetics. The latter also contribute to μ– and κ-opioid receptor-mediated analgesia (Ikeda et al., 2000).

Synergistic effects as described for the supraspinal interaction of opioids with either KCOs or serotonin are interesting in terms of providing a rationale for reducing opioid doses by using co-administration regimes, e.g. with tricyclic antidepressants which are known to increase serotonin levels in the synaptic cleft.

Keeping in mind the large number of known K^+ channels, our current knowledge on their involvement in analgesia is undoubtedly very limited. This is mainly due to methodological reasons such as the relative paucity of modulators specific for individual K^+ channels. Consequently, it cannot be ruled out that other K^+ channels might also participate in mediating antinociceptive effects.

However, the ubiquitous expression of the vast majority of them does not allow the intended antinociceptive effects to

be dissociated from a plethora of side-effects when they are modulated. This will probably prevent common K^+ channel openers to be used as analgesics to a large extent. On the other hand, due to their segregated expression pattern KCNQ2/3 and homomeric Kv1.4 channels have proven promising targets for treating chronic pain states.

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6.3 Calcium Channels

Hagen-Heinrich Hennies and Bernd Sundermann

Introduction

Calcium channel antagonists - also known as Ca^{2+} channel inhibitors or Ca^{2+} entry blockers - have now played a pivotal role in the treatment of hypertension for more than 20 years. Nifedipine, diltiazem and verapamil, the three prototype drugs of the L-type Ca^{2+} channel, were introduced in the late 1970s and early 1980s. But in addition to the interaction with the cardiovascular system, voltage-dependent ion channels also are attractive drug targets for analgesia and neuroprotection (Cox and Denyer, 1998; Williams et al., 1999). Voltage-dependent calcium channels (VDCC) are involved in the regulation of many physiological functions of excitable cells such as neurite outgrowth, enzyme regulation, neurotransmitter release, hormone release and gene expression in cell bodies (Kater et al., 1988; Tsien et al., 1988; Cox and Denyer, 1998). Molecular cloning, biochemical and pharmacological studies have shown that there are several subtypes of VDCC.

VDCC subtypes can be categorised by α 1-subunit families

VDCC form hetero-oligomeric complexes. The α 1-subunit is pore-forming and provides the extracellular binding site(s) for virtually all agonists and antagonists. The α 1-subunit belongs to a heterogeneous family. Ten cloned α-subunits of 1610 - 2424 amino acids in length are known (α 1S, α 1A, α 1B, α 1C, α 1D, α 1E, α 1F, α 1G, α 1H and α 1I). From these 10 α 1-subunits three families emerge:

1. L-type channels (high-voltage activated dihydropyridine- (DHP) sensitive channels): α 1C, α 1D, α 1S, α 1F.
2. N-, P/Q-, R-type channels (high-voltage and moderate-voltage activated DHP-insensitive channels): α 1B, α 1A, α 1E.
3. T-type channels (low-voltage activated channels): α 1G, α 1H, α 1I.

The structure of VDCCs

Each subunit has four homologous repeats (I - IV), each having six transmembrane domains (see Fig. 1). Gating is thought to be associated with the membrane-spanning S4 segment, which contains highly conserved positive charges. Many of the α 1-subunit genes give rise to alternatively spliced products. When expressed alone many α 1-subunits produce functional Ca^{2+} channels. However, at least for high-voltage activated channels, it is likely that native channels comprise co-assemblies of α 1-,

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β-, α 2-, δ- and possibly γ-subunits (reviews: Tsien et al., 1991; Catterall and Striessnig, 1992; Catterall, 1993; Isom et al., 1994; Striessnig et al., 1998; Alexander and Peters, 2000).

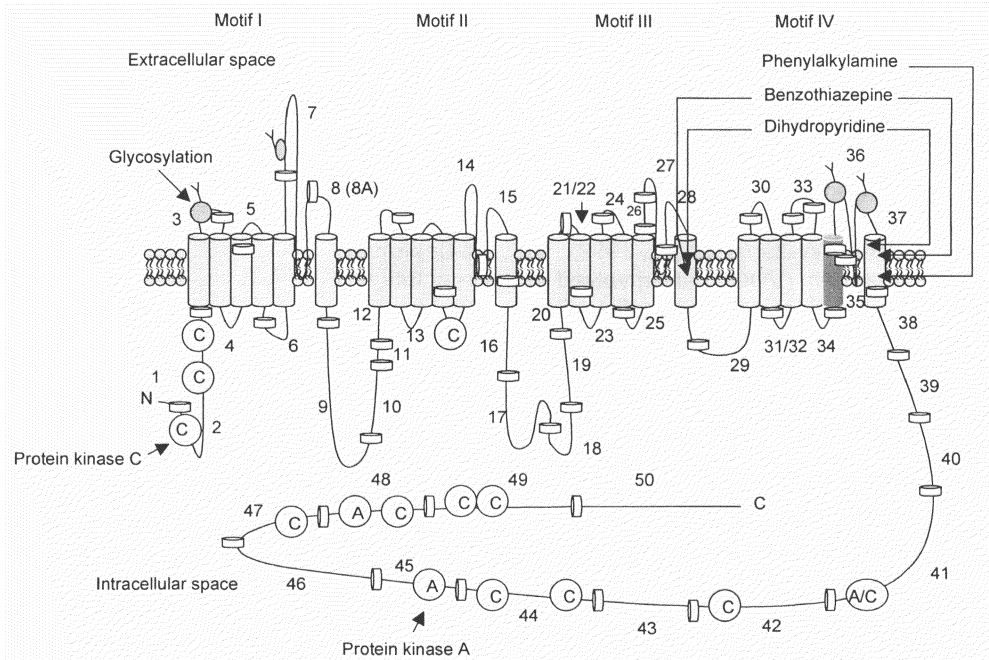


Figure 1: Proposed arrangement of the polypeptide chain of the channel-forming α1c subunit of the L-type calcium channel in humans.

The four repetitive motifs (I, II, III, IV) each consist of six putative transmembrane segments. Both the N terminal and C terminal point into the cytoplasm. White rings separate the segments encoded by numbered exons. The transmembrane segments encoded by alternative exons 8 or 8A, 21 or 22, and 31 or 32 are shown. Sequences encoded by invariant exons which are subject to constitutive splicing are 7, 33, and 45. Exons 40, 41, and 42 are subject to alternative splicing. Putative sites of glycosylation and of phosphorylation involving protein kinase C (C) and protein kinase A (A) are shown, as are the discrete binding areas of the three types of calcium antagonists – phenylalkylamine (verapamil-like), benzothiazepine (diltiazem-like), and dihydropyridine (nifedipine-like) (adapted from Abernethy and Schwartz, 1999).

Activation and localization of calcium channels

Arrival of the nerve impulse at a nerve terminal leads to the opening of voltage-gated Ca^{2+} channels and rapid influx of Ca^{2+}. The increase in Ca^{2+} concentration at the active zone from a basal level of 100 nM to more than 200 µM results in an appropriate neurotransmitter release within 200 µs (Barrett and Stevens, 1972; Linas et al., 1981; 1992; Augustine and Neher, 1992; Zucker, 1993; Heidelberger et al., 1994).

N-type Ca^{2+} channels for instance are located at presynaptic termini of neurons where they are directly involved in the regulation of neurotransmitter release. Staining of the dorsal laminae of the rat spinal cord revealed a complementary distribution of class A and class B Ca^{2+} channels in nerve terminals in the deeper versus the superficial laminae. Many of the nerve terminals immunoreactive for class B N-type Ca^{2+} channels also contain substance P, an important neuropeptide in pain pathways, suggesting the N-type Ca^{2+} channels are predominant at synapses that carry nociceptive information to the spinal cord (Westernbroek et al., 1998).

L-type Ca^{2+} channels can be detected in peripheral neurons, central neurons, synaptosomes as well as in non-neuronal cells (review: Tsien et al., 1988). In general the following gene products were found to be expressed, at least in part, in the central nervous system (CNS) (Birnbaumer et al., 1994; Alexander and Peters, 2000):

Predominant distribution of the α 1-subunit of the L-type channel:

α 1S: skeletal muscle

α 1C: cardiac and smooth muscle, brain

α 1D: endocrine, kidney and brain

α 1F: appears to be confined to the retina

α 1A (P/Q-type Ca^{2+} channel)
α 1B (N-type Ca^{2+} channel)
α 1C (L-type Ca^{2+} channel)
α 1D (L-type Ca^{2+} channel)
α 1E (R-type Ca^{2+} channel)

Preclinical Data

Antinociceptive Effects of L-type Ca^{2+} Channel Antagonists

Enhancement of opioid mediated antinociception by L-type Ca^{2+} channel antagonists

There are several reports in the literature showing that L-type blockers are more or less active in pharmacological testing systems such as the formalin, writhing, hot plate and tail flick assays (see Table 1). In addition to this data there are further publications reporting an enhancement of opioid-mediated antinociception by L-type Ca^{2+} channel antagonists. All scientific groups listed in Table 2 report that L-type Ca^{2+} channel blockers enhance the antinociceptive effect of μ-opioid agonists without exception. Positive results were reported with all three types of L-type Ca^{2+} channel inhibitors in combination with several μ-opioid receptor agonists such as morphine, DAMGO ((D-Ala^2,N-Me-Phe^4,Gly-ol^5)-enkephalin), fentanyl or sufentanyl. These pharmacological pain models employed mice as well as rats. In general the combination of μ-opioid agonists with an L-type Ca^{2+} entry blocker leads to an enhancement or potentiation of the antinociceptive effect. The dose - response curve of the opioid is shifted to the left. At the spinal level this is also

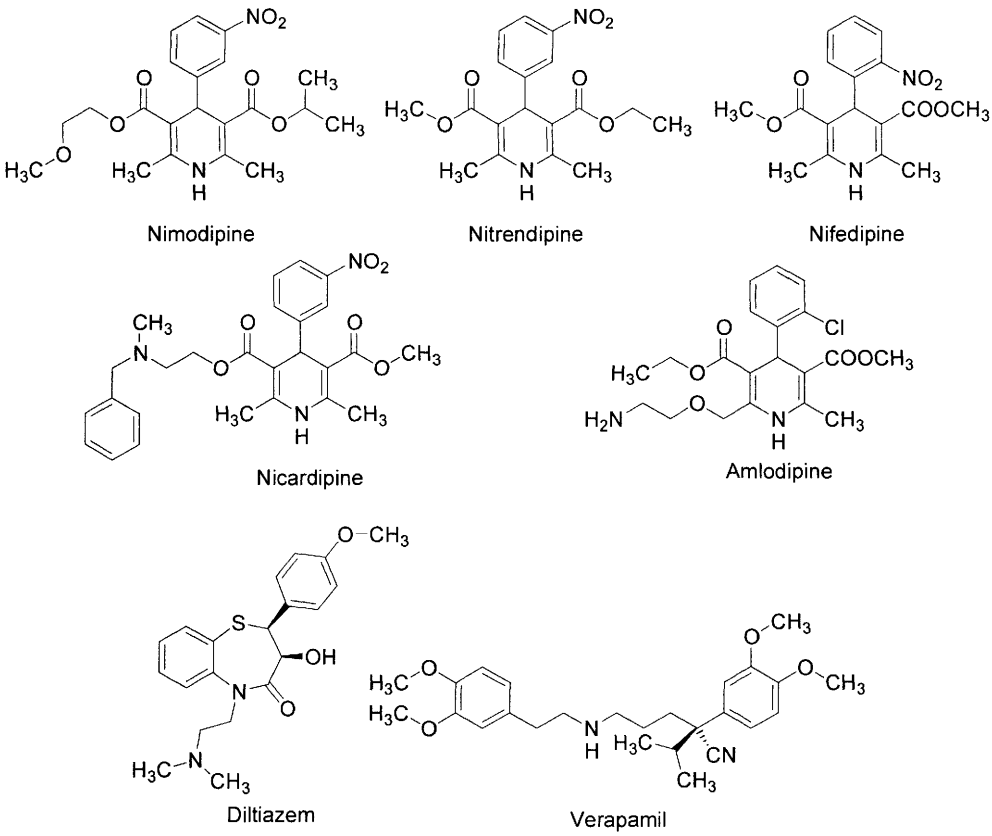
true for μ-agonists, but an enhancement of δ- or κ-opioid agonist-induced antinociception by an L-type VDCC inhibitor was not detectable (Table 2, Dogrul et al., 2001).

Table 1: Antinociceptive activity of L-type Ca^{2+} channel antagonists in specified pharmacological assays.

Model	Species	Compound	Application	Effect	References
Formalin test	Rat	Nifedipine, Nimodipine, Verapamil, Diltiazem	1 – 30 mg/kg i.p.	all drugs are active	Miranda et al. (1992)
Formalin test	Rat	Nifedipine	5 and 15 mg/kg i.p.	active at 15 mg/kg i.p.	Bustamante et al. (1989)
Formalin test	Rat	Nicardipine, Nitrendipine, Diltiazem, Verapamil	10-40 mg/kg i.p.	all drugs are active	Gürdal et al. (1992)
Writhing (acetic acid)	Mouse	Verapamil, Nimodipine, Nifedipine, Diltiazem	0.5 – 400 μg/kg i.c.v. depending on the substance	V > Nim. > D > Nif.	Miranda et al. (1993)
Writhing (phenyl-quinone)	Mouse	Diltiazem Verapamil Nifedipine	10 mg/kg s.c. 5 mg/kg s.c. 10 mg/kg s.c.	all comps. are active	Al-Humayyd (1991)
Writhing (acetic acid)	Mouse	Nifedipine, Nimodipine, Verapamil, Diltiazem	1-30 mg/kg i.p.	Nim. and Nif. are more active than V and D	Miranda et al. (1992)
Hot plate (55°C)	Mouse	Verapamil Diltiazem	15 – 120 μg 60 – 120 μg i.c.v.	significant antinocic. effects	Del Pozo et al. (1990)
Hot plate (63°C)	Mouse	Nifedipine Nimodipine Verapamil Diltiazem	1 – 30 mg/kg i.p.	only Nim. is active	Miranda et al. (1992)
Hot plate (55° C)	Rat	Nifedipine	2.5 – 20 μM epidurally	active at 20 μM, but short duration of action	Wong et al. (1994)
Tail flick	Rat	Nifedipine	2.5 – 20 μM epidurally	active at 5-20 μM	Wong et al. (1994)

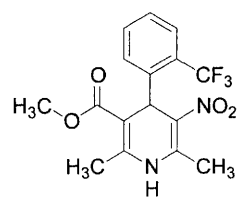
Table 1 continued:

Model	Species	Compound	Application	Effect	References
Tail flick	Mouse	Nifedipine	2.5 and 5 mg/kg i.p.	N has no effect on TF-latencies	Pavone et al. (1992)



Scheme 1: Structures of several clinically relevant L-type Ca^{2+} channel antagonists.

In contrast, a low dose of the Ca^{2+} channel agonist BAY K 8644, a dihydropyridine derivative, antagonizes the antinociceptive effect of μ-opioids. This is in agreement with results from Smith and Stevens (1995), who reported that Ca^{2+}, when administered i.c.v., antagonizes morphine-induced antinociception in the mouse tail flick assay. The dose - response curve of morphine is shifted to the right by i.c.v. administration of calcium ions.



BAY K 8644, a Ca^{2+}channel agonistic dihydropyridine

Table 2: Enhancement of opioid antinociception by L-type Ca^{2+} channel antagonist.

Model	Species	Opioid	L-type Ca^{2+} channel antagonist	Application	Effect	References
Hot plate (55°C)	Mouse	Morphine	Diltiazem or Verapamil	M: 1 mg/kg s.c.; D and V: 30-120 µg/kg i.c.v.	Both Ca^{2+} antag. lead to an enhancement of the M effect ≥ 30 µg/kg	Del Pozo et al. (1990)
Hot plate (55°C)	Mouse	Morphine	Diltiazem or Verapamil	M: 1-8 mg/kg s.c.; D and V: 40 µg/kg s.c.	The dose - response curve of M is shifted to the left by an additional application of D or V	Del Pozo et al. (1990)
Tail flick	Rat	Morphine	Verapamil or Nicardipine or Diltiazem	M: 2 µg i.t. plus: V: 50 µg i.t. or N: 20 µg i.t. or D: 100 µg i.t.	M alone and the Ca^{2+} antag. alone have no effect. In combination there is a very pronounced enhancement of the tail flick latencies	Omote et al. (1993)
Tail flick	Rat	Morphine	Verapamil or Nicardipine or Diltiazem	i.t.	The dose - response curve of M is shifted to the left by V, N or D	Omote et al. (1993)
Tail flick	Rat	DAMGO	Verapamil	D: 0.4 nmol i.c.v. V: 20 nmol i.c.v.	Large enhancement of the AUC of D by additional V application	Spampinato et al. (1994)
Hot plate (53.5°C)	Rat	Morphine	Diltiazem	M: 4, 6 and 8 mg/kg s.c.; D: 20 mg/kg s.c.	Potentiation and prolongation of the M effect by additional application of D	Beredek and Szikszay (1984)
Tail flick	Mouse	Morphine	Nifedipine	M: 5 mg/kg i.p.; N: 2.5 and 5 mg/kg i.p.	N prolongs M induced TF latencies	Pavone, et al. (1992)
Tail flick	Mouse	Morphine DAMGO DPDPE U-50,488H	Amlodipine	i.t.	I.t. administration of A potentiated M- and DAMGO -induced antinoc. by shifting their dose response curves to the left. However i.t. admin. of A did not effect DPDPE and U-induced tail flick latencies. These data indicate that L-type VDCC blockers potentiate the analgesic effects of µ-opioid receptor agonists, but not δ an κ receptor agonists, at the spinal level	Dogrul et al. (2001)

Table 2 continued:

Model	Species	Opioid	L-type Ca^{2+} channel antagonist	Application	Effect	References
Tail flick	Rat	Sufentanil	Nimodipine	S: 0.26 µg/kg s.c. (~ED_{50}); N: 200 µg/kg i.p.	N potentiated the antinociceptive effect of S by reducing the ED_{50} from 0.26 to 0.08 µg/kg s.c.	Dierssen et al. (1990)
Tail flick	Rat	Sufentanil	BAY K 8644 (Agonist)	S: 0.26 µg/kg s.c. (~ED_{50}); BAY K 8644: 20 µg/kg i.p.	BAY K 8644 in this low dose behaves as a calcium agonist and antagonized the effect of S (ED_{50} = 0.26 → 0.58 µg/kg s.c.), whereas at a high dose (200 µg/kg i.p.) it potentiated this action (ED_{50} = 0.26 → 0.15 µg/kg s.c.)	Dierssen et al. (1990)
Hot plate	Mouse	Fentanyl	Dihydro-pyridines (DHPs)	F: i.v.; DHPs: i.v.	F antinociceptive effects are potentiated by simultaneous i.v. administration of the Ca^{2+} antagonists	Hoffmeister and Tettenborn (1986)
Hot plate	Mouse	Fentanyl	BAY K 8644 (Agonist)		BAY K 8644 increases reaction time in the hot plate test dose-dependently (1-10 mg/kg p.o.)	Hoffmeister and Tettenborn (1986)
several	Rat	Fentanyl	BAY K 8644 (Agonist)		The influence on F antinociception in the rat of the Ca^{2+} agonist BAY K 8644 is biphasic: low doses attenuate, high doses potentiate F antinociception. It is concluded that calcium antagonism potentiates µ-receptor agonist antinociceptive effects, whereas calcium agonism antagonizes µ-receptor agonist antinociception	Hoffmeister and Tettenborn (1986)

Crosstalk between opioid receptors and Ca^{2+} channels

Due to the crosstalk between G-proteins of metabotropic receptors and the α 1 subunit of Ca^{2+} channels a highly regulated and dynamic control of neurotransmitter release results (Herlitze et al., 1996; Zamponi et al., 1997). This crosstalk has also been documented with respect to opioid receptors and Ca^{2+} channels (Moises et al., 1994; Bourinet et al., 1996). There are several publications

claiming that co-administration of L-type Ca^{2+} channel blockers has a positive result with respect to opioid-induced tolerance and/or withdrawal symptoms (Bongianni et al., 1985; 1986; Baeyens et al., 1987; Barrios and Baeyens, 1988; Contreras et al., 1988; Alfaro et al., 1990; Antkiewicz-Michaluk et al., 1990; 1993; Welch and Olson, 1991; Ruiz et al., 1993; Diaz et al., 1995; Garaulet et al., 1996; Tokuyama and Ho, 1996; Michaluk et al., 1998).

Evidence suggesting a fundamental role for VDCCs in the development of opioid tolerance and dependence

In morphine-tolerant mice an increased number of [^{3}H]-nitrendipine binding sites (B_{max}) was determined while the dissociation constant was unchanged (K_d; Ramkumar and El-Fakahany, 1984). In rats an increased DHP binding induced by prolonged opioid treatment was localized to the cortex, hippocampus and brainstem but not to the cerebellum and striatum (Ramkumar and El-Fakahany, 1988). These data were confirmed by Zharkovsky et al. (1993), who reported that in rats concurrent nimodipine treatment prevented the rise in the density of central DHP binding sites which occurred during chronic treatment. The authors suggest that chronic nimodipine treatment attenuates the development of withdrawal symptoms which occur on termination of chronic morphine treatment by preventing the upregulation of central DHP-sensitive binding sites. These pharmacological effects still have to be confirmed clinically.

Low antinociceptive strength of L-type Ca^{2+} channel antagonists for the treatment of pain

We reevaluated these experiments to determine the relationship between antinociceptive efficacy and possible side-effects which were only poorly reported in the literature. The first approach - application of L-type Ca^{2+} channel antagonists alone - was not convincing. The antinociceptive effect was rather poor and, since L-type Ca^{2+} channel antagonists have been used for the treatment of hypertension for many years, side-effects like hypotension are to be expected.

Potent antinociception and reduced side-effects through the combination of μ-opioid agonists with L-type Ca^{2+} channel antagonists

The second approach however - enhancement of opioid antinociception by L-type Ca^{2+} channel antagonists - offers the possibility for very effective antinociception with a reduced spectrum of side effects due to the opioid-sparing effect (Reimann (Grünenthal GmbH), 1998).

The rationale of this concept is:

- Ca^{2+}-antagonists are able to enhance the antinociceptive effect of opioids
- for this reason the μ-component can be reduced by an additional Ca^{2+} antagonistic component to achieve equipotent analgesia
- lowering the opioid component has the advantage that opioid mediated side-effects are no longer prominent

Combining μ-opioid agonism and L-type Ca^{2+} channel antagonism in one molecule

The aim of this concept was to synthesize drugs which combine both activities in one molecule. Before *in vitro* screening was started, excellent correlation between pharmacological effects and L-type channel as well as μ opioid binding affinity was ensured (data not shown).

L-type Ca^{2+} channels are the only VDCC which have three different drug binding sites, the

- Dihydropyridine (DHP)
- Phenylalkylamine (PAA)
- Benzothiazepine (BTZ)

sites (Glossmann and Striessnig, 1990; Catterall and Striessnig, 1992; Varadi et al., 1995; Striessnig et al., 1998).

The biochemical screening procedure consisted of a μ-opioid-, DHP-, PAA-, BTZ- and BTX- (Batrachotoxinin A 20-α-benzoate - binding site 2 of the Na^+ channel) radioligand binding assay essentially according to Frink et al. (1996), Murphy and Snyder (1982), Reynolds et al. (1983), Schoemaker and Langer (1985) and Pauwels et al. (1986). In contrast to DHPs, compounds binding to the PAA- and BTZ-site of the L-type Ca^{2+} channel exhibit a more or less pronounced interaction with the BTX-binding site of the Na^+ channel. For structural reasons we nevertheless decided to combine an agonistic μ-opioid affinity with an L-type Ca^{2+} channel blocking affinity mediated by the PAA and/or BTZ site(s).

Pronounced interaction with binding site 2 of the Na^+ channel leads to an insufficient safety index

During the drug discovery campaign we found that all compounds with a very high affinity for the BTX-site of the sodium channel had an insufficient safety index - the LD_{50}/ED_{50} ratio was too narrow. In this respect the BTX-assay was used as an early safety indicator.

Compounds with dual mode of action (Ki/μM):

μ-opioid affinity	affinty to PAA / BTZ site
0.1 - 0.0001	1 - 0.1

Selected orally active compounds (Graudums et al. (Grünenthal GmbH), 1997; Sundermann et al. (Grünenthal GmbH), 2000) exhibited a wide range of μ-opioid affinity (Ki 0.1 to 0.0001 μM) but affinities to the PAA and/or BTZ site were always quite constant (Ki 1 to 0.1 μM). Compounds with very pronounced μ-affinities are at least 10 times more potent than morphine *in vivo*, have an excellent safety index and a relatively mild effect on respiration in comparison to other very strong opioids, e.g. fentanyl.

Compounds with moderate μ-affinities are very potent in a variety of pain models in mice and rats. In addition to anti-nociceptive efficacy in models of acute pain (tail flick, writhing) these compounds inhibit acute and persistent inflammatory pain (Randall Selitto, formalin test). Furthermore, they show strong inhibition of acute visceral pain (colorectal distension) and of tactile and cold allodynia in models of neuropathic pain (spinal nerve ligation (Chung), chronic constriction injury (Bennett)). The data suggest these compounds to be potential candidates for the management of clinical pain indications. Somatic and visceral pain with and without inflammatory conditions as well as neuropathic pain might be addressed with this approach.

Antinociceptive Effects of N- and P/Q- type Ca^{2+} Channel Antagonists

Peptides from cone snails and spiders – molecular probes for N- and P/Q- type Ca^{2+} channels

Unlike DHP-sensitive L-type channels ω-conotoxin-sensitive N-type Ca^{2+} channels are exclusively expressed in the CNS. L-type channels inactivate very slowly whereas N-type channels inactivate more rapidly and are blocked by ω-conotoxin GVIA. ω-[^{125}I]conotoxin GVIA is an ideal ligand for binding experiments. The dissociation constant (K_D) for this toxin in rat brain membranes is 60 pM (Wagner et al., 1988).

P-type channels inactivate extremely slowly and are insensitive to both DHPs and ω-conotoxin GVIA, but are blocked by the spider venom peptide ω-agatoxin IVA, a peptide consisting of 48 amino acids isolated from the American funnel-web spider *Agelenopsis aperta*.

The inactivation kinetics of the Q-type channel are similar to the N-type channel but are resistant to DHPs and ω-conotoxin GVIA. Q-type channels are inhibited by ω-agatoxin IVA but less effectively than ω-agatoxin IVA blocks P-type channels (review: Miljanich and Ramachandran, 1995).

Table 3: Sequences of naturally-occurring ω-conopeptides.
(from Miljanich and Ramachandran, 1995)

Name	Sequence (I)	Species
SNX-124 (GVIA)	C K S X G S S C S X T S Y N C C R - S C N X Y T K R C Y	*Conus geographus*
SNX-178 (GVIIA)	C K S X G T X C S R G M R D C C T - S C L L Y S N K C R R Y	*C. geographus*
SNX-111 (MVIIA)	C K G K G A K C S R L M Y D C C T G S C - R - S G K C	*Conus magus*
SNX-159 (MVIIB)	C K G K G A S C H R T S Y D C C T G S C N R - - G K C	*C. magus*
SNX-230 (MVIIC)	C K G K G A P C R K T M Y D C C S G S C G R - R G K C	*C. magus*
SNX-238 (MVIID)	C Q G R G A S C R K T M Y N C C S G S C N R - - G R C	*C. magus*
SNX-157 (SVIA)	C R S S G S X C G V T S I - C C - G R C - - Y R G K C T	*Conus striatus*
SNX-183 (SVIB)	C K L K G Q S C R K T S Y D C C S G S C G R - S G K C	*C. striatus*
SNX-185 (TVIA)	C L S X G S S C S X T S Y N C C R - S C N X Y S R K C	*Conus tulipa*

(I) All synthetic peptides are amidated at the carboxy terminus. Dashes indicate gaps. X: Hydroxyproline.

Cone snails are found in tropical waters, often in the neighborhood of coral reefs. These molluscs produce a complex venom delivered through a specialized radular tooth that serves as a harpoon to immobilize their prey (Olivera et al., 1990; 1991). Complete immobilisation of the prey takes only a few seconds (Terlau et al., 1996). The venom from a single cone snail can contain up to 200 different biologically-active components (review: Shen et al., 2000). The primary structure of the naturally-occurring ω-conopeptides derived from several species of Conus are

shown in Table 3 (taken from Miljanich and Ramachandran, 1995). The ω-conopeptides are simple peptides built from 24 to 29 amino acids. All contain six cysteine residues linked to form three disulfide bridges. The SNX numbers refer to synthetically prepared peptides.

ω-conotoxins and -agatoxins

Table 4 summarizes the antinociceptive effects of several ω-conotoxins and ω-agatoxin IVA in different animal models. The peptides are administered by the i.t. or i.c.v. route.

SNX-111 (Ziconotide)

Concerning the ω-conopeptides, SNX-111 (Ziconotide) seems to be one of the proteins with the highest intrinsic antinociceptive activity in the formalin test (Table 4, Malmberg and Yaksh, 1994). This may be the main reason why most of the papers cited deal with this special synthetic peptide.

SNX-111, when given alone, is active in the Chung model (spinal nerve ligation), tactile allodynia test (hindpaw UV burn) and paw pressure test. In the hot plate assay there is only a small but significant effect of about 20% increase in response latency (Table 4, Malmberg and Yaksh, 1994).

ω-agatoxin IVA

ω-agatoxin IVA has been reported to be active in the formalin test whereas it is inactive in the hot plate assay (Table 4, Malmberg and Yaksh, 1994).

Combination of SNX-111 with morphine - additive to synergistic antinociception

SNX-111 combined with morphine in the hot plate assay results in response latencies greatly exceeding those produced by either compound alone (Table 4, Bowersox et al., 1998). In the formalin test a combination of both drugs leads to an additive effect (Table 4, Wang et al., 2000). In the tail immersion test the effect of both compounds together is higher than those produced by each drug alone. Morphine, when administered for 7 days leads to rapid tolerance. SNX-111 in combination with this μ-opioid agonist did not prevent tolerance to morphine analgesia (Table 4, Wang et al., 2000). In contrast to opioids only minimal development of tolerance was observed in the formalin test after chronic administration of SNX-111 over 7 days (Malmberg and Yaksh, 1995).

In the tail flick assay ω-conotoxin GVIA in combination with morphine leads to an additive effect similar to the combination of morphine with SNX-111 in the formalin test. But when ω-conotoxin GVIA was applied 24 h before morphine, antinociception was greatly reduced. In morphine-dependent rats, ω-conotoxin GVIA given i.c.v. 15 min before naloxone challenge (2 mg/kg i.p.), significantly attenuated the withdrawal symptoms (Table 4, Basilico et al., 1992).

Table 4: Antinociceptive activity of N-, P- and/or Q-type VDCC inhibitors in rats.

Model	Opioid	Compound	Application	Effect	References
Formalin test	-		i.t.	ED_{50} (nmol)	Malmberg and Yaksh (1994)
		N-type blocker		Phase 1 Phase 2	
		SNX-111		0.003 0,003	
		SNX-159		>0.26 0.12	
		SNX-183		0.010 0.009	
		SNX-199		>0.30 0.23	
		SNX-239		0.54 0.052	
		P- and/or Q-type blocker			
		ω-Agat.-IVA		>0.006 0.001	
		SNX-231		>0.24 >0.24	
Hot plate (52.5°C)	-	SNX-111	0.008 nmol i.t.	Small, but sign. effect	Malmberg and Yaksh (1994)
	-	ω-Agat.-IVA	0.006 nmol i.t.	Very small insign. effect	
Paw pressure	-	SNX-111	0.1, 0.3 and 1.0 µg i.t.	Sign. antinoc. effect at 0.3 and 1.0 µg	Bowersox et al. (1998)
Tactile allodynia (UV burn, hindpaw)	-	SNX-111	0.3 µg i.t.	≈ 4-fold increase in the hindpaw withdrawal threshold to mechan.stim.	Bowersox et al. (1998)
Formalin test (2nd phase)	-	SNX-111	0.1 µg i.t.	Suppression of nociceptive responses (flinch behavior)	Bowersox et al. (1998)
Chung model (spinal nerve ligation)	-	SNX-111	0.03, 0.1 and 0.3 µg i.t.	Dose-dependent blockade of mechan. allodynia in neuropathy rats	Bowersox et al. (1998)
Hot plate	Morphine	SNX-111 (Ziconotide)	M: 15 µg/hr S: 30 ng/hr; continuous spinal infusion for 7 days	Both drugs alone at this dose = little effect; in combination = response latencies greatly exceed those produced by either compound alone	Bowersox et al. (1998)
Chung model	-	SNX-111	3.0 µg i.t.	Significant suppression of allodynia	Chaplan et al. (1994)
		SNX-159	1.0 µg i.t.		
		SNX-239	3.3 µg i.t.		
Extracellular recording of dorsal horn neurones after s.c. formalin injection	-	ω-Con.-GVIA	0.1 and 0.4 µg i.t.	Both phases of the formalin response were inhibited by ω-Con-GVIA, ω-Agat-IVA blocks only the second phase	Diaz and Dickenson (1997)
		w-Agat.-IVA	0.125 and 0.5 µg i.t.		

Table 4 continued:

Model	Opioid	Compound	Application	Effect	References
Formalin test	Morphine	SNX-111 (Ziconotide)	dose-response curves alone and in combination i.t.	Isobolographical analysis: combination of both drugs results in an additive effect	Wang et al. (2000)
Tail immersion test	Morphine	SNX-111 (Ziconotide)	M: 15 µg/h S: 30 ng/h; continuous spinal infusion for 7 days	The effect of both compounds together exceed those produced by either compound alone. Morphine alone leads to rapid tolerance. SNX-111 in combination with M did not prevent tolerance to M analgesia	Wang et al. (2000)
Tail flick	Morphine	ω-Con.-GVIA	M: 1.5 µg/rat ω-C.: 20 ng/rat i.c.v.; M: 3.0 µg/rat ω-C.: 20 ng/rat i.c.v.	When ω-C. was given immediately before M, analgesic effect was additive. But, when the toxin was given 24 h before M, M analgesia was greatly reduced	Basilico et al. (1992)
Morphine-dependent rats	Morphine	ω-Con.-GVIA	two M. pellets (75mg each) implanted s.c. for 72 h; ω-C.: 20 ng/rat i.c.v.	ω-C injected in M dependent rats 15 min before naloxone challenge (2 mg/kg i.p.) signif. attenuated the abstinence syndrome	Basilico et al. (1992)

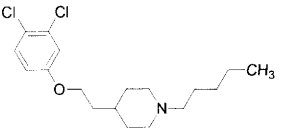
Side-effects of N-type VDCC inhibitors

High doses of the N-type VDCC inhibitors produce characteristic shaking behavior, serpent-like tail movements, and impaired coordination. At antinociceptive doses however there is no significant motor effect although some of the N-type Ca^{2+} channel antagonists produce tail movements (Malmberg and Yaksh, 1994).

Small molecule N-type VDCC inhibitors

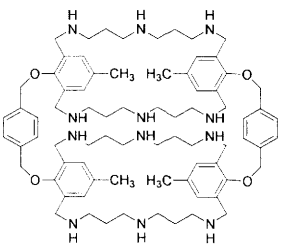
ω-Conotoxins (as well as ω-agatoxin) cannot be applied orally but the intrathecal route is, at any rate, far from ideal. To overcome the disadvantages of intrathecal administration the pharmaceutical industry has endeavored to develop orally-active small molecule inhibitors of the N-type VDCC. There is a large list of small molecules having a pronounced affinity for the N-type VDCC (review: Cox and Denyer, 1998). The majority of these compounds are not really selective and interact with other targets like the dopamine D_2 receptor (neuroleptics

like fluspirilene and pimozide), the NMDA receptor (eliprodil) and others.



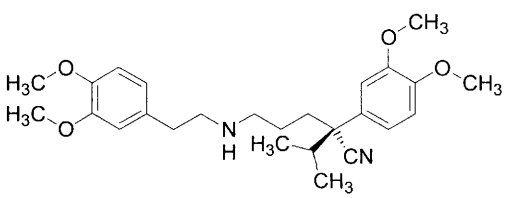
SB -201823

Since 1992 SmithKlineBeecham have published more than 20 patents on a range of piperidine and cycloalkylamine derivatives such as SB-201823 which are claimed to be non-DHP-like VDCC modulators. The main indication for these compounds is stroke (review including structures: Cox and Denyer, 1998) but no compound has yet been reported to be in clinical trials for the treatment of stroke or any other indication.

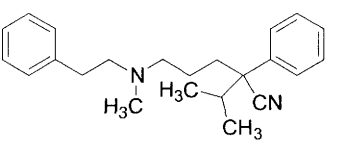


LY310315

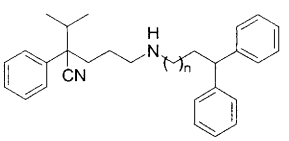
McNaughton et al. (1999) published data on LY310315, a synthetic macrocyclic polyamine. Effects of this compound were investigated on recombinant human N-type Ca^{2+} channels expressed in HEK293 cells. The electrophysiological characterization revealed that LY310315 is a potent and reversible N-type Ca^{2+} channel antagonist with an IC_{50} of approximately 0.4 µM at pH 7.35. Although most effective on N-type Ca^{2+} channels LY310315 also inhibits P-type and L-type Ca^{2+} channels.



Verapamil

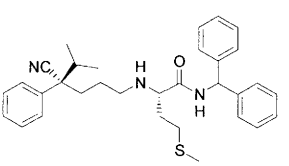


Emopamil



Compound 1

Through the modification of the known L-type VDCC inhibitors verapamil and its desmethoxy analog emopanil, Eli Lilly identified a novel series of amino acid containing phenylalkylamines which demonstrate submicromolar inhibition of neuronal non-L-type VDCC while showing markedly decreased activity on L-type channels. They initially investigated the modification of the phenethylamine moiety and found that chain elongation and incorporation of a second phenyl substituent (compound 1) markedly shifted activity away from L- towards non-L-type inhibition.

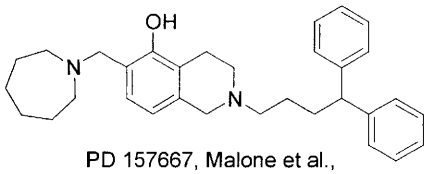


Compound 2

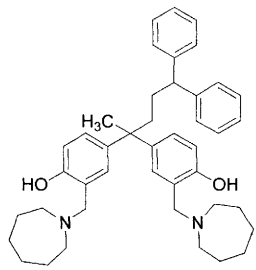
One of the best compounds of this series is the methionine-derived compound 2. It shows preferential inhibition of human α 1B construct (N-channel) with respect to α 1A (P/Q-channel) and α 1E (R-channel) (Ambler et al., 1997).

Warner-Lambert/ParkeDavis have published data on several N-type compounds for the treatment of stroke and pain (review: Cox and Denyer, 1998). PD157667, although

potent at the N-type Ca^{2+} channel, was found to have significant Na^+ and K^+ channel blocking activity at 10 μM. PD158143 however is selective with respect to sodium channel, potassium channel or L-type calcium channel activities.

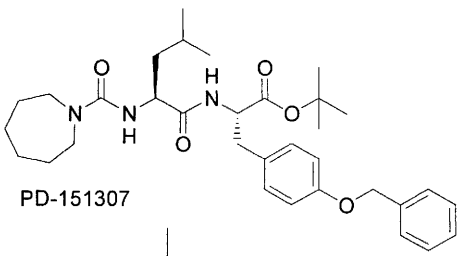


PD 157667, Malone et al., Warner-Lambert, WO9705125

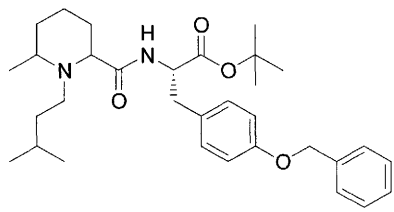


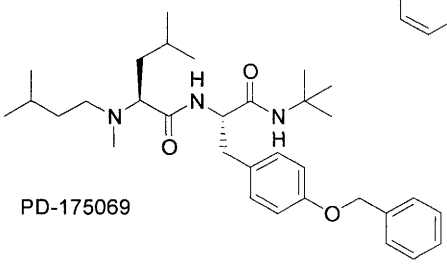
PD158143, Yuen, Warner-Lambert, US5767129, 1998

Neurex/Warner-Lambert and Elan/Warner-Lambert have published additional data on PD-151307 and PD-175069 and analogs thereof (Drug Data Report **1999**, *21*, 403, 576, 589):

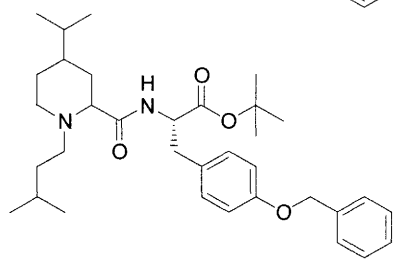


PD-151307



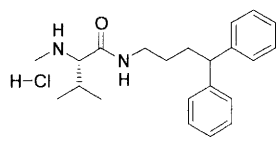


PD-175069



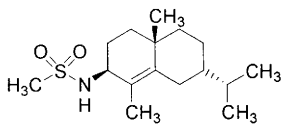
These compounds block N-type calcium channels in human neuroblastoma IMR-32 cells. PD-151307 (IC_{50} = 0.22 μM) shows about 40-fold selectivity for N- over L-type calcium channels (IC_{50} = 9.1 μM in GH3 cells). These compounds may also be potentially useful in the treatment of cerebral ischemia and chronic intractable pain.

Pfizer reported on a small-molecule calcium channel antagonist (compound 3) with potential for the treatment of pain (Song et al., 2000). Its antagonistic activity toward neuronal N-type calcium channels was confirmed by electrophysiology studies. It has an IC_{50} value of 1.3 μM against N-type calcium channels in superior cervical ganglion neurons. But it is also active, although to a lesser extent, against Na^+ channels (IC_{50} = 5.1 μM) and K^+



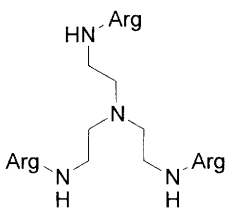
Compound 3

channels (IC_{50} = 9.9 µM). Furthermore this compound inhibits the activity of L-type calcium channels in smooth muscles. Fluorescence measurements, using Oregon Greens 488 Bapta-1 dye in the A10 smooth muscle cell line (FLIPR-assay) revealed an IC_{50} value of 0.4 µM. This compound penetrates into the CNS after i.v. and p.o. administration.



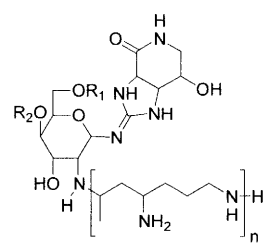
Compound 4,
Kazuyuki et al., Shionogi & Co., WO9801121

Shinogi and Co. has filed a patent application on P/Q-type calcium channel antagonists such as compound 4. Suppression of Ca^{2+} ion influx was evaluated using rat cerebellar synaptosomes. An IC_{50} value of 1.0 µM was obtained. Compounds in this series are claimed to be useful as antiischemic nerve cell disorder agents, anticonvulsants, migraine agents and analgesics and for the treatment of diseases caused by excessive release of neurotransmitters in the CNS.



Compound 5

Cypros Pharm. Corp. describes the use of polyguanidino derivatives as presynaptic N- and P/Q-type calcium channel blockers for i.v. (or i.c.v.) administration (Marangos et al. (Cypros Pharmaceutical Corp.), WO9836743). Compound 5 was administered to gerbils (7.5 mg/kg i.v.) prior to bilateral carotid occlusion. After 72 h the animals were sacrificed. Brains were perfusion-fixed and sections were stained to enable quantitative cell counts of live and dead neurons. The number of damaged neurons in the subiculum was 91.5 compared to 214 for a control treated with saline. It has been claimed that this compound can be used for the treatment of neuropathic pain and for the protection of neurons from excitatory damage under conditions of cerebral hypoxia.



	R_1	R_2	n
A-53930A	$CONH_2$	H	4
A-53930B	$CONH_2$	H	5
A-53930C	H	$CONH_2$	5

The Sankyo compounds A-53930A, B and C are natural products and were isolated from the culture broth of *Streptomyces vinaceusdrappus*. A-53930A and B are new compounds (JP08208690), whereas A-53930C is identical to streptothricin B. A-53930A, B and C inhibit [^{125}I]ω-conotoxin MVIIA binding to N-type Ca^{2+} channels (IC_{50} = 0.17, 0.091 and 0.071 µM respectively). There is no interaction with the DHP binding site of the L-type Ca^{2+} channel. It was also revealed that although A-53930C has antimicrobial activity against Gram-negative and -positive bacteria and fungi, A-53930A and B only show weak activity against Gram-negative bacteria (Hisamoto et al., 1998).

Clinical Data

L-type VDCC Inhibitors in Combination with a µ-Opioid Agonist

To demonstrate the enhancement of μ-opioid-mediated antinociception by L-type VDCC a nearly ineffective dose (threshold dose) of an opioid agonist can be used. All three types of L-type VDCC inhibitors enhance the opioid-mediated effect in an additive or synergistic manner, depending upon the pharmacological model used. For clinical investigations, however, such a study design may not be approved by the ethics commission. From this point of view clinical investigations are not as easy to carry out as the pharmacological investigations described in the section 'Antinociceptive effects of L-type Ca^{2+} channel antagonists'. By and large the following clinical trials reflect the results described in 'Preclinical data'. Nevertheless there are some reports with insignificant effects.

Fentanyl and nimodipine in cardiovascular surgery

To the best of our knowledge the first clinical study in this field was carried out at the universities of Giessen and Marburg (Germany) and reported by Bormann et al. in 1985. Twenty patients undergoing cardiovascular surgery were investigated in two groups. The 10 patients in the first group received high-dose fentanyl anesthesia (mean: 2.45 mg/patient), whereas the 10 patients in the second group were treated with only 0.1 mg fentanyl/patient in addition to nimodipine 1.0 μg/kg/min. Although the patients in the second group received only ≈ 1/25 of the μ-opioid dose compared to the first group, there were no differences with respect to perioperative course and postoperative demand for analgesics. So nimodipine can be used very effectively to reduce the necessary dose of fentanyl in cardiovascular surgery.

Fentanyl and nimodipine in aorto-coronary bypass operations

In a second paper, published 2 years later, the same group confirmed the data concerning the positive interaction of fentanyl with nimodipine. Forty-five men scheduled for aorto-coronary bypass operation received fentanyl according to their individual demands. Nimodipine at infusion rate of 1.0 μg/kg/min reduced the demand for fentanyl significantly. Astonishingly, nifedipine at infusion rate of 0.7 μg/kg/min failed to reduce the need for fentanyl during surgical procedures without influencing the quality of anesthesia (Boldt et al., 1987).

Fentanyl and nimodipine in elective hysterectomy

A randomized double-blind study including 40 patients undergoing elective hysterectomy under standardized balanced anesthesia were reported by Lehmann et al. in 1989. In a recovery room patients were allowed to self-administer fentanyl by means of the On-Demand Analgesia Computer. Demand dose was 34.5 μg, infusion rate 4 μg/h, lockout time 1 min, hourly max. dose = 250 μg. The patients were randomly and double-blindly assigned to have an additional infusion of either placebo

or nimodipine(N) (N = 15 µg/kg/h during the first 2 h, 30 µg/kg/h from the third to the 12th hour). Fentanyl consumption, pain scores, blood pressure, heart rate, respiratory rate and side-effects were monitored. At each time interval (4 h) when the fentanyl consumption was calculated, the nimodipine group had a lower consumption rate in comparison with the placebo group. Nevertheless, none of these differences were statistically significant. Further studies are necessary to evaluate this potential drug interaction at different dose ranges.

Morphine and nifedipine in orthopedic surgery and hysterectomy

Additional data concerning the influence of nifedipine on morphine analgesia was reported by Carta et al. (1990). In a double-blind, placebo-controlled experimental design, slow-release 20 mg tablets of nifedipine or identical placebo control tablets were used. The half-life of slow-release nifedipine is 15.2 ± 4.3 h (standard nifedipine tablets = 3.9 ± 2.3 h). Per randomization, persons were divided into a placebo (P) and a verum (V) group. Nifedipine significantly increased the analgesic effect of morphine (orthopedic surgery: P = 5; V = 5; hysterectomy: P = 8; V = 8). Respiratory and cardiovascular functions were not significantly changed by this L-type VDCC inhibitor.

Epidural morphine and sublingual nifedipine

Pereira et al. (1993) evaluated postoperative pain relief and incidence of side-effects of the combination of epidural morphine (0.5 mg) and sublingual nifedipine (10 mg). In this double-blind, placebo-controlled study 36 women were submitted to elective operations (hysterectomy and colpoperineoplasty). The nifedipine-treated group showed a significant drop in blood pressure which was controlled by rehydration. The results indicate that epidural morphine-induced postoperative pain relief may be enhanced by systemic administration of nifedipine with easily controlled side-effects.

Morphine and diltiazem, verapamil or nimodipine

Negative results were reported by Hasegawa and Zacny (1997). They examined the effects of three L-type VDCC inhibitors (diltiazem 30 mg p.o.; nimodipine 60 mg p.o. and verapamil 80 mg p.o.) on morphine (10 mg/70 kg i.v.) in a cold-pressor test with nine healthy volunteers. Subjects first ingested the oral drugs or placebo and 120 min later were injected with morphine or saline. Morphine alone and in combination with the VDCC inhibitors reduced pain ratings. No statistically significant differences in the pain measures between the morphine and the L-type VDCC blockers/morphine conditions were observed. Other conditions e.g. altered dose or time regimes of the p.o. and i.v. drug administration were not tested.

Morphine and nimodipine in cancer pain

Another negative result was reported by Roca et al. (1996). They assessed the ability of nimodipine to increase the analgesic effect of morphine in 32 patients suffering from cancer pain. In this double-blind, placebo-controlled cross-over study morphine administration began a few days before the start of the study. The analgesic effects of two combinations were compared: morphine plus placebo and morphine plus 90 mg/24 h nimodipine. The study spanned 8 days, including the wash-out period. No significant statistical differences were found in analgesic effect between the two groups. Higher doses of the L-type VDCC inhibitor were not tested.

In contrast to this study Santillán et al. (1994) used a higher dose of nimodipine and reported positive results for nimodipine - morphine association in a non-placebo controlled trial with cancer patients. Nimodipine succeeded in reducing the daily dose of morphine in 16 of 23 patients (p.o. n = 13; i.t.: n = 3), and failed to modify it in two patients. Total oral daily dose of morphine was significantly reduced by nimodipine (120 mg/day) from 283 to 159 mg. Intrathecal morphine was also reduced. This data was confirmed by a randomized, double-blind, placebo-controlled study which was published 4 years later (Santillán et al., 1998). The study started with 54 cancer patients. A total of 30 patients completed the study (14 in the nimodipine group and 16 in the placebo group). The dose of morphine was reduced from 313 to 174 mg/day ($p < 0.001$) in the nimodipine group (120 mg/day), and non-significantly from 254 to 218 mg/day in the placebo group. The authors conclude that the introduction of nimodipine in patients chronically treated with morphine may be a safe alternative to reducing the daily requirement of the μ-opioid agonist. It is further suggested that interference with Ca^{2+}-related events may attenuate the development and/or expression of tolerance to morphine in a clinically relevant way.

N-type VDCC Inhibitors

Clinical studies of N-type VDCC inhibitors are limited to SNX-111 (Ziconotide), a synthetic peptide related to the naturally-occurring ω-conopeptide MVIIA (see Table 3; for clinical reviews see Hunter, 1999 and Prado, 2001).

Clinical trials with SNX-111 (Ziconotide)

In 1997 Brose et al. published the result of a single case study. SNX-111, administered i.t. by continuous, constant-rate infusion, produced dose-dependent pain relief in a 43-year-old male patient with a 23-year history of intractable deafferentation and phantom limb pain secondary to brachial plexus avulsion and subsequent amputation. Dizziness, blurred vision, and lateral-gaze nystagmus

were dose-dependent side-effects that resolved with decreasing dose levels. Complete pain relief was achieved in this patient without side-effects after dose adjustment.

A second report was published by Atanassoff et al. (2000). This was a randomized, double-blind, placebo-controlled study including patients undergoing elective total abdominal hysterectomy, radical prostatectomy, or total hip replacement. Ziconotide was administered as a continuous i.t. infusion at a rate of 0.7 µg/h or 7.0 µg/h. Thirty patients received the study drug, 26 were evaluable for efficacy. Mean daily morphine consumption was less in patients receiving Ziconotide than in placebo-treated patients ($p = 0.040$). Four of six patients who received the high dose of Ziconotide (7.0 µg/h) developed adverse events such as dizziness, blurred vision, nystagmus and sedation. The conclusion of the report was that an i.t. infusion of Ziconotide results in a significant morphine-sparing effect. The dose of 0.7 µg/h may be closer to the ideal dose than 7.0 µg/h.

Adverse effects of SNX-111 (Ziconotide)

The first study dealing with the adverse effects after i.t. administration of Ziconotide was published by Penn and Paice (2000). This clinical report described the experiences of three patients suffering from chronic pain, who developed very serious side-effects after continuous i.t. infusion of the drug. In addition to the side-effects described in previous papers, nasal congestion, urinary retention, bradycardia, orthostatic hypotension, nausea and vomiting, coma, ataxia, dysmetria, agitation, hallucination, rash, hypoglycemia and diarrhoea were reported by the authors. Penn and Paice (2000) point out that these complications occurred within a highly monitored environment. Widespread use in general clinical conditions are likely to lead to an even greater prevalence of adverse effects, with potentially more serious outcomes.

Conclusions

L-type VDCC inhibitors

In pharmacological experiments L-type VDCC inhibitors are not convincingly effective for the treatment of pain. In combination with a µ-opioid agonist, however, all three classes of L-type inhibitors lead to an enhancement of opioid-induced antinociception. This enhancement may be additive or even over-additive (synergistic), depending on the species and pain model under investigation. To achieve equipotent antinociception the µ-component can be reduced. This opioid-sparing effect has also been documented in double-blind, placebo-controlled clinical studies without drastic changes to respiratory and cardiovascular function. Observed side-effects can be easily controlled. A large body of evidence points to the

possibility that L-type VDCC blockers may prevent the development of opioid tolerance. Thus, a new generation of drugs which exhibit a μ-agonistic and an L-type VDCC antagonistic activity in the same molecule is of great interest.

N - and/or P/Q - type VDCC inhibitors

N - and/or P/Q - type VDCC inhibitors (ω-conotoxins and ω-agatoxin IVA) are active in a variety of pharmacological pain models. In contrast to opioids, only minimal development of tolerance was observed when SNX-111 (Ziconotide), a synthetic peptide related to the naturally-occurring ω-conopeptide MVIIA, was administered chronically over 7 days. Ziconotide and related peptides can only be administered i.t. or i.c.v. An opioid-sparing effect is also evident. The enhancement of opioid antinociception with N-type VDCC blockers was reported to be additive. Clinical studies with N-type VDCC inhibitors are limited to SNX-111 (Ziconotide). Although positive results have been reported in patients suffering from chronic pain after continuous constant i.t. infusion of Ziconotide, severe side-effects seem to limit the usefulness of this peptide. To overcome the disadvantages of intrathecal administration, small molecule N -, or P/Q - type VDCC blockers for i.v. and/or p.o. application are under investigation.

