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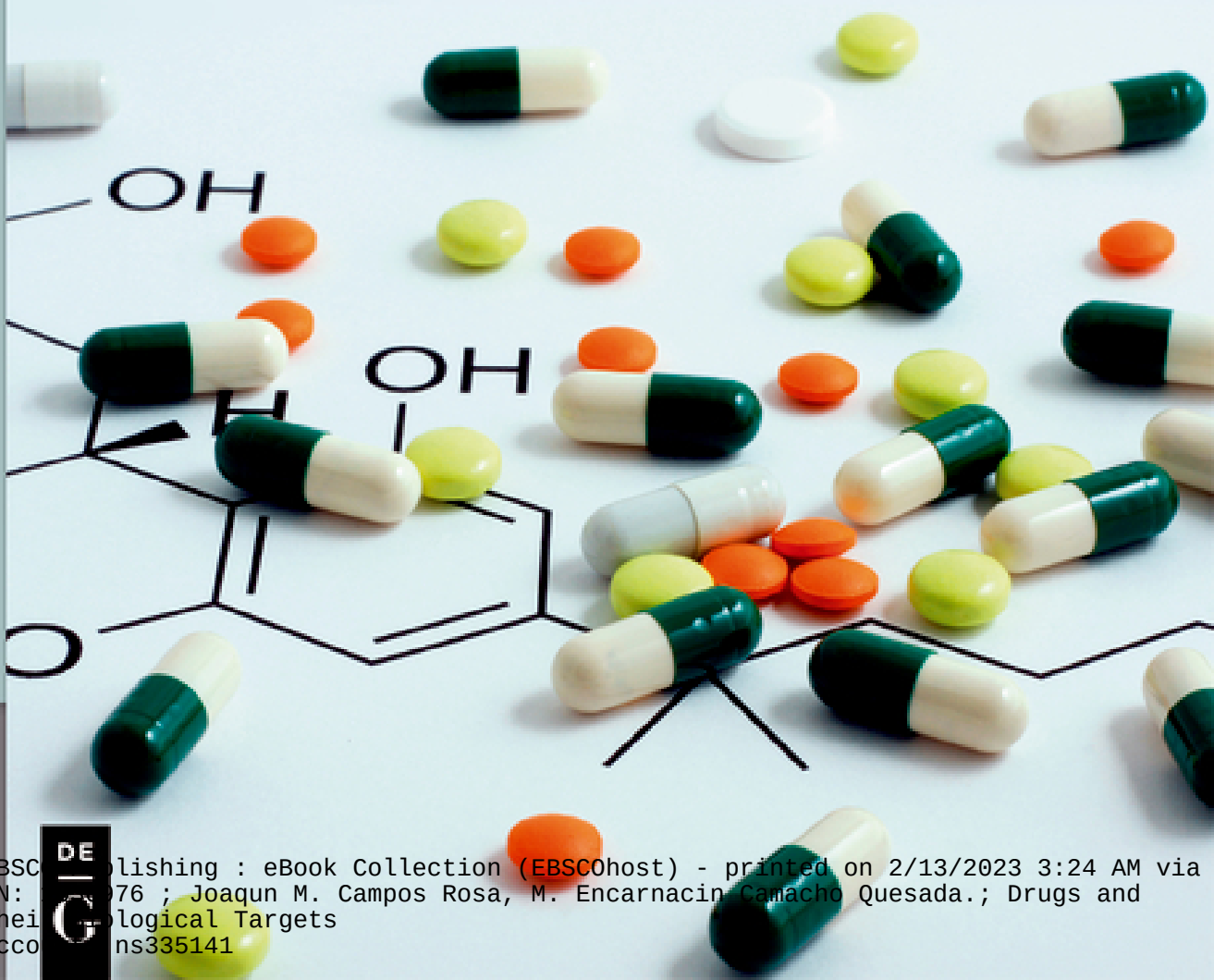

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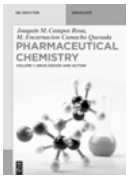

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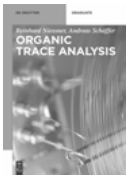

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Pharmaceutical Chemistry

Volume 2: Drugs and Their Biological Targets

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The whole of science is nothing more than a refinement of everyday thinking

"Science is the attempt to make the chaotic diversity of our sense-experience correspond to a logically uniform system of thought. In this system single experiences must be correlated with the theoretic structure in such a way that the resulting coordination is unique and convincing"

Albert Einstein (1879–1955)

Prologue

Pharmaceutical chemistry

In recent years, there have been enormous advances in the fields of biochemistry and molecular biology, organic synthesis, techniques of separation and instrumental analysis, especially HPLC, NMR, MS and X-ray crystallography. Moreover, the spectacular development of information technology and robotics has greatly contributed to changes in the approach to the development of new drugs by the pharmaceutical industry and centers dedicated to research in this field. This suggests that the drugs of the future will probably not resemble those used in the past, but what is absolutely certain is that they will have been developed on a more solid basis of knowledge, that is based less and less on serendipity and more on the biochemical disorders produced by the disease, on the rational design of prototypes and on biotechnology.

This volume is divided into thirteen chapters conveying a systematic study of different drugs, based on the systems they act upon. The drugs will be discussed as a function of the interference that they cause in ionic transport, changes of permeability or alterations of the membrane structure, and those whose mechanism is a consequence of its binding to a membrane or intracellular receptors, which motivates a signal transduction through second messengers. In addition, these chapters will study the chemical synthesis of structures used as prototypes of the different drug families, as well as those drugs of great therapeutic and/or chemical interest.

We will begin with the study of the classical neurotransmitters, acetylcholine and noradrenaline. The chemical transmitter found in all ganglia, neuromotor junctions and postganglionic terminations of the parasympathetic nervous system is acetylcholine, whereas noradrenaline is only found in postganglionic sympathetic terminations. The study of acetylcholine is addressed in Chapter 1, which describes the effects of agonist drugs on muscarinic receptors and of antagonistic drugs on the muscarinic and nicotinic receptors respectively. An attempt is made to explain the muscarinic response with the study of cholinergic or parasympathetic-mimic agonists. A distinction is made between direct and indirect agonists, as well as acetylcholinesterase inhibitors, which can be used as pesticides, and have great military and economic implications.

The nicotinic receptor, which regulates the nonselective channel of certain cations (Na^+, K^+, Ca^{2+}), thereby allowing a depolarization during nerve transmission, is described. There follows a brief study of the agonists, which have no therapeutic relevance, to describe their antagonistic activities as blockers of the neuromuscular junction. The chapter concludes with a description of the antimuscarinic agents, with the natural tropanic structure and the synthetic derivatives, with mytriatic, spasmolytic and antiulcerous utility.

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We then study the adrenergic system, to which Chapter 2 is devoted. In it, we study the different stages of the neurotransmitter cycle (biosynthesis, storage, release, association to presynaptic receptors that regulate biosynthesis and release, reuptake and metabolism). Drugs that may act at nonreceptor levels are described, such as false neurotransmitters, adrenergic blockers, enzyme inhibitors, reuptake inhibitors and indirect adrenergic agonists, such as *Ephedra* alkaloids, amphetamines, and related compounds. This is followed by a study of the adrenergic drugs at the receptor level, establishing the division of receptors suggested by Ahlquist and describing the different physiological responses. A profound analysis is made of both agonists and antagonists, marking the structural aspects that give receptor selectivity and stereospecificity, with great therapeutic applications, such as bronchodilators, antihypertensives and nasal decongestants.

The study of dopamine is discussed in Chapter 3, where the central (D_1 and D_2) and peripheral receptors are described; the different neurotransmitter conformers are discussed and agonist therapy, especially as antiparkinsonian agents, and the antagonists, such as neuroleptic drugs. Phenothiazine derivatives and other tricyclic systems, butyrophenones and their derivatives, benzamides and derivatives of ergot alkaloids are also discussed. A study of drugs with antiparkinsonian action, whose mechanism is to modulate the biosynthesis, release and metabolism of dopamine, is also carried out.

Chapter 4 deals with serotonin, or 5-hydroxytryptamine (5-HT), and its receptors, describing a series of antidepressant drugs whose mechanism of action is to inhibit the reuptake, or the metabolism, of serotonin. It also addresses a study of agonists and antagonists that are widely dispersed structurally, and sometimes also linked to dopaminergic receptors, which have pharmacological application, such as anxiolytics, antidepressants, anorexics, antimigraine and antiemetics. Lysergic acid derivatives and other ergoline compounds, whose structural features are linked to D_2 and 5-HT_{1D} antagonists, are also studied. Among the 5-HT_2 and 5-HT_3 antagonists, the indole derivatives (of the ondasetron type), widely used as antiemetics in patients undergoing radiotherapy treatment, are very useful.

In Chapter 5, the inhibitory amino acids (neutral amino acids: GABA, glycine and taurine) and excitators (acid amino acids: glutamic and aspartic acid) of the central nervous system are introduced. When studying the GABA receptor as a regulator of the chloride ion channel, the structure-activity relationships of benzodiazepines are analyzed because of their importance as anxiolytic drugs.

Chapter 6 is devoted to the study of narcotic analgesics, developing the concept of endogenous agonists (enkephalins and endorphins) and the types of opioid receptors (μ, δ, κ and σ). After a description of the opium alkaloids used as narcotic analgesics and antitussives, we deal with the stereochemical aspects of morphine and derivatives that preserve the pentacyclic system, with agonist and antagonistic activity. Next, semisynthetic derivatives are produced by the technique of structural disjunctive vari-

ation, indicating their structure-activity relationships. Finally, the structure-activity relationships are established with the endogenous peptides.

Chapter 7 begins the study of histamine. It is a widely distributed chemical messenger, which plays an important role in certain intracellular communication processes. The chapter describes the biological cycle of histamine and addresses its structural aspects. The different receptors and more importantly, from a pharmacological point of view, the different H_1 and H_2 antagonists are also described. They begin with the processes of structural variation made to improve the action and avoid side effects such as sedation. The second part of the chapter deals with the systematic study of a classic example of rational development of a drug, such as cimetidine.

Many clinically useful agents act by inhibiting the organism's own enzymes involved in the biosynthesis or metabolic degradation of endogenous substances that perform important functions. In Chapter 8, we will discuss some examples of drugs useful in therapeutics, including carbonic anhydrase inhibitors. In the second part of this chapter, starting with the renin-angiotensin system and the importance of the inhibition of the angiotensin-converting enzyme (ACE) for the design of antihypertensive drugs, the focus will be on the study of the peptide mediators. It describes how from teprotide, a nonapeptide found in the venom of the viper *Bothrops jararaca*, and taking as a model the metallo-protease carboxypeptidase, captopril was reached and then extended to two large families: carboxyalkanoyl and mercaptoalkanoyl amino acids.

Chapter 9 describes the different metabolites of arachidonic acid to then proceeds to the development of prostaglandins and analogues with therapeutic utility. Antagonists of the cyclooxygenase enzyme of great therapeutic utility, such as antalgesic drugs (which increase sensitivity to pain or reduce the pain threshold), antipyretics and nonsteroidal anti-inflammatory drugs (NSAIDs) are also described. Next, the different prototypes (derivatives of salicylic acid, *p*-aminophenol, *N*-arylanthranilic, arylacetic or propionic acids, etc.) used as anti-anxiety, antipyretic drugs are described. Finally, the selectivity of action on the isoenzyme cyclooxygenase-2 (COX-2) as a source of anti-inflammatory drugs with a lower incidence of side effects is discussed.

In Chapter 10 some families of drugs will be considered whose mode of action consists in the modulation of certain ion channels that, unlike those considered until now, are not regulated by specific ligands but by potential differences: the arrival of a nerve impulse (change in membrane potential) is able to regulate the opening or closing of an ion channel. These channels, known as *potential-dependent channels* generally result in faster changes in the membrane potential than those caused by the activation of the ion channels dependent on a ligand. Local anesthetics are compounds that decrease the excitability of cells by blocking the potential-dependent sodium channels. From a therapeutic point of view, the design of calcium channel *blockers* has become important because these blockers treat a variety of conditions and are useful as *antiarrhythmics* for the regulation of the rhythm of cardiac contraction. Moreover, they are also useful as *hypotensors*, for their ability to relax the heart muscle and the smooth

fiber of blood vessels, and as *antianginals*, for their ability to counteract the coronary ischemia associated with *angina pectoris*. Although the role of potassium channels in the transmission of membrane potential and in cellular excitability processes has long been known, the design of selective drugs at this level is a relatively unexplored field, partly due to the scarcity of ligands that may allow the study of the electrophysiology of the channels. The chapter concludes with the study of inhibitors of ATPase H^+/K^+ as antiulcer drugs, among which omeprazole stands out.

Penicillins and cephalosporins are studied in Chapter 11. The development of penicillins is responsible for the dramatic increase in the life expectancy of the population in western countries, which previously remained below the age of fifty. Many scientists consider penicillins the most important achievement of medicine in the last century. Since the commercialization of cephalothin in 1962, cephalosporins have risen to a position of distinction in the world of antibiotics. The modification of the side chains to the cephalosporin nucleus has produced an extraordinary proliferation of new compounds for clinical use which have become very important in the treatment of bacterial infections due to their relatively low toxicity, broad antibacterial spectrum, bactericidal activity and activity against β-lactamases.

Chapter 12 presents the therapeutic antibacterial arsenal belonging to other fields and their mechanisms of action, such as sulfonamides, agents affecting protein synthesis, aminoglycosides, tetracyclines, chloramphenicol, aminoacridines and fluoroquinolones. Aminoglycosides, tetracyclines, chloramphenicol and macrolides interfere with the synthesis of proteins at the ribosome level. Sulfamides and trimethoprim act as antimetabolites preventing the synthesis of purines. Fluoroquinolones act at the level of DNA strands, preventing supercoiling by inhibition of a topoisomerase such as DNA gyrase.

In Chapter 13, we will study anticancer and antiviral drugs. More than 100 chemotherapy drugs are currently being used in cancer treatment, either alone or in combination with other medications or treatments. From the antimetabolites we will study 5-fluorouracil and one of its prodrugs, capecitabine, while from the mitosis inhibitors, paclitaxel and docetaxel will be the objects of our study. From the most modern drugs, we will cover tyrosine kinase inhibitors, such as gefitinib.

Advances in the chemotherapy of viral diseases are much fewer than those achieved in the treatment of bacterial infections. In the USA, only a few antiviral agents of demonstrated clinical value are available. However, we will address some examples of extensive medical treatment.

The final version of this work is the responsibility of the authors. We apologize to the readers for any errors and omissions that may exist in advance, and we look forward to your generous help to overcome them in the future.

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Contents

1 Acetylcholine

1.1 Goals

- To introduce the student to the concept of neurotransmitter.
- To study the concept of pharmacophore group in more depth through the interactions between acetylcholine and its receptors.
- To broaden the student's knowledge about the most important drugs that act at this level and about their structure-activity correlations.
- To introduce the student to enzymatic inhibition exemplified by the enzyme acetylcholinesterase and its importance in certain pathologies.

1.2 Nerve transmission through the synapse

The physiological mission of chemical mediators of nerve transmission, or neurotransmitters, is communication of the nerve impulse, both voluntary and autonomous or vegetative, from one cell to the next.

Nerve transmission can be propagated along the axon of a nerve cell without the competition of more chemical compounds than those constituting the inflow and outflow of ions, resulting in a depolarization wave. However, when it reaches the end of the axon (generally highly branched in dendrites), the cell membrane represents an insurmountable barrier to ion exchange with the next cell. Nerve transmission can be propagated along the axon of a nerve cell. There must therefore exist some mechanism to selectively trigger the depolarization of the next cell. In general, this physiological mechanism consists of the arrival of the impulse at the end of the axon and the release of a chemical compound capable of being biosynthesized in the nerve cell and that was stored inside the axon. This compound, called neurotransmitter, diffuses through the small space that separates the end of the axon from the next cell, called the synaptic synapse or cleft. When it reaches the surface of the postsynaptic cell, it binds to specific receptors. When the receptors are occupied by the neurotransmitter, this causes a biochemical change on the other side of the membrane, which ultimately leads to the depolarization of the cell (if it is nervous), to its contraction (if it is muscular), or the release of hormones or other products (if it is glandular). Once its action is completed, the neurotransmitter must be removed from the synaptic zone in order for it to recover its excitability, either through the process of its enzymatic metabolism or through its recovery (reuptake) towards the presynaptic nervous termination (Fig. 1.1).

A chemical transmitter must meet three conditions:

1. The substance must be released at the presynaptic termination after stimulation (the neurotransmitter can be synthesized at the active site or transported there).

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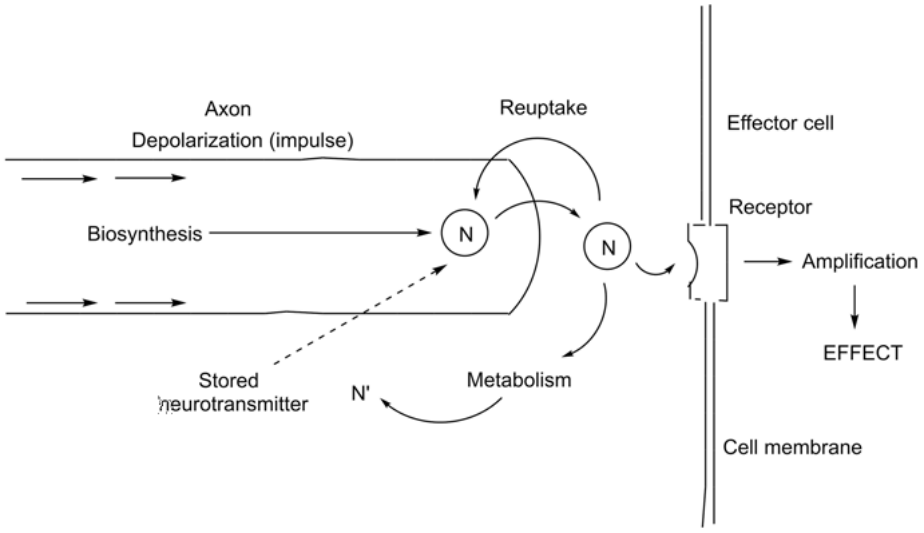


Fig. 1.1: Processes in which the neurotransmitters take part once the nervous transmission has occurred.

2. The substance applied exogenously should have a nerve stimulation effect on postsynaptic termination identical to that produced by a synaptic excitation.
3. The substance should be removed or inactivated at the site of action, and specific antagonists should block their synaptic action.

Amino acids (aspartic acid, GABA, glycine, taurine and glutamic acid), ammonium salts or amines (acetylcholine, dopamine, norepinephrine, tyramine, epinephrine, histamine and serotonin) and other substances, such as prostaglandins and monophosphate of cyclic adenosine, have been identified as chemical transmitters.

All these processes (biosynthesis, release, receptor binding, metabolization and reuptake) are capable of being mimicked or blocked by compounds external to the organism, thus producing important pharmacological effects by imitating the neurotransmitter or interrupting the nerve transmission. Of course, from the point of view of structural variation, the transmitters themselves (N, in Fig. 1.1) will be molecules of great interest, useful as prototypes for synthetic drugs that regulate, i.e., block or imitate their action in any of the indicated points.

1.3 Cholinergic nervous system: muscarinic and nicotinic receptors

Cholinergic nerves are those whose synaptic transmission is mediated by acetylcholine. By extension, cholinergic drugs are those that can mimic acetylcholine or replace it at its specific receptors. Physiologically, cholinergic impulses tend to regulate vital processes, such as circulation, breathing, peristalsis, secretions, urinary bladder, pupil dilation, etc. The synapses in which acetylcholine intervenes are the most abundant synapses within the body.

In the body, information is transmitted through the nervous system (NS). Its main parts include the brain and spinal cord, in addition to the peripheral NS, which consists of nerves and ganglia. A ganglion is a cluster of cell bodies of neurons. The autonomic nervous system (ANS, also called vegetative) innervates almost all the tissues of the body, except for the skeletal musculature. It consists of nerves, ganglia and plexuses (nerve networks), and it helps control the so-called vegetative functions, such as blood pressure, motility, gastrointestinal secretions, body temperature, urine elimination and other bodily functions. Autonomous impulses are transmitted through the body via the sympathetic and parasympathetic systems, the two branches of the ANS.

The sympathetic system tends to discharge generically or in mass. It enters into action during emotional crises or in case of necessity and prepares the body for fight or flight. This sympathetic-adrenal discharge (also involving the adrenal medulla with adrenaline secretion) causes tachycardia, arterial hypertension, mydriasis (pupil openness) and hyperglycemia (Fig 1.2).

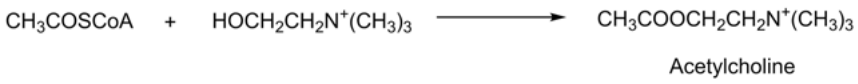
Fig. 1.2: Stimulation of the sympathetic nervous system due to fear prepares the body for fight or flight: it increases the speed of the heart, dilates the bronchi, dilates the pupils, produces constriction of the peripheral blood vessels with the physical consequence of pallor, etc.

On the contrary, the parasympathetic system unloads in a localized form and intervenes in the functions of conservation and restoration. It leads to a delayed heart rate, protects the retina from light (miosis), and stimulates gastrointestinal functions (Fig. 1.3). It has been shown that acetylcholine mediates the synapses between effec-

Fig. 1.3: In a relaxed state, the parasympathetic nervous system slows the pumping rate of the heart and increases the activity of the gastrointestinal tract.

tor nerves and voluntary musculature (skeletal muscle), as well as in the ANS ganglia (both sympathetic and parasympathetic) and in the termination of the effector (postganglionar) neurons of parasympathetic ANS (Fig. 1.4).

Acetylcholine is biosynthesized in the nerve cell by acetylation of choline (a constituent of many phospholipids, such as lecithins) (Scheme 1.1):



Scheme 1.1: Biosynthesis of acetylcholine (AcC).

The neurotransmitter is stored at the nerve end and released at the onset of the impulse. Once released, it reaches its specific receptors, where it gives rise to a conformational change, leading to the opening of an ion channel, which results in a rapid flow of sodium ions along the gradient (i.e., from outside to inside). Once it has exerted its action, AcC is rapidly inactivated through hydrolysis by the acetylcholinesterase enzyme, which is present in the cholinergic synapses (Scheme 1.2).

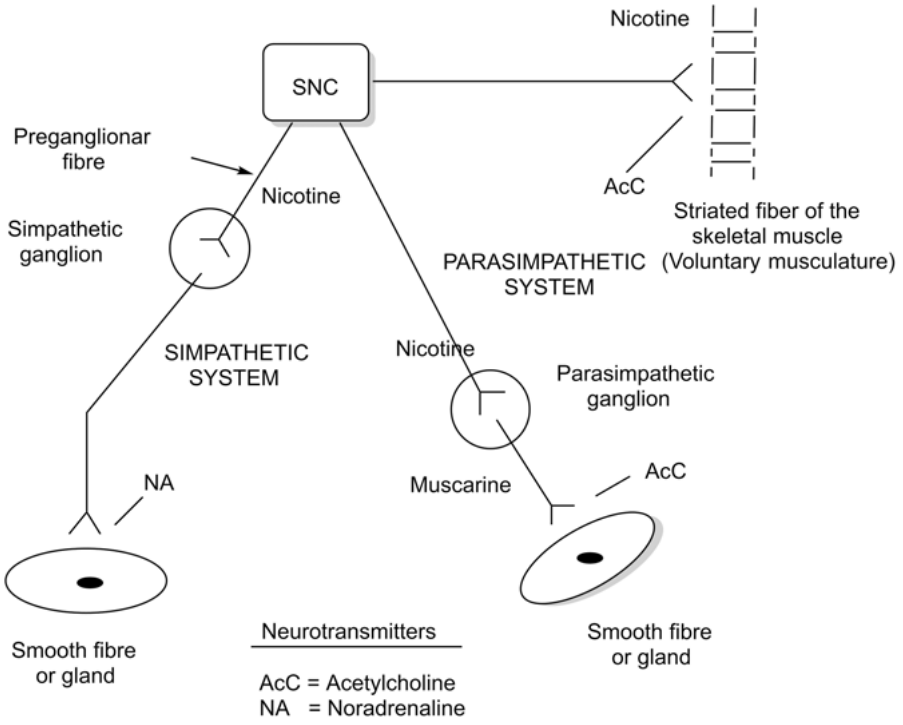
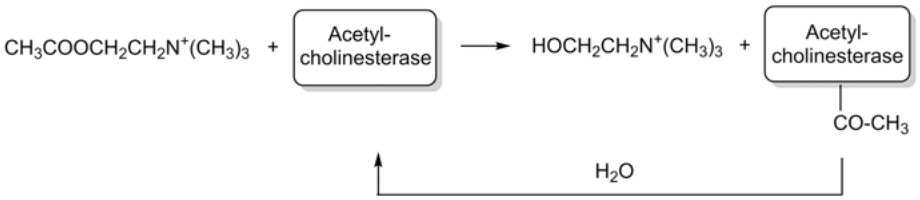


Fig. 1.4: Sympathetic and parasympathetic systems and sites of action of the neurotransmitters, nicotine and muscarine.



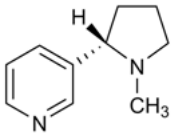
Scheme 1.2: Hydrolysis of AcC by acetylcholinesterase.

Once the AcC is hydrolyzed, choline activates a transport system by the consumption of ATP, which brings about its reuptake into the presynaptic neuron, where it will be acetylated again. Of course, any of these processes are susceptible to modification by appropriate drugs. However, drugs that can bind to cholinergic receptors and acetylcholinesterase are the best studied and most interesting because of their specific nature.

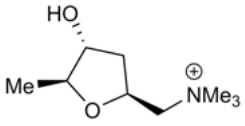
Not all cholinergic receptors are the same: nicotine tobacco alkaloid (Fig. 1.5) acts only as a mimetic for CcA in the voluntary neuromuscular junctions and in the ANS

(sympathetic and parasympathetic) ganglia. The nicotinic response consists of an increase in muscle tone that can lead to convulsions and death by asphyxia.

In contrast, the alkaloid of the *Amanita muscaria*, muscarine (Fig. 1.5) acts exclusively by replacing the AcC in the receptors of the postganglionic synapses of the parasympathetic system (that is, in the smooth muscle and glands), giving rise to typical parasympathomimetic actions: cardiac inhibition, vasodilation, pupillary contraction, increase of secretions, increase of peristalsis, etc. These effects have been called muscarinic to distinguish them from the nicotinic ones. AcC presents both at the same time.



Nicotine



L-(+)-Muscarine

Fig. 1.5: Nicotine and muscarine.

1.4 Direct agonist drugs

In practice, AcC turns out to be an ineffective therapeutic agent: it is inactive when administered orally and a little more active when administered parenterally (effected by means other than the digestive or intestinal route) due to the existence of plasma esterases that rapidly hydrolyze the acetate group. The only application that has been observed is to achieve a brief miosis (contraction) through a topical ophthalmic use.

Cholinergics are useful drugs in the treatment of tachycardia (cholinergic stimulus decreases the rate of the heart beat), urinary retention (urine bladder atony) and glaucoma (pupil contraction reduces intraocular pressure). The only useful cholinergics with direct action are those which can withstand enzymatic hydrolysis for some time, a property that can be achieved through a structural modification of AcC.

1.5 Molecular modifications of AcC

In view of the limitations associated with the use of AcC, extensive study of its structure-activity relationships has been carried out:

1.5.1 Modifications in the ammonium moiety

These have led to negative results in all cases. Studies of SARs (structure-activity relationships) have shown that the trimethylammonium group is optimal for cholinergic action and is conserved when only one of the methyl groups is replaced by an ethyl. When the trimethylammonium group ($-N^+Me_3$) present in the AcC is substituted by the triethylammonium ($-N^+Et_3$) in the corresponding analogue, a reduction of 2,000 times in the power occurs. This large variation is not exclusively due to the increase in distance between the N^+ and the anionic center of the cholinergic receptor. The most important factor seems to be the increase of the ion size, which entails an increase of the dielectric constant between both charges (Fig. 1.6).

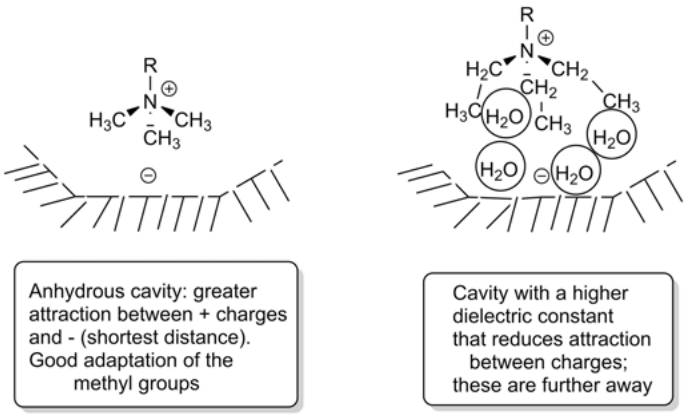


Fig. 1.6: Explanation of the lower activity of the AcC analogue when the trimethylammonium group is replaced by triethylammonium.

The optimal action of the trimethylammonium group seems to indicate that there is either a hydrophobic or a van der Waals bond, which creates a hollow with a low dielectric constant microenvironment. In contrast, when the groups are larger than the methyl one, the cavity is enlarged, water molecules enter, and the dielectric constant increases greatly, while the attraction force between the charges is proportionally reduced.

1.5.2 Modifications of the ethylenic bridge

The increase in chain length (homologues) has in all cases resulted in inactive compounds (↑ chain ≡↓power). However, the introduction of methyl substituents on the ethylenic chain (branching) has led to interesting results (Fig. 1.7). This can be carried out in two positions and in both cases, the resulting molecule is chiral.

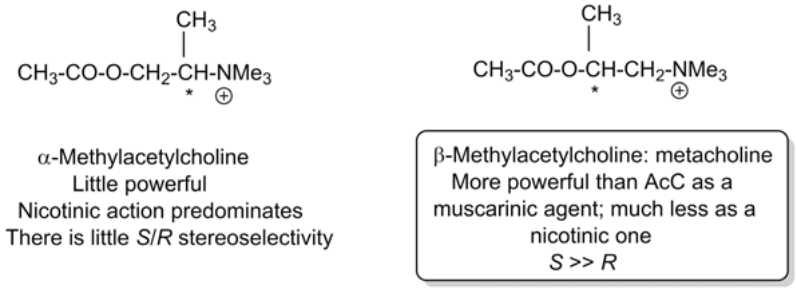


Fig. 1.7: α-Methylacetylcholine and β-methylacetylcholine.

The higher activity of methacholine relative to the natural neurotransmitter is mainly due to a reduction, approximately by half, in the rate of hydrolysis by acetylcholinesterase, and it can be attributed to a steric effect of the methyl group next to the ester function (Fig. 1.8).

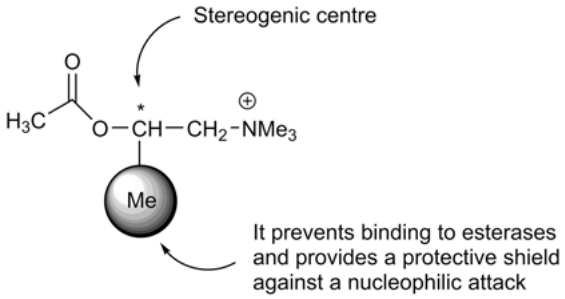
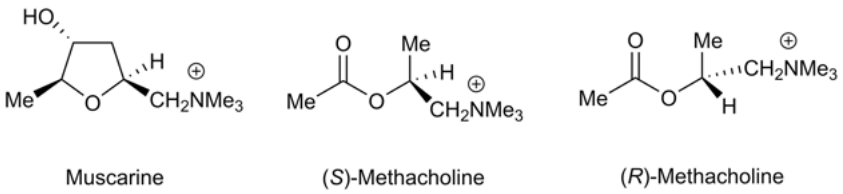


Fig. 1.8: Methacholine.

It is interesting to note that, of the two enantiomers of methacholine, the muscarinic activity resides in the one with the absolute configuration *S*, the same as that of muscarine in carbon 5. Methacholine can therefore be considered an open analogue of muscarine (Fig. 1.9).

1.5.3 Modifications of the acyloxy group

The change of the ester function by thioester, amide, ketone, ether or thioether has given rise to analogues devoid of cholinergic activity. However, attempts to reduce the rate of hydrolysis of AcC by varying electron effects around the carbonyl group have led to interesting results. Let us look at another previous aspect that will help us understand the electronic effects (Fig. 1.10).



Muscarine (*S*)-Methacholine (*R*)-Methacholine

Fig. 1.9: Comparison between muscarine and (*R*)- and (*S*)- enantiomers of methacholine.

Neighboring-group participation: As previously discussed, AcC is very prone to hydrolysis, and this can be explained by considering one of the conformations that the molecule can adopt (Fig. 1.10). There are four possible conformations, visualized as the Newman projections, with the following denominations:

- *anti* prefix is used when the bonds of the bulky groups form angles greater than 90°.
- *syn* prefix is used when the bonds of the most bulky groups form angles less than 90°.
- *periplanar* termination is applied when the two larger groups are in the same plane.
- *clinal* termination is when the two most bulky groups are in different planes.

The synclinal conformation is the one that is produced most often (Fig. 1.10).

In the synclinal conformation, positively charged nitrogen interacts with carbonyl oxygen and exerts an attracting effect on electrons. To compensate for this, the oxygen atom removes electrons from the neighboring carbon atom by means of an inductive effect, leading it to an electronically deficient atom and that is therefore more prone to nucleophilic attack. Water is a weak nucleophile, but because the carbon in the carbonyl group is more electrophilic, hydrolysis takes place easily. Such influence of the ammonium ion is known as neighboring-group participation.

Electronic effects: Attempts to reduce the rate of hydrolysis of AcC by varying electron effects around the carbonyl group have led to the design of carbachol, a carbamate analogous to AcC with greater resistance to hydrolysis (Fig. 1.11).

The resistance of the carbamate or urethane groups to hydrolysis can be rationalized by considering their resonant forms: the participation of the electron pair of the nitrogen atom partially compensates for the positive charge density of the carbonyl group, making it less susceptible to the nucleophilic attack.

Bethanechol appeared from the combination of steric effects (introduction of a methyl group at β position) and electronic effects (substitution of the acetate group for a carbamate), Bethanechol is a selective muscarinic drug, orally active and with therapeutic utility. Just like in methacholine, the muscarinic activity of bethanechol resides in the (*S*)-(+) enantiomer.

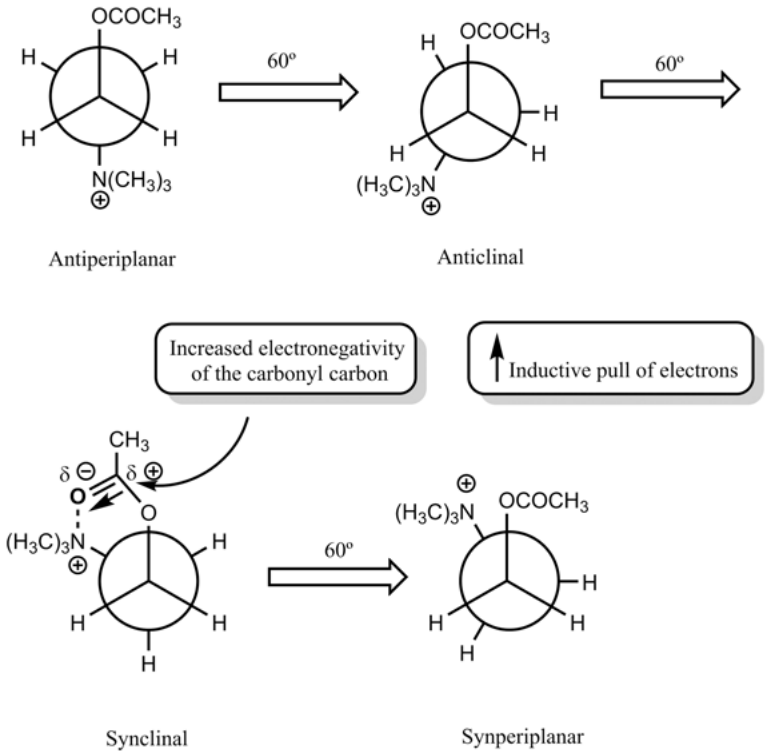


Fig. 1.10: Neighboring-group participation. The arrow indicates the electronic delivery of oxygen, which leads to an increase in the electrophilicity of carbonyl carbon.

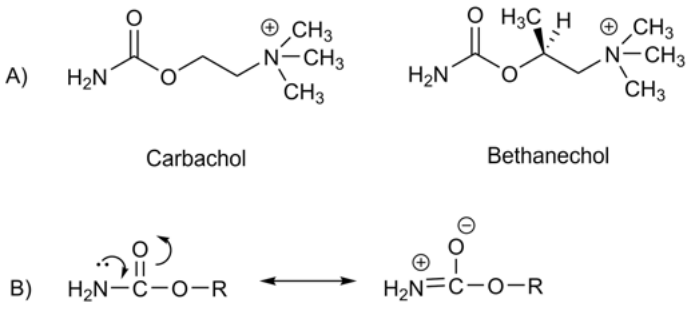
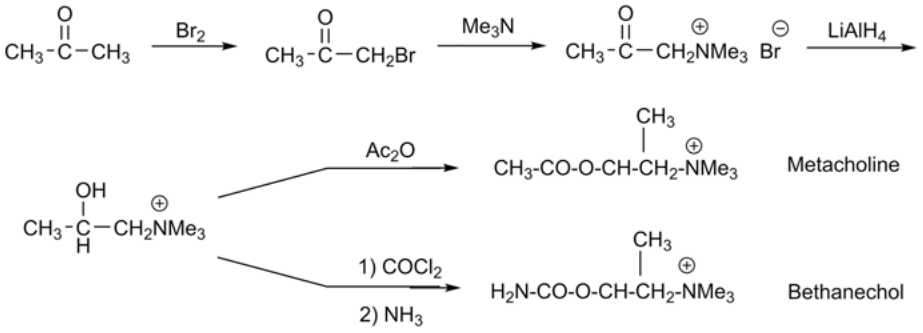


Fig. 1.11: (A) Analogous carbamates of AcC; (B) resonant forms of the carbamate or urethane function.

1.6 Synthesis of methacholine and bethanechol

Both syntheses have a common intermediate (Scheme 1.3):



Scheme 1.3: Synthesis of methacholine and bethanechol.

1.7 Muscarinic drugs derived from other models

Pilocarpine is an alkaloid that finds application in ophthalmology, namely in the treatment of glaucoma. Its muscarinic activity has been rationalized according to the presence of functional groups capable of interacting on the muscarinic receptors in an analogous way to AcC (Fig. 1.12).

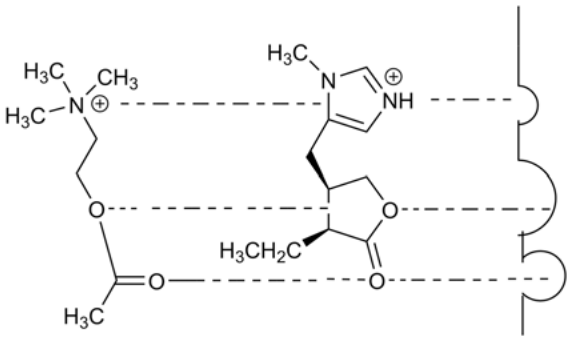


Fig. 1.12: Interactions of pilocarpine and AcC with a muscarinic receptor.

1.8 Clinical uses of cholinergic agonists

1.8.1 Muscarinic agonists

- Treatment of glaucoma.
- Activation of the gastrointestinal and urinary tracts after an operation.
- Treatment of certain heart defects that are due to decreased muscle activity and speed.

1.8.2 Nicotinic agonists

- Treatment of *myasthenia gravis*, an autoimmune and chronic neuromuscular disease characterized by varying degrees of weakness of the body's skeletal (volunteer) muscles. The denomination comes from Latin and Greek, and literally means "severe muscle weakness".

1.9 Muscarinic antagonists. Clinical effects

- Reduction of salivary and gastric secretions.
- Reduction of the motility of the gastrointestinal and urinary tracts (GIT and UT) by smooth muscle relaxation.
- Dilation of the pupils of the eyes.
- Effects on the CNS.

1.9.1 Clinical uses

- Deactivation of the GIT and UT during surgical operations.
- Ophthalmic exams.
- Relief of peptic ulcers.
- Treatment of Parkinson's disease.
- To counteract anticholinesterase poisoning.
- To counteract dizziness.

1.10 Muscarinic antagonists

1.10.1 Atropine

Atropine (Fig. 1.13) is obtained from the roots of belladonna (*Atropa belladona*) and was used to dilate the pupils of women's eyes to appear more beautiful (hence the

belladonna name). Clinically, it has been used to decrease the motility of GIT and to counteract anticholinesterase poisoning.

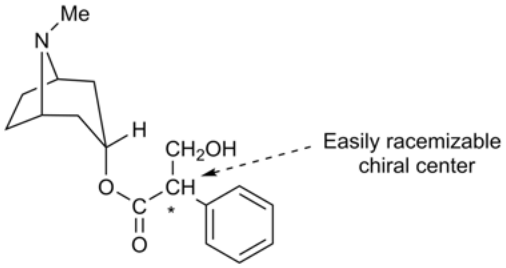


Fig. 1.13: (±)-Atropine and (*S*)-(-)-hyoscyamine.

Atropine has a stereocenter (*) and therefore two enantiomers are possible. Generally, natural products exist exclusively as a single enantiomer. This is also true for atropine, which is present in the *Solanaceae* plant species as a single enantiomer called (*S*)-(-)-hyoscyamine. However, as soon as the natural product is extracted in solution, the stereocenter racemizes, so that atropine is obtained as a racemic mixture and not as the only enantiomer. The stereocenter of atropine is easily racemized since it is adjacent to a carbonyl group, whereby the proton bound to the stereocenter is acidic and easily substituted.

1.10.2 Hyoscine

(±)-Atroscin, (*S*)-(-)-scopolamine or hyoscine (Fig. 1.14) is obtained from the estramonium, and its structure is very similar to that of atropine. Hyoscine has been used to treat motion sickness and postoperative nausea and vomiting.

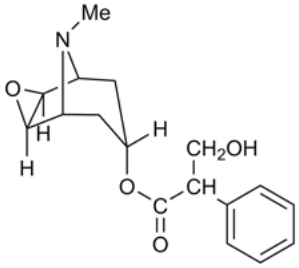


Fig. 1.14: (±)-Atroscin, (*S*)-(-)-escopolamine (hyoscine).

These two compounds can bind and block the cholinergic receptor. However, at first glance, they have nothing to do with AcC. If we look more closely, we can see a basic nitrogen and an ester group, and if we overlap the skeleton of AcC over that of atropine,

we see that the distances between the ester and the nitrogen of both molecules are similar (Fig. 1.15).

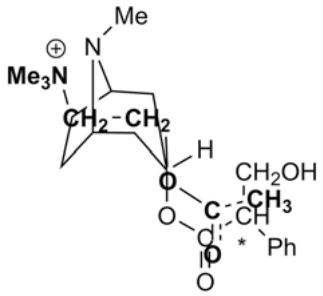


Fig. 1.15: Skeleton of AcC overlapping that of atropine.

However, the problem is that the nitrogen of atropine has no charge, whereas that of the AcC is a quaternary nitrogen and, accordingly, it has a positive charge. This implies that the nitrogen atom of atropine protonates when it binds to the cholinergic receptor.

As a consequence, atropine appears to have two important binding characteristics of AcC: a nitrogen, charged when protonated, and an ester group. Therefore, it can bind to the receptor, but it is not able to activate it. Because atropine is larger than AcC, it is able to bind to other binding groups outside the binding site of AcC. As a result, it interacts differently with the receptor, so that it does not induce the same conformational changes that AcC produces.

1.11 Structural analogues based on atropine

High lipophilicity, both of atropine and hyoscine, allows them to cross the blood-brain barrier (BBB) and distribute them effectively in the brain, where they give rise to various stimulation symptoms, which can lead to hallucinations. These side effects greatly limit their use as antispasmodic and antiulcer drugs. For this reason, the first semisynthetic derivatives were quaternary ammonium salts or amine *N*-oxides (Fig. 1.16). Although quaternization does not necessarily increase the action, it reduces penetration into the CNS and reduces gastrointestinal absorption (they are not lipophilic drugs), favoring the local actors of quaternary derivatives local action in the digestive tract. *N*-oxides are obtained by treatment of the base with hydrogen peroxide (H_2O_2) in an acid medium; in vivo they turn into the bases, so that the modification prolongs the antimuscarinic action.

These derivatives have poor systemic absorption and are mainly used in the treatment of gastrointestinal ulcers (associated with antacids to prolong their action), as well as in all those disorders that require a general reduction of secretions and contraction of smooth fiber. Muscarinic receptors are divided into five subtypes: M1–M5.

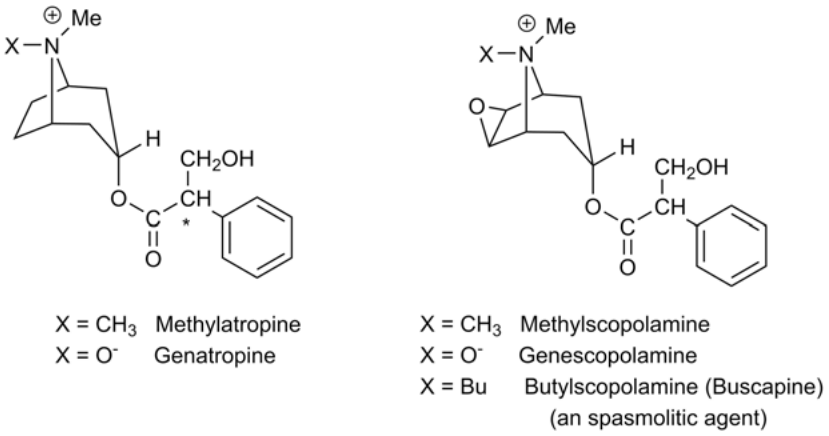


Fig. 1.16: Semisynthetic derivatives from atropine and scopolamine.

1.12 Main anticholinergic drugs obtained by synthesis. Structure-activity relationships

Synthetic atropinic anticholinergic drugs were designed from the natural model of atropine (by cycle excision and simplification) or from AcC, by introducing "blocking", bulky and lipophilic groups in their acetic ester moiety. Of course, the useful antimuscarinics found in this way became in turn, models of new drugs, that are not all anticholinergics (Fig. 1.17).

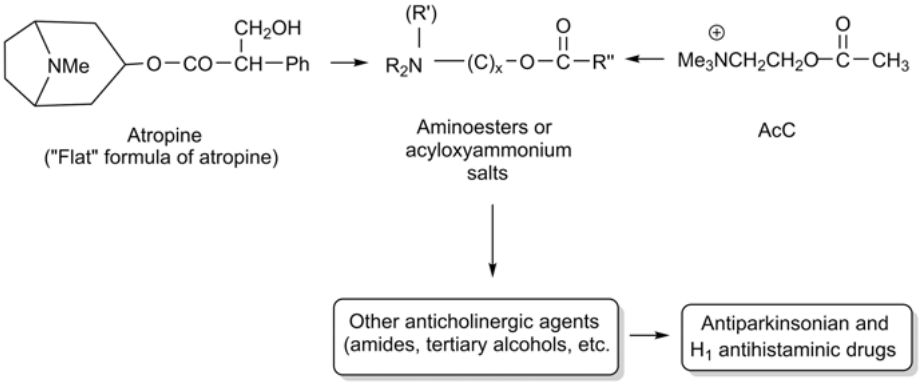


Fig. 1.17: Modification of AcC and atropine as a source of other drugs.

The common structural features of antimuscarinics can be found after rotating the structure 180°, as shown in Fig. 1.18.