Clinical trials with new L- type and especially N - or P/Q - type VDCC inhibitors are eagerly awaited.

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7 Glutamate Receptors

7.1 Metabotropic Glutamate Receptors

Klaus Schiene and Corinna Maul

Glutamate plays a significant role in nociceptive processing

Evidence from the last several decades indicates that the excitatory amino acid glutamate plays a significant role in nociceptive processing. Glutamate and glutamate receptors are located in areas of the brain, spinal cord and periphery which are involved in pain sensation and transmission (for a review see Fundytus 2001). Glutamate acts at several types of receptors, including ionotropic (directly coupled to ion channels) and metabotropic (directly coupled to intracellular second messengers via guanine nucleotide regulatory (G) proteins) receptors. In this chapter we are focussing on metabotropic glutamate receptors which modulate a variety of neuronal effects at both pre- and post-synaptic level in several brain regions.

Molecular cloning and pharmacological studies revealed the existence of at least eight mGlu receptor subtypes (mGlu1-mGlu8) which are classified into 3 groups based on sequence homology, signal transduction mechanisms and receptor pharmacology. Group I mGluRs which include mGluR1 and mGluR5 stimulate phosphatidylinositol (PI) hydrolysis, and activation of these receptors ultimately leads to activation of PKC, and increases in the level of intracellular Ca^{2+}. The increase in the level of intracellular Ca^{2+} may in turn trigger production of NO via Ca^{2+}/calmodulin activation of NOS. Group II (mGluR2 and mGluR3) and group III (mGluR 4,6,7,8) mGluRs are negatively coupled to adenylate cyclase, and activation of these receptors inhibits the production of cyclic adenosine-3',5'-monophosphate (cAMP).

group I	group II	group III
mGluR 1,5	mGluR 2,3	mGluR 4,6,7,8
PI-coupled	cAMP-coupled	cAMP-coupled

All mGluRs are characterized by a putative signal peptide, an unusually large (470 - 510 amino acids) extracellular amino terminal domain (ATD), seven membrane-spanning regions characteristic of the G protein-coupled receptor (GPCR) superfamily, and an intracellular carboxy terminal domain variable in size and amino acid composition among the various members of the family. Many of the mGlu receptors exist as various isoforms with different intracellular carboxy termini generated by alternative splicing of their pre-messenger RNA (Conn and Pin, 1997).

Sequence homology is in the range 65 - 70% between mGluRs belonging to the same group, but falls to 40 – 45 % among members of different groups. Conserved regions are found in the membrane-spanning regions and in a hydrophobic region located in the extracellular ATD. It

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is now generally accepted that mGluR receptors constitute, together with the Ca^{2+}-sensing receptor, a putative pheromone receptor, and $GABA_B$ receptors a distinct family (type C family) of GPCRs. Distinctive features of type C family are: an unusually large extracellular ATD, no homology with other GPCR families at the level of the transmembrane regions, and coupling with G-proteins localized at the level of the second and not the third intracellular loop. For group I mGluRs a molecular mechanism leading to domain closure has been postulated (Constantino et al., 1999).

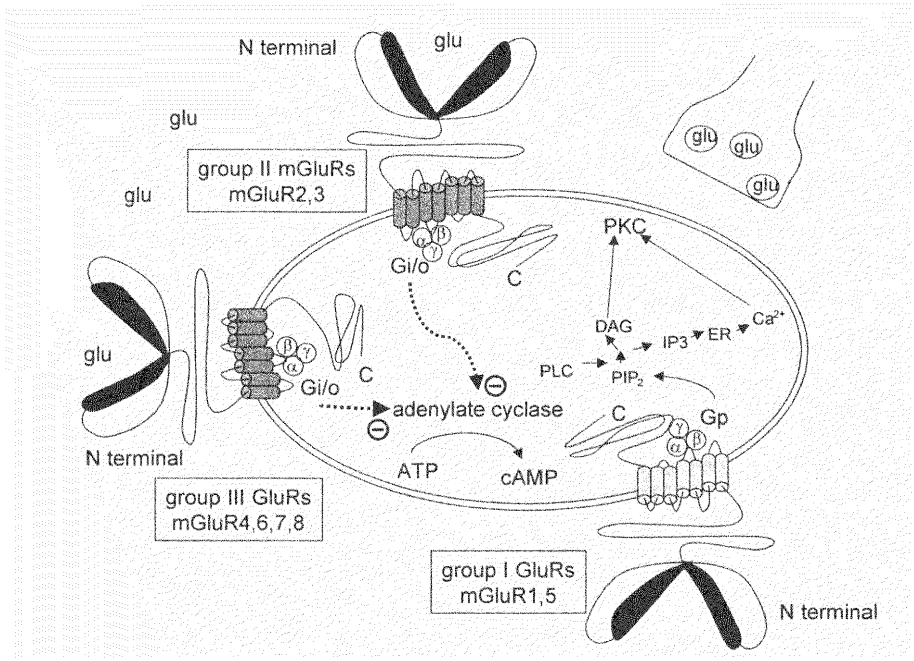
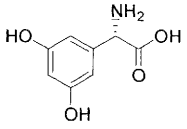


Figure 1: Second messenger coupling of mGluRs. Group II and group III mGluRs are negatively coupled to adenylate cyclase. Activity at group I mGluRs stimulates a phospholipase C (PLC)-catalyzed phosphoinositide (PIP_2) hydrolysis which leads to the production of inositol triphosphate (IP_3) and diacylglycerol (DAG). The production of IP_3 promotes the release of intracellular Ca^{2+} from its stores within the endoplasmatic reticulum (ER). The increased Ca^{2+} influx from either extracellular or intracellular sources, and production of DAG, are essential elements for the stimulation of the enzyme PKC.

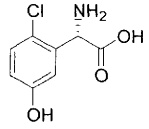
Selective compounds for all groups as well as some subtype-selective compounds have been found which have been very useful in mGluR research during the last

decade. A selection of mGluR modulators is given in fig. 2 (for a review see Pin et al., 1999).

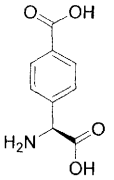
Group I mGluR modulators:



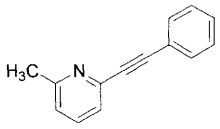
(S)-3,5-DHPG, selective group I mGlu receptor agonist, 3,5-dihydroxy-phenylglycine



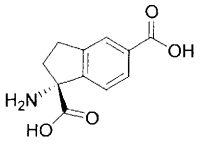
CHPG, selective mGlu5 receptor agonist, 2-chloro-5-hydroxy-phenylglycine



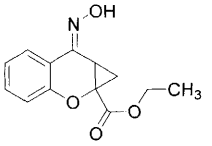
(S)-4-CPG, selective group I antagonist, 4-Carboxy-phenylglycine



MPEP, selective mGluR5 antagonist, 2-Methyl-6-phenyl-ethynyl-pyridine

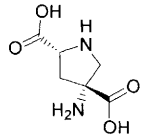


AIDA, selective mGluR1 antagonist 1-Amino-indan-1,5-dicarboxylic acid

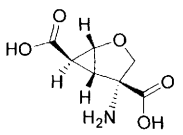


CPCCOEt, selective mGluR1 antagonist, 2-Hydroxyimino-1a,2-dihydro-1*H*-7-oxa-cyclopropa[*b*]naphthalene-7a-carboxylic acid ethyl ester

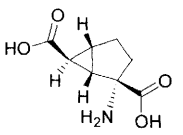
Group II mGluR modulators:



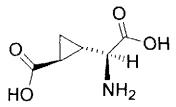
(2*R*,4*R*)-APDC ((2*R*,4*R*)-4-aminopyrrolidine-2,4-carboxylic acid), selective group II mGluR agonist



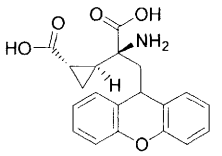
LY-379268 selective group II mGluR agonist (Monn et al. 1999)



LY-354740 selective group II mGluR agonist (Monn et al. 1997)

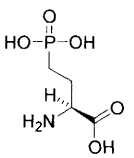


L-CCG 1, selective group II mGluR agonist, 2-(Amino-carboxy-methyl)-cyclopropane-carboxylic acid

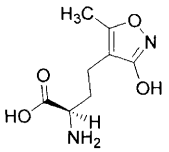


LY 341495, group II mGluR antagonist (Ornstein et al. 1998)

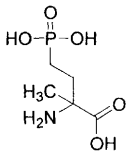
Group II mGluR modulators:



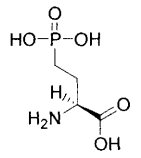
(*S*)-SOP, group III mGluR agonist, (*S*)-serine O-phosphate



(S)-homo-AMPA, mGluR6-selective agonist



MSOP, group III mGluR selective antagonist, (RS)-α-Methylserine-O-phosphate



L-AP4, selective group III mGluR agonist (Monaghan et al. 1983)

Figure 2: Group I, II and II mGluR modulators.

Varying distributions of expression of the mRNA for the various subunits of mGluRs have been detected throughout the dorsal horn of the spinal cord, with the exception of mGluR2, mGluR6 and mGluR8, which are undetectable in rat spinal cord (Yashpal et al., 2001).

The coupling of group I mGluRs to PI hydrolysis suggests an influence on pain perception because of PKC activation

The coupling of group I mGluRs directly to PI hydrolysis suggests an influence on pain perception because this leads to the activation of PKC. PKC has been shown to contribute significantly to the development of persistent pain. Inhibition of PKC attenuates formalin-induced pain scores and mechanical hyperalgesia associated with a thermal injury (Coderre, 1992, Yashpal et al., 1995). Following nerve constriction injury, there is an increase in membrane-associated PKC in the spinal cord (Mao et al., 1992). PKCγ knockout mice were shown to have a significant reduction of mechanical and thermal allodynia following nerve constriction injury (Malmberg et al., 1997).

Electrophysiological data have shown that application of mGluR agonists to the brain, spinal cord and periphery induces neuronal depolarization (Zheng and Gallagher, 1992, Boxall et al., 1996) and resulted in long-lasting potentiation of synaptic transmission as well as acute neuronal cell death (Zheng et al., 1996). Iontophoretic application of the mGluR antagonist (R,S)-CHPG attenuates rat dorsal horn activity associated with repeated mustard oil application (Young et al., 1994, 1995). However, *in vivo* experiments do not show clear results in acute pain models. It has been shown that the effects of mGluR agonists may be different in normal animals compared with animals with carrageenan-induced inflammation. The non-selective mGluR agonist (1S,3R)-ACPD facilitates neuronal responses evoked by noxious stimuli in normal animals, but inhibits these responses in animals suffering from inflammation. The group I mGluR selective agonist 3,5-Dihydroxyphenylglycine (DHPG) produces mixed effects in normal animals and inhibition in animals with inflammation (Stanfa and Dickensen, 1998; Maione et al., 2000). However, antisense oligonucleotide knockdown of spinal mGluR1 significantly reduced cold hyperalgesia, heat hyperalgesia and mechanical allodynia in the ipsilateral (injured) hindpaw of neuropathic rats (Fundytus et al., 2001).

mGluR agonists do not show clear results in acute pain models

In a model of acute postoperative pain, several mGluR antagonists do not produce any reduction in hyperalgesia or allodynia indicating that mGluR antagonists play only a minor role in acute pain (Zahn and Brennan, 1998). In a recent study it was shown that intrathecal administration of the mGluR1/5 antagonist (*S*)-4CPG was unable to significantly reduce the nociceptive responses induced by formalin injection in the hindpaw. It was shown that (*S*)-

4CPG (i.t. administration) did not influence formalin-induced activation of spinal PKC. On the other hand it has been reported that group I mGluR antagonists show antinociceptive in the second phase of the formalin test (s.c. administration) (Bhave et al., 2001). However, Bhave et al. presented results indicating that peripheral group I mGluR activation is necessary for full expression of inflammatory hyperalgesia, and blockage of these receptors is sufficient to completely eliminate the effects of increased glutamate levels in the periphery. Peripherally applied glutamate, the endogenous agonist of mGluRs, increased thermal sensitivity. Injection of the group I mGluR antagonists MPEP or CPCOEt 15 min before glutamate injection completely blocked glutamate-induced thermal hypersensitivity. NMDA and group II and III mGluR antagonists had no effect. This suggests that glutamate released during inflammation may activate group I mGluRs leading to thermal hyperalgesia.

Antagonism of group I mGluRs appears to be particularly useful in the reduction of hyperalgesia and allodynia associated with chronic pain

In contrast to its non-significant effects when administered i.t. in the formalin-test, i.t. treatment with (*S*)-4CPG was effective at significantly reducing neuropathic-like pain behavior in nerve-injured rats (Fisher et al., 1998; Yashpal et al., 2001; for a review see Fundytus, 2001). Consistent with the behavioural effects, there is evidence that chronic constriction injury induced increases in the translocation and activation of spinal PKC dependent on activity at mGlu1/5 receptors (Yashpal et al., 2001).

In summary, it has been shown that group I mGluR antagonists can lead to antinociception, antihyperalgesia or anti-allodynia in models of chronic and inflammatory pain.

Group II and III mGluRs represent potential targets for new analgesics

Inhibitory metabotropic GluRs (group II and III) also represent potential targets for new analgesics. Group II and group III mGluRs are present in the superficial dorsal horn, thalamus and cortical areas involved in pain processing. Furthermore, subtypes of mGluR II and III and their mRNA respectively have been shown to be upregulated in chronic pain states (Boxall et al., 1998; Azkue et al., 2001; Neto et al., 2001). There is growing evidence to suggest that selective agonists of group II and group III mGluRs have a potential for treatment of pain (Dolan and Nolan, 2000).

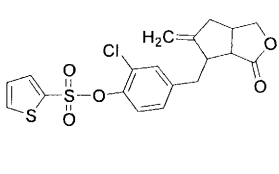
Agonists at group II and group III mGlu receptors may be useful in downregulating the enhanced responses of nociceptive neurons during neuronal sensitization which involves the cAMP - PKA pathway

Activation of the G-protein-coupled mGluR group II and group III has been shown to inhibit cAMP formation and reduce neuronal excitability and synaptic transmission (Gereau and Conn, 1995; Macek et al., 1996; Neugebauer et al., 1997; Miller, 1998; Bushell et al., 1999; Schoepp et al., 1999). Therefore agonists at these receptors may be useful in downregulating the enhanced responses of nociceptive neurons during the stages of the neuronal

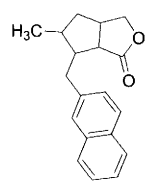
sensitization which involve the cAMP - PKA pathway. The group II agonists LCCG1 ((2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)glycine), the highly potent, selective and systemically active ligand LY379268 ((-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate) and the group III agonist L-AP4 (L(+)-2-amino-4-phosphonobutyric acid) reversed the capsaicin-induced sensitization in primate spinothalamic tract cells. The group II GluRs may modulate the response of sensitized neurons, because, in contrast to L-AP4, the group II agonists have no effect on responses to cutaneous stimuli under control conditions but reversed the enhanced responses of sensitized spinothalamic tract cells (Neugebauer et al., 2000). Furthermore the inflammatory hyperalgesia after intraplantar carrageenan injection as well as capsaicin-induced neurogenic thermal hyperalgesia was reduced after administration of LY379268 (Sharpe et al., 2002). Group II mGluRs may also play a role in the development of antinociceptive morphine tolerance. The systemically active group II mGluR LY354740 ((+)-2-aminobicyclo [3,1,0]hexane-2,6-dicarboxylic acid) inhibited the development of morphine tolerance in mice (Popik et al., 2000) and naloxone-induced symptoms of morphine withdrawal (Klodzinska et al., 1999).

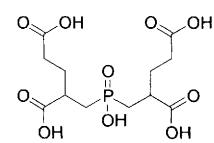
Furthermore, the group III agonist L-AP4 attenuated changes in mechanical thresholds after spinal cord injury (Mills et al., 2002) and produced dose dependent reductions in spontaneous nociceptive behavior of rats induced by intrathecal (i.t.) administration of the selective group I mGluR agonist (*RS*)-3,5-dihydroxyphenylglycine ((*RS*)-DHPG) (Lefebvre et al., 2000).

Therefore mGluRs, particularly group I mGluRs may be useful targets for therapy of chronic inflammatory pain, neuropathic pain and as an adjunct to opioid analgesic treatment. During the last couple of years a number of patents claiming new analgesics affecting metabotropic glutamate receptors have been filed. A selection is given in fig. 3:

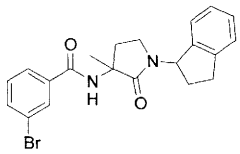


Stolle et al. (Bayer AG), WO 0104107, WO 9936418, WO 9936417, WO 9936419, mGluR1 antagonists

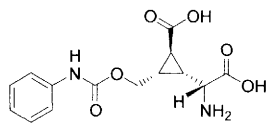




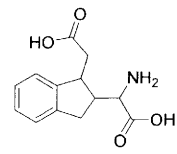
Kozikowski et al., WO 0064911 ligands for mGluRs



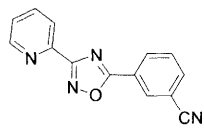
Clark et al. (Eli Lilly) WO 0069816
mGluR5 antagonists



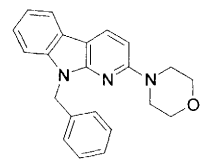
Colladocano et al. (Eli Lilly), WO 0192213,
mGluR agonists



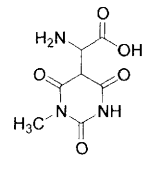
Curry (IGT Pharma),
WO 0102342, mGluR
modulators



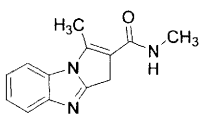
van Wagenen et al. (NPS Pharmaceuticals)
WO 0112627, mGluR5 antagonists



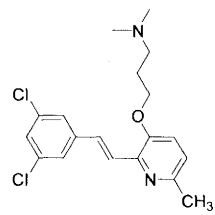
Jakobsen, P. (Novo Nordisk) US 5783575
mGluR1a antagonists



Curry, WO 0179185,
mGluR modulators



Hayashibe et al. (Yamanouchi)
WO 0059913



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mGluR5 antagonists

Figure 3: Further mGluR ligands.

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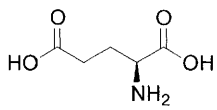
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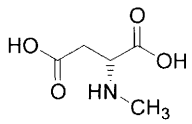
7.2 NMDA Receptor Antagonists

Werner Englberger, Michael Przewosny and Corinna Maul

The amino acid L-glutamate is the main excitatory neurotransmitter of the central nervous system (Fonnum, 1984). Glutamate exerts its excitatory effects either by activation of several G-protein-coupled metabotropic glutamate receptors or by induction of ion fluxes by different classes of ionotropic receptors. The NMDA receptor is one of those glutamate-gated ion channels which got its name from its selective artificial agonist NMDA (N-methyl-D-aspartate) and which controls slow but persistent ion fluxes of Na^+, K^+, and Ca^{2+} across the cell membrane.



Glutamate (glutamic acid) (2*S*)-2-Amino-pentanedioic acid



NMDA (N-methyl-D-aspartate) (2*R*)-2-Methylamino-succinic acid

Kainate or AMPA receptors are further glutamate-gated, fast-conducting cation channels which owe their designations to their selective agonists kainate or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid), respectively (for review see: Chapter 7.3, Collingridge and Lester, 1989; Seeburg 1993; Hollmann and Heinemann 1994; Ozawa et al. 1998; Parsons et al. 1998; Dingledine et al. 1999).

Molecular Diversity of NMDA Receptors

In addition to the multiplicity of receptor sites for glutamate, the NMDA receptors bear their own complexity as they are constructed as multimers from three distinguishable subunit classes (i.e. NR1, NR2 and NR3 subunit class). With regard to the stoichiometry of the NMDA receptor there is still some debate as to whether a native NMDA-gated ion channel within the cell membrane consists of either a tetramer or pentamer. More recently, it has been suggested that the tetramic stoichiometry is more probable (Laube et al., 1998; Hollmann, 1999).

From electrophysiological studies with *in vitro* expressed NMDA subunits in cellular systems, it has been concluded that a conventional NMDA receptor must consist of a mixed combination of NR1 splice variants and $NR2_{A-D}$ subunits in order to have full physiological activity (Monyer et al., 1992). This NR1/NR2 expression pattern has also been reported to be a prerequisite for adequate cell surface expression of NMDA receptors (McIlhinney et al., 1996). Given the tetrameric stoichiometry, any conventional NMDA receptor might consist of two NR1 and two NR2 subunits.

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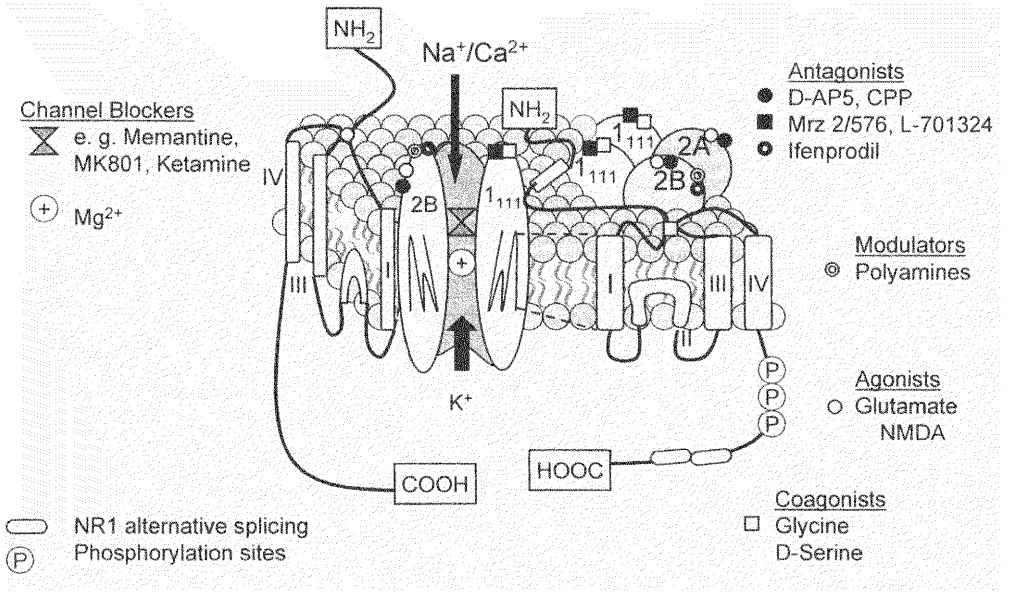


Figure 1: Model of NMDA receptor tetramer complex including the different pharmacological effector sites (adapted from Danysz and Parsons (1998))

NR1 subunit class - one gene encoding for eight alternative splice variants

From rodent and human genomes one gene which encodes a single member of the NR1 subunit class (Moriyoshi et al., 1991; Zimmer et al., 1995) and four genes encoding $NR2_{A-D}$ of the NR2 subunit class have been cloned and have been assumed as essential NMDA receptor subunits (Ikeda et al., 1992; Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992). The mRNA of the NR1 subunit is further posttranslationally modified by alternative splicing of three exons yielding eight different splice variants (Nakanishi et al., 1992). In cDNA libraries different splice variants have been found. They show a varying expression pattern in rat brain and contribute to a different physiology of NMDA receptors (for review see Zukin and Bennett, 1995). Recently differential expression of splice variants after spinal cord injury has been reported (Prybylowski et al., 2001) and there is evidence for an interaction of the spliced N1 cassette of the NR1 subunit with zinc, protons and spermine as modulators of the NMDA receptor (for review see Dingledine, 1999). Yet the pharmacological relevance of the alternative NR1 splice variants is still relatively unclear.

NR2 subunit class four genes encoding for $NR2_{A-D}$

From molecular mutagenesis studies the glycine binding site has been found to be expressed on the NR1 subunit (Kuryatov et al., 1994; Wafford et al., 1995; Ivanovic et al.,

1998), whereas the NR2 subunits provide the binding sites for glutamate (Laube et al., 1997, Anson et al., 1998).

In addition, it can be assumed that there are further cooperative effects between NR1 and NR2 subunits as the affinity of ligands for the glycine$_B$ binding site of the NMDA receptor is dependent on the structure of the NR2 subunit (Honer et al., 1998).

More details regarding the physiological and pharmacological relevance of NR2$_{A–D}$ subunits have been obtained, at least in part from studies using NR2 subtype-selective antagonists. A high NR2$_B$ subtype-selectivity has been reported for ifenprodil-like antagonists (for review see: Chizh et al., 2001 and Zhuo, 2002). NR2$_B$-selective antagonists show a favorable efficacy to side-effect ratio which is thought to be partially due to a restricted distribution of the NR2$_B$-subtype. The NR2$_B$ subtype was found in the superficial layer of the spinal dorsal horn where it is thought to be putatively engaged in the transmission of nociceptive inputs. Additionally supraspinal sites seemed to be involved (Chizh et al., 2001). In contrast the NR2$_B$ subtype was not found in the cerebellum, which might in turn be responsible for the good tolerability in as much as no or rather little ataxia is caused by NR2$_B$-specific antagonists. Other NMDA antagonists have been shown to have at least a preference for inhibition of certain NR2 subunits (Sucher et al., 1996). The high affinity non-competitive antagonists MK-801 or phencyclidine with a preference for NR2$_A$ and therefore putatively with a high risk for psychotomimetic side-effects should be mentioned here. Whereas some low affinity non-competitive antagonists rather show a preference for NR2$_C$ with better tolerability in this respect (Monaghan et al., 1997). Further information about the pharmacology of the NMDA antagonists is given below in more detail.

NR3 subunit class 2 genes encoding for NR3$_A$ or NR3$_B$

Furthermore, in rodents a member of a third class of NMDA receptor subunits (NR3$_A$) has been cloned (Sucher et al., 1995). Expression of this NR3$_A$ subunit together with NR1 and NR2 subunits has been shown to cause *in vitro* an attenuation of the NMDA-induced ion fluxes and consequently NR3$_A$ knock-out mice show *in vivo* enhanced NMDA receptor activity (Das et al., 1998). Meanwhile a second member (NR3$_B$) of this third class of subunit with comparable features has been cloned from mouse genome (Nishi et al., 2001).

Very recently, Chatterton et al. (2002) reported that expression of NR1 with NR3$_A$ or NR3$_B$ without any NR2 subunit in xenopus oocytes results in the generation of

functional active ion channels with unique physiological features. In particular, glycine alone seems to control the gating of the NR1/NR3 ion channel for small cations such as Na^+. Neither glutamate nor NMDA are needed as co-agonists for the activation of the channel.

Whereas the physiological role of the neurotransmitter glycine has so far been considered to be inhibitory (via strychnine-sensitive glycine receptors) the above interaction with NR1/NR3 ion channels indicates an excitatory role for glycine in addition to its co-agonistic function at the NMDA receptor. Further pecularities of the NR1/NR3 oligomers include the lack of voltage-dependent Mg^{2+} block (as shown subsequently for the conventional NMDA receptor combinations) and the antagonistic action of D-serine to these NR1/NR3 oligomers, whereas D-serine behaves as an alternative endogeneous co-agonist at the glycine binding site of conventional NR1/NR2 receptors (Chatterton et al., 2002). Since these findings are very recent, there is not sufficient data to discuss in more detail the physiology or even pharmacology of these novel NR1/NR3 oligomers.

Preliminary data support the existence of comparable sequences in the human genome, however, functional expression of human NR3 subunits has not been reported so far as far as we are aware.

In situ expression of NMDA receptors might be even more sophisticated in as much as NMDA receptor complexes might consist of a submixture of each different subunit class (for review see Béhé et al., 1999) possibly with new characteristic features. With the advent of the third NR3 subclass there might exist any mixture of all three subclasses of NR1, NR2, and NR3 subunits. In this respect NMDA receptor complexes might gain new functional characteristics as shown for the NR1/NR3 oligomers and there is obviously an urgent need for reconsideration of the NR3 contribution *in vivo*.

Functional Role of NMDA Receptors in Pain

Unfortunately a global knock-out of NR1 subunits (i.e. a total deficit of NMDA receptor functionality) produces non-viable offspring which die perinatally within hours (Forrest et al., 1994; Li et al., 1994). The non-viable offspring suffer from respiratory distress, cyanosis, and severe ataxia. In addition, due to neurophysiological abnormalities they do not develop any suckling reflex.

In this respect the development of conditional or regional knock-out mice proved to be a powerful tool for the

elucidation of the functional role of NMDA receptors. By means of these techniques have provided significant evidence for the essential role of NMDA receptors in learning and memory processes within the hippocampus (Tsien et al., 1996) and thus those effects should therefore also be considered as typical adverse drug reaction of NMDA antagonists. Very recently a conditional knock-out of NR1 in the spinal cord has been reported to attenuate hyperalgesia in formalin-induced persistent pain models or in partial nerve injury models (South et al., 2001). These data are consistent with the previously shown attenuation of formalin-induced pain in regional NR1 knock-downs induced by intrathecal injection of NR1 antisense polynucleotides in rats (Garry et al., 2000).

Knock-out mice with depletion of $NR2_A$, $NR2_C$, or $NR2_D$ proved to be viable although global $NR2_B$ knock-outs also die perinatally due obviously to a missing suckling response (Kutsuwada et al., 1996). The $NR2_A$, $NR2_C$, or $NR2_D$ global knock-out mice show no severe symptomatology, are fertile, and have a normal life expectancy (Ikeda et al., 1995; Sakimura et al., 1995; Ebralidze et al., 1996). Most obvious in these knock-out mice is the deficit in spatial learning or memory (Morris water maze) obviously associated with an impaired synaptic plasticity (Sakimura et al., 1995). However, the combined knock-out of $NR2_A$ and $NR2_C$ worsens the symptomatology and causes severe motor impairment (Kadotani et al., 1996). So far only sparse data are available regarding the impact of the genetic NR2 knock-outs or genetic modifications on pain perception in animal models. A knock-out of $NR2_A$ abolishes a mechanical allodynia inducible by intrathecal injection of PGE_2 or NMDA totally, whereas $NR2_D$ knock-out mice behave like wild-type mice. However, the same authors reported that a comparable, mechanical allodynia induced by intrathecal injection of $PGF_{2\alpha}$ or AMPA showed the reverse phenomenon in as much as $NR2_A$ knock-out mice show allodynia like wild-type mice while the allodynic behavior is totally abolished in $NR2_D$ knock-outs (Minami et al., 2001). An overexpression of $NR2_B$ subunits in the forebrain results in an enhanced inflammatory pain perception (Wei et al., 2001) in addition to enhanced learning and memory capabilities (Tang et al., 2001). In addition, knocking out D-amino-acid oxidase, an enzyme involved in the catabolic degradation of D-amino acids and thus in the accumulation of the co-agonist D-serine of the NMDA receptor, results in potentiation of NMDA receptor function and in enhanced nocicepion (Wake et al., 2001).

Taken together there is some direct proof for a functional contribution of spinal NMDA receptors in the process of induction of hyperalgesia by conditional spinal NR1 knock-out animals or spinal NR1 knock-down experiments. While at least indirect evidence suggests a correlation between upregulation of the NMDA receptor activity and nociception, the interpretation of the impact of knocking out NR2 subunits needs further detailed research.

By far the most evidence for an essential role of NMDA receptor activation especially in chronic pain has been provided by pharmacological studies with NMDA receptor antagonists which is discussed below.

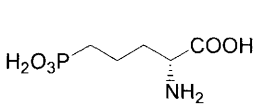
Physiology of NMDA Receptors Offers Several Therapeutic Target Approaches

Several functionally relevant binding sites at the NMDA receptor ion channel are suitable for therapeutical targeting (Wroblewski et al., 1989). These target sites comprise the primary recognition sites for the agonist glutamate or its artificial subsitute NMDA and the co-agonists glycine or D-serine as well as the modulatory polyamine sites or binding sites within the channel pore. They are discussed in more detail in the following sections.

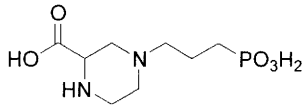
(a) Competitive NMDA receptor antagonists

Obviously glutamate or its artificial substitute NMDA is a prerequisite for the activation of a conventional NMDA receptor. In the case of a postsynaptic NMDA receptor of a centrally-projecting neuron or interneuron within the substantia gelatinosa of the spinal dorsal horn, this might be provided by an innervating glutamatergic primary afferent fiber and a substantial release of glutamate into the synaptic cleft. This offers the glutamate binding site as a first target site for the so-called competitive glutamate antagonists which exert their antagonistic actions by competively displacing glutamate. The structures of those antagonists resemble closely the pharmacophores of glutamate. Usually they consist of an amino group which may be part of a heterocyclic system and an acidic moiety such as carboxylic acid or phosphoric acid.

AP5 and CPP are antagonists having a binding affinity for the NMDA receptor comparable to glutamate (Lehmann et al., 1987; Murphy et al., 1987). Spinally administered, both compounds exert antinociceptive effects (Coderre et al., 1994; Goettl et al., 1994; Kristensen et al., 1994; Leem et al., 1996). The limitation of such structures is of course their low lipophilicity which restricts their availability mainly to the periphery and usually requires spinal administration to obtain therapeutic efficacy.



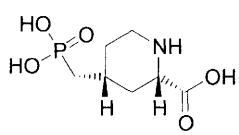
D-AP5 (D-norvaline)



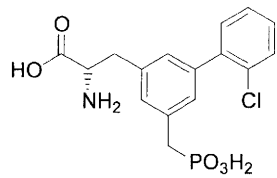
CPP, 4-(3-Phosphono-propyl)
-piperazine-2-carboxylic acid

Scheme 1: Structures of D-AP5 and CPP.

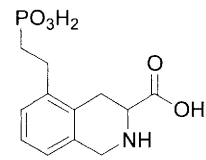
Incorporation of a nitrogen into heterocyclic structures (e.g. CPP, D-CPPene, or Selfotel) offers more lipophilic structures which in turn show a better blood - brain barrier (BBB) penetration (Kristensen et al., 1995; Herrling et al., 1997; Schmutz et al., 1997). Attempts to derivatize AP5/AP7 by introducing heterocyclic systems led to interesting structures with CNS availability and high potency. Examples are biphenylic derivatives such as SDZ-220,581 from the former Sandoz Pharma Ltd. (Urwyler et al., 1996), or others like PD-134,705 from the former Warner-Lambert Inc. (Ortwine et al., 1992), LY274614 from E. Lilly Inc. (Cheung et al.,1996), or WAY-126,090 from Wyeth-Ayerst (AHP) (Kinney et al., 1998) which is according to a company communication under preclinical development for neuropathic pain.



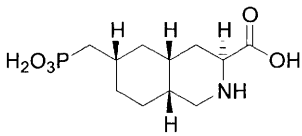
Selfotel (CGS-19,755)



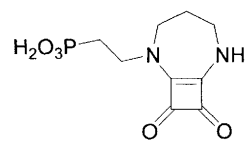
SDZ-220581



PD-134705



LY-274614



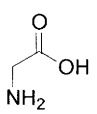
WAY-126090

Scheme 2: Examples for competitive NMDA receptor antagonists.

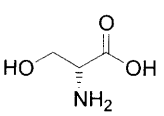
In a human case study concerning a severe intractable chronic pain state, racemic CPP (i.t.) has been shown to have analgesic efficacy (Kristensen et al., 1992). However, increasing the lipophilicity of competitive NMDA antagonists and hence their CNS availability in turn increases the risk of central adverse effects. Therefore administration of this class of antagonists should be restricted to local sites (Kristensen, 1997). This was also emphasized in clinical studies with the systemic administration of competitive NMDA antagonists (the main focus of drug development has been stroke therapy) which have been stopped because of intolerable CNS effects such as psychotomimesis among other reasons (Muir et al., 1995; Davis et al., 1997). Recently, inorganic iron complexes derived from degradation products of the nitric oxide donor nitroprusside have been shown to exert competitive NMDA antagonism with selective affinities in the nanomolar range. The inhibition does not seem to be due to generation of NO or to any interaction with a redox site but is probably the result of a steric hindrance mechanism. A new mechanism has been proposed involving an exchange of loosely bound H_2O/NH_3 species in the coordination sphere of the iron complex with an amino acid side chain in the vicinity of the competitive binding_site of glutamate. However, the significance of the latter findings for the pharmacology of NMDA receptors needs further evaluation (Neijt et al., 2001).

(b) Glycine$_B$ site antagonists

In addition, the presence of glycine as a further co-agonist is necessary for activation of conventional NR1/NR2 ion channels. Neither glutamate nor glycine on their own cause any essential activation so the simultaneous presence of both amino acids is an obligate requirement for activation (Johnson et al.1987, Kleckner et al.1988).



Glycine

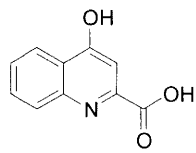


D-Serine

This co-agonistic excitatory glycine binding site is often designated as 'strychnine-insensitive glycine binding site of the NMDA receptor' to distinguish it from the inhibitory strychnine-sensitive glycine binding site. Following a pragmatic proposal by Danysz and Parsons (1998) in their comprehensive review of the physiology and prospective therapeutic significance of this glycine binding site of the NMDA receptor we prefer the terms glycine$_B$ site or glycine$_B$ antagonist or agonist for competitive effectors of this site. Besides glycine the non-proteinogenic amino acid D-serine has been found as a further endogenous agonist of the glycine$_B$ site (Danysz et al., 1990; Mothet et al., 2000). The distribution of endogeneous D-serine and its generating enzyme D-serine racemase in the CNS resembles the distribution of NMDA receptors (Wolosker et al., 1999; Mothet et al., 2000). Evidence for the

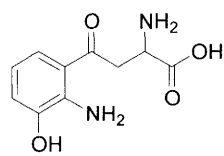
physiological relevance of D-serine for nociception might be provided by the enhanced nociceptive behavior of mutant mice lacking the enzyme D-amino-acid oxidase, an enzyme involved in the catabolic degradation of D-amino acids (Wake et al., 2001). An interesting new approach for inhibition of NMDA receptor activation might therefore be any attenuation of D-serine concentrations for example by inhibition of D-serine racemase (Panizzutti et al., 2001).

Glycine$_B$ site antagonists which have been shown to be effective in analgesic models can be divided into different chemical classes although so far any high affinity effector molecule of the glycine$_B$ site bears more or less the structural motif of glycine within its molecular structure, as can be observed in the first example, kynurenic acid.



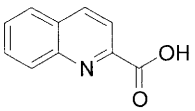
Kynurenic acid

Because kynurenic acid is an endogeneous metabolite of tryptophan metabolism and has been found in mammalian brain (Moroni et al., 1988) it has been suggested that it might be a putative endogeneous antagonist (Stone, 1993). In the later course of the tryptophan metabolic pathway there are further putative harmful metabolites generated such as the oxygen radical generating 3-hydroxy-kynurenine and the NMDA receptor agonist quinolinic acid. Therefore inhibition of the generation of these harmful metabolites has been attempted by blocking kynurenine-3-hydroxylase activity thus allowing kynurenic acid to accumulate (for review see Stone, 2000). However, relatively high concentrations of kynurenic acid are necessary for inhibition of glycine$_B$ site receptor binding or NMDA receptor ion fluxes (Bertolino et al.,1989; Danysz et al., 1989). But even metabolic inhibition of kynurenine-3-hydroxylase which provokes accumulation of kynurenic acid seems to have little physiological relevance (Urenjak et al., 2000). Kynurenic acid is further hampered by its relatively low selectivity.

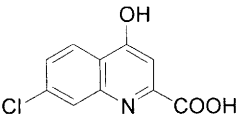


3-Hydroxy-kynurenine

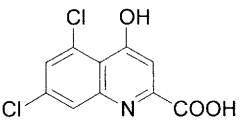
Derivatization of kynurenic acid leads to far more potent and selective antagonists such as 7-chloro-kynurenic acid or 5,7-dichlorokynurenic acid (Kemp et al., 1988; Baron et al., 1990) which have been shown to inhibit glycine-induced tailflick facilitation (i.e attenuation of glycine-induced hyperalgesia) or the late phase of formalin-induced nociception when given intrathecally (Kolhekar et al., 1994, Chapman et al., 1995). Nevertheless kynurenic acid derivatives are still hampered by a poor blood - brain barrier penetration and hence a low CNS availability.



Quinolinic acid

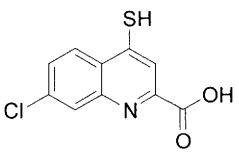


7-Chlorokynurenic acid



5,7-Dichlorokynurenic acid

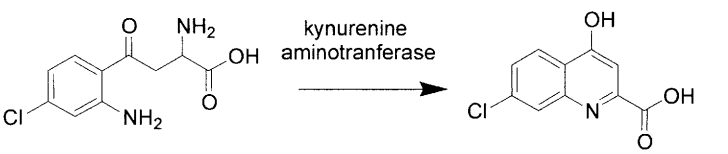
Scheme 3: Structures of kynurenic acid derivatives.



7-Chlorothiokynurenic acid

There have been further chemical attempts to improve CNS penetration such as the development of thio-kynurenic acids with improved CNS availability (Moroni et al., 1991; Chen et al., 1993).

Other attempts make use of the better CNS penetration of kynurenine which is converted enzymatically within the brain to kynurenic acid by kynurenine aminotransferase (Hokari et al., 1996) or the design of pro-drugs which enter the CNS more easily and are hydrolyzed within the brain e.g. glucosidic-linked 7-chlorokynurenic acid (Bonina et al., 2000).



4-chlorokynurenine

7-chlorokynurenic acid

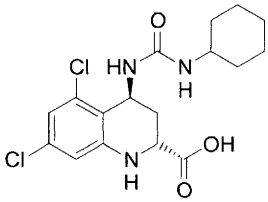
Scheme 4: Enzyme-catalyzed formation of 7-chlorokynurenic acid.

Further systematic derivatization leads to selective and high affinity compounds such as the 4-aminotetrahydro-chinolin-carboxylate L-689,560 (Leeson et al., 1992) and to 4-hydroxyquinolin-2(1H)-ones (Rowley et al., 1993) of which L-701,324 has become the best known prototype which combines selective and high $glycine_B$ affinity and oral bioavailability (Kulagowski et al., 1994; Priestley et al., 1996).

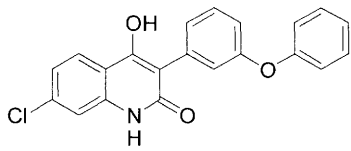
L-701,324 has been shown to reverse the inflammation-induced mechanical hyperalgesia in rats without affecting the accompanying carrageenan-induced paw edema (Laird et al., 1996).

1,4-Dihydro-2,3-quinoxalinediones comprise another scaffold for $glycine_B$ antagonists with antinociceptive activity (Vaccarino et al., 1993; Woodward et al., 1995a; Lutfy and Weber, 1996; Lutfy and Weber, 1998; Lutfy et al., 1999) of which licostinel is a good example of a highly selective

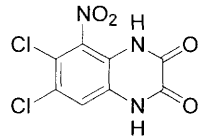
and highly potent member of this series (Woodward et al., 1995b).



L-689560



L-701,324



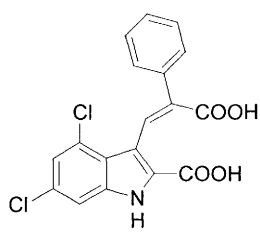
Licostinel (ACEA-1021)

Scheme 5: Examples for glycine$_B$ antagonists.

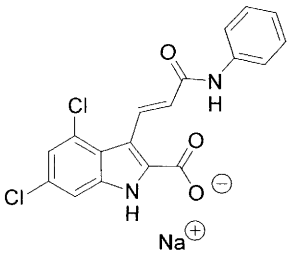
Whereas earlier quinoxalinediones derivatives comprise relatively similiar compounds which differ only in their side chain substitution pattern, the condensation of heteroarylic ring systems to the quinoxalinediones scaffold might offer the development of a broader range of highly potent and selective glycine$_B$ antagonists such as CGP-68,730A (Pozza et al., 2000).

Indole-2-carboxylic acids represent another scaffold known for more than a decade as competitive glycine$_B$ antagonists (Huettner, 1989). Structure - activity relationships favor the C4 and C6 position for substitutions with chlorine or other small electrophilic substituents to increase the binding affinity (Gray et al., 1991; Salituro et al., 1992) thus yielding for example the potent 4,6-dichloro-indole-2-carboxylate derivatives which closely resemble the 5,7-dichlorokynurenic acid pharmacophor.

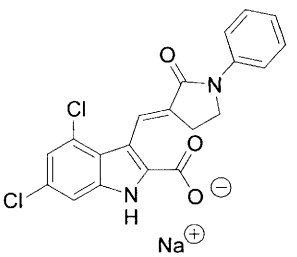
A well-known, selective and high affinity example is MDL-105,519 which also represents the most frequently used radioligand for the glycine$_B$ binding site (Siegel et al., 1996; Baron et al., 1997). Whereas the position of the halogenic substitutions and the carboxyl group seems to be restricted to fixed areas within the indole-2-carboxylate scaffold, a relatively bulky substitution at C3 is obviously possible or even favorable for high glycine$_B$ affinity. This is also the case for the two advanced glycine$_B$ antagonists synthesized by Glaxo Wellcome plc, Gavestinel (GV-150,526) and GV-196,771A from which the latter at least has been considered for clinical development in chronic pain therapy (Di Fabio et al., 2000).



MDL-105519



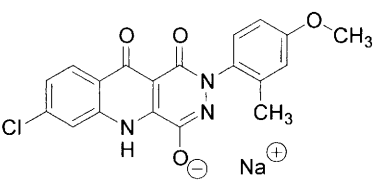
Gavestinel



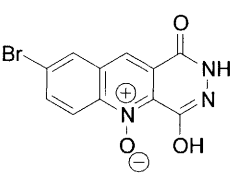
GV-196,771A

For GV-196,771 a basic schematic description of its synthesis is also given by Di Fabio et al. (2000). Both glycine$_B$ antagonists show a better fit with a two binding site model with Hill coefficients signifcantly lower than 1 (Chopra et al., 2000). The latter authors discuss possible steric hindrance of the binding of a second inhibitor molecule to the receptor oligomers thus resulting in negative cooperativity. GV-196,771A has been described to exert antihyperalgesic activity in either persistent formalin-induced pain models or in neuropathic sciatic nerve constriction models (Quartaroli et al., 1999, 2001). The modulation of nociceptive transmission in thalamic nuclei is obviously restricted to those neuropathic injury states which might result in an otherwise overall good tolerability of this compound (Bordi et al., 2000).

Quite diverse from the previously described chemical classes there are a series of more complex tricyclic glycine$_B$ antagonists of which the compound ZD-9,379 from Zeneca and MRZ-2/576 from Merz are good examples (Quiu et al., 1997; Parsons et al., 1997).



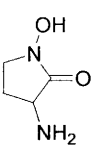
ZD-9379



MRZ-2/576

Scheme 6: Examples for glycine$_B$ antagonists.

MRZ-2/576 has been demonstrated to cross the blood - brain barrier rapidly and to function centrally as a glycine$_B$ antagonist after systemic administration of doses within the relevant antinociceptive dose range. However, the compound is only short-acting within the CNS because of a relatively short half-life within this compartment (Parsons et al., 1997). The compound has antinociceptive properties and shows exceptionally good efficacy in a visceral pain model (McClean et al., 1998; Olivar et al., 1999).



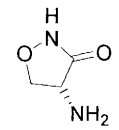
HA-966

Last but not least, an interesting class of functionally antagonistic glycine$_B$ effector molecules are the partial agonists of which (+)-HA-966 is the most important example. (+)-HA-966 has a low intrinsic agonistic activity (i.e. it yields on its own only a partial agonistic effect of about 10 - 40% of the possible maximal agonistic effect) but it has strong antagonistic activity which can 'neutralize' the effect of high efficacy agonists, such as glycine or D-

serine (Hendersen et al., 1990; Singh et al., 1990; Danysz et al., 1998).

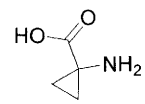
Thus partial agonists may allow some physiologically low level NMDA receptor activation while antagonizing high level excessive NMDA receptor activation and this may be the reason for the relatively good tolerability of such compounds.

The partial agonism of D-cycloserine has also been reported to be NR2-subunit dependent. Whereas D-cycloserine shows partial agonism in NR1-1a/$NR2_A$- or NR1-1a/$NR2_B$-expressing oocytes it has a higher intrinsic efficacy in NR1-1a/$NR2_C$-expressing oocytes in comparison to glycine (Sheinin et al., 2001).



D-Cycloserine

(+)-HA-966 or D-cycloserine have been reported to exert antinociception in the late tonic pain phase of formalin-induced pain in rats and mice or to attenuate the thermal hyperalgesia in peripheral mononeuropathy in rats by sciatic nerve ligation (Mao et al., 1992; Millan and Seguin, 1993; Hunter and Singh, 1994; Millan and Seguin, 1994). Obviously partial agonists also reinforce the antinociceptive activity of either opioids or tachykinin NK1 antagonists (Seguin et al., 1994; Christensen et al., 1998) or might attenuate the development of morphine dependence during chronic treatment of neuropathic rats (Christensen et al., 2000).



1-aminocyclo-propane-carboxylic acid

Previously reported as a putative partial agonist at the $glycine_B$-site (Fossom et al., 1995) 1-aminocyclopropane-carboxylic acid has more recently been shown to act as a $glycine_B$-site agonist and concurrently as a glutamate-site antagonist (Nahum-Levy et al., 1999).

c) Blockade of the channel pore by Mg^{2+} and non-competitive NMDA channel blockers

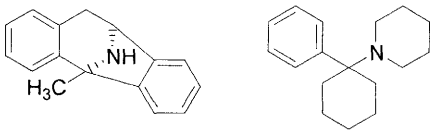
Of particular importance for the functionality of NMDA receptors is their voltage-dependent blockade by Mg^{2+}. At resting negative cell membrane potential in the presence of glutamate, glycine, and physiological Mg^{2+} concentrations the channel`s ion flux is still blocked due to Mg^{2+} ions at a Mg^{2+} binding site within the ion channel pore. This block is not relieved until the cell membrane potential becomes sufficiently depolarized and in turn Mg^{2+} is released from its binding site within the pore (Novak et al., 1984; Li-Smerin et al., 1996). By mutagenesis studies one asparagine within the M2-segment of all NMDA subunits has been identified as being essential for this feature as an exchance of this asparagine strongly reduces the voltage-dependent block by Mg^{2+} ions (Burnashev et al.1992; Mori et al., 1992). In addition the channel conductivity for Ca^{2+} is also influenced by the same asparagine at this position (Burnashev et al., 1992; Sakurada et al., 1993). This Mg^{2+} block also varies with

the NR2 subunit composition of the NMDA receptor, indicating a stronger voltage dependency of NR1-$NR2_A$ or NR1-$NR2_B$ NMDA receptors compared to their $NR2_C$ or $NR2_D$ counterparts (Kuner et al., 1996; for review see Dingledine et al., 1999). A similiar pattern of NR2 subunit sensitivity has been found in the attenuation of the Mg^{2+} block by phosphorylation of NMDA receptors by protein kinase C (PKC) which has previously been proposed as a mechanism for the modulation of NMDA receptors (Chen et al., 1992; Wagner et al., 1996). However further detailed studies have raised doubts that this is the principal mechanism of the enhanced NMDA channel conductance (Zheng et al., 1999; for review see Dingledine et al., 1999). In addition a broad variety of other kinases besides PKC have been shown to phosphorylate multiple sites in the NMDA receptors (Lau et al., 1995; Suzuki et al., 1995; Omkumar et al., 1996; Leonard et al., 1997; Nakazawa et al., 2001).

Spatial or temporal summation of excitatory postsynaptic potentials from adjacent synapses activated by non-NMDA glutamate receptors or by other co-released neuropeptides of the sensory afferent fibers may be responsible for the depolarization which reverses the Mg^{2+} block. NMDA receptor-induced ion fluxes therefore need the simultaneous synaptical release of glutamate and its co-agonists glycine or D-serine in addition to the coincident activation of nearby excitatory synapses. Although the physiological Mg^{2+} concentrations are considered to cause an efficient NMDA receptor block at normal resting cell membrane potential there are several reports that high additional administration of Mg^{2+} might further attenuate the excitability of NMDA receptors and that Mg^{2+} can be used for treatment of hyperalgesic states. The significance of this Mg^{2+} block for chronic pain has been shown by the antihyperalgesic effects of spinally and also systemically administered Mg^{2+} in neuropathic pain or the second pain phase of the formalin-induced pain model in rats (Xiao and Bennett, 1994; et al., 2000; Takano et al., 2000). Spinally-administered, Mg^{2+} potentiates the antinociceptive effect of morphine (Kroin et al., 2000). Consequently, a Mg^{2+} deficiency in turn induces hyperalgesia which may be reversed by an NMDA antagonist (Dubray et al., 1997). Mg^{2+} has also been shown to mediate clinical analgesia in postoperative or neuropathic pain caused by cancer and other etiologies (Felsby et al., 1995; Traber et al., 1996; Koing et al., 1998; Crosby et al., 2000).

Within the channel pore there is also the binding site for the so-called open channel blockers which is also able to

control ion currents through the receptor pore and is known as either MK-801 or phencyclidine (PCP) binding site, reflecting the nature of its well-known high-affinity ligands.



Dizocilpine (MK-801) Phencyclidine

Scheme 7: Structures of MK-801 and phencyclidine.

Ligands of this binding site are called open channel blockers or non-competitive NMDA antagonists and due to their putative functionality i.e. steric hindrance of the ion fluxes only antagonism by such ligands has been described so far. An important feature of these antagonists is their so-called use-dependency (Huettner and Bean, 1988). In order to reach their binding site within the NMDA ion channel pore non-competitive NMDA antagonists (e.g. MK801, PCP, ketamine, memantine, see below) require an activated and open ion channel. In turn, once bound the channel blockers may become trapped within the channel pore when the NMDA receptor channel returns to its closed state (Sobolevsky et al., 2002).

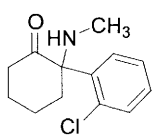
High affinity open channel blockers like MK-801 or PCP (also known as 'angel dust' among drug abusers) cause intolerable psychotomimetic side-effects (Rogawski and Porter, 1990; Jentsch et al., 2000). In addition PCP and MK801 have been shown to cause neuronal vacuolization at least in rodents (Olney et al., 1989). There exists an inverse correlation between high binding affinity and the speed of the on and off kinetics among NMDA antagonists i.e. the higher the binding affinity the slower the on and off kinetics (Parsons et al., 1995; Black et al., 1996). Low affinity channel blockers with fast channel blocking kinetics and strong voltage dependency such as memantine, dextromethorphan, or remacemide offer a far more favorable efficacy to side-effect ratio than high affinity blockers with slow onset and offset kinetics such as MK-801 (Kemp et al., 1987; for review see Rogawski, 2000). The better tolerability of low to medium affinity, fast channel blockers is also emphasized by the fact that all clinically used non-competitive NMDA antagonists belong to this class (e.g. amantadine, memantine, dextromethorphan, ketamine). In addition there are some low

affinity antagonists under clinical development for pain (Palmer and Widzowski, 2000).

The high affinity non-competitive antagonist MK-801 will be considered first (Wong et al., 1986; Huettner and Bean, 1988). Several analogs have been synthesized (Leeson et al., 1990) which have in common with other structural classes an amino nitrogen which can be protonated. MK-801 has been shown to inhibit thermal hyperalgesia, to reduce the hyperactivity and hyper-responsiveness of spinal dorsal horn neurons in neuropathic rats with unilateral sciatic nerve ligation, and to attenuate formalin-induced persistent pain in rats (Haley et al., 1990; Davar et al., 1991; Yamamoto et al., 1992; Chaplan et al., 1997; Sotgiu et al., 2000). However due to intolerable side-effects MK-801 has not been developed further (Koek et al., 1988; Tricklebank et al., 1989; Wozniak et al., 1990).

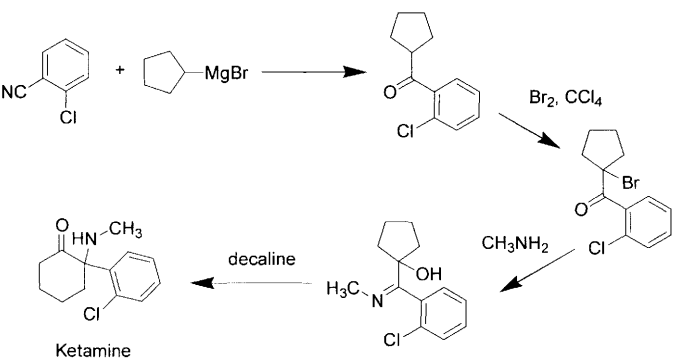
Ketamine

Ketamine



[6740-88-1]; 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone, $C_{13}H_{16}ClNO$, MW 237.73

Synthesis (Kleemann et al., 1999):



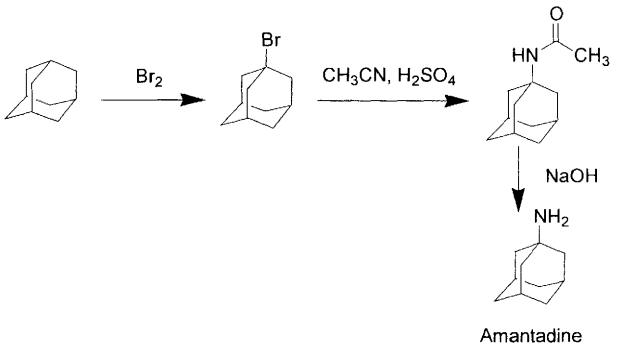
Scheme 8: Synthesis of ketamine.

The structure of ketamine is similar to that of PCP. Ketamine is an intermediate affinity fast channel blocker with a stereospecific preference for the S-(+)-enantiomer but with no selectivity for the NR1/NR2$_{A-D}$ heteromeric subunits (Yamakura et al., 2000). The majority of the clinical literature regarding NMDA antagonists is devoted to ketamine. In addition to evidence of its preclinical antinociceptive efficacy in animal pain models, there are many clinical studies and case reports substantiating the analgesic efficacy of ketamine. The therapeutic value of ketamine, either used alone or as an adjuvant to other pain relief substances, seems to be in the treatment of chronic pain states, neuropathic pain, post-herpetic neuralgia, phantom limb pain, fibromyalgia, opioid-intractable cancer

pain, burn-induced hyperalgesia, central post-stroke pain and other pain states (e.g. Eide et al., 1994; Max et al., 1995; Ilkjaer et al., 1996; Nicolajsen et al., 1996; Warncke et al., 1997; Davis, 1999; Fine, 1999; Finlay, 1999; Rabben et al., 1999; Graven-Nielsen et al., 2000; Mercadante et al., 2000; Vick et al., 2001). Despite the well-proven efficacy in several pain states the use of ketamine is hampered by psychotomimetic side-effects such as hallucinations, or sensory distortions and attenuation of memory (Davis, 1999; Newcomer et al., 1999; Shiigi et al., 1999). However one should also bear in mind that the pharmacology of ketamine is complex in as much as there are interactions with other binding sites (e.g. nicotinic and muscarinergic cholinergic and opioid sites, Na^+ and L-type Ca^{2+} ion channels) in addition to those involved in NMDA antagonism (for review see Kohrs et al., 1998).

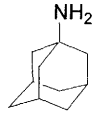
Amantadine

Synthesis (Kleemann et al., 1999):



Scheme 9: Synthesis of amantadine.

Amantadine

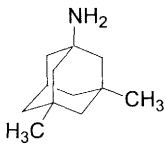


(1-amino-adamantane), Tricyclo[3.3.1.$1^{3,7}$]decan-1-amine, $C_{10}H_{17}N$, MW151.25, [768-94-5],

A further class of compounds comprises the amino-adamantanes from which amantadine and memantine produced by Merz are well-known examples of drugs used for the long-term therapy of Parkinson's syndrome. Both compounds are examples of low to medium affinity, fast channel blockers with a strong voltage-dependency and a favorable efficacy to side-effect ratio (Parsons et al., 1995; Parsons et al., 1999a). Although amantadine has a low affinity for the NMDA receptor channel site and relatively high concentrations are necessary for specific interaction with the NMDA receptor (Parsons et al., 1995), amantadine reaches concentrations in the human brain which are considered to be high enough for NMDA antagonism

(Kornhuber et al., 1995) and has been described to relieve clinical neuropathic pain states (Pud et al., 1998).

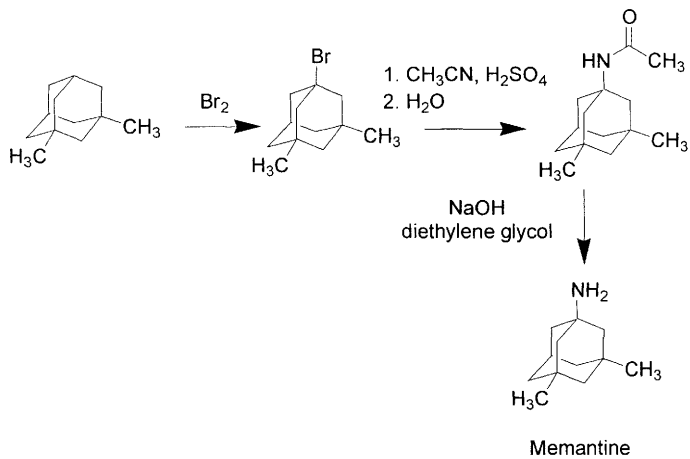
Memantine



(1-amino-3,5-dimethyladamantane), 3,5-dimethyl-tricyclo[3.3.1.1^{3,7}]decan-1-amine, $C_{12}H_{21}N$, MW 179,31, [19982-08-02];

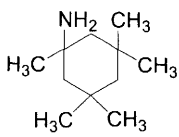
Memantine

Synthesis (Kleemann et al., 1999):



Scheme 10: Synthesis of memantine.

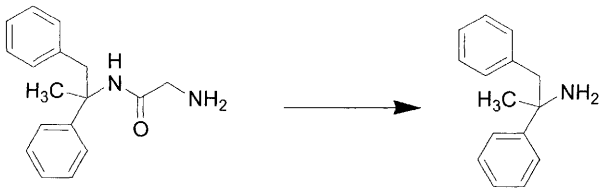
In addition to antinociceptive activity in preclinical pain models such as carrageenan-induced thermal hyperalgesia or sciatic nerve constriction mononeuropathy in rats (Eisenberg et al., 1994; Suzuki et al., 2001) memantine has also shown analgesic efficacy in clinical studies and is under clinical development for neuropathic pain treatment (Eisenberg et al., 1998; Headley, 1999; Nikolajsen et al., 2000). There is evidence to show that memantine concentrations in the CNS reach levels which can effectively produce NMDA antagonism (Wesemann et al., 1980). In addition there is also evidence showing in situ NMDA antagonism of spinal neurons after iontophoretic administration of NMDA and systemic administration of memantine (Herrero et al., 1994).



MRZ-2/579

A series of amino-alkyl-cyclohexanes (e.g. MRZ-2/579) under recent development by Merz have been shown to attenuate the development of tolerance towards antinociception by morphine in rats (Parsons et al., 1999b; Houghton et al., 2001).

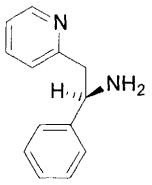
Remacemide from AstraZeneca is a pro-drug which is converted by hydrolysis and removal of glycine to its active desglycinyl derivative FPL-12,495 which is a low affinity fast kinetics channel blocker (Heyn et al., 1994; Subramaniam et al., 1996; Monaghan et al., 1997; Ahmed et al., 1999).



Remacemide FPL-12,495

Scheme 11: Hydrolysis of remacemide.

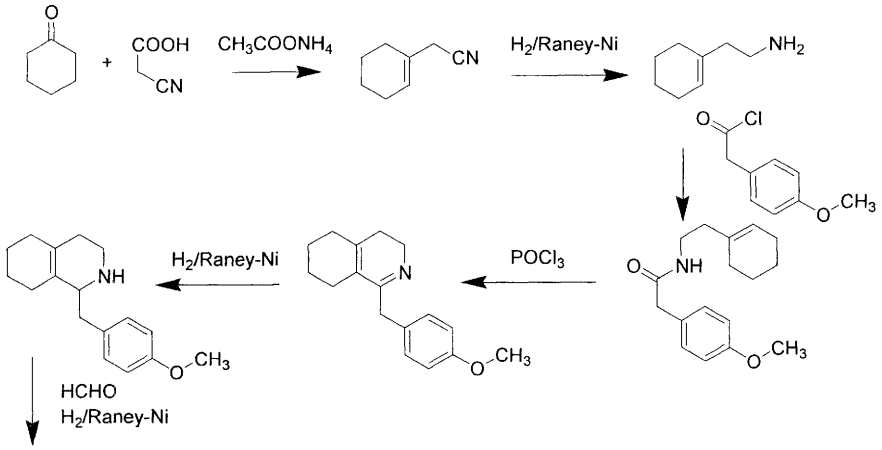
Both remacemide and its des-glycinyl-metabolite inhibit the inflammatory, mechanical hyperalgesia as well as the edema induced by injection of carrageenan or complete Freunds adjuvant into the rat hind paw (Asghar et al., 2000). As reported in a company communication, remacemide and its des-glycinyl-metabolite FPL-12,495 are not under further development although no obvious reason for this withdrawal is known (Schachter et al., 2000). A putative back-up of this structural class might be AR-R-15,896 which is at least in preclinical development by AstraZeneca (company communication). Compared to ketamine and memantine, AR-R-15,896 might have more favorable pharmacodynamic features (Mealing et al., 1999) which may be due to a stronger inhibition of $NR1/NR2_C$ and $NR1/NR2_B$ in comparison to $NR1/NR2_A$ NMDA receptor subtypes. This may also be the case for remacemide and its des-glycinyl-metabolite FPL-12,495 (Monaghan et al., 1997).

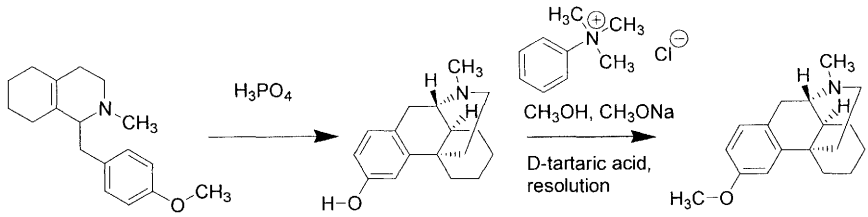


AR-R-15,896

Dextromethorphan

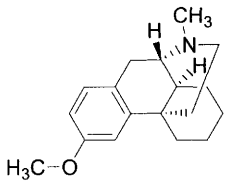
Synthesis (Kleemann et al., 1999):





Scheme 12: Synthesis of dextromethorphan.

Dextromethorphan



[125-71-3]; (9α,13α,14α)-3-methoxy-17-methyl-morphinan, MF $C_{18}H_{25}NO$, MW 271,40

Dextromethorphan and its O-demethylated metabolite dextrorphan (morphinans), are also low to medium affinity NMDA channel blockers. The former has been in clinical use as an antitussive for about 40 years and could therefore be considered as a very safe drug (Bem et al., 1992).

In addition to its NMDA antagonism in the micromolar range (Church et al., 1985; Wong et al., 1988; Netzer et al., 1993) dextromethorphan also inhibits 5HT and norepinephrine uptake in the submicromolar concentration range (Codd et al., 1995). It also has a high binding affinity for the sigma 1 binding site, a somewhat weaker affinity for the μ-opioid receptor and shows some inhibition of Na^+ and Ca^{2+} channels (Tortella et al., 1989; Zhou et al., 1991; Netzer et al., 1993; Codd et al., 1995). Dextromethorphan and its metabolite dextrorphan are antinociceptive in a variety of visceral, chronic neuropathic and inflammatory animal models (Mao et al., 1993; Tal et al., 1993; Elliott et al., 1995; Hao et al., 1996; Davidson et al., 1998). Dextromethorphan showed clinical analgesic efficacy in an experimental pain study and in the treatment of diabetic neuropathy, however it failed in postherpetic neuralgia and a further neuropathic pain study, a cancer pain treatment study, and in an experimental model for ischemic pain (McQuay et al., 1994; Price et al., 1994; Nelson et al., 1997; Mercadante et al., 1998; Plesan et al., 2000).

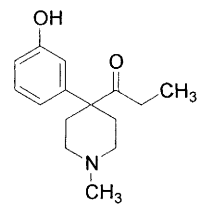
A further series which also originates from the opioid-descending structures comprises the benzomorphan scaffold as exemplified by BIII-277CL synthesized by Boehringer Ingelheim. This structural class includes both an affinity for the μ-opioid receptor site and the PCP-binding site of the NMDA receptor and has been optimized structurally to give selective NMDA interaction e.g. BIII-277CL (Carter et al., 1995; Grauert et al., 1997).

(d) Bifunctional molecules: μ-opioids with NMDA antagonistic activity

However, the above-mentioned benzomorphans and morphinans are not the only compounds reported to have affinity for both the NMDA receptor and the μ-opioid receptor. There have been several reports on the NMDA

antagonistic features of known opioid compounds which have also been suggested to add favorable properties in addition to their analgesic effects to the respective opioids. In that respect dextropropoxyphen, ketobemidone, dextromethadone, and D-morphine have been reported to have affinity for the PCP-binding site within the NMDA receptor channel and to exert NMDA antagonistic effects (Ebert et al., 1995; Andersen et al., 1996; Gorman et al., 1997; Ebert et al., 1998; Davis et al., 1999; Stringer et al., 2000).

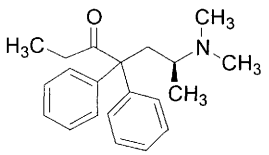
It has been proposed that both analgesic principles might act in a synergistic or at least additive manner thus reducing the necessary dose and in turn minimize adverse effects (Dickenson, 1997; Wiesenfeld-Hallin, 1998; Mao, 1999; Price et al., 2000). Following these assumptions there are combinations of dextromethorphan with opioids under clinical development (e.g. Morphidex™, a fixed combination of morphine with dextromethorphan) by the former Algos Pharmaceutical Corp. (Caruso, 2000; Katz, 2000). Many of the animal studies could be taken as a preclinical scientific basis for such clinical combination studies (Chapman et al., 1992; Advokat et al., 1995; Bernardi et al., 1996; Grass et al., 1996; Hoffmann et al., 1996; Honoré et al., 1996; Bhargava, 1997; Kauppila et al., 1998; Plesan et al., 1998; Allen et al., 2001).



Ketobemidone (see chapter 3.4)

Numerous reports have documented the favorable influence of NMDA antagonists (either non-competitive or competitive glycine$_B$ or glutamate antagonists) on the development of opioid tolerance and dependency during chronic use of opioids in a variety of different preclinical pain models or experimental paradigms (Marek et al., 1991; Tiseo et al., 1993; Mao et al., 1994; Elliott et al., 1994; Lutfy et al., 1995, 1996; Bilsky et al., 1996; Mao et al., 1996, 1999). The first clinical studies in humans for treatment or attenuation of physical dependency with NMDA antagonists are meanwhile under way (Bisaga et al., 2001). However, one should also bear in mind, that a combination of opioid agonism and NMDA antagonism might enhance the risk of developing a drug dependency. It is of particular importance to identify precisely the paradigms which are to be tested in the experimental models used to determine such relationships (Tzschentke et al., 1998). The reader is referred to some recently published reviews on this subject (Wolf, 1998; Vanderschuren et al., 2000; Trujillo, 2000).

As demonstrated for dextromethadone the pharmacological relevance of this additional NMDA component in addition to the remaining opioid component for antinociception after systemic administration needs further investigation. Dextromethadone has been shown to

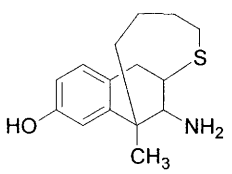


D-Methadone

(see chapter 3.4)

reverse NMDA-induced hyperalgesia after intrathecal administration (Davis et al., 1999). However, following intravenous injection within the appropriate antinociceptive dose range, it could not be shown that dextromethadone specifically inhibited the electrically- or mechanically-induced spinal wind-up phenomenon or to inhibit the activity of spinal dorsal horn neurons evoked by iontophoretic administration of NMDA, whereas its antinociceptive and non-specific effects on the detectable inhibition of spinal neuronal activity could be fully antagonized by naloxone (Chizh et al., 2000).

However, methadone is considered by some authors to be a very useful drug because of its NMDA antagonistic effects for example for the treatment of cancer pain (Ayonrinde et al., 2000; Mancini et al., 2000).



Sulfazocine

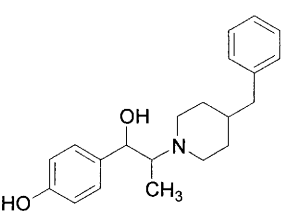
A very interesting approach might be the development of compounds whose structures give rise to a well-balanced NMDA antagonism and μ-opioid agonism. The former Biochem Pharma Corp. (today a subsidary of Shire) have developed such compounds of which sulfazocine is an example (Dimaio, WO 9703978; Dimaio et al., WO 9703979).

(e) Ifenprodil-like, $NR2_B$ specific antagonists

Recently there has been much interest in ifenprodil-like antagonists with regard to their potential role in pain relief, primarily because of the excellent tolerability of these $NR2_B$ subtype-specific drugs. Ifenprodil was the prototypical example of this $NR2_B$-subclass-specific type of NMDA receptor antagonists (Williams, 1993; Avenet et al., 1996) and is the most often used high affinity ligand for this target site (Grimwood et al., 2000; Coughenour et al., 2001).

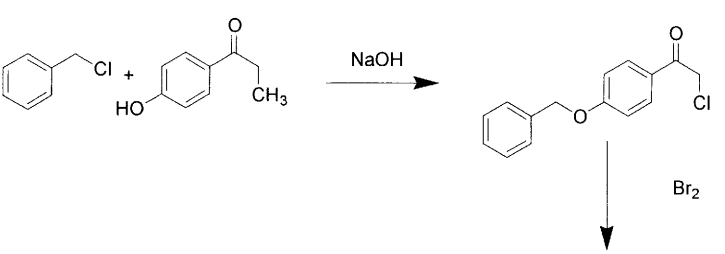
Ifenprodil

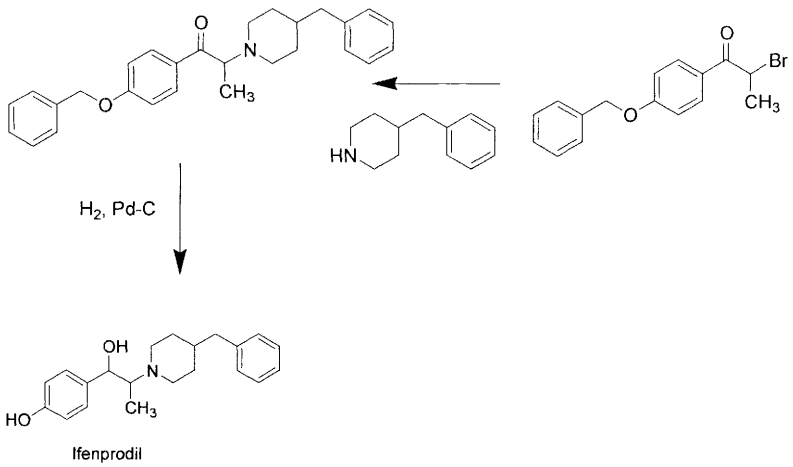
Ifenprodil



[23210-58-4]; 4-[2-(4-Benzyl-piperidin-1-yl)-1-hydroxy-propyl]-phenol, $C_{21}H_{27}NO_2$, MW 325.45

Synthesis (Kleemann et al., 1999):

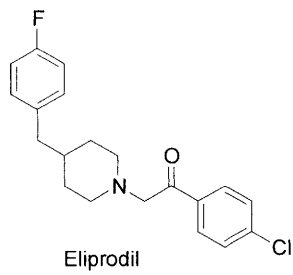




Ifenprodil

Scheme 13: Synthesis of ifenprodil.

In addition to its NMDA antagonistic activity, ifenprodil also interacts non-specifically with other receptor systems (e.g. sigma, 5HT3, α1; McCool et al., 1995; Moebius et al., 1997; Chenard et al., 1999). However, eliprodil, a back-up compound for ifenprodil and the newer $NR2_B$-specific antagonists proved to be far more specific (Chenard et al., 1991).

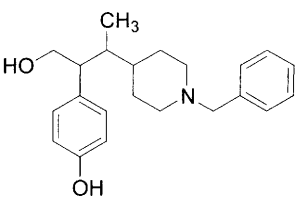


Eliprodil

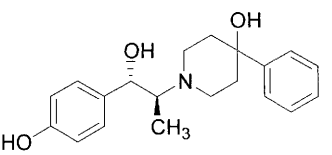
The inhibitory modulation of these drugs is restricted to NMDA receptor oligomers with at least one $NR2_B$ subunit. These subunit-specific antagonists might offer better efficacy and tolerability for pain treatment with particular emphasis on a lower risk of motor impairment in comparison to other NMDA receptor antagonist classes (Boyce et al., 1999; for a recent review see Chizh et al., 2001). In addition, genetic evidence has suggested that overexpression of $NR2_B$ in the forebrain enhances the nociception of formalin-induced pain (Wei et al., 2001; Zhuo, 2002). Since spinally mediated mechanisms appear to be unchanged in transgenic animals, this may provide evidence that central NMDA receptors are of significance in persistent inflammatory pain. Consistent with supraspinal pain modulation, ifenprodil could only be shown to inhibit single motor unit wind-up in non-spinalized rats, emphasizing the involvement of supraspinal mechanisms (Chizh et al., 2001). $NR2_B$ overexpression in the forebrain also enhances the processes of learning and memory (Tang et al., 1999, 2001). Therefore an attenuation might be expected as a putative side-effect of $NR2_B$-specific antagonists, although experimental studies have not shown any evidence for such side-effects so far (Fraser et al., 1996; Doyle et al., 1998). Yung (1998) provided evidence for the selective distri-

bution of the $NR2_B$ subunit within laminae I - III in the dorsal spinal horn, but no $NR2_A$ or $NR2_C$ was detectable using immunohistological techniques. Obviously the $NR2_B$ subunit was expressed on the sensory presynaptic afferent fibers (Yung, 1998; Ma and Hargreaves, 2000). The lack of $NR2_B$ expression in the ventral spinal horn and in the cerebellum (Portera-Cailliau et al., 1996; Sasner et al., 1996) has been proposed as an explanation for the reduced motor side-effect profile of $NR2_B$ selective antagonists (Boyce et al., 1999). Obviously this class of NMDA antagonists bear no or only low potential for abuse in humans since eliprodil showed no evidence of reinforcing effects in non-human primates or PCP-discriminative stimulus effects in rats (Balster et al., 1994).

Ifenprodil as well as the $NR2_B$-specific antagonists CP-101,606 and Ro-25-6981 have been shown to be antinociceptive in a variety of pain models (Bernardi et al., 1996; Taniguchi et al., 1997; Boyce et al., 1999; Chizh et al., 2001; Minami et al., 2001). Of particular importance are their good putative therapeutic indices comparing analgesic and side-effect dose ranges. They inhibit mechanical allodynia in a neuropathic chronic constriction injury model in rats and reduce carrageenan-induced hyperalgesia. In comparison to other NMDA antagonists they produce little or no motor impairment within the respective antinociceptive dose ranges (Boyce et al., 1999).



Ro-25-6981



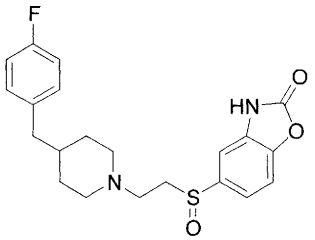
CP-101606

Scheme 14: Examples for $NR2_B$-selective antagonists.

Obviously most of the known $NR2_B$-selective antagonists very closely resemble ifenprodil thus belonging to the same structural class. Well known examples of $NR2_B$-selective antagonists are eliprodil which was the follow-up compound to ifenprodil synthesized by Synthelabo (Avenet et al., 1997), CP-101,606 and analogs synthesized by Pfizer, and Roche´s Ro 25-6981 (Fischer et al., 1997; Butler et al., 1998; Lynch et al., 2001).

Ifenprodil and its back-up compound eliprodil were under clinical development by Synthelabo but have been withdrawn because of cardiovascular side-effects. (i.e. HERG channel inhibition (Soldo et al., 2000)). According to communications by the respective companies the development of CP-101,606 and Ro 25-6981 has also been halted.

PD-196,860 is another $NR2_B$-specific antagonist which is under development by Purdue Pharma but still has the basic ifenprodil-like structure. It was only recently that new basic structures (e.g. iminopyrimidine) of $NR2_B$-specific antagonists were published (Claremon et al., 2002).



PD-196860

There is a broad literature concerning the involvement of NMDA receptor channels and the efficacy of NMDA antagonists in chronic pain treatment. Compounds like ketamine or dextromethorphan are well-characterized NMDA antagonists which have been in clinical use for decades. It has been shown in many case reports and in some clinical studies too, that these compounds might be clinically effective analgesics, however they are often associated with intolerable adverse effects. Thus the task is to develop safer NMDA antagonists with a better efficacy to side-effect ratio. The more recent advent of subtype-specific antagonists with strong analgesia but fewer side-effects looks to be a promising path towards new analgesics, especially for pain states which do not respond well to the conventional analgesics of today.

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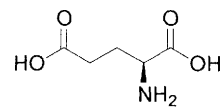
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7.3 AMPA and Kainate Receptors

Corinna Maul and Bernd Sundermann

Introduction

Glutamate is the major exitatory neurotransmitter in the mammalian central nervous system and its interactions with membrane receptors play a critical role in nearly every aspect of brain function, including cognition, memory and sensation.

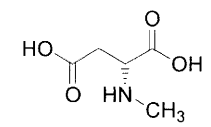


Glutamate (Glutamic acid)
(2*S*)-2-Amino-pentanedioic acid

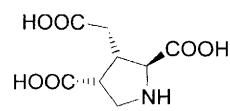
Glutamate receptors are classified in two main categories:

- ionotropic receptors (iGluRs) directly coupled to ion channels
- metabotropic receptors (mGluRs) coupled to intracellular second messengers via guanine nucleotide regulatory proteins (G-proteins; see Chapter 7.1)

iGluRs include those selectively sensitive to N-methyl-D-aspartate (NMDA; see Chapter 7.2), AMPA and kainate. AMPA and kainate selective receptors are often referred to as non-NMDA receptors. Their potential use in pain therapy is the topic of this chapter (for a comprehensive overview see Fundytus, 2001).

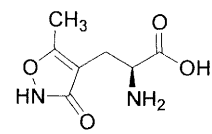


NMDA
(2*R*)-2-Methylamino-succinic acid



Kainate (Kainic acid)
(2*S*,3*S*,4*R*)-3-Carboxy-methylpyrrolidine-2,4-dicarboxylic acid

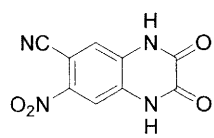
Expression studies have verified that there are further subtypes of all non-NMDA receptors. The subunits GluR1 to GluR4 bind AMPA with higher affinity than kainate, while GluR5 to GluR7 are kainate-selective (Gasic and Holleman, 1992). There is also a family of kainate-binding proteins (KA1, KA2) which bind kainate with high affinity, but are not functional receptor channels and have little sequence similarity with the GluR genes. AMPA and kainate receptors have four transmembrane-spanning domains, similar to other ligand-gated ion channels. In neurons expressing GluR2 with other subunits there are no Ca^{2+} currents, but in the absence of the GluR2 subunit glutamate can trigger Ca^{2+} influx.



AMPA
(2*S*)-2-Amino-3-(5-methyl-3-oxo-2,3-dihydro-isoxazol-4-yl)-propionic acid

AMPA/Kainate and Pain

The role of non-NMDA receptor modulators in pain is not as clear as the role of NMDA receptors, but there is evidence to indicate that AMPA and kainate receptors may be involved in nociceptive processing.

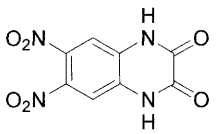


CNQX (7-Nitro-2,3-dioxo-1,2,3,4-tetrahydro-quinoxaline-6-carbonitrile)

Recently, it has been shown that spinal neurons express functional kainate receptors which contribute to synaptic transmission between primary afferent fibers and dorsal horn neurons (Li et al., 1999). Administration of the AMPA/kainate antagonist CNQX to the spinal cord

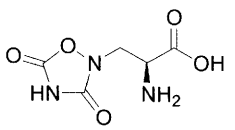
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diminishes dorsal horn neuronal responses induced by non-noxious and noxious mechanical stimulation (Neugebauer et al., 1993).



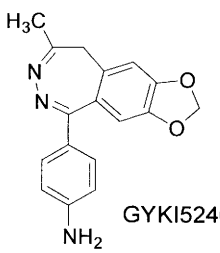
DNQX (6,7-Dinitro-1,4-dihydroquinoxaline-2,3-dione)

Following inflammation there is a 10-fold increase in axons expressing AMPA or kainate receptors (Coggeshall and Carlton, 1999). In anaesthetized rats, antagonism of AMPA/kainate receptors inhibits dorsal horn neuronal responses induced by innocuous and noxious mechanical stimulation of a chronically inflamed ankle (Neugebauer et al., 1994). Application of kainate to the rat hindpaw induces activation of primary afferent neurons, an effect that is reduced by the AMPA/kainate antagonist DNQX (Ault and Hildebrand, 1993).



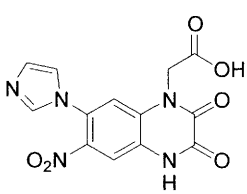
Quisqualic acid
glutamate receptor agonist
acting at AMPA receptors
and group I mGluRs

Several animal studies suggest that modulation of AMPA and kainate receptors can significantly affect pain transmission at diverse targets in the nervous system (Coderre and Melzack, 1992, Procter et al., 1998). Intrathecal administration of AMPA or kainate receptor agonists (e.g. AMPA, kainate, quisqualic acid) produce spontanous nociceptive behaviors, thermal and mechanical hyperalgesia, and allodynia (Sun et al., 1991; Okano et al., 1993; Yezierski et al., 1998). The AMPA/kainate receptor antagonist GYKI52466 was found to be fairly potent in rat tail flick and mouse phenylquinone writhing assays (Szekely et al., 1997). YM872, another AMPA receptor antagonist, also showed effects on acute pain (Nishiyama et al., 1999).

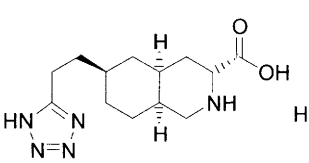


GYKI52466

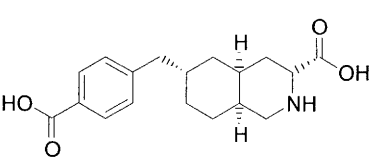
The AMPA/kainate antagonist LY-293558 (GluR5/GluR2) caused antinociceptive and ataxic effects in the formalin test, whereas the selective GluR5 antagonist LY-382884 exhibited antinociceptive effects without ataxia, while the GluR2-preferring antagonist LY-302679 caused ataxia but did not produce antinociceptive effects. These findings suggest an involvement of GluR5 in the processing of nociceptive information (Simmons et al., 1998). Moreover, there is evidence that kainate receptors can act as the induction trigger for long-term changes in synaptic transmission (Bortolotto et al., 1999).



YM-872



LY-293558

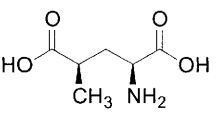


LY-382884

Scheme 1: AMPA/Kainate antagonists.

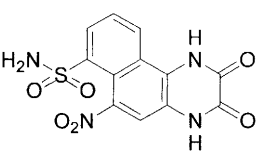
There is also evidence that AMPA/kainate receptors may be involved in chronic pain: following peripheral nerve injury AMPA receptor expression is upregulated and peaks 2 weeks after nerve ligation (Harris et al., 1996). Furthermore, GYKI52466 potently inhibits hyperalgesia in Freund adjuvant-induced chronic arthritis (Szekely et al., 1997).

(2*S*,4*R*)-Methylglutamate (SYM2081), a selective GluR5 and GluR6 desensitizing agonist showing 500 - 2000-fold selectivity for homomeric kainate receptors (composed of GluR5 and GluR6 subunits) over AMPA receptors (composed of GluR1, GluR2 or GluR3 subunits) (Zhou et al., 1997), reduces allodynia and hyperalgesia in CCI rats (Sutton et al., 1999) and freeze injury model of neuropathic pain (Ta et al., 2000). The location of the methyl group at the 4 position of glutamate is critical for kainate receptor agonist activity as glutamate analogs with the methyl group at the 2 or 3 position had negligible activity (Donevan et al., 1998).



SYM-2081

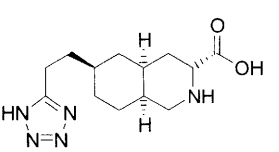
Analgesia after intrathecal administration of the glutamate antagonist NBQX supports a site of action at AMPA/kainate receptors in the superficial laminae of the spinal dorsal horn (Furuyama et al., 1993). In addition to this spinal action, injection of the glutamate antagonist CNQX into the rat hindpaw reverses hyperalgesia and allodynia caused by pharmacologic activation of AMPA and kainate receptors, thus suggesting a peripheral action (Zhou et al., 1996). Finally, the ubiquity of AMPA/kainate receptors in the brain leaves open the possibility that their antagonists may mediate analgesia by blocking excitatory neurotransmission at supraspinal sites.



NBQX (6-Nitro-2,3-dioxo-1,2,3,4-tetrahydro-benzo[f]quinoxaline-7-sulfonic acid amide)

There is evidence that AMPA/kainate antagonists also reduce clinical pain. Intravenous LY293558 (0.4 or 1.2 mg/kg) proved to reduce postoperative pain in human volunteers (Gilron et al., 2000). It strongly antagonizes both AMPA receptors (particularly GluR2) and kainate receptors (particularly GluR5). By January 2000, LY-293558 was undergoing phase II trials for pain and other CNS indications. Data from the first studies provide early evidence for its analgesic efficacy at doses that are well tolerated. The most striking side-effect was hazy vision, described as white clouds in the periphery with sparing central vision (Gilron, 2001).

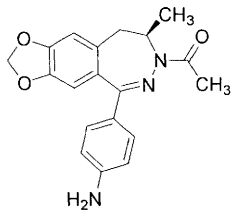
LY-293558



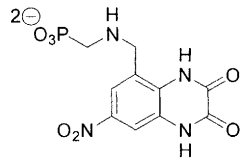
6-[2-(1H-Tetrazol-5-yl)-ethyl]-decahydro-isoquinoline-3-carboxylic acid, $C_{13}H_{21}N_5O_2$, MW 279.34, 154652-83-2

Further Compounds

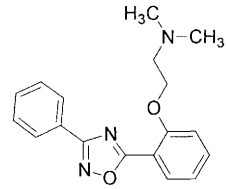
Some AMPA/kainate antagonists are in clinical trials for several indications based on their neuroprotective and anticonvulsive properties. A selection is given below.



Talampanel, phase II clinical trials for epilepsy and Parkinson's disease (Eli Lilly)

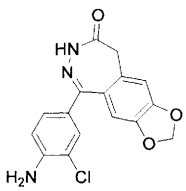


AMP-397A, phase I clinical trials for epilepsy (Novartis)

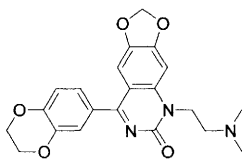


Irampanel, phase II clinical trials for cerebral ischemia, combined AMPA receptor/sodium channel antagonist (Weiser et al. (Boehringer Ingelheim), 1999)

Scheme 2: Compounds in clinical trials.



AMPA receptor antagonist
Xia et al., 1999 (CoCensys)



AMPA receptor modulator
Upasani et al., 1999 (CoCensys)

Although AMPA and kainate antagonists have been less extensively studied in pain models than NMDA receptor antagonists, recent evidence suggests that they may also be useful in the treatment of clinical pain. Studies suggest that antagonism of multiple types of glutamate receptors might be necessary to achieve effective pain relief since NMDA, AMPA and kainate are all involved in peripheral pain transmission (Lutfy et al., 1997).

With respect to recent patent applications the neuroprotective properties of non-NMDA antagonists are still in the focus of pharmacetical research, but the majority of patents does not propose that they should be used for pain relief. However, the continuing interest in AMPA/kainate receptor modulators might also lead to further investigations of their analgesic properties. Two examples of AMPA receptor modulators recently claimed for pain are shown.

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8 Acetylcholine Receptors

8.1 Nicotinic Acetylcholine Receptors

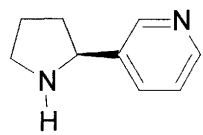
Bernd Sundermann and Corinna Maul

Introduction

Nicotinic acetylcholine receptors (nAChRs) form a family of pentameric ligand-gated ion channels (Hucho and Weise, 2001). A range of subtypes is known due to the occurrence of different subunits forming the ion channel (Jones et al., 1999; Clementi et al., 2000; Cordero-Erausquin et al., 2000; Picciotto et al., 2000). α- and β-subunits are most common, especially for neuronal nACh receptors, but γ-, δ- and ε-subunits have also been described. Tissue distribution of specific receptor subtypes varies distinctively, especially between the CNS and peripheral tissues. Neuronal nAChRs interact with various neurotransmitter systems besides the cholinergic system, including noradrenergic, GABAergic and dopaminergic systems (Williams and Arneric, 1996; Soreq, 1998). How neuronal nAChRs influence pain perception and signaling is not completely understood (Bai et al., 1997; Khan et al., 1997; Flores, 2000).

Although the antinociceptive potential of the natural product nicotine, an unselective nAChR agonist, was noticed as early as 1932 (Davis et al., 1932; Sahley and Berntson, 1979; Aceto et al., 1997; Rao et al., 1996; review: Badio et al., 1995) interest in antinociceptive nicotinic agents was virtually non-existent for several decades. This changed dramatically in 1992 when another natural nACh receptor agonist was discovered and reported to be at least 200 times more potent than morphine (Spande et al., 1992; Holladay et al., 1997; Flores et al., 1999; Decker et al., 2001):

(*S*)-Nicotine, a tobacco alkaloid:



Starting in 1964 Daly and his co-workers at the National Institute of Health collected poison-dart frogs in the Pacific highlands of Ecuador in collaboration with Myers' group. They were looking for pharmacologically active alkaloids (Spande et al., 1992; Daly et al., 2000) and in 1974 collected and extracted alkaloid-containing samples from the skins of *Epipedobates tricolor* specimens among others. The pooled samples were fractioned by HPLC (Daly et al., 1980) and when mice were injected with specific fractions they swiftly raised and arched their tails. This was interpreted as a typical Straub-tail effect characteristic of μ-opioids (Spande et al., 1992; Daly, 1993). Two years later Daly collected more frogs, but subsequent extractions of the frog skins yielded less than1 mg of the active compound, so it was not possible to

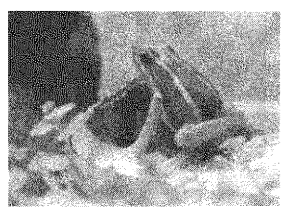


Figure 1: *Epipedobates tricolor.*

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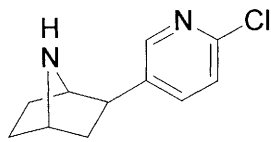
elucidate its chemical structure at that time. Coincidentally a newly signed international treaty to protect endangered species prohibited further collection of *Epipedobates tricolor* specimens. On the other hand skin extracts from frogs collected from other venues or raised in captivity did not contain the desired compound, so it was assumed that this compound - or its precursor - was derived from an unknown specific dietary source (Daly et al., 1997; 2000).

Identification of a natural compound as the first potent nicotinic analgesic – discovery of EPIBATIDINE

The extraordinary antinociceptive potency of the unknown compound was determined in a mouse model of acute nociception, the hot plate test. Despite the Straub-tail effect observed earlier, the antinociceptive effect of the compound could not be not antagonized with the μ-opioid receptor antagonist naloxone but with the nAChR antagonist mecamylamine, so the unknown compound was deduced to be a potent nicotinic analgesic (Spande et al., 1992; Qian et al., 1993; Badio and Daly, 1994; Sullivan and Bannon, 1996).

Elucidation of the structure of epibatidine – more than a decade after its initial discovery

Only in 1990 were the remains of the original extract stored by Daly subjected to modern analytical methods and the structure of the compound - which was named *epibatidine* - finally elucidated by spectroscopic methods, especially IR and NMR techniques (Spande et al., 1992; Daly, 1993; Daly et al., 2000). This was published in 1992 - 12 years after the initial publication on *Epipedobates tricolor* extracts (Daly et al., 1980) and very shortly after Daly and co-workers had filed patents on epibatidine and close structural analogs (see *Epibatidine*).



Epibatidine

The race for nicotinic analgesics

With the discovery of this first potent (Ki ≤ 100 pm) but non-selective neuronal nAChR agonist, being extremely efficacious in animal models of pain, a race not only for the synthesis of the scarce compound (Dehmlow, 1995) but for nicotinic agonists as potential analgesics ensued. Although several dozen publications on the total synthesis of epibatidine were published in the 1990s, original total syntheses of epibatidine are still a stimulating topic of academic research (Roy et al. 2001).

Boyce et al. 2000: Analgesic and toxic effects of ABT-594 resemble epibatidine and nicotine in rats

While the findings of Daly et al. where initially met with euphoria, today only limited work is carried out with respect to nicotinic analgesics. The promise of finding a non-opioid treatment for severe acute pain with a potency that is at least comparable to μ-opioid full agonists has not been fulfilled. This is in general due to either dose limiting toxic effects or insufficient potency (Boyce et al., 2000; also see: Bai et al., 1997; Francis 1999; Kesingland et al., 2000). It has been speculated, but not proven, that these problems might be overcome with nAChR subtype-selective compounds (Marks et al., 1998; Bannon et al.,

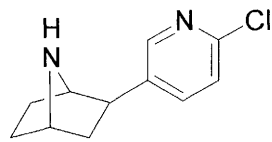
1998; Marubio et al., 1999; Williams et al., 1999). This speculation is corroborated by data suggesting that only neuronal nAChRs are important for the antinociceptive effects of epibatidine, while toxic effects might be mediated by peripheral nAChRs (Sullivan et al., 1994).

Can neuropathic pain be effectively treated with nicotinic agonists?

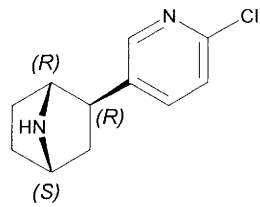
On the other hand ABT-594, a nicotinic agonist presumed to be selective for the $\alpha_2\beta_4$-subtype of the neuronal nACh receptor (Khan et al., 1997, 1998; Kowaluk and Arneric, 1998; Thatte, 2000), is reported to be in clinical development against neuropathic pain, a pain state that often cannot be effectively treated with opioids or other medications (Lawand et al., 1999; Gilbert et al., 2001).

Epibatidine

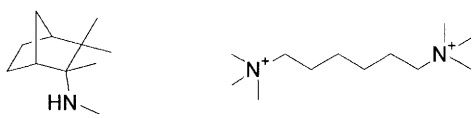
The natural product epibatidine was the starting point for worldwide activities towards the discovery of nicotinic analgesics. Besides its unusual antinociceptive potency (review: Bai et al., 1997) its exceptional structure combines a scarce chloropyridine moiety with a 7-aza-norbornane (7-azabicyclo[2.2.1]heptane) bicycle which has never before been found in a natural product before. In 1994 the absolute configuration of natural (+)-epibatidine was determined to be 1*R*,2*R*,4*S* (Fletcher et al., 1994). Initial patents on epibatidine and close structural analogs were filed by the U.S. Department of Health (Daly et al., 1993, 1995).



[*140111-52-0*];
(+)-(1*R*,2*R*,4*S*)-2-(6-Chloropyridin-3-yl)-7-azabicyclo[2.2.1]heptane,
$C_{11}H_{13}ClN_2$, M_r 208.69



Epibatidine's antinociceptive effect can be antagonized by pretreatment with the centrally active nAChR antagonist mecamylamine, but not with the peripheral antagonist hexamethonium, so the activation of central nAChRs is presumed to be essential for nicotinic analgesics (Sullivan et al., 1994). The high toxicity of epibatidine has been attributed to its lack of selectivity for specific neuronal nAChR subtypes and has precluded its development as a therapeutic agent.



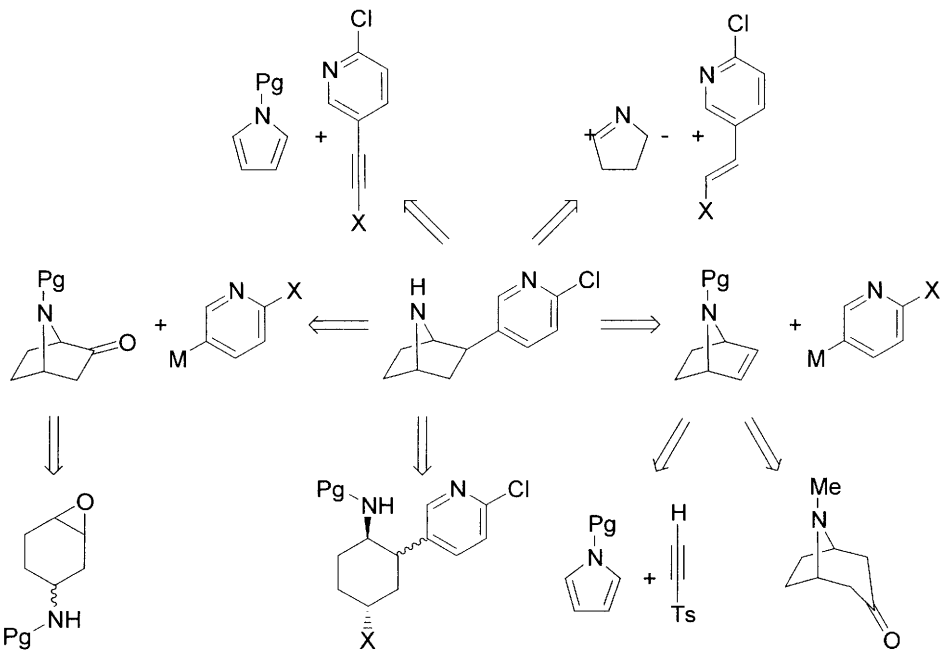
Mecamylamine Hexamethonium

Figure 2: nAChR antagonists.

Total syntheses of *epibatidine*

While Dehmlow's review details a variety of efforts towards the total synthesis of epibatidine (Dehmlow, 1995) the synthesis of this natural product is still an intriguing challenge for academic research. More than three dozen

(formal) total syntheses have been published in the past decade (Roy et al., 2001). A production route has not been developed so the following scheme is intended only to give an exemplary overview of successful early synthetic strategies:



Pg, protecting group; M, metal (complex); X, activating/leaving group.

Figure 3: Retrosynthetic strategies for the total synthesis of epibatidine.

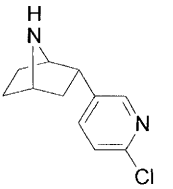
Epibatidine – a very potent but highly toxic nicotinic analgesic

Epibatidine was shown to be a very potent and selective agonistic ligand of nicotinic acetylcholine receptors. This natural product is effective in various animal models of pain through a pronounced nAChR agonistic mechanism (Ki < 100 pm) which is accompanied by severe and nACh-related side-effects (Corey et al. 1993; Rupniak et al., 1994; Boyce et al., 2000). A clear differentiation between antinociceptive activity in animal models of pain and toxic side-effects cannot be determined. Nevertheless there is some activity directed towards the development of epibatidine as an analgesic (Bai et al., 1997).

Structure-activity relationship of epibatidine and structural analogs

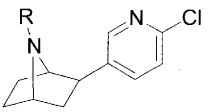
Remarkably, there is very little difference between the natural and unnatural enantiomer of epibatidine with respect to their antinociceptive potency in various animal models (Sullivan et al., 1994; Bai et al., 1997).

The *exo* position of the chloropyridine is essential for the antinociceptive potency of epibatidine – its racemic *endo* diastereoisomer is inactive. Also inactive are amides derived from epibatidine through acylation of the secondary amine function (R = C(O)R'). On the other hand the potency of 7-methylepibatidine (R = Me) is comparable to epibatidine itself, so a basic nitrogen but not necessarily a secondary amine is needed for activity (Li et al., 1993).

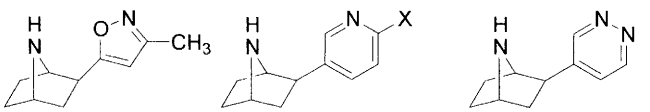


endo-epibatidine

Several attempts have been made to synthesize less toxic and/or more potent epibatidine analogs through the variation of its aromatic moiety. Selected examples are displayed in the following scheme (Badio et al., 1997; Carroll et al., 2001; Che et al., 2001):



epibatidine derivatives



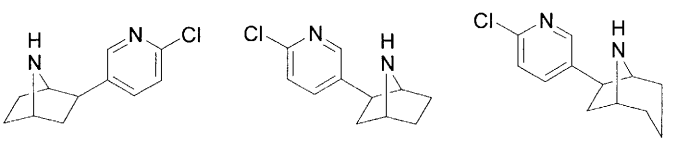
(±)-eboxidine, Badio et al., 1997 | epibatidine derivatives, Carroll et al., 2001 | epibatidine derivatives, Che et al., 2001

Figure 4: Variations in the aromatic moiety of epibatidine.

The analogs synthesized by Che et al. (Bayer AG) are reported to be in the preclinical stage directed towards the development of new analgesics (Pharmaprojects No. 31767).

Modifications to the azabicycloalkane skeleton

The biochemical properties of epibatidine have been compared to the ring-enlarged 8-azabicyclo[3.2.1]octane derivative homoepibatidine and several 2-azabicyclo-[2.2.1]heptanes (Cox et al., 2001).

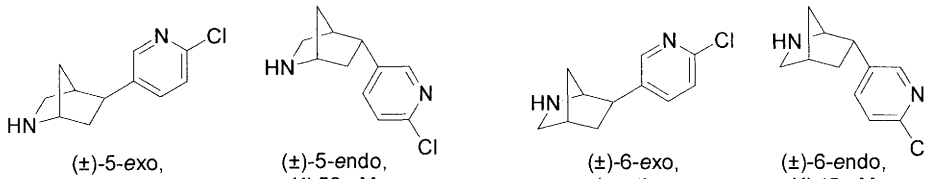


(+)-epibatidine, Ki 19 pM | (-)-epibatidine, Ki 20 pM | (±)-homoepibatidine, Ki 230 pM

Figure 5: nACh receptor affinities of the epibatidine enantiomers and (±)-homoepibatidine ($\alpha 2\beta 4$ subtype).

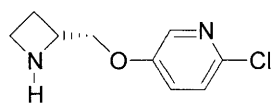
Biochemical properties of 2-azabicyclo[2.2.1]heptane analogs of epibatidine

exo-Homoepibatidine was found to be only slightly less active than epibatidine itself, but the 2-azabicyclo[2.2.1]-heptane derivatives behave differently: both 5-*exo*- and 6-*exo*-(6-Chloropyridin-3-yl)-2-azabicyclo[2.2.1]heptane are inactive while both *endo*-diastereoisomers are active. This was hypothesized to result from the spatial relationship between the nitrogen atoms.



(±)-5-*exo*, inactive | (±)-5-*endo*, Ki 56 pM | (±)-6-*exo*, inactive | (±)-6-*endo*, Ki 45 pM

Figure 6: nACh receptor affinities of 5- and 6-(6-Chloropyridin-3-yl)-2-azabicyclo[2.2.1]-heptanes ($\alpha_2\beta_4$ subtype).



[198283-73-7];
(*R*)-5-(Azetidin-2-yl-methoxy)-2-chloropyridine,
$C_9H_{11}ClN_2O$, M_r 198,65

ABT-594

ABT-594 is reported to be in clinical trials for the treatment of neuropathic pain (Thatte, 2000; Sorbero et al. 2001). Its precursor (*R*)-N-Boc-azetidin-2-yl-methanol is accessible in a short sequence starting from commercially available *D*-aspartic acid dibenzyl ester. The synthesis is concluded by Mitsunobu coupling with 6-chloropyridin-3-ol and subsequent acidic deprotection. On a larger scale the primary alcohol is activated as a mesylate prior to coupling with 6-chloropyridin-3-ol in the presence of potassium hydroxide, so that Mitsunobu conditions can be avoided (Meyer et al., 2000).

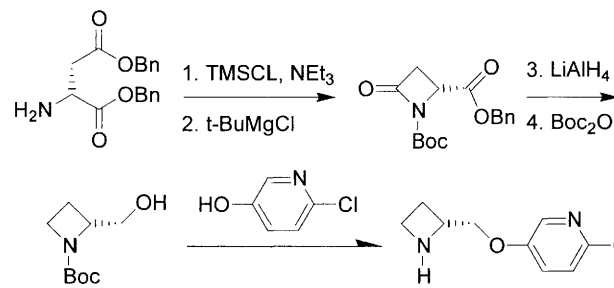


Figure 7: Synthesis of ABT-594.

The properties of ABT-594, the most advanced compound derived from the discovery of epibatidine, are well documented in the literature (Decker et al., 1998; Thatte, 2000). Its antinociceptive potential has been proven in various animal models, but according to Boyce et al. (2001) the side-effect profile of ABT-594 does not represent a significant improvement compared to other potential nicotinic analgesics.

Other Compounds

The race to find nicotinic analgesics has - in a very few years - resulted in a cornucopia of potential clinical candidates. The following scheme is intended to give an overview of the most striking examples:

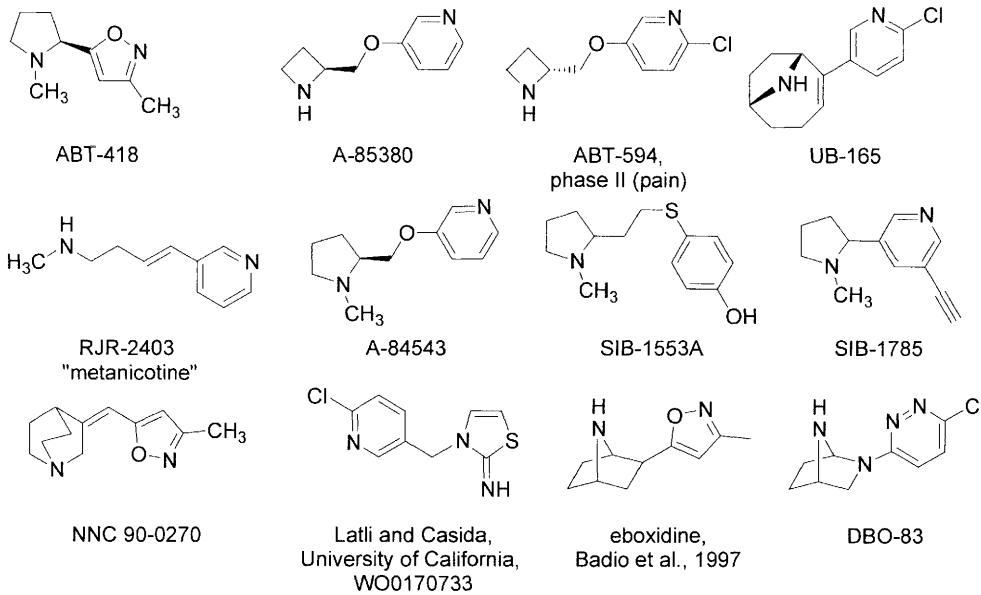
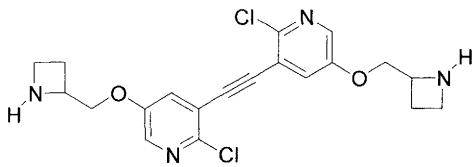
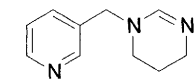


Figure 8: Potential nicotinic analgesics.