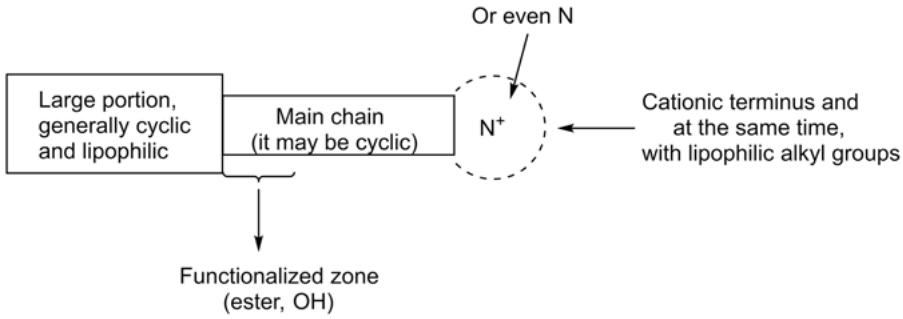


Fig. 1.18: Structural characteristics of antimuscarinics.

This Fig. 1.18 also corresponds to other groups of drugs, so that each molecule of a synthetic anticholinergic, in addition to its antimuscarinic action, will present other actions to a greater or lesser degree: CNS stimulation or depression, antihistaminic, local anesthetic, etc. In addition, in the anticholinergic action, one of the three main components may be predominant: antispasmodic, antisecretory or mydriatic. This set of pharmacological factors determines the clinical use of each of the specific drugs. We now turn to M2 antimuscarinics, which are the most important among the anticholinergics.

1.13 M2 Antimuscarinics

They are synthetic compounds structurally related to AcC. They incorporate an accessory area of high volume and lipophilicity in the neighborhood of the ester group, as evidenced in the alkaloids related to atropine. M2 antimuscarinics can also have voluminous substituents on the nitrogen atom, which shows the presence of complementary hydrophobic zones at that level. M2 antimuscarinics are structurally classified into aminoalkyl esters, aminopropanols and aminoamides (or amidoammonium derivatives). The general structures of these drug families are shown in Fig. 1.19. In all cases, the quaternization of the nitrogen atom leads to compounds devoid of central action.

1.14 Antagonist drugs on nicotinic receptors

The first known compound with antagonistic activity on nicotinic receptors of the neuromuscular junction was tubocurarine chloride (Fig. 1.20), the active ingredient contained in curare, extract of the *Chonchodendron tomentosum* plant, used by the Amazonian Indians in the hunting arts for its paralyzing properties.

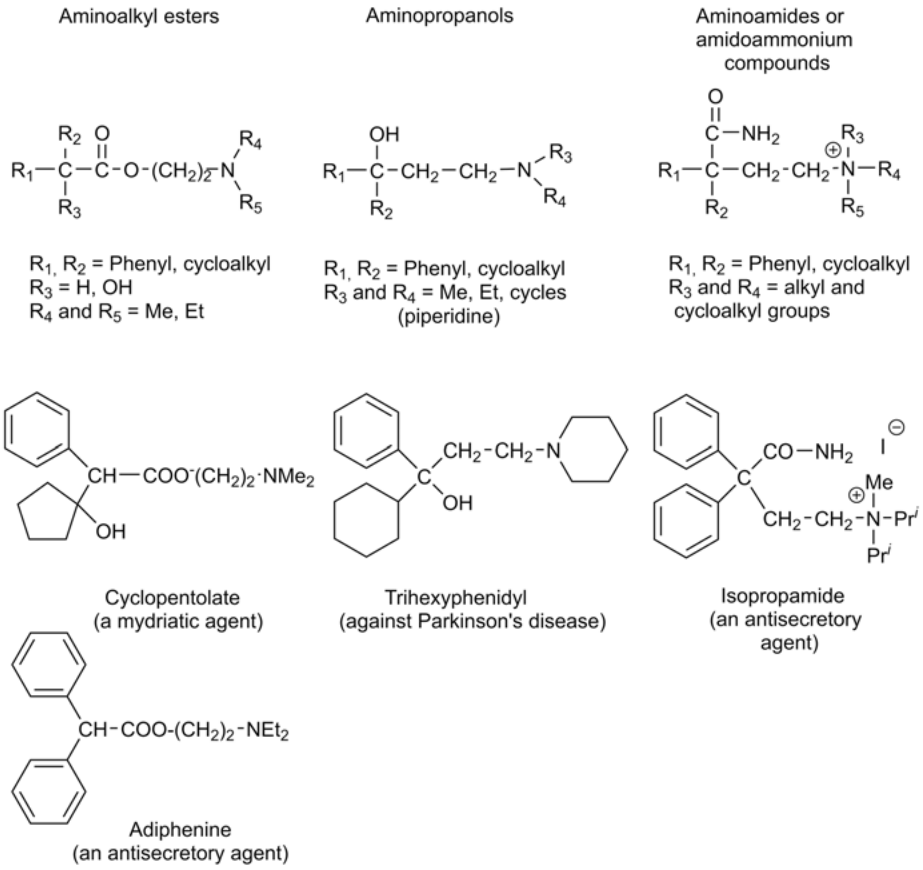


Fig. 1.19: Structural families and some M2 antimuscarinics.

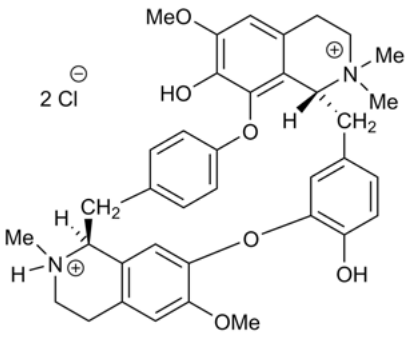


Fig. 1.20: Tubocurarine chloride.

Curare is actually a mixture of compounds, and its active substance (tubocurarine) was not isolated until 1935. The determination of its structure took even longer and was not established until 1970. Tubocurarine was used clinically as a neuromuscular blocker. However, since it has side effects (it is an antagonist in the nicotinic receptors of the autonomic nervous system), better neuromuscular blockers are now available.

One problem associated with tubocurarine is the absence of the ester moiety, which would not explain its strong blocking action on the nicotinic receptor. The blocking action of tubocurarine, as well as that of synthetic analogues with a double ammonium salt structure, is due to the presence of two cationic centers. They are separated by 1.4 nm (*nicotinic distance in the neuromuscular junctions*), anchoring the molecule to one of the binding sites of AcC and over an accessory zone, in which a cysteine residue is probably present (Fig. 1.21), located 0.9–1.2 nm away.

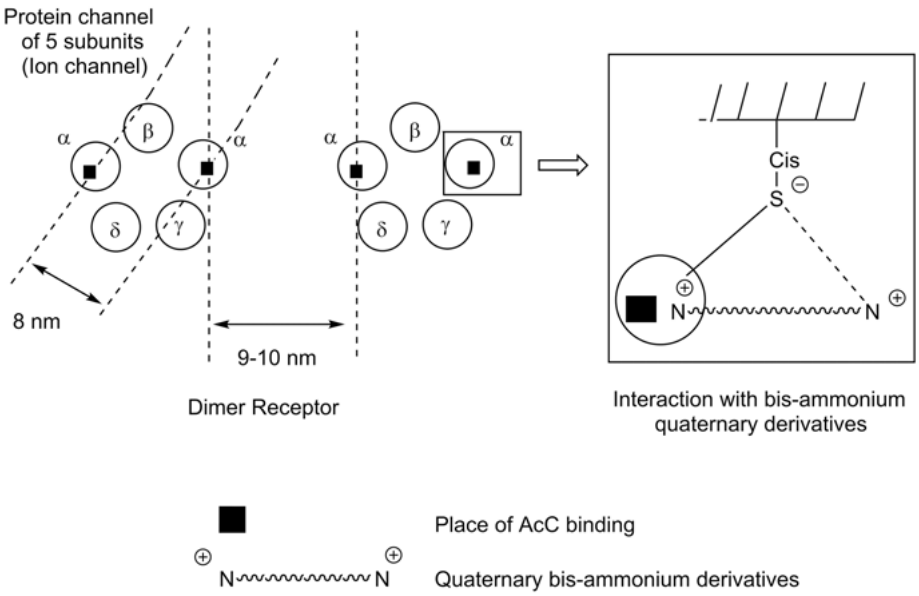


Fig. 1.21: Interaction model of the quaternary bisammonium derivatives with the nicotinic receptor.

The possibility that the quaternary bisammonium derivatives simultaneously bind to the two subunits of the ionic channel is not feasible because of the great distance between them (8 nm). On the other hand, neither is it possible between subunits of different monomers, since they are separated by a distance of 10 nm. Therefore, it is clear is that the distance between the two positively charged nitrogen atoms is crucial for the activity. Consequently, analogues that maintain this distance should be good antagonists.

1.14.1 Decamethonium and suxamethonium

Decamethonium (Fig. 1.22) is a simple analogue of tubocurarine. It is a linear molecule, and as such it is capable of achieving a large number of conformations. The fully extended conformation would have the nitrogen atoms separated by a distance of 1.35 nm, which is analogous to the distance of tubocurarine (1.4 nm).

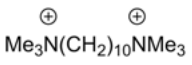


Fig. 1.22: Decamethonium.

The drug binds strongly to nicotinic receptors, but has several drawbacks: when it binds to the AcC receptor, it acts as an agonist rather than an antagonist. In other words, it activates the receptor, leading to a brief contraction of the muscle. Once this effect has passed, the drug remains bound to the receptor (blocking access for AcC) and then acts as an antagonist. Unfortunately, it binds very strongly, and accordingly, patients take a long time to recover from its effects. It is also not completely selective for neuromuscular junctions and has effects on the AcC receptors in the heart. This leads to a decrease in heart rate (bradycardia) and a drop in blood pressure.

As decamethonium is very stable, the introduction of some form of instability into the drug was considered. This was achieved by introducing ester groups in the chain, while maintaining the correct distance between both positively charged nitrogen atoms; suxamethonium (Fig. 1.23) was designed in such a way.

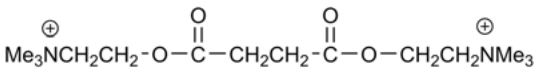


Fig. 1.23: Suxamethonium or succinylcholine.

The ester groups are susceptible to chemical and enzymatic hydrolysis. Once hydrolysis takes place, the molecule cannot bind to the receptor and becomes inactive. Drugs designed to be metabolized through a single, nonoxidative pathway are known as soft drugs. Suxamethonium's duration of action lasts only 5–10 minutes, but it has other side effects on the autonomic ganglia.

1.14.2 Atracurium

The design of atracurium (Fig. 1.24) was based on tubocurarine and suxametonium structures. It is superior to both, because it lacks cardiac side effects and rapidly degrades in the blood.

This rapid degradation allows the drug to be administered intravenously. Rapid degradation was designed by incorporating a self-destructive mechanism. At the pH

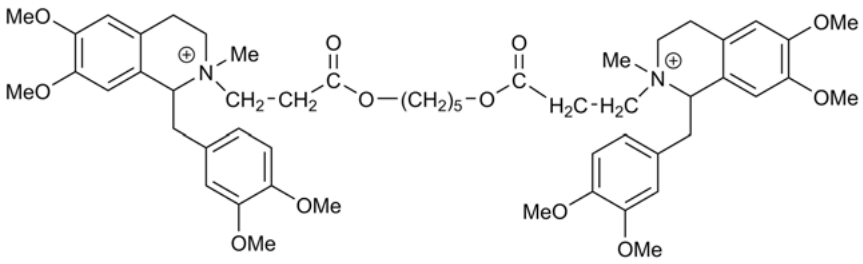


Fig. 1.24: Atracurium.

of blood (7.4), the molecule can undergo Hofmann elimination. Once this occurs, the compound is deactivated as the positive charge on the nitrogen atom is lost (Fig. 1.25).

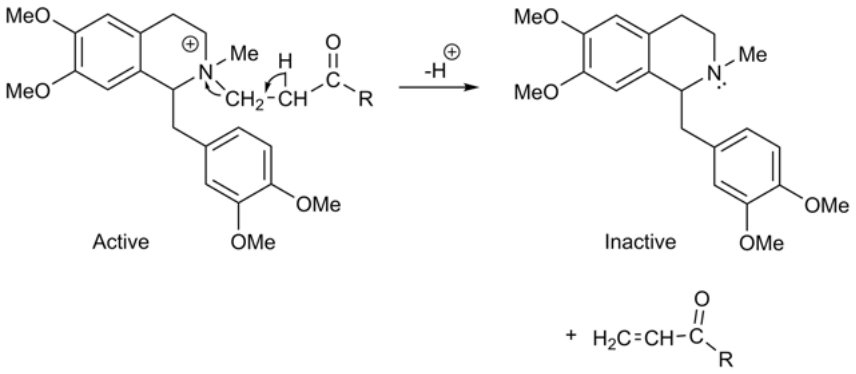


Fig. 1.25: Hofmann elimination on atracurium.

The characteristics of atracurium are as follows:

- The spacer: A chain of 13 atomic units connecting the two quaternary centers.
- Blocking units: Cyclic structures at both ends of the molecule block the AcC receptor site.
- Quaternary centers.
- Hofmann elimination: This usually requires strong alkaline conditions and high temperatures. However, if an electron-withdrawing group is present at the β-carbon in relation to N^+ (as is the acetate group), it allows the reaction to proceed under much milder conditions. The electron-withdrawing group increases the acidity of the hydrogen atoms in the β-carbon atom, so that they are easily lost.

Since the drug only acts very briefly, it has to be administered intravenously for as long as required. As soon as the surgical operation ceases, the drip stops, and the

antagonism ceases almost instantaneously. Another advantage of the drug is that it is deactivated by a chemical mechanism and not by an enzymatic mechanism, so the deactivation rate is constant across patients.

1.15 Anticholinesterases and acetylcholinesterase

1.15.1 Effect of anticholinesterases

Anticholinesterases are antagonists of the enzyme acetylcholinesterase (the enzyme that hydrolyzes AcC). If AcC was not destroyed, it could re-activate the cholinergic receptor, so the effect of anticholinesterase would be to increase the AcC levels, with a consequent increase in the cholinergic effects. Therefore, an acetylcholinesterase enzyme antagonist will have the same biological effect as a cholinergic receptor agonist.

1.15.2 Acetylcholinesterase active center

The design of anticholinesterases depends on the shape of the active site of the enzyme, the binding interactions involved with the ACC and, finally, the mechanism of hydrolysis.

1.15.2.1 Binding interactions at the active site

There are two important areas to be considered: the anionic binding site and the ester binding site (Fig. 1.26).

Acetylcholine binds to the cholinesterase enzyme by:

- An ionic bond to an Asp residue.
- A hydrogen bond to a tyrosine residue.

Histidine and serine residues at the catalytic site are involved in the mechanism of hydrolysis.

1.15.2.2 The mechanism of hydrolysis

Histidine acts as an acid/base catalyst, while serine plays the role of a nucleophile. Normally an aliphatic alcohol is a poor nucleophile, and, in fact, the serine is unable to hydrolyze an ester. However, the fact that histidine is close to exerting the role of acid/base catalytic agent far outweighs the drawback. Fig. 1.27 schematizes the mechanism of hydrolysis.

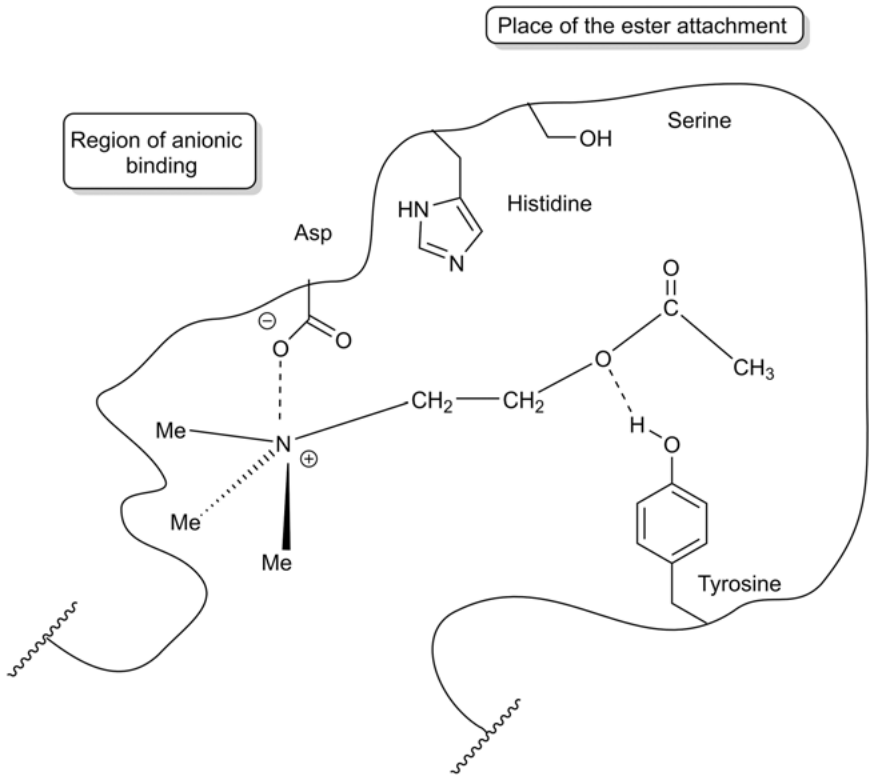


Fig. 1.26: Binding interactions in the active center.

1.15.3 Anticholinesterase drugs

There are two types of anticholinesterase drugs: carbamates and organophosphorus agents.

1.15.3.1 Carbamates

Physostigmine

A natural product again provided the foundation for would be better this group of compounds. The product was physostigmine (also called eserin), which was discovered in 1864 and whose structure was established in 1925 (Fig. 1.28).

SARs:

- The carbamate group is essential for activity.
- The benzene ring is important.
- The pyrrolidine N (which is ionized at the pH of blood) is important.

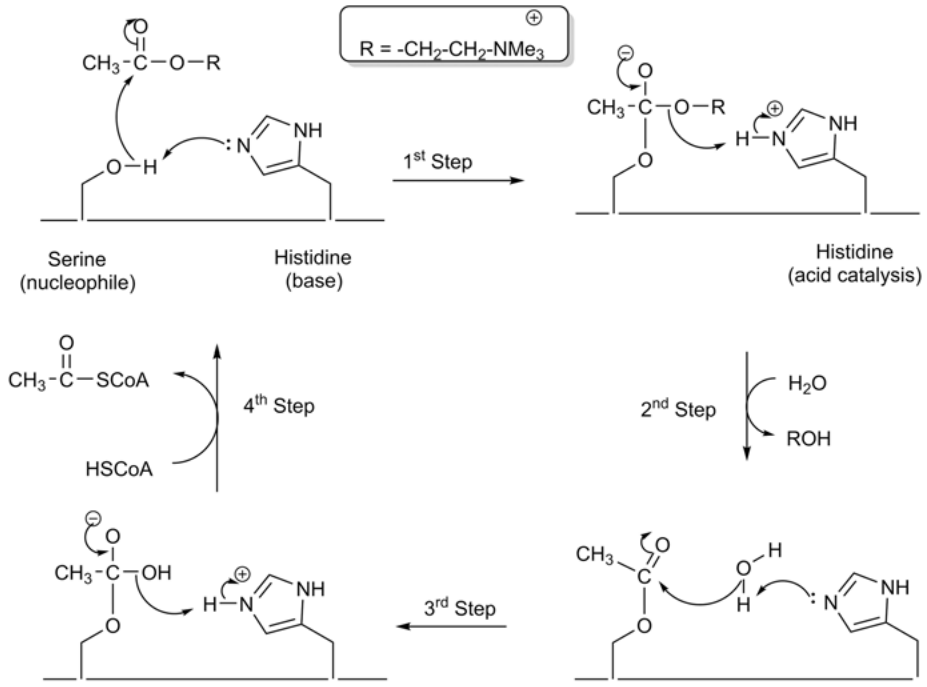


Fig. 1.27: Mechanism of acetylcholinesterase hydrolysis.

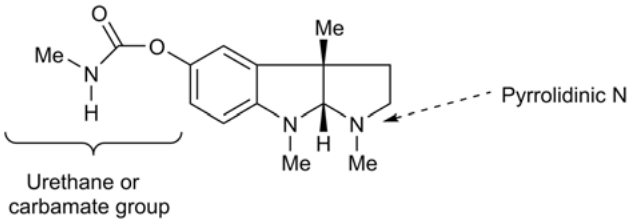


Fig. 1.28: Physostigmine.

The mechanism of hydrolysis

Considering the mechanism of hydrolysis of acetylcholinesterase (see Fig. 1.27), the presence of the carbamate group leads to the carbamoylation of the serine residue in the active site of the enzyme (Fig. 1.29), following a mechanism very similar to that shown in Fig. 1.27.

The hydrolysis of the carbamate moiety bound to the serine is extremely slow, with the rate of hydrolysis of the physostigmine being 40×10^6 times slower than that of AcC. As a result, the active site of the cholinesterase is blocked and unable to react with AcC. The reason that this stage is so slow is that the carbamoyl-enzyme complex

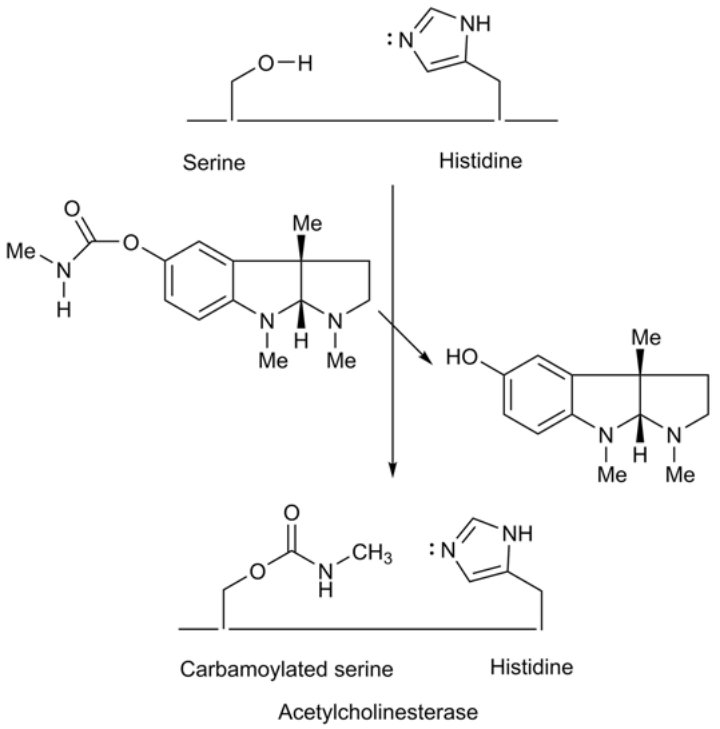


Fig. 1.29: Carbamoylation of the active site of the enzyme acetylcholinesterase.

is stabilized because the nitrogen supplies a pair of electrons to the carbonyl group by a resonance effect and drastically reduces its electrophilic character (Fig. 1.30).

Physostigmine Analogues

Myotin

It has the necessary carbamate group, the aromatic ring and a tertiary amine (Fig. 1.31). It is active as an antagonist but has the following disadvantages:

- It is susceptible to chemical hydrolysis.
- It can cross the BBB as a free base (undesirable effects).

Neostigmine

Neostigmine was designed to avoid the above problems: (a) it has a quaternary nitrogen atom, so that there is no possibility of forming the free base; (b) it has a dimethylcarbamate group (instead of a methylcarbamate group), making it more stable against hydrolysis (Fig. 1.32). There are two possible explanations, which are based on two possible hydrolysis mechanisms.

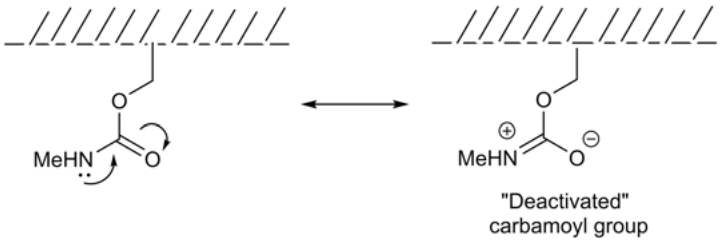


Fig. 1.30: Stabilization of the carbamoyl-enzyme intermediate.

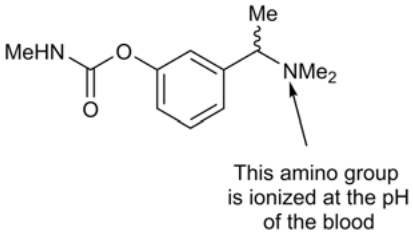


Fig. 1.31: Myotin.

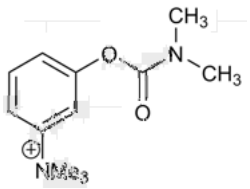


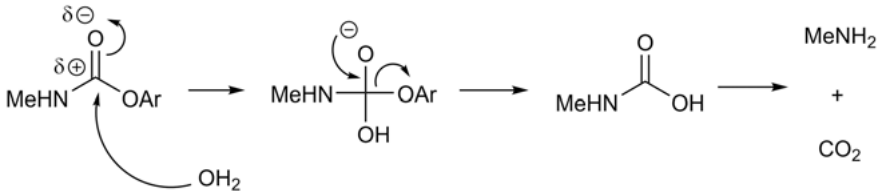
Fig. 1.32: Neostigmine.

Mechanism 1 involves a nucleophilic substitution by water. The reaction rate depends on the electrophilic character of the carbonyl group, and, if it is decreased, the rate of hydrolysis is also slowed down. The presence of a methyl on the nitrogen atom exerts an electron-releasing effect by inductive effect and increases the electron density on the nitrogen. This produces a greater interaction of the electronic pair of the nitrogen atom with the carbonyl group (Scheme 1.4).

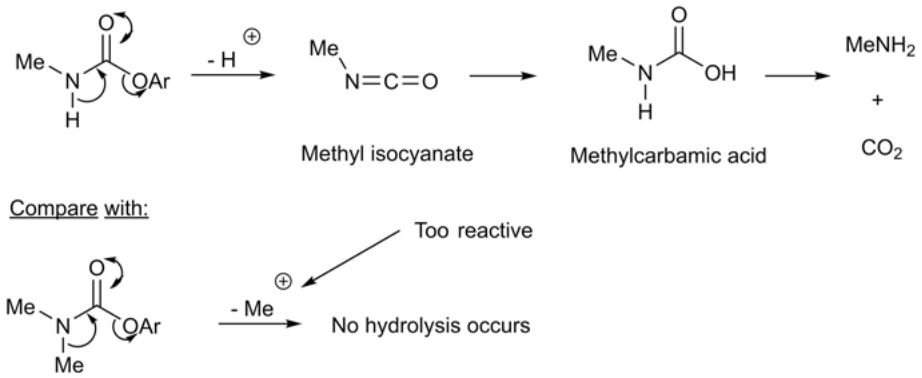
Mechanism 2 (Scheme 1.5) is a fragmentation involving the loss of the phenolic group prior to the addition of water.

Mechanism 2 would require the loss of a proton from the nitrogen atom. The reaction would be inhibited if the hydrogen was replaced by a methyl group. This is because the mechanism would require the loss of a methyl group, which is a highly unfavorable process.

Neostigmine is used orally in the treatment of *myasthenia gravis*.



Scheme 1.4: Mechanism 1.



Scheme 1.5: Mechanism 2.

1.15.3.2 Organophosphorus compounds

Organophosphorus agents were designed as toxic gases affecting the nervous system during World War II but, fortunately, were not used. In times of peace, they have been used as insecticides.

There are three types of acid phosphorus derivatives (Fig. 1.33):

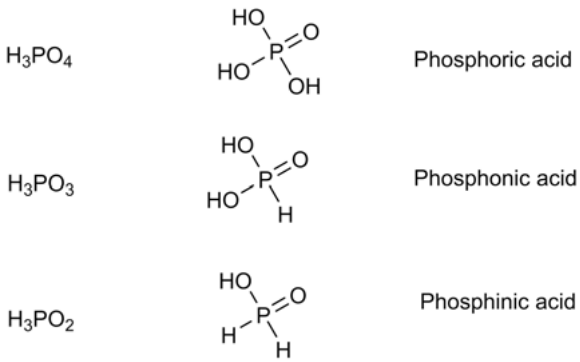


Fig. 1.33: Phosphorus acid derivatives.

Neurotoxic gases

The neurotoxic gases, diflos and sarin (Fig. 1.34), were described long before their mode of action was known. Diflos's denomination corresponds to an acronym of its chemical name, while sarin's name is derived from the names of its discoverers, scientists Schrader, Ambros, Rüdiger and Van der Linde.

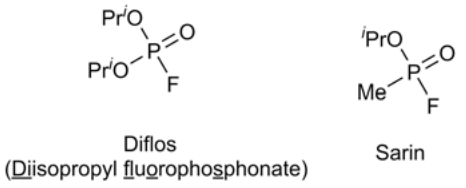


Fig. 1.34: Examples of neurotoxic gases.

Both agents inhibit acetylcholinesterase by irreversible phosphorylation of the serine residue at the active site (Fig. 1.35).

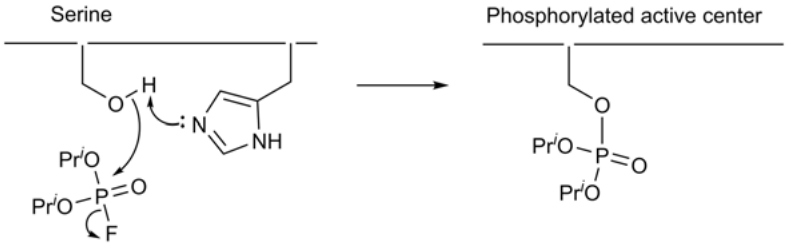


Fig. 1.35: Mechanism of action of diflos in the active center of acetylcholinesterase.

The toxicity of these substances is caused by the irreversible inhibition of the acetylcholinesterase by phosphorylation of the serine residue at the active site. The phosphorylated adduct is extremely resistant to hydrolysis, whereby the enzyme is permanently inactivated. Acetylcholine is not hydrolyzed, whereby the cholinergic system is continuously stimulated. This results in a permanent contraction of the skeletal musculature, which leads to death.

Ecotiopate (Fig. 1.36) was designed to be more accurately fixed to the anionic region of the active site by the quaternary ammonium group. It is used in medicine in the form of eye drops for the treatment of glaucoma. It hydrolyzes slowly, in a matter of days.

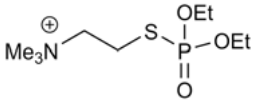


Fig. 1.36: Ecothiopate.

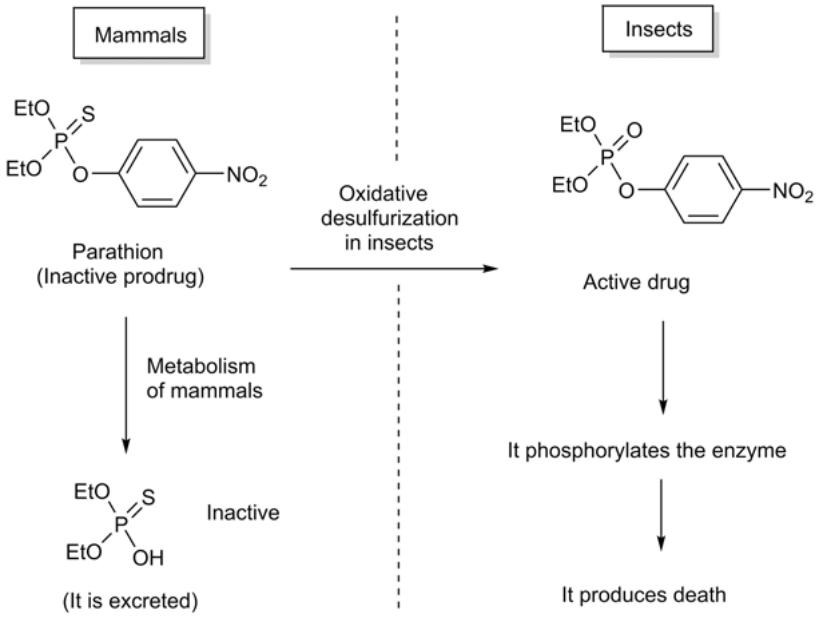


Fig. 1.37: Metabolism of insecticides in mammals and insects.

Insecticides

Compared to the neurotoxic gases, the insecticide parathion is nontoxic because the double bond P=S prevents this molecule from inhibiting acetylcholinesterase. On the other hand, the equivalent compound containing the double bond P=O is lethal.

Fortunately, in mammals, there are no metabolic pathways that can convert the P=S double bond into another P=O double bond; however, such a route exists in insects, meaning that the parathion acts as a prodrug in these species. It is metabolized by oxidative desulfurization, producing an active drug that irreversibly binds to the anticholinesterase enzyme of the insects, leading to their death. In mammals, the same compounds are metabolized differently, producing inactive compounds that are excreted (Fig. 1.37).

Parathion is a very lipophilic structure that is easily absorbed through the skin and mucous membranes.

Pralidoxime: an antidote to organophosphorus derivatives

Pralidoxime (Fig. 1.38) represents one of the best examples of rational drug design. It is an antidote to organophosphate poisoning. In this case, the problem, nonetheless, is to find a drug that displaces the organophosphorus molecule of the serine, since the hydrolysis of the phosphate-serine bond, which is a strong bond, is needed. Therefore, a nucleophile stronger than water is necessary. The scientific literature revealed that

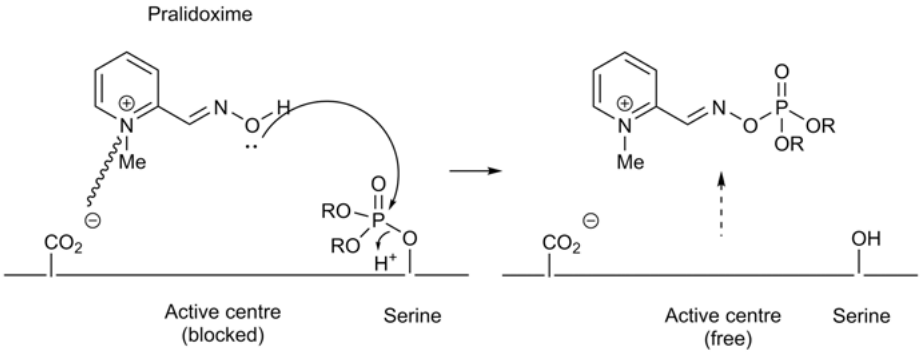


Fig. 1.38: Pralidoxime as an antidote in organophosphate poisoning.

phosphates can be hydrolyzed by means of hydroxylamine. However, this was found to be too toxic to be used in humans, so the next step was to design an equally reactive nucleophile group that specifically targeted the enzyme acetylcholinesterase. It was known that the organophosphorus group does not fill the active site of the enzyme, and that, in addition, the anionic binding site is vacant. Consequently, it was logical to find an appropriate group that would bind to this anionic center and attach to the resulting molecule a hydroxylamine moiety. Thus, once the drug is "anchored" at the active site, the hydroxylamine group can react with the phosphoric ester.

With these starting hypotheses, pralidoxime (or 2-PAM) was designed. The + charge was provided by a methylated pyridine ring, and the nucleophilic side group was attached to its 2-position. This is because it was calculated that this design would place the hydroxyl group in the correct position to react with the phosphoric ester.

The results were spectacular, since they showed that pralidoxime had a 10^6 times greater potency than hydroxylamine as an antidote.

Pralidoxime is permanently charged and does not cross the blood-brain barrier (BBB) to access the CNS. This implies that the antidote cannot act on any enzyme that has been inhibited in the brain. Pro-2-PAM (Fig. 1.39) is a prodrug that solves this problem. As a tertiary amine, it is sufficiently apolar to cross the BBB and once in the brain, it is oxidized to pralidoxime.

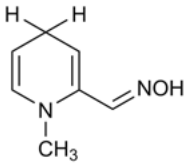


Fig. 1.39: Pro-2-PAM.

2 Noradrenaline

2.1 Goals

- To introduce the reader to the concept of false neurotransmitter.
- To introduce the reader to the concept of structure-activity correlation as a tool for designing new drugs (response β versus α, size of the substituents on the nitrogen atom, etc., from norepinephrine).
- To introduce the reader to antihypertensive drugs, peripheral vasodilators, etc.

2.2 Introduction

As indicated in the previous chapter, the ANS is divided into:

1. Cholinergic nerves (releasing AcC in their synapses). They cover all the preganglionic, parasympathetic postganglionic and some sympathetic postganglionic fibers, such as those innervating the sweat glands and certain peripheral blood vessels.
2. Adrenergic nerves, in which the mediating action was attributed for a long time to adrenaline (A), secretion of the adrenal medulla, to which Barger and Dale assigned sympathomimetic properties in 1910. It is now known that the neurotransmitter is noradrenaline (NA) or norepinephrine. It covers only the postganglionic sympathetic fibers.

2.3 Adrenergic synapses

The sympathetic postganglionic nerve endings branch at their end and come into contact with the effector organ (smooth muscle, in general) through a series of varicosities (Fig. 2.1).

We can mimic or block the NA action at different levels:

(1) Biosynthesis,
(2) release,
(3) storage in vesicles,
(4) acting on receptors,
(5) metabolism (inactivation) by monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) enzymes, and (6) reuptake.

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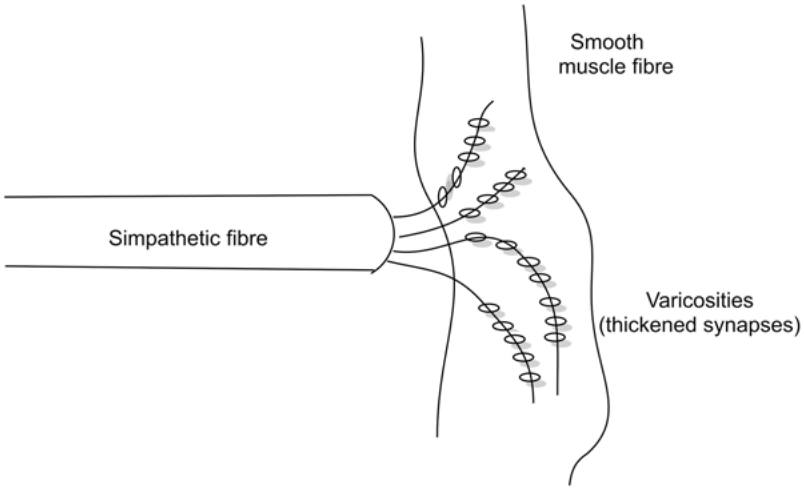
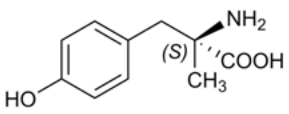


Fig. 2.1: Adrenergic synapses.

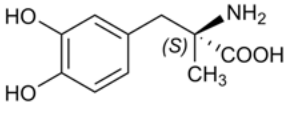
2.4 Drugs that focus on the noradrenaline biosynthesis. False transmitters

The biosynthesis of catecholamines starts from the L-tyrosine (Scheme 2.1).

Compounds capable of inhibiting each of these steps are known, although very few may have clinical utility. The most successful molecular modification is the introduction of a methyl group at the α-position of the amino acid (Fig. 2.2):



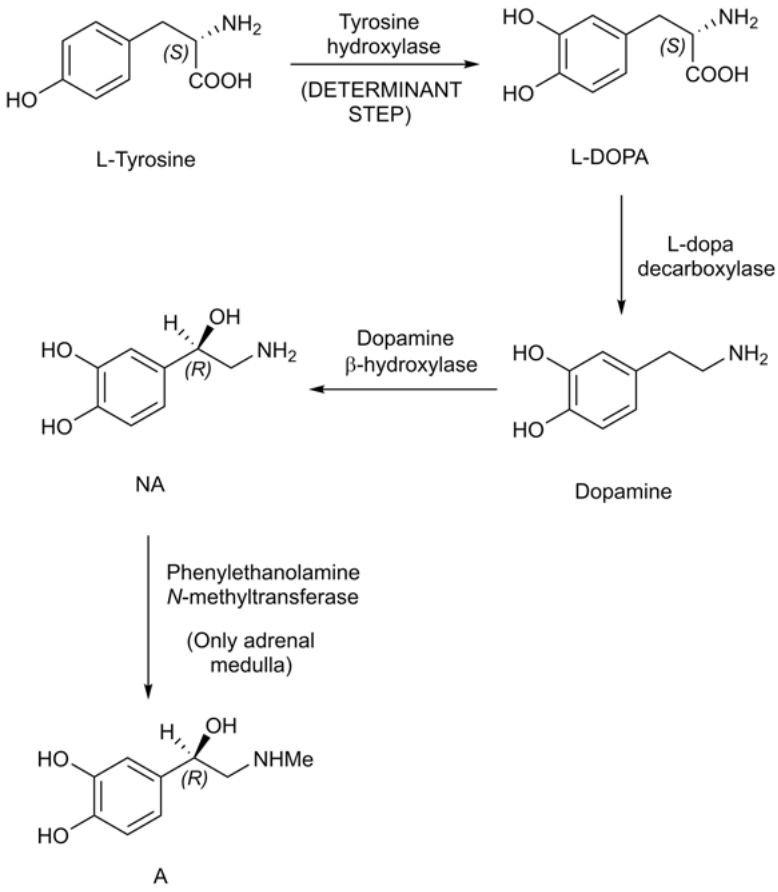
α-(*S*)-Methyltyrosine



α-(*S*)-Methydopa

Fig. 2.2: α-(*S*)-methyltyrosine and α-(*S*)-methyldopa.

α-Methyltyrosine inhibits tyrosine hydroxylase in the first step, which is the determining step of the rate of the sequence and, therefore, the ideal step for inhibition. Although not useful in clinical practice, it is used as an experimental drug. α-Methyldopa is a competitive inhibitor of L-dopa decarboxylase. It is a substrate of this enzyme, although its decarboxylation is slower than in the L-dopa. Upon transformation into α-methyldopamine and subsequently into α-methylnoradrenaline, a false-transmitter is produced. False transmitters are defined as substances that are not normally synthesized at the nerve endings. Instead, they are able to accumulate by the same transport processes, to be released under the effect of the nerve impulse and



Scheme 2.1: Biosynthesis of catecholamines.

to act on the postsynaptic receptors, although with less potency than the natural compound. Consequently, the storage of a false transmitter will lead to a reduction of the pulse passing through the synapse. Methyldopa may be used as an antihypertensive drug.

Dopamine-β-hydroxylase can be inhibited by disulfiram, which is a nonspecific blocker of oxiditive enzymes (Fig. 2.3). It causes an unpleasant reaction when alcohol intake occurs. It is used as a vulcanizer, seed disinfectant, fungicide and, specifically, in the treatment of alcoholism.

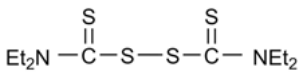
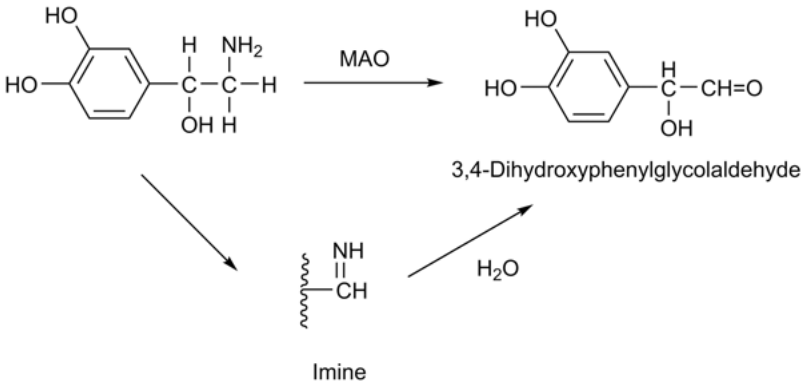


Fig. 2.3: Disulfiram.



Scheme 2.2: Oxidative deamination caused by MAO.

2.5 Drugs that affect the release of stored noradrenaline

Since stimulation of the adrenergic system requires two consecutive processes of NA release (from their sites of accumulation and then from the neuron to the synapse), drugs that facilitate or block these processes offer an interesting opportunity for therapeutic control.

The alkaloid reserpine is a drug capable of emptying the NA stored inside the neuron (the biochemical mechanism is not known), so that the neurotransmitter is exposed to oxidation by the MAO and ends up depleting it after a certain time (Scheme 2.2).

MAO, known since 1928, is an oxidizing enzyme that converts α-unsubstituted primary amines into aldehydes.

Adrenergic transmission is interrupted because of the lack of neurotransmitter at presynaptic terminations. This is the basis of the antihypertensive action of reserpine, which is accompanied by an important sedative effect (CNS depression). A certain separation of the hypotensive and sedative actions has been achieved by molecular modification. Thus, mediodespidine is a synthetic drug, equipotent to reserpine as a hypotensive, but less potent as a sedative (Fig. 2.4).

In addition to reserpine, the antihypertensive drug rescinamine is also found in the roots of different Asian, American or African species of the genus *Rauwolfia*. In rescinamine, the acyl group that esterifies to hydroxyl in C_{18} is the 3,4,5-trimethoxyicamoamoyl group.

Another drug that seems to empty the NA stored inside the neuron is guanethidine (1960) (Scheme 2.4). Its main advantage over reserpine is that it does not produce CNS depression, since it does not cross the BBB because of its strong positive ionic charge.

The protonation of the guanidino group gives rise to a cation that has a large resonance stabilization (Scheme 2.3). Guanidines are among the strongest organic bases.

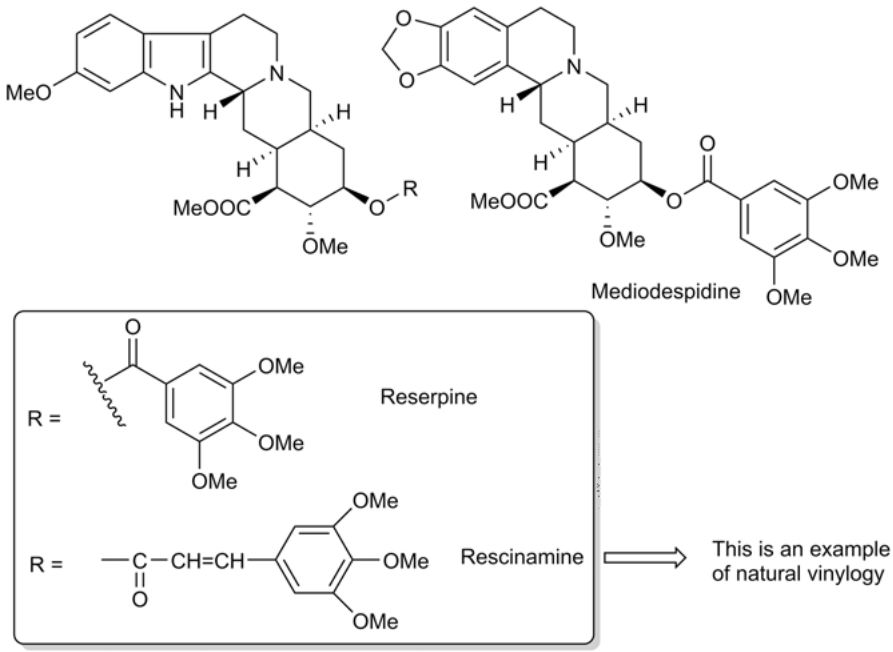
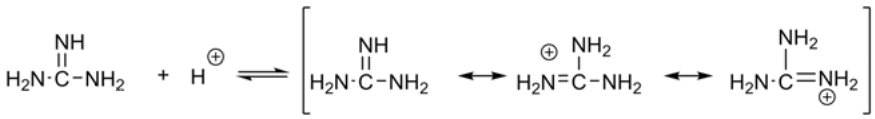


Fig. 2.4: Reserpine and mediodespidine.