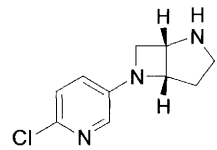
Recently disclosed patent applications comprise related structures designated to be nAChR modulators/antagonist. Also recently a patent was granted to Advanced Medicine Inc. covering so-called multibinding compounds for the treatment of pain, consisting of two to 10 covalently bound known nAChR agonists (Natarajan et al. (Advanced Medicine), US6288055 (2001)).



Natarajan, Advanced Medicine Inc., US6288055, 2001



Imoto et al.,
Suntory, WO0181334



Schrimpf et al.,
Abbott, WO0181347

An overview of available biochemical data on more than 20 nACh receptor ligands has recently been published (Sharples and Wonnacott, 2001).

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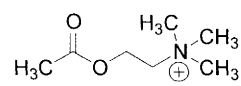
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8.2 Muscarinic acetylcholine receptors

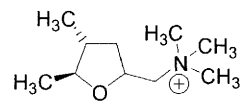
Corinna Maul and Bernd Sundermann

Introduction

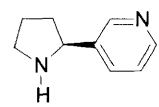
The neurotransmitter acetylcholine (ACh) binds to two types of cholinergic receptors: the ionotropic family of nicotinic receptors and the metabotropic family of muscarinic receptors. Nicotinic receptors are ligand-gated ion-channels which modulate cell membrane potentials. Muscarinic acetylcholine receptors (mAChRs) belong to the large superfamily of membrane-bound G-protein coupled receptors (GPCRs). Five subtypes of muscarinic receptors (M_1-M_5) have been cloned and sequenced from a variety of mammalian and nonmammalian species. They show a remarkably high degree of sequence similarities across species as well as across receptor subtypes. Like all GPCRs, muscarinic acetylcholine receptors are characterized by seven transmembrane regions connected by intra- and extracellular loops. Between the fifth and sixth transmembrane region muscarinic receptors possess a large intracytoplasmatic loop which is considered to be responsible for G-protein coupling selectivity and which exhibits high divergence between the different subtypes.



Acetylcholine



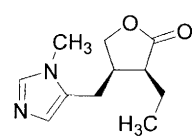
Muscarine



Nicotine

Receptor Localization

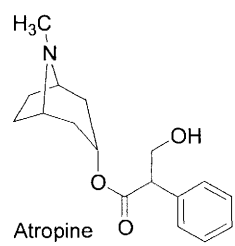
The M_1-M_5 muscarinic receptors are expressed predominantly within the parasympathetic nervous system, which exerts excitatory and inhibitory control over central and peripheral tissues, and participate in a number of physiological functions such as the function of heart and smooth muscles, glandular secretion, release of neurotransmitters, gene expression and cognitive functions such as learning and memory. Availability of molecular probes and receptor subtype-selective antibodies has provided detailed knowledge of receptor distribution. A significant presence in a wide variety of brain regions and peripheral tissues has been described.



Pilocarpine

Muscarinic Agonists and Antagonists

Muscarinic agonists such as muscarine and pilocarpine and antagonists such as atropine, the racemic form of natural hyoscyamine, have been known for more than a century. Only recently more selective ligands have been found that will hopefully generate a better understanding of the role mAChR subtypes in physiological processes and especially brain function.



Atropine

Analgesics. Edited by H. Buschmann, T. Christoph, E. Friderichs, C. Maul, B. Sundermann
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mAChR Subtypes

Discovery of mAChR subtypes

First pharmacological evidence for the presence of multiple subtypes of muscarinic receptors appeared in the early 1950s and became more evident as differences in tissue responses to various muscarinic ligands were observed. On pharmacological criteria, only four distinct subtypes of muscarinic acetylcholine receptors have been identified by use of selective antagonists.

Genes encoding M_1-M_5 receptors were identified in the mid 1980s (Kubo et al., 1986; Bonner et al., 1987); numbers were assigned in the order of discovery. The M_5 muscarinic receptor gene was the last to be found. It is present in the brain and viscera, but only in very low concentrations. No selective high-affinity ligands or toxins for the M_5 receptor have been found, and no tissues with predominant concentrations of M_5 receptors have been identified (Yeomans et al., 2001).

mACh Receptor Function

mAChR subtypes activate different second messenger transduction systems, with M_1, M_3 and M_5 acting through the phosphoinositol cascade via the α subunits of the G_q family, whereas M_2 and M_4 mainly lower cAMP levels via G_i and $G_{0\alpha}$ (Caulfield and Birdsall, 1998).

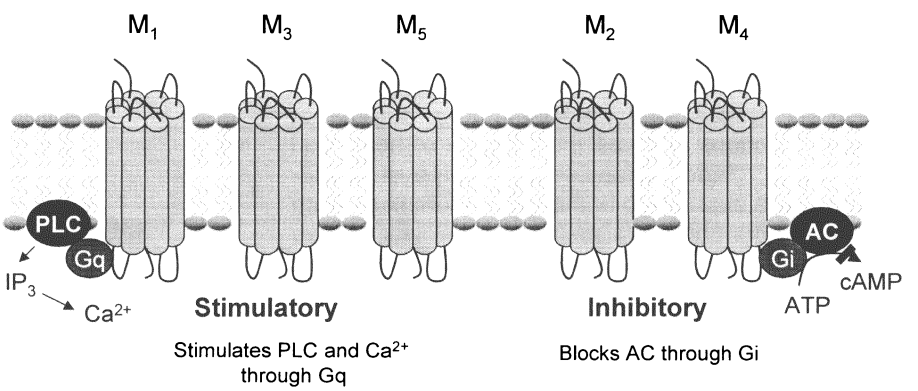


Figure 1: The muscarinic acetylcholine receptor family. IP_3, inositol triphosphate; PLC, phospholipase C; AC, adenylate cyclase (adapted from Felder et al. 2000).

Muscarinic receptors play a key role in many functions in the periphery and the central nervous system. In the periphery, muscarinic receptors are involved in cardiac function, glandular secretion, and smooth muscle contractility. Central muscarinic receptors modulate pain

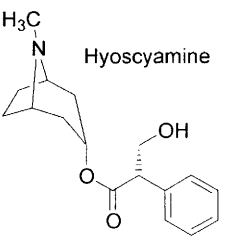
perception, motor control and memory processes (Bymaster et al., 2001).

Abuse of muscarinics

The psychological effects of muscarinic drugs have been exploited for religious or recreational purposes for a long time (Shulgin, 1982). The recreational use probably requires some deeper interests in psychedelics - there seems to be a general consensus that the effects are not purely pleasurable.

Natural Muscarinics

Many of the earliest known ligands for muscarinic acetylcholine receptors were of natural origin, typically from plants such as deadly nightshade, thorn apple and tobacco, which is particularly rich in muscarinic toxins such as the closely related antagonists atropine (see above), scopolamine (see below) and hyoscyamine. Other natural muscarinic acetylcholine receptor ligands are toxins from snakes, particularly mambas, which are selective for M_1, M_2 and M_4 receptors. They consist of 63 to 66 amino acids and four disulfide bridges which form loops. They are members of a large group of snake toxins which are called three-finger toxins: three loops are extended like the middle fingers of a hand, and the disulfides and the shortest loop are in the palm of the hand. For example M_1-toxin1, isolated from the venom of the East African green mamba, is highly specific for M_1 receptors and binds irreversibly to it. It appears to be completely specific for M_1 receptors and is one of the most specific ligands ever found for any target protein (Potter, 2001). A possible explanation for the good selectivity is that the toxins bind to an allosteric site, but because of their size they probably also bind to extracellular parts of the receptors which are rather different in the various subtypes (Karlsson, 2000).

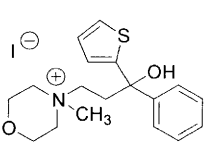


Hyoscyamine

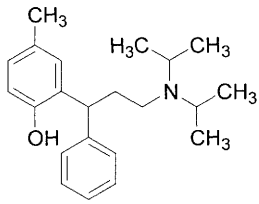
Snake toxins are potent (irreversible) muscarinic ligands

Muscarinics in Clinical Use

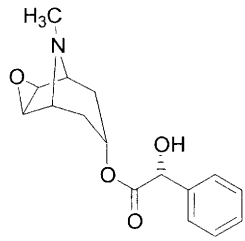
A number of muscarinic agonists and antagonists are launched or in clinical trials, especially as antiemetics (e.g. scopolamine), as treatment for urinary incontinence (e.g. tolterodine), glaucoma (pilocarpine), and airway diseases (e.g. ipratropium bromide), but, to the best of our knowledge, only few are used as adiuvants in analgesic compositions, e.g. tiemonium iodide which is used in various combinations with analgesics like paracetamol or metamizole (Coffalon®, Viscéralgine®).



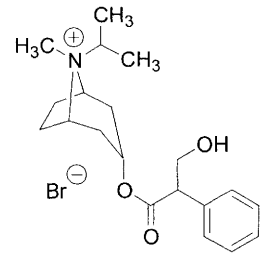
Tiemonium iodide used as an adiuvant in analgesic compositions (Labrid et al., 1976)



Tolterodine, muscarinic antagonist, launched for the treatment of urinary incontinence (Crandall, 2001; Nilvebrant, 2001)



Scopolamine, muscarinic antagonist, used clinically as an antispasmodic, antiemetic drug (Kovac, 2000)



Ipratropium bromide, bronchodilator (Disse, 2001)

Figure 2: Muscarinics drugs.

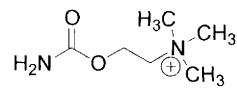
Potential Use of Subtype Selective mAChR Agonists and Antagonists

In the last couple of years, subtype-selective ligands of mAChRs were investigated for further therapeutic areas: M_1 (M_3) agonists as well as M_2 antagonists attracted interest in the treatment of Alzheimer's disease (Davis et al., 1995), which is accompanied by a shortage of acetylcholine and therefore an understimulation of muscarinic receptors (Zlotos et al., 1999; Lachowicz et al., 2001; Wienrich et al., 2001). For subtype-selective antagonists, the treatment of peripheral smooth muscle disorders such as bladder, airway and bowel disorders with M_3 antagonists has been of particular interest, while antagonists of M_2 and M_4 receptors have been suggested as treatments for movement disorders (Salamone et al., 2001), dementia, cardiac disorders, and pain (Felder et al., 2000).

Analgesic potential of mAChR modulators

Acetylcholine is generally considered to be an algogenic agent because it has been shown to produce burning pain when applied to human skin. Nonetheless, it is still unknown whether ACh appears in inflammatory exudates or under other painful conditions, but possible extraneuronal sources of ACh in the close vicinity of primary afferent terminals have been identified. In the cornal epithelial cells for example, high concentrations of ACh have been found that may be released after injury, and ACh has been shown to excite corneal nerve endings. Furthermore it has been shown that human keratinocytes are able to synthesize and secrete ACh. Here ACh plays a role in regulating cell-cell attachment, but in addition it may be released in larger amounts after cutanous injury. Moreover, the ability of dorsal root ganglia (DRG) to synthesize ACh has been reported (Tata et al., 1994).

An electrophysiological study has shown that muscarine treatment of C-units left them with a marked and sustained desensitization to mechanical and heat stimuli (Bernadini et al., 2001). The mechanical desensitization is in agreement with preceeding results where the ACh analog carbachol was shown to excite C-nociceptors and at the same time produced desensitization to mechanical stimulation lasting up to 45 minutes (Steen and Reeh, 1993).



Carbachol

Modulation of central nociception through mAChRs

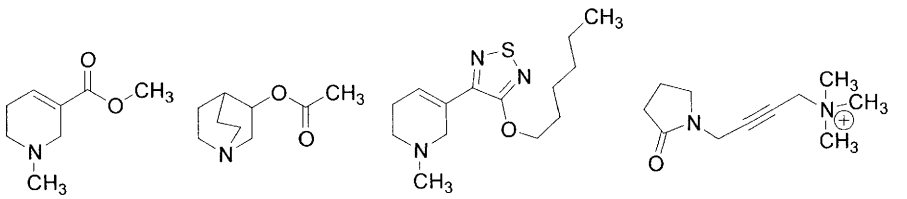
Furthermore, it is well documented that mAChRs are involved in the modulation of central nociception (see Yaksh et al., 1985, Hartvig et al., 1989, Bartolini et al., 1992):

Muscarinic agonists and pain - M_1 and M_3 receptors

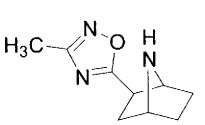
Centrally administered acetylcholine has been found to cause antinociception, which was proposed to depend on the activation of descending pathways (Hartvig et al., 1989). M_1 as well as M_3 muscarinic receptors have been shown to play an essential role in the modulation of pain perception (Naguib and Yaksh, 1997). Cholinergic antinociception induced both directly, through muscarinic agonists, and indirectly, by enhancing extracellular ACh levels through cholinesterase inhibitors, is prevented, in a dose-related manner, by i.c.v. administration of antisense DNA to the M_1 gene coding for the mouse M_1 receptor (Ghelardini et al., 2000). On the other hand, studies using muscarinic agonists lacking M_1 agonistic activity in tissues and M_1 cell lines showed that M_1 agonistic activity is not required for antinociception (Sheardown et al., 1997).

Muscarinic agonists and pain - M_2 and M_4

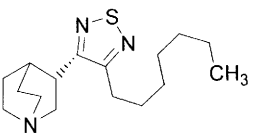
Experiments with muscarinic M_2 and M_4 knock-out mice indicate a crucial role of especially M_2 receptors in mediating antinociception. Analgesic activity of oxotremorine, an unselective muscarinic agonist, was reduced significantly when administered to M_2 knock-out mice (tail-flick, hot plate). However, oxotremorine-induced analgesia was not completely abolished in M_2 -/- mice indicating that the M_2 receptor is not the only muscarinic receptor subtype involved in 'muscarinic analgesia' (Gomeza et al., 2001). In another study it was shown by antagonization with a specific toxin that antinociception induced by muscarinic agonists, e.g. CMI-936 derived from epibatidine (see nAChRs), is mediated via muscarinic M_4 receptors (Ellis et al., 1999). The following figure shows muscarinic agonists with antinociceptive properties. Among others, the compounds arecoline, aceclidine, xanomyeline, and oxotremorine are active in the tail-flick, hot plate, writhing, and grid-shock assay (Sheardown et al., 1997):



Arecoline | Aceclidine | Xanomeline | Oxotremorine



CMI-936
(Ellis et al., 1999)

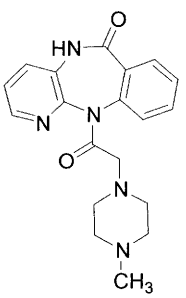


LY297802, agonist at M_1, partial agonist/antagonist at M_2/M_3, equiefficacious to morphine in producing antinociception in animals (Shannon et al., 1997)

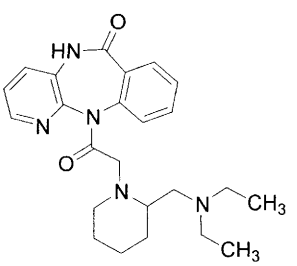
Figure 3: Muscarinic agonists with antinociceptive properties.

Muscarinic antagonists and pain

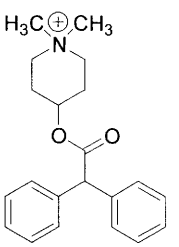
On the other hand, investigations on the antinociceptive effects of muscarinic antagonists, namely pirenzepine (M_1), AF-DX116 (M_2) and 4-DAMP (M_3), showed that the M_3 antagonist can inhibit the second phase of formalin-induced nociception (Honda et al., 2000).



Pirenzepine
M_1 muscarinic receptor antagonist



AF-DX 116
M_2 muscarinic receptor antagonist



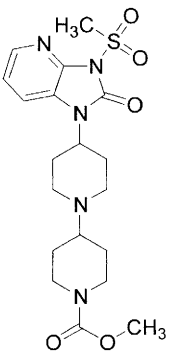
4-DAMP
M_3 muscarinic receptor antagonist

Potential use of allosteric modulators

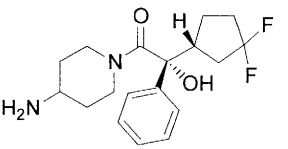
A further possibility for affecting muscarinic receptors are allosteric modulators. They act at a site apart from the common binding site of the receptor protein. Depending on the allosteric modulator, the type of ligand and the receptor subtype, ligand binding can be elevated, reduced or remain unchanged. Used as enhancers of acetylcholine action, allosteric modulators might be benificial for the treatment of pain (Holzgrabe and Mohr, 1998).

Outlook

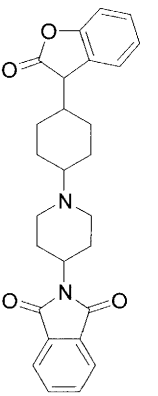
Although serious efforts have been directed towards the discovery of muscarinic acetylcholine receptor modulators, to the best of our knowledge the promising results concerning antinociceptive properties, especially of muscarinic agonists, in animal models have not led to a marketed drug by now. During the last years only few patent applications on muscarinic modulators have been filed for the treatment of pain. A selection is given in the following figure:



Yamawaka et al., Banyu, M_4 agonist, WO0127104



Tsuchiya et al., Banyu, M_3 antagonist, useful in pain accompanied by smooth muscle twitch of digestive organs, WO9940070



Dantanarayana, Alcon, muscarinic modulator, WO9932479

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9 Further Opioid Receptors[1]

9.1 The δ Opioid Receptor

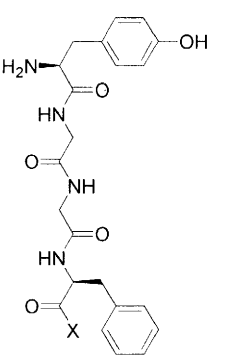
Claudia Pütz

Introduction

Among the variety of pharmacological effects related to the activation of the opioid system the ability of opioids to relieve pain for centuries was considered to be the most relevant clinical aspect. Drugs selectively activating μ receptors are well known to be potent analgesics and still represent the gold standard for the treatment of a variety of pain conditions. The clinical utility of these drugs is, however, limited by undesirable side effects including respiratory depression, constipation and physical dependence (Zenz and Willweber-Strumpf, 1993; Dondio et al., 1997). In the search for potent and safe analgesics to replace the existing μ agonists a great deal of research effort has been dedicated to the discovery of drugs selective for the δ opioid receptor (Dondio et al., 1997, 1999).

Discovery of the δ opioid receptor

The existence of the δ opioid receptor was first demonstrated by Lord, Waterfield, Hughes and Kosterlitz in 1977 (Lord et al., 1977). Their experiments, which showed a differential rank order of potency between morphine alkaloids and the opioid peptides Leu-enkephalin, Met-enkephalin (Hughes, 1975) and β-endorphin in bioassays on guinea pig ileum (GPI) and mouse vas deferens (MVD), led them to postulate that the peptides acted at the δ opioid receptors in the mouse vas deferens and μ opioid receptors in the guinea pig ileum. This receptor differentiation was supported by studies showing that approximately 10 times as much of the opioid antagonist naloxone was needed to antagonize actions at the δ opioid receptor compared with the μ opioid receptor. However, naloxone is a rather nonselective antagonist, so the synthesis of the δ opioid receptor selective antagonist naltrindole (NTI, see below) spawned the hope that pharmacologists would uncover the secrets of the δ opioid receptor and perhaps allow an assignment of different opiate effects to the activation of different opioid receptor types.



H-Tyr-Gly-Gly-Phe-X
Leu-enkephalin: X = Leu
Met-enkephalin: X = Met

[1] The so far unsuccessful attempts towards the development of selective κ-opioid agonists are summarised in Chapter 3.1. Clinically relevant opioids with affinity to the κ-opioid receptor are discussed in Chapter 3.4.

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Cloning of the δ receptor

Attempts to clone opioid receptors failed for a long time due to the paucity of their mRNAs in brain tissues and the lack of an appropriate cloning strategy. Two groups (Evans et al., 1992; Kieffer et al., 1992) simultaneously used very similar expression cloning strategies to identify the δ receptor gene. The advent of a powerful expression cloning method in mammalian cells allowed both groups to succeed in cloning the mouse δ opioid receptor from the mouse neuroblastoma/rat glioma hybrid cell NG108-15.

The δ receptor belongs to the GPCR superfamily

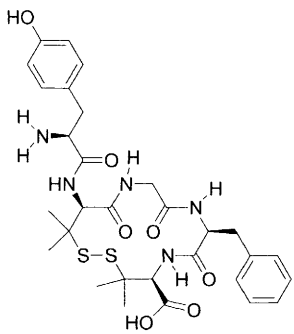
The δ opioid receptor sequence comprises 372 amino acids with seven putative transmembrane domains. The δ receptor belongs to the GPCR superfamily. The alignment of mouse, rat and human δ opioid receptor sequences shows highly homologous sequences among these species (Knapp et al., 1994; Jin et al., 1999).

Receptor distribution

The distribution of δ receptors appears to be best conserved across mammalian species. δ Receptors are most densely distributed in forebrain structures and sparse to non-existent in most midbrain and brainstem areas. This relationship is particularly evident in the nigrostriatal system where moderate to dense δ receptor binding is observed in the caudate-putamen of the rat, guinea pig, hamster, and monkey (Yaksh et al., 1997).

Evidence for two δ receptor subtypes

The availability of a number of potent and selective δ ligands, agonists and antagonists, gave great impact to the investigation of δ opioid receptor pharmacology. A wide array of pharmacological evidence supports the existence of two subtypes within the δ receptor class.



DPDPE
H-Tyr-D-Pen-Gly-Phe-D-Pen-OH
[D-Pen^2,D-Pen^5]enkephalin

Studies on the antinociceptive effects of various highly selective peptidic δ agonists, particularly [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (Mosberg et al., 1983) and [D-Ala^2,Glu^4]deltorphin, sometimes referred to as deltorphin II (Erspamer et al., 1989; Kreil et al., 1989; Jiang et al., 1990; Lazarus et al., 1999) and the distinct reversal of their action by the two nonequilibrium δ-opioid antagonists [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE) (Bowen et al., 1987) and naltrindole-5´-isothiocyanate (5´NTII) or by the reversible antagonists 7-benzylidenenaltrexone (BNTX) and naltriben (NTB), have provided the basis for the δ receptor subtypes δ_1 and δ_2. Further studies on cross-tolerance, electrophysiological recordings and measurements of adenylate cyclase activity in brain tissues strengthened the hypothesis of a δ_1 receptor that would be preferentially activated by DPDPE and blocked by BNTX, and a δ_2 receptor that responds to deltorphin II and is inhibited by NTB (Jiang et al., 1991; Sofuoglu et al., 1991).

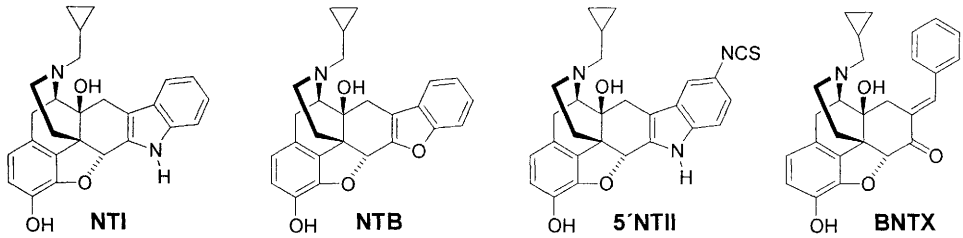


Figure 1: Non-peptidic tool compounds for the δ opioid receptor (antagonists).

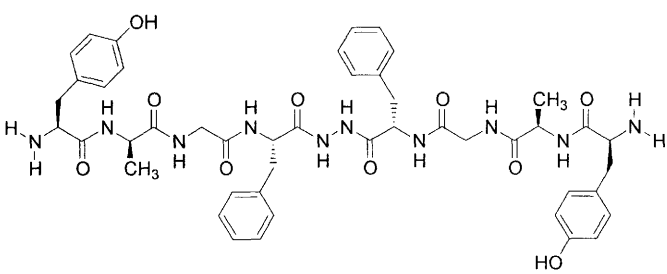
While *in vivo* data supports the existence of δ receptor subtypes, the results from radioligand binding studies have generally been less conclusive. The possibility of a single receptor with two affinity states cannot be excluded (Negri et al., 1991; Xu et al., 1991; Fang et al., 1994). Furthermore, only a single molecular form of the δ receptor has been isolated so far. Based upon binding and adenylate cyclase inhibition experiments with the cloned receptor, the latter seems to represent the δ_2 subtype. Another line of evidence based on antisense oligonucleotide experiments again supports the hypothesis that the cloned δ opioid receptor corresponds to that classified pharmacologically as δ_2 (Hul et al., 1994; Zaki et al., 1996).

Antisense oligonucleotide experiments supports the hypothesis that the cloned δ opioid receptor corresponds to the subtypes classified pharmacologically as δ_2

An additional aspect of the pharmacology of δ opioid receptor ligands has been referred to as a modulatory action on the effects of μ opioid receptor agonists such as morphine (Heyman et al., 1986, 1989; Porreca et al., 1992) in a variety of endpoints, including antinociception in mice and rats. Porreca and coworkers, using the mouse tail-immersion test, have confirmed that δ receptor agonists at sub-antinociceptive doses increase antinociceptive responses to μ opioid receptor agonists. Thus administration of the selective δ agonist DPDPE, at a dose that produces no antinociception, potentiates the analgesic activity of i.c.v. morphine (Porreca et al., 1992).

δ opioid receptor ligands modulatate the effects of μ opioid receptor agonists

Biphalin



Biphalin - one of the most potent opioids

The peptide biphalin, a dimeric enkephalin analog, shows equal affinity for both μ and δ receptors. Biphalin is bioactive and crosses the blood brain barrier but is not very stable. Biphalin has been shown to be one of the most potent opioids ever identified in eliciting antinociception after central administration in the mouse: its potency in the tail flick test was almost seven times greater than that of i.c.v. etorphine, at least two orders of magnitude greater than that of i.c.v. carfentanyl, sufentanyl or fentanyl, and three orders of magnitude greater than the antinociceptive potency of i.c.v. morphine or alfentanyl. Nevertheless, after i.t. administration it only produces a 60% maximal antinociceptive effect in the tail flick test, even at doses three orders of magnitude higher than those effective i.c.v., suggesting that it may in part act on the putative opioid receptor complex of physically or functionally interacting μ and δ opioid receptors (Horan et al., 1993).

Potential Clinical Applications of δ Opioid Agonists

Furthermore, studies with agonists at the δ opioid receptor uncovered that the δ opioid system is involved in many biological processes, and thus δ opioid based medications may have great therapeutic potential for the treatment of a variety of disorders (Coop and Rice, 2000).

- respiratory disorders

Those related to the respiratory apparatus are of special interest: DPDPE causes a dose dependent increase in foetal respiratory activity which was blocked by administration of naloxone (Cheng et al., 1992). These stimulatory effects of selective δ agonists may be of clinical value in treating respiratory disorders such as apnoea.

- gastrointestinal disorders

I.c.v. and i.t. administration of the δ opioid agonist deltorphin II in rodents inhibits diarrhea and colonic bead expulsion in a dose-dependent manner but does not delay small intestine transit time. δ agonists have been found to mediate mainly antisecretory effects and influence gut motility only marginally. These effects can be antagonized by pretreatment with NTI (Shook et al.1989).

- immunological disorders

DPDPE has been found to have marked *in vitro* immunostimulant activity in patients suffering from leprosy and tuberculosis, enhancing antigen-stimulated proliferation of peripheral blood mononuclear cells and T-cell rosetting. DPDPE has been found to enhance cytokine production by T-helper cells, IL-6 production by macrophages and NK cell activity in murine splenocytes, suggesting immunostimulatory activity at low *in vitro*

concentrations (Mazumder et al., 1993; House et al., 1996; Rogers et al., 2000).

- urinary incontinence

I.c.v. administration of DPDPE results in inhibition of spontaneous bladder contraction in the rat (Tsushima et al., 1993).

- hypoxia

In mice DPDPE enhances the hypoxic conditioning-induced increase in survival time. Furthermore, DPDPE also increases survival time in naive mice, independent of hypoxic conditioning. This effect can be blocked by BNTX but not by NTI, indicating that the mechanism of acute hypoxic adaptation involves an endogenous δ_1 opioid pathway. δ agonists therefore can be considered as promising therapeutic agents to reduce the morbidity and mortality associated with clinical hypoxia in settings such as drowning, head injury, apnoea and complicated childbirths. The mechanism of neuroprotection induced by activation of the δ opioid receptors seems to involve decreasing body temperature, thereby mimicking the natural acute adaptation to hypoxia (Kalivas and Stewart, 1991; Suzuki et al., 1994).

- cardioprotective effects

Selective δ agonists have been shown to exert potent cardioprotective effects in intact animals and cardiac myocytes via activation of $G_{i/o}$ proteins, protein kinase C, and ultimately the mitochondrial K_{ATP} channel (Warltier et al., 2000; Schultz et al., 2001).

Potential clinical applications of δ opioid antagonists

In addition to their use as pharmacological tools, selective δ opioid antagonists may have clinical potential in the treatment of a variety of disorders where endogenous opioids play a modulatory role, e.g. disorders of food intake, shock, constipation, mental disorders, CNS injury, alcoholism, drug addiction and immune function (Spetea et al., 2001). It is also worth mentioning that δ antagonists have been shown to possess an antitussive effects in rodents, thus indicating another possible clinical application for these compounds (Kamei et al., 1994).

Prevention of μ-opioid tolerance and physical dependence by δ antagonists

The development of acute tolerance and dependence evoked in mice by morphine can be suppressed by pretreatment with NTI. Multiple administration of either NTI or 5′NTII before and during chronic implantation with morphine pellets also substantially inhibits the development of morphine tolerance and dependence. These results suggest the use of δ antagonists to be useful for the prevention of opioid tolerance and physical dependence without compromising the antinociceptive potency of μ opioid receptor agonists (Abdelhamid et al., 1991).

Non-peptide δ ligands

Following a flurry of medicinal chemistry activity in the late 1980s, a number of non-peptide pharmacological tools selective for the δ opioid receptors, became available to challenge the pre-eminent position occupied by the existing peptide δ ligands. The first non-peptide δ antagonist NTI represented a breakthrough in this field that was followed by the discovery of several selective δ agonists - TAN-67, BW373U86 and SNC80 (Scheideler, 2000).

Rational design of non-peptide δ opioid ligands - the message address concept

Essential progress for the discovery of non-peptide δ ligands came from rational design: the message address concept proposed by Schwyzer in 1977 that subsequently was re-elaborated by Portoghese (Portoghese et al., 1988). This concept attributes the role of the opiate message to the N-terminal Tyr^1 present in the endogenous peptide δ ligands Leu-enkephalin and Met-enkephalin (Tyr-Gly-Gly-Phe-X), whereas the δ address resides in the amino acid sequence starting from Phe-Leu or Phe-Met respectively. The Gly-Gly sequence is interpreted as a spacer maintaining an adequate distance between Tyr and Phe. Based on this rationale, the first non-peptide selective δ opioid ligand naltrindole (NTI) was discovered (Portoghese et al., 1988).

NTI shows subnanomolar affinity for the δ opioid receptor along with a very potent antagonistic activity in the mouse vas deferens

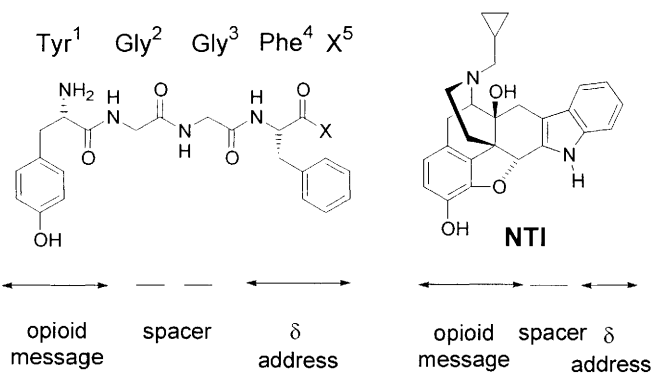


Figure 2: The message address concept.

NTI – starting point for the design of novel potent and selective δ opioid ligands

Portoghese and coworkers also considered other naltrindole derivatives substituted in position 6 or 7 with aryl, benzyl or aniline moieties to evaluate the effect of flexible aryl groups on selectivity, but obtained non-selective compounds only. This result demonstrates that the conformational flexibility of these aryl groups causes them to reside preferentially in regions of space that are not accepted by the δ opioid receptor (Portoghese et al., 1994). A more rigid compound however, 7-benzylidene-naltrindole (BNTX) proved to be a potent and highly

selective δ_1 antagonist ($K_i(\delta_1)$ = 0.1 nM; $K_i(\delta_2)$ = 10.8 nM; Portoghese et al., 1992). The isothiocyanate derivative 5′-NTII was designed as a non-equilibrium δ-selective antagonist (Portoghese et al., 1990).

Another approach to producing δ-selective antagonists is the fragmentation of the indolomorphinan framework. Resulting indolooctahydroisoquinoline derivatives were first disclosed by Toray. Eight months later, SmithKline Beecham disclosed the same compounds, but also derivatives featuring diverse five-membered ring spacers and different substitution patterns at the phenolic moiety (Dondio et al. (SmithKline Beecham), 1994).

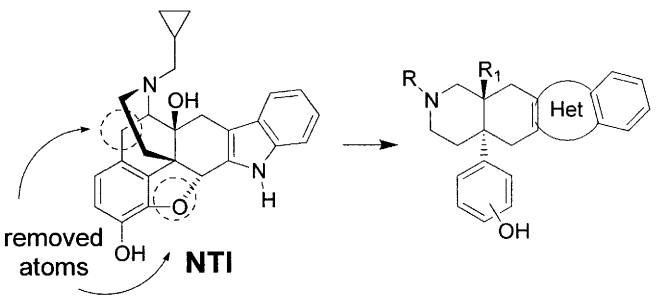
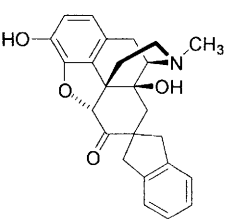


Figure 3: Fragmentation of the indolomorphinan framework.

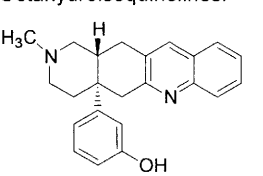
Substitution of N-cyclopropylmethyl (CPM) for N-methyl in general results in a shift from opioid antagonist to agonist. Applied to spiromorphinans the spiroindanyloxymorphone SIOM was discoverd, a potent δ ligand characterized as a full agonist in the MVD (IC_{50} 19 nM; antagonized by NTI). *In vivo* data however showed that SIOM possesses the unusual feature of acting as a δ antagonist at low dose and as a δ agonist at higher dose (Portoghese et al., 1993).



SIOM

Introduction of a six-membered ring into the octahydroisoquinoline series resulted in the discovery of a new class of potent and selective δ agonists (Dondio et al., 1995). Toray and SmithKline Beecham disclosed similar octahydroisoquinolines bearing an aromatic six-membered ring spacer. TAN-67 is the most interesting derivative to emerge from this (Nagase et al., 1994, 2001; Knapp et al., 1995). The pharmacological properties of the two enantiomers of TAN-67 were investigated by Dondio (Dondio et al., 1995). SB213698 (also named (-)-TAN-67 by Toray) is the active enantiomer displaying subnanomolar affinity for the δ receptor, being far less potent at μ and κ opioid receptors. SB213698 is a full δ agonist in the MVD (IC_{50} 62 nM). The corresponding (+)-

Octahydroisoquinolines:



TAN-67 – the prototype of a new class of potent and selective δ agonists

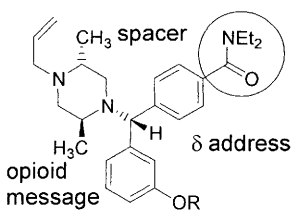
enantiomer is inactive in this test up to a concentration of 1 µM.

BW373U86: Extension of the message address concept to non-aromatic moieties

In 1992 Glaxo Wellcome disclosed BW373U86 (Lee et al., 1992), a new selective δ agonist structurally unrelated to the previously known δ ligands. BW373U86, although not very selective in binding assays, is the most potent δ agonist in isolated tissue bioassays displaying a remarkable µ/δ selectivity ratio of 715 and is reported to be a potent analgesic that does not produce physical dependence (Lee et al., 1992).

SNC80 dose dependently produces antinociception in the mouse warm water tail flick test (i.c.v., i.t. and i.p.)

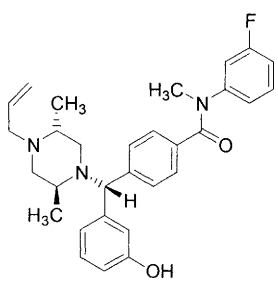
SNC80, the optically pure enantiomer of the methyl ether of BW373U86, exhibits a remarkable µ/δ selectivity in both receptor binding and *in vitro* bioassays (Calderon et al., 1994, 1997). In mice, SNC80 administered i.c.v., i.t. and i.p. dose dependently produces antinociception in the mouse warm water tail flick test with ED_{50} values of 105 and 69 nmol/mouse and 57 mg/kg, respectively. After i.c.v. administration SNC80 is active in the mouse hot plate test (ED_{50} 92 nmol/mouse). Studies with selective µ and δ opioid antagonists revealed that the antinociceptive activity elicited by SNC80 is mediated by the selective activation of the δ receptor. However, high doses caused brief, non-lethal seizures. Glaxo Wellcome has discontinued development of SNC80 for the treatment of pain. The project was terminated in 1995.

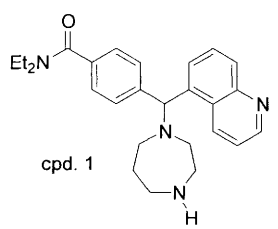


BW373U86 (R = H)
SNC80 (R = Me)

DPI-3290

DPI-3290 (Chang et al. (Ardent Pharmaceuticals), 1994), another compound from the piperazine series, is a mixed δ/µ opioid receptor agonist under development by Ardent Pharmaceuticals as an analgesic for the relief of severe intra- and post-operative pain. The i.v. formulation is licensed to Organon Teknika. In preclinical models DPI-3290 significantly reduces levels of respiratory depression, nausea and emesis of narcotic drugs. Phase II i.v. trials in severe pain in AIDS patients are underway. In Phase I trials DPI-3290 induced potent analgesia equal to morphine. A sublingual formulation is also in Phase I trials.

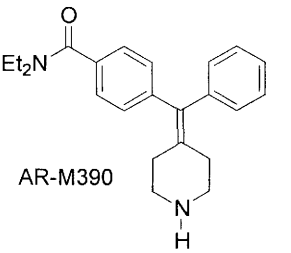




cpd. 1

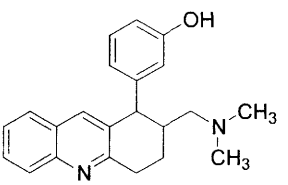
AstraZeneca is developing a series of selective non-peptidic δ opioid receptor agonists for the treatment of neuropathic pain. The compounds (e.g. cpd., 1) are in preclinical studies. *In vivo*, they are effective analgesics with negligible tolerance and dependence. They have parenteral and oral activity with suitable pharmacokinetics and pharmacodynamics.

The δ opioid receptor agonist AR-M390 is under development by AstraZeneca for the treatment of neuropathic pain. It is designed to overcome unwanted side-effects of opioid analgesics. In rats, it shows naltrindole-reversible antiallodynic activity but no efficacy against physiological pain. There is no seizure activity. AR-M390 has an oral bioavailability of 90 – 100% (221 ACS (San Diego), 2001, MEDI 185).

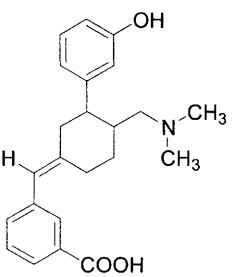


AR-M390

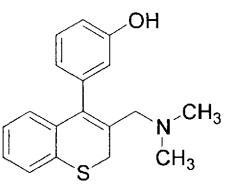
Other δ ligands that have been disclosed include aryl-tetrahydroacridinemethanamines (Pütz et al. (Grünenthal), 1998) like cpd. 2, aminomethylphenylcycohexanes (Pütz et al. (Grünenthal), 2000) like cpd. 3, but also compounds from a heterocyclic benzocycloalkene series (Zimmer et al. (Grünenthal), 1997) like cpd. 4. These compounds are claimed to be δ agonists and especially useful for the treatment of pain.



cpd. 2



cpd. 3



cpd. 4

In conclusion, the investigation of peptidic and non-peptidic tool compounds for the δ receptor have demonstrated the potential use of δ agonists and antagonists for a variety of clinical applications, especially for the treatment of pain. Full exploitation of this potential will however only be possible with ideal non-peptidic compounds having high potency, selectivity and, above all, optimal drug metabolism and pharmacodynamic characteristics.

Non-peptide δ compounds hold substantial potential for the mediation of a variety of pharmacological effects

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9.2 Opioid-Receptor-Like 1 (ORL1)

Bernd Sundermann and Corinna Maul

Introduction

Following the discovery of the orphan G-protein-coupled human receptor ORL1 (Mollereau et al., 1994), later classified as the fourth opioid receptor subtype in addition to μ, κ, and δ, its endogenous agonist, the heptadeka-peptide nociceptin (orphanin FQ) was identified simultaneously by Meunier et al. and Reinscheid et al. in 1995. Early studies indicated an important role for ORL1 in a range of physiological processes, implicating selective agonists or antagonists of this receptor to have potential for the treatment of pain and anxiety, the control of appetite, influencing memory and learning and other uses (Mogil et al., 1996a; Pomonis et al., 1996; Ueda et al., 1997; Jenck et al., 1997; Yu et al., 1997; Manabe et al., 1998; for reviews see: Meunier 1997, 2000; Darland et al. 1998, Barlocco et al. 2000; Mogil and Pasternak 2001). The pharmacology of nociceptin in particular has put ORL1 in the spotlight of pain research (see *Nociceptin*).

IUPHAR recommended nomenclature:

- **OP_1** (δ)
- **OP_2** (κ)
- **OP_3** (μ)
- **OP_4** (ORL1)

The nomenclature in this field is nonuniform. ORL1 designates the human receptor specifically; its equivalents in other species have different names. Use of the term OP_4 for all species has been recommended by IUPHAR; in a comprehensive review on ORL1 by Mogil and Pasternak (2001) the term NOP_1 is used. Herein the terms ORL1 and nociceptin will be used exclusively.

Some receptors similar to (or identical with) ORL1:

- rat: ROR-C, XOR, LC132
- mouse: MOR-C, KOR-3

Receptor Localization and Signaling

ORL1 is widely distributed in the CNS (Mollereau et al., 1994) and is also present in some peripheral tissues. The gene encoding the rat variant of ORL1 has been shown to give rise to several receptor forms by alternative splicing (Curró et al., 2001). Similar to other opioid receptors activation of ORL1 results in inhibition of cAMP synthesis, N-type voltage-gated calcium channels and neurotransmitter release as well as activation of inwardly rectifying potassium channels (review: Ronzoni et al., 2001).

In general the classical opioid ligands, e.g. morphine and fentanyl, do not bind to ORL1. Known exceptions include the μ-selective nonpeptidic ORL1 agonists buprenorphine (Wnendt et al., 1999), lofentanil and etorphine (Butour et al., 1997). Naloxone benzoylhydrazone (NalBzOH) has been found to be a non-selective ORL1 antagonist with moderate affinity (Ozaki et al., 2000b). In addition to nociceptin as a peptide agonist compound and certain

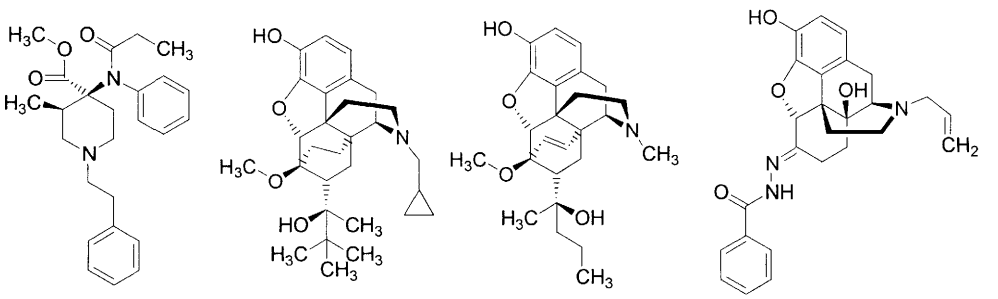
Table 1: Ligands for ORL1.

receptor affinity	ORL1 Ki/nM	μ Ki/nM
bupren-orphine	8.4	0.51
lofentanil	24	0.023
etorphine	530	0.18

(Thomsen and Hohlweg, 2000)

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other peptides (Topham et al., 1998, Bigoni et al., 2000, Ronzoni et al., 2001), drug discovery projects aimed at the identification of potent and selective nonpeptidic ligands for ORL1 have identified antagonists from two different structural classes (J-113397, JTC-801) and some closely structurally related agonists – spiropiperidines such as Ro 64-6198 and NNC 63-0532 – which can be used today to elaborate on the physiological role of ORL1 in preclinical studies (Ronzoni et al., 2001; Thomsen 2001; see *ORL1 Antagonists* and *ORL1 Agonists*).



Lofentanil Buprenorphine Etorphine NalBzOH

Figure 1: Non-selective ORL1 ligands.

Nociceptin (Orphanin FQ)

Nociceptin:
FGGFTGARKSARKLANQ
[170713-75-4]

The heptadecapeptide nociceptin has some structural similarity to dynorphin A (YGGFLRRIRPKLKWDNQ; Darland et al., 1998), but bears an N-terminal phenylalanine (F) instead of tyrosine (Y) which is essential for the activation of classical opioid receptors (μ, κ, δ). It was named nociceptin (NC) by Meunier et al. (1995) to indicate its initially observed hyperalgesic effect and is the endogenous agonist of ORL1.

Nociceptin's pain modulating properties

The role of nociceptin in pain modulation has been extensively studied, but opposite effects have been observed depending upon dose and route of administration (Barlocco et al., 2000):

Supraspinal anti-opioid effects of NC?

In mice NC has been reported to cause hyperalgesia in the hot-plate (Meunier et al., 1995) and tail-flick test (Reinscheid et al., 1995) after i.c.v. administration, but these observations may rather be due to a reduction of opioid-mediated stress-induced analgesia (attributable to the i.c.v. route of administration) than to a genuine hyperalgesic effect of NC (Mogil et al., 1996a). This anti-opioid function of supraspinal NC has been shown to antagonize antinociception produced by opioid agonists (Mogil et al., 1996b). On the other hand Rossi et al.,

(1996) have reported naloxone-sensitive analgesia after i.c.v. administration of NC in mice.

Spinal pro- and antinociception by NC?

After intrathecal (i.t.) administration of NC in rats hyperalgesia (Okuda-Ashitaka et al., 1996) was observed as well as naloxone- or naltrexone-reversible antinociception potentially caused by NC-induced release of endogenous opioids (King et al., 1997; Jhamandas et al., 1998). These bidirectional effects may be dose dependent with antinociception being predominant at higher doses (Tian et al., 1997). In mice both Reinscheid et al. (1995) and Grisel et al. (1996) did not observe antinociception but paralysis after i.t. administration of NC. Jhamandas et al., (1998) report an opioid-like antinociceptive effect of intrathecal NC as well as antagonism of morphine-induced antinociception. These and further contradicting early reports on the pharmacology of NC have been reviewed by Zaki and Evans (1998). Contradictory finding have also been reported with knockout mice (review: Mogil and Pasternak 2001).

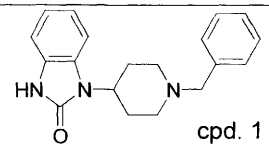
Potent and selective ORL1 ligands as potential analgesics

Keeping in mind the peptidic nature of NC, two basic conclusions can be drawn: ORL1 and NC do play an important role in pain transmission and thorough investigations of potent and selective small molecule ORL1 agonists and antagonists are needed to elucidate whether a potential new analgesic should block or activate the ORL1 receptor.

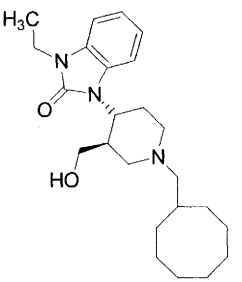
ORL1 Antagonists

The first potent and selective nonpeptidic ORL1 antagonist was described by Kawamoto et al. (1999, 2001; (Banyu Pharmaceutical Co.), 2000). J-113397 is the result of a lead optimization effort based on a screening hit (cpd. 1) with submicromolar affinity for ORL1.

Table 2: Opioid receptor affinities of J-113397 and related compounds.

receptor affinity (Ki/nM)	ORL1	μ	κ	δ
 cpd. 1	200	1700	110	>10000
J-113397	2.3	2200	1400	>10000
ent-J-113397	820	3300	2600	>10000

J-113397

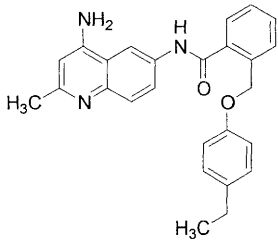


[256640-45-6] 1-[(3*R*,4*R*)1-Cyclooctylmethyl-3-hydroxymethyl-piperidin-4-yl]-3-ethyl-1,3-dihydro-benzoimidazol-2-one, $C_{24}H_{37}N_3O_2$, M_r 399.57

An alternative to the initial 11-step synthesis of J-113397 with improved yield was developed by De Risi et al. (2001)

Pharmacological investigations have proven J-113397 to be the most potent ORL1 antagonist known today (Ozaki et al., 2000a, b; Bigoni et al., 2000; Ichikawa et al., 2001). J-113397 is reported to be active in the formalin test, but inactive against pain responses to thermal and mechanical stimuli (Okuda et al., 2000).

JTC-801

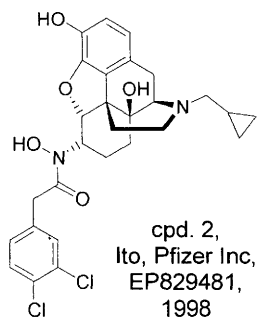


[244218-51-7] N-(8-Amino-6-methyl-naphthalen-2-yl)-2-(4-ethyl-phenoxymethyl)-benzamide, $C_{27}H_{26}N_2O_2$, M_r 410.20

The achiral ORL1 antagonist JTC-801 again is a result of lead optimisation efforts, in this case starting from a 4-aminoquinoline derivative that has not been disclosed. In pharmacological evaluations JTC-801 has been reported to antagonize nociceptin-induced allodynia and to show antinociceptive properties *in vivo* (mouse hot plate and rat formalin test; Shinkai et al. (Japan Tabacco), 1999; Shinkai et al., 2000; Yamada et al., 2002). The potential of JTC-801 as a novel type of analgesic is reported to be under clinical evaluation (Japan Tabacco web page).

Table 3: Opioid receptor affinities of JTC-801.

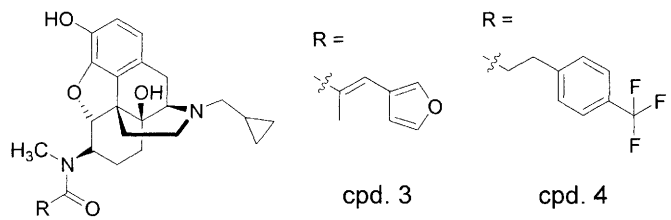
receptor affinity (Ki/nM)	ORL1	μ	κ	δ
JTC-801	8.1	103	1060	8650



cpd. 2, Ito, Pfizer Inc, EP829481, 1998

Further ORL1 antagonists include a series of NalBzOH analogs where the benzoylhydrazone moiety is replaced by hydroxylamides (cpd. 2). These compounds are also claimed to have agonistic activity at μ, κ and δ receptors.

Similar compounds have been disclosed by Toray Industries (Nagase et al., JP2000053572). Cpd. 3 and 4 are specifically claimed to be ORL1 antagonists:



cpd. 3 cpd. 4

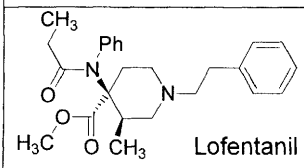
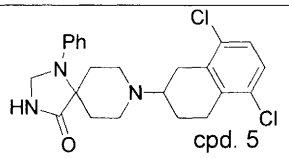
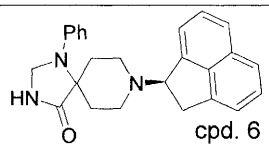
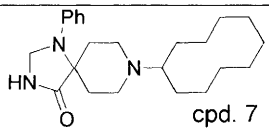
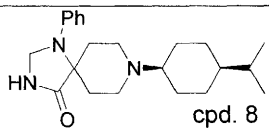
Figure 2: ORL-1 antagonists.

ORL1 Agonists

High throughput screening at Roche revealed cpd. 5, bearing some resemblance to lofentanil (see Introduction) and having pronounced affinity for ORL1. Optimization efforts led to the spiropiperidine cpd. 6 with 10-fold selectivity for ORL1 over μ and ultimately gave rise to the discovery of Ro 64-6198 (Wichmann et al., 1999; Röver et

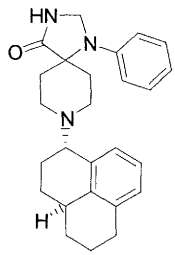
al., 2000; Adam et al. (Roche), 1998, 1999, 2000; Cesura et al. (Roche), 2000).

Table 4: Opioid receptor affinities of selected ORL-1 ligands.

receptor affinity (Ki/nM)	ORL1	μ	κ	δ
 Lofentanil	25	0.13	5.0	0.79
 cpd. 5	5.0	7.9	40	630
 cpd. 6	0.25	4.0	20	100
 cpd. 7	0.082	0.63	2.0	50
 cpd. 8	0.079	3.2	25	250
Ro 64-6198	0.40	50	79	>1000

Ro 64-6198 has 100-fold selectivity for ORL1 and thus is the most selective small molecule ORL1 agonist known today. Oral bioavailability is poor (4 %), but the compound crosses the blood-brain barrier. Ro 64-6198 is reported to be in clinical trials for the treatment of anxiety. At anxiolytic doses (~1 mg/kg) Ro 64-6198 has no effect on the perception of acute (tail-flick) or inflammatory pain in rats, at higher doses severe neurological side-effects dominate (Jenck et al., 2000; Wichmann et al., 2000; Higgins et al., 2001). Since no neurological impairment was observed in ORL1 knockout mice treated with Ro64-6198, these effects may well pose a general limitation to the clinical use of ORL1 agonists.