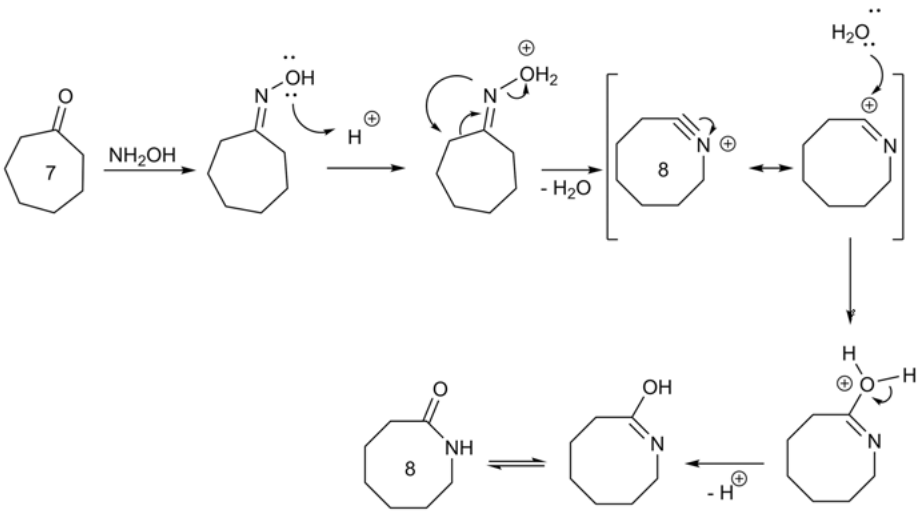
Scheme 2.3: High basicity of guanidine.

Guanethidine is an antihypertensive agent that acts by interfering with the adrenergic transmission. It can be prepared from cycloheptanone via a Beckmann rearrangement.

The Beckmann rearrangement is a reaction in which an oxime is converted into an amide through treatment with a strong concentrated acid, typically H_2SO_4. This reaction is used industrially in the transformation of cyclohexanone oxime into ε-caprolactam, from which nylon 6 is obtained. Preparation of guanethidine starts with a Beckmann rearrangement from the oxime of cycloheptanone (Scheme 2.4).

The total synthesis of guanethidine is shown in Scheme 2.5. A reaction of *S*-methylisothiouronium chloride with primary amines is the general procedure for the preparation of monosubstituted guanidines.

Guanethidine first causes a hypertensive reaction, followed by a long-term hypotension. It is used in cases of severe and malignant hypertension.



Scheme 2.4: Conversion of cycloheptanone into the eight-membered lactam by a Beckmann rearrangement.

2.6 Mechanism of action of the MAOs

Many enzymes require cofactors, which are metal ions, such as Zn^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, or organic molecules called coenzymes (in general, essential vitamins or their metabolites).

Vitamin B2, or riboflavin, is a benzopteridine derivative with a reduced ribose chain at the 10 position. As a component of flavin-adenine dinucleotide (FAD), it is an electron and proton carrier due to the stability of the radical anion that occurs when an electron is added (Fig. 2.5).

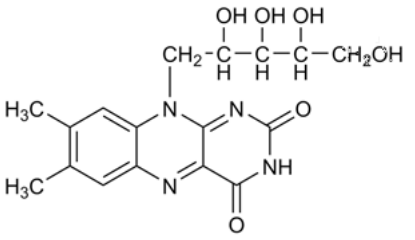
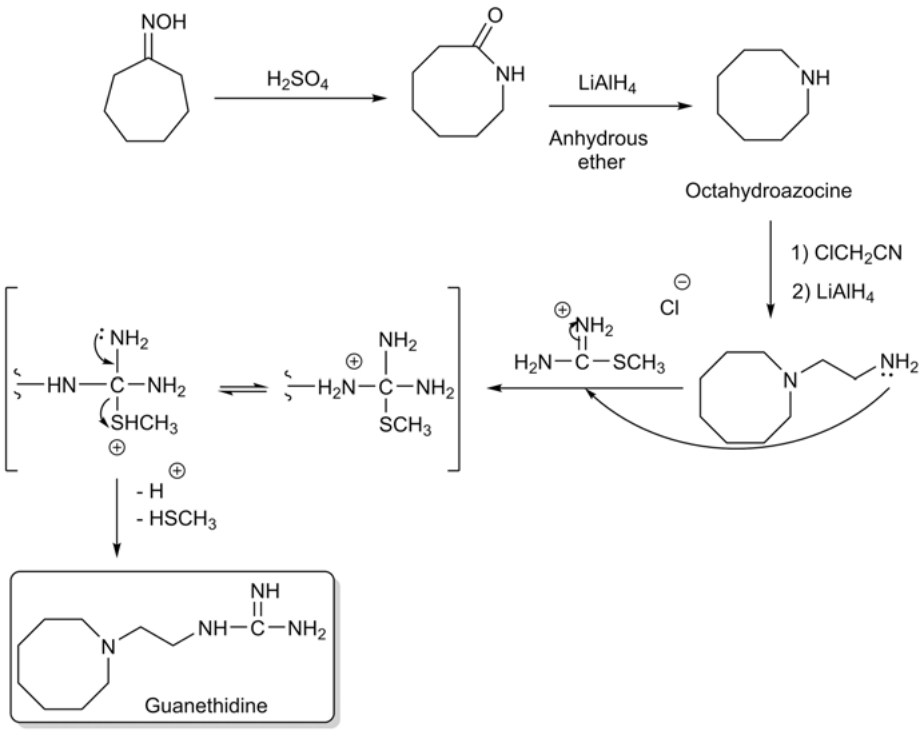


Fig. 2.5: Riboflavin (vitamin B2).

MAOs are flavoproteins that catalyze the oxidation of primary amines to aldehydes. The FAD (Flox) cofactor acts as an electron acceptor to give an anion-radical intermediate (Scheme 2.6), which adds a proton to yield the radical $FlH^{\bullet}$. Further addition of another electron yields the FlH^{-} anion, and the final addition of another proton



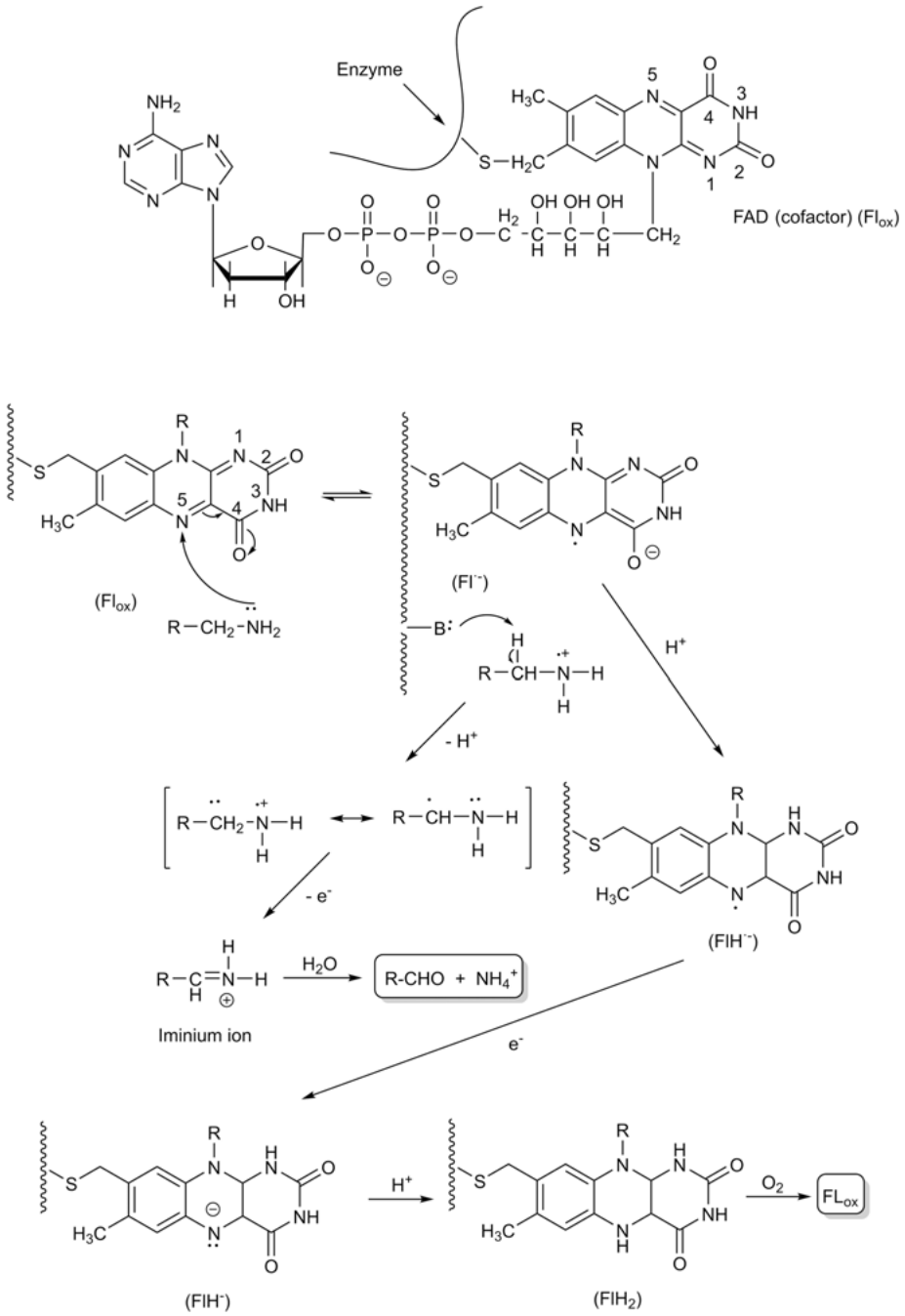
Scheme 2.5: Synthesis of guanethidine.

leads to the reduced form FlH_2 ($FADH_2$). This process is coupled with oxidation of the substrate. It involves the abstraction of an electron to yield a radical cation, the subsequent loss of a proton to yield a radical, and the loss of another electron to yield an iminium cation. The iminium cation is then spontaneously hydrolyzed to yield ammonia and the corresponding aldehyde. Reoxidation of the cofactor by molecular oxygen completes the catalytic cycle.

2.7 Adrenergic indirect drugs

Indirect stimulants, or adrenergic agonists, are compounds that do not bind to adrenergic receptors, but stimulate the sympathetic transmission through causing an increase in the NA concentration that reaches these receptors.

However, the mode of action is rarely strictly direct or indirect. Given the structural similarity between the agonists of both types, these drugs frequently have a mixed action. In addition, many of the compounds with indirect adrenergic action (especially those less polar) are also CNS stimulants. They are used clinically in several cases,



Scheme 2.6: The MAO redox mechanism.

such as anorexics or appetite depressants and as false transmitters. Either of these actions may predominate in each concrete compound.

From a structural point of view, most of the indirect adrenergic agents are phenethylamines that come either from a molecular modification of the NA neurotransmitter or from the ephedrine alkaloid, with a mixed adrenergic action (Fig. 2.6).

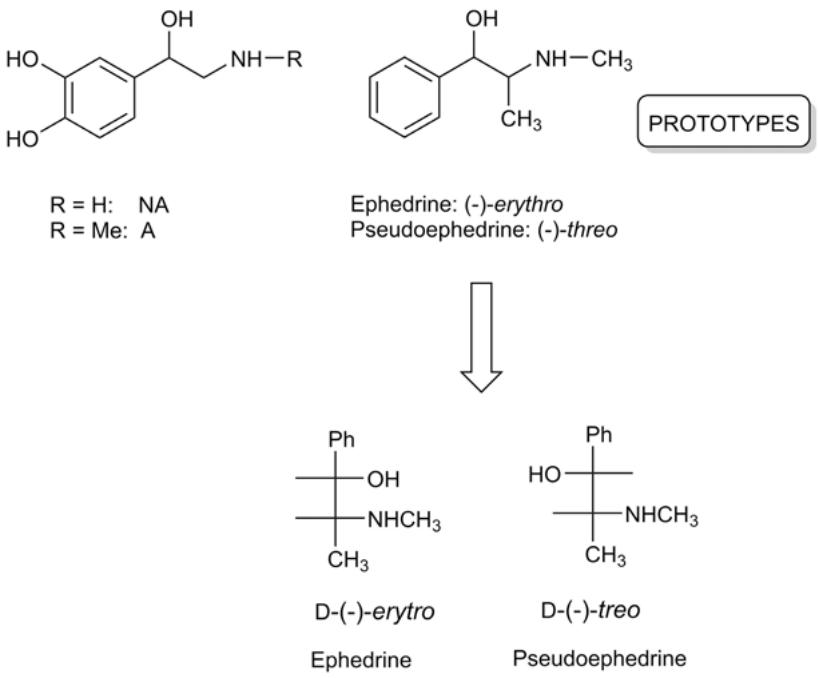


Fig. 2.6: Natural phenethylamines.

Natural ephedrine is the D-(-)-*erythro* isomer, which is 36 times more potent than D-(-)-pseudoephedrine. Synthetic compounds with an indirect or mixed action can be derived from them, such as those shown in Fig. 2.7.

Amphetamine differs from ephedrine in that it lacks both *N*-Me and hydroxyl groups. The lack of the latter group decreases its polarity, so that it is more easily transportable towards the CNS. The compound is thus used primarily as a CNS stimulant. Note the major structural differences between the indirect adrenergic drugs and NA:

1. Indirect andrenergic drugs do not have the phenolic OH group at 3 and 4 positions (hydroxyamphetamine has a mixed action). This increases their oral absorption and penetration into the CNS, so that most andrenergic drugs (amphetamines, phentermine) act as central stimulants.

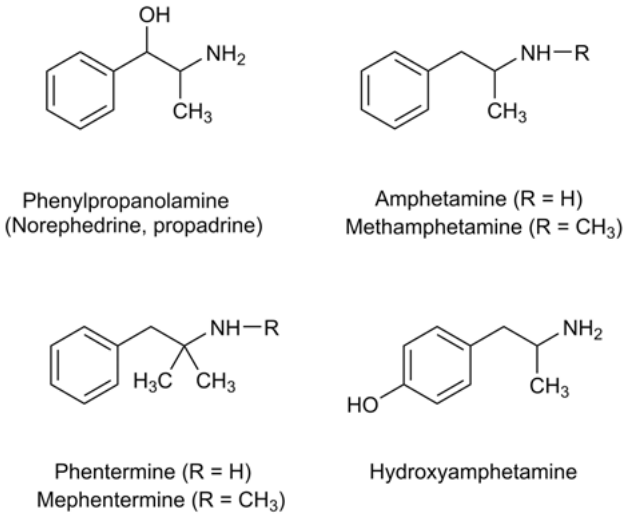


Fig. 2.7: Synthetic compounds with indirect or mixed actions.

2. In general, indirect andrenergic drugs lack the benzyl OH group, although in some (ephedrine, propadrine) it is still present. Those that have it, being more polar compounds, are always lesser CNS stimulants.
3. Indirect adrenergics usually have one methyl group (and sometimes two) on the nitrogen atom alpha. This methyl group, similar to that found in methyldopa that yields the false α-methylnoradrenaline transmitter, significantly reduces the direct action. At the same time, it increases the oral efficacy, because it prevents the degradation of the drug by MAO and increases the lipophilicity of the amine. A methyl group in beta eliminates the activity.
4. The phenyl group may be replaced by other aromatic and even cycloalkanic rings; for example, as is the case for the indirect adrenergic drugs cyclopentamine and propylhexedrine (Fig. 2.8).

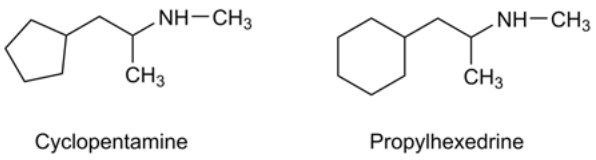
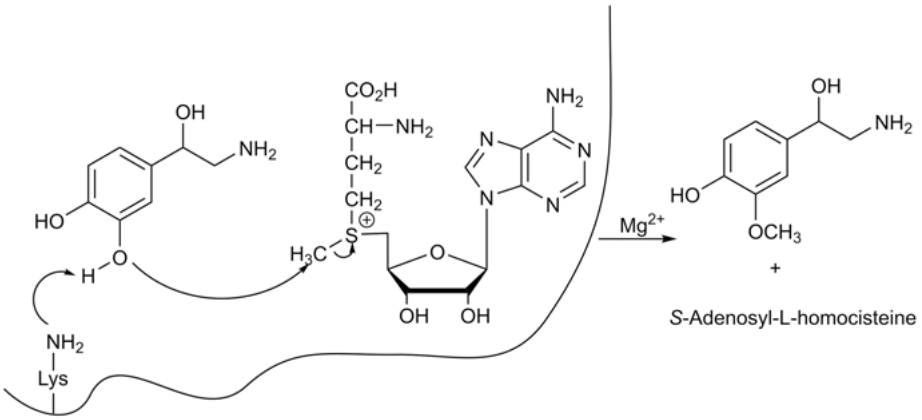


Fig. 2.8: Cyclopentamine and propylhexedrine.

2.8 Catechol-*O*-methyltransferase inhibitors (COMT)

Catechol-*O*-methyltransferase (COMT) catalyzes the methylation of the phenolic hydroxyl group in catechol (1,2-benzenediol) structures. The mechanism is shown in Scheme 2.7.



Scheme 2.7: *O*-methylation of NA, catalyzed by COMT.

2.9 Direct adrenergic (postsynaptic agonists) drugs

The first postsynaptic phenomenon that occurs during the adrenergic nerve transmission is the binding of norepinephrine to its receptors. Chemical groups necessary for the compound to mimic the action of NA have been determined through SARs. In a schematic form, these structural requirements are as follows (Fig. 2.9):

The most interesting modification was the substitution at the nitrogen atom. Catecholamines have a dual action on the tissues: a contractile effect, called α, and a relaxation effect, called β. The existence of two types of adrenergic receptors, also called α and β, responsible in the first approximation for those excitatory and inhibitory actions, was established. This division was established on the basis of their responses to a series of agonists with a different substitution at the nitrogen atom (Fig. 2.10):

- Receptor α has a decreasing A >NA >IP sensitivity.
- Receptor β has a decreasing IP >A >NA sensitivity.

Adrenergic receptors predominantly play a role in the smooth muscle excitation. ß-Adrenergics are associated with inhibition of the smooth muscle tone (vasodilatation and bronchodilation), even with that of the bowel and the myocardium stimulus.

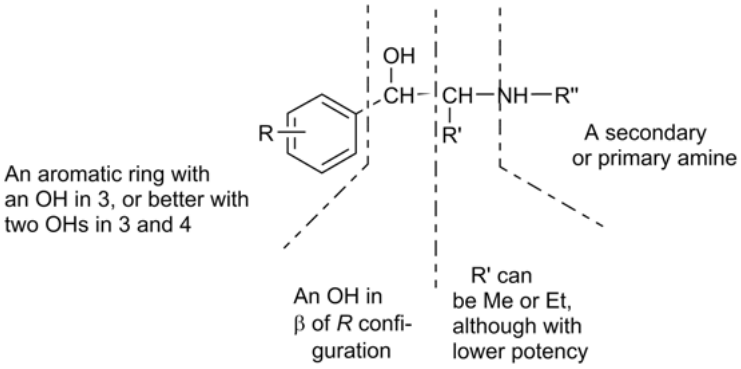
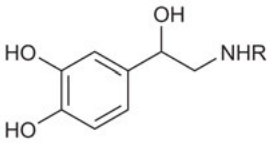


Fig. 2.9: SARs necessary for a compound to mimic the NA action.



R = H: NA
R = CH_3: A
R = Pr^i: IP (Isoprenaline)

Fig. 2.10: Noradrenaline, adrenaline and isoprenaline.

Adrenaline is a powerful as both an α- and a β-stimulant. It raises blood pressure, because it produces an increase in cardiac activity: (a) a + inotropic effect (energy of the muscular contraction); (b) a + chronotropic effect (frequency of contraction).

Noradrenaline is a potent vasoconstrictor by a predominantly α-action. It is used in acute cases of hypotension due to trauma, hemorrhage and vasomotor depression.

Isoproterenol (isoprenaline) preferentially stimulates the β-receptor, resulting in bronchodilation and an increase in heart rate. It is consequently used as an antiasthmatic and as a cardiac stimulant in hospital emergency rooms. There are now three known β-receptor subtypes, β_1, β_2 and β_3.

β_1-receptors are located in the heart and small intestine, while β_2-receptors are located in the bronchi and vessels (vascular bed). β_3-receptors stimulate the metabolism of carbohydrates and increase their metabolic rate.

There are two types of adrenomimetics, consequently:

(1) α-Adrenergics, whose prototype is NA.

(2) β-Adrenergics, whose prototype is IP.

The agonist drugs that recive most practical interest are those with a β_2-selectivity, useful in the treatment of asthma because they are bronchodilators. They are also more useful because they do not affect the heart and blood pressure unlike, for example, isoetharine and salbutamol (Fig. 2.11).

The primary clinical use of adrenergic agonists is for the treatment of asthma. The activation of β_2-adrenoreceptors causes relaxation of the smooth musculature of the bronchi, thereby widening the airways. Salbutamol, also known as albuterol and

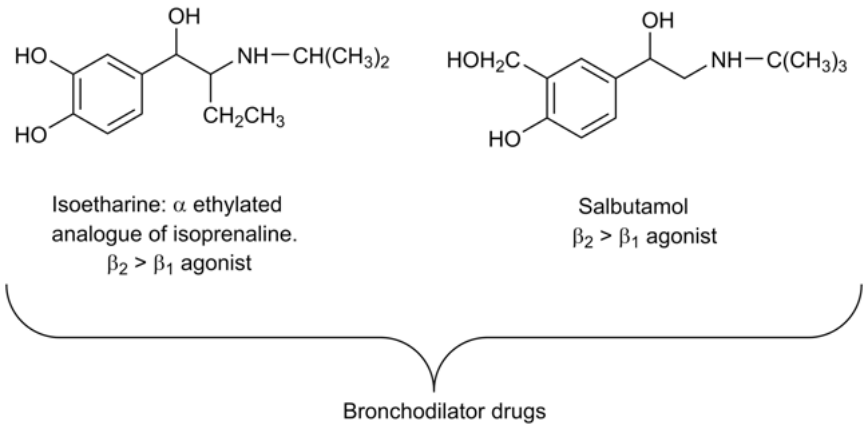


Fig. 2.11: Isoetharine and salbutamol.

marketed as Ventolin® among other names, was introduced in 1969 for the treatment of asthma.

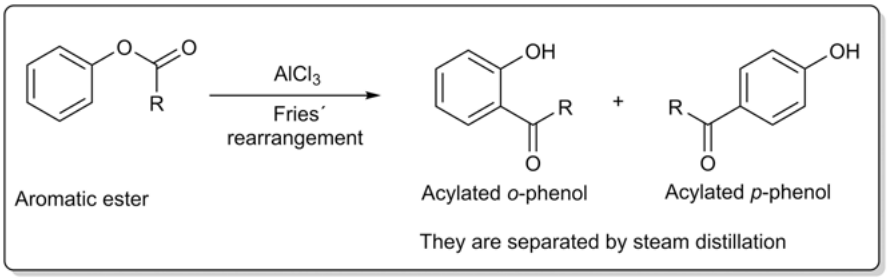
One of the problems presented by catecholic compounds is the metabolic methylation of the phenolic group in *meta* with respect to the side chain. If both phenolic groups are involved in hydrogen bonds with the receptor, the methylation of one of them makes this type of bond impossible and renders the compound inactive. For example, the NA analogue (**2.1** in Fig. 2.11) exhibits antiasthmatic activity, but its effect is short, because the compound is rapidly metabolized to the inactive methyl ether (**2.2** in Fig. 2.12).

Removal of the phenolic OH group in *meta* or its substitution by a methyl group not only prevents its metabolization, but also prevents the interaction through a hydrogen bond with the receptor-binding site. In order to solve this problem, it was suggested to separate the vulnerable OH group from the aromatic ring and intercalate a carbon atom between them. The resulting compound was not recognized by the metabolic enzyme and, at the same time, could continue to form the important hydrogen bond with the receptor.

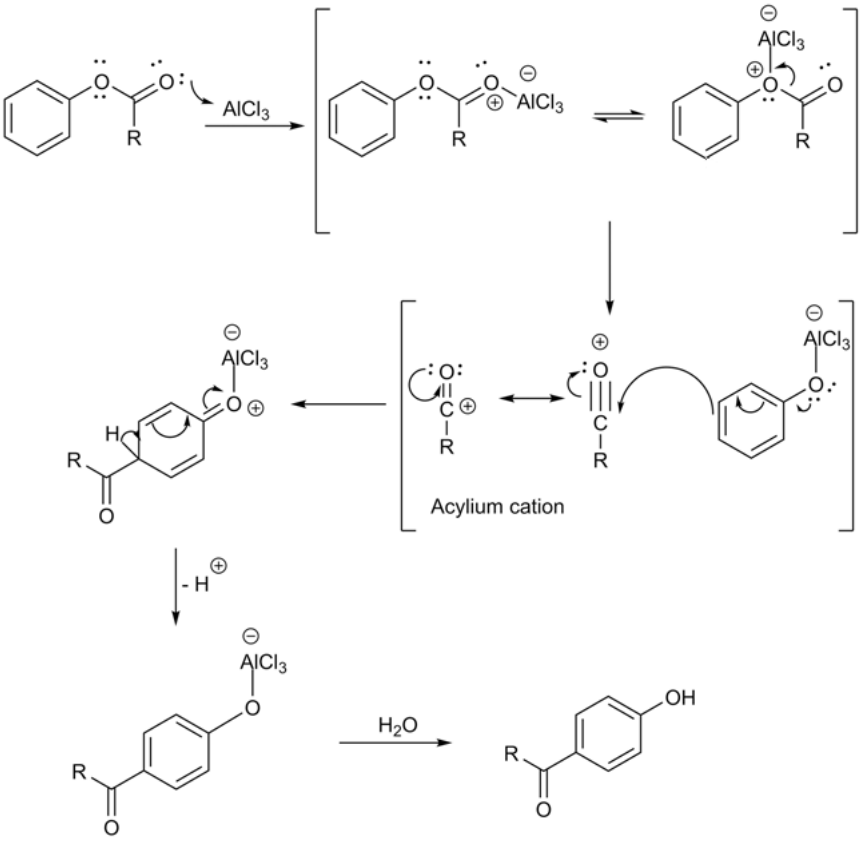
Fortunately, the receptor is quite condescending with respect to the position of the OH group that gives rise to the hydrogen bond. It is interesting to note that the hydroxyethyl group is also acceptable for the establishment of a corresponding bond. However, when OH binds to a larger alkyl moiety, it is too large to fit (Fig. 2.13).

These results show that it is better to consider a binding region within the receptor-binding site as an available volume, rather than imaging it as a point. With this approach a drug can be designed so that the binding group can be positioned anywhere in the available volume.

The (*R*)-enantiomer of salbutamol is 68 times more active than the (*S*) one. In the first step of the salbutamol synthesis, a Fries rearrangement is used (Scheme 2.8):



Mechanism:



Scheme 2.8: Mechanism of the Fries rearrangement.

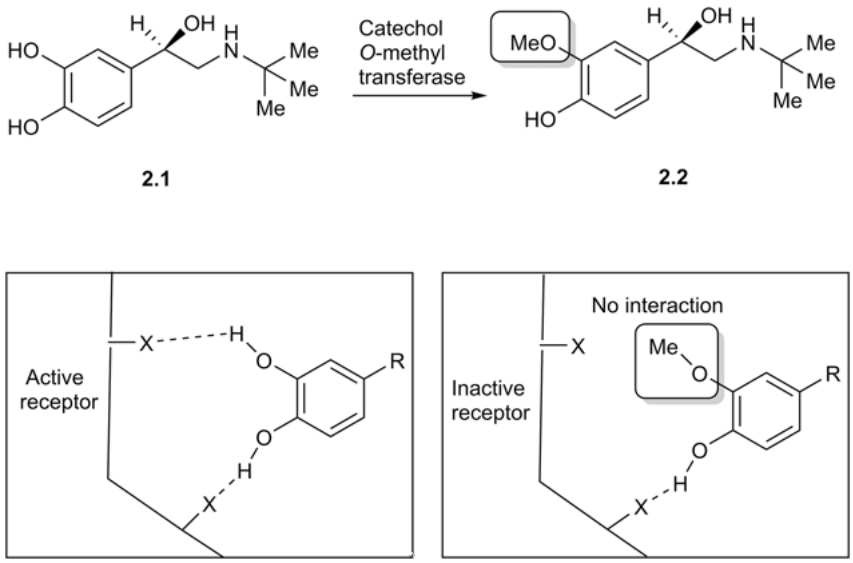


Fig. 2.12: Metabolic methylation of the NA analogue. X indicates an electronegative atom.

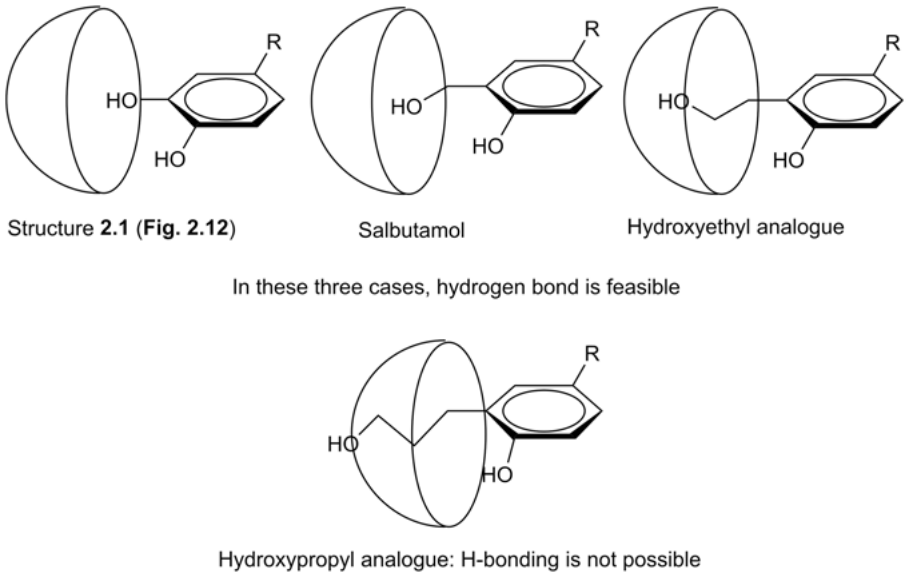
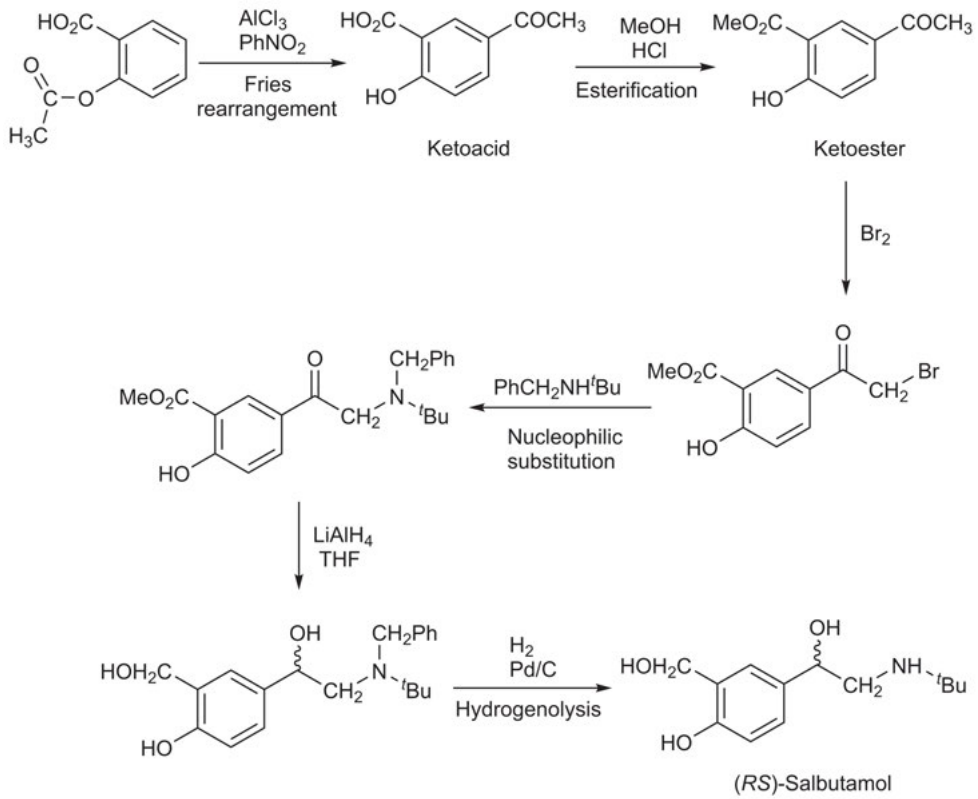


Fig. 2.13: View of a binding region as an available volume.



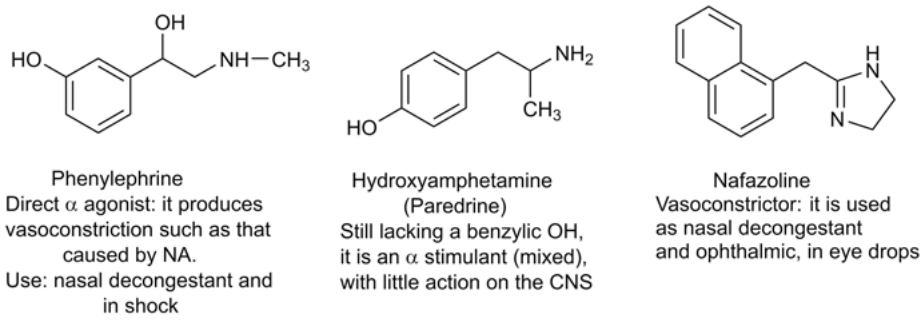
Scheme 2.9: Synthesis of racemic salbutamol.

Racemic salbutamol can be synthesized from Aspirin®, as shown in Scheme 2.9.

The Fries rearrangement of aspirin produces a ketoacid that is esterified. The bromoketone is then prepared, allowing for the introduction of the amino group by nucleophilic substitution. The methyl ester and the ketone are reduced and, finally, the *N*-benzyl group is removed by hydrogenolysis.

With regard to direct ß-adrenergic agonist drugs, they may also have a strong structural relationship with the neurotransmitters NA or A, but, in fact, not as restrictive as in the case of agonists. Compounds are thus found with a direct (or at least mixed) action, lacking some of the phenolic hydroxyls, benzylic OH groups or even structures completely different from those of catecholamines, as is the case of imidazoline derivatives (Fig. 2.14).

Naphazoline can be obtained from its iminoether or from naphthylacetic acid (Scheme 2.10).

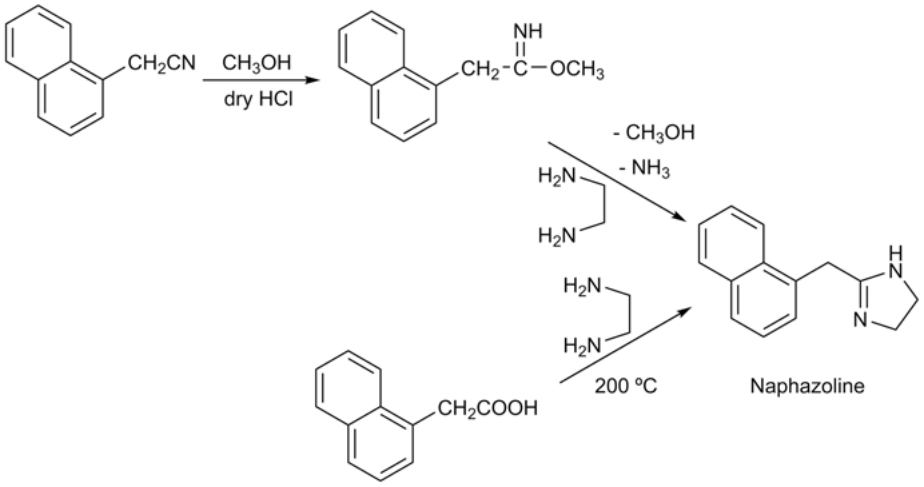


Phenylephrine
Direct α agonist: it produces vasoconstriction such as that caused by NA.
Use: nasal decongestant and in shock

Hydroxyamphetamine (Paredrine)
Still lacking a benzylic OH, it is an α stimulant (mixed), with little action on the CNS

Nafazoline
Vasoconstrictor: it is used as nasal decongestant and ophthalmic, in eye drops

Fig. 2.14: Direct α-adrenergic agonist drugs.

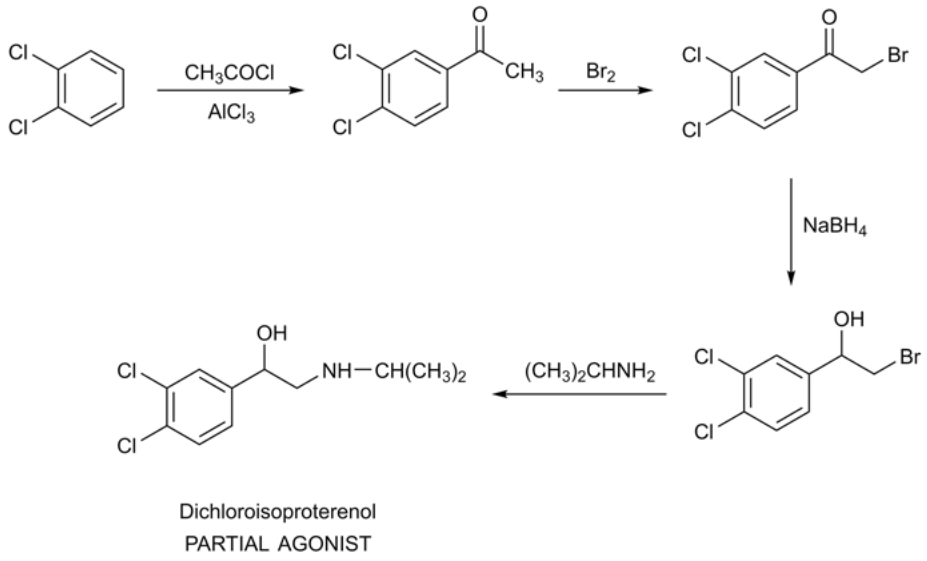


Scheme 2.10: Synthesis of naphazoline.

2.10 Adrenergic β-blockers

Drugs acting as receptor blockers only need to possess those structural requirements that impart affinity for the receptor, and not intrinsic activity. The first β-adrenergic blockers arose as molecular modifications of β-adrenergic agonists (especially isoprenaline). The most interesting variation was the substitution of catechol by a more lipophilic group. Thus, the first β-blocker was introduced by Powell and Slater in 1958 under the name dichloroisoproterenol, 1-(3,4-dichlorophenyl)-2-isopropylaminoethanol, in which the 1,2-dichlorobenzene moiety substituted the catechol one (Scheme 2.11).

However, dichloroisoproterenol is not a pure β-blocker, but a partial agonist, which markedly reduced its value as a possible antihypertensive drug. The reason is

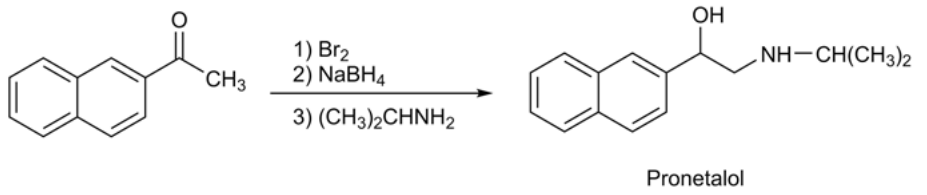


Dichloroisoproterenol
PARTIAL AGONIST

Scheme 2.11: Synthesis of dichloroisoproterenol.

that the partial agonist has affinity for receptors and occupies them, but it only exerts partial intrinsic activity; the receptors are therefore occupied and cannot react with the agonist.

In 1962, Black introduced a new structural variation, pronetalol, 1-(2-naphthyl)-2-isopropylaminoethanol, prepared in a sequence identical to that of dichloroisoproterenol, but starting from 2-naphthylmethylketone (Scheme 2.12).



Pronetalol

Scheme 2.12: Synthesis of pronetalol.

In pronetalol, the phenolic OH groups of isoproterenol (agonist) have been replaced by a second benzene ring. In fact, the aromaticity of this ring is not essential for its action, since the two possible tetrahydrogenated analogues are approximately equipotent with pronetalol (Fig. 2.15).

Although pronetalol was more active than dichloroisoproterenol, and it lacked partial agonism, its clinical utility was very limited since it caused the development

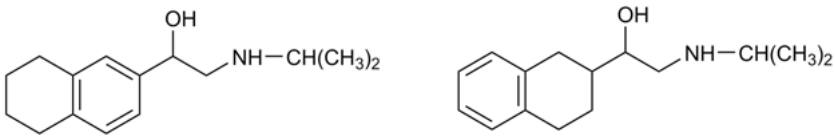


Fig. 2.15: Tetrahydrogenated pronetalol derivatives with the same activity as that of pronetalol.

of certain tumors in animals, which forced its withdrawal. It was replaced in 1964 by a new drug, propranolol, which was ten to twenty times more active, a pure β-blocker and a nontumorigenic drug.

Before showing the synthesis of propranolol, the different regioselectivity, depending on whether the medium is basic or acidic, of the opening of monosubstituted oxacyclopropanes or epoxides should be explained. In general, ethers are poorly reactive, used in many cases as inert solvents. This contrasts with the important reactivity of epoxides, which undergo ring opening reactions by nucleophilic attacks.

2.10.1 Regioselective opening of epoxides in basic media

The model is the opening of isobutylene oxide. In a basic medium, the nucleophile's attack occurs on the least substituted carbon if the ether is not symmetrical and oxygen acts as a leaving group, because the reaction goes through an S_N2 mechanism. The force that drives this reaction is the release of the ring's tension (Scheme 2.12 a). Larger rings do not open since they do not have this form of ring tension.

2.10.2 Regioselective opening of epoxides in acidic media

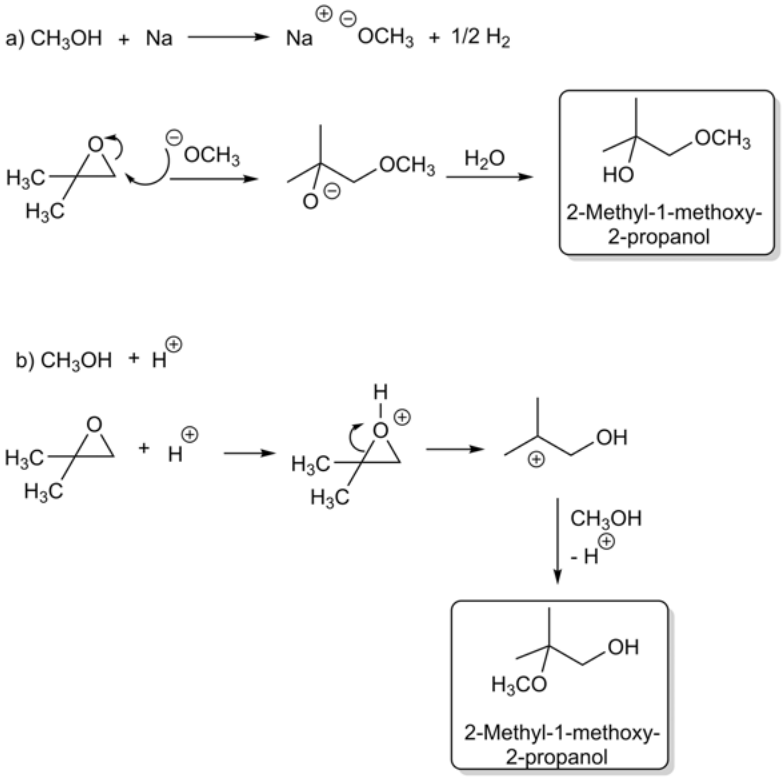
The attack of the nucleophile in an acidic medium occurs on the most substituted carbon of isobutylene oxide after protonation of oxygen, since the process is an S_N1 process, and it goes through the most stable tertiary carbocation. The opening is easier since the protonated oxygen behaves as a good leaving group (Scheme 2.13 b).

Propranolol synthesis is very simple and starts from 1-naphthol (Scheme 2.14). The opening of the epoxide ring of **2.1** takes place through an S_N2 process.

Fig. 2.16 demonstrates the development of propranolol.

2-Naphthol was not available at the time, but there was an amount of its isomer, 1-naphthol. This led to propranolol. The (*S*)-enantiomer is the active one, although propranolol is used clinically as racemic. Later, when the target molecule (first molecule of Fig. 2.16) was synthesized, it turned out to be less active than propranolol.

Propranolol was to become the most commonly used β-blocker, as a drug against angina pectoris (lack of blood supply), as an antiarrhythmic agent and as a pe-

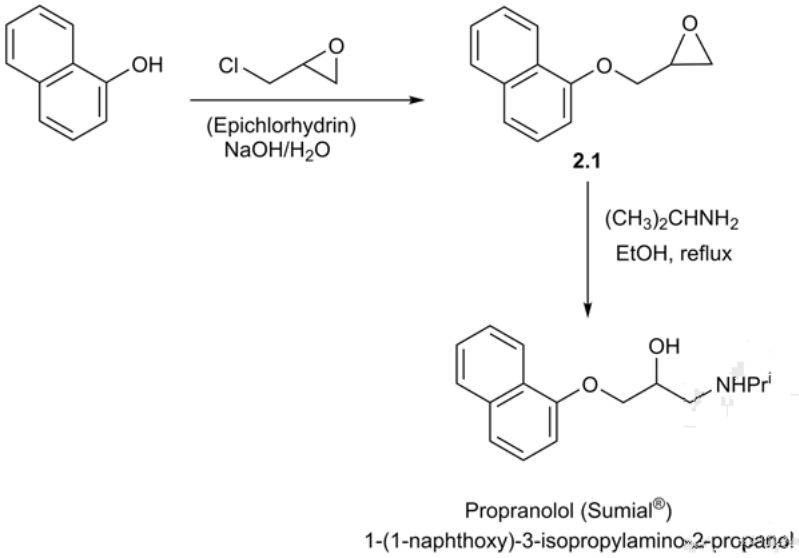


Scheme 2.13: Opening of isobutylene oxide with MeOH in basic and acidic media.

ripheral vasodilator. In addition, it has served as a model for a great number of aryloxypropanolamines, a structural family in which all the β-adrenergic blockers currently used clinically are included. Research in this field has been very active, because the usefulness of such drugs is not limited to cardiac arrhythmias, but they also alleviate pain in angina pectoris (transient ischemia, no necrosis and no sequelae) and myocardial infarction (there is necrosis and therefore, it leaves sequels). It seems that they can also prevent myocardial infarction. β-Blockers are also used, usually in combination with other drugs, in the control of hypertension, glaucoma and specific arrhythmias.

The main contraindications of β-blockers occur in patients with bradycardia (abnormal slowness of the pulse) and in patients with asthma, because β-blockers produce bronchoconstriction. The latter problem is solved by the use of β-selective blockers for cardiac receptors (β_1). Propranolol is a mixed β_1- and β_2-blocker.