Ro 64-6198



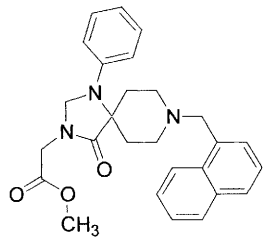
[280783-56-4] 8-[(1*S*,3a*S*)-2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl]-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one, $C_{26}H_{31}N_3O$, M_r 401.54

In this series of spiro-piperidines selectivity was achieved by replacement of the initial substituted tetra-hydronaphthalene moiety (cpd. 5) by acenaphthenyl (10-fold; cpd. 6) and finally by hexahydro-1H-phenalene (100-fold; Ro 64-6198)

The cyclodecyl- and *cis*-4-isopropylcyclohexyl derivatives (cpds. 7 and 8) are the most potent, but only moderately selective small molecule ORL1 agonists known today

In vitro characterization of Ro 64-6198: (Dautzenberg et al., 2001; Rizzi et al., 2001)

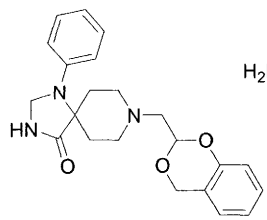
NNC 63-0532



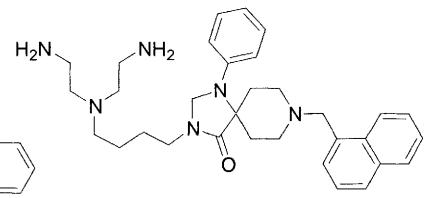
Watson et al.,
Novo Nordisk, WO9959997

[250685-44-0] (8-Naphthalen-1-ylmethyl-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl)-acetic acid methyl ester, $C_{27}H_{29}N_3O_3$, M_r 443.54

At Novo Nordisk, a 3D search performed on lofentanil predicted that the 5-HT_{1A} agonist spiroxatrine was also an ORL1 ligand. Actually spiroxatrine was found to have moderate affinity for ORL1 (Ki 118 nM). A lead optimization effort led to the potent ORL1 agonist NNC 63-0532 (Thomsen and Hohlweg, 2000). While its oral bioavailability (20%) is reported to be considerably improved with respect to Ro 64-6198 (4%), *in vivo* studies suggest NNC 63-0532 to be not sufficiently selective for ORL1 (Ki 7.3 nM) over μ (Ki 140 nM). Further synthetic studies have led to more selective compounds (cpd. 9), but in contrast to NNC 63-0532 these do not cross the blood-brain barrier (Thomsen, 2001).



Spiroxatrine

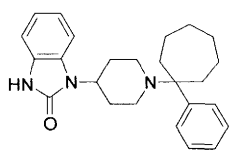


cpd. 9, Hohlweg et al.,
Novo Nordisk, WO0136418

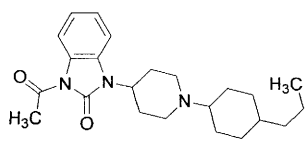
Scheme 3: ORL1 ligands discovered by Novo Nordisk.

Other Compounds

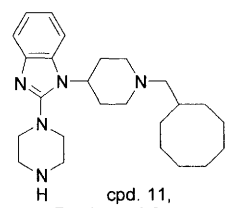
Other ORL1 ligands that have been disclosed with little details include several benzimidazoles (cpds. 10-12), but also compounds from other structural classes (cpds. 13-16). Some of these compounds are claimed to be ORL1 agonists and especially useful for the treatment of pain.



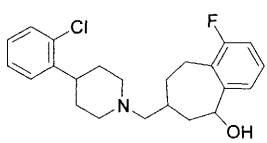
cpd. 10,
Ito et al., Pfizer Inc.,
WO9936421



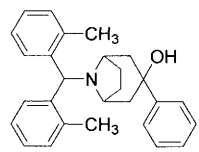
cpd. 12, Kyle et al.,
Euroceltique, WO0139775



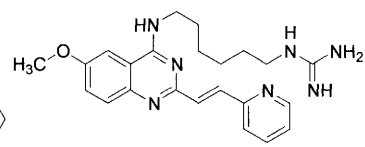
cpd. 11,
Fumitaka, Pfizer Inc.,
US6340681, 2001



cpd. 13, Barlocco et al.,
SmithKline Beecham, WO0183454



cpd. 15, Tulshian et al.,
Schering Corp., WO0006545



cpd. 16, Okana and Mori,
Nippon Shinyaku, WO0172710

Scheme 4: Further ORL-1 ligands.

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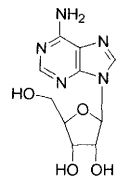
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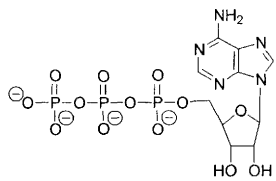
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10 Adenosine

Corinna Maul, Helmut Buschmann and Bernd Sundermann



Adenosine



ATP

In the 20 years since Daly reported the potential of adenosine receptors as drug targets (Daly, 1982), considerable advances have been made in the field of purinergic receptor-related research. Although a range of neurotransmitters is known, today there is no doubt that adenosine and adenosine 5'-triphosphate (ATP) also play an important role in the process of cell to cell communication. This function leads to multiple potential indications for research on adenosine and ATP, e.g. neurodegeneration or cardiovascular diseases, but here the focus lies on adenosine's role in pain (see also review by Salter and Sollevi, 2001).

Transmission of somatosensory information is normally initiated in the periphery through stimulation of endings of primary afferent neurons encoding information by an action potential discharge which is propargated into the central nervous system (CNS). The first level of central processing for most somatosensory information is the dorsal horn of the spinal cord or the homologous region of the trigeminal nucleus. The perception of this information as pain depends, among other things, on the actions and interactions of numerous neurotransmitter/neuromodulator systems at peripheral and central sites. Multiple lines of evidence support roles for adenosine and adenosine-5'-triphosphate (ATP) in the transmission of sensory information in the periphery and the dorsal horn (Salter and Sollevi, 2001).

Adenosine nucleosides and nucleotides exert a variety of effects through the activation of specific membrane receptors which are generally referred to as purinoreceptors. Burnstock defined two major classes of purinoreceptors named P1 and P2 (Burnstock, 1978). It was found that P1 purinoreceptors (adenosine receptors) are more responsive to adenosine. Adenosine receptors are the only extracellular nucleoside membrane receptors that have been described so far. P2 purinoreceptors (ATP-receptors) are more responsive to ATP and ADP as physiological agonists (Müller, 1996; Baraldi et al., 1999).

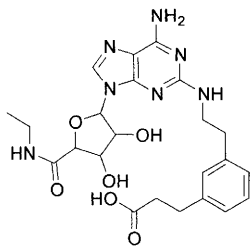
P1 receptors = adenosine receptors

P2-receptors = ATP-receptors

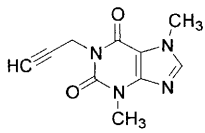
Today four subtypes of adenosine receptors - A_1, A_{2a}, A_{2b} and A_3 - are known, all of which are members of the G-protein coupled receptor (GPCR) family. While A_{2a} and A_{2b} receptors stimulate adenylyl cyclase and consequently lead to an increase of cAMP levels, A_1 and A_3 receptors produce the opposite effect upon activation.

Four adenosine receptor subtypes: A_1, A_{2a}, A_{2b} and A_3

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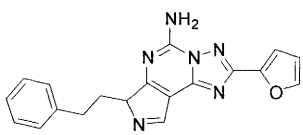


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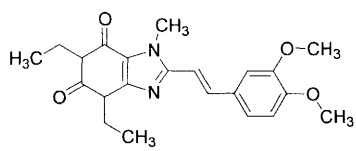


DMPX

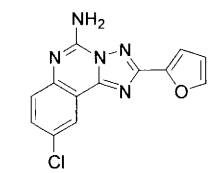
The peripheral actions of adenosine itself have been found to be pro- or antinociceptive in behavioral tests. In human subjects pain is evoked when adenosine is administered locally, e.g. into the coronary artery (Lagerquist et al., 1990). Algogenic or pronociceptive effects have also been seen in animal models (Karlsten et al., 1992). Pharmacological studies indicate that the pro-nociceptive effects of peripherally administered adenosine are mediated by interaction with A_2-like adenosine receptors. The A_{2A}-selective agonist CGS 21680 enhances formalin-induced nociceptive behavior only during the latter phase of the test, while the low affinity A2-selective antagonist DMPX has the opposite effect (Doak and Sawynok, 1995). A_{2A}-selective antagonists (Fig. 1) are well studied in neuroprotective indications (Phillis, 2002) and there are (or have been) compounds in clinical trials which makes them the most important class of potential future drugs influencing P1 receptors, and some of them are claimed to be useful against pain (Gillespie et al. (Vernalis Research)), while A_{2A} agonists are reported to be useful against inflammatory diseases (Chan et al. (Glaxo Group), 1999, Linden et al. (University of Virginia), 2000). A_{2B} receptors have not been investigated as thoroughly as A_{2A} receptors and their role in pain management has not yet been elucidated.



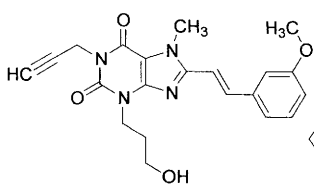
SCH-58261, neuroprotective, A_1 287 nM, A_{2A} 0,6 nM (human) (Schering-Plough), for analogs see Baraldi et al., 2002



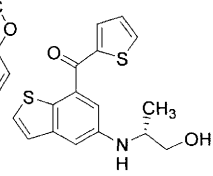
KW-6002, Phase II Parkinson, A_1 580 nM, A_{2A} 13 nM (rat) (Kyowa Hakko)



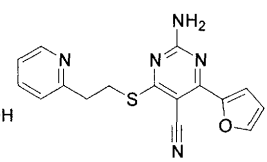
CGS 15943, discontinued, vasoprotectant , A_1 3,5 nM, A_{2A} 0,4 nM (human) (Novartis)



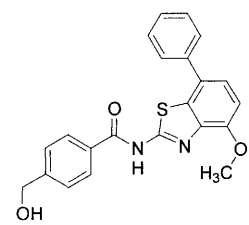
MSX-2, A_1 2500 nM, A_{2A} 8 nM (human) (Sauer et al., 2000)



A_{2A} 2 nM (Gillespie et al., Vernalis Res., WO 0102409)



A_{2A} 0,8 nM (Borroni et al.,Hoffmann La Roche, WO 0162233)



A_{2A} (Alanine et al., Hoffmann La Roche, WO 0197786)

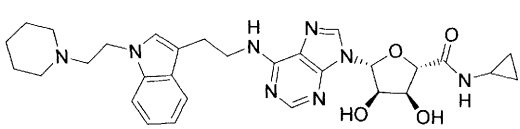
Figure 1: A_{2A} receptor antagonists

Additional antinociceptive potential of adenosine may arise from its antiinflammatory effects. These effects have been attributed to peripheral activation of A_2-like receptors which, among other actions, inhibits neutrophil adhesion and prevents the secretion of proinflammatory cytokines. Thus, while the activation of A_2 receptors might be involved in pronociception, antiinflammatory effects which may lead to diminished post-inflammatory pain have also been observed.

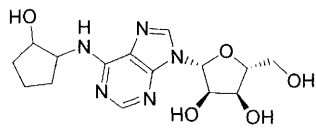
The A_1 receptor is distributed preferentially in most regions of the CNS, where adenosine acts as a neuromodulator inhibiting the release of neurotransmitters via prejunctional A_1 receptors. In electrophysiological recordings from DRG cell bodies adenosine has been shown to inhibit high voltage-gated Ca^{2+} currents, an effect mediated mainly through the activation of A_1 receptors. Presynaptic inhibitory effects of adenosine in the CNS are well known and may suppress Ca^{2+} currents as well as transmitter release processes not dependent upon Ca^{2+} influx (Dolphin et al., 1986; Macdonald et al., 1986).

Evidence for A_1 receptor agonists to be analgesics

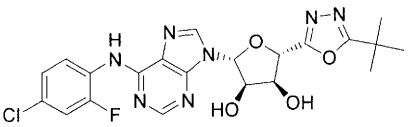
In contrast to the local algogenic effects of adenosine, systemic administration of adenosine at low dosage has been shown to induce analgesia in humans. The activation of peripheral A_1 adenosine receptors induces antinociception in animal models of inflammatory or neuropathic pain. Tissue adenosine levels are elevated in conditions of ischemia and inflammation. The peripheral administration of A_1 antagonists increases pain behavior in nociceptive tests while A_1 agonists can produce antinociception, so it has been suggested that adenosine may activate peripheral A_1 receptors which then participate in reducing post-inflammatory pain. Some A_1 receptor agonists, all of them adenosine analogs with an intact sugar moiety, have proven to be analgesics. The adenine binding site at the A1 receptor has been investigated intensively (IJzerman et al., 1995; Rivkees et al., 1999).



UP202-32, analgesic, no development reported since 1997 (BMS)



GR 79236, analgesic, discontinued after phase 1 (Glaxo Wellcome)

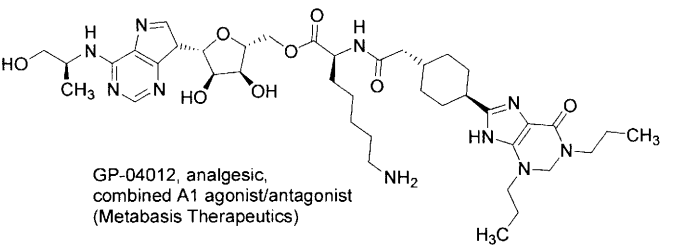


Glaxo Group WO99/67262, analgesic, several patent applications for combinations with other analgesics recently published (Bountra et al 2001)

Figure 2: A_1 receptor agonists.

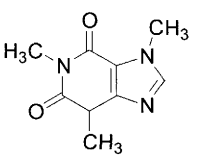
Combination of A_1 agonistic and antagonistic action in one molecule

Some developments have been made towards the combination of A_1 agonist and antagonist structures in one molecule and these molecules were reported to have analgesic properties (Reddy et al., 1998), but further progress has not yet been published.

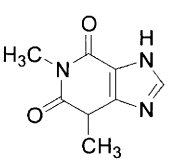


GP-04012, analgesic, combined A1 agonist/antagonist (Metabasis Therapeutics)

Figure 3: Combination of A_1 receptor agonist and antagonist.

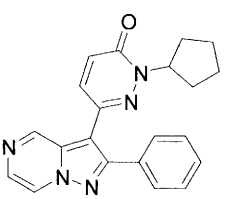


caffeine



theophylline

The most prominent mixed A_1/A_2 receptor antagonist is caffeine which is currently used clinically as an adjunctive analgesic in combination with acetaminophen and other NSAIDs. Its adjuvant activity has been demonstrated in both clinical and preclinical studies and it is extremely successful on the OTC market. Other mixed A_1/A_2 receptor antagonist are currently being investigated in preclinical studies (Akahane et al. (Fujisawa Pharm Co), 2001).



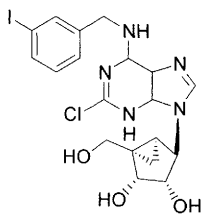
A_1 0,1nM, A_{2a} 0,84 nM
(Akahane et al., Fujisawa, WO 0140230)

Figure 4: A_1/A_{2A} antagonist.

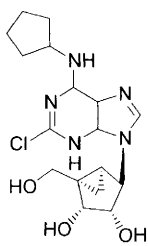
The fourth receptor of the adenosine receptor family - A_3 - has not yet been thoroughly investigated. The activation of A_3 receptors is not antagonized by xanthines like theophylline. The A_3 receptor shows low sequence homology between species: while the amino acid sequence homology of other adenosine receptors is usually in the range of 85% up to >90%, homology of the rat and human A_3 receptor is only about 74%. This may cause problems in drug development because data transfer from animal models to humans might be difficult.

There is some evidence that adenosine also participates in modulating peripheral somatosensory function through A_3 receptors on immune cells. A predominant response to the activation of A_3 receptors is degranulation of mast cells causing the release of multiple proinflammatory mediators (IL-6/IL-10/IL-12). Further involvement of A_3 receptors in pain and inflammation may be a result of adenosine-mediated inhibition of the release of tumor necrosis factor α (TNF-α), a proinflammatory cytokine produced by monocytes and macrophages.

The A_1 and A_3 receptor agonists known today are adenosine analogs and the sugar moiety has usually only minor modifications at 3'- and 5'-position. Moreover, there are various examples of N^6-substituted A_1-selective adenosine analogs reported in the literature. Exceptions which do not possess a sugar moiety are methanocarbocyclic analogs of adenosine where the A_1 and A_3 receptor agonists are claimed to be useful against pain (Jacobsen et al., 2001).

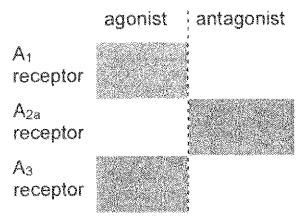


K_i values [nM]:
A_1 141, A_{2A} 732, A_3 2,2



K_i values [nM]:
A_1 8,7, A_{2A} 3390, A_3 466

Figure 5: A_3/A_1 receptor agonists.



Functionality at P1 receptors of compounds useful for pain management (grey)

Indirect mechanisms influencing adenosine levels

Adenosine levels can also be influenced through indirect mechanisms mediated by adenosine kinase, adenosine deaminase and the nucleoside transport protein.

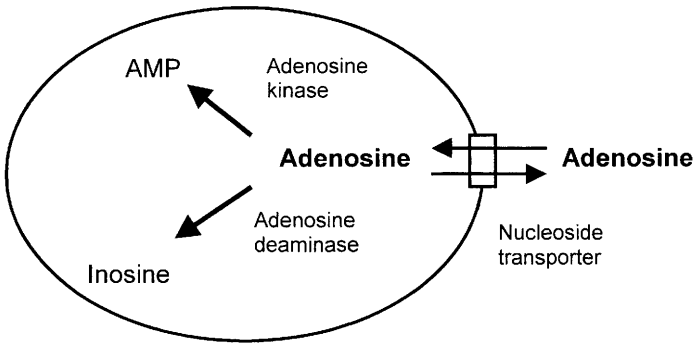
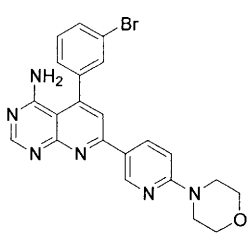


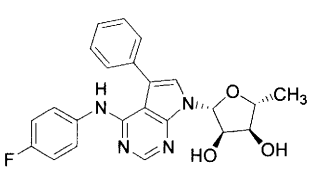
Figure 6: Indirect mechanisms for influencing adenosine levels.

Adenosine kinase

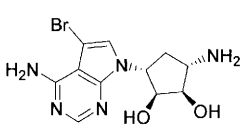
Among these potential pain targets only adenosine kinase has been intensively investigated preclinically as well as clinically. Adenosine kinase is a cytosolic enzyme catalyzing the phosphorylation of adenosine to adenosine-5'-monophosphate (AMP) and thus plays an important role in adenosine metabolism. Inhibition of intracellular adenosine kinase decreases the cellular reuptake of adenosine, thereby increasing the concentration of adenosine in the extracellular compartement. Antinociceptive effects of adenosine kinase inhibitors have been demonstrated pharmacologically with 5-iodotubercidin and 5'-deoxy-5-iodotubercidin which are efficacious in animal models of acute pain. Some adenosine kinase inhibitors with adenosine-related structure have been reported to be in clinical trials as potential pain killers. Non-nucleoside adenosine kinase inhibitors are also known: ABT-702 is reported not only to produce antinociception in both acute and chronic animal models of pain but furthermore can be administered by the oral route.



ABT-702, analgesic, preclinical (Abbott)



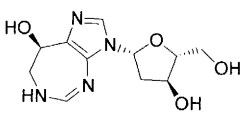
GP-3269, analgesic, discontinued after phase I (Metabasis Therapeutics)



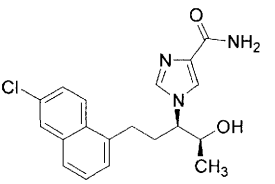
A-286501, analgesic, preclinical (Abbott) (Jarvis et al. 2002)

Figure 7: Adenosine kinase inhibitors

Severe side-effects (hemorrhages in rats and dogs brain) have been reported after systemic application of several selected adenosine kinase inhibitors (Erion, 2000). Nevertheless, ABT-702 in particular is still reported to be in preclinical development.



2'-deoxycoformycin



Tsuji et al.,
Fujisawa Pharmaceuticals,
WO 0153271

Figure 8: Adenosine deaminase inhibitors

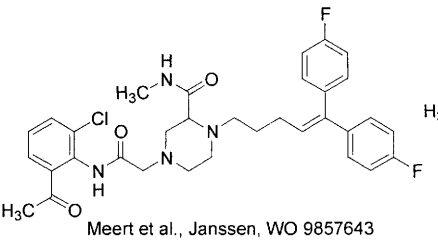
Adenosine deaminase: potential target for chronic pain

Adenosine deaminase catalyzes the hydrolytic deamination of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine respectively. Inhibition of adenosine deaminase leads to an accumulation of its substrates which results in adenosine receptor-mediated effects. Most inhibitors are not reported to have antinociceptive properties, but 2'-deoxycoformycin was proven to have an inhibitory effect on pain transmission (Poon and Sawynok, 1999), and Fujisawa Pharmaceuticals claim adenosine deaminase inhibitors to be active against chronic pain.

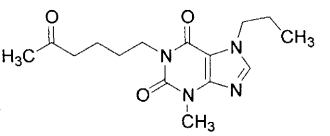
Nucleoside transport inhibitors, active in neuropathic pain?

An increase of intracellular adenosine levels can also be achieved by inhibition of nucleoside transport proteins. Mammalian nucleoside transport processes can be classified into two types on the basis of their thermodynamic properties. These classes are the concentrative, Na^+-dependent transport processes and the equilibrative, Na^+-independent processes. The corresponding transporters are called CNTs (concentrative nucleoside transporters) and ENTs (equilibrative nucleoside transporters) (Pastor-Anglada and Baldwin, 2001).

Some adenosine nucleoside transport inhibitors have been reported to be useful against pain, especially neuropathic pain (animal models) (Meert and van Belle (Janssen Pharm), 1998; Deleo and Schubert, 2000).



Meert et al., Janssen, WO 9857643
active in chronic pain

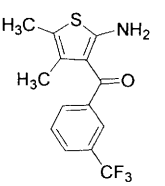


Propentofylline, neuroprotective, has
proven to be active in neuropathic pain

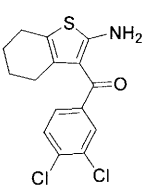
Figure 9: Nucleoside transport inhibitors.

Enhancement of the binding of agonists to the receptor by allosteric enhancers

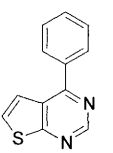
A further possibility to modulate physiological adenosine or agonistic effects could be allosteric enhancement. Allosteric enhancers described so far enhance the binding of several agonists by up to two-fold so they bind more efficiently, and lower concentrations of the agonist are needed (Bruns et al., 1990). Some of the compounds are claimed to have analgesic properties (Baraldi, 1999) or reported to be active in neuropathic pain (Li et al., 2002).



PD 81,723
(Parke-Davis)



van der Klein et al. 1999



Baraldi, US5939432
(1999)

Figure 10: Allosteric enhancers for the A1 receptor.

Clinical investigations to date have focussed on effects of adenosine in experimentally-induced pain as well as clinical pain states. These studies have shown that adenosine administration reduces pain primarily in situations that involve enhanced excitability and nociceptive transmission in the CNS. Since centrally-mediated enhanced excitability is considered to be an important factor in chronic pain conditions, adenosine-induced pain relief in patients with neuropathic pain suggests that adenosine and adenosine analogs are of special importance for future research.

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11 P2 Receptors

Hagen-Heinrich Hennies, Corinna Maul and Bernd Sundermann

The physiological activity of adenine compounds has been under investigation for many years. Very early studies were published by Drury and Szent-Györgyi in 1929. Evidence first implicating ATP as an excitatory neurotransmitter in the somatosensory system came from studies by Holton and Holton who demonstrated that ATP is released from peripheral endings of primary sensory neurons (Holton and Holton, 1953; Holton, 1959). Then, in 1970, it was proposed that nonadrenergic, noncholinergic (NANC) nerves supplying the gut and bladder use ATP as a motor neurotransmitter (Burnstock et al., 1970; 1972a). In a pharmacological review, Burnstock introduced the term 'purinergic' and presented the first evidence for purinergic transmission in a wide variety of systems (Burnstock, 1972b).

The release of ATP in the central nervous system

The central release of ATP from dorsal horn synaptosomes was proven by White et al. (1985). Further studies (Sawynok et al., 1993) suggest that ATP can be released from central terminals of primary afferent neurons as well as from terminals of non-primary afferents within the dorsal horn and that ATP and GABA are co-transmitters at many synapses in the dorsal horn (Jo and Schlichter, 1999). After being released ATP acts on specific receptors, designated as P2 purinoreceptors, on the cell surface.

P2 nomenclature:

P2X ligand-gated ion channels (ionotropic receptors)

P2Y G-protein coupled receptors (metabotropic receptors)

The P2 receptor nomenclature was prompted by evidence that extracellular ATP works through two different transduction mechanisms, namely intrinsic ion channels and G-protein coupled receptors (Benham and Tsien, 1987; Dubyak, 1991). In 1994 it was formally suggested that P2 receptors should be divided into two groups termed P2X and P2Y according to whether they are ligand-gated ion channels (Fig. 1) or are coupled to G-proteins - metabotropic receptors belonging to the heptahelical superfamily (Abbracchio and Burnstock, 1994; Barnard et al., 1994; Fredholm et al., 1994).

As intensively reviewed by Ralevic and Burnstock (1998), studies on purinoceptors were accompanied by many pitfalls:

1. P2X receptors are multi-subunit receptors. They may exist as homomers or as heteromers; heteromers may have a different pharmacology in comparison with homomers
2. Cations can affect P2X channel activity very profoundly

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3. Ligands previously regarded to be selective for P2Y receptors were found to be active at P2X channels also
4. Ecto-nucleotidases can alter agonist potencies (for review of extracellular metabolism of ATP see Zimmermann, 2000)
5. Antagonists used as P2 receptor blockers were found to be non-selective. In addition they are able to modulate ecto-nucleotidase activity also

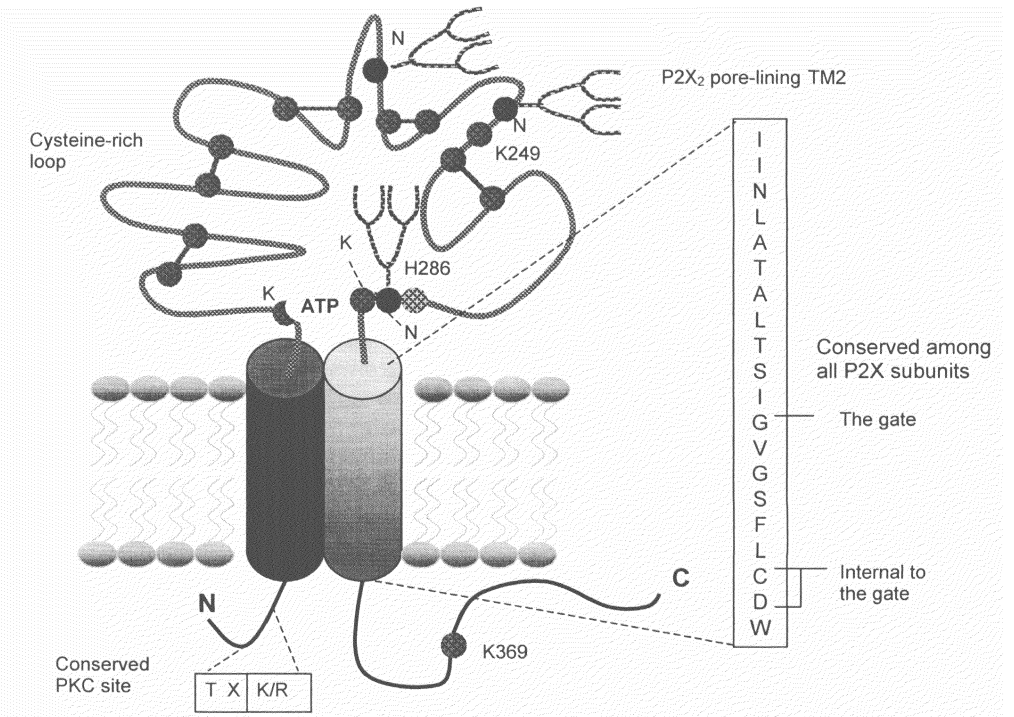
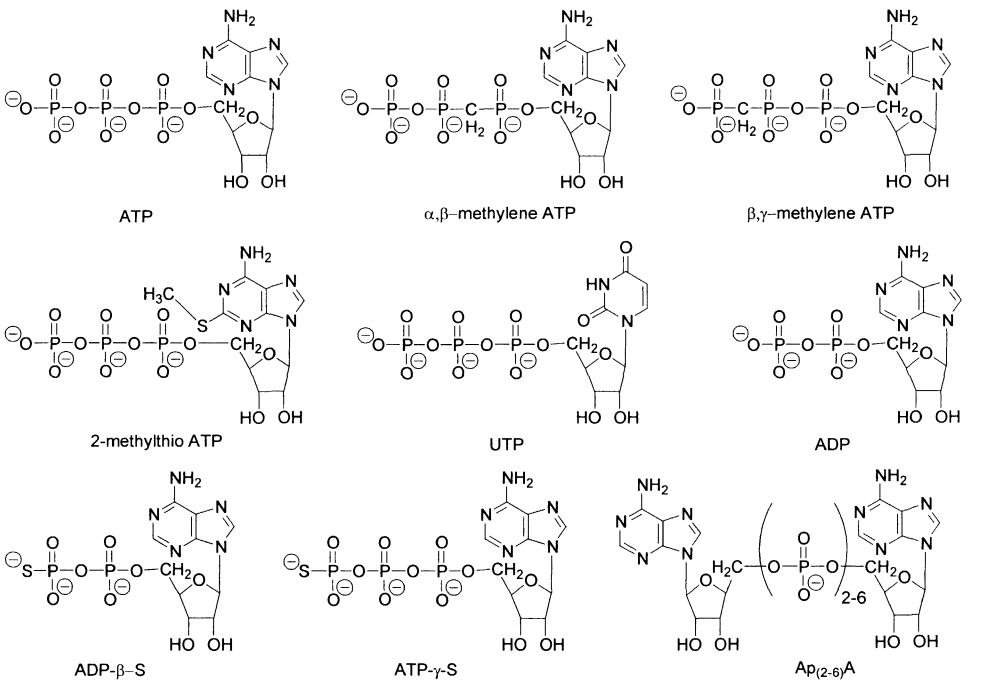


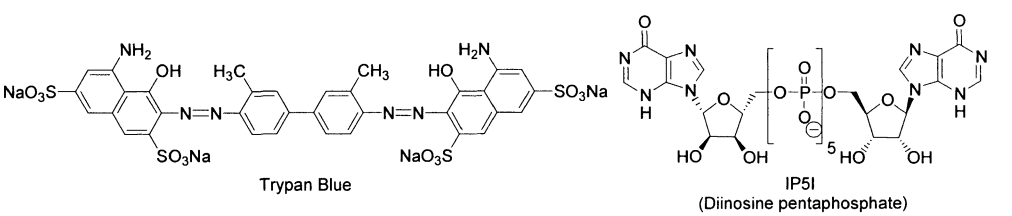
Figure 1: A representation of P2X subunit structure and function. P2X channels possess two transmembrane domains (TM); TM2 is believed to line the pore of the channel. The amino and carboxyl termini of the different P2X subunits are located intracellularly. The length of the amino termini is relatively constant among the different subunits and comprises about 20 - 30 residues. By contrast, the carboxy-terminal tails vary widely in length and range from 28 residues for $P2X_6$ to 242 for $P2X_7$. In the functional channel, the amino and carboxyl termini might be close to each other. P2X subunits have a conserved protein kinase C (PKC) site in the amino terminal tail, which is phosphorylated in $P2X_2$. Lysine residues extracellular to TM1 and TM2 contribute to the ATP-binding site, whereas K249 in $P2X_2$ forms a Schiff base with the antagonist PPADS. The extracellular loop of all P2X subunits contains 10 conserved cysteine residues. In addition, P2X subunits are glycosylated at three asparagine residues (N182, N239, N298). Finally, a histidine residue at 286 in human $P2X_4$ receptors contributes to H+ modulation, and the amino acids after splice site K369 determine desensitization in $P2X_2$ channels (adapted from Khakh, 2001).

The coexistence of different P2 receptors together with impure solutions caused by purine and pyrimidine degradation and interconversion as well as the lack of selective agonists and antagonists have led to some frustration in this field of research.

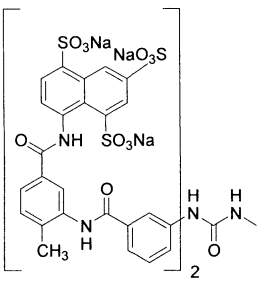
Recent reviews summarizing the functional properties of recombinant homomeric, recombinant heteromeric and native P2X receptors were published by Bianchi et al. (1999), Nörenberg and Illes (2000) and Khakh et al. (2001). In these publications the effects of the following agonists, antagonists (among others) as well as the ions Zn^{2+}, H^{+} and Ca^{2+} on specified P2X-subtypes were described:

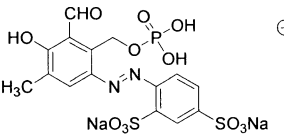


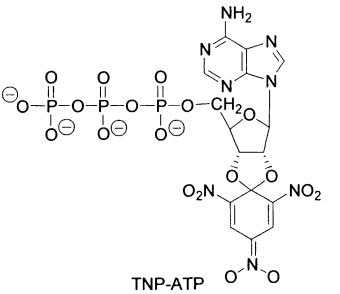
Scheme 1: P2X receptor agonists.

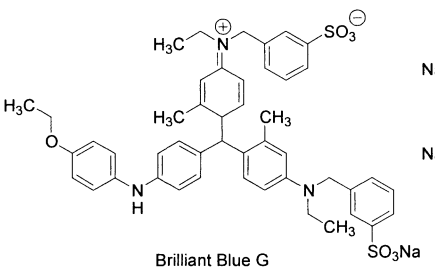


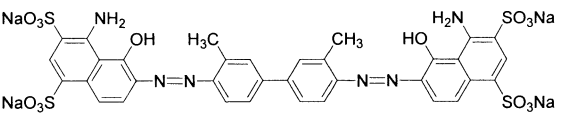
Scheme 2: P2X receptor antagonists.











Scheme 2 continued.

Peripherally administered, ATP has pro-nociceptive effects

In behavioral tests, the actions of peripherally-administered ATP are pro-nociceptive. These nociceptive responses have been suggested to be due to direct activation of peripheral nerve terminals (Illes and Nörenberg, 1993). ATP produces depolarization when applied to the cell bodies of primary afferent neurons located within the dorsal root ganglia (DRG) (Jahr and Jessell, 1983). The depolarizing effect of ATP results from the activation of a non-selective cation channel (Bean, 1990) and is blocked by P2 purinoreceptor antagonists (Tsuda et al., 1999), indicating that excitation is mediated via ionotropic P2X purinoreceptors.

$P2X_3$ is expressed in cell populations enriched in nociceptors

$P2X_{1-6}$ mRNA transcripts are expressed in sensory neurons of the dorsal root, nodose and trigeminal ganglia (Collo et al., 1996). Of these subunits one subtype, $P2X_3$, one of the seven P2X receptor subtypes known today (for overview see Alexander and Peters, 2000), has emerged as having a potentially important role in nociception because of its selective exprimation in cell populations enriched in nociceptors (Chen et al., 1995; Lewis et al. 1995, Tsuda et al., 1999, for minireview see Chizh et al., 2000). However, it is not clear if $P2X_3$ exists as a homomultimer or a heteromultimer with $P2X_2$ in sensory neurons *in vivo*. Evidence has been presented that capsaicin-sensitive, small DRG neurons may express

mainly the homomultimeric $P2X_3$ subunit showing co-localization with vanilloid VR-1 receptors, while capsaicin-insensitive, medium-sized neurons express the heteromultimeric $P2X_2/_3$ receptor. (Guo et al., 1999; Ueno et al., 1999).

As ATP seems to be involved in activating nociceptive sensory neurons through $P2X_3$-containing receptors, peripherally-acting antagonists selective for these receptors might be analgesics (Jarvis et al. (Abbott Laboratories), 2000). It is experimentally proven that pain induced by peripheral administration of ATP or the agonist α,β-methylene ATP is blocked by the non-selective P2 antagonists suramin and PPADS (Hamilton et al., 1999; Williams and Jarvis, 2000).

Pain induced by $P2X_3$ agonists is blocked by suramin and PPADS

Driessen et al. (1994) studied the effects of intrathecally applied P2 purinoceptor antagonists and agonists in the rat tail-flick test and rat formalin model. In the tail-flick test, the P2 antagonists suramin, Evans blue, Trypan blue and Reactive blue 2 (but not PPADS) caused moderate antinociception up to a doubling of the response latency. In contrast, the P2 agonists α,β-methylene ATP and 2-methylthio-ATP decreased the tail-flick latency up to 50%. In the formalin test, pretreatment with suramin 60 min prior to testing caused significant antinociception by decreasing the weighted pain intensity score up to 80 %.

Concerning human studies, only three reports have been reported (for review see Burnstock et al., 2000). Bleehen and Keele (1977) reported observations on the algogenic actions of adenosine compounds on blister base preparations. Coutts et al. (1981) injected ATP, ADP, AMP, adenosine, adenine and inosine intradermally. The area of erythema induced by the injection was delineated at 30 s. and again after a further 4.5 min when the size of the response was maximal. ATP, ADP and AMP evoked weal and flare responses in the skin in a dose-dependent manner. The rank order of potency was ATP > ADP > AMP; other metabolites were apparently inactive. Injections of ATP and high doses of ADP produced a sensation of persistent pain.

Pain studies on humans with ATP

In 2000 Hamilton et al. reported that ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia. The authors used iontophoresis to deliver ATP to the forearm skin of volunteers who rated the magnitude of the evoked pain on a visual analog scale. ATP consistently produced a modest burning pain, which began within 20 s. of starting iontophoresis and was maintained for several minutes. Persistent iontophoresis of ATP led to desensitization

within 12 min but recovery from this was almost complete 1 h later. The pain produced by ATP was dependent on capsaicin-sensitive neurons, since in skin treated repeatedly with topical capsaicin pain was reduced to less than 25% of that elicited on normal skin. Moreover, ATP iontophoresed into skin 24 h after solar simulated radiation resulted in double the pain rating of normal skin. The pain response to saline was not altered after UV irradiation. The authors conclude that ATP produces pain by activating capsaicin-sensitive nociceptive afferents when applied to the skin.

Regulatory role of $P2X_3$ receptors

Experiments with $P2X_3$-knockout mice confirmed that $P2X_3$ receptors have a significant regulatory role in persistent inflammatory pain (Souslova et al., 2000), but ATP-evoked nociceptive behavior in mice is only partially reduced by disruption of the $P2X_3$ gene.

The role of $P2Y_1$ in pain sensation

In a recent paper Tominaga et al. (2001) present evidence that $P2Y_1$ receptors may also be involved in pain sensation. An accompanying commentary to this paper is given by Premkumar (2001) offering a scheme (see Fig. 2) of known second-messenger pathways that possibly modulate the vanilloid receptor (VR). Tominaga et al. (2001) show that in human embryonic kidney (HEK293) cells, stimulation of the endogenous metabotropic purinergic receptor $P2Y_1$ enhances the sensitivity of the heterologously expressed VR1 to capsaicin, protons, and temperature in a protein kinase C (PKC)-dependent manner. To further substantiate these findings, the authors co-expressed M1 muscarinic acetylcholine receptors and VR1 and demonstrated that, when pretreated with acetylcholine, the VR response induced by capsaicin or protons was potentiated. Finally, a similar potentiation of VR response by ATP could be observed in neurons from dorsal root ganglia. The authors conclude that ATP-induced nociception is partly caused by a potentiation of the VR response.Thus $P2Y_1$ receptors may represent a fruitful target for the development of drugs against pain. $P2Y_1$ knock-out mice were shown to have defective platelet aggregation and increased resistance to thromboembolism suggesting that potentially analgesic $P2Y_1$ antagonists may have antithrombotic side-effects (Boeynaems et al., 2001).

ATP-induced nociception is partly caused by a potentiation of the VR response

In view of the early results which indicated that mainly phosphorylated ATP derivatives had activity at P2 receptors, the identification of selective agents for one of the P2 receptors seemed to cause problems because of the difficulty in preventing such molecules from being rapidly broken down in the ordinary course of ATP metabolism (Jacobsen et al., 1995; Zimmermann, 2000).

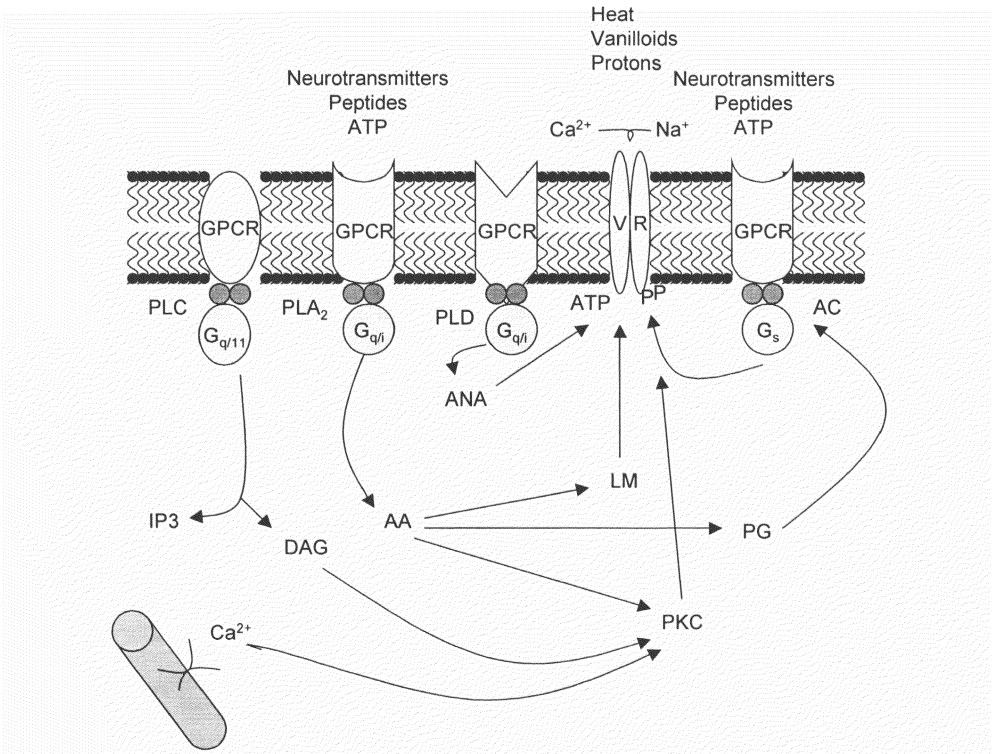


Figure 2: Known second-messenger pathways that modulate capsaicin or VRs. GPCR, G protein-coupled receptors; PLA_2, phospholipase A2; AC, adenylate cyclase; IP3, inositoltriphosphate; DAC diacylglycerol; LM, lipoxygenase metabolites; AA, arachidonic acid; ANA, anandamide; PG, prostaglandins; $G_{q/11}$, $G_{q/i}$, G_s, trimeric G-proteins (adapted from Premkumar, 2001)

The search for P2X receptor subtype selective ligands

Although a very reliable radioligand binding assay with use of a stable ATP analog (e.g. α, β-methylene ATP) and functional fluorescent assays suitable for a high throughput screening campaign are available, the search for a selective $P2X_3$ receptor antagonist has not been successful up to now. There are only few known compounds with agonistic or antagonistic activity (Lamprecht, 2000) for P2 receptors not selective for $P2X_3$ which can be used in assay characterization. Some ATP derivatives, e.g. 2', 3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) and diinosine pentaphosphate (IP_5I) are rather potent and selective antagonists of $P2X_1$ and $P2X_3$ receptors (the former, but not the latter compound, also blocks the heteromeric $P2X_2/P2X_3$ receptor, see Dunn et al., 2000); however, their *in vivo* stability is limited.

In conclusion, activation of certain types of P2 receptors by ATP seems to be an important factor in several pain states. $P2X_3$ receptors, and $P2Y_1$ receptors represent an attractive target for the treatment of pain. Further progress in this area is hampered by the lack of potent and selective antagonists with sufficient *in vivo* stability.

Addendum

In addition to pain, experiments with $P2X_3$ knock-out mice (Cockayne et al., 2000; Souslova et al., 2000) have revealed surprising results. Cockayne et al. found, that $P2X_3$-null mice exhibit a marked urinary bladder hyporeflexia, characterized by decreased voiding frequency and increased bladder capacity, but normal bladder pressure. Antagonists to $P2X_3$ may therefore have therapeutic potential in the treatment of disorders of urine storage and voiding such as overactive bladder (Cook and McCleskey, 2000; Burnstock, 2001).

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12 Natural and Synthetic Cannabinoids

Corinna Maul and Bernd Sundermann

For many thousand years *cannabis sativa* has been a valuable source of hemp fibre. The (ab)use of its psychoactive constituents has also been know in many cultures for a very long time. With the advent of superior alternative medications the medical use of cannabis extracts faded in the last century. Cannabis was removed from the US Pharmacopoeia in 1942 and from the British Pharmacopoeia in 1976 when it was classified as a drug with no therapeutic benefit.

Nevertheless and despite the widely illegal recreational use of cannabis the effects of cannabinoids on the brain still are under active investigation. The discovery of neuronal receptor proteins for cannabinoids and the existence of endogenous cannabinoid substances (Sullivan, 2000) are the most important milestones to date.

At least 60 bioactive compounds are contained in herbal cannabis. Δ^9-Tetrahydrocannabinol (Δ^9-THC) (Mechoulam and Gaoni, 1967), cannabidiol and cannabinol are the major psychoactive or adjuvant ingredients. Cannabinoids act through at least two different G-protein coupled receptors named CB_1 and CB_2 receptors.

Oral preparations of Δ^9-THC (dronabinol, marinol) and the synthetic structural analog nabilone are marketed as suppressants of nausea and vomiting provoked by antitumor agents. Furthermore Δ^9-THC is employed to stimulate the appetite of AIDS patients.

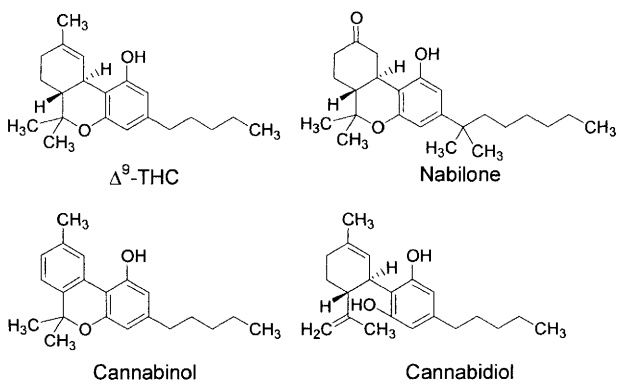


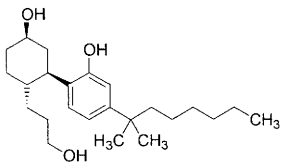
Figure 1: Structures of natural cannabinoids and nabilone.

In addition to these indications the pharmacology of cannabinoids promises a range of further potential applications. Cannabinoids have been shown to produce antinociception in animals and humans, so analgesia is

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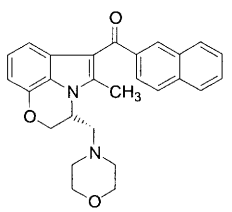
one of the important features of cannabinoids with therapeutic prospects (Segal, 1986; Fuentes et al., 1999).

Synthetic tool compounds:



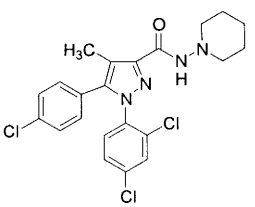
CP 55,940
(non-selective agonist)

One particular feature of plant-derived cannabinoids is their high lipid solubility, which indicates that limited gastrointestinal absorption and bioavailability are significant barriers to their development as therapeutics. For this reason cannabis is traditionally smoked, providing the most predictable and titratable route for administration. For therapeutic development pulmonary deliveries of cannabinoid aerosols are under investigation as an alternative.



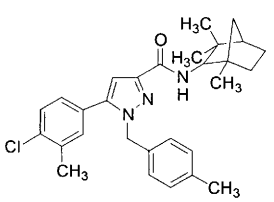
WIN 55,212-2
(moderately CB_2-selective agonist)

Besides the classical cannabinoids there are other structural classes with cannabinoid activity: the *non-classical cannabinoids* typified by CP-55,940 and the *aminoalkylindols* exemplified with WIN 55,212-2. The 1H-pyrazole-3-carboxylic acid amide derivatives SR 141716A (Rinaldi-Carmona et al., 1994) and SR 144528 (Rinaldi-Carmona et al., 1998) were discovered to be moderately selective cannabinoid receptor antagonists.



SR 141716A
(CB_1-selective antagonist)

The antinociceptive activity of cannabinoid receptor agonists has been widely investigated. Δ^9-THC has been most intensively studied. It exhibits antinociceptive activity in a wide range of animal models of acute pain (e.g. tail flick, hot plate) when administered orally, systemically or directly into brain or spinal cord. Δ^9-THC is also effective in models of inflammatory and neuropathic pain (overview: Pertwee, 2001). Other cannabinoids such as WIN 55,212 also show analgesic properties in acute and neuropathic pain models (Bridges et al. 2001). The antinociceptive effects could only be antagonized with the CB_1 selective antagonist SR 141716a and not with the CB_2 selective antagonist SR 144528. However, the motor effects of cannabinoids complicate interpretation of behavioral studies assessing motor reactions to noxious stimuli, but electrophysiological studies also support the behavioral findings. It was shown that cannabinoids selectively modulate the activity of nociceptive neurons in the spinal dorsal horn by actions at CB_1 receptors. This modulation represents a suppression of pain neurotransmission because the inhibitory effects are selective for pain-sensitive neurons and are observed with different modalities of noxious stimulation (Hohmann et al., 1999).



SR 144528
(CB_2-selective antagonist)

Cannabinoid receptors

CB_1 receptors are found in particularly high concentrations within the central nervous system, but also on some peripheral neurons as well as in certain non-neuronal tissues (Herkenham et al., 1990). CB_2 receptors mainly occur in immune cells where they can mediate an immunosuppressant effect (Iwamura et al., 2001). Both

CB_1 and CB_2 seem to couple to inhibitory G_i and/or G_o proteins. CB_1 receptors are known to effect adenylate cyclase, a variety of potassium and calcium currents, and the mitogen-activated protein kinase pathway. CB_1 receptors have been shown to activate at least six subtypes of G_i and G_o proteins, supporting reports that they effect a wide variety of intracellular signaling systems. In contrast to CB_1 receptors, CB_2 receptors do not seem to effect ion currents directly (Breivogel et al., 2001).

Influence of cannabinoids on Ca^{2+} currents

It has been demonstrated that cannabinoids act to suppress action potential-evoked calcium rises in the presynaptic terminal, thereby decreasing transmitter release. The action potential-evoked rise in intraterminal calcium was decreased by postsynaptic depolarization. This postsynaptic depolarization induced reduction of presynaptic calcium was prevented by application of antagonists to the CB_1 receptor (Kreitzer and Regehr, 2001). Cannabinoid-induced decreases in synaptic transmission have been shown to result from an inhibition of N- and P/Q-type calcium channels, the subtypes through which calcium influx occurs during evoked transmitter release (Twitchell et al., 1997).

Interactions with the opioid and glutamate systems

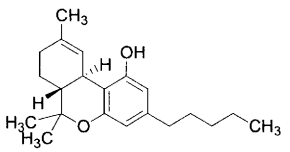
Several lines of investigation indicate an interaction between the opioid and cannabinoid system (Welch and Eads, 1999). There is also evidence that a physiological stimulation of metabotrobic glutamate and NMDA receptors is required for cannabinoid-induced analgesia (Palazzo et al., 2001).

Studies with CB_1 receptor knock-out mice suggest the existence of an as yet unknown neuronal cannabinoid receptor - potentially a new target for pain research

Investigations on CB_1 receptor knock-out mice have shown that the animals appear healthy and fertile, but with an increased mortality rate. They display reduced locomotor activity, increased ring catalepsy and hypoalgesia in the hot plate and formalin test. Interestingly in this study administration of Δ^9-THC still induced antinociception in the tail flick test, but not in the hot plate test, which indicates that not all CNS effects of Δ^9-THC are mediated by the CB_1 receptor. The molecular basis for the antinociceptive and other effects remains to be determined, but may involve an as yet unknown neuronal receptor (Zimmer et al., 1999). This hypothesis is supported by results from [^{35}S]GTPγS binding studies stimulated by anandamide and WIN 55,212-2 in brain membranes from both $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. Both ligands were able to activate an unknown receptor which should be related to CB_1 and CB_2 because two chemically unrelated cannabinoid agonists produced pharmacological effects and activate this receptor. Interestingly the classical cannabinoids like THC do not activate G-proteins

in brain membranes from $CB_1^{-/-}$ mice (Breivogel et al., 2001; Di Marzo et al., 2000a).

Δ^9-THC

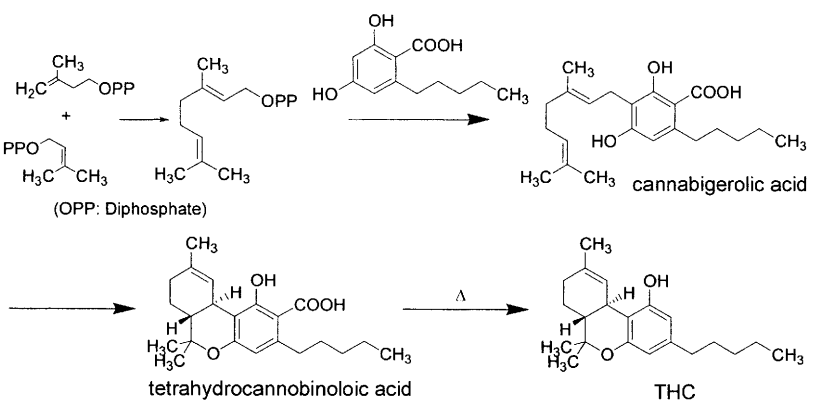


[*1972-08-3*], (-)-(*Z*)-(6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol, "(-)-trans-Δ^9-**t**etra**h**ydro-**c**annabinol" $C_{21}H_{30}O_2$

Trade names: Marinol, THC, Unimed, Compassia

Δ^9-THC is marketed as marinol or dronabinol for the treatment of chemotherapy-induced nausea and vomiting in Australia, Canada, Israel, South Africa and the USA. It was granted orphan drug status in the US for the stimulation of appetite and prevention of weight loss in patients with a confirmed diagnosis of AIDS. Δ^9-THC is in phase I trials for spasticity, multiple sclerosis and post-operative pain. Several small clinical studies have confirmed the effectiveness of Δ^9-THC as an analgesic, with doses of 15 to 20 mg being comparable to 60 to 120 mg of codeine (Williamson and Evans, 2000).