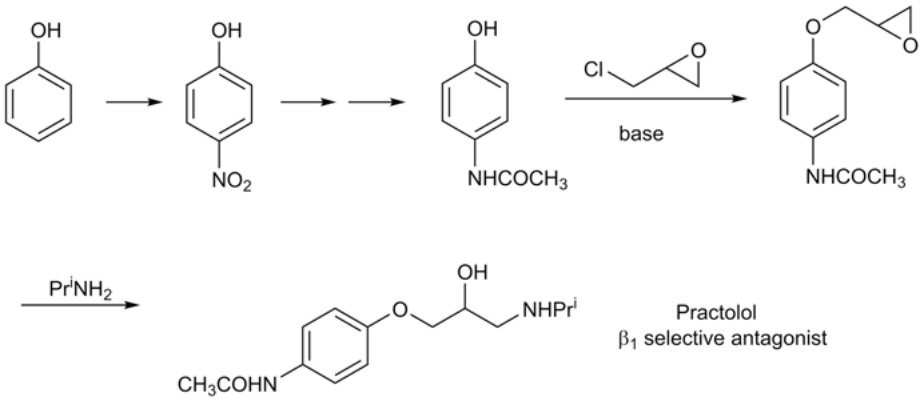
One aspect that may be misleading when comparing the structure of aryloxypropanolamines (such as propranolol) with that of arylethanolamines (such as pronetalol) is the stereochemical notation of the carbon atom, which supports the hydroxyl



Scheme 2.14: Synthesis of propranolol.

group in the side chain. Thus, because of the different priority of the substituents around said atom, the *S* configuration in the aryloxypropanolamines is equivalent to the *R* of the arylethanolamines. Consequently, the eutomers of the aryloxypropanolamines are those with configuration *S*, whose spatial arrangement is superimposable with that of the arylethanolamines with configuration *R* (Fig. 2.17).

Practolol is a drug that, at doses capable of blocking the β_1 cardiac receptors, exerts a much lesser effect on the β_2 receptors of the bronchi (Scheme 2.15).



Scheme 2.15: Synthesis of practolol.

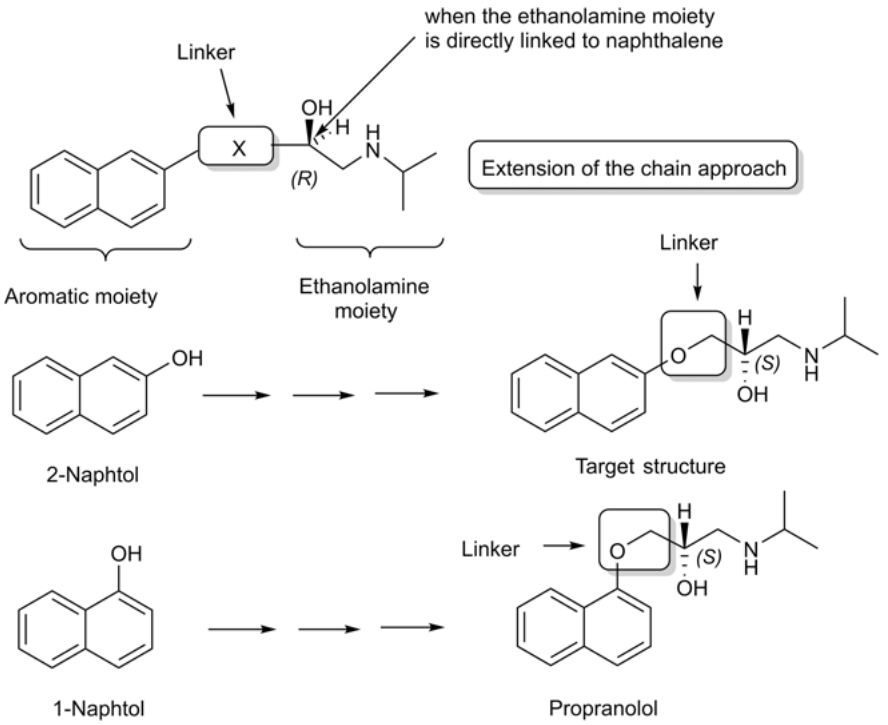


Fig. 2.16: Development of propranolol.

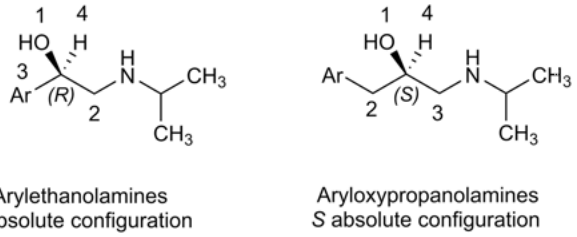


Fig. 2.17: Configurational equivalence between arylethanolamines with the absolute configuration *R* and the aryloxypropanolamines of the absolute configuration *S*.

Practolol is structurally characterized by having a substituent in *para* position of the aromatic ring capable of adopting an extended *transoid*-like conformation, which is important for its selectivity. Evidence of this is the absence of selectivity of the analogue, which results from the incorporation of the acetamido group in an additional cycle (Fig. 2.18).

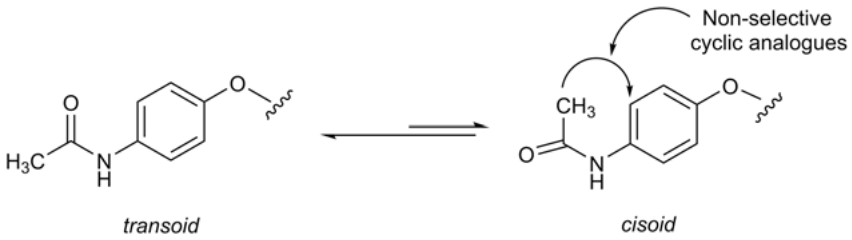


Fig. 2.18: Conformational equilibrium of the acetamido group of practolol.

Unlike cardioselectivity, a desirable feature when treating cardiac patients prone to bronchial spasm, the preparation of β_2-selective antagonists has no other interest than a theoretical one in pharmacological research or for the establishment of SARs.

2.11 α–Adrenergic blockers

α–Blockers were known and used in cardiovascular disorders long before the β-antagonists. Unlike the latter, which exhibit a great structural homogeneity, β-adrenergic antagonists are compounds with very diverse structures, suggesting that the adrenergic receptor has more than one zone capable of interacting with and blocking drugs.

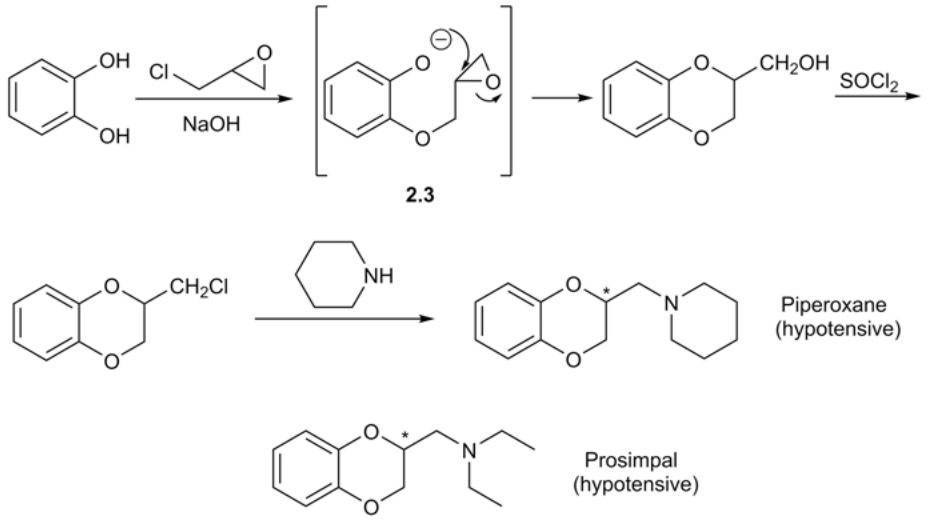
β–Blockers are divided into the following categories:

2.11.1 Competitive antagonists of the NA and A

1. Ergot alkaloids, especially the dihydrogenated ones.
2. Yohimbine and related alkaloids.
3. Benzodioxanes and other diverse structural drugs.
4. Various 2-substituted imidazolidines.

2.11.2 Noncompetitive antagonists of NA, especially β-haloethylamines, capable of irreversibly alkylating the receptor

They are of little or no therapeutic use, but have theoretical value, since they have allowed the establishment of a model of the α-adrenergic receptor.



Scheme 2.16: Piperoxane and prosimpal.

2.11.3 Benzodioxanes and other synthetic heterocycles. Imidazolines

α–Blockers (α_1 specifically) have been studied primarily as antihypertensive drugs. The benzodioxane ring system (currently in disuse), associated with an aminomethyl group, has a potent α-adrenergic blocking action. Compounds such as piperoxane and prosimpal are potent antagonists (Scheme 2.16).

One aspect worth highlighting is the regioselectivity of the epoxide ring opening of intermediate **2.3**. When carried out in a basic medium, the S_N2 process should have been produced by attack on the less hidered face. As a consequence a benzo-fused seven-membered ring with a secondary hydroxyl group should have been obtained. However, there is another process that modifies the regioselectivity of the epoxide opening, namely the stability of a six-membered ring (caused by the attack to the most hindered carbon atom of the epoxide) against the lesser stability of the nonproduced seven-membered ring, leading to the obtaining of the benzo-1,4-dioxane ring.

In general, benzodioxanes present stereoselectivity in their α-blockade; for example, the *S*(-)-isomer of prosimpal is about six times more active than its enantiomer. Benzodioxane is not the only heterocyclic system that is part of α-blockers. Currently, one of the most important ones is prazosin, 2-[4-(2-furoyl)piperazin-1-yl]-4-amino-6,7-dimethoxyquinazoline (Fig. 2.19).

The most extensive group of heterocyclic β-blockers is that of imidazolines (Scheme 2.17).

Tolazoline and phentolamine are antihypertensive agents. They are used in the diagnosis of pheochromocytoma, a tumor of the adrenal medulla that produces hyperten-

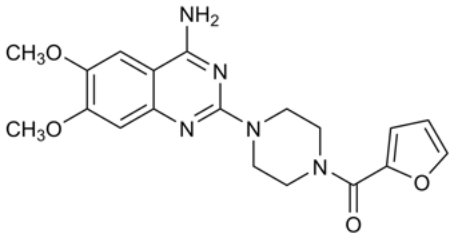
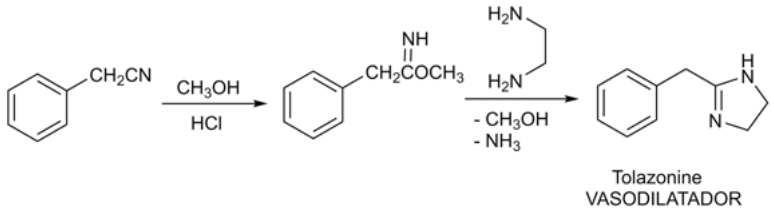
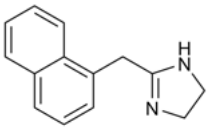


Fig. 2.19: Prazosin (hypotensive).



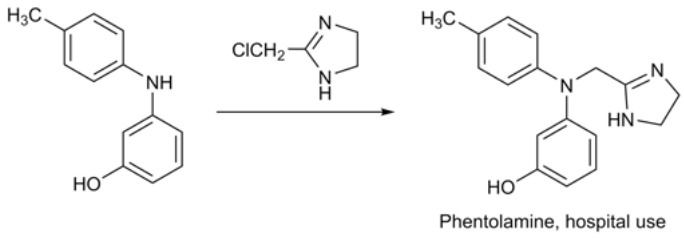
Note the great structural similarity between tolazoline (blocker) and naphazoline (sympathomimetic)



Naphazoline

This pair of drugs is one of the few exceptions to the rule that blockers are usually compounds whose lipophilic portion is more voluminous than in agonists

Phentolamine, another blocker in this group, has a higher potency than tolazoline



Phentolamine, hospital use

Scheme 2.17: Various imidazolines as β-blockers.

sion due to excessive secretion of adrenaline. After giving phentolamine or tolazoline, the presence of the tumor is can be detected by a sharp drop in blood pressure.

3 Dopamine

3.1 Goals

- To introduce the concept of double prodrug applied to dopamine.
- To introduce the student to the complex world of Parkinson's and its symptomatic treatment.
- To understand the structure-activity relationships in phenothiazines and butyrophenones.

3.2 Introduction: Antiparkinsonians related to the action or release of dopamine

Parkinson's disease is a neurological disorder characterized by muscle stiffness, tremors and akinesia (inability to initiate a movement when this movement is precise), accompanied by ANS disturbances, cognitive or memory deficits and other manifestations. Although it was discovered in 1817, its cause is still unknown. In some cases it follows a disease (encephalitis), in others it is a side effect of certain pharmacological treatments (especially when neuroleptics are used) and, finally, it may be idiopathic. In Greek it means "a disease of unknown etiology", which usually begins after the age of 50 and is degenerative, disabling and fatal.

The biochemical basis of Parkinson's disease is a deficiency of dopamine in the basal ganglia, which occurs as a result of a loss of pigmented cells in the substantia nigra. These cells synthesize dopamine, which is transported axonally to the caudate nucleus and other points of the CNS, where, among other functions, it is essential for the control of coordinated movements. Dopamine has to maintain a balance with another neurotransmitter, AcC. Modern treatment of Parkinson's disease is thus aimed at replacing dopamine, causing its release, or stimulating its brain receptors. Previously, anticholinergic drugs were used as antiparkinsonians. These would restore the dopamine/AcC balance by repressing the latter and not replacing the former (Fig. 3.1). Numerous dopaminergic blockers that are used as neuroleptics (a neuroleptic or antipsychotic is a drug that commonly, although not exclusively, is used for the treatment of psychoses) cause, as side effects, tremors similar to those caused by Parkinson's disease.

Another physiological action of dopamine on the CNS is to induce vomiting (emesis). Although this action is complex, antiemetic drugs, which are due in part to the ability to antagonize the emetic effects of dopamine at the central level, have been developed. At the peripheral level, specific dopamine receptors are also known, especially in the renal arteries, where dopaminergic stimulation leads to vasodilation.

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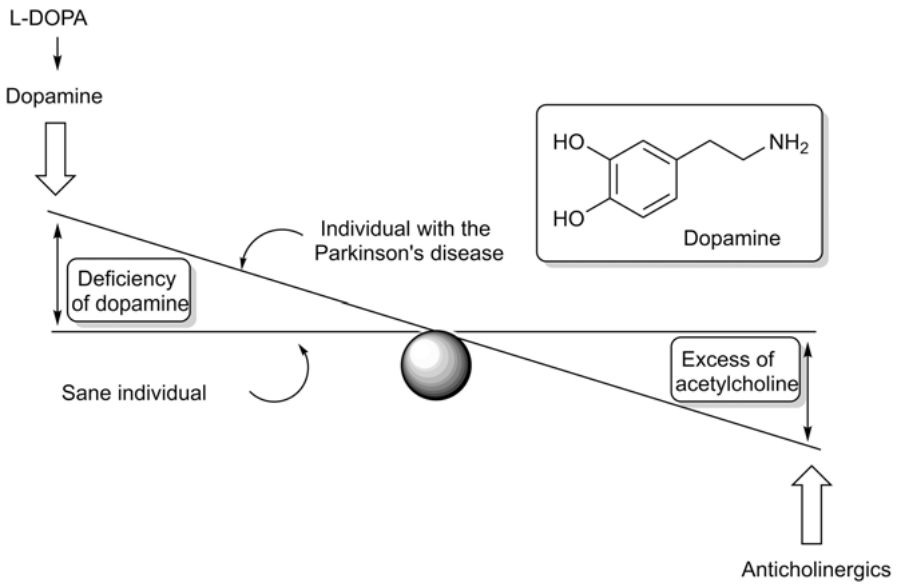


Fig. 3.1: Imbalance between dopamine and AcC in Parkinson's disease.

However, the development of selective drugs at this level has not yet gained much relevance from the therapeutic point of view.

3.2.1 Conformationally restricted analogues of dopamine

Dopamine is one of the most complex and poorly understood neurotransmitters, despite its structural simplicity. Epinin, on the other hand, is the *N*-methylated derivative of dopamine. Meanwhile, apomorphine can be considered the first rigid analogue of dopamine with peripheral partial agonist action (Fig. 3.2).

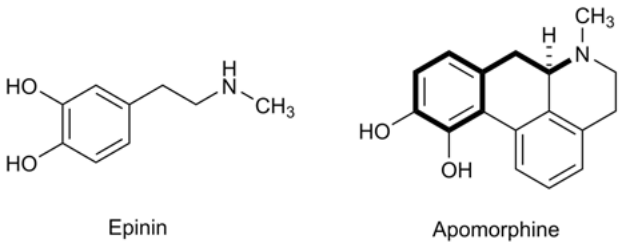


Fig. 3.2: Compounds structurally related to dopamine.

Molecular modifications of dopamine that have been studied are numerous. For example, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene is a potent dopaminergic agonist (Fig. 3.3).

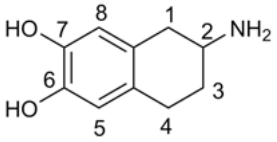


Fig. 3.3: 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

In dopamine and epinin there will be many conformations as a consequence of free rotation. However, in apomorphine, as well as in 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, the conformations are rigid. This is considered evidence that the "*active conformation*" of dopamine is the antiperiplanar one.

3.3 Direct agonists

Dopamine is very hydrophilic, which means that it can neither be absorbed well orally, nor does it cross the BBB. In contrast, L-dopa is properly absorbed by active transport mechanisms, and, once in the brain, it is decarboxylated and produces dopamine, thus behaving as a prodrug of dopamine (Fig. 3.4).

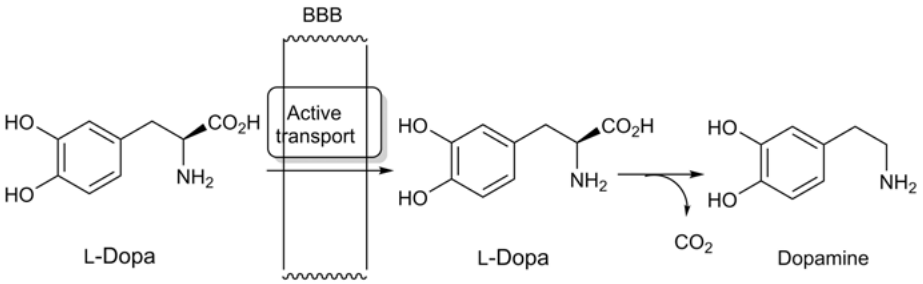


Fig. 3.4: L-dopa as a prodrug of dopamine.

In practice, to avoid side effects, it is necessary to combine L-dopa with peripheral dopa-decarboxylase inhibitors, such as α-(*S*)-methyl-DOPA, carbidopa or benserazide (Fig. 3.5).

Recently, a strategy that allows the passage of dopamine through the BBB, if it has been adequately latentized (Fig. 3.6) in the form of the di-pivaloyl dopamine prodrug has been developed.

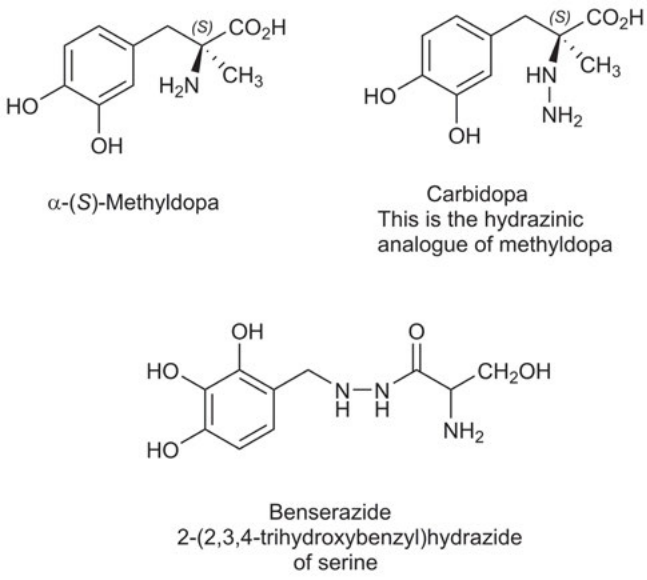


Fig. 3.5: Peripheral dopa decarboxylase inhibitors.

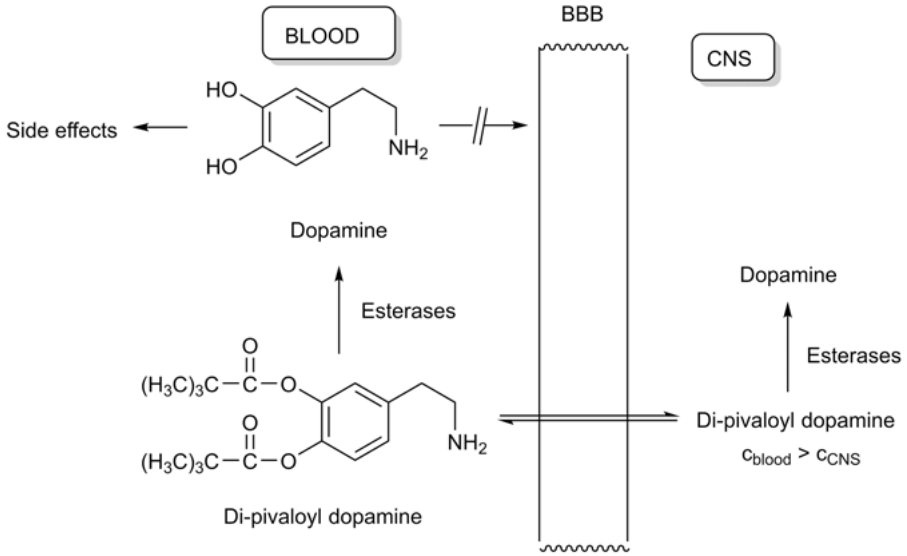
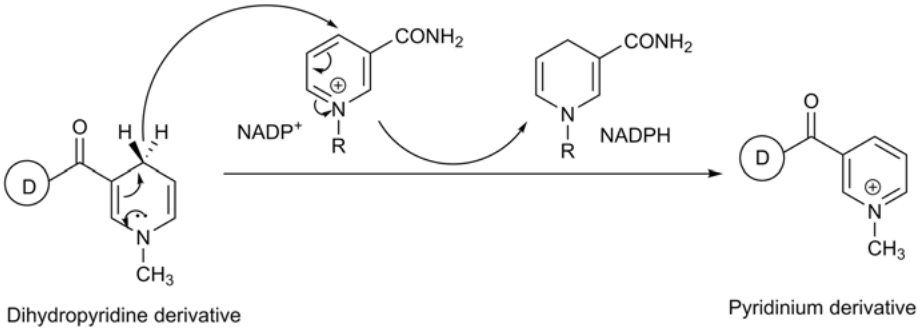


Fig. 3.6: Administration of the di-pivaloil dopamine prodrug.

Di-pivaloyl dopamine would be lipophilic enough to cross the BBB, but, since it is not retained in the brain, its action would be brief. In addition, hydrolysis of the prodrug by plasma esterases would produce elevated peripheral levels of dopamine, with the corresponding toxicity.

Another strategy is that a reaction takes place, reversing the polarity of the drugs and preventing its exit from the brain. The most frequently studied system is based on the ease with which dihydropyridines are transformed into pyridinium salts, with the intervention of $NADP^+$-dependent oxidoreductases. It has been used to selectively concentrate drugs in the CNS (Scheme 3.1).



Scheme 3.1: The $NADP^+$/NADPH cofactor pair is essential for reductive biosynthesis. D stands for drug.

1-Methyl-1,4-dihydropyridine-3-carboxamide, a more elaborate prodrug, in which the primary amine group is also blocked, also has a lipophilic structure which requires two hydrolytic reactions for its bioactivation to dopamine. The fraction that oxidizes in the brain is retained because it cannot diffuse into the plasma through the BBB. Trigonelline, the other degradation product of the double prodrug in the CNS, is non-toxic. The resulting situation is a high concentration of dopamine in the brain (action site) and a low concentration or none in the plasma (Fig. 3.7).

3.4 MAO and COMT inhibitors

In 1975, Youdin and Riedere introduced selegiline (N,α-dimethyl-*N*-propargylphenethylamine), which is a selective inhibitor of MAO-B (isoenzyme that together with MAO-A oxidizes monoamines). Of the two possible enantiomers, *R*(-)-selegiline, is the eutomer. This is because the *S* enantiomer is metabolized by dealkylation to the methamphetamine stimulant, which is responsible for the side effect of the drug. This isoenzyme is the predominant form in the striatum, and the one responsible for most of the oxidative metabolism of dopamine at this level. Unlike nonspecific MAO inhibitors, selegiline does not block the peripheral metabolism of catecholamines, meaning that it can be taken with levodopa, which allows a reduction in the dose of this drug. It also appears to protect patients from the loss of the remaining dopamine-

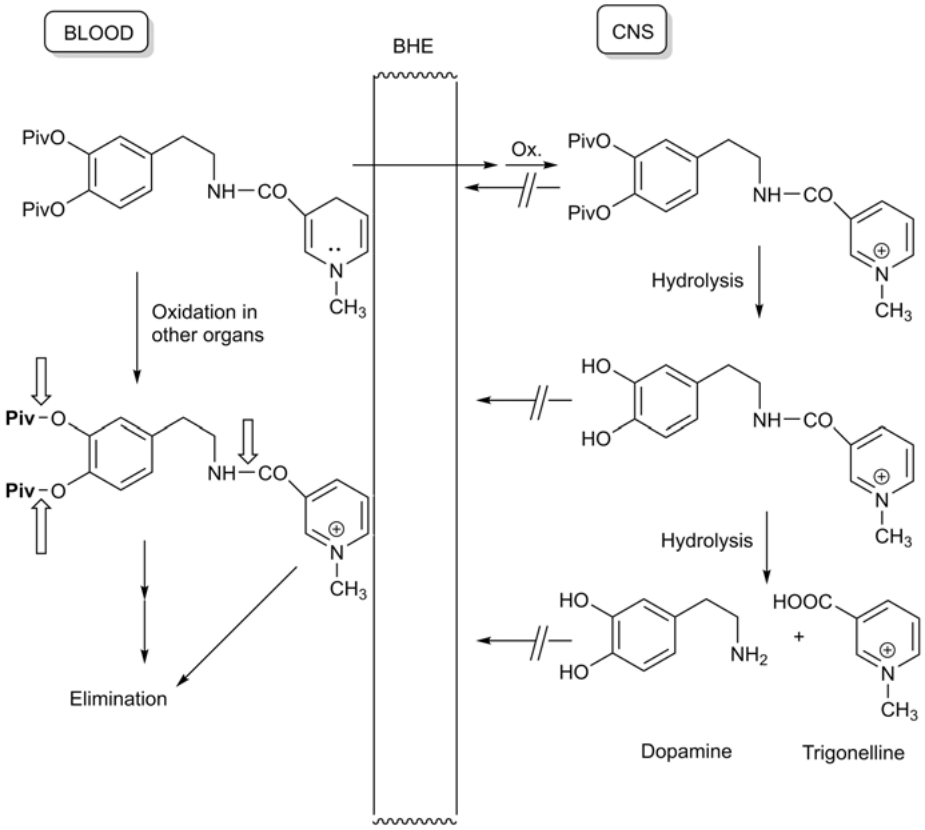


Fig. 3.7: Latentization of dopamine that allows its passage to the CNS.

producing neurons. A cyclic analogue of selegiline is rasagiline, a MAO-B inhibitor that is also useful in the treatment of Parkinson's disease (Fig. 3.8).

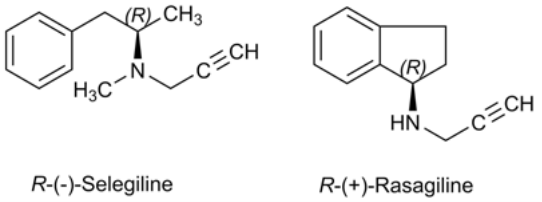


Fig. 3.8: Selective MAO-B antiparkinsonian drugs.

Another way to block the metabolic breakdown of dopamine is with selective COMT inhibitors, such as tolcapone. This inhibitor reduces the metabolism of dopamine in the brain and potentiates the action of levodopa, resulting in the reduction of the dose by a third (Fig. 3.9).

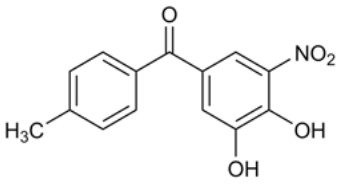


Fig. 3.9: Tolcapone.

3.5 Drugs capable of causing the release of dopamine from the peripheral neural sites at the presynaptic level

A third strategy for the treatment of Parkinson's disease is the use of drugs capable of causing the release of dopamine from the peripheral neural sites at the presynaptic level. The only compound of this type with clinical utility is amantadine (1-aminoadamantane), which is also used as a potent antiviral agent.

Tertiary alcohols alkylate the nitrogen atom of nitriles when they are dissolved in sulfuric acid. This process, known as the Ritter reaction, yields amides, which when hydrolyzed give *tert*-alkylamines. It should be noted that compounds such as *tert*-butylamine, which cannot be prepared from ammonia and *tert*-butyl chloride (because only elimination occurs) are synthesized by means of the Ritter reaction (Scheme 3.2).

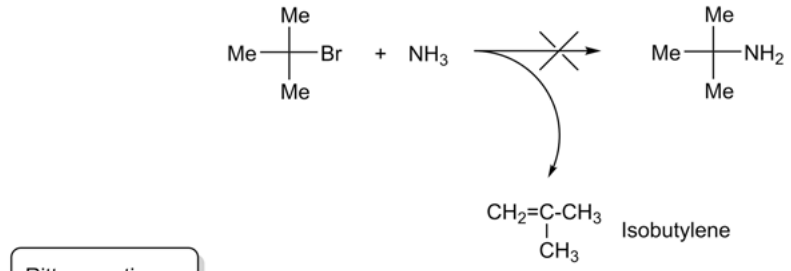
Amantadine (Scheme 3.3) is obtained from adamantine.

The four bridgehead positions are identical and surprisingly reactive. After halogenation with an excess of Cl_2 or Br_2, the subsequent reaction with acetonitrile in sulfuric acid leads to an amide through an apparent S_N1 reaction (Ritter reaction); subsequent hydrolysis yields the amantadine. Amantadine is a very basic amine and therefore fully protonated, but the extreme lipophilicity of the adamantanyl moiety allows its passage through the BBB. In addition, its volume and chemical inertia hinder the oxidative metabolism of the drug, and amantadine is excreted unchanged in the urine.

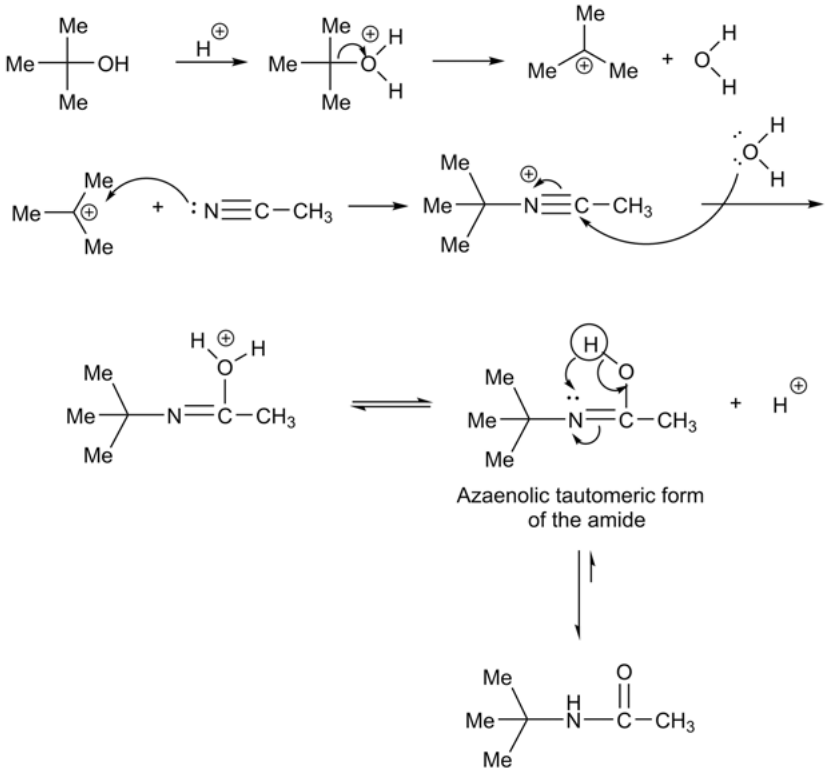
3.6 Other dopaminergic agonists

Finally, another dopaminergic alternative for treating Parkinson's disease can be by means of dopaminergic agonists, other than dopamine, among which apomorphine stands out. Apomorphine is obtained as a semisynthesis product of morphine by heating with concentrated HCl (Scheme 3.4).

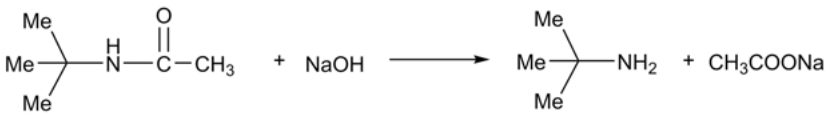
Apomorphine effectively controls Parkinson's symptoms, but it is only active parenterally, its action is brief, and it leads to violent vomiting.



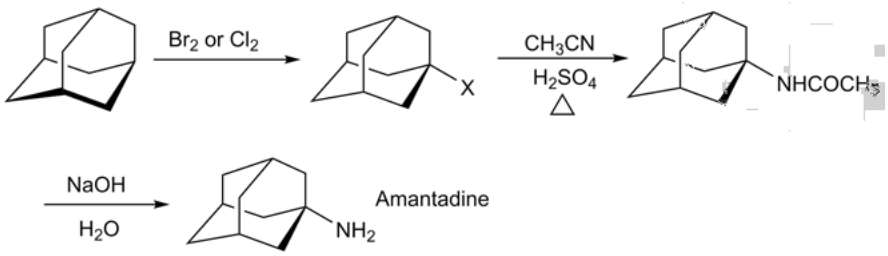
Ritter reaction



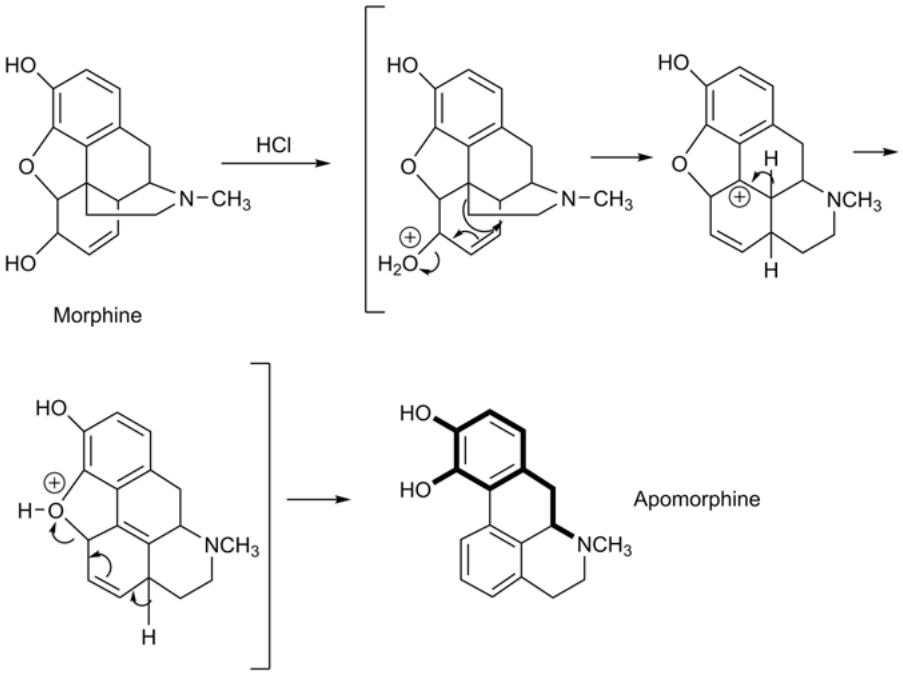
The amine is finally obtained by hydrolysis of the amide:



Scheme 3.2: Ritter reaction.



Scheme 3.3: Amantadine synthesis through the Ritter reaction.



Scheme 3.4: Semisynthesis of apomorphine.

3.7 Dopaminergic antagonists

Neuroleptic drugs are selective D_2 antagonists. The molecular level at which they act is not known. They comprise the following groups, of which we will study only the first two:

- Tricyclic neuroleptics
- Butyrophenones
- Benzamides
- Rauwolfia alkaloids

3.7.1 Tricyclic neuroleptics: Phenothiazines and thioxanthenes

Although phenothiazine has been used as an anthelmintic, the first known drugs derived from this heterocyclic system found utility as antihistaminics (see Chapter 7). An example is promethazine (Fig. 3.10), an antihistaminic designed by structural variation of ethylenediamines, compounds that constituted one of the first groups of antihistaminics, which are useful as antiallergics.

Promethazine is an antihistaminic that has sedative-like side effects. This effect became dominant in chlorpromazine, a neuroleptic prototype that comes from a structural variation of promethazine.

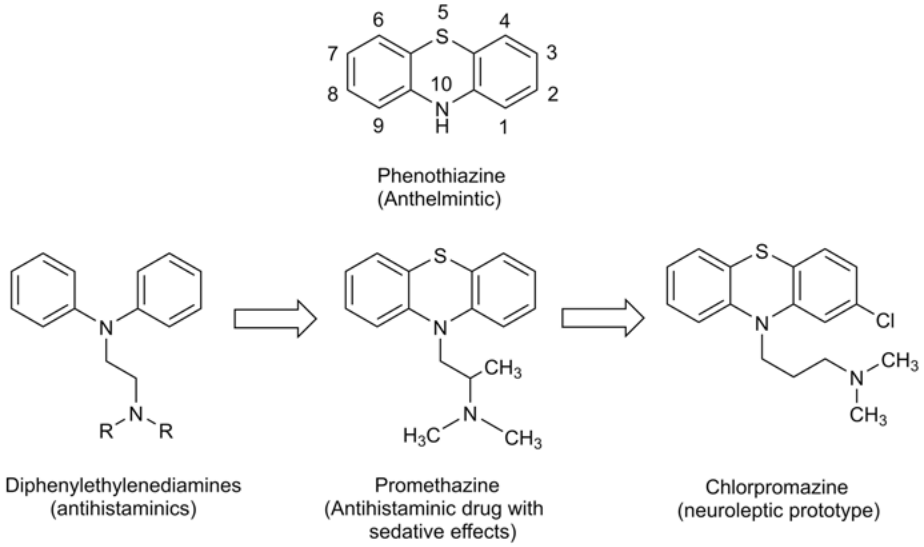


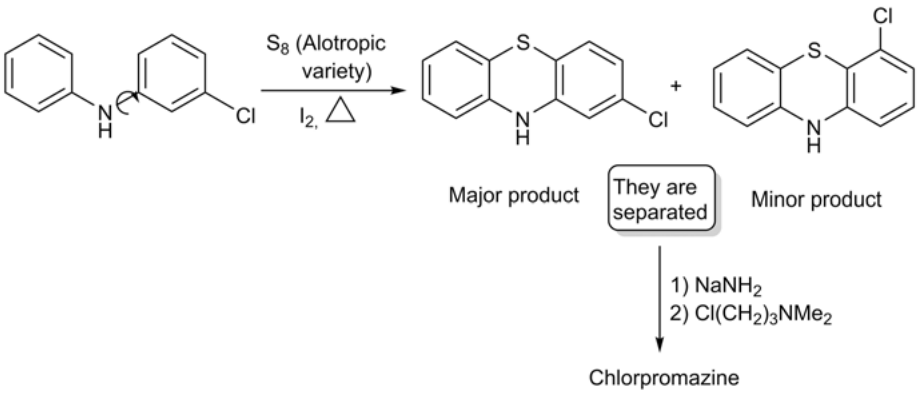
Fig. 3.10: Development of chlorpromazine from phenothiazine.

3.7.1.1 Synthesis of the tricyclic system

There are three syntheses:

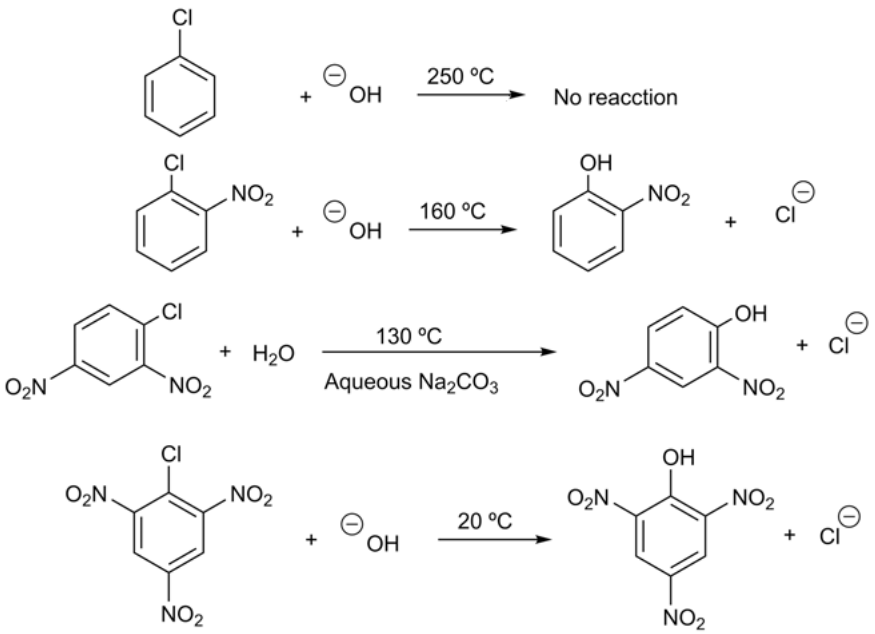
(a) **Sulfuration (or thionation) reaction of diphenylamine**, in which the heterocyclic nucleus is formed, and which must then be *N*-alkylated (Scheme 3.5). Note the formation of two isomers in the sulfuration step.

(b) **Synthesis of Ullmann**: **Aromatic S_N of halogens activated by nitro groups**. A halogen bound to a saturated carbon atom is often easily displaced by a suitable nucleophile at temperatures below 100°C. In contrast, chlorobenzene and bromobenzene are inert to this reaction under such conditions. They react with sodium hydroxide in water, for example, only at temperatures above 300°C. Thus, a halogen atom attached to an aromatic ring (or to an olefinic double bond, e.g.,

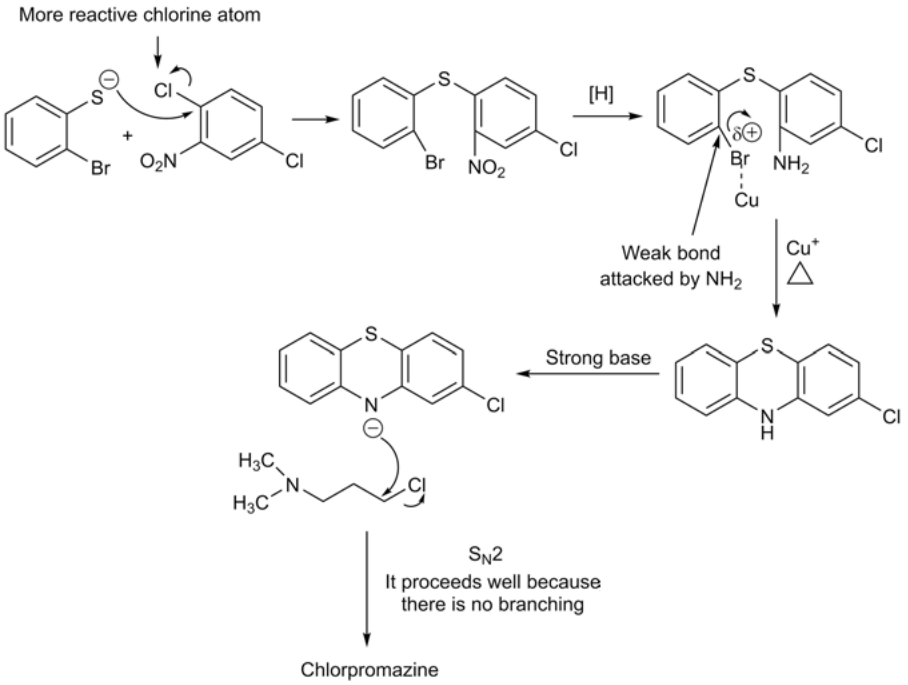


Scheme 3.5: Synthesis of chlorpromazine.

vinyl bromide) is generally reasonably inert to substitution by nucleophiles (in any of its mechanisms, S_N2 or S_N1). However, substitution can very easily occur if there is a strongly electron-withdrawing group attached to the aromatic ring at *ortho* or *para* positions with respect to the halogen (Scheme 3.6).



Scheme 3.6: Greater ease of substitution of the chloro atom, when there is a strongly electron-withdrawing group attached to the aromatic ring at *ortho* or *para* positions with respect to the halogen atom.



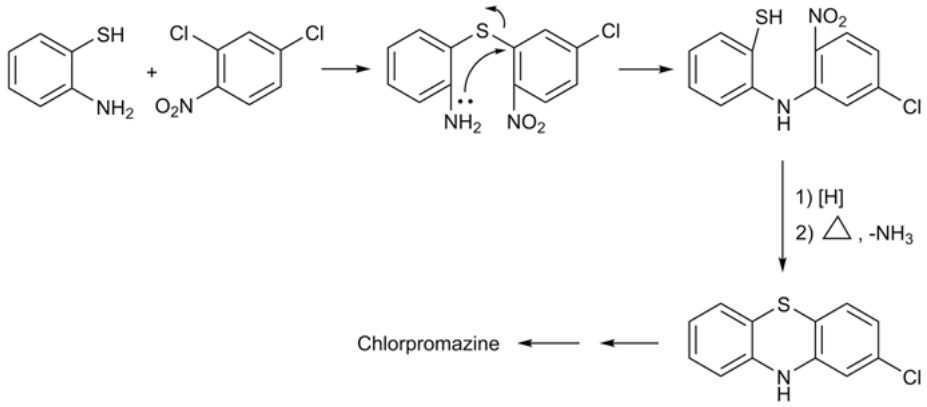
Scheme 3.7: Ullmann synthesis of chlorpromazine.

These are reactions of nucleophilic aromatic substitutions. The mechanism involves the formation of an intermediate that is analogous to that formed in electrophilic aromatic substitutions, but in which an anionic intermediate (Meisenheimer complex) is formed. If there are two or more electron-withdrawing groups at the *ortho* and *para* positions of the aromatic ring, the intermediate is even more stable, and the substitution reaction proceeds even more easily. Thus, any aromatic compound containing a good leaving group, such as halide, and a moderately strong (or rather several) "activating groups" at the *ortho* or *para* positions, will be exposed to substitution by an adequately effective nucleophile. Herein lies the synthesis of Ullmann (Scheme 3.7).

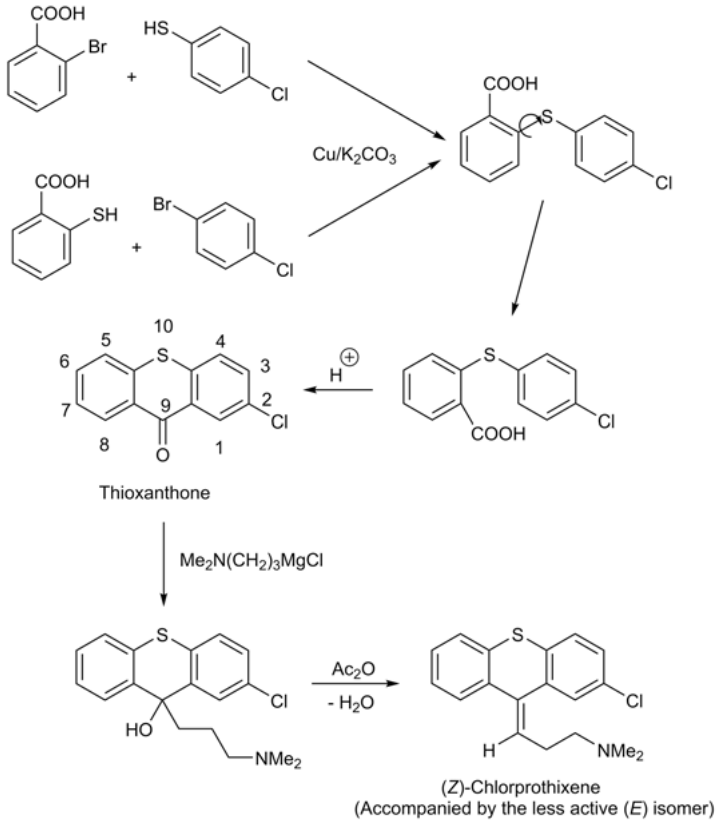
(c) **Smiles rearrangement** (it is simply an intramolecular nucleophilic substitution, Scheme 3.8).