In the 1970s the biosynthesis of cannabinoids was investigated with radiolabeling experiments. ^{14}C-labeled mevalonate and malonate were shown to be incorporated into tetrahydrocannabinolic acid and cannabichromenic acid at very low rates (< 0.02%). Until 1990 the precursors of all terpenoids, isopentenyl diphosphate and dimethylallyl diphosphate were believed to be biosynthesized via the mevalonate pathway. Subsequent studies, however, proved that many plant terpenoids are biosynthesized via the recently discovered deoxyxylulose phosphate pathway (Eisenreich et al., 1998; Rohmer, 1999). It was shown that the C_{10}-terpenoid moiety of cannabinoids is biosynthesized entirely or predominantly (>98%) via this pathway (Fellermeister et al., 2001). The phenolic moiety is generated by a polyketide-type reaction sequence.



Scheme 1: Biosynthesis of Δ^9-THC.

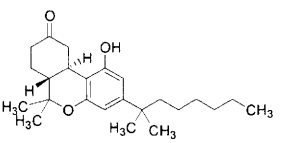
The oxidocyclization of cannabigerolic acid to tetrahydrocannabinolic acid is catalyzed by tetrahydrocannabinolic

acid synthase without any cofactors or coenzymes. This enzyme was recently identified together with two other specific enzymes, cannabidiolic acid and cannabichromenic acid synthase which transform cannabigerolic acid to cannabidiolic acid and cannabichromenic acid (Morimoto et al., 1999; Taura et al., 1996).

The psychoactive ingredients of *cannabis sativa* are finally formed when its leaves are dried at elevated temperature so that e.g. tetrahydrocannabinolic acid is decarboxylated to Δ^9-THC.

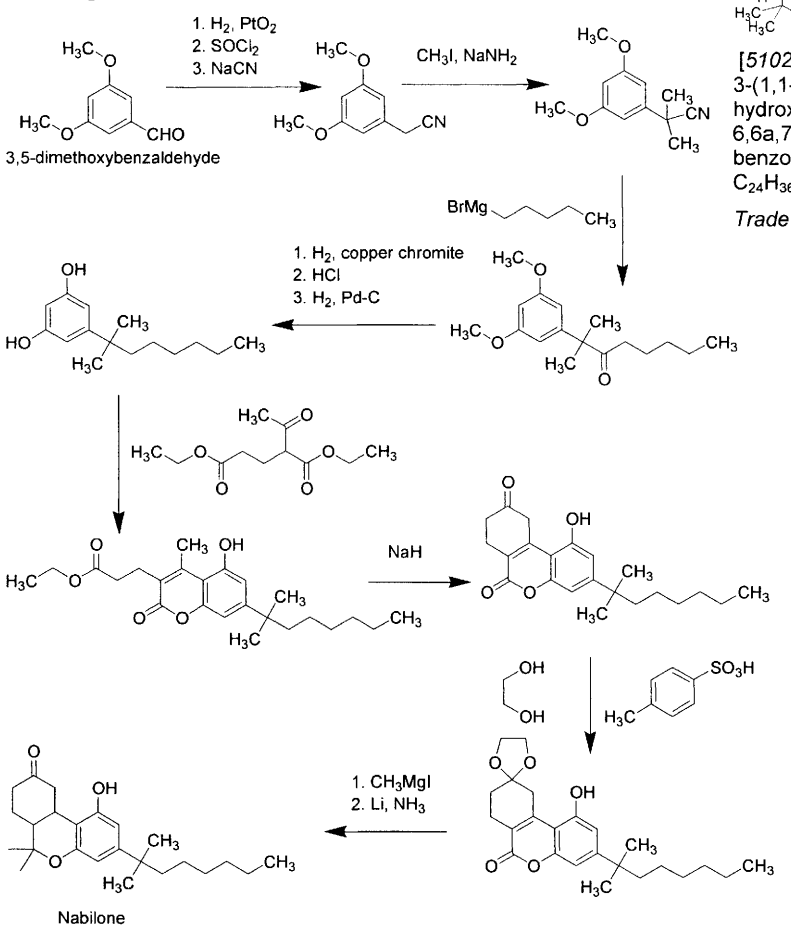
Nabilone

Nabilone, a synthetic cannabinoid, is marketed by Eli Lilly for the treatment of chemotherapy-induced nausea and vomiting in Canada and Great Britain.



[*51022-71-0*], (6a*R*,10a*R*)-3-(1,1-Dimethyl-heptyl)-1-hydroxy-6,6-dimethyl-6,6a,7,8,10,10a-hexahydro-benzo[c]chromen-9-one, $C_{24}H_{36}O_3$

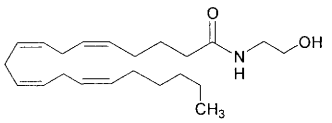
Trade name: Cesamet



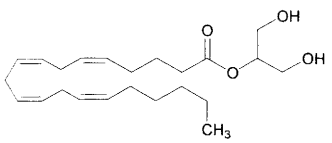
Scheme 2: Synthesis of nabilone.

Anandamide

Anandamide – the first endocannabinoid



[*94421-68-8*], all-(*Z*)-Eicosa-5,8,11,14-tetraenoic acid (2-hydroxy-ethyl)-amide, $C_{22}H_{37}O_2$



2-Arachidonyl-glycerol

The finding that endogenous derivatives of the non-oxidative metabolism of polyunsaturated fatty acids could bind to and activate the cannabinoid receptors opened a new era in research on the possible therapeutic use of cannabinoids. Endogenous cannabinomimetic compounds have pharmacological properties similar to the exogenous compounds and thus offer new strategies for the pharmacological modulation of cannabinoid receptor activity. The endocannabinoids discovered so far are the anandamides which are ethanolamides of n-6 poly-unsaturated fatty acids with at least three double bonds and 20 carbon atoms of which the C20:4 anandamide is simply known as anandamide, and 2-arachidonoyl glycerol, a well known intermediate of phosphoglyceride and di- or tri-glyceride metabolism. The pharmacology, particularly of anandamide, has been extensively studied over recent years.

The discovery of endocannabinoids

Anandamide was isolated from water-insoluble fractions of the porcine brain. It binds to CB_1 with rather moderate affinity (Ki 61 nM) and a low affinity for the CB_2 receptor (Ki 1930 nM). The name anandamide is based on its chemical nature (an amide) and the Sanskrit word ananda meaning bliss. The chemical structure of anandamide can be divided into two major molecular fragments: a polar ethanolamido head group and a hydrophobic arachidonyl chain. The polar head group comprises a secondary amide functionality with an N-hydroxyalkyl substituent while the lipophilic fragment is a non-conjugated *cis* tetraolefinic chain and an n-pentyl chain reminiscent of the lipophilic side chain found in the classical cannabinoids. A number of anandamide analogs have been synthesized and demonstrated to have considerable selectivity for the CB_1 receptor in comparison to the CB_2 receptor.

Pharmacological properties of anandamide – further evidence for an unknown cannabinoid receptor

Anandamide was shown to alleviate nociception in several behavioral animal models, e.g. the hot plate and formalin test (Calignano et al., 2001). A most interesting aspect of anandamide with respect to pain research is that it seems to bind to the hypothetical third cannabinoid receptor (Di Marzo et al., 2000b): In the hot plate test anandamide is still antinociceptive in $CB_1^{-/-}$ mice, which is consistent with the observation that the selective CB_1 receptor antagonist SR 141716A does not block motor inhibitory and antinociceptive effects of anandamide in wild-type mice.

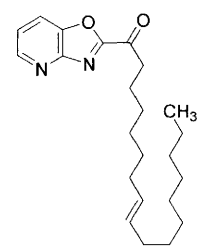
Biological functions of endocannabinoids – a topic for further research

Like other arachidonate-derived endogenous compounds the endocannabinoids seem to be synthesized and released by cells on demand. The hydrolysis of phospholipid precursors, also probably embedded in the

plasma membrane, seems to be necessary for the production of both anandamide and 2-arachidonyl-glycerol. The release of these compounds from cells does not appear to be vesicle mediated. However, unlike other eicosanoids no enzyme has been characterized so far as being uniquely responsible for anandamide or 2-achidonyl-glycerol formation. Anandamide is produced in neurons and leukocytes together with other N-acyl-ethanolamines from the hydrolysis of the corresponding N-acyl-phosphatidyl-ethanolamines. None of the enzymes responsible for the release of anandamide from membrane phospholipids appears to be used selectively for the formation of this endocannabinoid. The pathways leading to endocannabinoid biosynthesis seem to be part of more complex mechanisms regulating membrane phospholipid remodeling and intracellular second messenger levels, so the development of drugs selectively interfering with anandamide biosynthesis appears, at the moment, to be a difficult task.

The degradation of anandamide is catalyzed by fatty acid amide hydrolase (FAAH). Investigations on FAAH knock-out mice have shown that they cannot effectively degrade anandamide so they have 15-fold higher endogenous levels than wild-type mice. The mice seem normal until injected with anandamide, then they behave similar to wild-type mice treated with THC. The knock-out mice, even without anandamide treatment, have reduced pain sensitivity which can be reversed by a cannabinoid receptor antagonist, indicating that increased endogenous anandamide in FAAH knock-out mice affects their pain pathways. Therefore selective FAAH inhibitors might provide a selective method of using the cannabinoid signaling system for chronic pain relief.

Inhibition of fatty acid hydrolase (FAAH) – a new target for pain research?



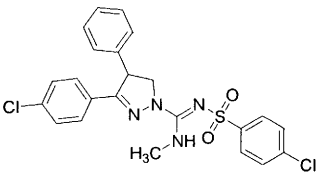
FAAH-inhibitor, 2 nM (Boger et al., 2001)

Anandamide is approved by the FDA as an appetite enhancer. Its analgesic activity is under preclinical investigation by Yissum.

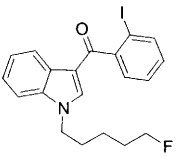
Current Developments

Other developments in the area of cannabinoid analgesics include a marijuana patch being investigated by the American Cancer Society for the relief of chronic pain, nausea and vomiting associated with chemotherapy (Cancer Drug News, Feb. 2000, 30). Several cannabinoid agonists are reported by Pharmaprojects to be in the stage of preclinical evaluation. A range of recently published patent applications reveals that new compounds with cannabimimetic activity are being intensively

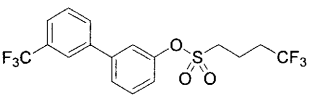
investigated. A selection of these new compounds is given in Figure 2:



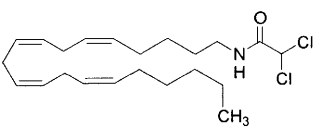
CB_1 receptor antagonist, claimed to be analgesic (Lange et al., Solvay, WO0170700)



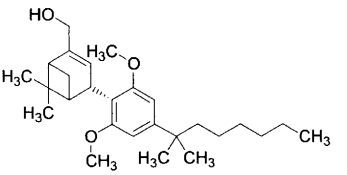
subnanomolar CB_1 receptor ligand (Makriannis and Deng, University of Conneticut, WO0128557)



analgesic (Heil et al., Bayer, WO0174763)



subnanomolar CB_1 receptor ligand (Makriannis and Deng, University of Conneticut, WO0128498)



HU-308, analgesic (Fride et al., Yissum, WO0132169)

Figure 2: Compounds recently disclosed in patent applications.

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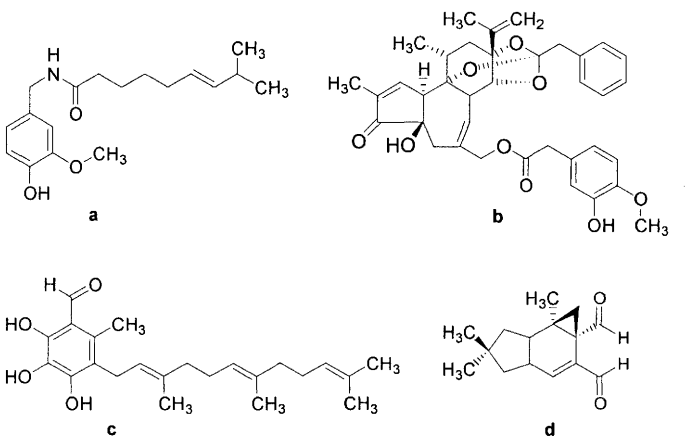
13 Vanilloids and the VR1 Receptor

Robert Frank

Introduction

Pain is initiated when the peripheral terminals of a subgroup of sensory neurons called nociceptors (Caterina and Julius, 1999) are activated. Nociceptor-specific cation channels, so-called vanilloid receptors, are the neuronal membrane recognition sites that serve as the molecular target for capsaicin, the main pungent ingredient in hot chilli peppers and related irritant compounds (Szallasi and Blumberg, 1990). A functional vanilloid receptor - called VR1 - activated not only by capsaicin but also by noxious heat (>43°C) and low pH (protons) has been cloned from rat (Caterina et al., 1997) and man (Hayes et al., 2000). So VR1 can be regarded as an integrator of chemical and physical stimuli that elicit pain.

Hot peppers and painful heat both activate sensory nerve fibers through an ion channel, known as vanilloid receptor subtype 1 (VR1). When activated, the channel opens, allowing an influx of calcium and sodium ions. The influx depolarizes neuronal pain fibers, initiating a nerve impulse through the dorsal root ganglion (DRG) to the brain. Noxious heat also activates VR1, explaining why our mouths feel hot when we eat chilli peppers.



Scheme 1: Chemical structures of natural vanilloid receptor agonists: **a**, capsaicin, the irritant principle in hot peppers; **b**, resiniferatoxin (RTX), isolated from the cactus-like plant *Euphorbia resinifera*; **c**, the triprenyl phenol scutigeral, found in an edible, non-pungent mushroom; **d**, the sesquiterpenoid dialdehyde isovelleral, found in pungent mushrooms.

VR1 Receptor Structure, Localization and Signaling

VR1 is homologous to members of TRP (Harteneck et al., 2000), the transient receptor potential family of putative store-operated Ca^{2+} channels first identified in the *Drosophila* phototransduction pathway (Montell and Rubin, 1989; Caterina et al., 1997). These ion channels comprise six transmembrane domains with N-terminal anykrin

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repeats (Clapham, 1996). The protein most likely exists in a multimeric form and forms a cation-selective ion channel with a preference for Ca^{2+} (Caterina et al., 1997). Although binding and Ca^{2+} uptake were initially believed to detect independent vanilloid receptor subtypes (Szallasi and Blumberg, 1996), VR1 can account for both the binding and functional activity of vanilloid ligands that show R- (resiniferatoxin-) and C- (capsaicin-) type structure-activity relations in rat dorsal root ganglion (DRG) neurons expressing native vanilloid receptors (Szallasi et al., 1999).

VR1 is a polytopic protein containing six transmembrane segments with an additional short hydrophobic stretch between transmembrane regions 5 and 6, which is believed to be associated with the channel pore. There are three possible protein kinase A phosphorylation sites on the VR1 that might play a role in receptor desensitisation.

Vanilloid receptors are expressed by a subset of primary sensory neurons in dorsal root and trigeminal ganglia where their expression is regulated by nerve growth factor (Winter et al., 1993) and by a subpopulation of vagal (nodose ganglion) neurons sensitive to brain-derived neurotrophic factor (Helliwell et al., 1998). They have also been detected in several brain nuclei including the pre-optic area, locus coeruleus, medial hypothalamus, reticular formation and ventral thalamus (Acs et al., 1996; Sasamura et al., 1998). A more widespread distribution of VR1 within the CNS and on blood mononuclear cells has been reported recently (Mezey et al., 2000). Thus, VRs in the brain (and putative endogenous vanilloids) have been suggested to be involved in control of emotions, learning and satiety, to name just a few exciting possibilities (Mezey et al., 2000).

Binding to VR1 can be measured using [^{3}H]RTX (Szallasi and Blumberg, 1990) or [^{125}I]RTX (Wahl et al., 2001). Furthermore, vanilloid receptor distribution can be visualized by [^{3}H]RTX autoradiography (Szallasi, 1995) *in situ* hybridization (Caterina et al., 1997) or immunostaining (Tominaga et al., 1998). The heterogeneity of vanilloid-induced biological responses predicts the existence of vanilloid receptor subtypes. Vanilloids have been found to evoke several kinetically distinct currents in sensory neurons (Liu and Simon, 1996; Petersen et al., 1996).

Potential vanilloid receptor subtypes

Homologs of VR1 with a high threshold (> 52°C) for activation by noxious heat, or sensitivity to membrane stretch, provisionally termed vanilloid receptor-like protein (VRL-1) (Caterina et al., 1999) and stretch-inactivated channel (SIC) (Suzuki et al., 1999), respectively, have been identified. Neither channel is activated by vanilloid agonists (Caterina et al., 1999; Suzuki et al., 1999). A mouse ortholog of VRL-1 acts as a growth factor regulated channel (GRC) permeable to Ca^{2+} ions (Kanzaki et al., 1999). A splice variant of VR1 (VR.5'sv) that lacks the majority of the intracellular N-terminal domain is refractory to activation by vanilloid agonists, protons or noxious

thermal stimuli (Schumacher et al., 2000). An additional homologue of VR1, variously termed OTRPC4 (Strotmann et al., 2000), VR-OAC (Liedtke et al., 2000), Trp12 (Wissenbach et al., 2000) or VRL-2 (Delany et al., 2000), is reported to be an osmotically regulated nonselective channel (Liedtke et al., 2000; Strotmann et al., 2000).

Effects of vanilloid agonists

Treatment with vanilloid agonists leads to the activation of distinct subpopulations of primary sensory neurons (nociceptors), with somata in dorsal root, trigeminal as well as nodose ganglia (Holzer, 1991). These neurons transmit nociceptive information back to the central nervous system (afferent function), whereas their peripheral terminals are sites of release for a variety of pro-inflammatory neuropeptides (efferent function) such as calcitonin gene-related peptide (CGRP) or the tachykinin substance P (SP, NK1). Excitation of these neurons by vanilloids is followed by a long-lasting refractory state referred to as desensitization (Jancsó, 1968) or, under certain conditions such as neonatal treatment (Jancsó et al., 1977), may lead to gross neurotoxicity.

Actual and Potential Applications of Natural and Synthetic Vanilloids

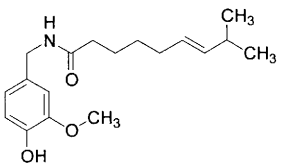
Human disorders which today are of interest in the context of vanilloid treatment can in general be divided into three categories (Winter et al., 1995):

(1) Disease states in which currently available capsaicin solutions or creams are clearly beneficial, such as non-allergic (vasomotor) rhinitis (Stjarne et al., 1989; Lacroix et al., 1991; Marabini et al., 1991; Filiaci et al., 1994), urinary bladder hyperreflexia (Fowler et al., 1992; Geirsson et al., 1995) and notalgia parestetica (Leibsohn, 1992).

(2) Pathological conditions in which capsaicin itself is not sufficiently active, but more potent vanilloids are expected to be of greater therapeutic value. For example: diabetic neuropathy (Ross and Varipapa, 1989), postherpetic neuralgia (Watson et al., 1988; Bernstein et al., 1989), chronic distal painful poly-neuropathy (Low et al., 1995), post-mastectomy pain syndrome (Watson et al., 1989), Guillain-Barré syndrome (Morgenlander et al., 1990), reflex sympathetic dystrophy (Cheshire and Snyder, 1990), vulvar vestibulitis (Friedrich, 1988).

(3) Innovative uses for novel, receptor subtype-selective vanilloids (see below), e.g. weight control.

Capsaicin

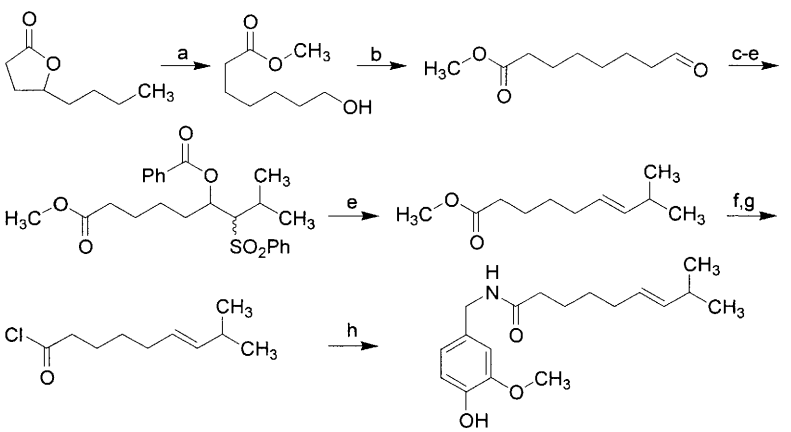


[404-86-4], trans-8-Methyl-N-vanillyl-6-nonenamide, $C_{18}H_{27}NO_3$, M_r 305.42, *mp* 62-65 °C

Trade names: Axsain (GB), Capsin, Capzasin, No pain (USA), Mioton (BG), Zacin (GB, IRL), Zostrix (AUS, CAN, USA), Capsacin (ES).

The vanilloid agonist capsaicin has been in use for decades as a pharmacological tool. In principle, all the three characteristic effects of capsaicin – excitation, desensitisation, neurotoxicity – can be used therapeutically, but desensitization to capsaicin is of particular interest as a novel approach to mitigate neuropathic pain insensitive to traditional pain-killers such as opiates (Szallasi and Blumberg, 1993; Winter et al., 1995). Capsaicin binding leads to Na^+ influx resulting in action potential generation (perceived as pain), and to Ca^{2+} accumulation (Wood, 1993). The increasing cytoplasmic Ca^{2+} concentration first impairs neuronal functions (desensitization) and may ultimately kill the affected neurons (Bleakman et al., 1990; Dray, 1992).

Synthesis: The synthesis of capsaicin is shown below (Gannett et al., 1988). For clinical use capsaicin is isolated from chilli pepper (*Capsicum annum)*.



(a) MeOH, H^+; (b) PCC, NaOAc, CH_2Cl_2; (c) Butylphenylsulphone, -78°C, *n*-BuLi; (d) -78°C, then 0°C; (e) -78°C, C_6H_5COCl; (f) Na(Hg), MeOH, -20°C; (g) KOH, EtOH; (h) $SOCl_2$; (i) Vanillylamine, pyridine, rt, 3 days (Gannett et al., 1988).

Scheme 2: Synthesis of capsaicin.

Clinical use: Being a vanilloid agonist capsaicin is used based on counter irritation and desensitization. It is a standard ingredient in a variety of over-the-counter drugs used worldwide to relieve muscle ache. Through the existence of vanilloid-sensitive nerves in the human urinary bladder, capsaicin is beneficial in the treatment of urge incontinence (motor form bladder hypersensitivity and sensory form detrusor hyperflexia). Whereas toothache is a traditional indication, topical capsaicin has additional therapeutic value in atypical odontalgia, burning mouth syndrome and vasomotor rhinitis. Topical capsaicin

has also been tried as an adjuvant analgesic in a variety of neuropathic pain conditions such as postherpetic neuralgia, painful diabetic neuropathy and postmastectomy pain syndrome, as well as in osteo- and rheumatoid arthritis (Winter et al. 1995; Sasamura et al., 1998; Szallasi and Blumberg, 1999).

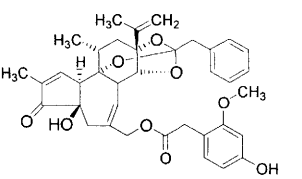
Adverse effects of capsaicin

The most important adverse effect of capsaicin is the initial burning sensation that it produces. Intravesical capsaicin induces intense suprapubic pain during intravesical instillation that may be made tolerable by lidocaine in some but not all patients. Capsaicin also frequently causes a transient worsening of the urinary conditions before improvement of symptoms due to desensitization of bladder afferents becoming evident. In patients with high spinal cord lesions capsaicin might provoke life-threatening autonomic dysreflexia.

Capsaicin is poorly absorbed through the human skin and is extensively metabolized (Szallasi and Blumberg, 1999), which explains why capsaicin creams are less active than expected on the basis of animal experimentation (Winter et al., 1995; Szallasi and Blumberg, 1999). When administered via a catheter into the human urinary bladder capsaicin is a powerful drug that relieves detrusor hyperreflexia (Winter et al., 1993; de Groat, 1997; Chancellor and de Groat, 1999). However, capsaicin administration is also very painful and might activate undesirable autonomic reflexes if absorbed into the circulation through the bladder mucosa (Cruz, 1998).

Resiniferatoxin

Resiniferatoxin (RTX) is a daphnane diterpenoid contained in the irritant latex of some succulent African *Euphorbias*. Its total synthesis has been described as a 40 step asymmetric synthesis (Wender et al., 1997; see below), but for clinical use RTX isolated from *Euphorbia resinifera*.

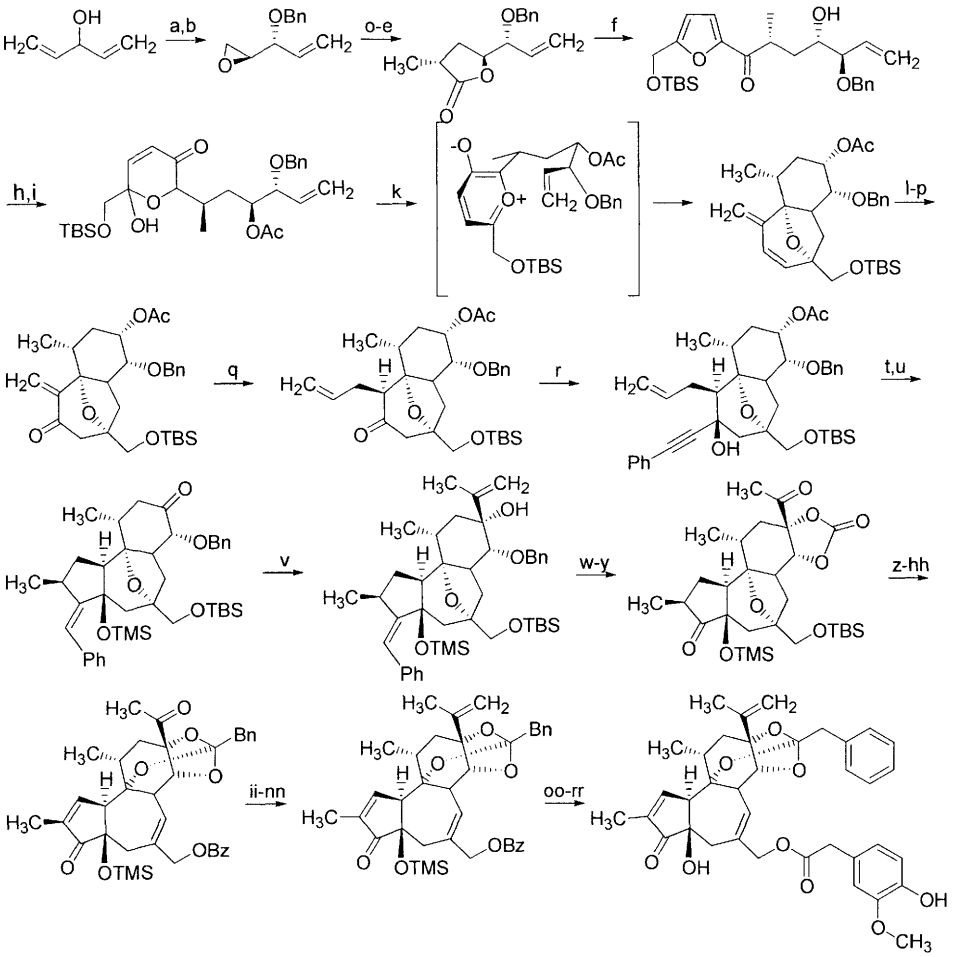


[57444-62-9], 4-Hydroxy-3-methoxy-[(2*S*,3a*R*,3b*S*,6a*R*,9a*R*,9b*R*,10*R*,11a*R*)-3a,3b,6,6a,9a,10,11,11a-octahydro-6a-hydroxy-8,10-dimethyl-11α-(1-methylethenyl)-7-oxo-2-(phenylmethyl)-7H-2,9β-epoxyazuleno[5,4-e]-1,3-benzodioxol-5-yl]benzeneacetic acid, $C_{37}H_{40}O_9$, M_r 628.71.

Trade names: Afferon RTX (USA, CH).

Clinical use: Resiniferatoxin has been characterized as an ultrapotent sensory neuron desensitizing agent. It is 100 to 10,000 times more potent than capsaicin, has a longer duration of action, is much less irritating, and is substantially more effective in producing neural desensitization. Clinical studies have demonstrated the utility of RTX in humans for the treatment of urinary urge incontinence, also known as detrusor hyperactivity, detrusor instability, detrusor hyperreflexia, or uninhibited bladder. It can also be used against pain associated with diabetic neuropathy and in the treatment of migraine and rhinitis. RTX is at present undergoing clinical trials investigating its ability to suppress detrusor instability

(Cruz et al., 1997; Cruz, 1998; Lazzeri et al., 1998), an important cause of urinary incontinence.



(a) $Ti(OPr)_4$, (-)-DIPT, *t*-BuO_2H, -15°C; (b) BnBr, NaH, *n*-Bu_4NI, THF; (c) EtOCCLi, $BF_3{\bullet}OEt_2$, THF, -78°C; (d) TsOH, CH_2Cl_2; (e) LDA, -78°C, then MeI; (f) TBS-protected furfuryl alcohol, *n*-BuLi, THF, -78°C; (g) AcCl, pyridine, CH_2Cl_2, 0°C; (h) $NaBH_4$, MeOH, 0°C; (i) *m*-CPBA, THF, 0°C; (j) Ac_2O, DMAP, pyridine; (k) DBU, CH_3CN, 80°C; (l) H_2 (49 psi), 10% Pd/C, EtOAc; (m) Ph_3PCH_2, THF, reflux; (n) AcCl, DMAP, pyridine, 0°C; (o) SeO_2, *t*-BuO_2H, CH_2Cl_2; (p) MnO_2, CH_2Cl_2; (q) CH_2=CHLi, CuCN, Et_2O, -60°C; (r) PhCCLi, LiBr, THF, -78°C; MSTFA; (s) TMSCl, imidazole, CH_2Cl_2; (t) Cp_2ZrBu_2, THF, -78°C; HOAc; (u) TPAP, NMO, CH_2Cl_2; (v) CH_2=C(Me)MgBr, THF, 0°C; (w) O_3, CH_2Cl_2-MeOH, -78°C; $(NH_2)_2CS$, -78°C; (x) H_2 (45 psi), 20% $Pd(OH)_2$/C, EtOAc, MeOH; (y) triphosgene, pyridine, CH_2Cl_2, 0°C; (z) 49% HF, CH_3CN, 0°C; (aa) Tf_2O, pyridine, CH_2Cl_2, 0°C; *n*-Bu_4NI, CH_3CN; (bb) Rieke Zn, EtOH, reflux; (cc) SeO_2-*t*-BuO_2H, THF-HMPA (10:3), 80°C; (dd) $SOCl_2$, propylene oxide-Et_2O (1:2), 0°C; (ee) AgOBz-KOBz, 18-crown-6, CH_3CN; (ff) 0.5 M NaOH in aq. dioxane; (gg) DMAP, 2,4,6-Cl_3-PhC(O)C(O)CH_2Ph, toluene; (hh) 0.5% $HClO_4$ in MeOH; (ii) $TMSCH_2Li$, THF, -78°C; (jj) 49% HF, CH_3CN; (kk) BzCl, pyridine, DMAP, CH_2Cl_2; (ll) MSTFA, DMAP, DABCO, CH_3CN, 110°C; (mm) NBS, THF; (nn) Li_2CO_3, LiBr, DMF, 150°C; (oo) TBAF, THF, 0°C; (pp) $Ba(OH)_2$, MeOH; (qq) 2,4,6-Cl_3-PhC(O)OC(O)CH_2(4'-OAc)(3'-OMe)Ph, DMAP, toluene; (rr) pyrrolidine, CH_2Cl_2 (Wender et al., 1997).

Scheme 3: Synthesis of resiniferatoxin.

In comparison to capsaicin RTX has four major advantages:

(1) Because of its ultrapotency it may be used in much lower concentrations (Surh and Lee, 1995).

(2) Its use leads to desensitization (tachyphylaxis) rather than to irritation, which is the main factor limiting the therapeutic use of capsaicin.

(3) It has a much broader therapeutic window: full desensitization to pain or neurogenic inflammation may be achieved by means of a single RTX injection and without unacceptable toxicity.

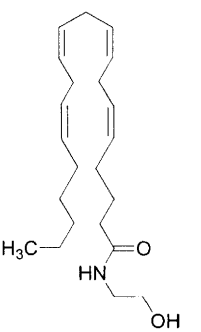
(4) Unlike capsaicin it not only suppresses chemogenic pain, but is effective against noxious heat-evoked pain in normal rats as well as cold-evoked pain in animals with spinal cord injury.

RTX has its own shortcomings. Most importantly, RTX is expensive to isolate from natural sources and is difficult to synthesize. So there is a need for structurally simplified, orally active vanilloids. Whether either unsaturated dialdehydes or triprenyl phenols could serve as templates for the synthesis of such improved vanilloids remains to be explored.

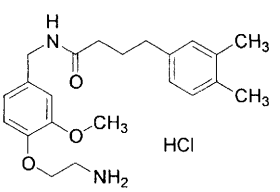
Shortcomings of resiniferatoxin

Other Compounds

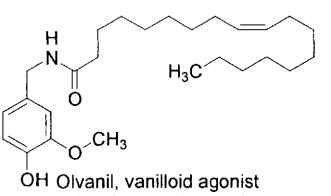
Other exogenous agonists at vanilloid receptors include novel capsaicin analogs that lack pungency or compounds that lack a vanilloid (o-hydroxymethoxy- or o-dimethoxyphenyl) moiety, for example unsaturated sesquiterpenoid dialdehydes or triprenyl phenols (see Introduction). Furthermore, the cannabinoid receptor agonist anandamide (see Chapter 11, *Cannabinoids*) and several eicosanoid products of lipoxgenases also activate vanilloid receptors (Hwang et al., 2000) and are putative endogenous agonists. Vanilloid receptor activity can also be induced by the activation of protein kinase C and the latter has been suggested to couple certain algesic stimuli, e.g. bradykinin, to signalling via vanilloid receptors (Cesare et al., 1999; Premkumar and Ahern, 2000).



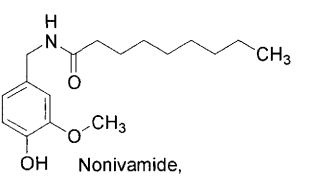
Anandamide



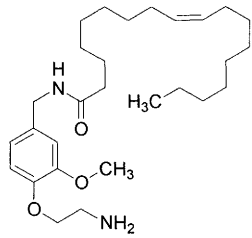
DA-5018, NK1 antagonist, Phase II (Dong-A)



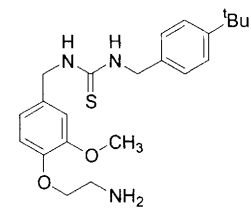
Olvanil, vanilloid agonist development ceased (Procter & Gamble)



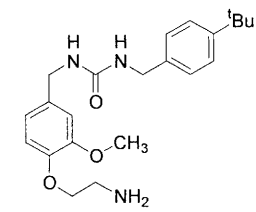
Nonivamide, NK1 antagonist (Procter & Gamble)



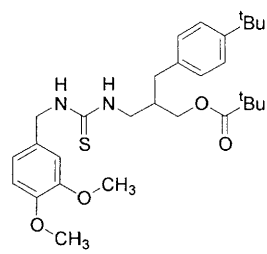
NE-21610, vanilloid agonist (Procter & Gamble)



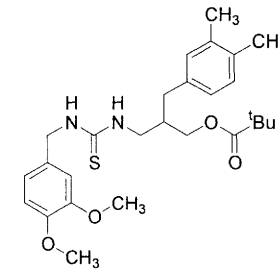
SDZ-249-482, unspecified vanilloid agonist, development ceased (Novartis)



SDZ-249-665, unspecified vanilloid agonist, preclinical studies against neuropathic and inflammatory pain (Novartis)



6d-I NIH, vanilloid antagonist, preclinical analgesic, (NIH)

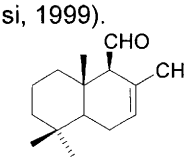


6b-I NIH, vanilloid antagonist, preclinical analgesic, (NIH)

Scheme 4: Structures of selected vanilloid like compounds.

The hot-tasting sesquiterpene dialdehyde polygodial was first isolated from water pepper (*Polygonum hydropiper*, Fukuyama et al., 1982).

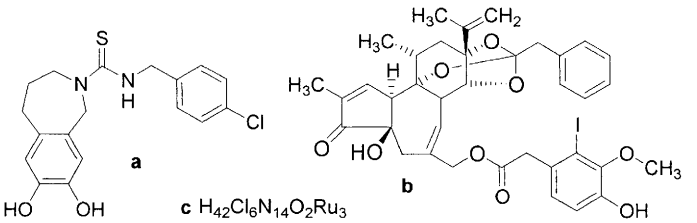
The only current therapeutic use of an unsaturated 1,4-dialdehyde in the Western hemisphere is the use of polygodial (Kolorex® Capsules and Cream) to control localized candidiasis. According to the producer polygodial damages the cell wall of *Candida albicans* and other fungi (Sterner and Szallasi, 1999).



Polygodial [6754-20-7], 5,5,8a-Trimethyl-1,4,4a,5,6, 7,8,8a-octahydronaphthalene-1,2-dicarbaldehyde, $C_{15}H_{22}O_2$, M_r 234.33. *Trade name*: Kolorex® (NZ).

VR1 Antagonists

Known antagonists of vanilloid receptors include capsazepine, which acts competitively but with low potency at the capsaicin binding sites (Bevan et al., 1992), iodo-RTX, which binds with high affinity (Wahl et al., 2001), the unselective antagonist ruthenium red (Amann and Maggi, 1991) and synthetic arginine-rich hexapeptides (Planells-Cases et al., 2000), which are putative channel blockers.



Scheme 5: Chemical structures of known vanilloid receptor antagonists: **a**, capsazepine, a weak but competitive synthetic compound from Novartis, **b**, iodo-resiniferatoxin, a potent and competitive synthetic compound from Novo Nordisk, **c**, ruthenium red, a weak, non-competitive and non-selective antagonists.

Potential use for potent and selective VR1 antagonists

Contrary to the currently used (rapidly) desensitizing VR1 agonists capsaicin and RTX, selective VR1 antagonists are potentially useful for the treatment of pain and other pathological conditions without pronounced adverse effects (e.g. burning sensation). But whether the blockade of VR1 by potent and selective antagonists is as useful still remains to be proven. The one potent and selective VR1 antagonist known today - iodo-RTX - has received much attention but is a scarce and potentially chemically unstable compound needing further characterization.

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14 Substance P/NK_1 Receptors

Gregor Bahrenberg and Corinna Maul

Introduction

In 1930, substance P (SP) was discovered by von Euler and Gaddum as an unidentified substance - referred to as P on tracings and protocols - present in alcoholic extracts of equine brain and intestine (von Euler and Gaddum, 1931). The early experiments were mostly concerned with its stimulating effect on smooth muscle and its vasodilator properties, later interest focussed on its role as a neurotransmitter or neuromodulator (von Euler, 1981). It took another 40 years to definitely establish the structure of hypothalamic SP (Chang et al., 1971). The development of SP receptor agonists and antagonists, and more recently the employment of transgenic mice, contributed to further elucidation of its sensory function (review: Harrison and Geppetti, 2001). This function and the anatomy of its expression (demyelinated sensory fibers, small and medium-sized neurons of spinal horn substantia gelatinosa) strongly suggest an important role as a mediator of pain and chronic inflammation (Otsuka and Yoshioka, 1993; Hill, 1994; Iversen, 1998; Zubrzycka and Janecka, 2000). Therefore, in the last 20 years, antagonists of SP and the SP-preferring receptor, called neurokinin-1 (NK_1) receptor, have been studied for their contribution in pain relief and a variety of conditions, including inflammation, asthma, emesis, anxiety and migraine (Wahlestedt, 1998). Herein, these efforts, especially with respect to pain, and their clinical rewards are critically reviewed.

Substance P and its Receptor

SP is an undecapeptide and the most abundant and best characterized of the neurokinin (tachykinin) group of peptides, which are defined by the common C-terminal amino acid sequence Phe-X-Gly-Leu-Met-NH_2. This group includes neurokinin A (NKA) and neurokinin B (NKB), but also neuropeptide K (NPK) and neuropeptide gamma (NPγ) (reviewed in: Saria, 1999; Hökfelt et al., 2001). The actions of SP, NKA and NKB are respectively mediated through G-protein linked receptors, designated NK_1, NK_2 and NK_3 and characterized by seven putative transmembrane helices (Maggio, 1988; Regoli et al., 1989; Nakanishi, 1991; Cascieri et al., 1992). The stimulation of tachykinin NK_1 receptors activates several second messenger systems that are stimulators of phosphatidyl inositol turnover via phospholipase C, arachidonic acid mobilization via phospholipase A_2 and cyclic adenosine

Substance P
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH_2

Neurokinin A
Hys-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-MetNH_2

Neurokinin B
Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-MetNH_2

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monophosphate accumulation via adenylyl cyclase (Mitsuhashi et al., 1992; Nakajima et al., 1992; Takeda et al., 1992; Seabrook and Fong, 1993; Garcia et al., 1994; Mochizuki et al., 1994; Saria, 1999). The rank order of potency of the neurokinin agonists for the neurokinin-1 (NK_1) receptor is SP>NKA>NKB, while the rank orders of potency for the other two subtypes (NK_2, NK_3) are NKA>NKB>SP and NKB>NKA>SP, respectively (Helke et al., 1990; Fong et al., 1992; Maggi and Schwartz, 1997). Substitutions of part of segments from NK_1 demonstrated that both the extracellular and transmembrane domains of the NK_1 receptor are involved in the binding of SP. The C-terminal sequence of SP is essential for activity at the NK_1 receptor, the minimum length of a fragment with reasonable affinity for the receptor is a C-terminal hexapeptide (Saria, 1999).

Evidence for a Role of SP in Nociception

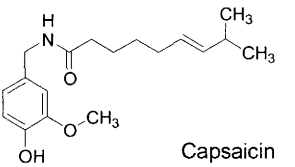
Distribution of SP and its preferred receptor indicates a role in nociception. SP and NKA are suggested as primary afferent neurotransmitters because the gene precursor for these (preprotachykinin A) is found in unmyelinated primary sensory afferent neurons (Weihe, 1990; Duggan and Weihe, 1991; Rupniak and Hill, 1999). After synthesis in the cell bodies of sensory nerve fibers (located in spinal or cranial sensory ganglia), SP and NKA are released from terminals within the CNS and within peripheral tissues (Hill, 1986). In the CNS, SP neurons are present in the tegmental nuclei of the medulla, the central nucleus of amygdala and in the spiny neurons of the striatum that project into the medial segment of the globus pallidus and the substantia nigra pars reticulata. Fewer SP neurons are present in the dentate gyrus of the hippocampus. Some neurons are also present in layers V and VI of the cortex and project into the upper layers (Penney, 1996).

Distribution of SP

Distribution of NK1 receptors

The striatum, the nucleus accumbens, the hippocampus, the lateral nucleus of the hypothalamus, the habenula, the interpeduncular nucleus, the nucleus of the tractus solitarius, the raphe nuclei and the medulla oblongata are rich in tachykinin NK_1 receptors (Otsuka and Yoshioka, 1993). The predominant expression of NK_1 receptors within the spinal dorsal horn is consistent with the assumption that SP and NKA are important messengers here (Bleazard et al., 1994). The distribution of NK_1 receptors in the peripheral nervous system and in the gut are discussed elsewhere (McLean, 1996; Quartara and Maggi, 1997, 1998).

Sensory function of SP



Capsaicin

Surprisingly, the vanilloid receptor ligand capsaicin supplied the first experimental evidence for the association between SP and nociception (Gasparovic et al., 1964). Capsaicin depletes small primary afferents of at least SP, if not all of their peptide content, and this was accompanied by hypoalgesia. SP depolarizes the ventral root of an isolated rat spinal cord preparation (Konishi and Otsuka, 1974), and has also been shown to excite and/or depolarize neurons in the dorsal root (Urban et al., 1985). Furthermore, in studies on the larger laminae IV and V neurons, the selective NK_1 agonist, [Sar^9, $Met(O_2)^{11}$]substance P, was the most potent ligand tested (Morris et al., 1992). Fleetwood-Walker et al. (1987) reported that the NK_1 selective agonist [Met-O-Me^{11}]substance P excited neurons in laminae IV and V of cat spinal cord. Salter and Henry (1991), using iontophoretic application, demonstrated that presumed NK_1 receptor activation preferentially excited nociceptive neurons. Intrathecal administration of SP to a decerebrate, spinalized rat was found to facilitate the hamstring flexion reflex (Wiesenfeld-Hallin and Duranti, 1987), indicating that release of SP in the spinal cord may mediate hyperalgesia following tissue injury. Prolonged or intense noxious stimulation caused the release of SP in the vicinity of NK_1 receptors in the dorsal horn (Shults et al., 1984; Duggan et al., 1987). In addition, SP induces specific patterns of c-Fos expression in distinct regions (paraventricular, dorsomedial, parabrachial nuclei, medial thalamus) of the rat brain (Spitznagel et al., 2001). To sum up, one of the functions of SP is thought to be related to transmission of pain information into the central nervous system.

SP and nociception

Other effects of SP

SP from primary afferents has a number of other effects on target cells besides pain transmission, e.g. vasodilatation, plasma protein extravasation, mast cell degranulation, recruitment of inflammatory cells, stimulation of secretion and muscle contraction (Maggi, 1997). The role of SP as an endogenous vasodilator in cerebral circulation and resulting effects on pain production have been discussed by Beattie et al. (1995a).

Evidence for the involvement of NK_1 receptors in nociceptive transmission

Quartara and Maggi (1998) summarize evidence for the involvement of NK_1 receptors in nociceptive transmission as follows: (1) NK_1 receptors are expressed at appropiate anatomical locations for noxious input in the spinal cord. (2) Spinal cord NK_1 receptor expression undergoes regulation after noxious manipulation. (3) The signal transmitted by activation of NK_1 receptors is a slowly-developing sustained depolarization, while the fast synaptic input to second order sensory neurons is

mediated by excitatory amino acids. (4) Responses of second order sensory neurons to NK_1 receptor activation are enhanced by peripheral tissue injury or inflammation. (5) Tachykinin NK_1 receptor antagonists act synergistically to inhibit N-methyl-D-aspartate (NMDA)-mediated nociceptive transmission.

SP and NK_1 receptor knock-out mice

Further experimental evidence for the involvement of SP in pain perception came from knock-out animals. Mice, in which the preprotachykinin A gene was disrupted, showed significantly reduced responses in tests that involved more intense noxious stimuli (Cao et al., 1998). De Felipe et al. (1998) disrupted the NK_1 receptor, and found the characteristic amplification ('wind up') and intensity coding of nociceptive reflexes to be absent. NK_1 receptor knock-out mice show no changes in *acute* nociception tests. In contrast, SP and NK_1 receptor knock-out mice show reduction in responses to inflammatory stimuli. Nerve injury-induced mechanical but not thermal hyperalgesia is attenuated in NK_1 receptor knock-out mice, when inducing chronic neuropathic pain by unilateral ligation of the L5 spinal nerve (Mansikka et al., 2000).

SP and noradrenaline

Recently published work suggests that SP acting at the NK_1 receptor, when unopposed by tonic release of noradrenaline, causes chronic thermal hyperalgesia (Jasmin et al., 2002; reviewed in: Hill, 2002) The authors used mice lacking the gene coding for dopamine β-hydroxylase (DBH), the enzyme responsible for synthesis of noradrenaline from dopamine. The DBH knock-out resulted in a decreased nociceptive threshold to thermal, but not mechanical, stimuli and decreased efficacy of morphine. NK_1 receptor antagonists reversed the hyperalgesia in the DBH knock-out mice, confirming that the responses are operated through SP and NK_1 receptors. From these data, it is proposed that (at least in mice) SP and opioids have opposite effects on pain behavior with the pronociceptive effects of substance P being balanced by the antinociceptive effects of opioids.

In addition, several painful clinical conditions, including peripheral neuropathy, fibromyalgia and osteoarthritis, are associated with increased levels of SP in human cerebrospinal fluid (Rupniak and Kramer, 1999).

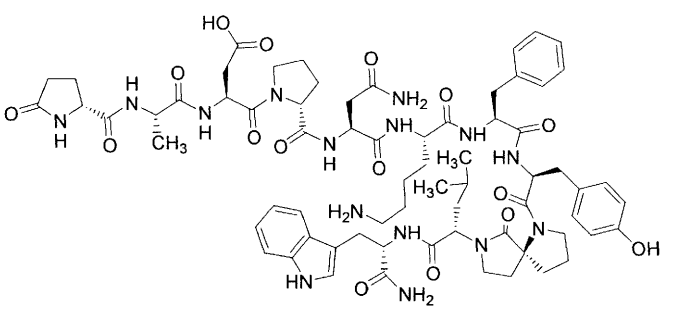
Peptidic SP antagonists

Following from the notion that an SP (or NK_1) antagonist might be useful for pain relief, several peptide and non-peptide antagonists have been discovered. The first peptide antagonists of SP were obtained in the early 1980s (review: Maggi et al., 1993). They have invariably

been developed through the modification of one or more of the 11 amino acids that comprise substance P, mainly based on substitution with D-amino acids (Folkers et al., 1981).

Spantide I & II

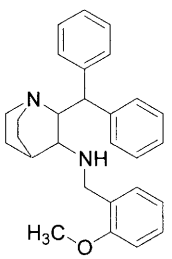
The most potent of this first generation of tachykinin antagonists is [D-Arg^1,D-$Trp^{7,9}$,Leu^{11}]SP, named spantide I (Folkers et al., 1984). An important advance was the discovery of SP analogs with N-terminal truncation of SP containing two or three D-Trp residues, such as in [D-Pro^4, D-$Trp^{7,9,10}$]SP (Regoli et al., 1984). For studies of the NK_1 receptor in the central and peripheral nervous system, [D-$NicLys^1$,3-Pal^3,D-Cl_2Phe^5,Asn^6,D-$Trp^{7,9}$,Nle^{11}]SP, also known as spantide II (Folkers et al., 1990), has been introduced (where D-$NicLys^1$ is N epsilon-nicotinoyllysine, Pal^3 is 3-(3-pyridyl)alanine, D- Cl_2Phe^5 is 3,4-dichloro-D-phenylalanine, and Nle is norleucine). Further examples of SP-derived antagonists are the peptidic antagonists [D-Pro^9,Pro^{10},Trp^{11}]-substance P, [D-Pro^9,$MeLeu^{10}$,Trp^{11}]-substance P, and the D-Pro^9-(S)-spirolactam derivatives GR82334 (see below) and GR72251 (Ward et al., 1990; Lavielle et al., 1994). Interestingly, these antagonists interact with different receptors in the guinea pig ileum bioassay, as they differ in their potencies to inhibit the spasmogenic activity evoked by the NK_1 receptor selective agonist [Pro^9]substance P or by septide, [pGlu^6,Pro^9]-substance P-(6-11) (Lavielle et al., 1994). Although peptidic antagonists proved useful as experimental tools to analyze SP or NK_1 receptor function (Hakanson and Sundler, 1985), they possess poor pharmacokinetic properties and relatively broad *in vivo* activities (residual agonist activity, degranulation of mast cells, local anesthetic properties, neurotoxic effects). It seemed likely that therapeutic effects would not be fully recognized until non-peptide, metabolically stable antagonists became available.



GR 82334

Non-Peptide Antagonists for NK_1 Receptors

Early attempts to synthesize non-peptide antagonists were hampered by the sequence diversity of the primary sequence of the NK_1 receptors from humans and rats, which influenced the potency of the compounds studied (Sachais et al., 1993; Saria, 1999). Appropriate models, with NK1 receptors closer to the human sequence, had to be established in gerbils, rabbits, and guinea pigs.



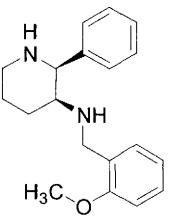
CP-96,345
[(2S,3S)-cis-2-(diphenyl methyl)-N-[(2-methoxyphenyl) -methyl]-1-aza-bi-cyclo[2.2.2] octan-3-amine]
$C_{28}H_{32}N_2O$, MW 412,57
[132746-60-2]

The first high-affinity, non-peptide antagonist of the tachykinin NK_1 receptor was discovered by researchers from Pfizer Central Research during a chemical file-screening approach and named CP-96,345 (Snider et al., 1991a; McLean et al., 1991; Watling, 1992; Ito et al., 1993). CP-96,345 displaced [^{3}H]substance P binding to NK_1 sites with a K_i of 0.6 nM (equipotent with SP), and yielded a pA_2 of 8.7 in the relaxation assay of the dog carotid artery previously contracted with noradrenaline. These effects are specific for the 2*S*,3*S* configuration of CP-96,345 and for competitive antagonism at NK_1 sites. SP-induced salivation (10 nmol/kg i.v. SP), which is mediated by the NK_1 receptor, was inhibited by CP-96,345 (3.4 mg/kg i.v.), while the acetylcholine-evoked salivation response was not impaired (Snider et al., 1991b). CP-96,345 readily blocked the excitation produced by iontophoretic substance P or by nociceptive inputs to dorsal horn neurons in cats and rhesus monkeys (Radhakrishnan and Henry, 1991; Dougherty et al., 1993). In guinea pig brain, CP-96,345 inhibited 100 nM SP-induced increases in the firing rate of locus coeruleus neurons with an IC_{50} value of 90 nM (McLean et al., 1991). In rats it antagonized the flexor reflex facilitation produced by intrathecal application of SP in a dose-dependent manner (Xu et al., 1992). CP-96,345 has shown its antinociceptive activity in several mouse and rat pain models. Racemic CP-96,345 (30 mg/kg i.p.) induced a long-lasting increase in reaction time using the mouse hotplate test (Lecci et al. 1991). Moreover, inflammatory pain models in rat (intraplantar injection of 2% carrageenan or 5% formalin) revealed the antinociceptive activity of CP-96,345 (Birch et al., 1992). CP-96,345 has been reported to be a potent inhibitor of tachykinin-mediated neurogenic inflammation in the rat, blocking the SP-evoked fall in blood pressure and the mustard oil-induced plasma extravasation (Lembeck et al., 1992). Unfortunately, a major drawback of CP-96,345 is that it blocks sodium and calcium channels at high concentrations (Caesar et al., 1993), which itself caused analgesia not resulting from antagonism of the binding of SP to NK_1 receptors (Nagahisa et al., 1992).

Binding sites for substance P and the non-peptide antagonists

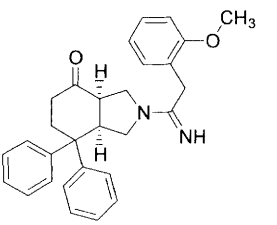
Nevertheless, with the development of CP-96,345 the binding site for non-peptide antagonists and SP could be analyzed in parallel. It was found that SP binds at the extracellular ends of the transmembrane helices, whereas the small hydrophobic, non-peptide antagonists bind deeper in between the transmembrane segments (Hökfelt et al., 2001).