After the synthesis of antipsychotic phenothiazines, a large number of molecular modifications were carried out, both in the aminoalkyl side chain and in the tricyclic nucleus.

Replacement of phenothiazine by a thioxanthene nucleus was the most successful, leading to chlorprothixene [*cis*-2-chloro-9-(3-dimethylaminopropylidene) thioxanthene], a drug prepared by the Ullmann condensation between *p*-chlorothiophenol and *o*-bromobenzoic acid (or between *p*-chlorobromobenzene and



Scheme 3.8: Synthesis of chlorpromazine through the Smiles rearrangement.



Scheme 3.9: Synthesis of chlorprothixene.

o-mercaptobenzoic acid), followed by cyclization and introduction of the side chain (Scheme 3.9).

3.7.1.2 Pharmacophore of tricyclic neuroleptics

The general structure is shown in Fig. 3.11.

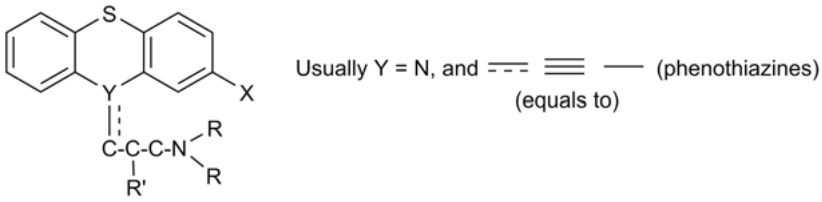


Fig. 3.11: Pharmacophore of phenothiazines and thioxanthene derivatives.

Gordon et al. postulated in 1964 that phenothiazines interact with the receptor in three specific zones, **A**, **B** and **C**, to produce the neuroleptic response (Fig. 3.12).

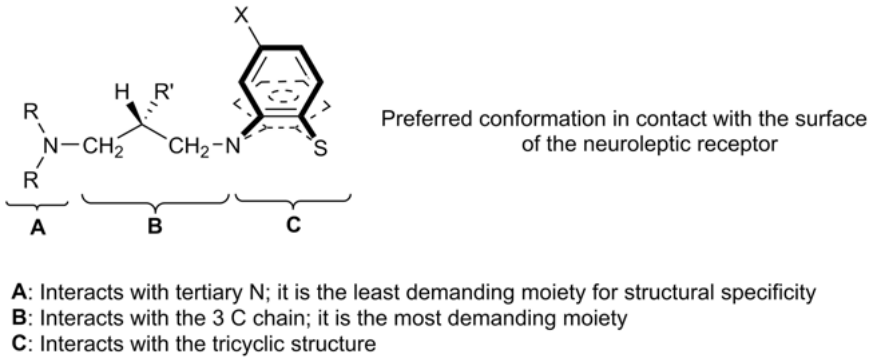
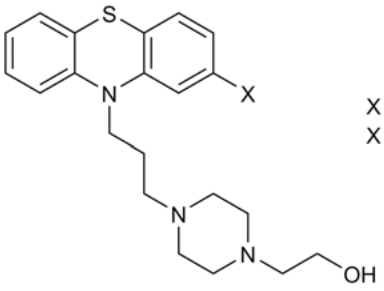


Fig. 3.12: Boat-like arrangement of the phenothiazine ring.

1. Normally the tranquilizing activity is optimal with X = Cl, CF_3, $SOCH_3$, etc., i.e., electron-withdrawing groups.
2. The optimal side chain has three carbon atoms between both nitrogen atoms. The depressant character is conserved with two carbon atoms, but the antihistaminic one predominates.
3. Something similar occurs with the branching in the side chain: if R'= CH_3, the tranquilizing character is reduced and the antihistaminic one increases. Other

substituents in the side chain (with the exception of inclusion in a piperidine cycle) greatly reduce the neuroleptic action.

4. As for the aminic nitrogen atom (basic), its quaternization cancels the activity (probably due to lack of distribution in the CNS). It is possible to include this N atom in a cycle (such as the piperazine ring, Fig. 3.13).



X = -CO-CH_2-CH_3: Carfenazine
X = -CF_3: Flufenazine

Fig. 3.13: Carfenazine and fluphenazine.

The presence of the OH group in carfenazine or fluphenazine allows its esterification with intermediate or long chain acids (heptanoic acid = enanic acid, decanoic acid = capric acid), thus obtaining latent drugs of very prolonged action (even several weeks). Scheme 3.10 illustrates the synthesis of carfenazine.
The hydroxyethyl derivative of piperazine can be prepared as outlined in Scheme 3.11.

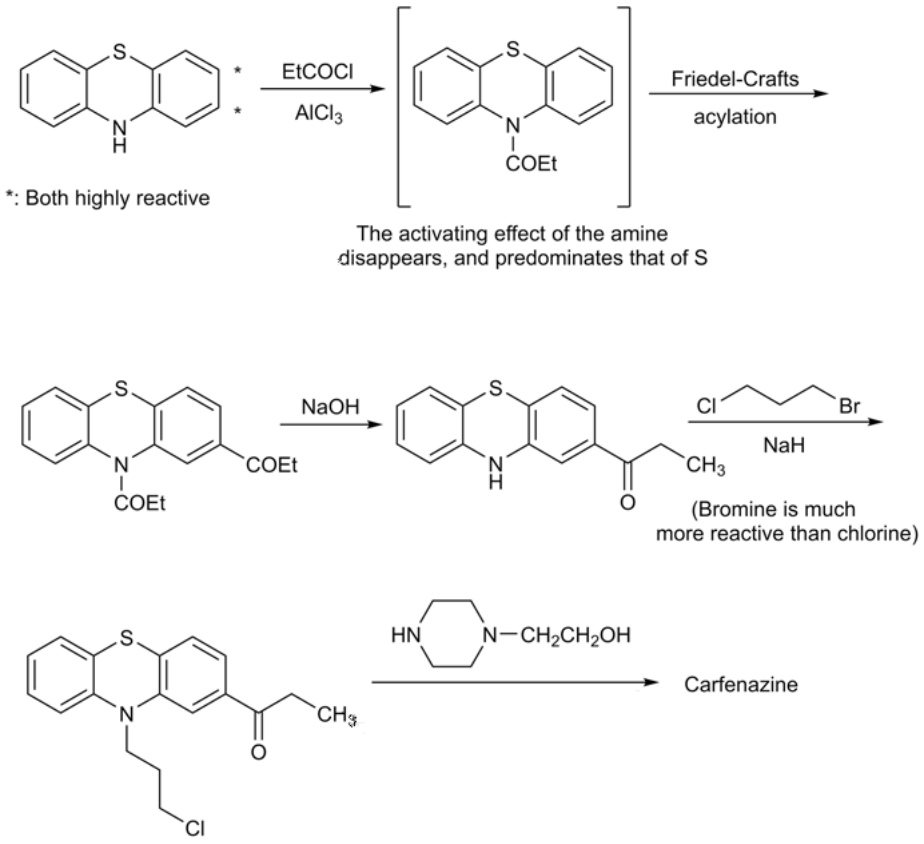
3.7.2 Butyrophenones and analogues

Development of butyrophenones is related to molecular modifications aimed at enhancing the analgesic effects of pethidine, an opioid-related analgesic (Fig. 3.14).

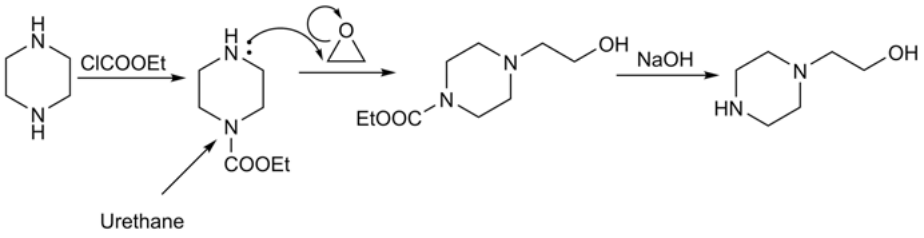
Modification of the butyrophenone analogue, by introducing fluoride at the *para* position of the acylated ring and changing the ester by the OH group, allowed the isolation of the neuroleptic action, separating it from the analgesic one. The prototype obtained was haloperidol, 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4'- fluorobutyrophenone (Fig. 3.15).

The synthesis of haloperidol is depicted in Scheme 3.12. The synthesis of the disubstituted piperidine at 4-position is initiated with a first transformation, which is a Mannich-type reaction.
Not all neuroleptics with a butyrophenone structure have the 4-arylpiperidinol grouping. This group can be changed for a tetrahydropyridine ring (Fig. 3.16).

Although the butyrophenone group, after which this class of compounds is named, was believed to be essential for the neuroleptic action, it was found that



Scheme 3.10: Synthesis of carfenazine.



Scheme 3.11: Preparation of the hydroxyethylpiperazine moiety.

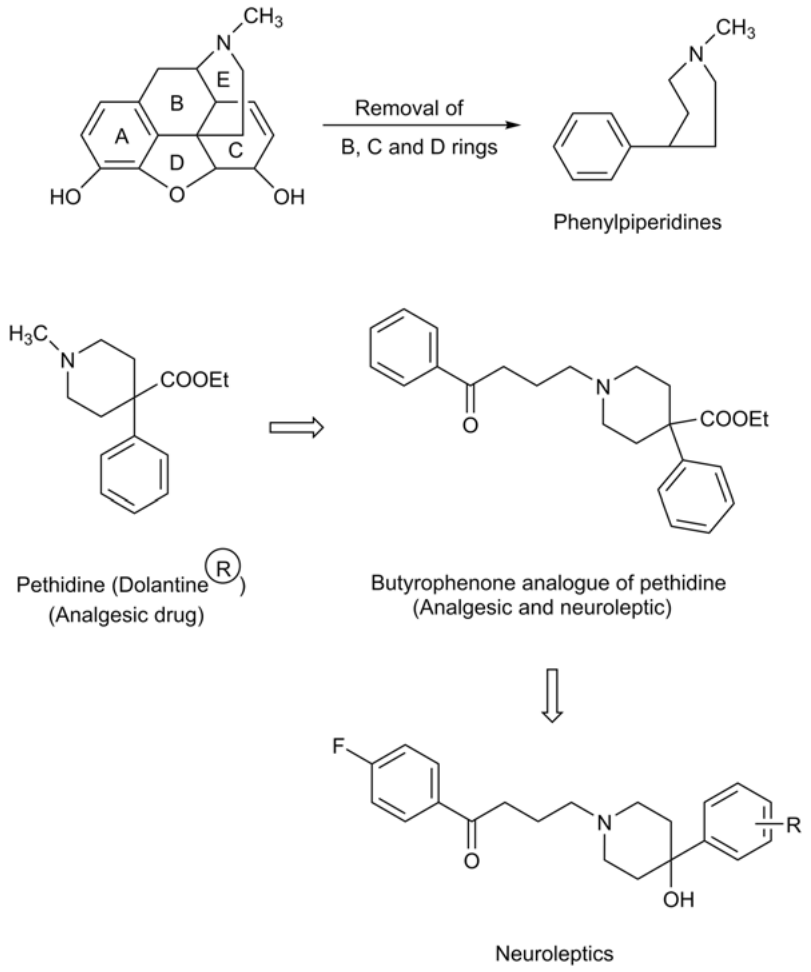


Fig. 3.14: Evolution in the design of neuroleptics of the butyrophenone family.

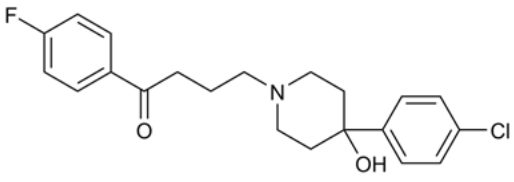
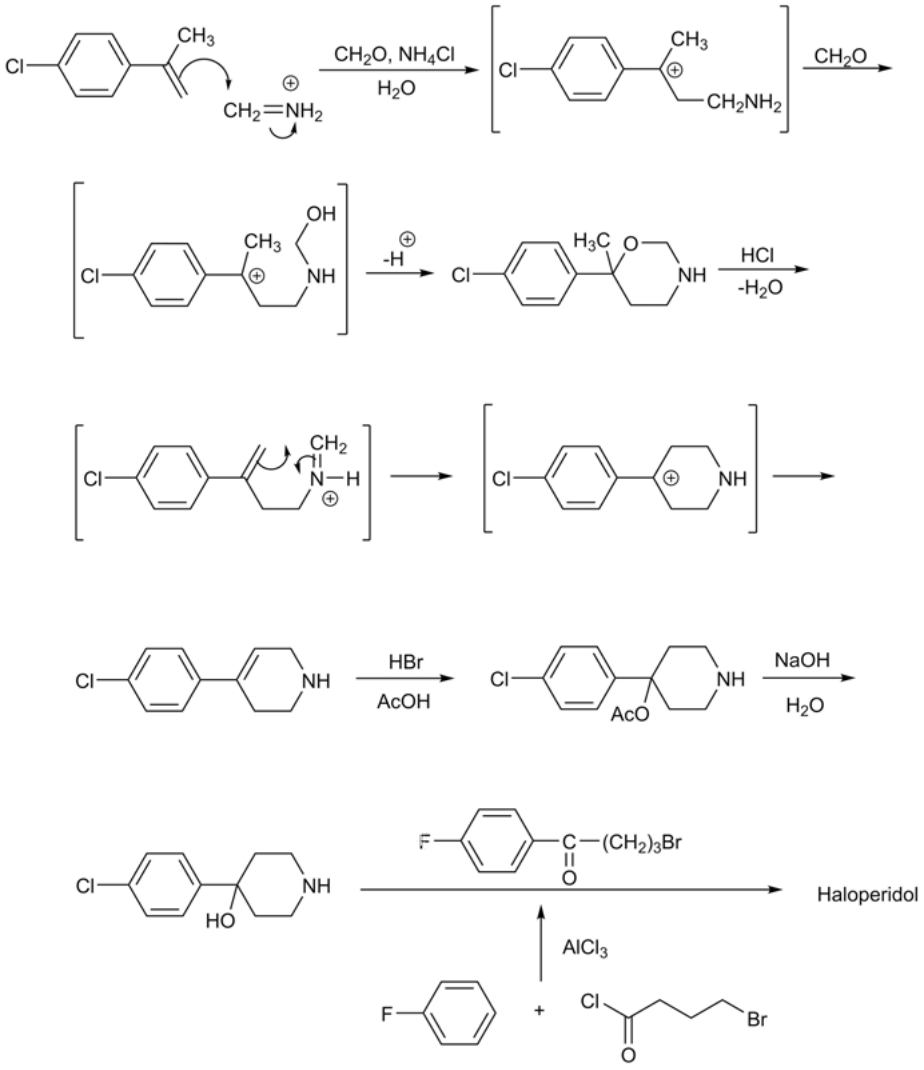


Fig. 3.15: Haloperidol.

it is possible to substitute the carbonyl group for a new fluorinated aromatic ring, thus obtaining diphenylbutylpiperidines, such as pimozide (Fig. 3.17).

In general, butyrophenones outweigh the phenothiazines, which possess a high antipsychotic potency and a relatively low toxicity.



Scheme 3.12: Synthesis of haloperidol.

3.7.3 *ortho*-Methoxybenzamides (orthopramides)

Orthopramides are obtained from the structural variation of local anesthetics. In particular, *ortho*-methoxyprocainamide (Fig. 3.18) showed, in addition to the expected local anesthetic action, a remarkable antiemetic action. For subsequent modifications of this structure, metoclopramide, a potent antiemetic with moderate local anesthetic activity, was obtained.

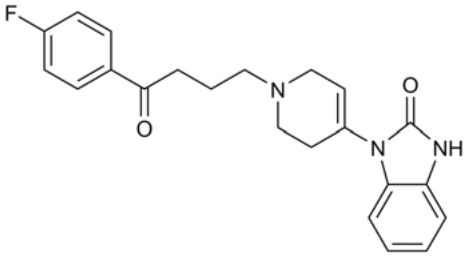
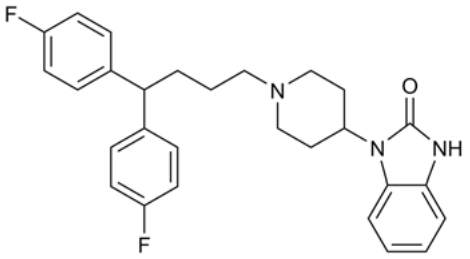


Fig. 3.16: Droperidol.



1-{1-[4,4-Bis(*p*-fluorophenyl)butyl]-4-piperidinyl}-2-benzimidazolone

Fig. 3.17: Pimozide.

Ortho-Methoxyprocainamide

Metoclopramide

Fig. 3.18: Ortopramides.

The antiemetic activity can also result from antagonism of the 5-HT_3 serotonergic receptors involved in chemotherapy-induced vomiting (see Chapter 4).

4 Serotonin and reuptake inhibitors of biogenic amines

4.1 Goals

- To introduce drugs modulating the reuptake and metabolism of biogenic amines and their therapeutic usefulness.
- To introduce the different serotonin receptors.
- To introduce the chemistry and structure-activity relationships of drugs that act on this neurotransmitter and its biochemical environment.

4.2 Introduction

Depression is a mood disorder and a mood disturbance (sadness) that is usually accompanied by anxiety. According to the hypothesis of monoamines initially formulated in 1965, depression is caused by a functional deficiency of neurotransmitting monoamines in certain brain areas. Thus, the absence or inability to act on these monoamines reduces nerve transmission at the central level and, along with it, the mood of the person.

Among the modulating drugs that help the reuptake and metabolism of CNS-related biogenic amines, especially NA, 5-HT and, to a lesser extent, DA (dopamine), antidepressants that inhibit the reuptake and metabolism of these amines are available. Thus, inhibition of reuptake is the mechanism of action of tricyclic antidepressants. On the other hand, the selective inhibition of MAO and COMT metabolizing enzymes has given rise to other drug families useful in the treatment of depression (COMT inhibitors such as tolcapone have found utility against Parkinson's disease).

4.3 Reuptake inhibitors: Tricyclic antidepressants

Most of the drugs in this group come from molecular modifications of neuroleptics (Fig. 4.1). Thus, imipramine (prototype) was synthesized in 1951 as an antihistaminic, sedative, analgesic or antiparkinsonian drug. Its usefulness as an antidepressant was observed in 1957.

Imipramine, 5-(3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine, has a dibenzazepine structure. Scheme 4.1 shows its synthesis: the corresponding carbanion is formed by treatment of *o*-nitrotoluene with sodium ethoxide. This anion is oxidized by air to give a radical, which dimerizes to produce the dinitro compound. By catalytic hydrogenation the diamine is obtained which, upon treatment with phos-

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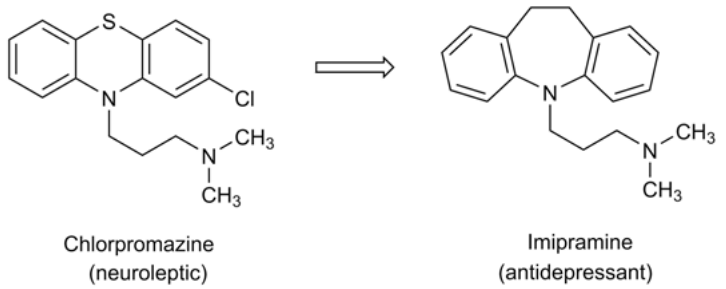
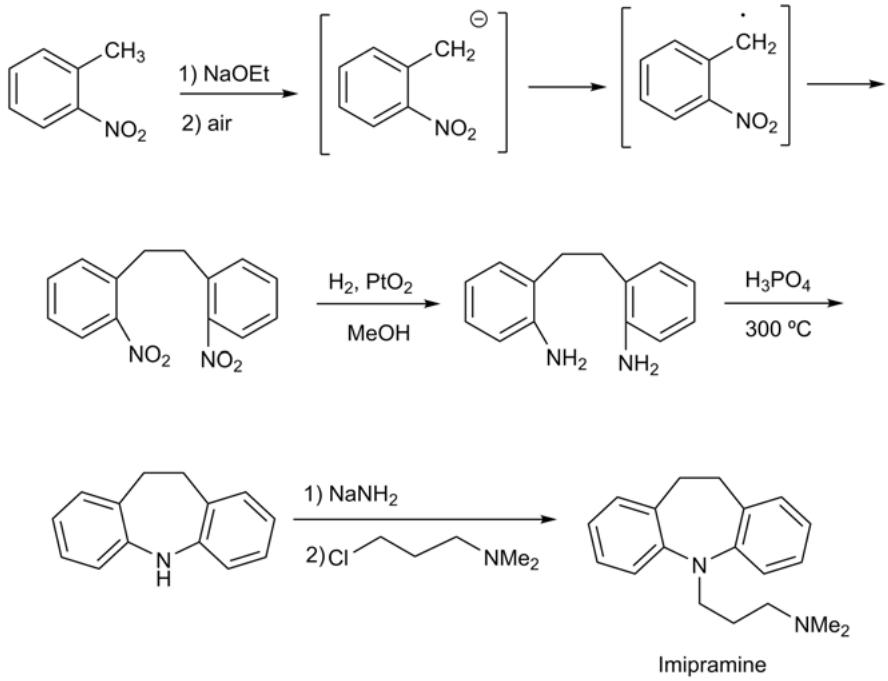


Fig. 4.1: Relationship between phenothiazines and tricyclic antidepressants.

phoric acid at an elevated temperature, loses ammonia in an S_NAr process to give the dibenzo[*b,f*]azepine. Alkylation of the nitrogen atom in the presence of a strong base yields imipramine.



Scheme 4.1: Synthesis of imipramine.

Interestingly, it is the metabolite of imipramine obtained after the *N*-dealkylation process that is responsible for its antidepressant activity (Fig.4.2). Consequently, many of the dibenzoazepines designed from imipramine are secondary amines, provided

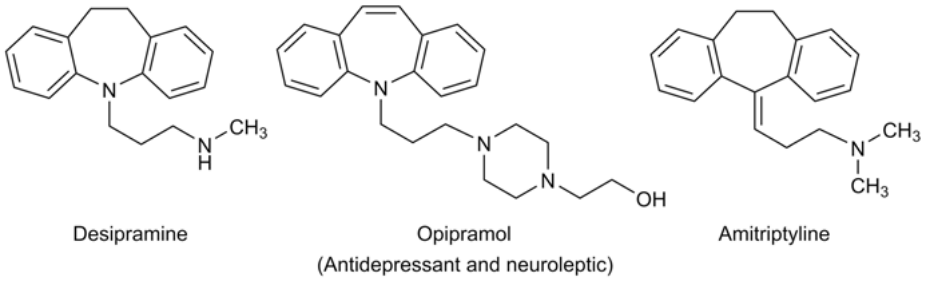


Fig. 4.2: Representative tricyclic antidepressants.

they have enough lipophilicity to ensure their entry into the CNS. Otherwise, tertiary amines are used and should be considered as prodrugs.

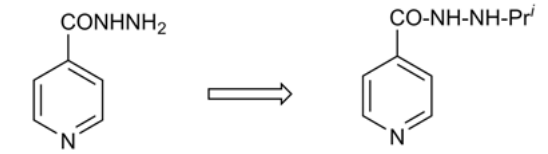
The introduction of a double bond between the carbon atoms of the ethylene bridge in the dibenzoazepines leads to neuroleptic compounds such as opipramol. The isosteric substitution of the cyclic N atom of imipramine by a methylidene group leads to useful antidepressants, such as amitriptyline (Fig. 4.2).

4.4 MAO inhibitors (MAOI)

MAO inhibitors (MAOI) are one of the first groups of drugs that are useful as antidepressants. MAO is actually a group of flavin-dependent isoenzymes that oxidize a wide variety of biogenic amines, whose mechanism of action has been studied in Chapter 2. Two MAO subtypes are known: MAO A, responsible for the oxidation of serotonin and noradrenaline, and MAO B, which oxidizes phenethylamine, dopamine, and tyramine. MAO subtypes are inhibited by different substances. Thus, antidepressant drugs are inhibitors of MAO A, and some antiparkinsonians are MAO B inhibitors.

The first MAO inhibitors were irreversible and nonselective against the different isoenzymes. Among them, iproniazide (a hydrazide designed from isoniazid tuberculostatic) (Fig. 4.3) and pargyline (a prototype of 2-propynylamines or propargylamines) (Fig 4.4) are available. Both drugs exert a suicide inhibition by irreversibly alkylating the flavin moiety. The lack of selectivity of these compounds has limited their therapeutic use, due to the side effects caused by the increase in levels of other biogenic amines, especially tyramine (2-*p*-hydroxyphenylethylamine). Tyramine is abundant in numerous foods, and the pressor effects of its accumulation by inhibition of MAO A can be dangerous.

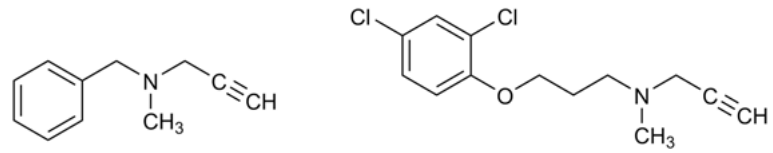
The second-generation MAOIs belong to the group of 2-propynylamines and are the result of molecular modification of pargyline. They are latent inhibitors that give rise to the irreversible alkylation of the flavin moiety of the enzyme. Clorgiline, a selective MAO-A inhibitor, is used as an antidepressant (Fig. 4.4).



Isoniazid
(tuberculostatic)

Iproniazid
(anti-depressant MAOI)

Fig. 4.3: Nonselective MAO inhibitors (MAOIs).



Pargyline
(Benzylmethylpropargylamine)

Clorgiline

Fig. 4.4: Antidepressant propargylamines.

Derivatives belonging to the third generation of MAOI have opened new perspectives in the development of antidepressants (MAOI A) and antiparkinsonians (MAOI B) by significantly reducing the side effects arising from the nonselective inhibition characteristic of classic MAOI. Meclobemide (MAOI A) (Fig. 4.5) is the first representative of this new generation of selective reversible inhibitors, in which the 2-aminoethylamine chain is essential for activity.

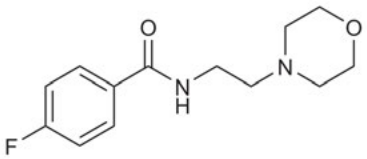
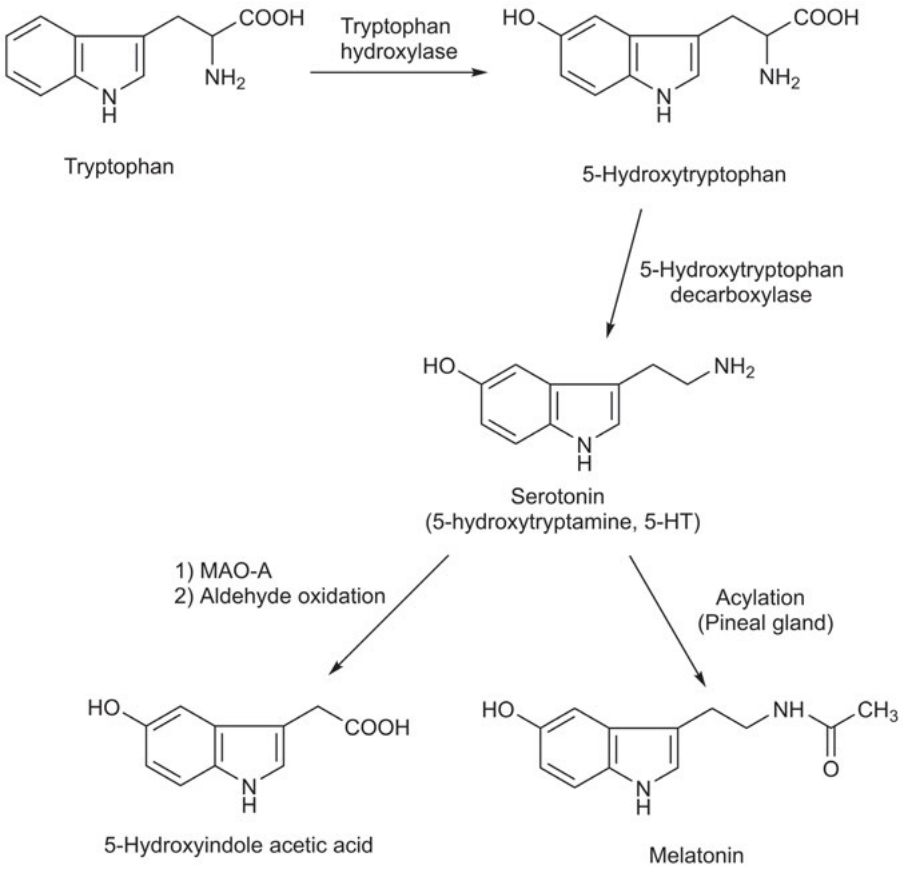


Fig. 4.5: Meclobemide as a third generation of MAOI.

4.5 Serotonin

Serotonin (5-hydroxytryptamine or 5-HT) is a neurotransmitter widely distributed in the body. It is derived biosynthetically from tryptophan in the diet by hydroxylation and subsequent decarboxylation, as indicated in Scheme 4.1. Upon its release and action on specific receptors, MAO A metabolizes it to an intermediate aldehyde, which is the 5-hydroxyindoleacetic acid precursor. Moreover, serotonin is converted into melatonin by *N*-acetylation in the pineal gland.

From a physiological point of view, serotonin has a wide range of effects, both at the peripheral and central levels. It is thus involved in the control of the pituitary gland function (hormonal production), in the regulation of temperature and in the perception of pain. It is related to migraine and various psychotic states. At the peripheral level, it affects the smooth muscles of the respiratory, the gastrointestinal and the cardiovascular systems (vasodilatation and hypotension). However, only specific drugs have been developed to modulate the action of this neurotransmitter on the CNS, which entails emptying the neurotransmitter deposits, neurotransmitter reuptake, and direct action on its receptors.



Scheme 4.2: Biosynthesis and metabolism of serotonin.

Drugs that modulate serotonin levels find application in various therapeutic fields: antidepressants, antihypertensives, antimigraine drugs, neuroleptics, anxiolytics, antiemetics and oxytocics related to this neurotransmitter.

4.6 Selective serotonin reuptake inhibitors (SSRIs)

Many of the drugs used in the treatment of depression inhibit the reuptake of NA or 5-HT, or both. Most tricyclic antidepressants are drugs of proven efficacy, but they are also sedatives and have side effects. Much more recent antidepressants are serotonin reuptake inhibitors (SSRIs), which have a wide margin of safety and fewer side effects.

Commonly, the SSRIs have a chiral phenylalkylamine structure, wherein the amino group may be forming part of a heterocycle. Fluoxetine (Prozac®), citalopram and sertraline are noteworthy. Prozac® is a racemic fluoxetine. (*S*)-Fluoxetine is used for migraine and (*R*)-fluoxetine for depression (Fig. 4.6).

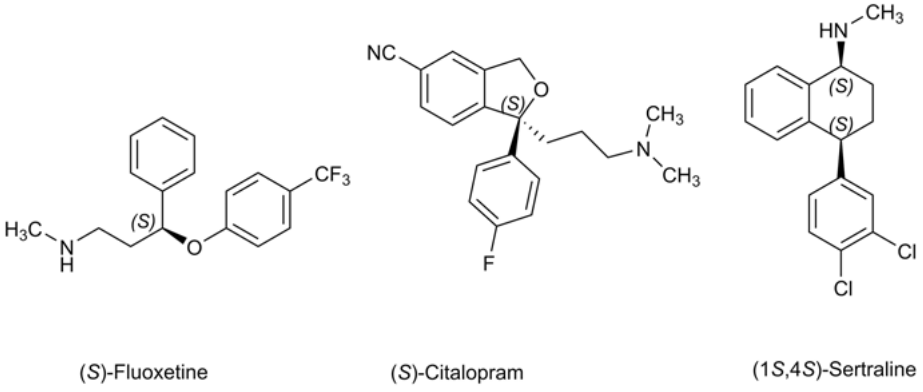


Fig. 4.6: Inhibitors of the serotonin reuptake with indication of the eutomer of each.

4.7 Direct action on serotonergic receptors

Seven types of serotonergic receptors are currently known, ranging from 5-HT_1 to 5-HT_7, and some are divided into subtypes.

4.7.1 5-HT_{1D} Agonists. Antimigraine drugs

A particular group of serotonin agonists is the selective 5-HT_{1D} agonists. This group of receptors presents a response opposite to the rest of the 5-HT_1 receptors. Thus, if the overall effect observable by the nonselective activation of such receptors is vasodilation, stimulation of the 5-HT_{1D} receptors results in selective vasoconstriction of the blood vessels of the CNS. This effect finds application in the treatment of a type of headache, generically called migraine. Migraines are characterized by localized pain. They are often hemicranial and may be accompanied by nausea, vomiting and

photophobia. Their etiology is not known, although certain factors increase their occurrence, such as a diet rich in cheese or wine, excessive noise, extreme temperatures and/or certain hormonal factors, have been detected. The symptoms of migraine are attributed to a central serotonin vasodilation. Migraine was frequently treated with drugs such as methysergide, which was initially considered a nonselective serotonin antagonist (Fig 4.7). However, the efficacy of this compound in the prevention of migraines was far superior to that of other more potent serotonin antagonists. This was one piece of data that allowed to postulate the selective agonist nature of methysergide on the 5-HT_{1D} receptor. Ergot alkaloids have a broad spectrum of activities. They are derived from a tetracyclic indole-quinolinic structure, known as ergoline, which contains the phenylethylamine and 3-indolylethylamine subunits that are characteristic of dopamine, adrenaline and serotonin (Fig. 4.7).

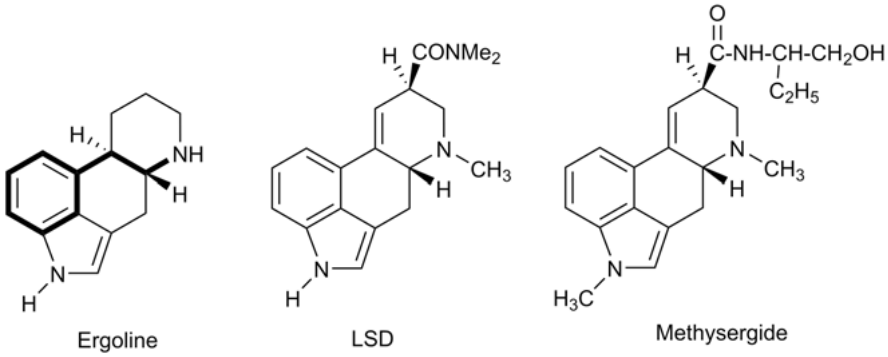


Fig. 4.7: Methysergide, the first selective 5-HT_{1D} receptor agonist.

4.7.1.1 Sumatriptan and other triptans

Sumatriptan (Fig. 4.8) of the Glaxo pharmaceutical company was the first serotonergic agonist and was introduced in the pharmaceutical market in 1991 (Scheme 4.3). It not only alleviates the intense migraine headache crisis, but also the associated symptoms such as vomiting, nausea, photophobia, etc. Since 1995, triptans such as zolmitriptan (Fig. 4.8) have emerged, which improve sumatriptan in terms of increased availability, increased CNS penetration, increased half-life etc.

Almotriptan (Fig. 4.8) has an absolute oral bioavailability of 70%, the highest of the currently known triptans.

The search for selective agonists began by manipulating the structure of 5-HT, starting with the modification of the hydroxyl group, because, when it is eliminated, antimigraine activity disappears. Positions 1, 2 and β of serotonin should not be substituted. 5-Carboxamidotryptamine (5-CT) was thus achieved, but unfortunately, its action was not selective, and it produced hypotension by interaction

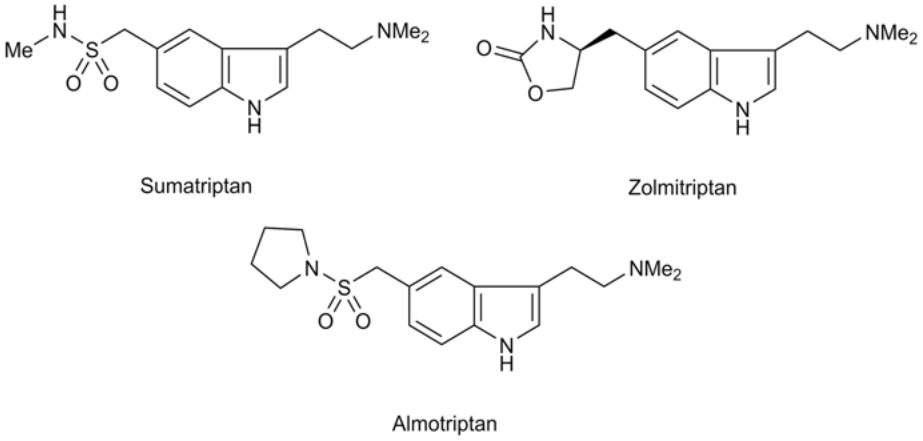
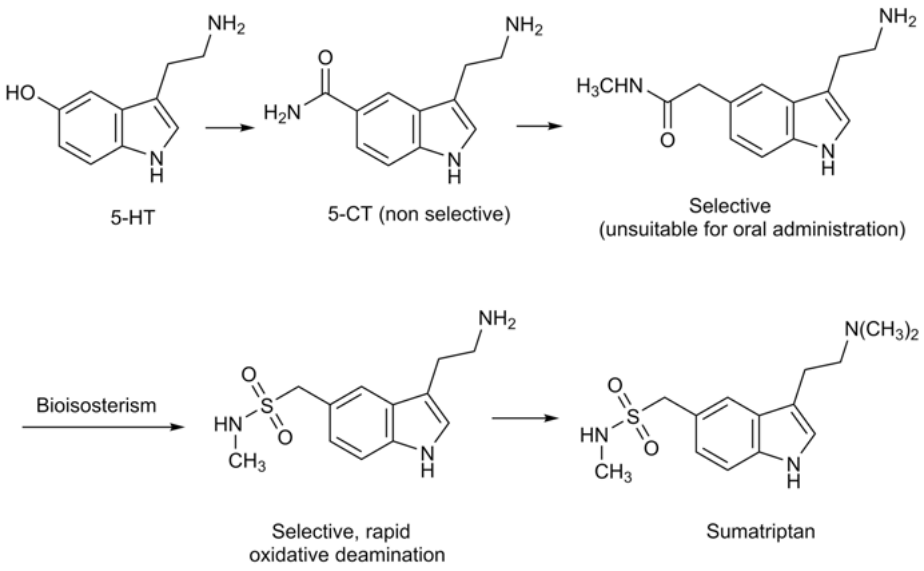


Fig. 4.8: 5-HT_{1D} agonists. Triptans.

with other 5-HT receptors. The substitution of the C-5 carbamoyl group for the *N*-methylcarbamoylmethyl group gave a compound that was selective, but unsuitable for oral administration. The bioisosteres of the carboxamide group were tested, highlighting the activity of the derivative with the *N*-methylsulfamoylmethyl group. Finally, to avoid its rapid oxidative deamination, the primary amino group was replaced by a tertiary amine. Sumatriptan was marketed in 1991.

The preparation of indole derivatives from nonheterocyclic precursors can be accomplished by Fischer synthesis, which takes place through a complex mechanism involving the cleavage of the N-N bond in phenylhydrazones. The choice of the carbonyl compound, which is condensed with phenylhydrazine, determines the nature of the R_1 and R_2 substituents. In this reaction, the phenylhydrazone of an aldehyde or a ketone with a strong acid is heated under nonhydrolytic conditions. As reagents of this type, molten zinc chloride and, more recently, polyphosphoric acid (H_3PO_4+P_2O_5) have been used. Although it is a very general reaction, it does not serve for the simplest case: the conversion of acetaldehyde phenylhydrazone to indole. Some of the proposed intermediates are shown in Scheme 4.4.

The aniline substituted at position 4 by an *N*-methylsulfamoylmethyl group is the starting reactant for the synthesis of sumatriptan. This aniline is transformed into a diazonium salt with nitrous acid. Its reduction with tin chloride gives rise to the corresponding phenylhydrazine, which is condensed with 3-cyanopropionaldehyde to yield hydrazone, which in acid medium undergoes the Fischer rearrangement to the corresponding indole. Finally, the reduction of the nitrile to the primary amine and its subsequent treatment with an excess of formaldehyde and sodium borohydride yields the *N,N*-dimethyl derivative (Scheme 4.5).



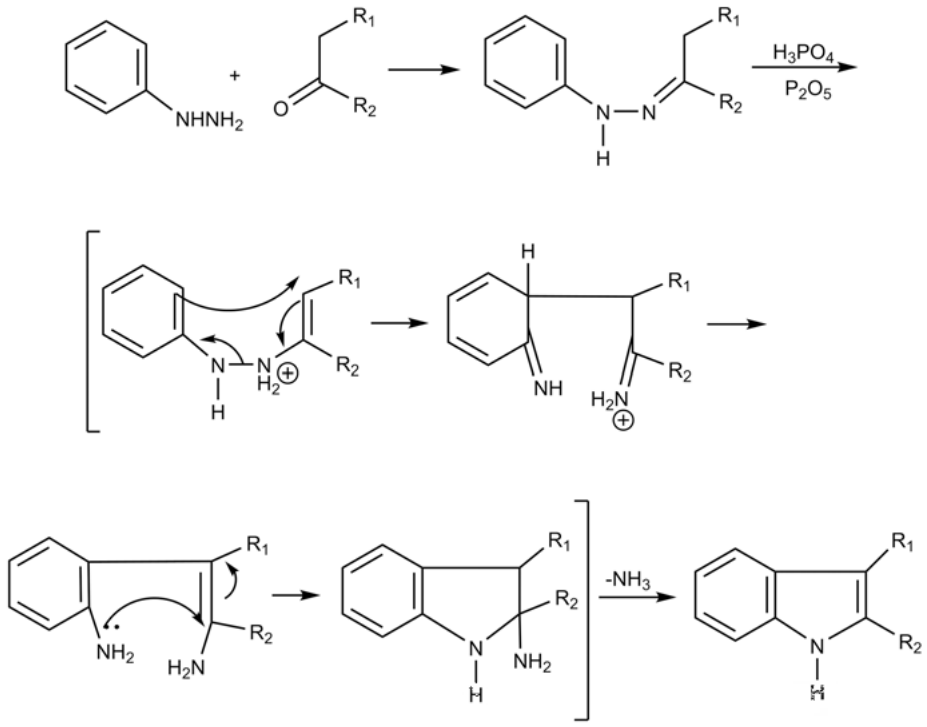
Scheme 4.3: Evolution in the development of sumatriptan.

4.7.2 5-HT$_{1A}$ Agonists

5-HT$_{1A}$ receptors are very abundant in certain areas of the brain, such as the hippocampus, and appear to be involved in the origin and development of anxiety. Although benzodiazepines, agonists of an allosteric site of the GABA-A receptor, are widely used as anxiolytics, they have a very short action, have anticonvulsive effects and, in their continued administration, they produce dependence phenomena and sedation. 8-Hydroxy-2-*N*,*N*-dipropylaminotetralin (8-OH DPAT) is a 5-HT$_{1A}$ agonist and has become a useful pharmacological tool to characterize several subtypes, since it has low affinity towards 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_2$ receptors, but very high (nanomolar range) towards 5-HT$_{1A}$ receptors. It has very low stereoselectivity [the (*R*) enantiomer shows only two times more affinity than the (*S*)]. Partial agonists of the 5-HT$_{1A}$ receptor, such as buspirone and ipsapirone, are *N*-arylpiperazine derivatives, separated by a polymethylene bridge of the nitrogen-containing heterocycle. They are also agonists of the α_1, D_2 and 5-HT$_{2A}$ (Fig. 4.9).

4.7.3 5-HT$_3$ Antagonists

They are used primarily as antiemetics in the treatment of nausea and vomiting induced by chemotherapy. Some orthopramids, such as metoclopramide (Fig. 3.18), are antiemetic, not only for their D_2 antagonistic activity, but also for blocking 5-HT$_3$ receptors. This finding motivated the search for pure 5-HT$_3$ antagonists. The application

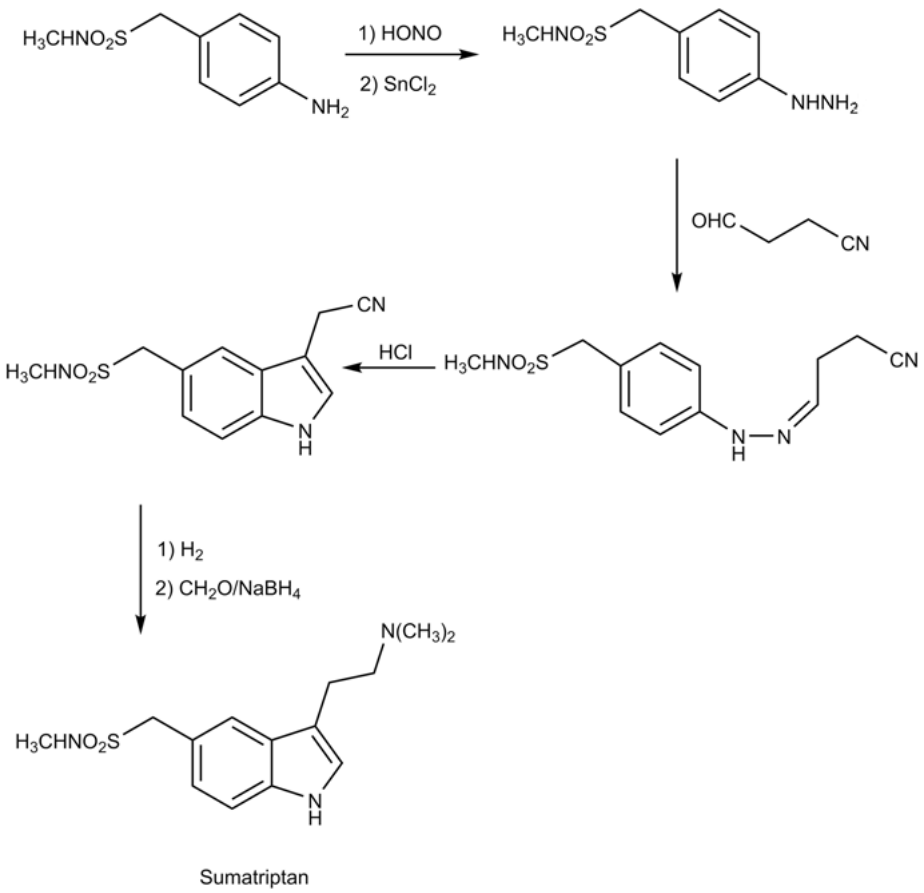


Scheme 4.4: Mechanism of the indole Fischer synthesis.

of molecular hybridization explains the structural genesis of ondansetron, a functionalized carbazole derivative with important antiemetic properties, and which acts as a selective antagonist of the 5-HT_3 receptor. The genesis of ondansetron was based on structural modifications of tropisetron (tropinyl indole-3-carboxylate, Fig. 4.10). This drug was designed through molecular hybridization between cocaine and serotonin. The weak 5-HT_3 antagonistic activity of cocaine has been known since 1970, so an ester derived from acid that includes the indole structure (present in triptans) was prepared with the alcohol derived from tropane (Fig. 4.10).

Tropisetron was the precursor of granisetron (Fig. 4.11), obtained by two isosteric substitutions of the original prototype and involving the change of the indolic ring by the indazole system, the ester function by an amide group, and in addition, the substitution of the tropane system by its higher homologous alkaloid of tropane, *N*-methylgranatanine. Tropane is a nitrogenous [3.2.1] bicyclic compound, whilst granatanine is a nitrogenous [3.3.1] bicyclic one. Granisetron had a longer half-life in the biophase than that of the initial prototype, tropisetron, due to the lower presence of amidases in the plasma.

Subsequently, the nonane system present in tropisetron was replaced by saturated monocyclic subunits and aza-aromatic rings, such as the 5-(4-methyl)imidazole



Scheme 4.5: Sumatriptan synthesis.

system, giving rise to GR-65630 (Fig. 4.11). This new compound was very active, and its structure included a benzylic carbonyl group attached to the C-3 of the indole ring. The benzylic carbonyl group occupies the same position as the ester carbonyl group of tropisetron (Fig. 4.11). Moreover, GR-65630 had a linker of three carbon atoms between the two aromatic rings, thereby maintaining a distance between the nitrogen ring of the terminal heterocyclic ring and the C-3 position of the *N*-methylated indole ring similar to that observed between the basic center of the tropane system and the C-3 of the indole ring of tropisetron (Fig. 4.11).

The introduction of a methyl group into the imidazole ring present in GR-65630 allows the tautomeric balance, with the tautomer exemplified in Fig 4.11, to be reduced. Finally, ondansetron was designed by application of the molecular annulation strategy as a way of restricting the flexibility of the carbonyl chain and thus contributing to the increase of the selectivity for 5-HT_3 receptors (Fig 4.11).

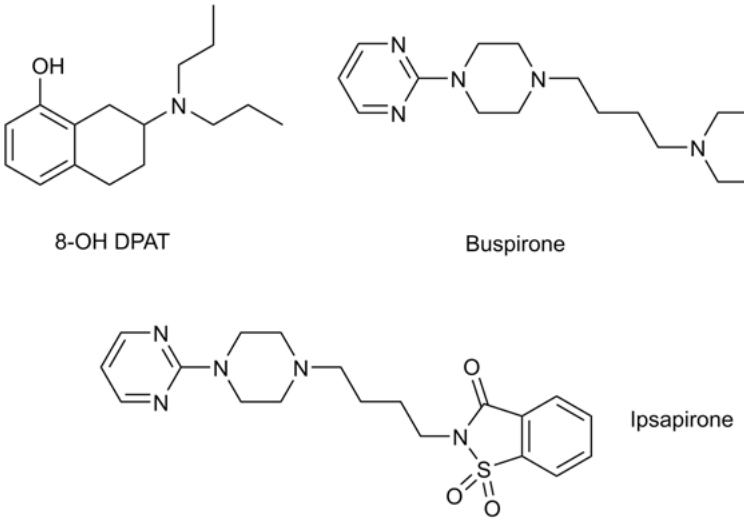


Fig. 4.9: 5-HT_{1A} Agonists.

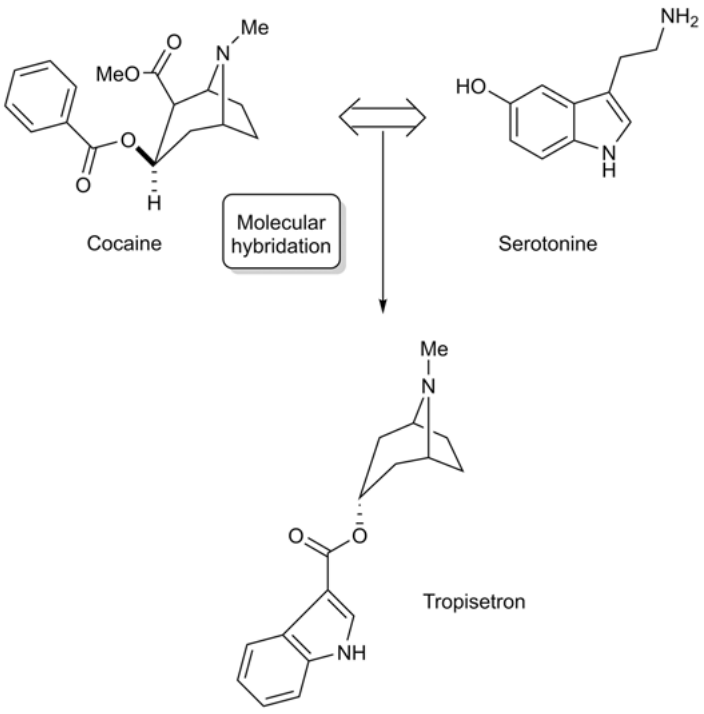


Fig. 4.10: Tropisetron: Molecular hybridation of cocaine and serotonin.

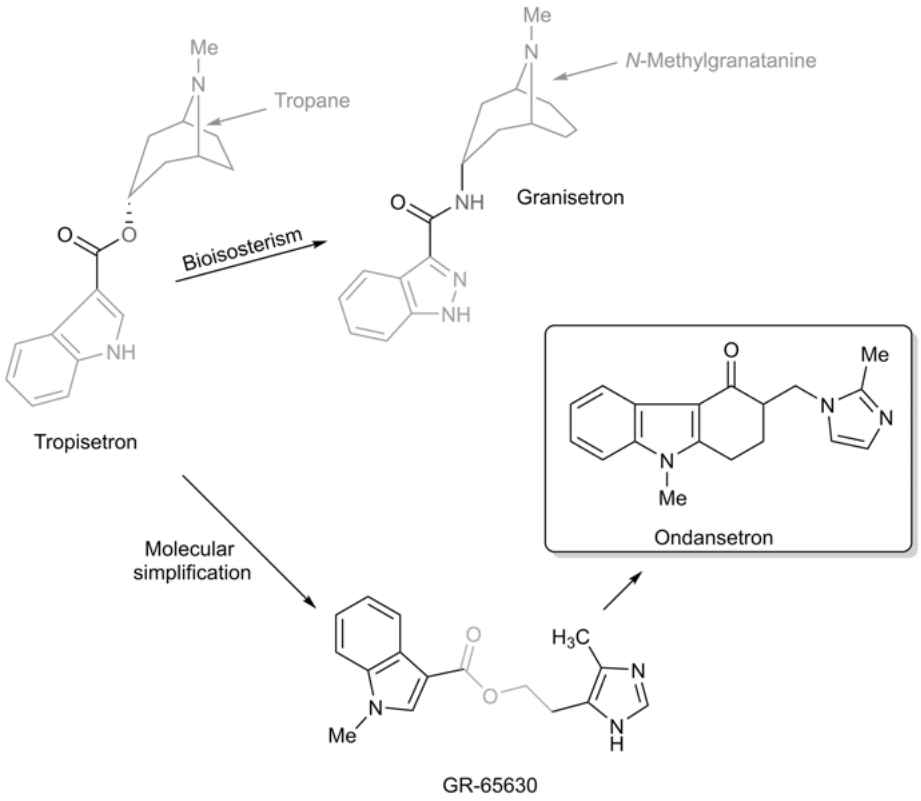


Fig. 4.11: 5-HT_3 Antagonists: Development of ondansetron.

4.8 Summary

Serotonin is the major neurotransmitter in the brain and is also involved in some peripheral actions. Seven families of 5-HT receptors (5-HT_1–$5HT_7$) have been identified, and some are divided into distinct subpopulations. In the last 20 years, selective agonists and antagonists have been discovered for some of these subpopulations. In addition to acting directly on 5-HT receptors, therapeutic agents are available with other mechanisms that influence the serotonergic transmission, such as SSRIs, tricyclic antidepressants, and MAO inhibitors. Studies with the 5-HT receptors have led to the introduction of agents useful for the treatment of anxiety (e.g., buspirone), migraine (e.g., sumatriptan), and vomiting caused by chemotherapy (e.g., ondansetron). Other drugs are currently under clinical trials for the treatment of depression, schizophrenia, obsessive-compulsive disorder (OCD) and other diseases. Research has also led to a better understanding of the cardiovascular pharmacology, obesity, neurodegenerative diseases, aggression, sexual behavior and drug abuse, among others. Serotonin

may even play an indirect role in techniques as diverse as acupuncture and transcendental meditation. All this has led to the comment that "it seems that serotonin is involved in everything." From the above, it can be deduced that the pharmacological profile of compounds modulating serotonin activity is extremely complex. In this chapter, we have presented a simplified view of the different families related to serotonin. Given the dynamism that characterizes this type of studies, it is foreseeable that in the coming years new prototypes will arise to allow the development of alternative therapies based on the selective modulation of some of the multiple actions of serotonin.

5 Amino acids as neurotransmitters

5.1 Goals

- To learn the different amino acids that behave as neurotransmitters.
- To learn the biological effects of the different ligands of the GABA receptors.
- To describe the different benzodiazepine structures and related compounds that behave as anxiolytics.
- To describe barbiturates and their therapeutic use.

5.2 Introduction

Neurotransmitter amino acids may be excitatory or inhibitory. The excitatory amino acids are acidic (two acid groups against a basic group, such as amino), while the inhibitors are neutral (an acid group and an amino group). L-Glutamic acid and L-aspartic acid are among the most representative excitatory amino acids, while γ-aminobutyric acid (GABA), glycine and taurine are examples of inhibitory amino acids.

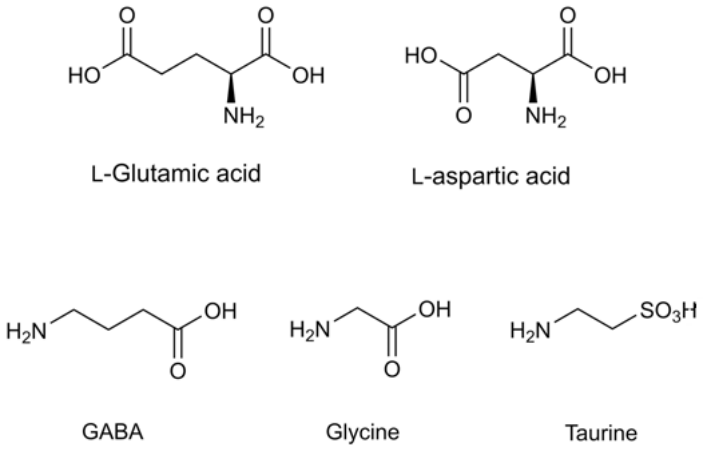


Fig. 5.1: Neurotransmitter amino acids.

5.3 Inhibitors of γ-aminobutyric acid (GABA)

This neurotransmitter amino acid is of high interest because it is the only one for which drugs are known that act as modulators of its activity (benzodiazepines and barbiturates).

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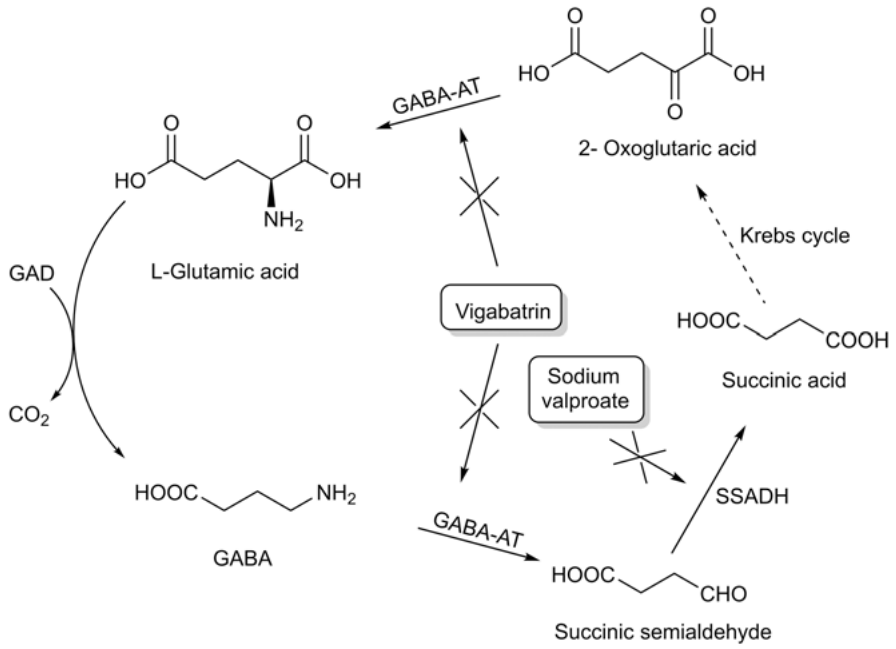


Scheme 5.1: Biosynthesis and metabolism of GABA.

Given the inhibitory nature of this neurotransmitter, low levels of GABA in the CNS are associated with pathologies such as epilepsy, schizophrenia, Parkinson's disease, Huntington's chorea and anxiety.

5.4 Presynaptic modulators

The biosynthesis of GABA occurs exclusively at the CNS level, since GABA cannot cross the blood-brain barrier (BBB) and its peripheral precursor are not known. GABA is derived from the decarboxylation of L-glutamic acid in a process that is catalyzed by the enzyme glutamate decarboxylase (GAD) (Fig. 5.1). Moreover, the biosynthesis of GABA is linked to the Krebs cycle through 2-oxoglutaric acid and succinic semialdehyde, a precursor of succinic acid in the Krebs cycle by the action of semialdehyde succinic dehydrogenase (SSADH).

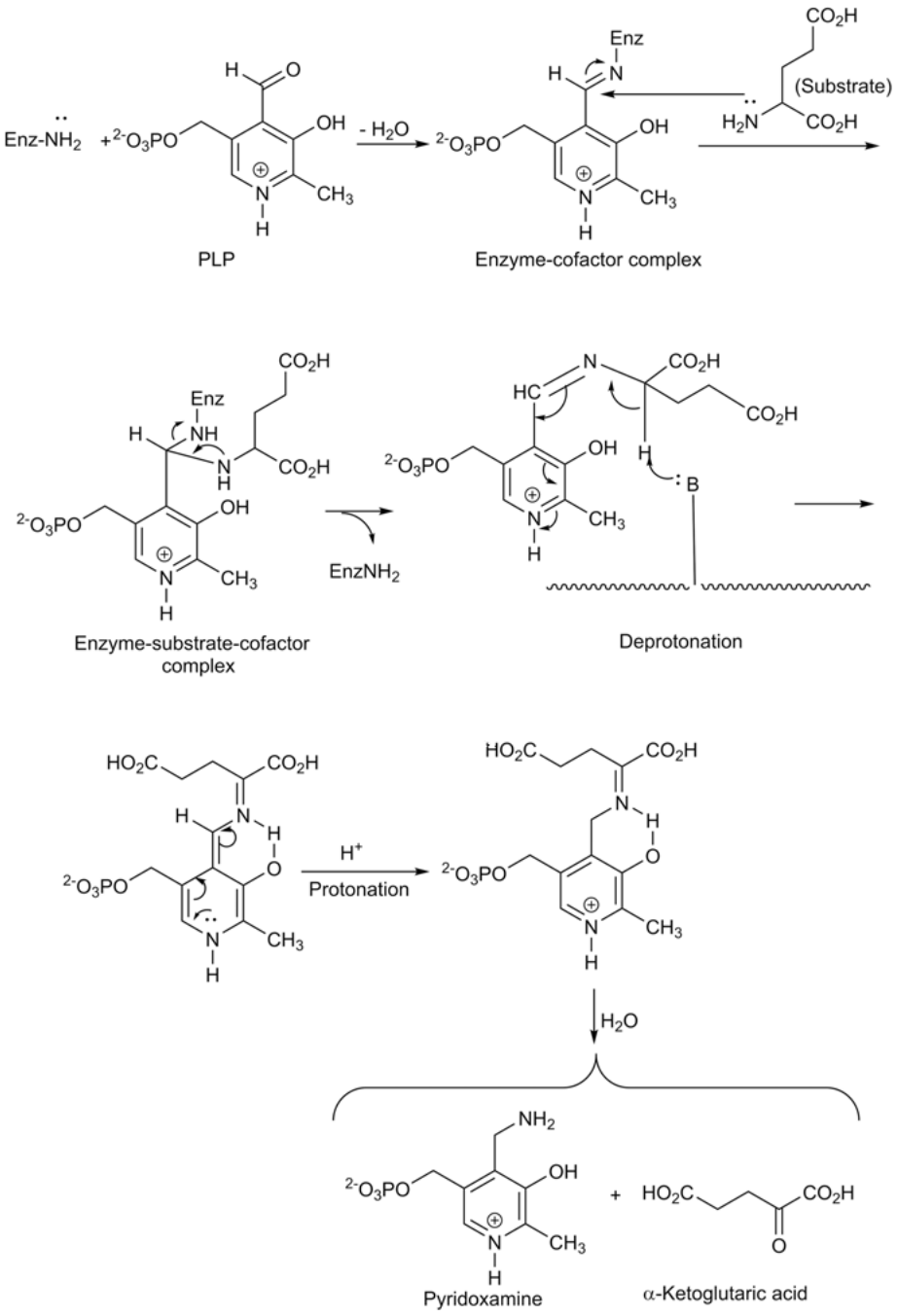
A key enzyme in the biosynthesis and metabolism of GABA is GABA-aminotransaminase (GABA-AT). This enzyme catalyzes both the oxidative deamination of GABA itself to succinic semialdehyde and the conversion of 2-oxoglutaric acid to glutaric acid, an immediate precursor of GABA through the enzyme GAD (Scheme 5.1). Given the key role in these processes, GABA-AT was considered the ideal enzyme to carry out the indirect regulation of GABA levels in the CNS.

Seizures occur due to the imbalance of the two most important neurotransmitters in the brain, namely L-glutamic acid, an excitatory amino acid, and GABA, an inhibitory neurotransmitter. The concentration of GABA is regulated by GABA aminotransferase (GABA-AT), which degrades GABA to succinic semialdehyde with the regeneration of glutamic acid (see the bottom and the top of Scheme 5.1). Although succinic semialdehyde is toxic to cells, there is no accumulation of this metabolite because it is efficiently oxidized to succinic acid by the semialdehyde succinic dehydrogenase (SSADH). When the concentration of GABA decreases below a certain threshold in the brain, seizures begin. If convulsions are induced in an animal and GABA is injected directly into the brain, the seizures cease. Therefore, GABA could be considered as the ideal anticonvulsant. However peripheral administration of GABA does not produce any anticonvulsant effect, since GABA cannot cross the BBB. Another approach to increase the concentration of GABA in the brain would be to design a compound capable of traversing the BBB and to inactivate GABA-AT, an enzyme that catalyzes the degradation of GABA. If glutamic amino decarboxylase (GAD) is not inhibited, the concentration of GABA should increase. In fact, this approach has been shown to be effective for the design of anticonvulsant drugs. Compounds that cross the BBB and inhibit GABA-AT in vitro have been shown to increase the GABA levels in the brain in vivo, and thus exhibit anticonvulsive activity. To understand Scheme 5.1, the mechanism of the amino group transfer reactions depending on the pyridoxal phosphate (Scheme 5.2) should be known.

5.5 Enzymic inhibitors that have pyridoxal phosphate as cofactor

Enzymes that require metal ions are called metalloenzymes. Pyridoxal phosphate (PLP, **P**yridoxa**L** 5'-**P**hosphate or vitamin B_6) is a cofactor of countless enzyme-catalyzed reactions that are involved in amino acid metabolism. All the transformations in which PLP is involved start with the formation of an aldimine by reacting a lysine residue in the enzyme with the formyl group at the 4-position of the cofactor. This derivative will serve as a reagent in metabolic reactions of transamination, decarboxylation and racemization among others, in which amino acids intervene as substrates. The condensation of an amino acid with the aforementioned aldimine gives rise to a new aldimine, or Schiff base, which is hydrolized. Scheme 5.3 depicts the transamination reaction catalyzed by glutamate transaminase that produces α-oxoglutaric acid.

For the transformations to take place, the complex-cofactor must have free rotation around the N-C_α enlace bond. In this case, the C_α-H bond is arranged orthogonally to the plane of the pyridinium ring, with the enzyme fixing such a conformation. In this arrangement, the maximum overlap occurs between the negative charge being developed by abstraction of the proton at that position and the electron deficient conjugate



Scheme 5.2: Transamination reaction catalyzed by glutamate transaminase, and PLP as cofactor.

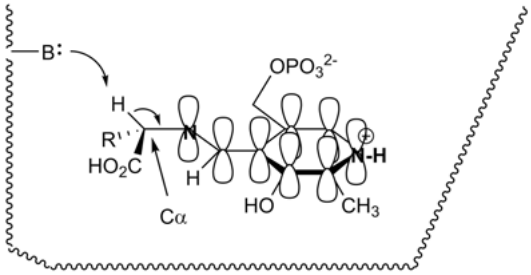
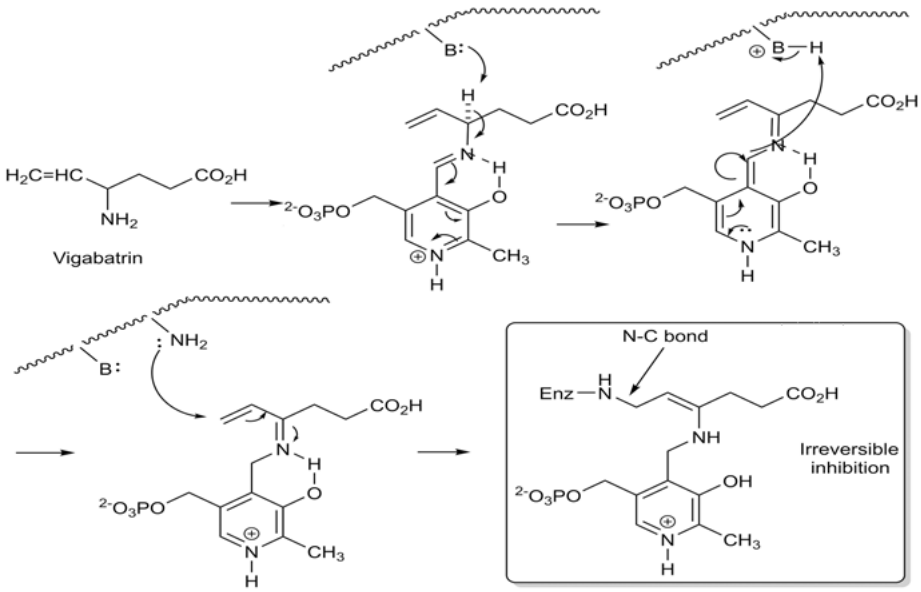


Fig. 5.2: Geometry required for deprotonation.

system (Fig. 5.2). Subsequent protonation produces ketamine (instead of aldimine), which upon hydrolysis leads to an α-oxoacid.

In general, PLP-dependent enzymes are inhibited by compounds similar to their natural substrates, with functional groups capable of attacking the formyl group at the 4-position of the cofactor. Many investigations have focused on the design of suicide inhibitors. Among the multiple inhibitors of GABA-AT, vigabatrin [(S) -γ-vinyl-GABA] is a suicide inhibitor whose mechanism of action is depicted in Scheme 5.3. Inhibitors with unsaturations are activated to α,β-unsaturated imines, which are subsequently covalently linked to the enzyme through a conjugated nucleophilic addition. Vigabatrin is currently used as an antiepileptic drug.



Scheme 5.3: Inhibition mechanism of glutamate transaminase by an unsaturated GABA analogue: Vigabatrin.

GABA levels may be increased indirectly by inhibition of semialdehyde succinic dehydrogenase. This is the basis of some classical antiepileptic drugs, such as sodium valproate (Scheme 5.1 and Fig. 5.3).

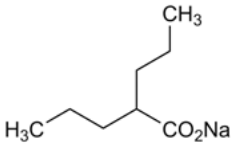
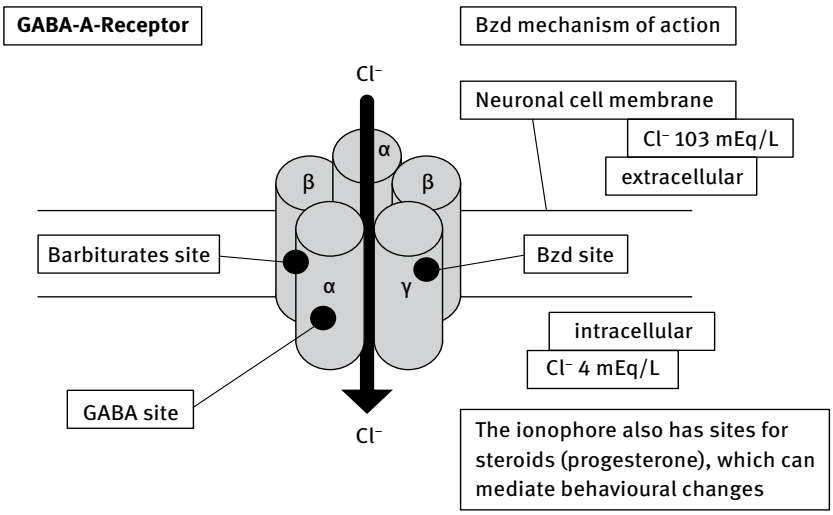


Fig. 5.3: Sodium valproate.

5.6 Postsinaptic modulators

The postsynaptic receptor of GABA ($GABA_A$) consists of several membrane proteins cooperating allosterically. Their purpose is the regulation of a channel that is selective for chloride ions (Fig. 5.4). Under resting conditions, the binding site of GABA is blocked by an accessory protein, GABA-modulin, which is displaced by an endogenous ligand to allow the interaction of GABA with its receptor.



GABA-A-Receptor coupled to Cl^- ionophore is pentameric with subunits α α β β γ
GABA binds to subunits α → conformational change → open Cl^- channel → hyperpolarization
Bzd probably bind γ subunit facilitating GAGA binding and ↑ Cl^- channel opening frequency
Barbiturates: ↑ duration Cl^- channel opening with or without GABA and ↑ Cl^- flow

Fig. 5.4: Model of the $GABA_A$ receptor complex associated with a chloride channel.

Benzodiazepines (Bzds) act by interaction with allosteric zones close to the GABA receptor, resulting in the dissociation of GABA-modulin from its binding site. Barbiturates act in a similar way, albeit in a different receptor zone. These families can be considered as GABA "coagonists" or stabilizers of the "open channel" (Fig. 5.5).

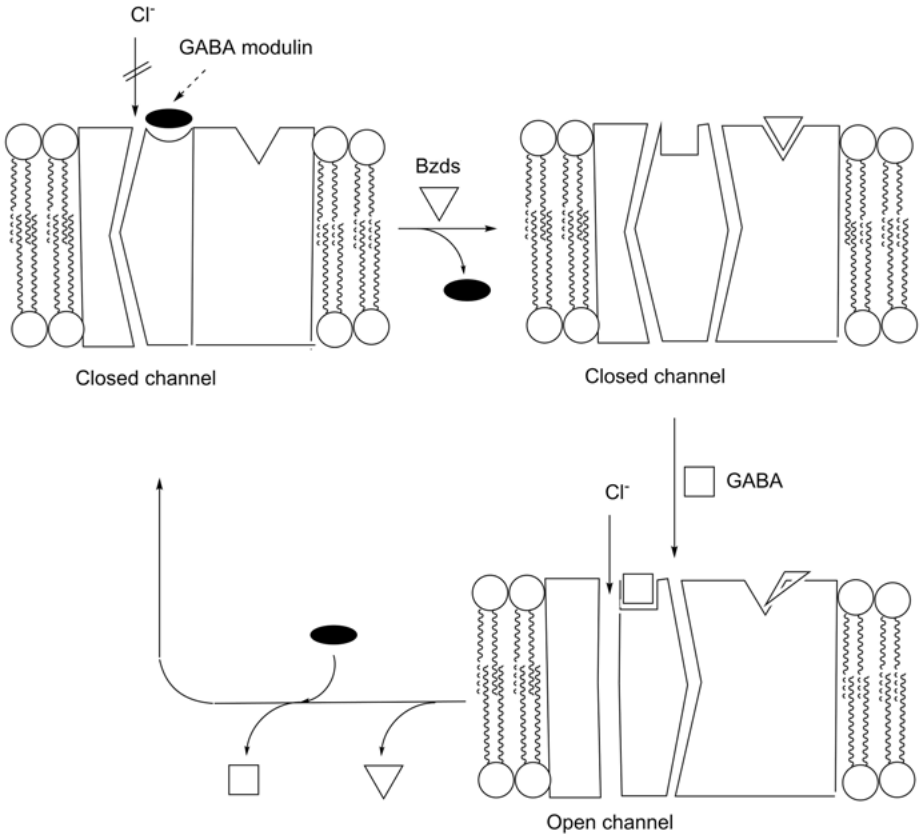
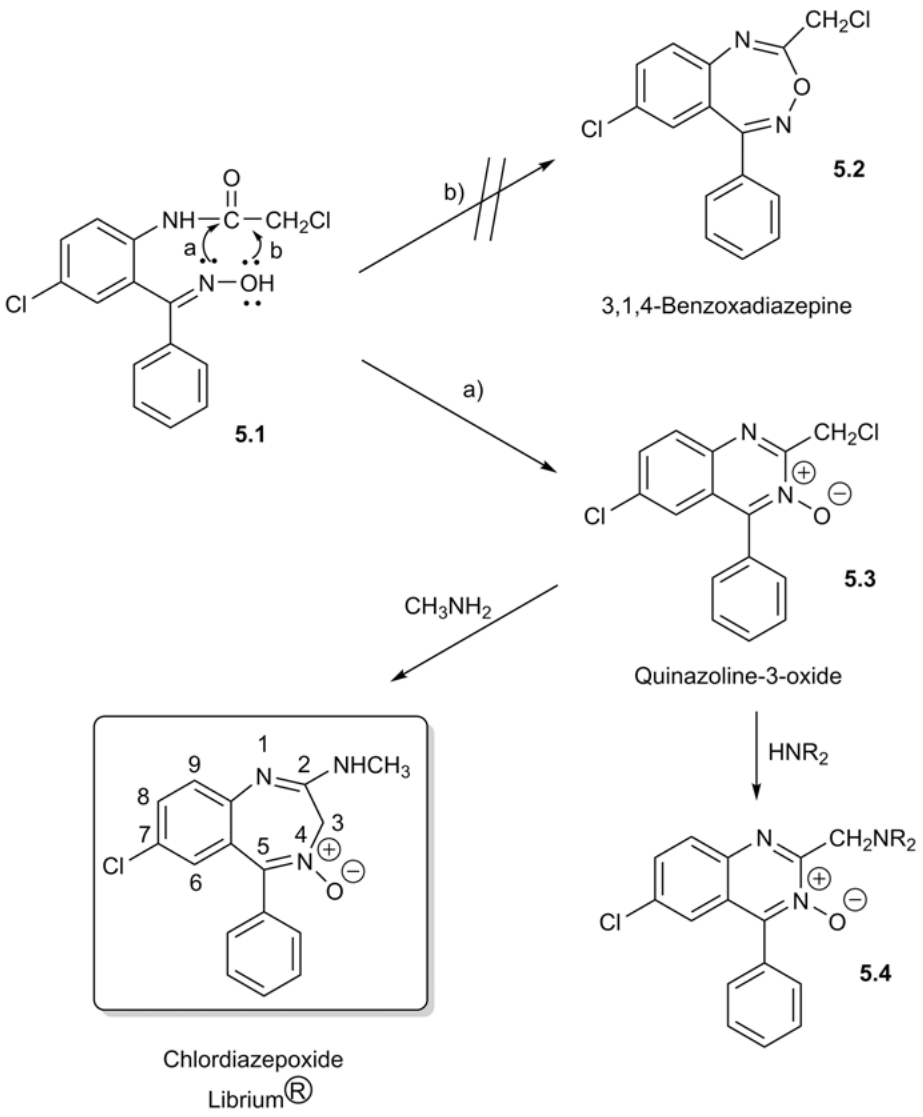


Fig. 5.5: Allosteric interaction of Bzds with the GABA receptor.

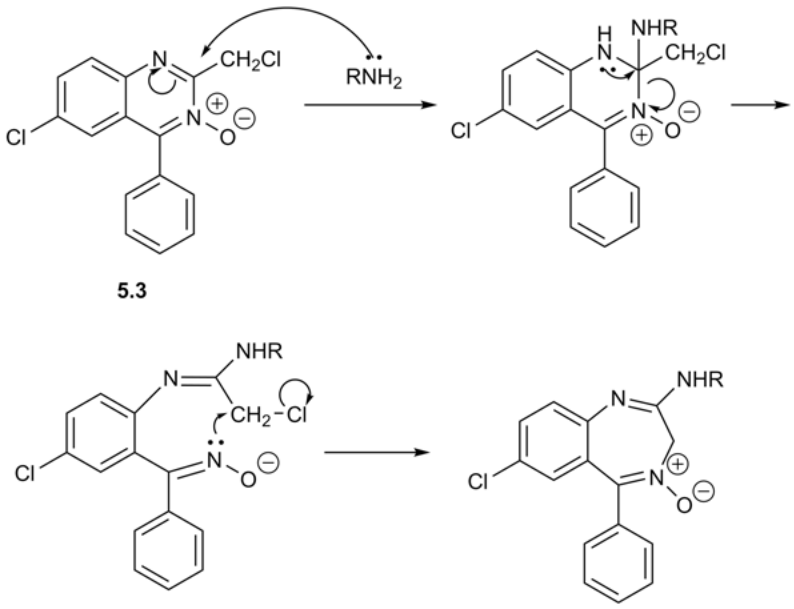
5.6.1 Benzodiazepines

The discovery of the Bzds is an example of the successful discovery of new drugs from a random pharmacological test of new synthetic compounds. By 1930–1940, Sternbach carried out postdoctoral work on the synthesis of heterocyclic systems to which the structure of 3,1,4-benzoxadiazepine (**5.2**) was initially attributed. Compound **5.2** should have been obtained from a reaction between the oxime of a benzophenone and chloroacetyl chloride (**5.1**, Scheme 5.4).



Scheme 5.4: Development of benzodiazepines (Bzds).

Twenty years later, Sternbach discovered that these compounds had a six-membered ring rather than a seven-membered heterocycle. They are, in fact, quinazoline-3-oxides with a chloromethyl function at 2-position (**5.3**, Scheme 5.4). These halides were then converted to aminomethylquinazoline-3-oxides by treatment with various secondary amines (e.g., dimethylamine). Such compounds gave negative results in



Scheme 5.5: Mechanism of the rearrangement leading to 1,4-benzodiazepines.

the general pharmacological test, and, therefore, investigations in this field were abandoned.

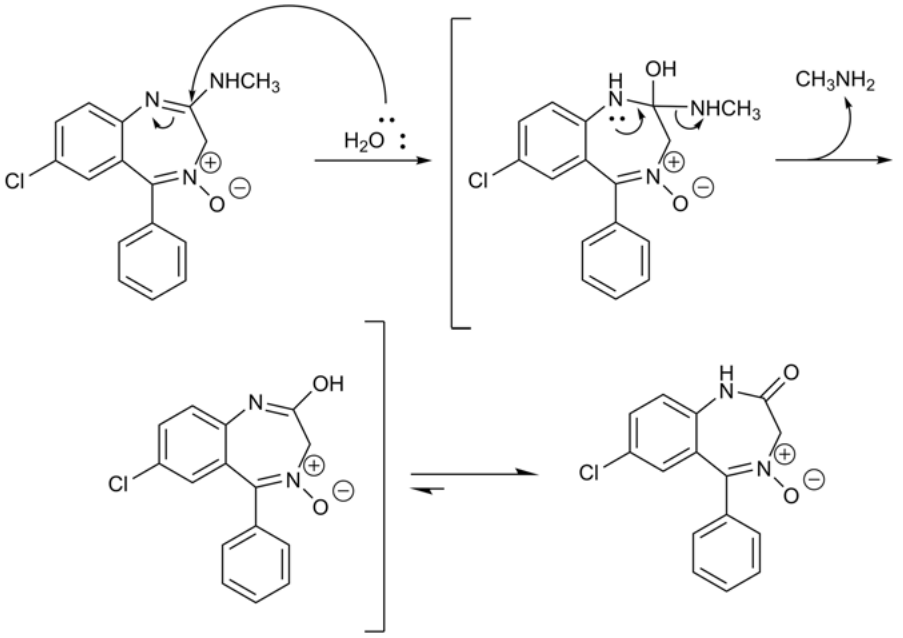
However, one of the members of the series was prepared by reaction with methylamine, a primary amine. After a time, in 1957, before finally abandoning it, this compound also underwent pharmacological evaluation, of its potent hypnotic-sedative effect. A careful study revealed a new mistake in its structural assignment, since the compound was not a quinazoline *N*-oxide, but a 1,4-benzodiazepin-4-oxide. In this way and after resolving the misunderstanding, the sedative and hypnotic drug chlordiazepoxide was discovered and became a prototype for an extraordinarily wide range of analogues.

5.6.1.1 Mechanism of the rearrangement reaction to 1,4-benzodiazepines

Scheme 5.5 shows the rearrangement process that takes place.

5.6.1.2 Mechanism of metabolic hydrolysis of chlordiazepoxide (Librium®, 1960)

It was later found that the chlordiazepoxide is metabolically converted to a 2-diazepinone (Scheme 5.6), with loss of the amine at that position. These lactams are of equal or greater potency and in addition, they are synthesized more easily, which is the reason why they have acquired a great therapeutic importance.



Scheme 5.6: Chlordiazepoxide metabolic hydrolysis.

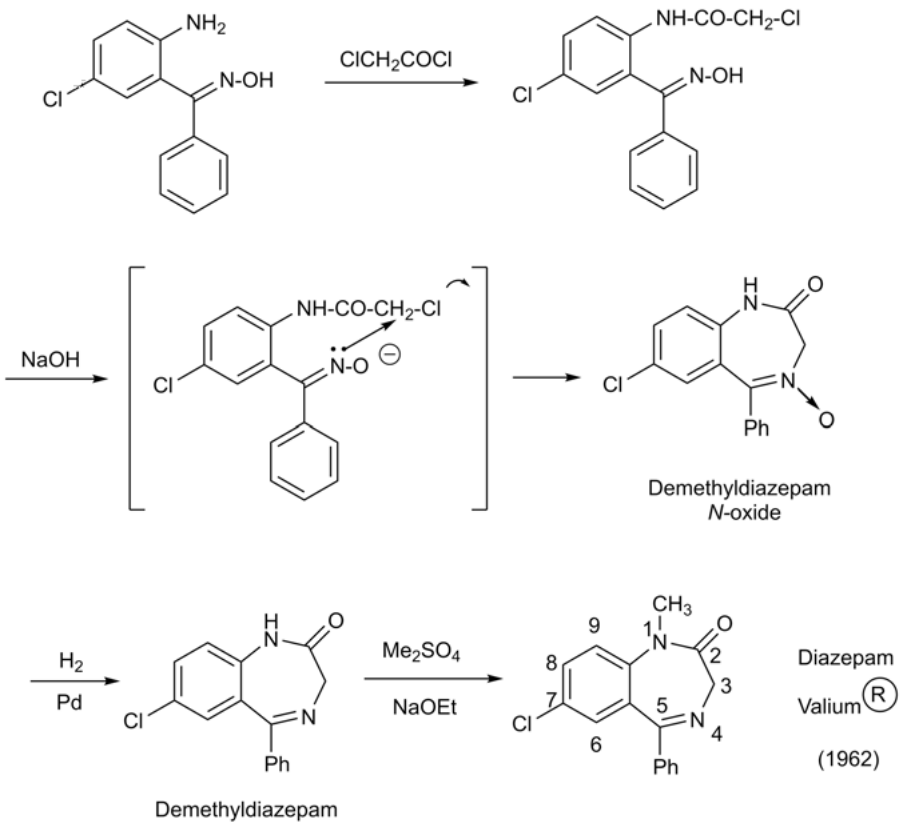
It was soon observed that the product of hydrolysis at carbon 2 has an activity equivalent to that of chlordiazepoxide, and that the *N*-oxide group was not essential, giving rise to benzodiazepines of the second generation.

5.6.1.3 Second-generation benzodiazepines [diazepam (Valium®)]

Diazepam, 7-chloro-5-phenyl-1-methyl-1,3-dihydro-1,4-benzodiazepin-2-one, was the first of the second generation of benzodiazepines (1,4-benzodiazepin-2-ones) and prototype of a large number of them (see Scheme 5.7 for its synthesis).

One of the metabolites of diazepam is oxazepam, a compound hydroxylated at position 3. It can also be used as an anxiolytic. It is obtained from the desmethyldiazepam *N*-oxide, by the Polonovski rearrangement (Scheme 5.8).

Not all the useful 1,4-benzodiazepine-2-ones have the NCH_3 group at the 1-position, or a 5-unsubstituted phenyl. Modifications at *N*-1 and on the 5-Ph group may allow a certain variation in action, always within the range of CNS depressant effects. For example, flurazepam is used more as an anticonvulsant and hypnotic than as an anxiety drug (see Scheme 5.9 for its synthesis).



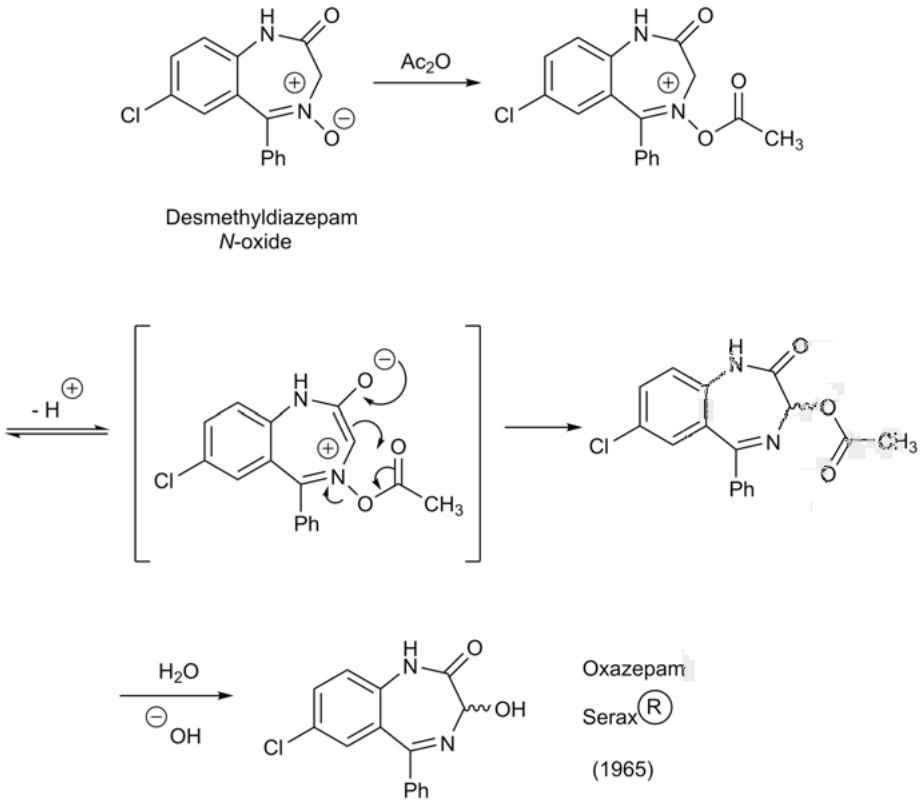
Scheme 5.7: Diazepam synthesis.

5.6.1.4 Structure-activity relationships

Thousands of benzodiazepine analogues, which have been synthesized and tested can be initially divided into two major groups: (a) 1,4-benzodiazepine-4-oxides (the only clinical drug: chlordiazepoxide, Librium®), and (b) 1,4-benzodiazepine-2-ones (diazepam, etc.). The most significant structure-activity relationships are as follows:

A-Ring

The presence of an electron-withdrawing substituent at 7-position is necessary. In the examples cited was a chlorine, but -NO_2, -CF_3, Br, -CN, etc. are also suitable. Replacement at 6, 8 or 9 positions reduces potency. One example is nitrazepam, a drug used primarily as a sedative-hypnotic (Fig. 5.6).



Scheme 5.8: Oxazepam synthesis.

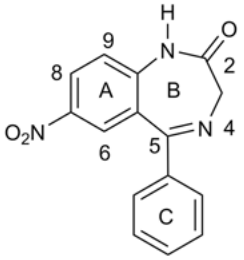
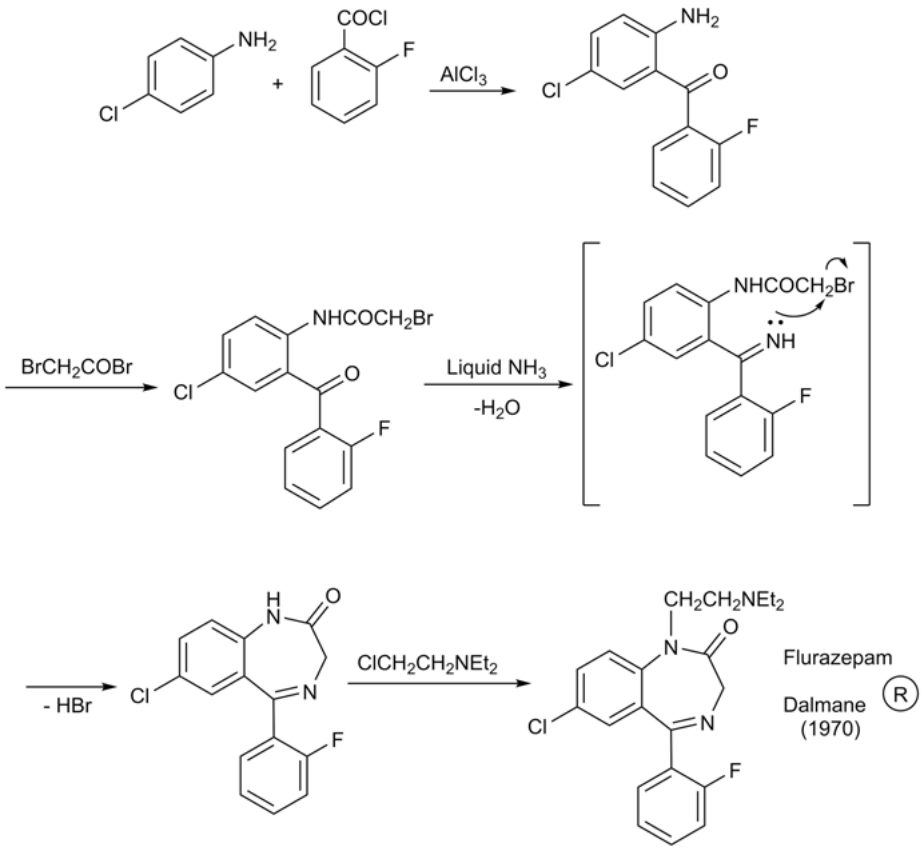


Fig. 5.6: Nitrazepam (1,3-dihydro-5-phenyl-7-nitro-1,4-benzodiazepine-2-one, Mogadon®)

B-Ring

The presence of a CH_3 group in *N*1 usually increases potency (e.g., diazepam). If substituents are bulkier, the activity is reduced. However, groups that can be removed by metabolyzation as $-CH_2-CH_2-NEt_2$ in flurazepam are acceptable.

The presence of an OH at the 3-position (oxazepam) reduces potency, but favors the anxiolytic action against undesirable side effects, such as fatigue and physical



Scheme 5.9: Flurazepam synthesis.

dependence. Furthermore, the drug is eliminated more rapidly. The esters and carbamates of this 3-OH are useful prodrugs. At position 3 a carboxylate group (clorazepate dipotassium, Tranxilium®) may also be introduced (Fig. 5.7).

C-Ring

At the 5-position of the benzodiazepines, a phenyl or aryl substituent must be found for optimum activity. The alkyl, cycloalkyl or heterocyclic groups usually give rise to less potent drugs, although there are exceptions such as bromazepam, a benzodiazepine in which a 2-pyridyl residue in 5 is found (Fig. 5.8).

Substitution in the 5-phenyl group for electron-withdrawing groups in *ortho* (F, Cl), or disubstitution in *ortho-ortho*', increases the activity (flurazepam). The presence of groups in *meta* or *para* invariably reduces potency.