Pfizer's follow-up compound, CP-99,994 (Desai and Rosen, 1993), has a high affinity for NK_1 sites present on human IM-9 cells (K_i 0.17nM), but is essentially devoid of affinity at NK_2 and NK_3 sites and L-type Ca^{2+} channel sites (Watling and Krause, 1993). Peripherally administered CP-99,994 (0.1-10 mg/kg s.c.) antagonized the locomotor response induced in guinea pigs by intracerebroventricular injection of the selective NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]SP. Previous studies suggest that CP-99,994 penetrates the blood brain barrier in rodents (McLean et al., 1993), and several arguments support a spinal action in the inhibition of prolonged chemical stimulation (Seguin et al., 1995).



CP-99,994

The non-peptide NK_1 antagonist RP-67580 [((3a*R*,7a*R*)-7,7-diphenyl-2(1-imino-2-(2-methoxyphenyl)ethyl)perhydroisoindole)] can block the mechanical allodynia induced by the conditioning of C-fiber stimulation (Ma and Woolf, 1995), and attenuates progressive hypersensitivity of flexor reflex during experimental inflammation in rats (Ma and Woolf, 1997). Furthermore, RP-67580 in a range of 20-200 nmol antagonizes in a dose-dependent manner the sensitizing effect of SP in inflamed knee joints (Pawlak et al., 2001), and, when infused into the ventral tegmental area, prevents footshock stress-induced analgesia in the formalin test (Altier and Stewart, 1999). RP-67580 was as potent as morphine in various analgesic tests (Garret et al.,1991). RP-67580 displayed no activity at either NK_2 or NK_3 receptors in binding and *in vitro* functional assays (Betancur et al., 1997). However, at high concentrations, RP-67580 also exerts non-specific effects on Ca^{2+} channels.



RP-67580

For CP-96,345 and RP-67580, and also other NK_1 antagonists, marked species variants were observed in pharmacological studies. RP-67580 has a higher affinity for the rat and mouse NK_1 receptors, whereas CP-96,345 preferentially binds to human and guinea pig NK_1 receptors. CP-96,345 has a 90-fold selectivity for the human NK_1 receptor over the rat NK_1 receptor, while the agonist SP shows no such selectivity (Sachais et al., 1993).

SR140,333, WIN51,708, WIN62,577

In mice, three further NK_1 antagonists - SR140,333, WIN51,708 and WIN62,577 - inhibited the late phase of formalin-induced licking, but failed to modify the tail-flick response at non-ataxic doses (Seguin et al., 1995). Examples of non-peptide antagonists for tachykinin receptors are listed in Table 1:

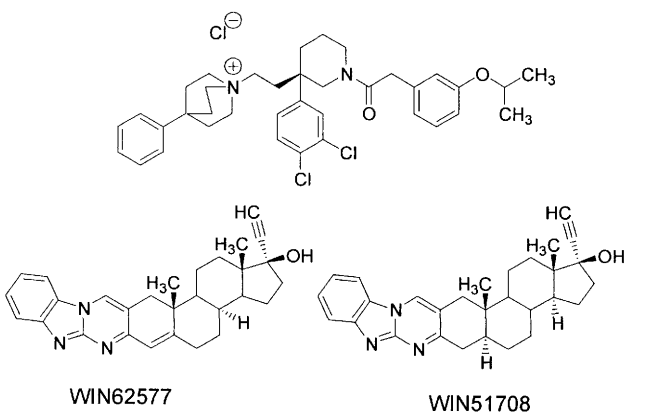
Table 1: Non-peptide antagonists for tachykinin receptors (adapted from Betancur et al.,1997).

Receptor subtypes	Endogenous ligands	Selective antagonists	
NK_1	SP>NKA>NKB	CP96349, SR140333, WIN51708, CGP49823, LY303870, L161664, L742694, CP122721, CAM4750, GR205171,	RP67580, CP99994, WIN62577, PD154075, LY306740, L733060, RPR100893, CAM4515, GR203040, MEN10930
NK_2	NKA>NKB»SP	SR48968,	GR159897
NK_3	NKB>NKA>SP	SR142801, SB223412	PD161182,
		Mixed NK_1/NK_2 antagonist: MDL105212A	

NKA, neurokinin A; NKB, neurokinin B; SP, substance P.

SR140,333, Nolpitantium chloride

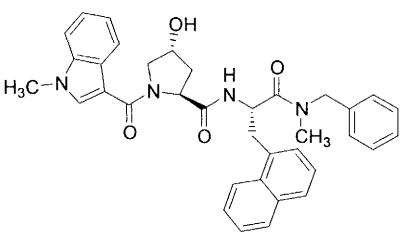
1-(2-{3-(3,4-Dichloro-phenyl)-1-[2-(3-isopropoxy-phenyl)-acetyl]-piperidin-3-yl}-ethyl)-4-phenyl-1-azonia-bicyclo[2.2.2]octane; chloride, $C_{37}H_{45}Cl_3N_2O_2$, MW 656,12, [153050-21-6]



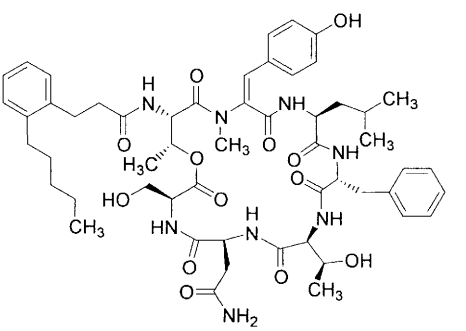
WIN62577 WIN51708

Scheme 1: Examples for non-peptide NK_1 antagonists.

Two further NK_1 receptor antagonists, described for the first time in the early 1990s, are FK888 and FK224. FK888 is a dipeptide derivative, synthesized by the Fujisawa group via a rational design strategy from the lead compound [D-Pro^4,D-Trp^{7,9,10},Phe^{11}]SP_{4-11}. FK888 is a compound with extremely high affinity for NK_1 receptors which inhibits [^{3}H]SP binding to guinea pig lung membranes with a K_i of 0.69 nM and displays a pA_2 of 9.29 versus SP-induced contractions of the guinea pig ileum (Fujii et al., 1992; Watling and Krause, 1993). FK224 is a mixed NK_1/NK_2 antagonist (Morimoto et al., 1992), which passed first clinical evaluations in the treatment of obstructive airway disease (Soneoka et al., 1995). A 4-mg dose inhibited the bronchoconstrictor effect in 10 asthmatic patients when given by inhalation 20 min before challenge with bradykinin (Ichinose et al., 1992). In addition, FK224 may have therapeutic potential in the treatment of arthritis, as it blocks carragenenan- and SP-induced plasma leakage in the rat knee-joint in a dose-dependent manner.



FK888

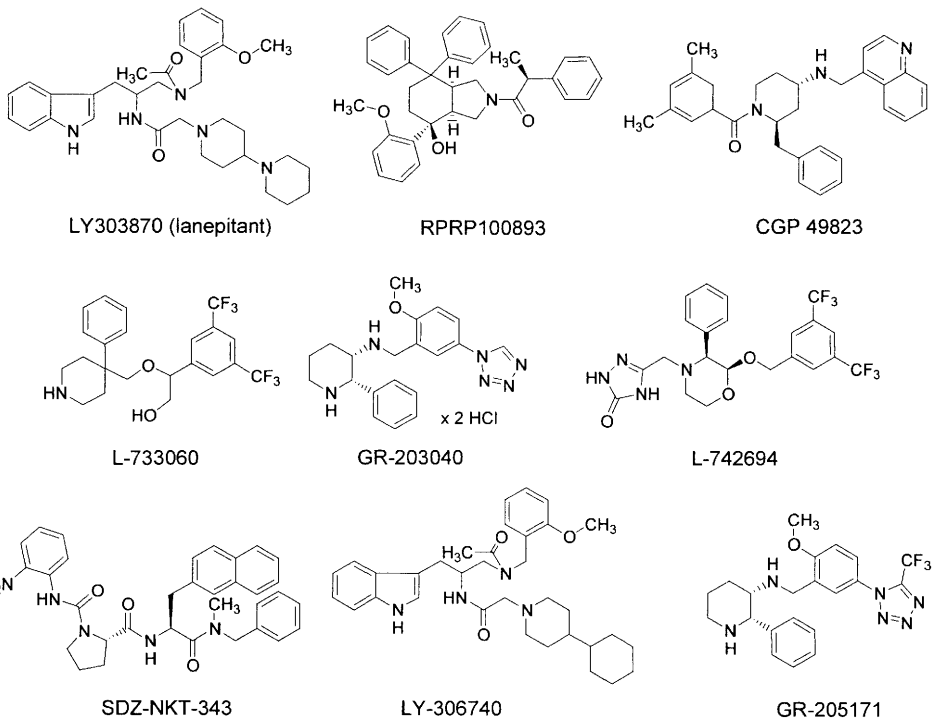


FK224

Scheme 2: Examples for synthetic peptide derivatives with NK_1 agonistic activity.

NK_1 receptor antagonists vary significantly in their abilities to penetrate the CNS following systemic administration. Poorly brain-penetrant compounds include SR140333, LY303870, RPR100893, and CGP 49823, whereas those with exceptionally good CNS penetration include the piperidines CP-99,994 and GR203040, the piperidine ether L-733,060, and morpholines such as L-742,694 (Rupniak and Hill, 1999). Putatively resulting from poor penetration of the blood brain barrier, LY303870 and RPR100893 are examples of NK_1 antagonists with poor efficacy and potency against inflammatory and

neuropathic hyperalgesia in the guinea pig after oral administration. In contrast, the NK_1 receptor antagonist SDZ NKT343 (Ko and Walpole, 1996) produced 67% and 100% reversal of inflammatory and neuropathic hyperalgesia, respectively, at comparatively low oral doses (Urban and Fox, 2000). Therefore, SDZ NKT343 is clearly the most effective and potent NK_1 receptor antagonist in animal models. Unfortunately, clinical confirmation of the antihyperalgesic effects of SDZ NKT343 is not available.

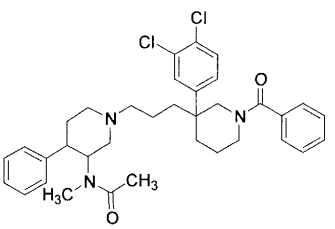


Scheme 3: Selection of non-peptide antagonists for NK_1 receptors.

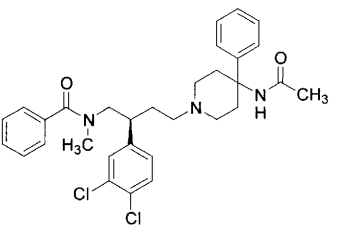
Moreover, duration of central NK_1 receptor blockade is a critical point, as anesthetic-like nerve block caused by non-specific effects on ion channels in peripheral tissues could mask the selective antinociceptive effects of blocking NK_1 receptors in the spinal cord. The long-acting NK_1 antagonist L-733,060 maintained blockade of central NK_1 receptors at a time when peak plasma drug levels had subsided. Therefore, in paw licking experiments, the inhibitory effect of L-733,060 appeared to be due to central NK_1 receptor blockade (Rupniak et al., 1996).

NK_1 receptor antagonists exhibit weak potency in acute pain, whereas antinociceptive effects can only be observed after persistent peripheral inflammation, i.e. models of chronic pain (Radhakrishnan et al., 1998; Saria, 1999). This view has been confirmed with the discovery of the even more selective SDZ NKT343, mentioned above (Walpole et al., 1998a,b).

It should be mentioned here that discovery of the tachykinin receptor antagonists has not been limited to the NK_1 receptor, as non-peptide antagonists for the NK_2 receptor (e.g SR48,968) and NK_3 receptor (e.g. SR142,801) are known.



SR-142801 (Osanetant)
phase II clinical trials for schizophrenia, depression, and anxiety
(Sanofi-Synthelabo)

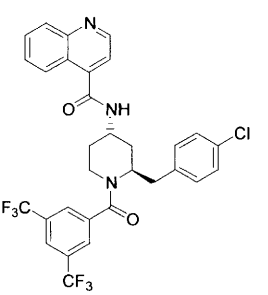


SR-48968 (Saredutant)
phase II clinical trials for asthma, incontinence, and depression
(Sanofi-Synthelabo)

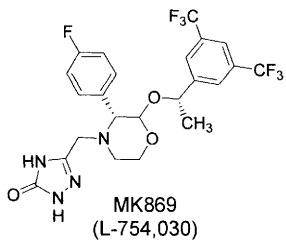
Scheme 4: NK_2 and NK_3 receptor antagonists in clinical trials.

Non-Peptide SP Antagonists

Non-peptide SP antagonists belong to a number of different structural classes: steroids, perhydroisoindolones, benzylamino and benzylether quinuclidine, benzylamino piperidines, benzylether piperidines, other piperidine-based structures and tryptophan-based antagonists (Quartara and Maggi, 1997; Argyropoulos and Nutt, 2000). From these, only two compounds progressed to Phase II trials in depression. The Novartis compound NKP608 is in Phase II trials for depression and social phobia, as well as chronic bronchitis (Argyropoulos and Nutt, 2000). MK869, also known as L754,030, from Merck was studied for depression. In clinical trials carried out at four sites, MK869 was found to be well tolerated, and its efficacy in major depressive disorder, was comparable to that of the serotonine uptake inhibitor paroxetine (20 mg daily, a moderate clinical dose; Kramer et al., 1998; Wahlestedt 1998). In addition, MK869 caused a lesser degree of sexual dysfunction. On the other hand, the high dose (300 mg) of MK869, producing micromolar plasma levels, and



NKP608



MK869
(L-754,030)

delayed onset of clinical efficacy (no effect before 2 to 3 weeks), presented no real progress compared to known monoamine uptake inhibitors. Trials with MK869 for treatment of depression were suspended in Phase II. Trials for anxiety disorders and schizophrenia continue. The compound has also been tested for emesis (Navari et al., 1999).

Clinical Use of SP or NK_1 Receptor Antagonists for the Treatment of Pain

Clinical pain studies

Before the first clinical studies started, there was a strong expectation that NK_1 antagonists would be clinically efficacious analgesics. But the clinical trials have been very disappointing in terms of confirming the efficacy of NK_1 receptor antagonists in alleviating pain. NK_1 receptor antagonists fail to provide the level of sensory blockade required to produce clinical analgesia in humans (Rupniak and Kramer, 1999; Hill, 2000). However, the number of published clinical pain trials for NK_1 receptor antagonists remains limited, and many of the studies are only presented in abstract form. Results for clinical pain trials with different NK_1 antagonists have been summarized in Table 2 (according to Rupniak and Hill (1999)).

Table 2: Analgesic activity of NK_1 receptor antagonists (Rupniak and Hill 1999; with preliminary findings).

Clinical condition	Compound	Effect
dental pain	CP99994 (0.75 mg/kg, i.v.)	analgesia equivalent to ibuprofen
	CP122721 (200 mg, p. o.)	weak analgesia
	MK869 (300 mg, p. o.)	no analgesia
ostheoarthritis	LY303870 (600 mg, p.o., b.i.d.)	no analgesia
neuropathic pain	CP99994 (0.1 mg/kg, i.v.)	no analgesia
	LY303870 (200 mg, b.i.d.)	no analgesia
	MK869 (300 mg, p. o.)	no analgesia
migraine	RPR100893 (20 mg, p.o.)	no analgesia
	LY303870 (240 mg, p.o.)	no analgesia
	L758298 (60 mg, i.v.)	no analgesia
	GR205171 (25 mg, i.v.)	no analgesia

Assessment of dental pain following molar extraction represents one of the clinical evaluation methods for

analgesic efficacy of NK_1 antagonists. During one study for postoperative dental pain, CP-99,994 (750 µg/kg i.v., over 5 h) was observed to be as effective as ibuprofen (Dionne et al., 1996). Findings with other NK_1 antagonists have been less encouraging. It is noteworthy that L-754,030 (300 mg p.o.) was reported to be ineffective as an analgesic for postoperative dental pain (Reinhardt et al., 1998), whereas it was clinically effective in antagonizing the effects of SP on forearm blood flow in humans (Newby et al., 1999). However, we should take into account that the dental pain model is considered to be a model of acute pain. Therefore, its use as a test setting for NK_1 receptor antagonism in humans is questionable, given that results from behavioral studies and knock-out mice suggest that a role for NK_1 receptor antagonists in acute pain is unlikely. For osteoarthritis with moderate joint pain, the effects of LY303870 (lanepitant) were compared against the reference analgesic, naproxen, in one clinical study (Goldstein, 1998). LY303870 was found to be ineffective when given as a twice daily treatment at doses up to 600 mg per-orally for 3 weeks.

In a study of painful peripheral neuropathy, CP99,994 ($\leq$ 100 µg/kg i.v., over 2 h) had no analgesic effect (Suarez et al., 1994). Thus, to date clinical studies indicate that NK1 receptor antagonists are unlikely to be general analgesics (Rupniak and Kramer, 1999).

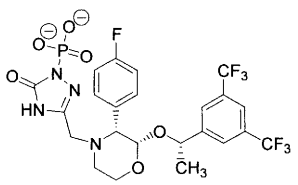
Clinical Use of NK_1 Receptor Antagonists for the Treatment of Migraine, Depression and Emesis

Clinical trials with NK_1 receptor antagonists for migraine, depression, and emesis were considerably more succesful than those for pain. These studies are excellently reviewed by May and Goadsby (2000, 2001).

Clinical studies for migraine

Migraine headache is characterized by an intense unilateral throbbing pain, likely caused by diameter changes in extracranial, and most likely intracranial, arteries (Wolff, 1963). The source of the migraine headache is still not clear. The dura mater and its small vessels are suggested as an important parameter of headache pain. Mechanisms during initiation of migraine attacks may be explained by the model of neurogenic inflammation in the dura, characterized by plasma protein extravasation, vasodilatation, increased endothelial permeability and mast cell degranulation (Buzzi and Moskowitz, 1990; Moskowitz, 1992). In animal models of neurogenic inflammation, GR-203040 (Beattie et al. (Glaxo Wellcome), 1995b), lanepitant (LY-303870, Phebus et al. (EliLilly & Co), 1997), GR-82334 (O´Shaughnessy

and Connor (Glaxo Wellcome), 1994), dapitant (RPR-100893, Lee et al. (Aventis Pharma AG), 1994), RP-67580 (Shepheard et al. (Aventis), 1993) and CP-99994 (Shepheard et al. (Pfizer), 1995) are all highly potent in blocking plasma protein extravasation. These results provide a substantial part of the preclinical rationale for the study of NK_1 antagonists in migraine. The first study employed RPR 100,893 at doses up to 20 mg (Diener, 1995), no headache relief was found. Similarly, in a double-blind, placebo-controlled, three-way crossover study, the NK_1 antagonist lanepitant (LY 303870), in doses up to 240 mg orally, was inactive in migraine (Goldstein et al., 1996).



L-758298

Under the assumption that these compounds were not convincingly lipophilic, 25 mg GR205171 (Connor et al., 1998) and up to 60 mg L-758,298 (Norman et al., 1998) were administered intravenously. But again, these antagonists had no significant effect over placebo-controls in the double-blind studies. Most recent clinical evidence suggests, however, that perhaps vasoconstrictor properties are required to some degree to provide antimigraine effects, and this may be the reason why treatment with highly selective substance P receptor antagonists is insufficient (Cutler et al., 2000; May and Goadsby, 2001).

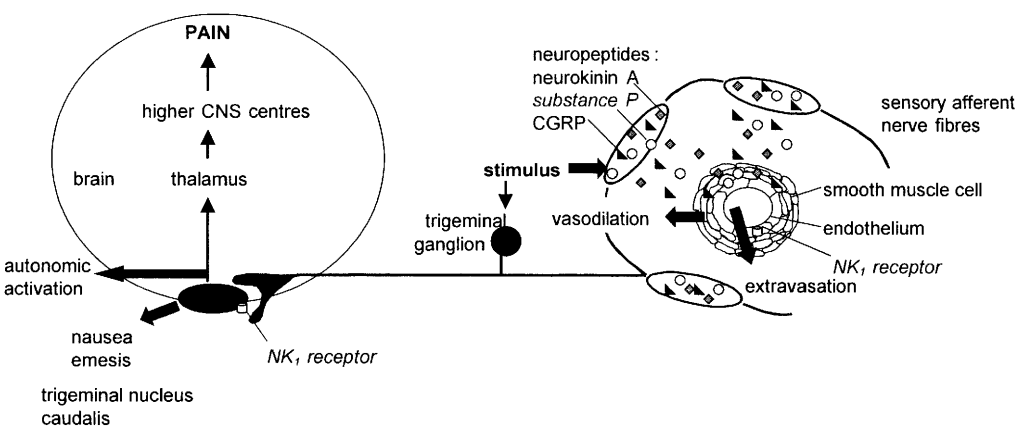


Figure 1: Diagrammatic representation of the putative pathophysiological mechanisms involved in the development of migraine headache and the potential sites of action of NK_1 receptor antagonists (adapted from Longmore et al., 1997).

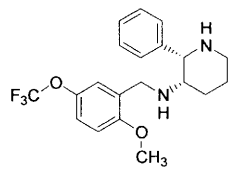
Clinical studies for depression

The localization of SP in brain regions that coordinate stress responses, as well as behavioral studies with mice

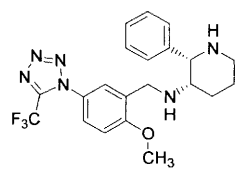
lacking the receptor for SP (De Felipe, 1998), suggest that NK_1 receptor antagonists might have psychotherapeutic properties. CGP49823 possesses significant anxiolytic properties (File, 1997). MK869 (non-peptide substance P antagonist) was tested in the treatment of moderate to severe depression, in a proof-of-concept study, as already described. The efficacy of MK869, as with other antidepressants, was expressed 2 to 3 weeks following the onset of treatment, suggesting the possibility of a common pathway for the action of antidepressants (Nutt, 1998; Wahlestedt, 1998). But the real progress was that co-morbid anxiety was treated very effectively with MK869 (Kramer et al., 1998; Nutt, 1998).

Several NK_1 receptor antagonists (including CP-99,994, GR203040, GR205171 and CP-122,721), due to their anti-emetic activity, offer the prospect of a novel approach for the control of emesis associated with, for instance, cancer chemotherapy. L754,030 has been shown to prevent emesis after treatment with cisplatin (Navari et al., 1999). Oral CP-122,721 200 mg decreased emetic episodes compared with ondansetron (4 mg i.v.) during the first 24 h after gynecologic surgery (Gesztesi et al., 2000). The NK_1 antagonists appear to be no more effective than $5HT_3$ receptor antagonists in preventing acute emesis. But they are well tolerated, and could prove useful, in combination with other compounds, as an alternative for delayed emesis (Hesketh, 2001). Effects of SP/NK_1 receptor antogonists on schizophrenia and affective disorders are reviewed by Rupniak and Kramer (1999).

Clinical studies for emesis



CP-122721



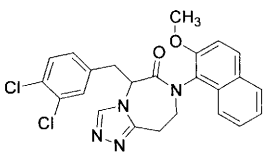
GR-205171

Conclusions and Implications for Future Studies

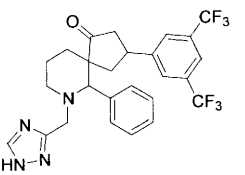
The trials described so far have commonly shown a lack of usefulness of NK_1 receptor antagonists in the treatment of pain. But we do not know whether the failure of the selected compounds is a matter of pharmacodynamics (e.g. poor penetration of the blood brain barrier) or a genuine discrepancy between animal and human pain pathophysiology (Urban and Fox, 2000). Hence, animal tests should carefully be chosen whether they are predictive or not, and it would be helpful if a wider range of conditions could be examined (Hill, 2000a). Therefore, new preclinical analysis methods should be developed for a more effective judgement of likely clinical outcomes.

Furthermore, the discovery of novel compounds with higher therapeutic potential can be foreseen. A selection of compounds which were filed recently for the treatment of pain and migraine is given below. As all of the non-peptide tachykinin receptor antagonists arose from

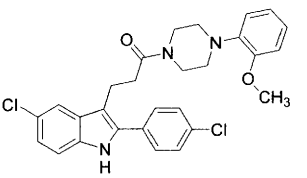
targeted screening of large substance libraries using a robust radioligand binding assay, future high throughput techniques will provide further compounds with better pharmacological properties. Combination of such substances with existing analgesics could prove to be beneficial. Such co-medications will prove helpful in patients affected not only by chronic pain but also depression. As a result, we should not give up the studies with NK_1 receptor antagonists with respect to analgesis!



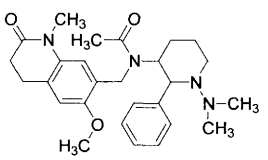
Galley et al. (Hoffmann-La Roche) WO 0190083



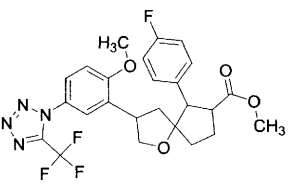
Kulagowski (MSD), WO 0047562



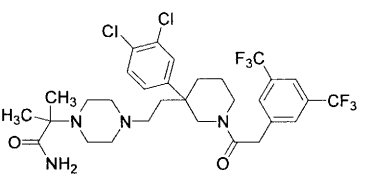
Chapman et al. (MSD), WO 0051984



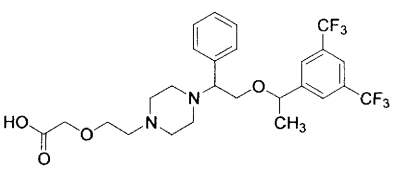
Arnold et al. (Pfizer), WO 0177100



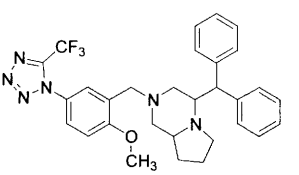
Durette et al. 1999 (MSD) US 5929094



Ducoux et al. (Sanofi-Synthelabo) WO 0047572



Stiernet et al. (UCB), WO 0146167



Take et al. (Fujisawa), WO 0200631

Scheme 5: Selection of substanceP/NK1-antagonists filed for the treatment of pain and migraine.

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15 $CGRP_1$-Receptor Antagonists

Clemens Gillen

Introduction

Calcitonin gene-related peptide αCGRP is a 37-amino acid neuropeptide that was first identified in 1982 as an extremely potent vasodilator (Amara et al., 1982). It is generated by alternative splicing from the calcitonin gene and has a characteristic 7-amino acid ring formed by a disulfide bridge between position 2 and 7 and an amidated N-terminus. A second CGRP homolog, βCGRP, with high sequence homology was subsequently isolated and is derived from a different gene. Both peptides display rather similar biological effects that include vasodilatation, increased regional blood flow, hypotension and tachycardia.

First identification of CGRP as an extremely potent vasodilator

The high expression in pain-relevant areas of the nervous system together with its dual role as potent vasodilator and excitatory neuromodulator suggested a role for CGRP in pain or migraine and led to intense research on this topic.

CGRP Family of Peptides

The CGRP family of peptides includes the 37-amino acid neurotransmitter CGRP, the 52-amino acid adrenomedullin, found predominantly in vascular cells and adrenal tissue and the 37-amino acid amylin found in pancreatic islet β-cells. These peptides share a 6-amino acid ring structure, formed by an intramolecular disulfide bridge.