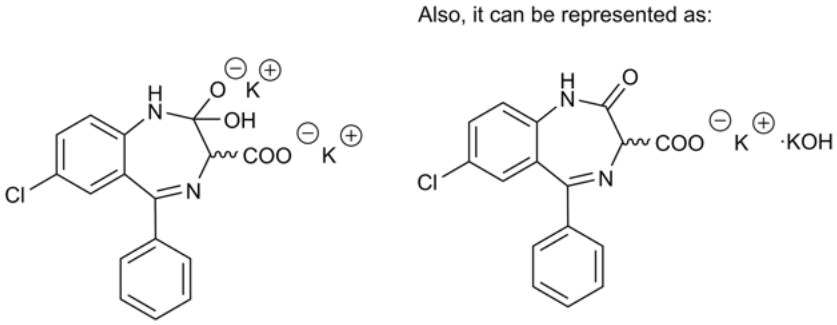


Fig. 5.7: Clorazepate, also known as clorazepate dipotassium (Tranxilium®, 1972).

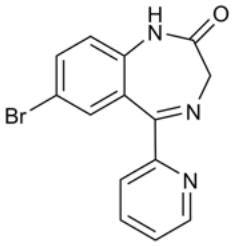
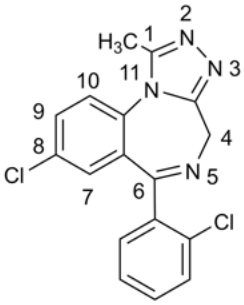


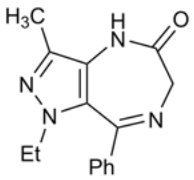
Fig. 5.8: Bromazepam (ansiolitic).

Other rings (fusion of new rings)

In recent years, research in the field of benzodiazepines has been directed mainly at the fusion of new rings to the basic system, as well as the substitution of ring A by nuclei of heterocyclic nature. Triazolam and ripazepam are examples of these modifications (Fig. 5.9).



Triazolam
8-Chloro-6-(*o*-chlorophenyl)-1-methyl-
4*H*-[1,2,4]-triazolo[4,3-*a*]-1,4-benzodiazepine



Ripazepam
(pyrazolodiazepine)

Fig. 5.9: Triazolam and ripazepam.

5.6.2 Fixation of steroids to $GABA_A$

It has been observed that many steroids, such as progesterone (Fig. 5.10) and some glucocorticoids produce actions in the CNS through their interaction with a binding site located at the $GABA_A$ receptor, which is not the same as that of barbiturates, but producing very similar effects.

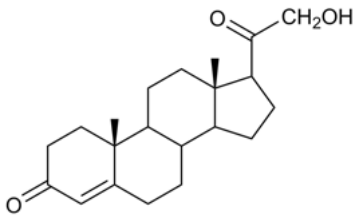


Fig. 5.10: Progesterone.

5.6.3 Other drugs related to benzodiazepines

Zopiclone and zolpidem (Fig. 5.11) are two drugs that act as agonists on the Bzd receptors. They are anxiolytic although, unlike Bzds, present more pronounced hypnotic and sedative effects.

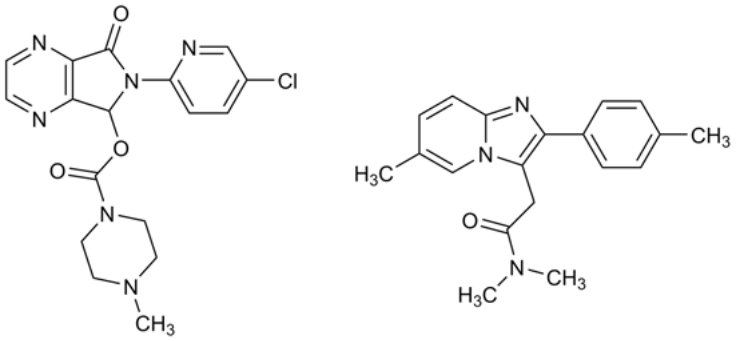
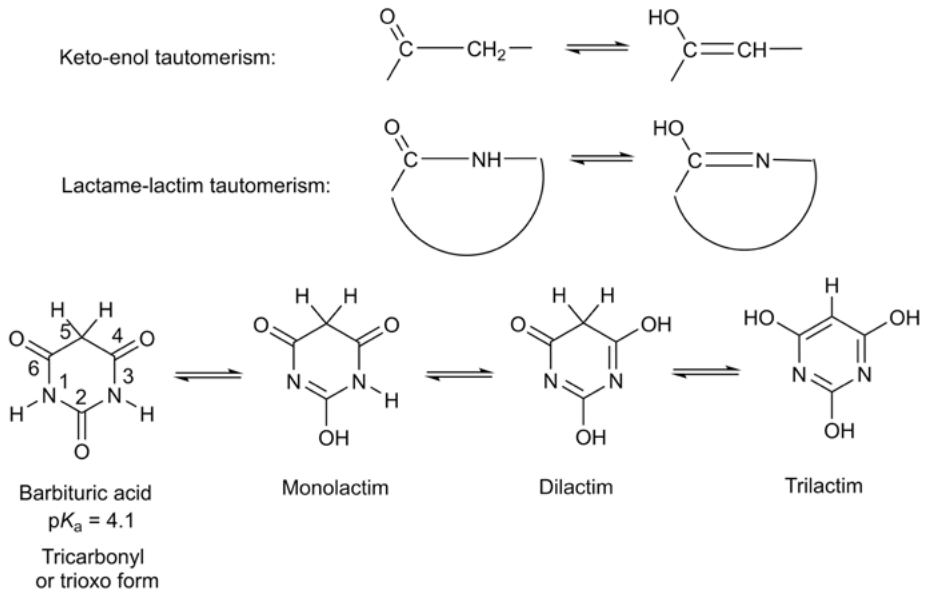


Fig. 5.11: Agonists of the Bzd receptor.

5.6.4 Barbituric acids (or barbiturates)

Although they are still in use, the addiction and toxicity problems associated with barbiturates suggest their gradual substitution with benzodiazepines. Although the activity of barbituric acids was initially considered to be related only to their physico-



Scheme 5.10: Tautomeric equilibrium in 5,5-disubstituted derivatives.

chemical properties (especially lipophilicity), they are now known to act by stabilizing the open form of the chloride channel in the vicinity of the allosteric receptor of GABA, analogously to benzodiazepines.

Barbiturates are derivatives of 2,4,6-trihydroxypyrimidine, for which we can consider the tautomeric equilibrium shown in Scheme 5.10.

The barbituric acid itself lacks hypnotic properties due to its high acidity ($pK_a = 4.1$), which causes it to be totally ionized at physiological pH, so it will not pass the BBB. Also fully alkylated derivatives at positions 1,3,5,5 are lacking in hypnotic effects. Therefore, the sedative action requires the presence of lipophilic substituents at the 5-position of the ring (5,5-disubstituted derivatives) and moreover, the existence of at least one H that allows tautomerism and ionization. In the 5,5-disubstituted and 1,5,5-trisubstituted derivatives, the tautomer with the trilactim structure is not possible so the acidity is lower ($pK_a = 7.6 - 8.5$, Scheme 5.10), and at the same time, depending on the nature of the substituents they may present the ideal lipophilic characteristics suitable for their pharmacological activity (Fig. 5.12).

Sanberg enunciated a fundamental postulate: for a barbiturate to have good hypnotic-sedative activity, it must be a weak acid and have a lipid/water partition coefficient between certain limits ($\log P_0 \approx 2$). This fact agrees with the need for a double lipophilic-hydrophilic character. Consequently, for a barbiturate to be active it must meet the following requirements:

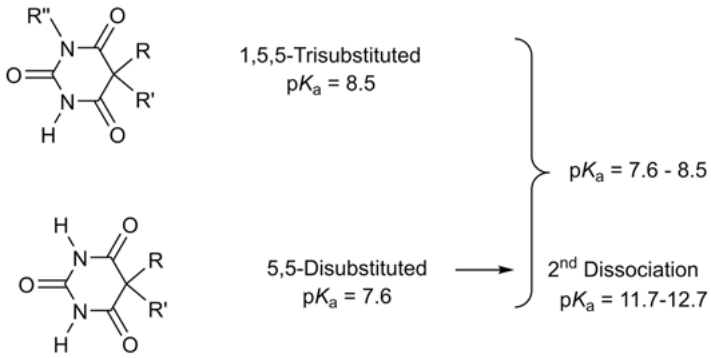
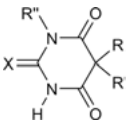


Fig. 5.12: Active derivatives of barbituric acid.

1. It must be disubstituted at the position 5,5.
2. For each substituent, each of which must independently have at most 6 C → R, R ' ≤ 6 C.
3. The sum of the substituents must not be greater than 10 → R + R '≤ 10 C.

Depending on the nature of the lipophilic groups, the effect of barbituric acids may vary and even be accompanied by an anticonvulsive effect. The intensity effect can reach the degree of general anesthesia, when the dose is sufficiently high. However, these types of compounds should be treated with caution, as the dose increases the respiratory depression they cause. Table 5.1 shows the most frequent barbiturates, classified according to the duration of the effect (See Volume 1, Chapter 8 for some representative syntheses).

Table 5.1: Classification of barbiturates according to their structures and effects.

		R	R′	R″	X
	*Prolonged effect*				
	Phenobarbital	Et	Ph	H	O
	Metarbital	Et	Et	CH_3	O
	Intermediate effect				
	Pentobarbital	-CHPr CH_3	Et	H	O
	Ultra short effect				
	Hexobarbital		CH_3	CH_3	O
	Thiopental	-CHPr CH_3	Et	H	S

Substitution of the carbonyl group at the 2-position by a thiocarbonyl group leads to the thiobarbituric acids, which are characterized by their ultra-short effect. They are

usually used as anesthetics intravenously for this reason. Long-acting barbiturates are used in the treatment of epilepsy and the preservation of the sedative character during the day, in states of anxiety and tension. Those of intermediate and short effects are used as hypnotics and sedatives in insomnia and for pre-anesthesia. Barbiturates have an effect on basal anesthesia. See Chapter 8 of Volume 1 for some characteristic syntheses.

Their action is attributed to the level of thalamus and ascending reticular formation that interfere with the transmission of the nerve impulses towards the cortex.

6 Peptides as neurotransmitters: Hypnoanalgesics

6.1 Goals

- To introduce the reader to the need for pure analgesic drugs.
- Morphine as a prototype of new analgesics.
- To introduce the reader to the research in this field, and to the future development of μ-antagonists, κ-agonists that do not have activity against the σ-receptor.

6.2 Morphine

The active principle of opium is morphine (Fig. 6.1) and this compound is still one of the most effective analgesics available in medicine. Morphine is especially suited to the treatment of constant, dull pain rather than periodic and sharp pain.

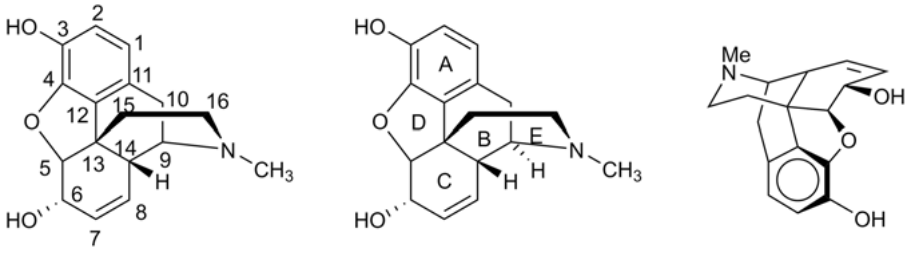


Fig. 6.1: Morphine.

It acts on the brain and seems to raise the threshold for pain, decreasing the awareness of pain in the brain. It has many side effects, such as:

- depression of the respiratory center
- excitement
- euphoria
- nausea
- tolerance
- dependence

One of the salient side effects of morphine use is the physical or psychological dependence on drug symptoms and the ensuing withdrawal symptoms when drug consumption is interrupted. Tolerance occurs when a person needs to take a drug in ever higher doses to achieve the same desired effect, such as euphoria or pain relief, than before.

https://doi.org/10.1515/9783110528527-007

Morphine possesses five stereogenic centers of configurations 5*R*, 6*S*, 9*R*, 13*S* and 14*R*. The molecule contains five rings (called A–E) and has a T-shape. It is basic because of the tertiary amino group, but also contains a phenolic group, an alcoholic group, an aromatic ring, an ethereal bridge and a double bond (Fig. 6.1).

6.2.1 SARs

6.2.1.1 The phenolic group

Codeine (Fig. 6.2) is used for the treatment of moderate pain and coughs. When methylating the phenolic group, analgesic activity drops drastically (0.1% of that of morphine, on isolated receptors).

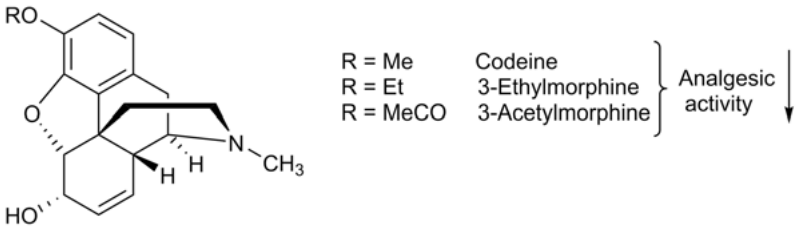


Fig. 6.2: Decrease of the analgesic activity as the size of the R group increases at position 3.

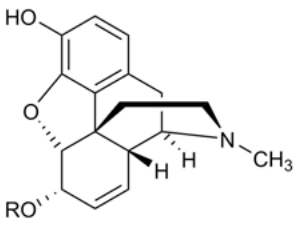
However, the analgesic effect of codeine when administered to patients is 20% of that of morphine: codeine can be metabolized in the liver to yield morphine (it is a prodrug).

6.2.1.2 Alcohol at 6-position

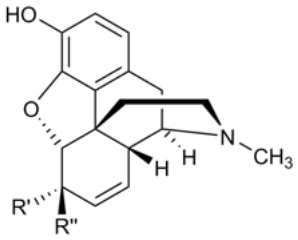
The results displayed in Fig. 6.3 show that neither masking nor the complete loss of the alcohol group decrease analgesic activity and in fact exert the opposite effect.

It is interesting to compare the activities of morphine, 6-acetylmorphine and heroin (Fig. 6.4). The most active compound of the three (and the most dangerous) is 6-acetylmorphine, which is four times more active than morphine. Heroin is twice as active as morphine, but less active than 6-acetylmorphin. Let us try to explain these facts.

6-Acetylmorphine is less polar than morphine and will enter the brain more easily than morphine. The phenolic group is free and will interact immediately with the analgesic receptors. Heroin is the least polar and will be the most effective of the three to cross the BBB. However, before it can act, the acetyl group of the phenolic group has to be eliminated by esterases in the brain.



R		Analgesia against that of morphine
Me	Heterocodein	5x
Et	6-Ethylmorfine	Higher
MeCO	6-Acetylmorphine	4x



R'	R"	Analgesia against that of morphine
H	OH	Augmented
H	H	or
Ketone		similar

Fig. 6.3: Effect of the loss of the alcoholic group on analgesic activity.

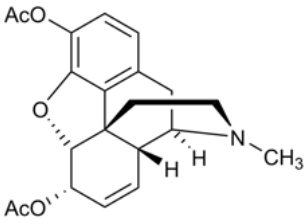


Fig. 6.4: Heroin.

In short, the 6-hydroxyl group is not required for analgesic activity and its elimination may be beneficial for such activity.

6.2.1.3 The 7-8 double bond

Several analogues, including dihydromorphine (Fig. 6.5), demonstrate that the double bond is not necessary for analgesic activity.

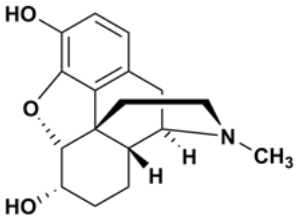
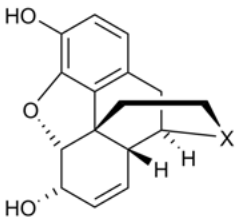


Fig. 6.5: Dihydromorphine.

6.2.1.4 The *N*-methyl group

The *N*-oxide and the quaternary salts of morphine are inactive, from which it could be inferred that the introduction of charge destroys the analgesic activity (Fig. 6.6). However, these results refer to in vivo assays. If injected directly into the brain, these compounds exhibit an activity similar to that of morphine. This, coupled with the fact that these compounds cannot lose their charge, shows that the nitrogen of the morphine is ionized when bound to the receptor.



X		Analgesic activity
NH	Normorphine	25%
	*N*-Oxide	0%
	Ammonium salt	0%

Fig. 6.6: Effect of introducing a charge on analgesic activity.

Substitution of the *N*-methyl group for an NH reduces the activity, but does not eliminate it: the *N*Me group is not essential for activity. However, the nitrogen atom is crucial. If eliminated, all analgesic activity would be lost.

6.2.1.5 E-ring and the ethereal bridge

They are not essential.

6.2.1.6 Stereochemistry

Morphine is a chiral molecule containing several stereocenters, and exists as a single enantiomer. When morphine was first synthesized, a racemic mixture of the enantiomer that exists in nature was obtained, in addition to its specular image. The racemic was resolved and the unnatural enantiomer was tested as an analgesic. This compound was totally inactive. This is not surprising when analyzing the interactions that take place between morphine and its receptor These interactions involve the phenolic group, the aromatic ring and the amine of morphine. Consider a schematic representation of the T-shape of morphine, the three important groups standing out (Fig. 6.7). The receptor has complementary binding groups, placed in such a way that they can interact with the three important groups of morphine. On the contrary,

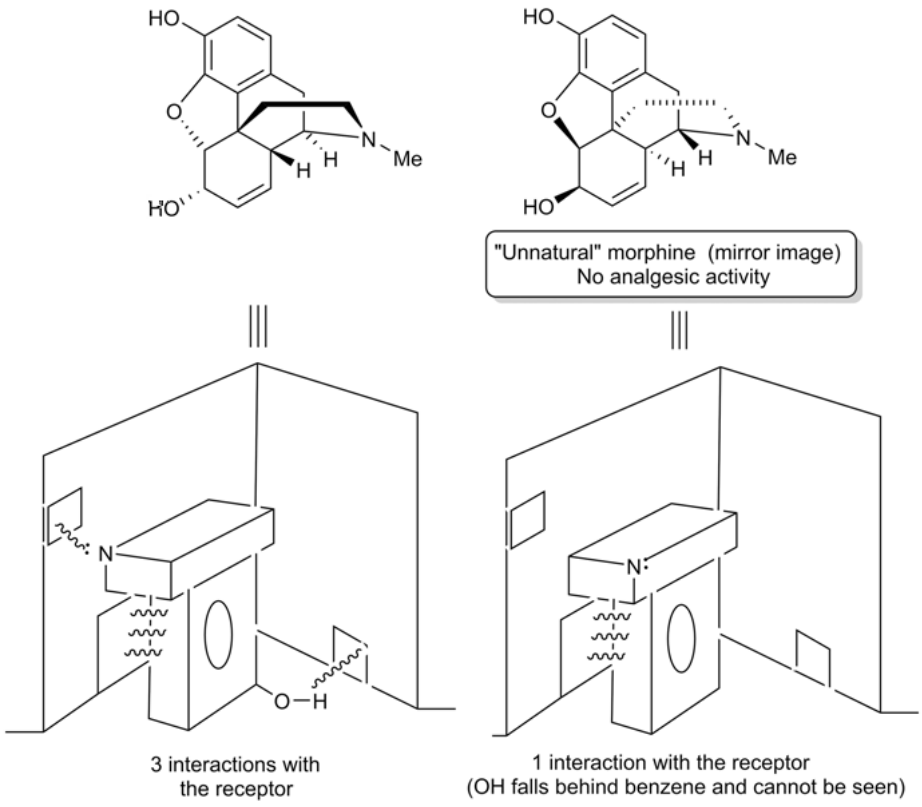


Fig. 6.7: Morphine and "non-natural" morphine.

if we consider the mirror image of natural morphine, only one interaction can be established (corresponding to the aromatic ring A, Fig. 6.7).

Epimerization of a single stereocenter such as that at position 14, is not beneficial, as it results in a drastic change in the shape of morphine (Fig. 6.8), making it impossible for the molecule to bind to analgesic receptors.

To summarize, the groups important for analgesic activity are shown in Fig. 6.9.

6.2.2 Development of morphine analogues: Strategies

The following strategies have been particularly useful in the development of morphine analogues:

- variation of substituents and elongation (or extent) of the drug
- simplification
- increase of rigidity

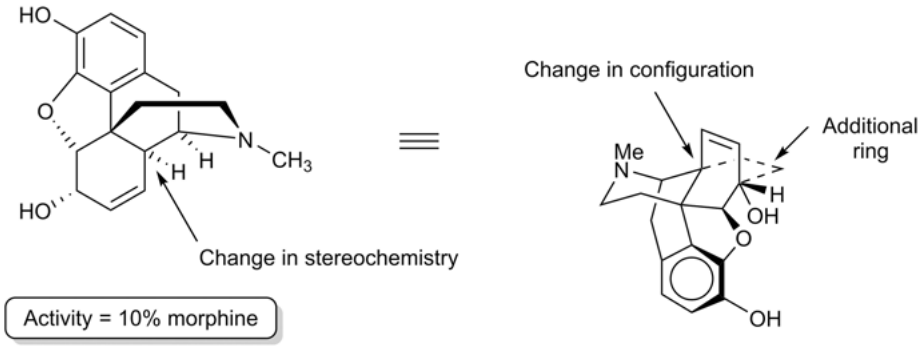


Fig. 6.8: Carbon epimerization at position 14.

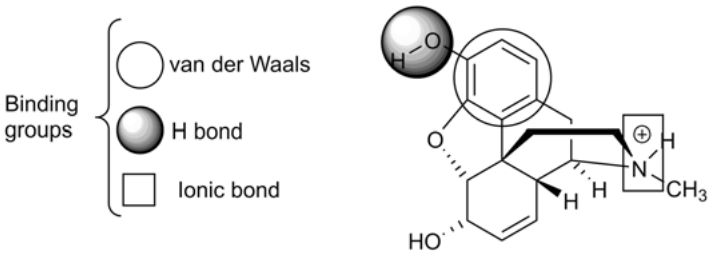


Fig. 6.9: Groups important for the analgesic activity of morphine.

6.2.2.1 Extension of the drug: addition of "extra" binding groups (Fig. 6.10)

Introduction of a hydroxyl group at the 14-position has been particularly useful. It could suggest that there is a possible interaction between the 14-OH group and an amino acid of the receptor (example: oxymorphone or 14-hydroxydihydromorphinone, Fig. 6.11).

Variation of substituents and elongation (or extension) of the drug

The easiest and most advantageous position to add substituents is the nitrogen atom. The synthesis is carried out by removing the *N*-methyl group from morphine to yield the normorphine and by alkylating the secondary amino group with an alkyl halide (Scheme 6.1).

If the alkyl group increases in size from Me to Bu, the activity drops to zero. With a larger group such as amyl or hexyl, the activity recovers slightly. However, when a phenethyl group is attached, the activity increases 14-fold (a strong indication that a hydrophobic binding site has been located that interacts favorably with the new aromatic ring).

Fig. 6.12 indicates the change in activity with the size of the alkyl group.

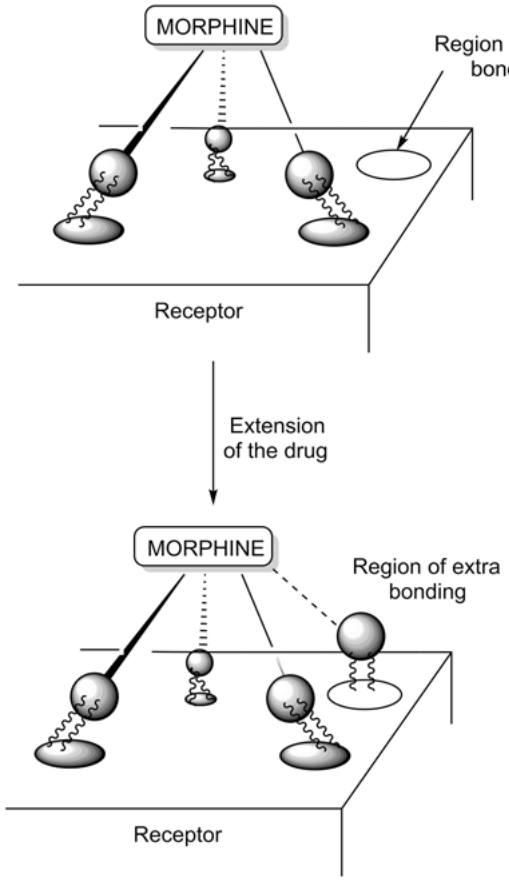


Fig. 6.10: Extension or elongation of morphine.

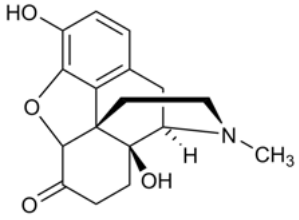
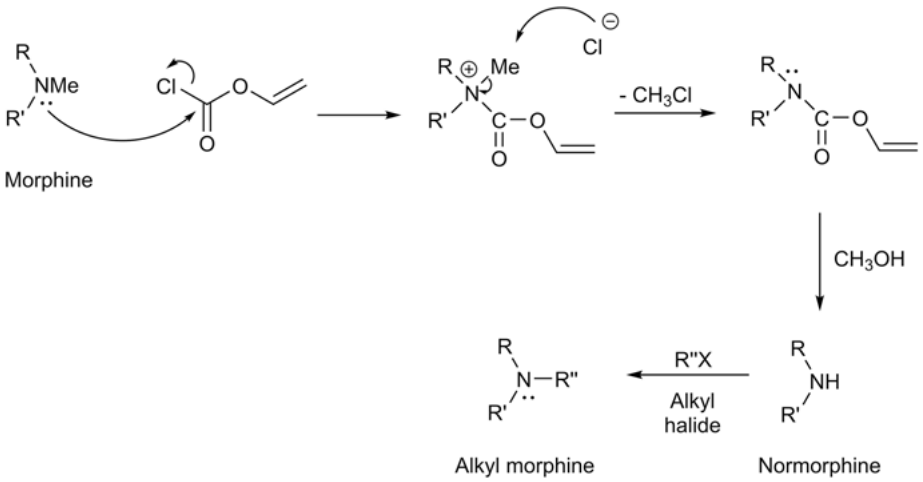


Fig. 6.11: Oxymorphone (5 × the activity of morphine).

Spectacular results were obtained when an allyl or cyclopropylmethylenic group was attached to the nitrogen atom (Fig. 6.13).

Naloxone, for example, has no analgesic activity, whereas nalorphine has weak analgesic activity. The most important feature is that they act as morphine antagonists. The fact that they block morphine from accessing their receptors implies that they do not produce any of the side effects of morphine and it is the blocking of these effects that makes these antagonists extremely useful.



Scheme 6.1: Desmethylation and alkylation of the basic center of morphine.

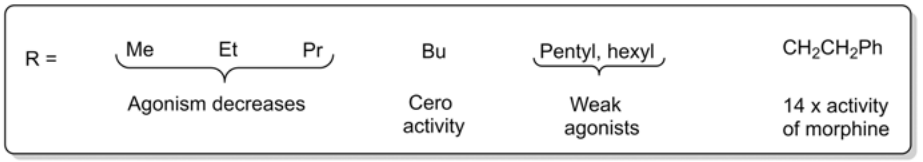


Fig. 6.12: Change in activity with respect to the size of the alkyl group.

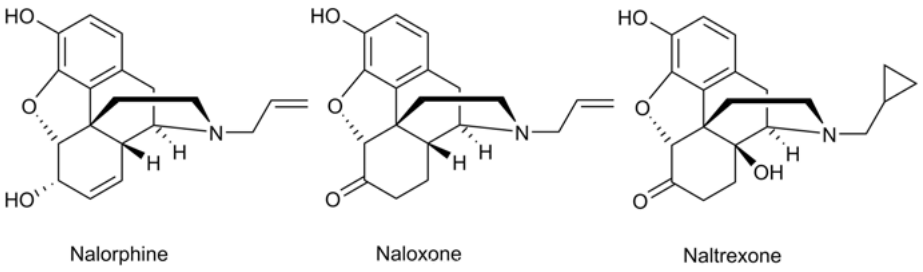


Fig. 6.13: Morphine antagonists.

Nalorphine is a strong antagonist and blocks morphine from its receptors. Therefore, analgesic activity should not be observed. However, a very weak analgesic activity is perceived and in addition, this analgesia seems to be free of undesirable side effects. This was the first indication that a nonaddictive analgesic activity might be possible. The explanation is that there is no a single type of receptor. Morphine activates three types of analgesic receptors (Fig. 6.14).

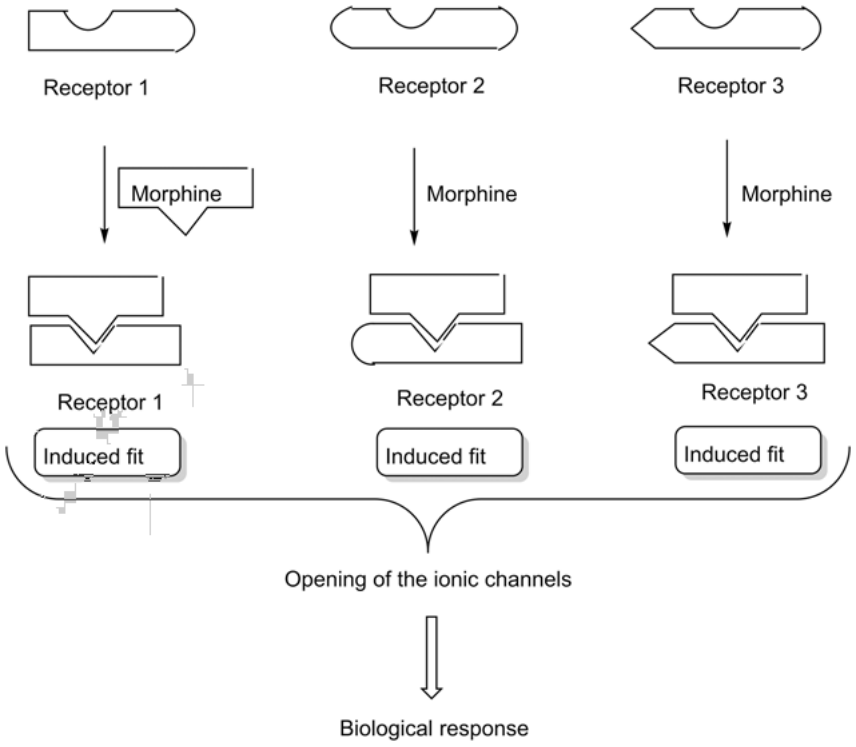


Fig. 6.14: Interactions of morphine with the analgesic receptors.

Nalorphine binds to all three types of analgesic receptors and therefore blocks morphine in these three receptors. Nalorphine is unable to activate two of the receptors and is therefore a true antagonist in these receptors. However, in the third type, nalorphine acts as a weak or partial agonist. Morphine is a strong agonist of all three receptor types. Naloxone is a pure antagonist.

The results observed with nalorphine show that the activation of this third type of analgesic receptor leads to analgesia without the undesirable side effects associated with the other two types of analgesic receptors (Fig. 6.15).

Unfortunately, nalorphine exhibits hallucinogenic side effects resulting from activation of a nonanalgesic receptor. For the first time, a certain degree of analgesia was obtained without the side effects of respiratory depression and tolerance.

6.2.2.2 Simplification or dissection of the drug

Removal of the E- (piperidine) ring

Removal leads to a complete loss of activity. This result emphasizes the importance of the basic nitrogen atom in the analgesic activity.

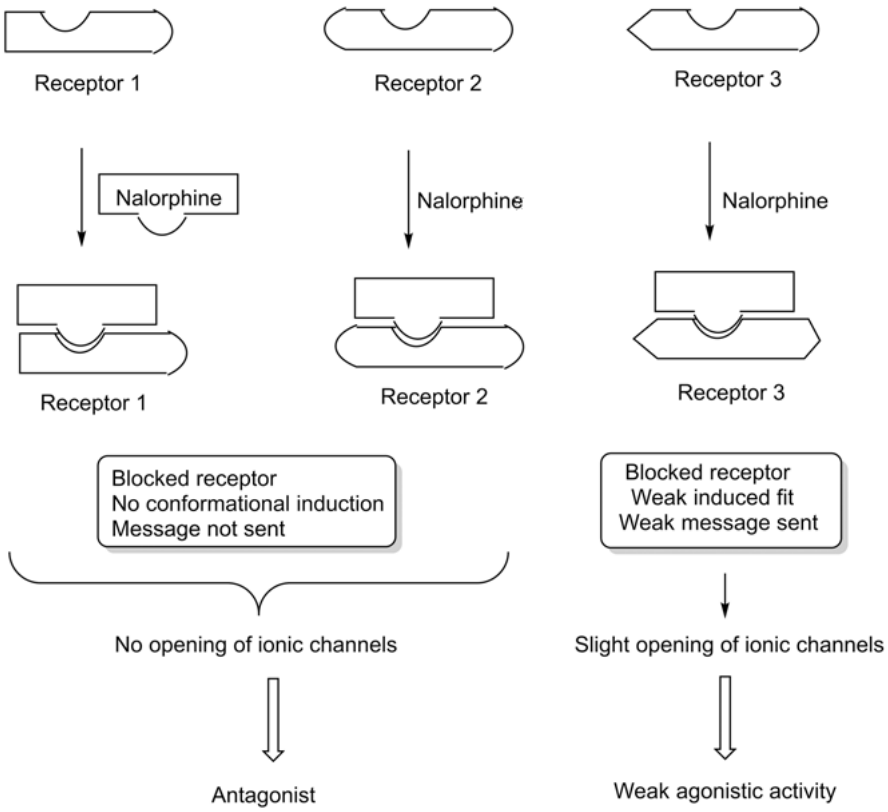


Fig. 6.15: Interactions of nalorphine with the analgesic receptors.

Removal of the D-ring

Removal of the oxygenated bridge gives rise to a series of compounds called morphinans, which have useful analgesic activity (Fig. 6.16).

The biological activity of *N*-methylmorphinan proceeded as expected, because it lacks the phenolic group. Levorphanol is five times more active than morphine and although side effects have also increased, levorphanol can be taken orally and lasts much longer in the body because it is not metabolized in the liver to the same extent as morphine. The specular image of levorphanol (dextrorphan) exhibits a negligible analgesic activity.

The same strategy of drug extension already described for morphine was implemented for morphinans with similar results. For example, the addition of an allyl group to the nitrogen atom produces antagonists (→ levalorphan); addition of a phenethyl group to N greatly increases potency. Addition of a 14-OH group also increases activity. This implies that both types of molecules interact with the same receptors in the same way.

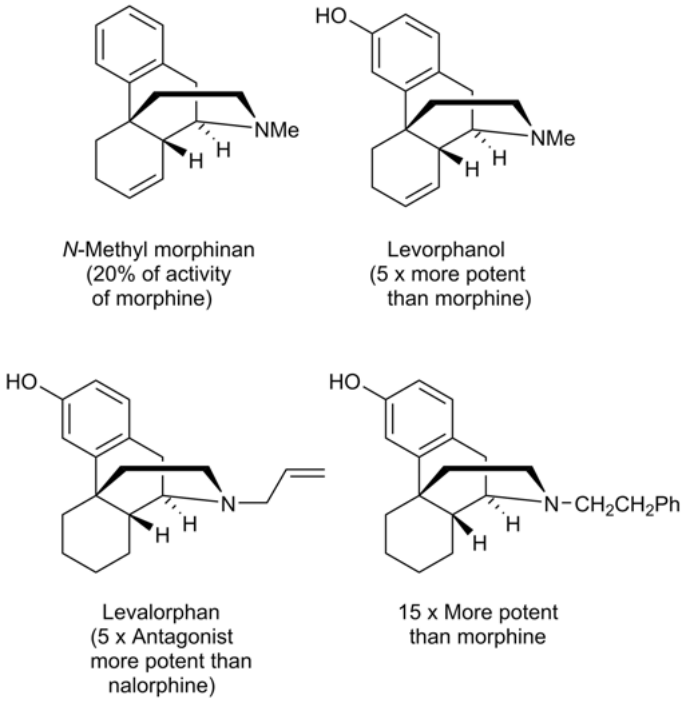


Fig. 6.16: Examples of morphinans.

Removal of C and D-rings

This gives rise to an interesting group of compounds called 6,7-benzomorphans that retain the analgesic activity (Fig. 6.17).

Note that the two methyl groups of the metazocine are *cis* to each other and represent the "stumps" of the C-ring. Further development led to pentazocine, which turned out to be a short-acting analgesic with very little risk of addiction. Bremazocine (more modern) has a longer duration of action, has no addictive properties and does not depress breathing (Fig. 6.18).

These compounds appear to be similar in their action to morphine. They act as antagonists in two of the three types of analgesic receptors, but act as agonists in the third type. The major difference between nalorphine and compounds like pentazocine is that the latter is a much stronger agonist, resulting in a higher level of analgesia. Unfortunately, many of these compounds exhibit hallucinogenic side effects, due to interactions with a nonanalgesic receptor.

Removal of B, C and D-rings

This leads to a series of compounds known as 4-phenylpiperidines. Activity can be increased 6-fold by introducing a phenolic group and altering the ester to a ketone to yield ketobemidone (Fig. 6.19).

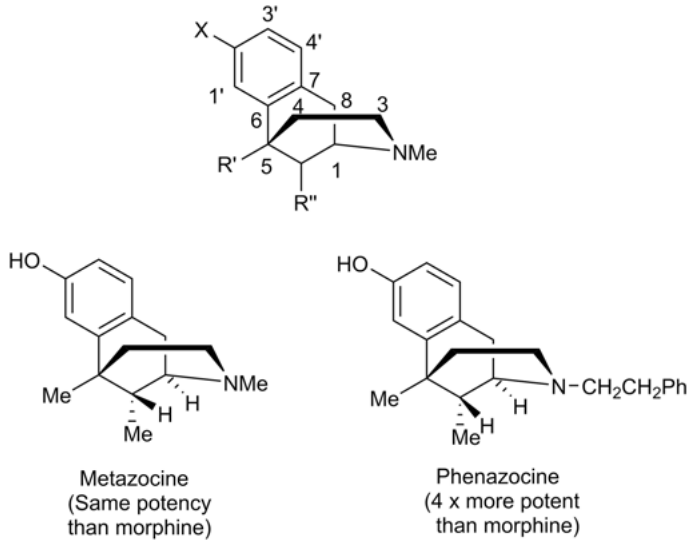


Fig. 6.17: Benzomorphans.

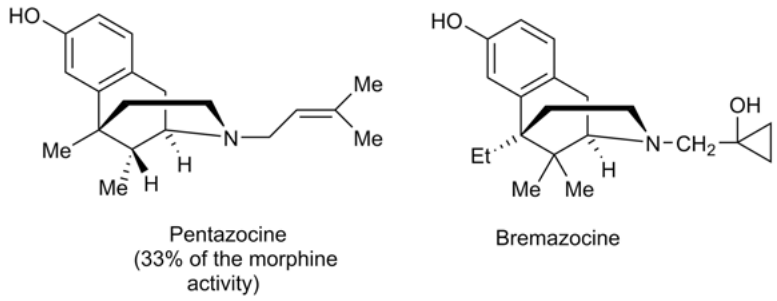


Fig. 6.18: Pentazocine and bremazocine.

The synthesis of meperidine and ketobemidone has many features in common. Both begin with a double alkylation of a phenylacetonitrile, to form the piperidine ring (Scheme 6.2). The basic hydrolysis of the resulting nitrile followed by esterification, leads to meperidine, while the treatment of the nitrile with ethylmagnesium bromide, followed by addition of hydrogen bromide, yields the ketobemidone.

There is some doubt as to whether they act in the same way as morphine in analgesic receptors since some of the chemical modifications described for morphine do not lead to comparable results. For example, addition of allyl and cyclopropylmethyl groups does not yield antagonists. The methyl substitution of meperidine by the cinnamoyl group increases the activity 30-fold, whereas this same group in morphine eliminates the activity (Fig. 6.20).

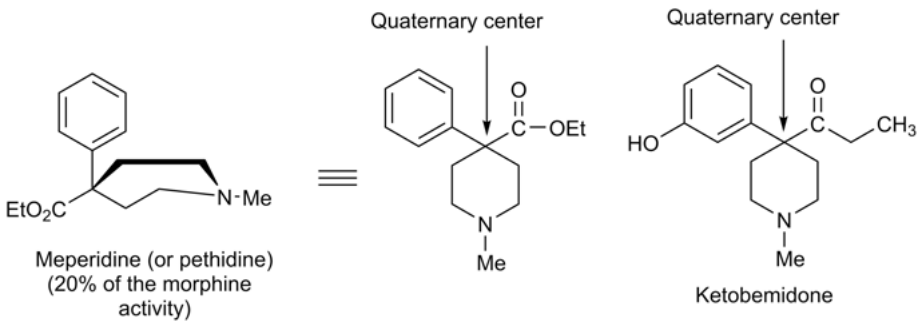
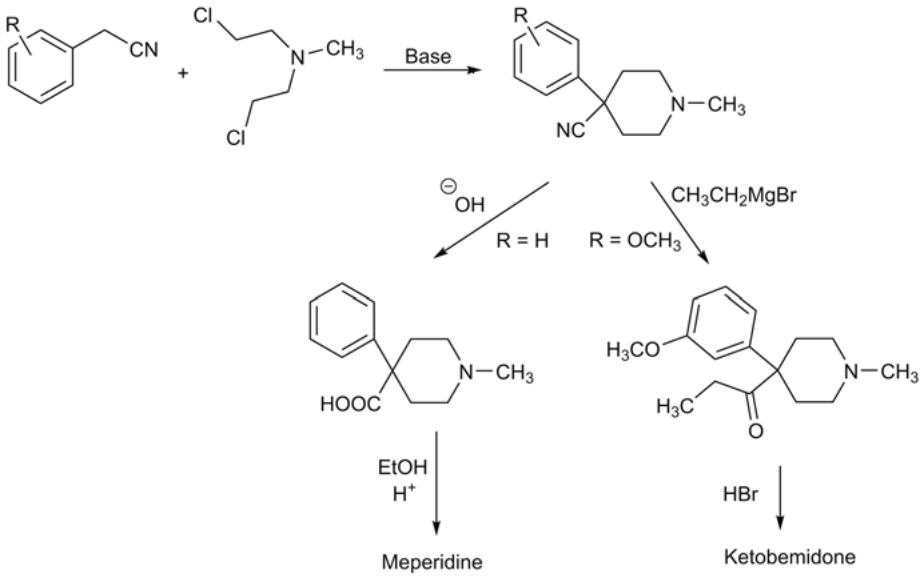


Fig. 6.19: 4-Phenylpiperidines.



Scheme 6.2: Synthesis of meperidine and ketobemidone.

These results could have to do with the fact that the piperidines are more flexible molecules than the previous structures and can interact with the receptors in a different way.

One of the best derivatives of piperidine is fentanyl. The drug lacks a phenolic group and is very lipophilic (Fig. 6.21).

Piperidinic analgesics appear to interact with analgesic receptors differently than previous groups do.

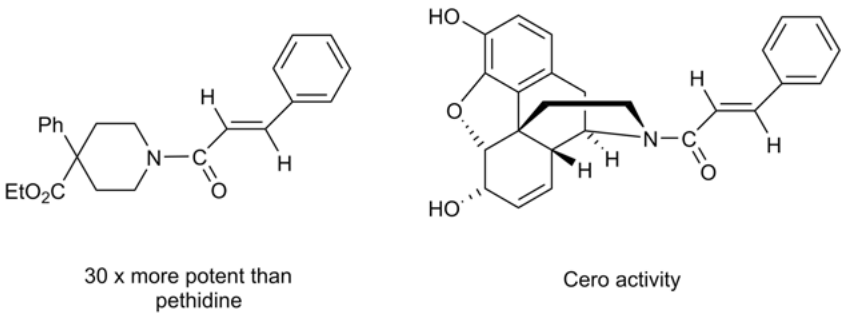


Fig. 6.20: Effects of the addition of the cinnamoyl residue to meperidine and morphine.

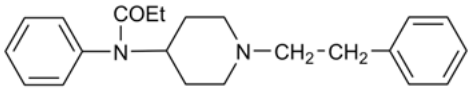


Fig. 6.21: Fentanyl.

Removal of B, C, D and E-rings

Methadone was discovered in Germany during World War II. Unfortunately, it retains the undesirable effects of morphine but it is orally active and has fewer emetic effects. Side effects such as sedation, euphoria and withdrawal syndrome are also less severe and therefore the compound is supplied to drug addicts as a substitute for morphine (or heroin) to achieve the cessation of these drugs. It is not a complete cure because it simply changes an addiction to morphine (or heroin) into another to methadone, which is less dangerous. The molecule has a single stereocenter and when drawn in the same way as morphine, one should expect the *R*-enantiomer to be the most active enantiomer. Indeed, the (*R*) enantiomer is two times more potent than morphine, whereas the (*S*) enantiomer is inactive (Fig. 6.22).

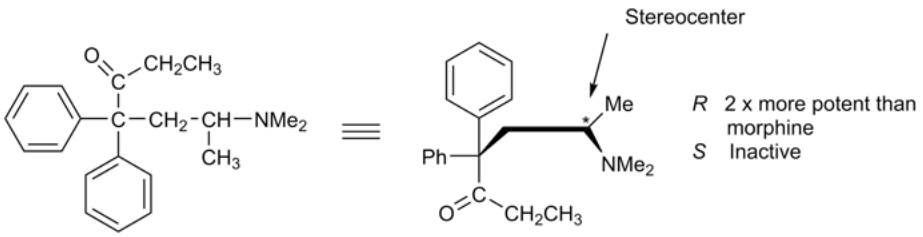
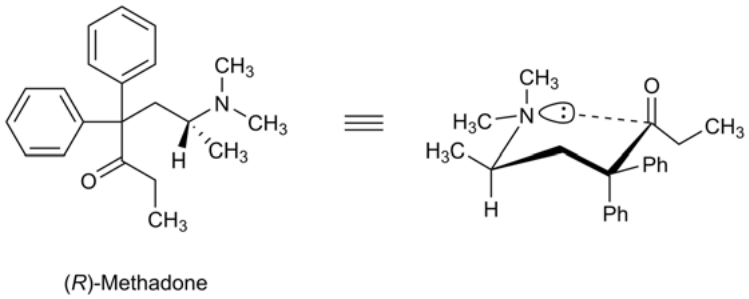


Fig. 6.22: Methadone.

From the structural point of view, it is interesting to note that the most stable conformation of methadone is a folded one (chair-like), stabilized by dipole interactions

between the amino and carbonyl groups (Fig. 6.23). This conformation demonstrates the similarity of methadone to the piperidine derivatives indicated above.



(*R*)-Methadone

Fig. 6.23: Conformational analogy of methadone with phenylpiperidine derivatives.