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human_CGRPα                    ACDTATCVTHRLAGLLSRSGGVVKNNFVPTN-VGSKAF-NH2 37
human_CGRPβ                    ACNTATCVTHRLAGLLSRSGGMVKSNFVPTN-VGSKAF-NH2 37
rat_CGRPα                      SCNTATCVTHRLAGLLSRSGGVVKDNFVPTN-VGSEAF-NH2 37
rat_CGRPβ                      SCNTATCVTHRLAGLLSRSGGVVKDNFVPTN-VGSKAF-NH2 37
rat_amylin                     KCNTATCATQRLANFLVHSSNNFGAILSSTN-VGSNTY-NH2 37
rat_adrenomedullin YRQSMNQGSRSTGCRFGTCTMQKLAHQIYQFTDKDKDGMAPRNKISPQGY-NH2 50
                                 *  .**. ::**  : :  .    : . * :..: :
```

Figure 1: Comparison of the CGRP family peptides. The amino acids are annotated in the following way: small and hydrophobic in plain text, acidic in bold, basic in bold italics and hydroxyl or amine in italics. "*" Indicates identical or conserved residues in all peptides; ":" indicates conserved substitutions and "." indicates semi-conserved substitutions.

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Receptor Pharmacology

The first pharmacological tools available for studying the CGRP receptor were derivatives of CGRP. The N-terminally truncated fragment CGRP(8-37) is antagonistic, whereas a linear peptide like ([Acetamidomethyl-cysteine^{2,7}]CGRP (Cys(ACM^{2,7})CGRP)) behaves as an agonist. The analysis of these CGRP peptide analogs *in vitro* and *in vivo* allowed the subdivision into a $CGRP_1$ and a $CGRP_2$ receptor subtype, a nomenclature initially proposed by Dennies et al. (1989). The $CGRP_1$ receptor subtype is antagonized by CGRP(8-37) ($pA_2 = 7.7$) and Cys(ACM^{2,7})CGRP is weak or inactive as an agonist. As a functional example for the $CGRP_1$ receptor the guinea pig atrium is used.

$CGRP_1$ and $CGRP_2$ receptor subtypes

The $CRGP_2$ receptor subtype is selectively activated by Cys(ACM^{2,7})CGRP, but only weakly antagonized by CGRP(8-37) ($pA_2 = 6.5$). A prototypical *in vitro* bioassay to study the $CGRP_2$ receptor is the rat vas deferens. The above-mentioned nomenclature is under discussion, because it is based on poorly selective and weak agonists and antagonists but the $CGRP_1$/$CGRP_2$ subdivision is still generally used. Evidence for further CGRP receptor subtypes comes from studies demonstrating CGRP(8-37)-insensitive βCGRP-activated receptors. More recently, the use of the non-peptide antagonist BIBN-4096BS suggests a further subtype expressed in rat vas deferens. Although CGRP was one of first neuropeptides to be identified, the molecular basis of the two CGRP receptors is still under debate.

Evidence for further CGRP receptor subtypes

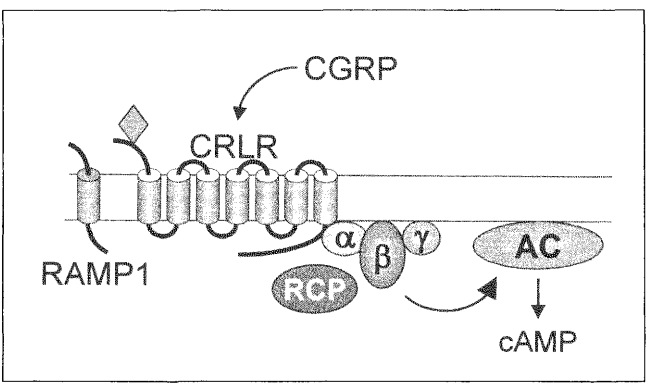


Figure 2: Model of the $CGRP_1$ receptor complex. The $CGRP_1$ receptor is a heteromer of CRLR and RAMP1 that couples predominantly via G_S proteins to adenylate cyclase (AC). The receptor component protein (RCP) is essential for G protein activation.

Molecular characterization of $CGRP_1$

The reason for these difficulties became apparent after successful molecular characterization of the $CGRP_1$

receptor. The $CGRP_1$ receptor has a unique phenotype since it is a receptor complex built up by a classical heptahelical G protein-coupled receptor, the so called calcitonin receptor-like receptor (CRLR) and the receptor-associated modifying protein 1 (RAMP-1). In addition another protein, the receptor component protein (RCP) binds intracellularly to the CRLR/RAMP-1 complex and is essential for the efficient activation of G_S proteins (Juaneda et al., 2000; see Fig. 2). The molecular basis of the $CGRP_2$ receptor and the additional subtypes is currently unknown.

Signaling

Activation of the CGRP receptor is linked to several intracellular pathways. The CRLR/RAMP1 heteromer can couple to pertussis toxin- (PTX-) insensitive G proteins, leading to production of cAMP (Main et al., 1998). The same authors also found coupling through PTX-sensitive G proteins, suggesting that the recombinant $CGRP_1$ receptor may signal through G_i and G_o proteins. Using primary cultures of rat neonatal spinal cord an increase in cGMP could be observed, suggesting that the CGRP receptor also couples to the guanylcyclase (Parsons and Seybold, 1997) although this effect only became apparent at high CGRP concentrations. In non-neuronal cells a coupling of the native CGRP receptor to the MAP kinase signaling pathway (Kawase et al., 1999) and to phospholipase C leading to increased intracellular Ca^{2+} concentrations was found (Drissi et al., 1998). In conclusion, the most important signaling mechanism is undoubtedly the G_s protein-mediated increase in cAMP concentrations.

Evidence for a Role of CGRP in Migraine

The pathophysiology of migraine is still under debate. The predominant current opinion is that migraine is a neurovascular disorder with the primary trigger potentially occurring in the CNS. This trigger ultimately leads to vasodilatation with a subsequent activation of trigeminal afferent sensory neurons activating central `nociceptive´ neurons projecting to higher pain centers (see Fig. 3; for review see: Hargreaves and Shepheard, 1999).

Animal studies supporting a role for CGRP in migraine

Several lines of investigation have provided evidence to support the theory that CGRP plays a role in migraine. CGRP is the most abundant primary afferent peptide in trigeminal sensory nerves. Since CGRP is one of the most potent vasodilators known and the $CGRP_1$ receptor is

expressed on the endothelial cells of the meningeal arteries CGRP is presumed to be a key mediator of meningeal vasodilatation. In addition to these physiological considerations several animal studies support the proposal that $CGRP_1$ receptors play a role in migraine. Stimulation of the trigeminal ganglion in anesthetized rats leads to meningeal vasodilatation, which could be inhibited by the CGRP1 antagonist CGRP(8-37) (Kurosawa et al., 1995). This experiment implied that CGRP were elevated after trigeminal stimulation, an observation that had been reported by Goadsby and Edvinsson (1993). Interestingly, these elevated CGRP levels could be reduced by treatment with migraine drugs like sumatriptan or dihydroergotamine (Goadsby and Edvinsson, 1993).

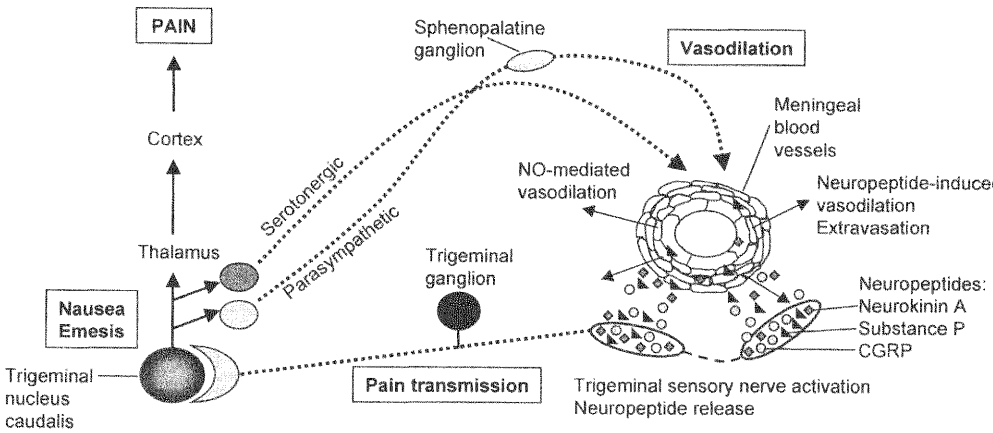


Figure 3: Integrated hypothesis of pathogenesis of migraine headache and associated symptoms, and role of trigeminovascular system (adapted from P.J. Goadsby and R.J. Hargreaves). Intense neurometabolic cortical activity such as cortical spreading depression (CSD) is a trigger for migraine. The release of potassium ions and protons, neurotransmitters and metabolites into the extracellular space causes transient hyperemia and vasodilation in cortex, pia vessels and dura mater. This sensitizes and ultimately activates perivascular trigeminal afferents which transmit impulses to the trigeminal ganglia and the trigeminal nucleus caudalis (TNC). Impulses from the TNC are carried rostrally to brain structures involved in pain processing and perception. Depolarization of the trigeminal afferents leads to the retrograde perivascular release of vasoactive neuropeptides such as neurokinin A, substance P and CGRP. Activation of the ipsilateral TNC leads to stimulation of the superior salivatory nucleus (SSN) and parasympathetic efferents via the spenopalatine ganglion. Postganglionic parasympathetics promote vasodilation and augment flow releasing vasoactive intestinal peptide, nitric oxide and acetylcholine into the dura mater.

Another study described the CGRP-induced histamine release from dural mast cells that could be blocked by CGRP(8-37) (Ottoson and Edvinsson, 1997). Therefore

CGRP together with substance P may also play a role in migraine-associated neurogenic inflammation.

Elevated CGRP levels in patients with migraine or cluster headache

The analysis of CGRP plasma levels in human volonteers was found to be a important diagnostic parameter, since elevated plasma levels were found in patients with acute migraine and in patients with cluster headache (Edvinsson and Goadsby, 1994) as well as in humans after trigeminovascular stimulation. In accordance with the above-mentioned animal studies the elevated CGRP levels in migraineurs were reported to be reduced by sumatriptan or dihydroergotamine (Goadsby and Edvinsson, 1993). A mechanistic explanation was given by Durham and Rosso (1999) who found that sumatriptan can directly repress CGRP secretion from cultured trigeminal neurons. Here the activation of $5HT_1$ receptors inhibits the release via an increase in phosphorylase activity that is likely mediated by a sustained elevated level of intracellular calcium. In conclusion, there is strong evidence that a $CGRP_1$ antagonist should provide effective treatment for migraine and cluster headache.

In Vivo Evidence for a Role in Pain

CGRP is highly abundant in somatic sensory nerves. Here CGRP often coexists with other neuropeptides such as substance P. Immunocytochemical studies have shown that substance P is nearly always associated with CGRP in small type DRG neurons, whereas CGRP can be localized without substance P (Wiesenfeld-Hallin et al., 1984). In the periphery the CGRP released by sensory neurons leads to vasodilatation and together with neuropeptides like substance P mediates neurogenic inflammation. CGRP is also released in the dorsal horn following peripheral noxious stimulation. Like SP, it also produces slow depolarization of spinal dorsal horn neurons and can potentiate the depolarizing effect of SP. Three different strategies were used to clarify the role of the CGRP receptors in pain. The $CGRP_1$ antagonist CGRP(8-37) was tested in pain models. In order to antagonize endogenous CGRP an anti-CGRP antiserum was given intrathecally in several pain models. The generation and behavioral analysis of αCGRP-deficient mice completes the picture.

Antinociceptive effect of the CGRP antagonist CGRP(8-37) in several pain models

The antinociceptive effect of CGRP(8-37) was observed in different pain types of such as abdominal pain (p-phenylquinone induced writhing, Saxen et al., 1994; acetic acid-induced writhing, Friese et al., 1997), visceral pain (colorectal distension model, Plourde et al., 1997), burn pain (heat-induced hyperalgesia; Lofgren et al., 1997), and

central neuropathic pain (spinal hemisection; Bennett et al., 2000). No effect was observed in mouse tail-flick as a model of acute pain (Saxen et al., 1994).

In order to block the effects of spinally released CGRP, an antiserum against CGRP was tested in several models of chronic pain and was found to be antinociceptive in models of chronic inflammatory pain such as adjuvant-arthritis (Kuraishi et al., 1988) or carrageenan-induced hyperalgesia (Kawamura et al., 1989). In addition this antiserum inhibited the repeated cold stress-induced hyperalgesia in rats (Satoh et al., 1992).

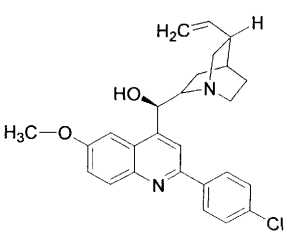
$CGRP^{-/-}$ mice show reduced hyperalgesia

The antinociceptive effects seen with CGRP(8-37) or anti-CGRP antiserum are in line with the hyperalgesia found in αCGRP-deficient mice produced by Salmon et al. (1999). The $CGRP^{-/-}$ mice showed in comparison with $CGRP^{+/+}$ mice a reduced hyperalgesia during chronic inflammatory pain, elicited by injection of formalin or capsaicin into the hindpaw (Salmon et al., 2001). The authors also described reduction in abdominal pain, since the intraperitoneal injection of acetic acid yielded fewer writhing reactions. The injection of carrageenan into the hind paw of $CGRP^{-/-}$ mice showed reduced edema formation and indicates a role for CGRP in neurogenic inflammation.

A second αCGRP-deficient mouse was produced by Hoff et al. (1998) in order to study the role of calcitonin. $CGRP^{-/-}$ mice are born normally, are fertile and live a normal life span. These mice were tested in a model of chronic arthritis, where a mixture of kaolin/carrageenan was injected into the knee joint and in comparison to wild type mice failed to develop secondary hyperalgesia (Zhang et al., 2001).

Assuming that we can apply these results to the human situation, $CGRP_1$ antagonists may also be effective for treatment of chronic inflammatory and visceral pain but not for acute pain. A thorough evaluation of this receptor in neuropathic pain is still missing.

Nonpeptidic Antagonists and their Current Status

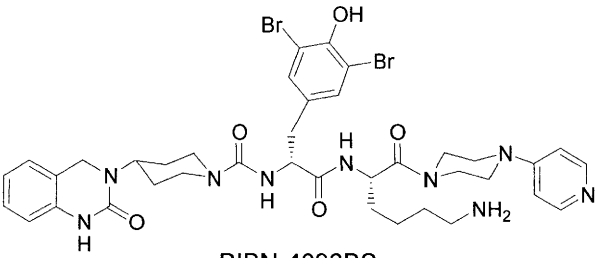


SmithKline Beecham, WO9709046

In 1997 scientists from SmithKline Beecham published a series of quinine analogs as CGRP antagonists (Daines et al.1997, WO9709046). These compounds displayed only weak affinities for the human CGRP receptor in the micromolar range and were therefore not of great importance (Daines et al., 1997).

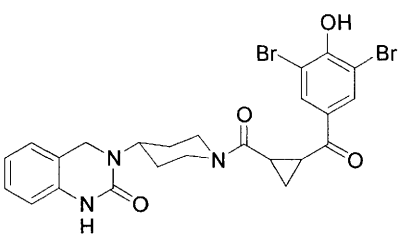
A major breakthrough in the field was the development of the first highly potent non-peptide CGRP receptor antagonist BIBN-4096BS (Eberlein et al. (Boehringer

Ingelheim) DE19911039, 2000; for a review see: Doods et al., 2001). This compound, a Tyr-Lys dipeptide derivative, is a pure antagonist with high affinity towards the human $CGRP_1$ receptor (K_i = 14.4 pM).

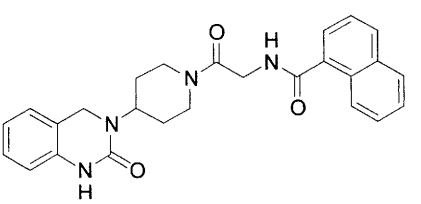


BIBN-4096BS

In studies using human cerebral vessels BIBN-4096BS was able to reverse CGRP-mediated vasodilatation (Doods et al., 2000). Stimulation of the marmoset trigeminal ganglion leads to an increase in facial blood flow. In this model of neurogenic inflammation, BIBN-4096BS showed a rapid and dose-dependent inhibition of the increased blood flow (Doods et al., 2000). Furthermore, in anesthetized marmosets BIBN-4096BS inhibited CGRP-mediated neurogenic vasodilatation in doses from 0.001 to 0.03 mg/kg, whereas concentrations up to 1 mg/kg BIBN-4096BS did not affect cardiovascular parameters (Doods et al., 2000). This may be explained by low basal CGRP levels. Although high doses of CGRP are cardioprotective, the $CGRP_1$ antagonist BIBN-4096BS had no negative effect on myocardial infarct size or release of creatine phosphate kinase. In conclusion BIBN-4096BS may provide a therapeutic migraine intervention without cardiovascular side-effects (Wu et al., 2001). In a recent publication it was shown that the CGRP-induced relaxation of human coronary arteries was blocked by BIBN-4096BS with a pA2 value of 10.4 compared to an pA2 value of 10.2 in human cerebral arteries. In contrast to the results in marmosets described above, this study assumes cardiovascular side-effects for this compound (Edvinsson et al., 2002). BIBN-496BS is currently in phase II clinical studies for the treatment of migraine.

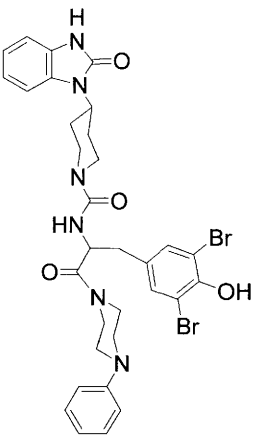


Boehringer Ingelheim, WO0132648



Boehringer Ingelheim, WO0132649

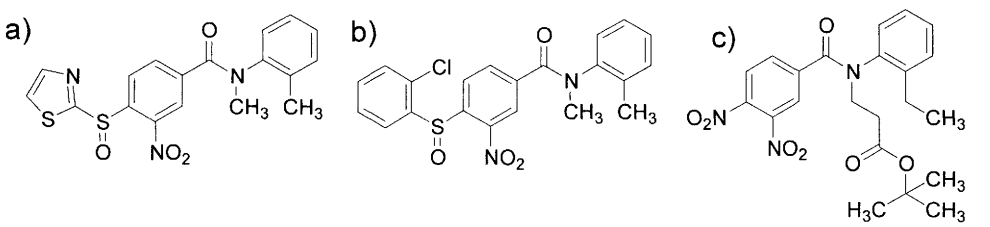
Based on this structure Boehringer Ingelheim synthesised and filed patents on compounds where the dipeptide core of BIBN-496BS is replaced by a cyclopropyl ring (Eberlein et al., WO0132648). In a recent patent application Boehringer Ingelheim also claimed naphtalenes, piperidines, imidazoles, and quinazolines as CGRP receptor antagonists without disclosing any functional properties of the molecules (Rudolf et al., WO0132649). In addition piperidine-substituted amino acids have been filed by Boehringer Ingelheim (Rudolf et al., WO0149676).



Boehringer Ingelheim, WO9811128

Interestingly Boehringer Ingelheim has also filed a patent application on a structure that is derived directly from BIBN-4096BS and can be described as a De-Lys-BIBN-4096BS-derivative (Rudolf et al., WO9811128). This so called 'compound 1' (WO9811128) was resynthesized by scientists from Merck, Sharp and Dohme and presented as a functional CGRP receptor blocker in human SK-N-MC cells with a pK_i value of 7.8 and in human cerebral arteries with a pK_i value of 7.7 (Edvinsson et al., 2001). In porcine coronary arteries 'compound1' demonstrated no antagonistic effect (Hasback et al., 2001) that may be explained by a species-specific binding profile.

SmithKline Beecham is another company with interests in CGRP receptor antagonists and has filed patent applications for two structural classes, the 3,4-dinitrobenzamides (Daines et al., WO9809630) and 4-sulfinyl benzanilides (Daines et al., WO9856779).



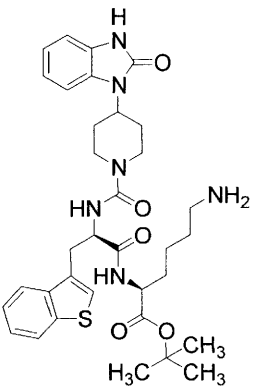
CGRP antagonists from SmithKline Beecham:
a) SB-(+)-273779, b) WO9809630, c) WO9856779.

One of these 4-sulfinyl benzanilides, SB-(+)-273779 inhibited ^{125}I-labeled CGRP binding to SK-N-MC cells and human cloned $CGRP_1$ receptor with K_i values of 310 nM and 250 nM, respectively (Aiyar et al., 2001). Detailed binding analysis suggests that this compound has irreversible binding characteristics. The authors demonstrated that SB-(+)-273779 inhibits several CGRP-mediated effects such as vasodilation of the pulmonary artery, [^{14}C]deoxyglucose uptake in L6 cells or decrease in

blood pressure in anesthetized rats (Aiyar et al., 2001). Unfortunately SB-(+)-273779 exhibits several limitations including poor solubility, poor oral availability and a short half life of ~10 min that precluded an extensive *in vivo* characterization (Aiyar et al., 2001).

The activities of Merck, Sharp and Dohme have also led to a patent application disclosing benzamidoyl-piperidine derivatives to be CGRP antagonists (Hill et al., WO0018764). The compounds are claimed to inhibit [^{125}I]CGRP binding to SK-N-MC cell membranes with K_i values below 10 µM.

It is interesting to note that although several major pharmaceutical companies have tried to develop CGRP antagonists, only few compounds have been published. Together with the fact that most of them are bulky structures - like BIBN-4096BS or 'compound 1' (WO9811128) - this may indicate a general structural hindrance in the development of small molecule CGRP antagonists. At the moment BIBN-4096BS remains to be the only compound that is suitable for studying the role of CGRP in clinical trials.



Merck, Sharp and Dohme, WO0018764

Perspectives

There are several lines of evidence that propose a key role for CGRP in the pathophysiology of migraine. In current practice, the first line treatment for migraine are NSAIDS but the triptans as mixed 5HT1b/D agonists are the gold standard. Several triptans are on the market now that differ mainly in onset of action and oral availability. However there is a high percentage of non-responders (up to 30%) and a high recurrency rate (25-35%) with all these compounds. For this reason new and effective drugs are needed. After the failure of several new approaches such as NK1 antagonists, endothelin antagonists and inhibitors of protein plasma extravasation, potential migraine treatment is based on targets like CGRP or NO (for review see: Doods et al., 2001). Therefore the results of the clinical trials with BIBN-4096BS for acute migraine attacks are awaited with great interest. The evidence for the role of CGRP antagonists in chronic pain is quite convincing, since an antinociceptive/hypoalgesic effect has been shown with the peptidic antagonist CGP(8-37), an anti-CGRP antiserum and αCGRP-deficient mice. However, a thorough evaluation of the non-peptidic tools in different animal models of pain is essential but hampered by the fact that a central acting analgesic is needed here. On the molecular level the nature of the different CGRP receptor subtypes has to be elucidated. For this purpose the non-

peptidic antagonists are also useful. Current clinical studies using BIBN-4096BS will show if CGRP antagonists provide effective treatment for migriane with fewer side effects. If these studies have a positive outcome, this class of drugs may be of use in further indications like chronic pain or inflammation.

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16 Nitric oxide : Potential of NO Donors and NO Synthase Inhibitors for the Treatment of Pain

Corinna Maul, Hagen-Heinrich Hennies and Bernd Sundermann

In recent years, nitric oxide (NO) has emerged as one of the most interesting mediators of normal and patho physiological processes. NO is a highly reactive free radical, a lipophilic gas with a very short half-life in the range of 5 - 30 s under bioassay conditions (Palmer et al., 1987). NO is rapidly converted to nitrogen dioxide (NO_2), which again rapidly forms the more stable metabolites nitrite (NO_2^-) and nitrate (NO_3^-).

Direct vascular effects of NO

As Furchgott, Ignarro and Murad have shown, NO is essential for keeping blood vessels wide open to maintain blood flow and pressure. In atherosclerosis, in which plaques occlude the coronary arteries, the cells lining the blood vessels produce less NO. The work that led to the Nobel prize in 1998 explains why patients with chest pain (angina pectoris) caused by atherosclerosis get relief from pills containing nitroglycerine, which, once it has entered the smooth muscle cells, releases NO (Ignarro, 1999; Furchgott, 1999; Murad, 1999).

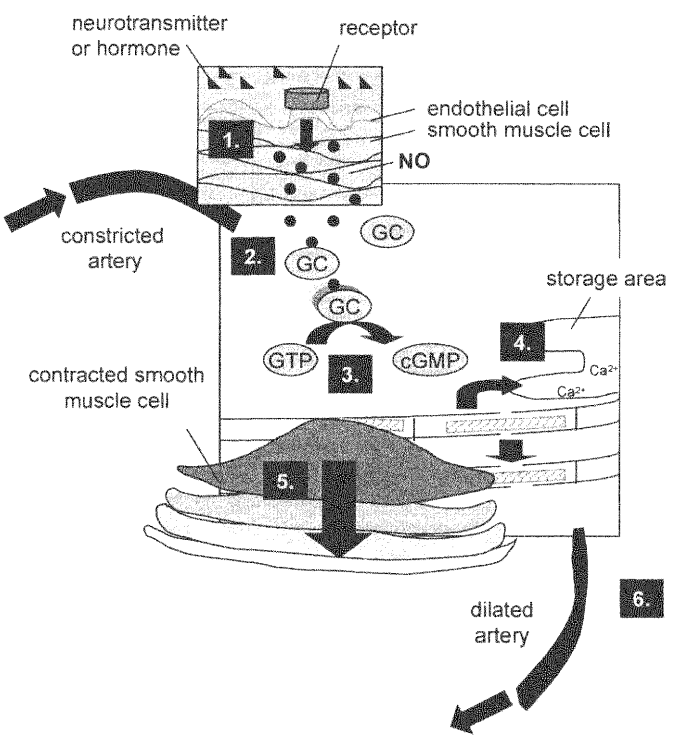
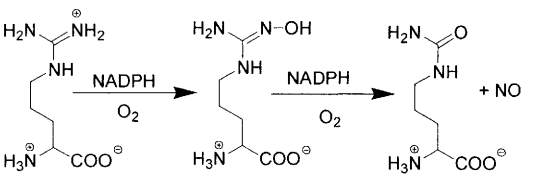


Figure 1: Mechanism of action of NO.
1. Neurotransmitter or hormone bind to receptors on endothelial cells lining the artery, which in response releases nitric oxide (NO). 2. NO molecules from the endothelium travel into smooth muscle cells, where they activate the enzyme, guanylyl cyclase (GC). 3. GC converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). 4. cGMP causes calcium ions to enter storage areas of the cell. The lowered concentrations of calcium ions (Ca^{++}) set off a cascade of cellular reactions that cause the cell's contractile filaments (myosin and actin) to slide apart. 5. Smooth muscle cells relax. 6. Blood vessel dilates.

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Arginine: source of endogenous NO

In mammalian cells NO is produced by the oxidation of the terminal guanidino nitrogen of L-arginine (L-arg) by nitric oxide synthase (NOS). According to patent literature, NOS inhibitors have been one of the most intensively investigated research areas in industry of the last couple of years.



Scheme 1: NO formation from L-arg catalyzed by NOS.

Identification of three isoforms: nNOS, eNOS, iNOS

nNOS and the NMDA receptor are closely coupled: in nNOS-deficient mice, effects induced by PCP, an NMDA receptor antagonist, were not obtained (Bird et al. 2001)

Three distinct isoforms of NOS have been identified (Knowles and Moncada, 1994). Molecular cloning has shown these to share 50 - 60% homology. There is a constitutive form (neuronal NOS, nNOS), whose activity is regulated by Ca^{2+} and calmodulin, and which is found in neuronal tissue, both centrally and peripherally. nNOS is believed to play a role in the production of NO as a neurotransmitter. NO production in the brain is initiated by glutamate binding to the NMDA receptor, which is closely coupled to nNOS. A second, Ca^{2+}/calmodulin-requiring, constitutive enzyme (endothelial cell NOS, eNOS) is present in vascular endothelium where it regulates blood pressure and vascular tone. A third, Ca^{2+}-independent isoform (inducable NOS, iNOS) is induced in activated macrophages and other cell types by numerous inflammatory stimuli including lipopolysaccharide (LPS) and cytokines (e.g. IL-1) (Hobbs et al., 1999). Once induced, iNOS manufactures large amounts of NO over many hours and can have either a beneficial effect (e.g. host defense response) or a harmful effective induction is uncontrolled. iNOS has been implicated in the pathology of a large number of inflammatory conditions.

Localization of constitutive NOS isoforms

The constitutive isoforms of NOS are mainly, but not only, localized in the tissues where they were originally identified. In some brain regions, eNOS and nNOS occur in the same cell populations (Dinerman et al., 1994). In mice it was shown that hippocampal neurons express eNOS, and nNOS was found in human bronchi as well as in human skeletal muscle (Hobbs et al., 1999). In coronary arteries of eNOS knock-out mice, nNOS-derived NO, via activation of cGMP, takes over the role of eNOS and maintains blood flow-induced vessel dilation (Huang et al., 2002).

All NOS proteins possess a bi-domain structure, and dimerization to homodimers (≥ 260 kDa each), is required for enzyme activity (see Table1).

Activation of NOS through dimerization

Table 1: Characteristics of human NOS isoforms.
(Adapted from Wattanapitayakul, Young et al., 2000).

Isoform	MW (kDA)	Chromosome localization (Gene structure)	Gene size (kb)	Expressional regulation	Calcium dependence
NOS1 (nNOS)	160	12q24.2 (29 exons, 28 introns)	> 200	constitutive but highly regulated	yes
NOS2 (iNOS)	130	17q11.2-q12 (26 exons, 25 introns)	37	inducible by cytokines	no
NOS3 (ecNOS)	135	7q35-q36 (26 exons, 25 introns)	21	constitutive but highly regulated	yes

Known Features of NOS Protein Structure

The C-terminal portion of the NOS protein closely resembles to cytochrome P-450 reductase, possesses many of the same cofactor binding sites, and basically performs the same functions. Consequently, this portion is often referred to as the reductase domain. At the extreme C-terminus is an NADPH binding region, which is conserved in all NOS and aligns perfectly with that of cytochrome P-450 reductase. The NADPH binding site is followed, in turn, by flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) consensus sequences.

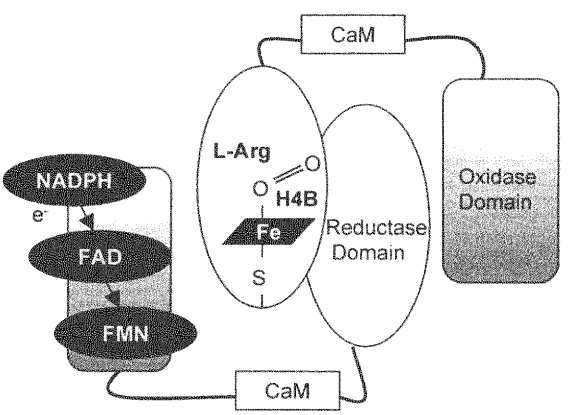


Figure 2: Hypothetical structure of NOS.

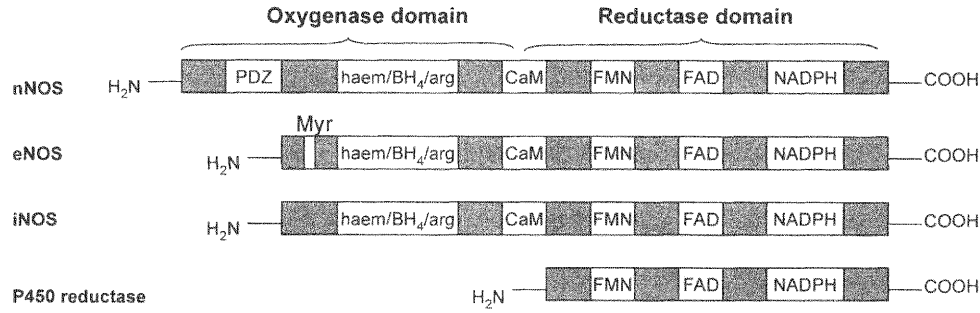


Figure 3: Schematic representation of nitric oxide synthase isoforms and cytochrome P450 reductase. Haem, heme; PDZ, PDZ domain (GLGF repeats); CaM, calmodulin; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide (adapted from Hobbs et al., 1999).

Unlike cytochrome P-450 reductase, NOS is a self-sufficient enzyme in that the oxygenation of its substrate, L-arginine, occurs at the heme-site in the N-terminal portion, termed the oxygenase domain, of the protein. Stoichiometric amounts of heme are present in NOS and are required for catalytic activity.

Nitroglycerine – an NO Donor Causing Severe Headache

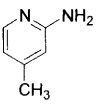
The analgesic activity of NO synthase inhibitors has not been known for a long time, although a connection was suggested. The discoverer of nitroglycerine, Ascanio Sobrero, had noted that exposure to this chemical can cause severe headaches. Alfred Nobel, who spent much of his time experimenting with this substance, must have had experienced this effect and later on, when nitroglycerine was produced on an industrial scale, it is reasonable to assume that it posed a serious medical and environmental problem, especially for his collaborators (Ringertz, 2000). On the other hand, Lauder Brunton, a distinguished British physician, had found in 1867 that organic nitrates were effective in relieving angina pectoris pain. When in 1890 Nobel's physicians recommended nitroglycerine as a remedy for his heart disease he declined it because he knew about the headaches caused by this compound.

NOS Inhibitors as Potential Analgesics

The analgesic effect of 2-amino-4-methylpyridine was reported in 1958 (Fastier and McDowall). In 1980 the

compound was described as a "morphine-like analgesic" (Bergman and Elam). A recent study published by Pfizer suggests that 2-amino-4-methylpyrdine acts by inhibiting neuronal NO synthesis (Pettipher et al. 1997).

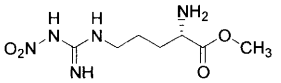
The analgesic effect of NOS inhibitors refers to the inhibition of nNOS and, in the case of inflammation, iNOS. Knock-out experiments support this thesis: mice lacking iNOS have a delay in the expression of neuropathic pain following a chronic constriction injury of nerves associated with neuropathic pain (Levy et al., 2001). For physiological changes observed in nNOS-, iNOS- and eNOS-knock-out mice, see Table 2.



2-Amino-4-methyl-pyridine, NOS inhibitor with analgesic activity [695-34-1]

Table 2: Physiological changes observed in nNOS-, iNOS- and eNOS-knock-out mice (adapted from Wattanapitayakul, Young and Bauer 2000).

Knock-out-mice	Physiological changes and responses to diseases	References
NOS1 (nNOS)	Resistance to cerebral ischemia	Huang et al. (1994)
	Reduction in dendrite numbers	Inglis et al. (1998)
	Resistance to NMDA-induced neurotoxicity *in vitro* and *in vivo*	Ayata et al. (1997); Dawson et al. (1996)
	Resistance to MPTP-induced neurotoxicity in the brain	Przedborski et al. (1996)
	Enhanced aggression in male mice	Nelson and Young (1998)
	Reduced aggression in female mice	Gammie and Nelson (1999)
	Deficiency in nocturnal motor coordination	Kreigfeld et al. (1999)
	Altered urinary bladder structure	Burnett et al. (1997)
	Pyloric sphincter hypertrophy, enlarged stomach	Huang et al. (1993)
NOS2 (iNOS)	Increased susceptibility to bacterial infection	Shiloh et al. (1999)
	Increased susceptibility to tuberculosis	MacMicking et al. (1997)
	Susceptible to experimental autoimmune encephalitis	Fenyk-Melody et al. (1998)
	Ssceptible to endotoxin-induced uveitis	Smith et al. (1998)
	Neuroprotective after endogenous traumatic brain injury	Sinz et al. (1999)
NOS3 (eNOS)	Hypertension and decreased heart rate Increased plasma renin activity Lack of response to ACh and calcium ionophore Increased sensivity to phenylephrine, serotonin, and nitroglycerine	Shesely et al. (1996); Kojda et al. (1999)
	Impaired ovulation and oocyte meiotic maturation	Jablonka-Shariff and Olson (1998)



L-NAME (N-Nitro-L-arginine-methylester), [50903-99-6]

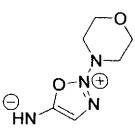
Studies to elucidate the functional role of NO have used inhibitors of NO synthase such as L-NAME and L-NMMA (see below). Systemic and intrathecal injections of NO synthase inhibitors have been shown to reduce noxious responses to formalin and carrageenan-induced hyperalgesia (Sakurada et al., 2001). Furthermore, NO-induced mechanical hyperalgesia has been reported to be mediated by supraspinal centers and does not occur in *in vitro* preparations of the spinal cord.

Antinociceptive properties of NO

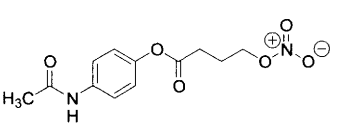
On the other hand, NO can also be antinociceptive in that it exerts a tonically inhibitory action on the background activity of dorsal horn neurons. The increase in background activity occurs almost exclusively in nociceptive neurons (Trudrung et al., 2000).

dual effect of nitric oxide donors in nociception

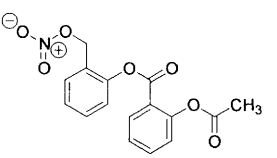
This may explain the conflicting effects observed with NO or NO donors on laboratory animals or on humans. Transdermal nitroglycerine was used successfully in the management of shoulder pain syndrome due to supraspinatus tendinitis, and as a co-adjuvant in opiate therapy for the control of cancer pain. NSAIDs releasing NO, e.g. nitroparacetamol, are described to exhibit greater antinociceptive activities than their parent compounds (al-Sawayeh et al., 2000). In a study investigating the antinociceptive effect of the NO donor SIN-1 it was shown that SIN-1 has an antinociceptive effect at low doses but a pronociceptive effect at higher doses (Sousa and Prado, 2001).



SIN-1 (NO-donor) [26687-79-6]



Nitroparacetamol, phase I clinical trials (NicOx 2001)



NO-ASA, has been in phase I clinical trials for pain, and is now developed for cardiovascular indications (phase II, NicOx) [175033-36-0]

Scheme 2: NO donors in clinical trials.

Nitric Oxide Inhibitors and Migraine

Nitric oxide inhibition has an analgesic effect in patients with chronic tension-type headache, probably due to a reduction in central sensitization at the level of the spinal dorsal horn, trigeminal nucleus or both (Ashina, 2002).

Increasing scientific evidence suggests a key role for NO in migraine. This evidence is mainly based on experimental studies using two different human headache models (Olesen and Jansen-Olesen, 2000).

Clinical studies have suggested that migraine patients more often experience a migraine-like headache in association with nitroglycerine administration than do non-migraineurs. In controlled double-blind trials it has been shown that migraine-sufferers develop a genuine migraine

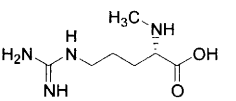
attack following nitroglycerine infusion after a time lapse of several hours. This induced migraine headache is preceded by an immediate headache response during the infusion (Olesen et al. 1993).

Moreover, migraine sufferers have been found to be hypersensitive to histamine in controlled trials. The activation of endothelial H1-receptors induces the formation of NO (Jansen-Olesen et al., 1997). Thus, the increased sensitivity to histamine in migraine sufferers may also been explained by hypersensitivity to activation of the NO pathway.

Targinine (L-NMMA)

The L-arginine derivative targinine was investigated by GlaxoWellcome for the treatment of severe hypotension resulting from septic shock. Phase III clinical trials were discontinued due to concerns from an interim analysis which showed a higher mortality rate among patients treated with targinine compared to the placebo group. Targinine had previously been in development for migraine. In 15 migraine patients (phase II, 6 mg/kg i.v. infusion, 15 min), targinine significantly reduced migraine pain, phonophobia and photophobia, and although it increased mean arterial pressure by 17% and decreased the heart rate by 21%, patients were unaffected by these changes (Lassen et al., 1997).

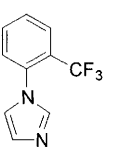
Targinine (L-NMMA)



5-Guanidino-2-methylamino-pentanoic acid, $C_7H_{16}N_4O_2$, MW 188,23

TRIM

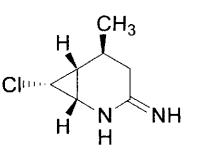
TRIM is an extremely weak NOS inhibitor. The compound was reported to be 40-fold more selective for nNOS and eNOS over iNOS (Moore, King's College London). Potential for the treatment of chronic pain was suggested, but, as far as we know, no development has been reported since 1997.



TRIM, 1-(2-Trifluoromethyl-phenyl)-1H-imidazole, $C_{10}H_7F_3N_2$, MW 212,17

ONO-1714

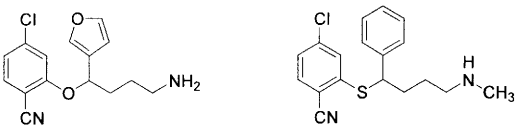
ONO-1714 has reached phase II clinical trials for the treatment of hypotension and septic shock. The compound is a very potent and competitive inhibitor of the human iNOS with no selectivity over the neuronal form and 10-fold selectivity over eNOS. The compound is very active *in vivo*, reducing LPS-stimulated NO production in a mouse model with an ID_{50} value of 0.1 mg/kg s.c.. In 2000, the analgesic activity of ONO-1714 was filed by Ono Pharmaceuticals (Naka and Kobayashi).



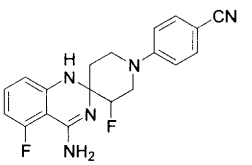
ONO-1714, 7-Chloro-5-methyl-2-aza-bi-cyclo[4.1.0]hept-3-ylidene-amine, $C_7H_{11}ClN_2$, MW 158,63, [214479-33-1]

A wide variety of new NOS inhibitors have been reported over the last couple of years, but clinical data have not been available up to now. A selection of NOS inhibitors patented recently is shown in the following scheme :

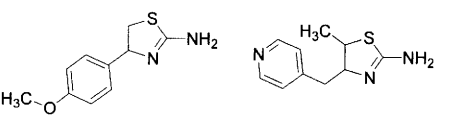
The NOS enzyme activities can be determined by different test systems cited in the literature (e.g. Green et al. 1982; Feelisch and Noak, 1987; Mayer et al., 1989; Bredt and Snyder 1990; Archer, 1993; etc.).



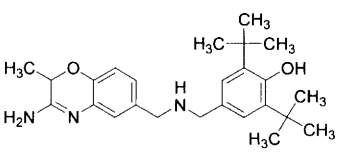
Cheshire et al., AstraZeneca, WO 0162713; WO 0162714; WO 0162721; WO 0162704



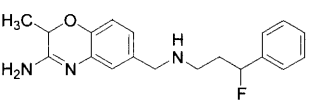
Walpole et al., AstraZeneca, WO 0158867



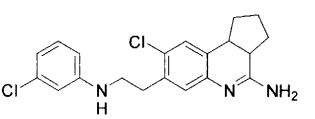
Carry et al., Aventis Pharma, WO 0193867; WO 0194325



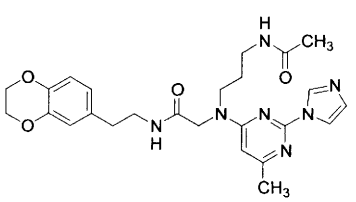
Hölscher et al., Schering, WO 0181324



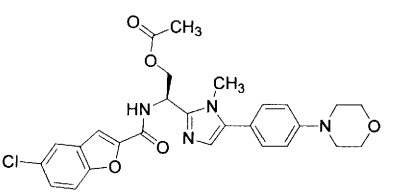
Rehwinkel et al., Schering, WO 0181323



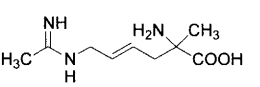
Jaroch et al., Schering, WO 0017167



Arnaiz et al., Berlex, WO 0114371



Sakai et al., Fujisawa, WO 0102387



Durley et al., Pharmacia, WO 0222559

Scheme 3: NOS-inhibitors patented recently.

There is no doubt that NO plays a key role in pain sensation and migraine, which makes NO synthase an interesting target for pain research. Recent overviews have been published by Lowe (2000) and Cheshire (2001).

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Part IV

Outlook

17 The Future of Pain Management

Ulrich Jahnel and Clemens Gillen

Introduction

The current pharmacotherapy of pain is based mainly on two well established principles: non-steroidal anti-inflammatory drugs (NSAIDS) and opioids. Both of these classes suffer from drawbacks in clinical use. For some types of pain, where the efficacy of existing pain therapies is relatively high (e.g. opioids for perioperative pain or NSAIDS for inflammatory pain) , the need for new drugs is dictated by side-effect liabilities. Some of the NSAIDS are associated with gastric damage as well as kidney and liver toxicity, while the opioids can produce addiction, tolerance and dependence along with constipation, nausea, respiratory depression and sedation. Furthermore, for some indications such as neuropathic pain these classical strategies appear to be ineffective in a substantial number of patients. The urgent medical need for novel and safe analgesics with high efficacy has led to intense research for new targets, and those with the greatest potential are reviewed in this book.

In addition to the target- and substance-orientated chapters, this chapter presents additional strategies for pain relief along with the classification of different strategies within a hypothetical time schedule.

Near-term Improvements of Pain Therapy

An optimization of the two classical principles mentioned above was achieved by the generation of more selective compounds. For example, the cyclooxygenase-2- (COX-2) selective NSAIDS (Celecoxib, Rofecoxib) with improved gastric tolerance have entered the market with tremendous success (see Chapter 2). In addition,

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research in the opioid field has been directed towards the identification of selective ligands for the individual opioid receptor subtypes. At present a number of κ- and δ-receptor agonists are being analyzed in clinical trials (see Chapters 3, 9.1).

An alternative strategy uses the combination of known drugs such as tramadol and acetaminophen, thereby targeting multiple components of the pain pathway (Silverfield et al., 2002). This combination of opioids with NSAIDs is a well-established strategy that is part of the WHO scale for treatment of chronic pain.

Another strategy which exploits already established drugs is the development of new drug delivery systems. The transdermal delivery of opioids such as fentanyl (Durogesic®) or buprenorphine (Transtec®) using patch formulations has proven to be very successful. The continuous, long-lasting delivery of a strong opioid is especially suited to the treatment of severe chronic pain in elderly patients.

The intense research for additional pain targets resulted in the identification of several compounds which have entered the clinic, including glutamate receptor antagonists (see Chapter 7), nicotinic receptor agonists (see Chapter 8.1), muscarinic receptor agonists (see Chapter 8.2), sodium channel modulators (see Chapter 6.1), excitability blockers such as gabapentin (see Chapter 5) and adenosine receptor modulators (see Chapter 10). At present there are approximately 70 new analgesic compounds in clinical studies which will surely lead to substantial improvements within the next few years.

Midterm Improvements: The Visible Horizon

In order to gain more insight into the mechanisms underlying pain perception and in order to identify new targets for pain treatment, several strategies are currently used.

Genomics

The search for genes that are differentially expressed in certain pain-relevant tissues, e.g. dorsal root ganglia, versus other tissues or in pain-suffering animals versus control animals led to the identification of several pain-relevant target genes. For example the search for nociceptor-specific genes led to the identification of the TTX-resistant sodium channel SNS/$Na_{v1.8}$ (Akopian et al., 1996) and the purinergic receptor P2X3 (Chen et al., 1995). A different molecular procedure includes the use of degenerate primers and low-stringency homology screening using PCR to clone novel members of particular

protein families. Using primers for G protein-coupled receptors and DRG-cDNA as a template, a new class of sensory neuron-specific receptors was recently identified (Lembo et al., 2002).

Proteomics

After completion of the human genome sequence with the reduced estimation of the number of human genes the focus has now switched to proteomic strategies. Future studies will focus on the pain-associated modification or translocation of pain-relevant proteins, and will elucidate signaling mechanisms by identifying protein-protein interactions.

Deorphanizing of G protein-coupled receptors

These strategies led to the identification of several receptors for which the endogenous ligands were not known. The subsequent screening of peptidic compound libraries using functional receptor assays was successfully used for deorphanizing. Following this strategy the heptadecapetide nociceptin/orphanin FQ could be identified as the endogenous ligand for the orphan receptor ORL1 (see Chapter 9.2). A further pain-relevant example is the receptor for the neuropeptide FF (NPFF) that was deorphanized simultaneously by Bonini et al. and Elshourbagy et al. (2000).

Identification of targets for known analgesics

In a strategy complementary to that mentioned above, several compounds with well-known analgesic activity were used to identify their molecular targets. Successful examples include the isolation of the $GABA_B$ receptor using baclofen (Kaupmann et al., 1997) and the vanilloid receptor 1 using capsaicin (Caterina et al., 1997). In both cases an expression-cloning strategy was used that will be described briefly for the capsaicin receptor. Based on the fact that capsaicin causes a large influx of calcium into cells which contain its receptor, a DRG cDNA library was expressed in HEK293 cells which were subsequently screened for calcium influx after capsaicin treatment using the flurorescent calcium-sensitive dye Fura-2. The positive cells were shown to contain a novel cation-channel called vanilloid receptor subtype 1 (VR1, see Chapter 13).

Strategies for target validation

In order to validate this increasing number of potential pain targets, several strategies have been pursued by the pharmaceutical industry. An HTS screening of compound libraries is carried out in order to identify low molecular weight compounds that are subsequently tested in animal models of nociception. In the last few years, molecular genetic approaches have become more significant. In the knock-out approach, the specific deletion of the target gene leads to so-called knock-out mice which can be tested in models of nociception. Although this approach has its limitations due to developmental changes and

compensatory alterations, it allows significant insights into the functional role of several pain targets such as VR1, adenosine receptors, or muscarinic receptors (see corresponding chapters). In the knock-down approach the target protein levels are reduced *in vivo* by the administration of antisense oligonucleotides into certain CNS regions. Pain targets analyzed with this strategy include the δ-opioid receptor (Wahlestedt et al., 2000), the TTX-R sodium channel $Na_{v1.8}$ (Lai et al., 2002) and the cannabinoid-receptor CB1 (Edsall et al., 1996).

As a result of the strategies described above for target identification and target validation, several new pain targets are in preclinical research with promising compounds like the ORL1 receptor antagonists (see Chapter 9.2), capsaicin receptor antagonists (see Chapter 13) or metabotropic glutamate receptor modulators (see Chapter 7.1). For these targets their relevance to the treatment of pain in man is still unknown and has to be proven by the forthcoming clinical studies. Nevertheless it is to be expected that some of these compounds will be successful and reach the market within the next 5 to 10 years.

Molecular Aspects of the Future

Given the intense research carried out in this field by several pharmaceutical companies and numerous academic groups it is to be expected that the list of new pain targets will increase further and will result in new pharmacological interventions.

There are several molecular approaches that are currently used with great success for target validation in animals and might also be used in humans. Three of these approaches will be discussed below.

Selective ablation of spinal pain-relevant neurons

This method which was developed by Mantyh et al. (1997) uses a conjugate of substance P and the ribosome-inactivating protein saporin for selective ablation of neurons in the dorsal spinal cord. After intrathecal administration of SP-SAP in adult rats, it is internalized and cytotoxic to the lamina I spinal cord neurons which express the substance P receptor. Histochemical analysis showed a reduction of NK1-positive lamina I neurons of approx. 85%. The animals showed normal behaviour, but a strongly reduced thermal and mechanical hyperalgesia after an intraplantar injection of capsaicin. In a second publication (Nichols et al., 1999) the authors demonstrated that this effect was dose-dependent, long-lasting (up to 200 days) and affected mechanical and thermal hyperalgesia during inflammatory as well as neuropathic

pain. This method can be regarded as pain-reducing molecular surgery that may also be used for the treatment of incurable severe chronic pain in humans.

Antisense approaches for knock-down of pain relevant genes

As described earlier, the antisense strategy was used successfully in animals for the validation of pain-associated target genes. This knock-down strategy could be directed against pronociceptive gene products, such as the NMDA receptor subunits, the NK1 receptors or the sodium channel $Na_{v1.8}$. However, it must be taken into account that the antisense oligonucleotides need to be injected directly into the CNS (e.g. intrathecally). It is still questionable whether antisense constructs will find a place in pharmacotherapy, given the fact that only one antisense ODN is currently on the market (Formivirsen® for treatment of cytomegalovirus-induced retinitis). The substantial improvements in nucleotide chemistry leading to ODNs of increased stability and reduced toxicity could open the way for a broader application of antisense-ODNs and may include their use as analgesics.

Gene transfer approaches to control pain

The current report of the U.S. recombinant DNA advisory committee lists 509 approved human gene therapy protocols. Most of these protocols are for cancer followed by infectious diseases and virus infections. At the moment there is no human gene therapy protocol which targets chronic pain. In contrast to the antisense strategy, this molecular approach should lead to the overexpression of antinociceptive gene products, such as endogenous opioids. Indeed, overproduction of opioid peptides in primary sensory neurons or spinal cord-induced antihyperalgesic effects in various animal models of persistent pain (Finegold et al., 1999; Wilson et al., 1999; Braz et al., 2001; Goss et al., 2001). The recent significant and constant advances in vector system design suggest that these techniques will be available in the future for safe application in humans.

Mechanism-Based Diagnosis and Therapy of Pain

The pursuit of the pharmaceutical industry for a powerful wide-spectrum analgesic mechanism may find its limitations in the complexity of the mechanisms underlying chronic pain. In recent years the increasing availability of information regarding the molecular basis of pain perception and transmission has led to the proposal of a mechanism-based diagnosis and therapy for pain (Woolf and Decosterd, 1999; Dallel and Voisin, 2001; Woolf and Max, 2001). The induction of pain encompasses multiple components acting on different levels of the neuraxis and are inherently dynamic. A certain syndrome such as

neuropathic pain may be accompanied by several symptoms such as spontaneous chronic or paroxysmal pain or allodynia. For instance, allodynia, which includes all situations in which the threshold of pain is lowered, may be due to one or several of the following mechanisms: nociceptor sensitization, central sensitization or regenerative sprouting of Aβ fibers. Therefore it is of great importance to identify in individual patients which mechanisms are responsible for their pain and to target treatment specifically at those mechanisms. This mechanism-based pain assessment can only be established by the concerted efforts of physicians, academic pain researchers, industry and government research funders. Together with the discovery of targets specific to particular pain mechanisms, a bridge between molecular neurobiology and the patient arriving at a clinic seems possible.

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Glossary

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Absorption

Process of taking in. Chemicals can be absorbed into the bloodstream after breathing or swallowing.

Active transport

Active transport is the carriage of a solute across a biological membrane from low to high concentration that requires the expenditure of (metabolic) energy.

Acute pain

A normal biological response to help protect the body against potentially harmful environmental stimuli. Typically acute pain is caused by identifiable stimuli, is short-lived and stops when the tissue injury that caused it has healed.

Addiction

The compulsive use of drugs for non-medical purpose. It is characterised by a craving for mood-altering drug effects, not painrelief. Addiction refers to a dysfunctional behaviour as opposed to the improved function and quality of life that result from pain relief. In cancer patients who use opioids for long-term pain relief addiction is extremely rare.

Adjuvants

Agents used as adjuncts or adjunctive therapies to opioid analgesics in total management of moderate-to-severe pain. They can directly diminish pain, counteract opioid side effects, or help manage concurrent psychiatric symptoms.

Address-message concept

Address-message concept refers to compounds in which part of the molecule is required for binding, (address) and part for the biological action (message) (IUPAC).

ADME

Absorption, Distribution, Metabolism, Excretion. See Pharmacokinetics.

Adverse effect

Undesirable and unintended, although not necessarily unexpected, result of therapy or other treatment.

A-fibres

A specific type of nerve fibre involved in the conduction of nociceptive impulses.

Affinity

Affinity is the tendency of a substance to associate with another. The affinity of a drug is its abillty to bind to its biological target (receptor, enzyme, transport system, etc. For pharmacological receptors it can be thought of as the frequency with which the drug, when brought into the proximity of a receptor by diffusion, will reside at a position of minimum free energy within the force, field of that receptor. For an agonist (or for an antagonist) the numerical representation of affinity is the reciprocal of the equilibrium dissociation constant of the ligand-receptor complex denoted KA,

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	calculated as the rate constant for offset (k_{-1}) divided by the rate constant for onset (k_1).
Agonist	A substance that can stimulate a receptor type to transmit an intracellular message and thus initiate a cellular biochemical change. An agonist is an endogenous substance or a drug that can interact with receptors and initiate a physiological or a pharmacological response (contraction, relaxation, secretion, enzyme activation, etc). An agonist is a Drug that binds cellular receptors which are ordinarily stimulated by naturally occurring substances, triggering a response.
Allodynia	Pain due to a stimulus that does not usually provoke pain.
Alzheimer's disease	Progressive, neurodegenerative disease characterized (AD) by loss of function and death of nerve cells in several areas of the brain leading to loss of cognitive function such as memory and language.
Anaesthesia	Absence of all sensory modalities.
Anaesthetic	A compound that reversibly depresses nerve function, producing loss of ability to perceive pain or other sensations.
Analgesia	Absence of pain in response to a stimulation that would normally be painful.
Analgesic	A drug used primarily for relieving pain.
Analogue	An analogue is a drug whose structure is inspired by that of another drug but whose chemical and biological properties may be quite different (IUPAC). See Congener.
Animal Models of Pain	Animal models of nociception can be divided according to the therapeutic indication: Acute Pain, Migraine Pain, Inflammatory Pain, Visceral Pain, Neuropathic Pain. Different degrees of chronification (up to weeks in neuropathic pain models) and different stimuli (mechanical, thermal, chemical, electrical) are used depending on the experimental question. In most cases a nociceptive threshold (e.g. withdrawal latency of a paw) is determined. Sometimes, nociceptive intensities are determined e.g. in order to quantify hyperalgesia.
Antagonist	A substance that binds to a receptor type without activating it but which blocks the attachment of agonists to the receptor. An antagonist is according to this definition a drug-or a chemical entity that opposes the physiological effects of another. At the receptor level, it is a chemical entity that opposes the receptor

associated responses normally induced by another agent. An antagonist is a Drug that binds a receptor without triggering a response.

Anticonvulsant

A compound commonly used for treating epilepsy but also has applications in treating pain (e.g. phenytoin, carbamezapine, gabapentin and sodium valproate).

Antisense molecule

An antisense molecule is an oligonucleotide or analogue thereof that is complementary to a segment of RNA or DNA and that binds to it and inhibits its normal function. (IUPAC).

Assay

Any combination of targets and compounds which is exposed to a detection device to measure chemical or biological activity.

Backache

Probably the second most common and probably the most expensive form of chronic pain in industrialised societies behind headache. It can be acute or chronic and has many causes. It is sometimes treated with epidural injections or injections into joints.

Bennet model of neuropathic pain

Nerve injury, followed by pain related behaviour, is induced by loose ligation of the ischiatic nerve of one hind paw of the rat. 3-4 weeks after ligation, neuropathic pain-like behaviour is seen as increased sensitivity towards heat and pressure stimuli (hyperalgesia). Also pain reactions toward non-noxious tactile (mechanical allodynia) or cold stimuli (cold allodynia) can be observed. Mechanical allodynia is tested with von Frey hairs and cold allodynia by putting the animals on metal plate cooled to 4 °C. The number of paw liftings is counted (Bennett and Xie, Pain **1988**, *33*, 87-107).

Bioinformatics

the use of search programmes (public domain, proprietary or in-house programmes) to analyse DNA and protein sequences to predict the function of a gene sequence.

Bioisostere

A bioisostere is a compound resulting from the exchange of an atom or of a group of atoms with another, broadly similar, atom or group of atoms. The objective of a bioisosteric replacement is to create a new compound presenting similar biological properties to the parent compound. The biolsosteric replacement may be physicochemically or topologically based (IUPAC).

Biotransformation

Biotransformation is the chemical conversion of substances by living, organisms or enzyme preparations derived therefrom (IUPAC).

Bone pain Pain experienced in the bones either caused by osteoporosis or bone metastases in cancer patients.

Cell Smallest membrane-bound biological unit capable of replication.

Cell membrane The phospholipid bilayer that surrounds a cell, forming a selectively-permeable barrier.

Cellular assay Assay run on whole living cells.

Central pain Pain associated with a lesion of the central nervous system.

C-fibres Afferent (inward) nerve fibres; most C-fibres are nociceptive, carrying pain impulses to the central nervous system.

Chemoinformatics A generic term that encompasses the design, creation, organisation, storage, management, retrieval, analysis, dissemination, visualisation and use of chemical information, not only in its own right, but as a surrogate or index for other data, information and knowledge.

Chronic pain Pain which lasts more than 6 months and which may continue in the absence of any identifiable tissue injury. Typically this type of pain can severely affect the patient's quality of life by causing intensive physical suffering. Chronic pain can also be divided into chronic cancer-related pain and chronic non-cancer pain, which have different methods of management.

Chung model of neuropathic pain Nerve injury in rats is induced by a tight ligation of the root of the spinal nerve at L5-L6. Animals show hyperalgesia and allodynia similar to the Bennet model. Tactile allodynia, measured with von Frey hairs, is the most reliable parameter of pain intensity in this model (Kim and Chung, Pain **1992**, *50*, 355-363).

Clinical Candidate A compound (small molecule) that has achieved the first ever dose administered to the first human (including patients if they are the first humans to receive the compound).

Clinical trials Research studies that involve patients.

Clone Group of identical genes, cells, or organisms derived from a single ancestor.

Cloned DNA Any DNA fragment that passively replicates in the host organism after it has been joined to a cloning vector.

Cloning Process of making genetically identical copies.

Coenzyme A coenzyme is a dissociable, low-molecular weight, non-proteinaceous organic compound (often

nucleotide) participating in enzymatic reactions as acceptor or donor of chernical groups or electrons.

Computer-aided/structural drug design

Rational drug design - use of high resolution molecular imaging techniques (NMR, x-ray crystallography) to identify the active site of the target molecule and construct an new active substance which binds to this active site.

Congener

A congener is a substance literally con- (with) *generated* or synthesized by essentially the same synthetic chemical reactions and the same procedures. Analogues are substances that are analogous in some respect to the prototype and in chemical structure. Clearly congeners may be analogues or vice versa but not necessarily. The term congener, while most often a synonym for homologue, has become somewhat more diffuse in meaning so that the terms congener and analogue are frequently used interchangeably in the literature.

Dependence

Inability to do without, in this context, a drug; a problem which can occur in particular with the long-term use of opioids.

Diabetic neuropathy

A complication of diabetes where painful nerve damage can affect the limbs (especially causing ulcers in the feet if the patient has poor blood circulation), intestinal and cardiovascular system.

Docking studies

Docking studies are molecular modeling studies aiming at finding a proper fit between a ligand and its binding site (IUPAC).

Double-blind study

A double-blind study is a clinical study of potential and marketed drugs, where neither the investigators nor the subjects know which subjects will be treated with the active principle and which ones will receive a placebo (IUPAC).

Drug

Any chemical compound that may be used on humans to help in diagnosis, treatment, cure, mitigation, or prevention of disease or other abnormal conditions.

Drug targeting

Drug targeting is a strategy aiming at the delivery of a compound to a particular tissue of the body (IUPAC).

Dual action drug

A dual action drug is a compound which combines two desired different, pharmacological actions at a similarly efficacious dose (IUPAC).

Efficacy

Efficacy is the property that enables drugs to produce responses. It is convenient to differentiate the properties of drugs into two groups; those which cause them to associate with the receptors (affinity) and those that produce stimulus (efficacy). This term is

often used to characterize the level of maximal responses induced by agonists. In fact, not all agonists of a receptor- are capable of inducing identical levels of maximal responses. It depends on the efficiency of receptor coupling, i.e., from the cascade of events, which, from the binding- of the drug to the receptor, leads to the observed biological effect. Efficacy describes the relative intensity with which agonists vary in the response they produce even when they occupy the same number of receptors and with the same affinity. Efficacy is *not* synonymous to intrinsic activity (IUPAC).

Enzyme Protein that acts as a catalyst, affecting the rate at which chemical reactions occur in cells. An enzyme is any molecular structure that catalyses a physiological chemical reaction.

Epidural A form of intraspinal analgesia where the agent is injected into the epidural space that surrounds the dura mater, which is the membrane that contains the cerebo-spinal fluid directly outside the spinal cord.

Fibromyalgia A disease characteristically affecting depressed, middle-aged women complaining of diffuse, symmetrical and persistent musculoskeletal pain/tenderness and sleep disturbance with no organic basis, or a real pathological entity. It requires more than a psychological/psychatric approach to treat effectively.

Formalin test A small amount of formalin solution is injected into the hind paw of mice or rats. This induces a bi-phasic pain reaction and a specific pain-related behaviour. The first phase represents acute nociceptive pain, whereas the second phase indicates more persistent pain associated with inflammation and tissue damage. Pain behaviour is observed in both phases and measured by means of a scoring system. Besides opioids, compounds active against inflammatory and neuropathic pain can be detected (Dubuisson and Dennis, Pain **1977**, *4*, 161-174).

Gene Unit of inheritance; a working subunit of DNA containing the code for a specific product, typically, a protein such as an enzyme.

Gene expression Process by which a gene's coded information is translated into the structures present and operating in the cell (either proteins or RNAs).

Genetics Scientific study of heredity how particular qualities or traits are transmitted from parents to offspring.

Genome

All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genome projects

Research and technology development efforts aimed at mapping and sequencing some or all of the genorne of human beings and other organisms.

Genomics

Identification and functional characterization of genes; Genomics is the identification of previously unknown human DNA sequences encoding natural human proteins with previously unknown medical use that can be used as targets in Drug Discovery to discover novel therapeutic agents or administered for therapeutic benefit.

Genotype

Genetic constitution of an organism.

General anaesthetic

An anaesthetising compound which results in a loss of consciousness; usually a gas or intravenously injected liquid. Often used together with a neuromuscular blocker.

G-protein coupled receptor (GPCR)

any cellular macromolecule to which a ligand binds initiating an effect via a G-protein mechanism (Note that it will include only binding activity through binding domain and will not account for any further event that the protein may perform through its effector domain).

Heteroreceptor

A heteroreceptor is a receptor regulating the synthesis and/or the release of mediators other than its own ligand (IUPAC).

High-throughput-screening (HTS)

Technique of rapidly searching for molecules with desired biological effects from very large compound libraries.

Hit (compound)

Compound found by screening to have a desired biological effect.

Homologue

The term homologue is used to describe a compound belonging to a series of compounds differing from each other by a repeating unit, usually a methylene group (IUPAC).

Hot plate test

A model of thermal pain. Mice or rats are placed on a heated metal plate of variable temperature. Depending on the temperature (48-58 °C), a weak or strong pain stimulus is induced. Animals respond either by licking their paws or by jumping. Analgesics increase the latency for this pain reaction. The low temperature hot plate detects a broader spectrum of less efficacious analgesics in comparison to its high temperature modification or the tail flick test (Eddy and Leimbach; J. Pharmacol. **1953**, *107*, 385).

Human Genome Project
International research effort aimed at mapping and sequencing all of the genome of the human beings and other organisms.

Hydrophilicity
Hydrophilicity is the tendency of a molecule to be solvated by water (IUPAC).

Hydrophobicity
Hydrophobicity is the association of non polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non polar molecules (IUPAC). See Lipophilicity.

Hyperalgesia
An increased response to a stimulus that is normally painful. Many cases of hyperalgesia have features of allodynia, however, the term hyperalgesia is used specifically when there is a response of increased pain to a stimulus that normally is painful.

Hyperesthesia
Increased sensitivity to stimulation, excluding special senses.

Hypoalgesia
Diminished sensitivity to a noxious stimulation.

ICH (International Conference on Harmonization)
The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to harmonise scientific and technical aspects of product registration. They make recommendations which will be adopted by the national / EU authorities after an approval process.

Intrathecal
A form of intraspinal anaesthesia or analgesia in which the agent is injected through the dura mater and arachnoid membrane into the cerebro-spinal fluid which surrounds the spinal cord.

IND
Investigational New Drug. Application must be approved by the Food and Drug Administration (FDA) before a drug can be tested in humans in clinical trials.

Intrinsic activity
Intrinsic activity is the maximal stimulatory response induced by a compound in relation to that of a criven reference compound. This term has evolved with common use. It was introduced by Ariens as a proportionality factor between tissue response and receptor occupancy. The numerical value of intrinsic activity (alpha) could ran from unity (for full agonists, i.e., agonist inducing the tissue maximal response) to zero (for antacyonists). The fractional values within this ran denoting partial agonists. Arien-8 original definition equates the molecular nature of alpha to maximal response only when response is a linear function of receptor occupancy. This function has

been verified. Thus, intrinsic activity, which is a drug and tissue parameter, cannot be used as a characteristic drug parameter for classification of drugs or drug receptors. For this purpose, a proportionality factor derived by null methods, namely, relative efficacy, should be used. Finally, "intrinsic activitv" should not be used instead of "intrinsic efficacy". A "parcial agonist" should be termed "agonist with intermediate intrinsic efficacy" in a given tissue (IUPAC).

Inverse agonist

An inverse agonist is a drug which acts at the same receptor as that of an agonist, yet produces an opposite effect. Also called negative antagonists.

In vitro

In a test tube.

In vivo

In the living cell or organism as opposed to in vitro.

Ion channel

Receptor or carrier proteins which, when activated, allows the passage of ions across cell membranes.

Lead / Lead compound

As a result of the screening process used during drug discovery, active substances will be identified. Of these active substances, the compound that best fits the desired characteristics profile (pharmacological activity, lack of early toxicity, patentability, etc) will be declared a lead compound. Development activities will then begin to shift from a broad discovery program to a more focused development program centred around the lead compound.

Lead Candidate

A chemical entity (small molecule) or series (a set of structural analogues) that has shown sufficient activity and selectivity for the target, to form the basis for focused medicinal chemistry and optimisation of pharmacological properties.

Lead discovery

Lead discovery is the process of identifying active new chemical entities, which by subsequent modification may be transformed into a clinically useful drug (IUPAC). This phase begins at the initiation of target screening "start of target screening" milestone and concludes with the identification of the first chemical lead compound (or lead series) selected for optimisation - "lead series selected" milestone. It involves the testing of compounds, either *in vitro* or *in vivo*, to determine their target effect (e.g. molecular interaction or biological effects).

Lead generation

Lead generation is the term applied to strategies developed to identify compounds which possess a desired but non-optimized biological (IUPAC).

Lead optimization

Lead optimisation is the synthetic modification of a biologically active compound, to fulfill all stereoelectronic, physicochemical, pharmacokinetic and toxicologic required for clinical usefulness (IUPAC). This phase begins with the first chemical lead or lead series selected for optimisation (i.e. the "lead series selected" milestone) and concludes with a decision for an optimized compound to enter preclinical development (i.e. the "pre-clinical candidate selected" milestone). This phase consists of testing of a compound to determine the chemical structure that has the optimum potency and selectivity for the target in question. The phase includes the search for back-up compounds and may also include early ADME and toxicity evaluation.

Ligand

Chemical messenger, usually released by one cell to communicate with a different cell by binding to specific receptors on the receiving cell's surface.

Lipophilicity

Lipophilicity represents the affinity of a molecule for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid-liquid (e.a. partition coefficient in 1-octanol / water) or solid-liquid (retention on reversed-phase high performance liquid chromatography *(RP-HPLC)* or thin-layer chromatography *(TLQ System)* (IUPAC). See Hydrophobicity.

Metabolism

The term metabolism comprises the entire physical and chemical processes involved in the maintenance and reproduction of life in which nutrients are broken down to generate energy and to give simpler molecules (catabolism) which by themselves may be used to form more complex molecules (anabolism). In case of heterotrophic organisms, the energy evolving from catabolic processes is made available for use by the organism (IUPAC).

Me-too drug

A me-too drug is a compound that is structurally very similar to already known drugs, with only minor pharmacological differences (IUPAC).

Molecular modeling

Molecular modeling is a technique for the investigation of molecular structures and properties using computational chemistry and graphical visualization techniques in order to provide a plausible three-dimensional representation under a given set of circumstances (IUPAC).

Ligand

Any atom or molecule attached to a central atom, usually a metallic element, in a co-ordination or complex compound.

Local anaesthetic

Anaethestising compound that only acts on the area in which it is applied; usually in the form of a spray, gel cream, local injection into the skin or adjacent nerves where they function to block nerve impulses.

Migraine

A vascular disease causing an intensely painful unilateral headache that can last from a few hours to 72 hours or longer.

Narcotic

Opioid is the preferred medical and pharmacological term for opium derivatives and analogues, narcotic having legal and other non medical connotations

NCE

New chemical entity. A new chemical entity is a compound not previously described in the literature.

NDA

New drug application. A document that combines all relevant data (with attachments) to allow the US FDA (or an other drug regulatory agency) to review and decide whether to approve marketing of a new drug. Detailed reports of chemistry; pharmacology, toxicology, metabolism, manufacturing, quality controls and clinical data along with proposed labelling are included.

Neuralgia

Pain occurring in the area served by a sensory nerve, either because of compression or disease of that nerve, or else occurring without any apparent organic cause.

Neurogenic pain

Pain resulting from non-inflammatory dysfunction of the peripheral or central nervous system without nociceptor stimulation or trauma. Examples include post-herpetic neuralgia, complex regional pain syndromes, phantom pain and trigeminal neuralgia.

Neuropathic pain

Pain caused by functional abnormalities or structural lesions in the peripheral or central nervous system frequently arising from injury (e.g. surgery, accident or amputation), diseases (e.g. diabetes, herpes zoster or cancer), infarction or dysfunction of the nervous system. A damaged nerve may initiate signals in other nerves not associated with the injured area. It may either have a burning sensation or an aching sensation.

Nociception

The response to excitation of nociceptors. Although nociception may give rise to the experience of pain, pain may arise in the absence of nociception. Conversely, nociception may also occur in the absence of pain.

Nociceptive pain

Pain which occurs when intact peripheral nerve endings (nociceptors) are stimulated by noxious

	mechanical, thermal or chemical stimuli (e.g. inflammatory pain).
Nociceptor	Peripheral nerve ending.
Noxious stimulus	A stimulus which is potentially or actually damaging to body tissue, however it does include instances where there is no lasting tissue damage, such as that which occurs as muscle pain due to excessive exercise.
nuclear receptor	Receptors which are associated to a cell nucleus.
Opiate	Refers to the drug whose origin is the opium poppy, including codeine and morphine.
Opioid	Any substance having activity at the opioid receptor. Opioids can also be grouped according to pharmacological activity: pure, partial agonists, antagonists or mixed agonist-antagonists.
Opioid receptors	The different types and subtypes of opioid receptors in the nervous system. The mu (μ), kappa (κ) and delta (δ) subtype are designated by corresponding Greek letters.
Orphan drug	An orphan drug is a drug for the treatment of a rare disease for which reasonable recovery of the sponsoring firm's research and development expenditure is not expected within a reasonable time. The term is also used to describe substances intended for such uses (IUPAC).
Orphan receptor	Receptor with unknown function binding known ligands.
Pain threshold	The least experience of pain that a person can recognise. It is the level at which 50% of stimuli are recognised as painfül; it must be understood that pain is the experience of the patient, which is difficult to measure, whereas the stimulus intensity can be measured by the psychophysicist as an extemal event.
Pain tolerance level	The greatest level of pain that a person is prepared to tolerate. It should be understood that this a subjective experience and so its clinical value is limited.
Palliative care	A form of care which attempts to provide at least superficial or temporary relief of pain and suffering such as that which is provided for cancer and/or terminally-ill patients.
Partial agonist	A substance which partially (in comparison to an agonist) activates a receptor type to transmit an intracellular message. A partial agonist is an agonist which is unable to induce maximal activation of a

receptor population, regardless of the amount of drug applied.

Peptidomimetic

Peptidomimetics are compounds containing non-peptidic structural elements that are capable of mimicking or antagonizing the biological action(s) of a natural parent peptide (IUPAC).

Phantom pain

Pain which occurs after a limb has been amputated or lost as if it were still there.

Pharmacokinetics

Pharmacokinetics refers to the study of absorption, distribution, metabolism and excretion (ADME) of bioactive compounds in a higher organism (IUPAC).

Pharmacophore (pharmacophorie patterrn)

A pharmacophore is the ensemble of steric and electronic features that are necessary to ensure the optimal supramolecular interactions with a specific biological taraget structure and to trigger (or to block) its biological response. A pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capacities of a group of compounds towards their target structure. The pharmacophore can be considered as the largest common denominator shared by a set of active molecules. This definition discards a misuse often found in the medicinal chemistry literature which consists of naming as a pharmacophore simple chemical functionalities such as guanidines, sulfamides or imidazolines, or typical structural skeletons such as flavones, phenothiazines, prostaglandins or steroids (IUPAC).

Phase I (clinical trial)

The first trials in humans that test a compound for safety, tolerance, and pharmacokinetics. The Phase I trials usually employ healthy volunteers and may expose up to about 50 individuals to the drug. For therapeutic biologics and known toxic compounds, e.g. anticancer agents, only patients with the targeted illness would be used. A Phase I study is a closely monitored clinical trial of a drug or vaccine conducted in a small number of healthy volunteers; used to determine toxicity, pharmacokinetics, preferred route of administration, and safe dosage range of a drug.

Phase II (clinical trial)

The first studies to define efficacy in patients. In general, 100-300 patients would be entered into. Various closely monitored clinical trials during this phase. Dose and dosing regimens are assessed for magnitude and duration of effect during this phase. Some companies further differentiate this phase into Phase 2A and 2B (proof of efficacy and dose finding). A phase II study is a controlied clinical study of a drug

	or vaccine to identify common short-term side-effects and risks associated with the drug or vaccine, to collect information on its immunogenicity and to demonstrate its efficacy conducted on a limited number of patients with disease.
Phase III (clinical trial)	Expanded controlled and uncontrolled clinical trials intended to gather additional evidence of effectiveness for specific indications and to better understand safety and drug-related adverse effects. Phase III trials are usually large multicenter trials which collect substantial safety experience and may also include specialized studies needed for labelling (e.g. paediatric or elderly, comparative agents). Thousands of patients may be included in the Phase III trials.
Placebo	A placebo is an inert substance or dosage form which is identical in appearance, flavor and odour to the active substance or dosage form. It is used as a active control in a bioassay or in a clinical study (IUPAC).
Physical dependence	Involves the development of a withdrawal syndrome following abrupt discontinuation of treatment or a substantial reduction in dose. It is a normal expected response to continuous opioid therapy and does not mean that the patient is addicted.
Potency	Potency is the dose of drug required to produce a specific effect of given intensity as compared to a standard reference. Potency is a comparative rather than an absolute expression of drug activity. Drug potency depends on both affinity and efficacy. Thus, two agonists can be equipotent, but have different intrinsic efficacies with compensating differences in affinity (IUPAC).
Pre-clinical Candidate	An optimised (having sufficient potential as a therapeutic candidate to be tested in humans) compound (small molecule) selected to enter pre-clinical development.
Prodrug	A prodrug is any compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule (IUPAC).
Protease	Any enzyme that catalyzes the cleavage of a peptide or protein.
Protease inhibitors	Class of drugs designed to inhibit the enzyme protease.

Protein

Large, complex molecule composed of amino-acids. Proteins are essential to the structure, function, and regulation of the body. Examples are hormones, enzymes, and antibodies.

Proteome

Complete profile of all expressed (produced) proteins within a cell, a tissue, or an entire organism at a given time.

Proteomics

Analysis of the functions and interactions of proteins in healthy tissue compared to tissue affected by a disease. Proteomics includes the the separation, identification & characterisation of proteins present in a biological sample and comparison of disease and control samples to identify "disease specific proteins". These proteins may have potential as targets for drugs or as molecular markers of disease.

Randall-Selitto test

This test, performed in rats, is the classical model of inflammatory pain. Intraplantar injection of inflammatory stimuli such as carrageenan, kaolin, or complete Freund adjuvants (CFA) induces paw swelling and increased pain sensitivity. As pain stimulus pressure is applied on the inflammed paw and gradually increased until the animal responds by vocalisation or withdrawal of the paw. Analgesics increase the pressure threshold (Randall and Selitto, Arch. Int. Pharmacodyn. **1957**, *111*, 409-419).

Receptor

Protein in a cell or on its surface that selectively binds a specific substance (ligand). Upon binding its ligand, the receptor triggers a specific response in the cell.

Respiratory depression

Decreased breathing rate as brought on by analgesia/anaesthesia.

Rheumatism

Any painful state of the supporting structures of the body such as bones, ligaments, joints, tendons or muscles. Arthritis is a form of rheumatism in which the joints have become inflamed.

Structure-activity relationship

Structure-activity relationship is the relationship between chemical structure and pharmacological activity for a series of compounds (IUPAC).

Strong opioid

Refers to a substance related to, or sharing the action of opium which has a strong action such as morphine (as opposed to weak action provided by codeine) and is used for treating chronic and moderate-to-severe pain only.

Tail flick test

This model represents intense nociceptive pain induced by heat. A hot lightbeam is focussed on the tail of a mouse or rat and the latency for the withdrawal of the tail, a brisk movement called 'tail

	flick', is determined. Only very potent analgesics such as opioids (e.g. morphine), α_2 adrenergic agonists (e.g. clonidine) and cholinomimetics (e.g. arecoline, epibatidine) dose-dependently increase tail flick latency up to a voluntarily fixed cut off time (D'Amour and Smith; J. Pharm. Exp. Ther. **1941**, *72*, 74-79).
Target	Specific biological molecule, such as an enzyme, receptor or ion channel, assumed to be relevant to a certain disease. Most drugs work by binding to a target, thereby affecting its biological function.
Target identification	Identifying a molecule (often a protein) that is instrumental to a disease process (though not necessarily directly involved), with the intention of finding a way to regulate that molecule's activity for therapeutic purposes.
Target validation	Crucial step in the drug discovery process. Following the identification of a potential disease target, target validation verifies that a drug that specifically acts on the target can have a significant therapeutic benefit in the treatment of a given disease.
Teratogen	A teratogen is a substance that produces a malformation in a foetus.
Tolerance	Associated with drug dependence, this phenomenona may occur with chronic administration of a drug. It is characterised by the necessity to progressively increase the dose of the drug to produce its original effect. Tolerance is mainly caused by neuroadaptive changes in the brain.
Transporters	Carrier proteins which transport molecules across a cell membrane.
Trigeminal neuralgia	Pain characterised by an agonising shooting pain that starts at one side of the face for no apparent reason and can last from a few seconds to a few minutes. May be associated with multiple sclerosis.
Weak opioid	Refers to a substance related to, or sharing the action of opium. They have a weak action, such as codeine (as opposed to the strong action provided by morphine), and are used for treating mild-to-moderate pain (some are available as over-the-counter products).
WHO analgesic ladder	Developed by the World Health Organization and widely regarded as the best approach to the management of acute pain, chronic non cancer pain and chronic cancer pain. The analgesic ladder ascends from non-opioids through weak opioids to strong opioids according to the severity of the pain.

Writhing test

A pain reaction is induced in mice or rats by intraperitoneal injection of an irritant (e.g. acetic acid, phenylquinone, acetylcholine). This induces stretching movements called 'writhings'. The number of writhings, counted during time intervals of 20-30 min, represents pain intensity and is dose-dependently reduced by analgesics. This test detects almost all types of analgesics but is sensitive to unspecific effects such as sedation (Hendershot and Forsaith, J. Pharmacol. Exp. Ther. **1959**, *125*, 237-240).

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