6.2.2.3 Increased rigidity

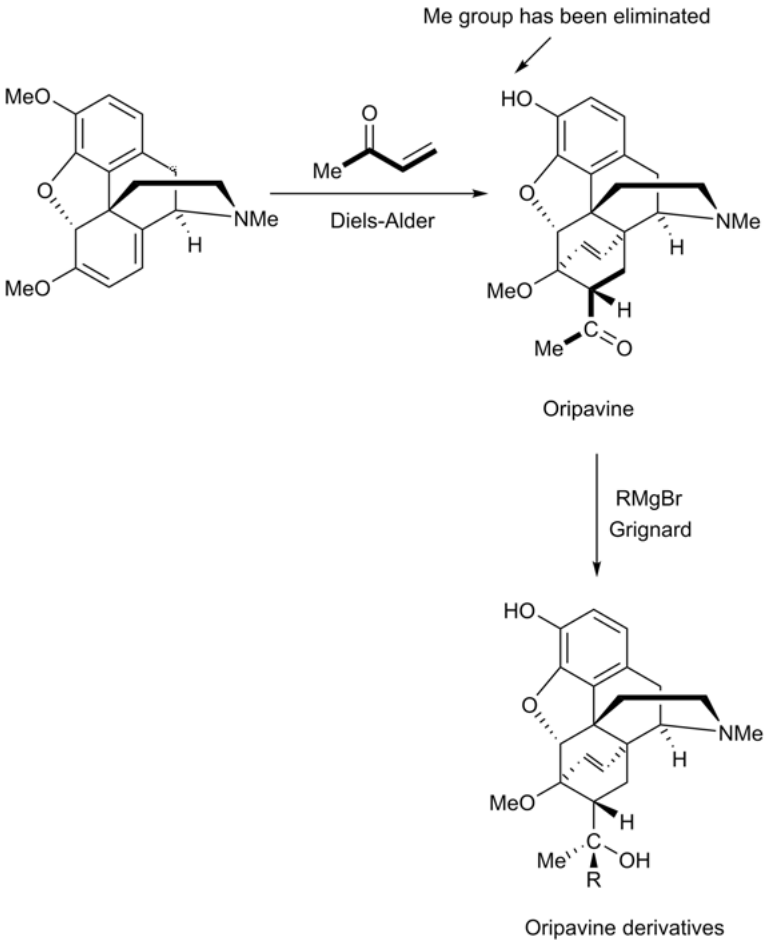
If the molecule is made more rigid, the conformations that are recognized by undesirable receptors can be eliminated and thus restrict the molecule to a specific conformation that fits with the desired receptor. Side effects, such as dependence and respiratory depression, may thus be eliminated. An increase in activity can also be expected, since the molecule could adopt the correct conformation to interact with the receptor.

Thebaine can be extracted from opium with codeine and morphine and is very similar to both compounds; however, it has no analgesic activity. There is a diene group in the C-ring of thebaine and when it is reacted with methylvinylketone, a Diels–Alder reaction takes place to yield an extra ring, which gives a greater rigidity to the structure (Scheme 6.3). The so-called Bentley or oripavine analgesics are the result of the formation of an additional six-carbon cycle between positions 6 and 14 of the opioid skeleton.

A comparison with morphine proves that the extra ring protrudes in the form of a crossbar of the T-structure (Fig. 6.24).

Since a ketone group has been introduced, it is now possible to attempt the strategy of drug extension by adding several groups to the ketone via a Grignard reaction. It should be noted that the Grignard reaction is stereospecific: he Grignard reagent is complexed with both methoxy and ketone groups and the carbanion ion attacks the less hindered side of the ketone, preferably yielding one of the enantiomers (Scheme 6.4).

By varying the groups added by the Grignard reaction, remarkably powerful compounds have been obtained. Etorphine is 10,000 times more potent than morphine (Fig. 6.25). It is used to immobilize large animals such as elephants or rhinos.



Scheme 6.3: Formation of oripavine derivatives.

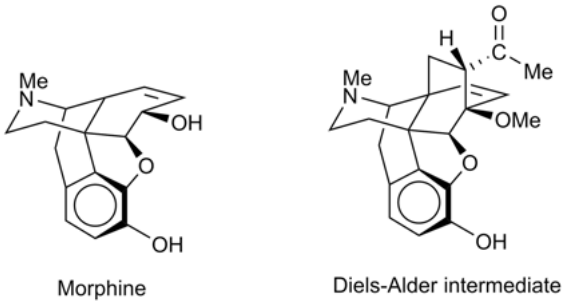
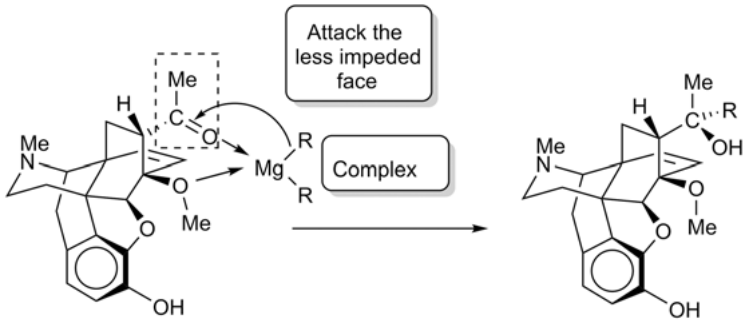


Fig. 6.24: Comparison of the shapes of morphine and oripavine.



Scheme 6.4: Grignard reaction leads to a stereocenter.

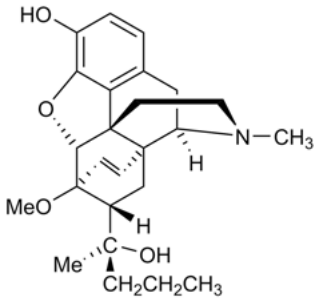


Fig. 6.25: Etorphine.

The increase in analgesic activity is also accompanied by unacceptable side effects. It was therefore decided that if substituents such as allyl or cyclopropylmethyl were placed on the nitrogen atom, they would yield antagonists as in the morphine, morphinan and benzomorphan series. If so, it would be possible to obtain equivalents of pentazocine or nalorphine (antagonists with some agonist activity and with reduced side effects). The introduction of a cyclopropylmethyl group yields an antagonist called diprenorphine (Fig. 6.26), which is more potent than nalorphine and can be used to reverse the immobilizing effects of etorphine. Diprenorphine has no analgesic activity.

Substitution of a methyl group with a *t*-butyl group gives buprenorphine (Buprex®, Fig. 6.26), which has drug-like properties like nalorphine and pentazocine. If pain levels are high, buprenorphine is unable to counteract pain and morphine should be used. However, buprenorphine provides another example of an opioid analogue in which analgesia has been separated from the dangerous side effects.

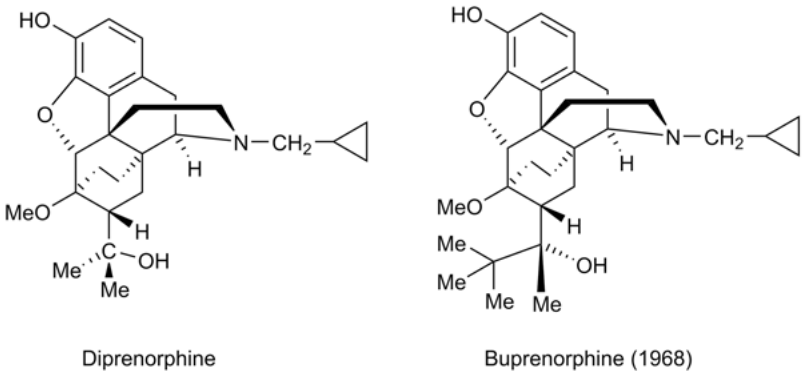


Fig. 6.26: Diprenorphine and buprenorphine.

6.2.3 Multiple analgesic receptors

6.2.3.1 Mu receptor (μ)

Morphine binds strongly to this receptor and produces analgesia. Binding to this receptor also leads to the undesirable effects of respiratory depression, euphoria and addiction.

6.2.3.2 Kappa receptor (κ)

The bond strength is less than that of the μ-receptor. The biological response is analgesia with sedation and none of the dangerous side effects. It is this receptor that provides the best hopes for safe analgesia. The results obtained with nalorphine, pentazocine and buprenorphine can now be understood. These compounds act as antagonists at the μ-receptor, blocking morphine. However, they act as weak agonists at the μ-receptor. Unfortunately, they present hallucinogenic side effects as a result of binding to a completely different receptor in the brain called the σ-receptor, where they act as agonists.

6.2.3.3 Delta receptor (δ)

The δ-receptor is where the natural brain analgesics act (see enkephalins below). Morphine binds strongly to this receptor.

Table 6.1 shows the relative activities of morphine, nalorphine, pentazocine, enkephalins, pethidine and naloxone.

Inclusion of the polymethylene chain of methadone within a ring gives rise to a compound with a restricted conformational freedom, such as tramadol (Fig. 6.27). Tramadol is an opioid analgesic that relieves pain by acting on specific nerve cells in the spinal cord and brain. Its behavior is atypical compared to other opioids of the

Table 6.1: Relative activities of opioid analgesics.

Rec.	Effect	Morphine	Nalorphine	Pentazocine	Enkefalinas	Petidine	Naloxone
μ	Analgesia Respiratory depression Euphoria Addiction	+ + +	–	–	+	++	– – –
κ	Analgesia Addiction	+	+	+	+	+	–
σ	Psychomimetic	0	+	+	0	0	0
δ	Analgesia	++	-	-	+ + +	+	-

+: Compound acting as an agonist; –: as an antagonist; 0: without activity.

morphine type, since despite having a relatively weak agonism over the μ-opioid receptors, its analgesic effect is largely due to its action in the neurotransmitter system, as it releases serotonin and inhibits the NA reuptake.

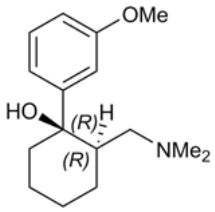


Fig. 6.27: Tramadol.

It is a pure, nonselective agonist on μ-, δ- and κ-opioid receptors, with higher affinity for μ-receptors. Other mechanisms contributing to its analgesic effect are inhibition of the NA neuronal reuptake, as well as enhancement of serotonin release. The potency of tramadol is 1/10-1/6 of that of morphine.

6.2.4 Agonists and antagonists

It has been suggested that there are two hydrophobic binding sites in an analgesic receptor. A structure will act as an agonist or antagonist depending on which of these binding sites is used; in other words, one of these hydrophobic binding sites is an agonist-binding site, while the other is antagonistic. Snyder proposed this model in 1954, before the multiplicity of the opioid subjects became known, and it still retains its validity and remains the basis for new agonist and antagonistic actions. In the model,

the agonist-binding site is further away from the nitrogen atom and placed axially with respect to it. The antagonist site is closer and placed equatorially (Fig. 6.28).

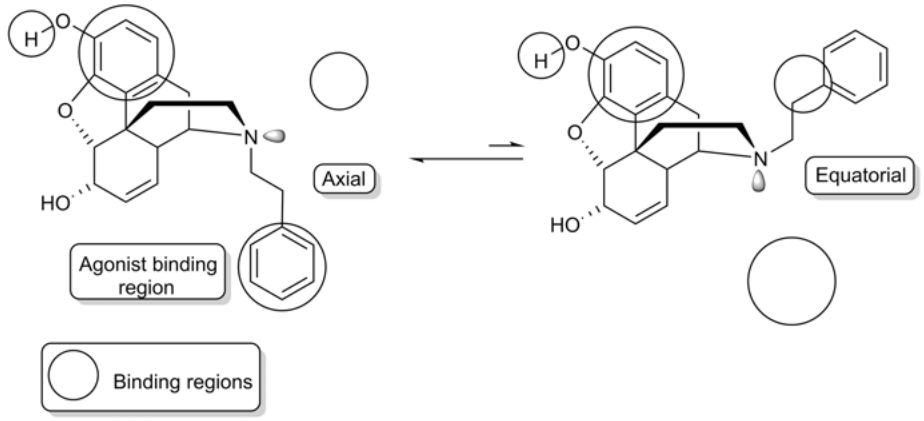


Fig. 6.28: *N*-Phenethylmorphine binding interactions.

If the phenethyl group is in an axial position, the aromatic ring is in the correct position to interact with the agonist binding site; however, if the phenethyl group is in an equatorial position, the aromatic ring is placed beyond and does not bind to the antagonist binding site. The overall result is an increase in agonist activity.

Consider what happens if the phenethyl group is replaced by an allyl group. If it is in equatorial position, the allyl group is able to bind strongly to the antagonist binding site, whereas in the axial position it will hardly reach the agonist site, resulting in a weak interaction (Fig. 6.29).

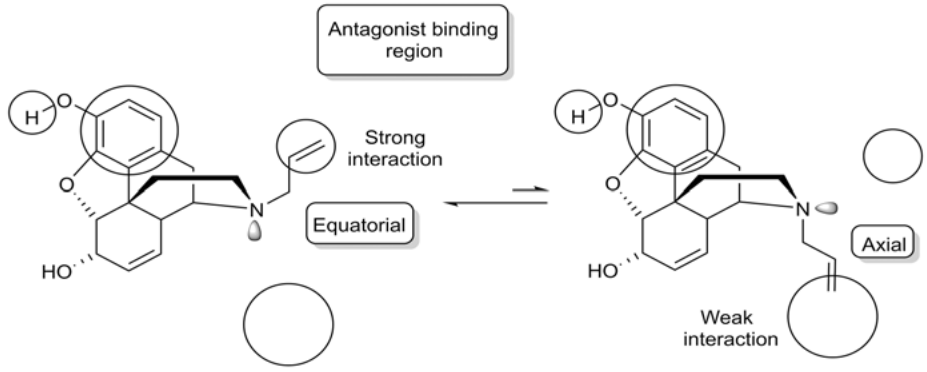


Fig. 6.29: *N*-allylmorphine binding interactions.

According to this theory, it is proposed that a molecule such as phenazocine (with a phenethyl group) acts as an agonist since it can bind only to the agonist site. A molecule such as nalorphine (with an allyl group) can bind to both agonist and antagonist sites. The relationship of these effects will depend on the relative equilibrium ratio of the axial and equatorial isomers.

A compound that is a pure antagonist would be forced to have an appropriate substituent at the equatorial position. The presence of a 14-OH group sterically hinders the isomer with the axial substituent and forces the substituent to remain equatorial (Fig. 6.30).

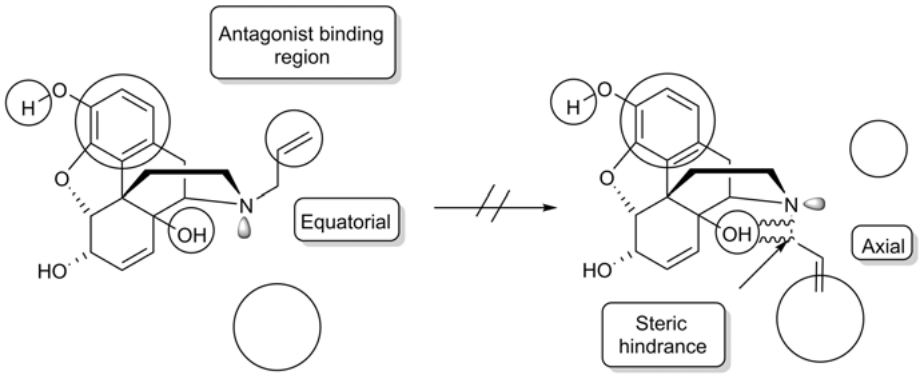


Fig. 6.30: Influence of the 14-OH group on binding interactions.

6.2.5 Enkephalins and endorphins

The following conclusions can be drawn from all of the above: (1) There must be analgesic receptors in the CNS; and (2) there must be chemicals produced in the body that interact with these receptors. The search led to the discovery of enkephalins and endorphins. The term enkephalin derives from the Greek and means "in the head", which is exactly where the enkephalins are produced. The first enkephalins discovered were the pentapeptides Met-enkephalin and Leu-enkephalin (Fig. 6.31).

H-Tyr-Gly-Gly-Phe-Met-OH

Met-enkephalin

H-Tyr-Gly-Gly-Phe-Leu-OH

Leu-enkephalin

Fig. 6.31: Met-enkephalin and Leu-enkephalin.

At least 15 endogenous peptides ranging in length from 5 to 33 amino acids have been discovered. The 15 compounds have the skeleton of Met- or Leu-enkephalin at their *N*-termini, which underscores the importance of the pentapeptide structure for anal-

gesic activity. These compounds are neurotransmitters or neurohormones in the brain and operate as natural painkillers. Enkephalins are thought to be responsible for the analgesia that results from acupuncture.

6.2.6 Enkephalin analogues

The SAR studies of enkephalins show the importance of the phenolic ring and the amino group tyrosine. Without them the activity is lost (Fig. 6.32).

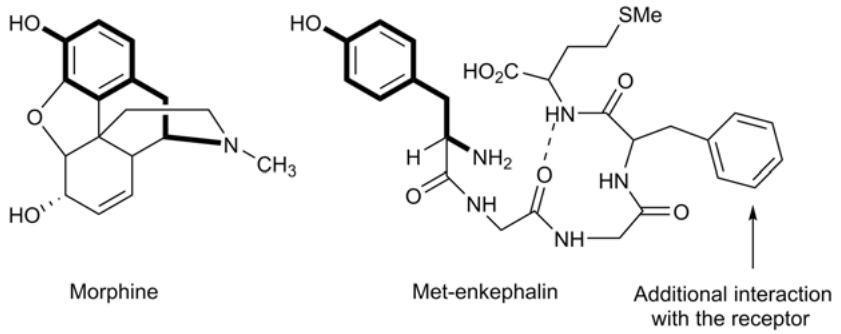


Fig. 6.32: Comparison between morphine and Met-enkephalin (the dotted line indicates a hydrogen bond.

Enkephalins have been found to be readily inactivated by peptidases in vivo. The most labile peptide bond in enkephalins is that between tyrosine and glycine. The alternative of replacing L-tyrosine with D-tyrosine is not possible since it completely alters the relative orientation of the aromatic ring of tyrosine in relation with the rest of the molecule. As a result, the analogue is unable to bind to the analgesic receptor and is inactive. If a methyl group is placed on the *N*-amidic acid, the hydrolysis by peptidases is also blocked.

Unfortunately, enkephalins also have some activity on the μ-receptor and therefore the search for selective analgesics continues.

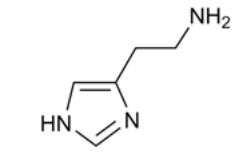
7 Histamine and antihistaminics

7.1 Goals

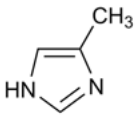
- To know the role of histamine as a cellular mediator.
- To describe histamine receptors and the drugs that interact with them, with a special emphasis on antiallergic and antiulcer drugs.
- To perform a systematic study of the factors involved in the design of the first antiulcer drug that revolutionized the treatment of the gastrointestinal ulcer.

7.2 Histamine

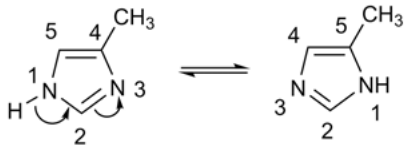
It is possible to have two tautomeric forms (Fig. 7.1) when one of the nitrogen atoms of the imidazole ring has hydrogen. In this case, when taking into account that the nitrogen atom carrying the hydrogen has the locator 1, the second equilibrium of Fig. 7.1 would force the correct name of the derivative to be 4(5)-methylimidazole.



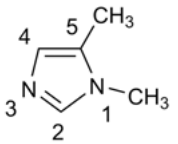
Histamine
4-(2-aminoethyl)imidazole



4-Methylimidazole, although the correct name would be 4(5)-methylimidazole, for the possibility of tautomerism



*N*1 always carries hydrogen. This tautomerism exists as long as one of the two Ns has a hydrogen atom



1,5-Dimethylimidazole: There is no possibility of tautomerism

Fig. 7.1: Possibility of tautomeric forms in the imidazole ring.

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If the hydrogen on the nitrogen is changed by a methyl group, there is no possibility of tautomerism, so that the chemical name of 1,5-dimethylimidazole corresponds to a single structure (Fig. 7.1). In the case of histamine, two tautomeric forms are possible. However, instead of labeling them 4(5), they will be labeled differently for historical reasons, as will be explained presently.

Histamine was isolated in 1927 from the liver and lungs of mammals. It is bound in the body by ionic forces to heparin (Fig. 7.2), a natural polymeric substance. Heparin is an anticoagulant; it acts on the blood by fluidizing it and is used to treat thrombosis.

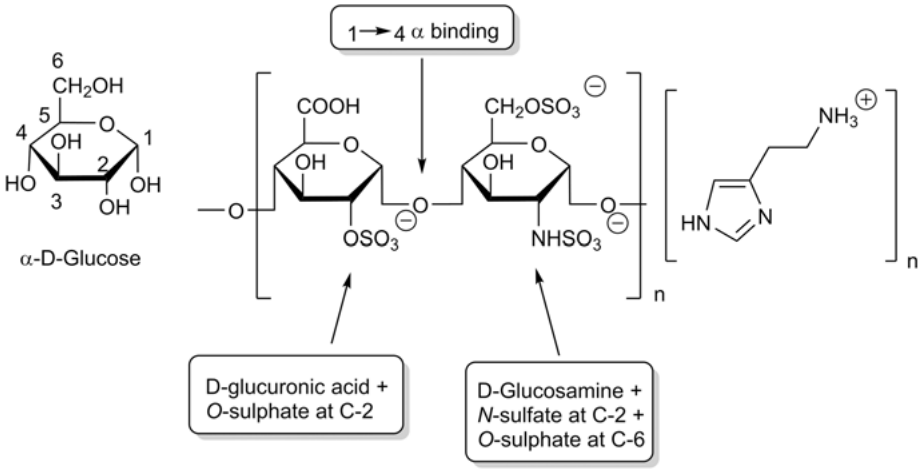


Fig. 7.2: Heparin bound by ionic forces to histamine.

Histamine is toxic: if all that is stored in the organism was released at the same time, it would induce death. It is biosynthesized by the decarboxylation of the amino acid histidine (Fig. 7.3), mainly in mast cells.

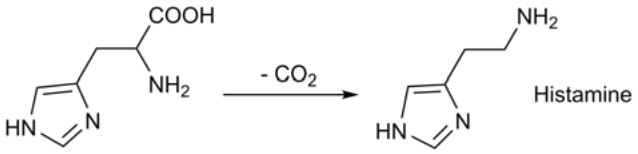


Fig. 7.3: Decarboxylation of histidine.

Histamine is a chemically complex molecule, since it can present several tautomeric forms in equilibrium and it also has great conformational freedom (Fig. 7.4). It is a polyvalent base, which gives rise to mono- and di-cations.

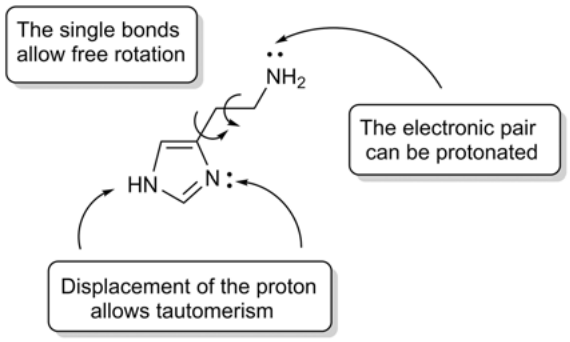


Fig. 7.4: Problems posed in histamine.

Four types of histaminic receptors are known: H_1, H_2, H_3 and H_4:

- When acting on the H_1 receptors (discovered in 1966), located mainly on the skin and membranes of the respiratory and digestive apparatus, histamine fulfills the physiological role of defending the organism against foreign agents, since its release occurs in response to antibodies, mechanical damage, burns or infections. The stimulation of H_1 receptors leads to several effects, such as the contraction of the smooth muscle of the intestine, uterus and bronchi (this effect can lead to asthma), the dilatation of the smooth muscle of the blood vessels (resulting in hypotension) and an increased permeability of the walls of the blood capillaries (which favors the outflow of plasma and the formation of edemas). These effects cause allergic reactions and therefore, H_1 antihistaminics are used as antiallergics.
- H_2 Receptors, established by Black et al. in 1972, are located in the walls of the stomach and are responsible for the production of HCl in the parietal cells. The development of H_2 antagonists has led to their application as antiulcer drugs.
- In 1987, the existence of H_3 receptors, which are autoreceptors capable of regulating the synthesis and release of histamine at the cerebral level, was confirmed. They are, apparently, responsible for nerve transmission.
- Currently, the H_4 receptor (discovered in 2000) is being studied and it is known that its function is regulated by the production of inflammatory cytokines, so it participates in inflammatory processes.

7.2.1 H_1 Antihistaminics: synthesis and SARs

Piperoxane (a ß-adrenergic blocker) antagonizes histamine effects: it is able to protect animals from the histaminic bronchospasm, thereby opening up new avenues for research.

H_1 Antihistaminics relax smooth muscle spasms or contractions. Piperoxane is an aryloxyethylamine and therefore aminoalkyl ethers were investigated (Fig. 7.5).

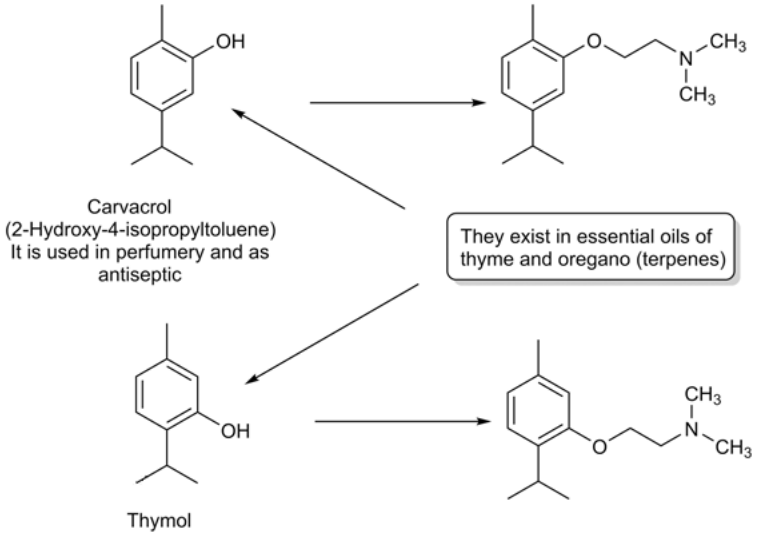


Fig. 7.5: Development of the first aminoalkyl ethers.

Subsequently, the oxygen atom was exchanged for the nitrogen atom, whereby the field of ethylenediamines was opened.

The first H_1 antihistaminic drug with clinical utility, fenbenzamine (Fig. 7.6), was prepared in 1942.

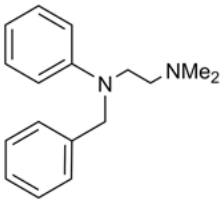
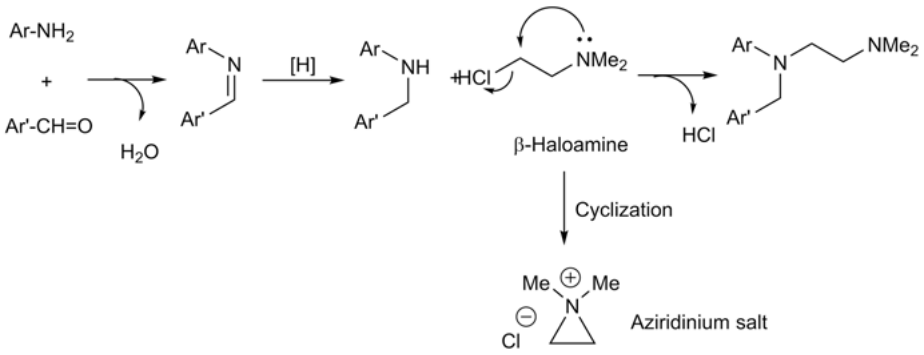


Fig. 7.6: Fenbenzamine or Antergan®.

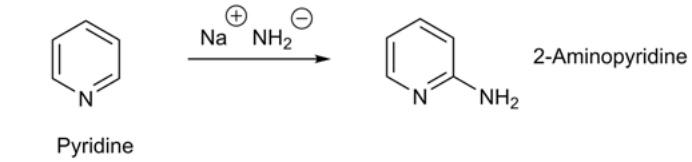
It has side effects due to its similarity to other pharmacological groups. Almost all of the H_1 antihistaminics are sedatives. They have structures closely related to neuroleptics or antiparkinsonians. They also have an enormous resemblance to anticholinergics, which results in several side effects, such as blurred vision or dry mouth. The general synthesis for the family is outlined in Scheme 7.1.

Ar and Ar', inexpensive heterocyclic rings, were introduced as shown in Scheme 7.2.

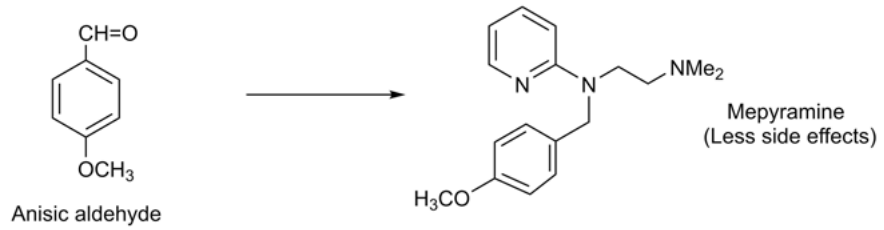
Simultaneously, between 1943 and 1954, antihistaminics of general structure RO-CH_2-CH_2-NR_2' were investigated. In 1946, diphenhydramine, and shortly after-

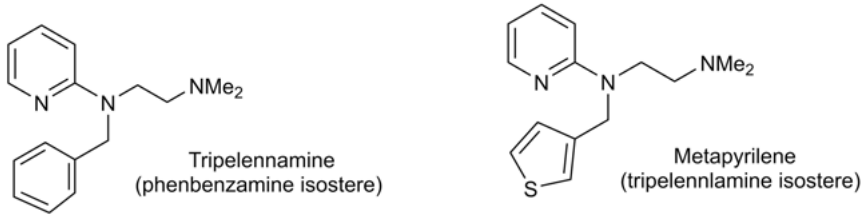


Scheme 7.1: General synthesis for the preparation of the ethylenediamines as H_1 antihistaminics.



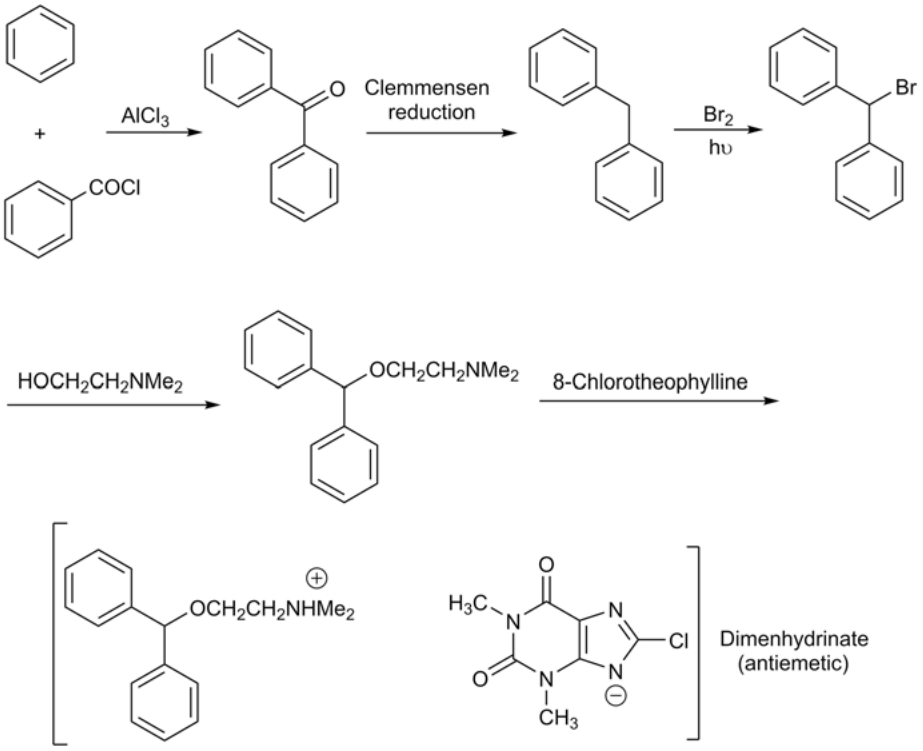
The nature of electronic deficiency of the pyridine ring is demonstrated in the S_N reactions against strong nucleophiles, such as sodium amide (Chichibabin reaction, 1914)





Scheme 7.2: Various ethylenediamines as H_1 antihistaminics.

wards its salt, were introduced with 8-chloroteophylline, called dimenhydrinate and began to be used extensively against motion sickness (Scheme 7.3).



Scheme 7.3: Synthesis of dimenhydrinate.

The antinauseous component is the antihistaminic cation, and not the anion. Like ethylenediamines, it has several side effects: sedation, anticholinergic, etc. Note the close structural relationship with atropinics. The NCH_2CH_2N side chain of the antihistaminic ethylenediamines may be included totally or partially in a heterocyclic system, as in the case of thenaldine (Fig. 7.7).

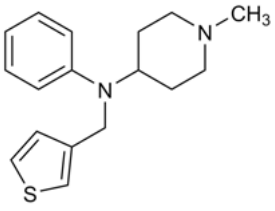


Fig. 7.7: Thenaldine.

At present, the structure of antihistaminics that act by means of a competitive blockade of the H_1 receptor has been generalized with the following pharmacophore (Fig. 7.8).

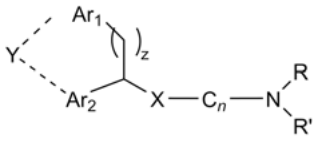


Fig. 7.8: Pharmacophore of the H_1 antihistaminics.

R and R' are methyl groups or small rings (pyrrolidine, etc.). Ar and Ar' are aromatic carbon- or hetero-cyclic rings, with or without substituents. X May be CH-O-, N- or CH (aminoethers, ethylenediamines and propylamines, respectively when $n = 2$). z can be 0 or 1. Y Is a bridge (optional) between the aromatic rings, which would give rise to a tricyclic system: CH_2, NH, O, S, CH_2O, etc. It must be sufficiently flexible and large enough to force a non-coplanar arrangement of the Ar_1 and Ar_2 rings. Finally, C_n is a linear chain or part of a cycle. Normally, $n = 2$, although in tricyclic antihistaminics n may be 2 or 3.

Two examples of widely used antihistaminics, belonging to the classes of propylamines and tricyclic derivatives, are chlorpheniramine and promethazine, respectively (Scheme 7.4).

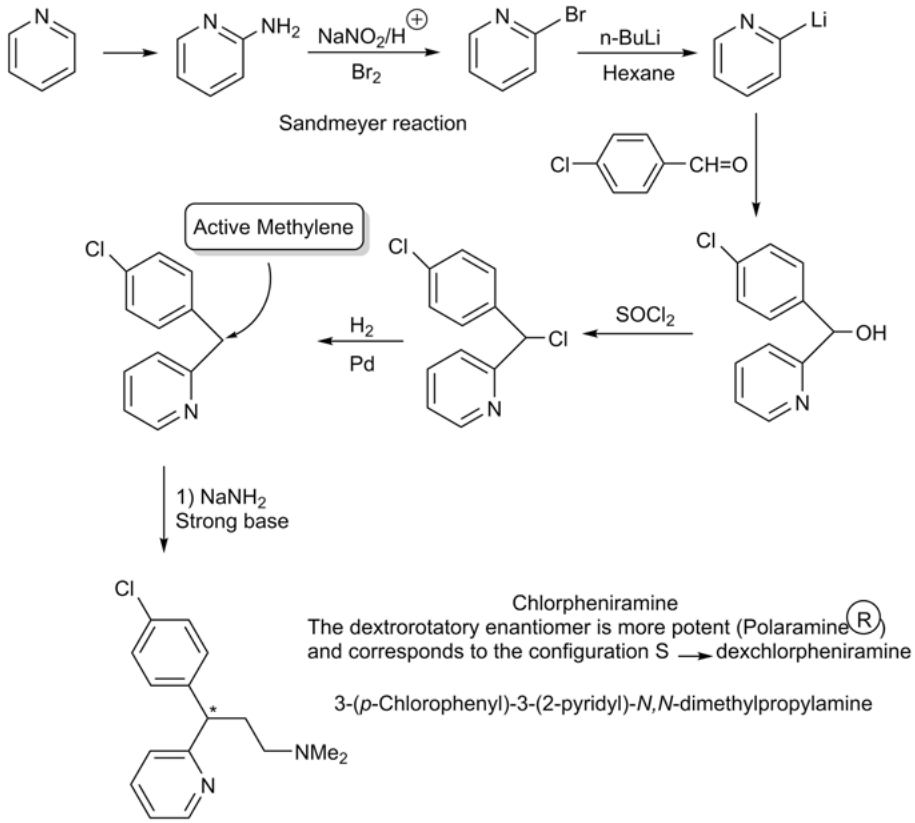
Promethazine belongs to the group of phenothiazines, the first tricyclic antihistaminics discovered in 1945 (Scheme 7.5). It can be obtained from diphenylamine, or directly through the alkylation of phenothiazine.

The aminoalkyl halide employed in the alkylation may be substituted for its 2-chloropropyldimethylamine isomer. By means of intramolecular displacement, both lead to the same aziridinium salt, which is the true alkylating agent of the reaction.

7.2.2 Second-generation H_1 antihistaminics

The applications of the first generation of antihistaminics are limited, because they cause significant side effects, such as sedation, memory problems and psychomotor dysfunction. However, the second-generation of antihistaminics have far fewer adverse CNS effects, because of their greater difficulty in traversing the BBB.

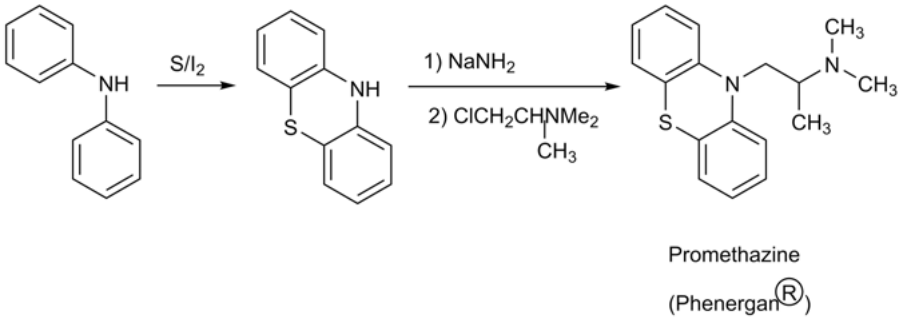
The evolution of azatidine towards loratadine and desloratadine is an excellent example of how a small change in chemical structure can have a huge impact on the pharmacological profile (Fig. 7.9). In 1973, Schering–Plow launched the first-generation antihistaminic azatadine. It was found that it crossed the BBB and consequently caused a profound drowsiness. The treatment of azatadine with ethyl chloroformate results in desloratadine, which has virtually no sedative effects.



Scheme 7.4: Synthesis of chlorpheniramine.

However, it has a duration of action of only 6-8 hours. The addition of a chlorine atom at position 8 of the fused tricyclic system results in loratadine {ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*] pyridin-11-ylidene)piperidine-1-carboxylate}. Loratadine is metabolized to desloratadine (decarboxyethoxyloratadine), which is its active metabolite and a potent H_1 antagonist. The half-life of loratadine is approximately 8 hours and that of its metabolite reaches 28 hours. Loratadine is four times more potent than desloratadine. In addition, loratadine shows little activity against cholinergic or adrenergic receptors. It has no anticholinergic effects at doses up to 320 mg/kg in animals. Research indicates that loratadine relieves the symptoms of urticaria and other allergic skin conditions. It also effectively treats and controls the symptoms of allergic rhinitis, such as sneezing, rhinorrhea and pruritus. It also helps to alleviate allergic conjunctivitis and its symptoms such as watering and itching of the eyes.

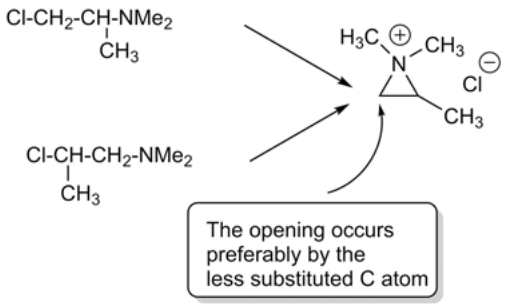
The small amounts of the anti-H_1 drugs actually reaching the brain of the anti-H_1 drugs to the brain explains the absence of adverse neurological symptoms. Recent



Promethazine

(Phenergan®)

The primary aminoalkyl halide employed in the alkylation may be substituted for its isomer, 2-chloropropyldimethylamine; both, by an intramolecular displacement, lead to the same aziridinium salt, which is the true alkylating agent in the reaction.



Scheme 7.5: Synthesis of promethazine.

studies have shown that the concentration of drugs in the central nervous system is controlled by the permeability glycoprotein transport system (P-gp), lipophilic characteristics and ionization. At recommended doses, novel anti-H_1 drugs do not usually induce sedation, perhaps because they are removed from the CNS by the P-gp system; these mechanisms however are complex and still not fully understood.

Now let us consider the synthesis of loratadine and desloratadine (Scheme 7.6). To begin with, the reaction of Ritter on 2-cyano-3-methylpyridine with *tert*-butanol is carried out with the aid of concentrated sulfuric acid to yield *tert*-butyl carboxamide **7.1**. Next, the deprotonation of the methyl group of **7.1** by means of *n*-BuLi and successive additions of a catalytic amount of sodium bromide and *m*-chlorobenzyl chloride yields the adduct **7.2**. The catalytic amount of sodium bromide carries out a Finkelstein reaction with *m*-chlorobenzyl chloride to give rise to *m*-chlorobenzyl bromide, which is a better electrophile for S_N2. The refluxing of **7.2** with $POCl_3$ again transforms the *tert*-butyl carboxamide in the nitrile group to give **7.3**. The addition of the *N*-methylpiperidyl magnesium chloride (**7.4**) to the nitrile group of **7.3** gives rise to the

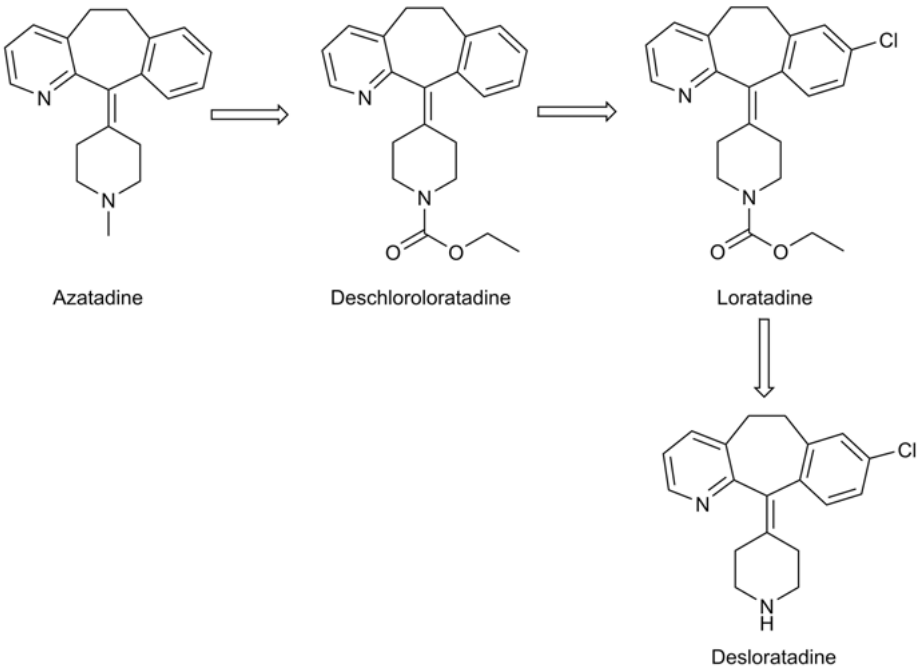


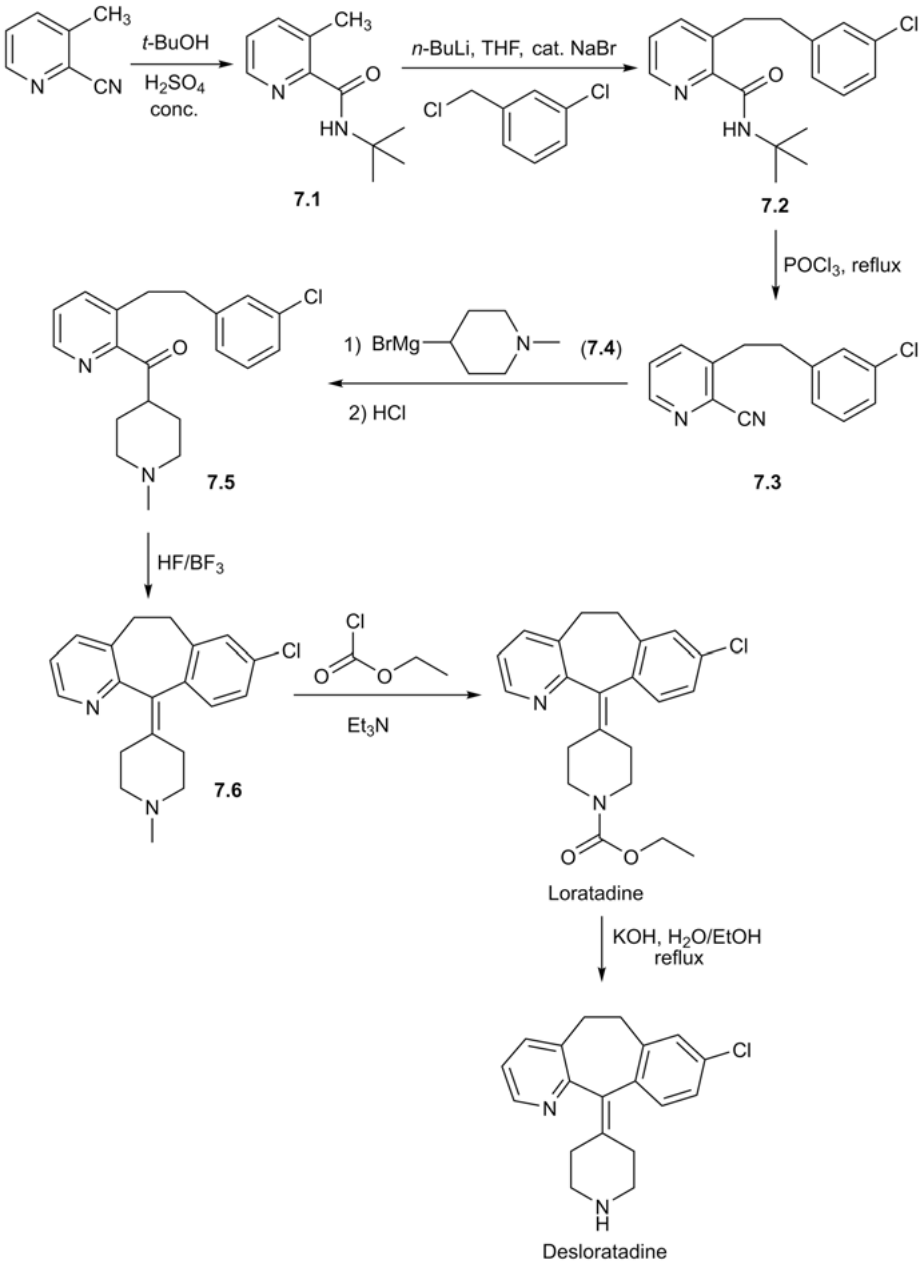
Fig. 7.9: Evolution of azatadine to loratadine and desloratadine.

corresponding imino magnesium bromide, which is hydrolyzed to yield ketone **7.5**. The cyclization of **7.5** using an excess of the superacid composed of HF and BF_3 leads to cycloheptene **7.6**.

The demethylation and formation of loratadine occurs when **7.6** is treated with ethyl chloroformate. The hydrolysis of loratadine is carried out using KOH in a refluxing H_2O/EtOH mixture to produce desloratadine.

7.3 Cimetidine: Example of a rational approach in the design of a drug

Ulcers are localized erosions in the mucous membranes of the stomach or duodenum. It is not known how they arise, but the presence of gastric acid aggravates the problem and delays recovery. In the early 1960s, the conventional treatment consisted of neutralizing gastric acid by administering bases such as sodium bicarbonate and calcium carbonate. However, the dose levels required for the neutralization were large and caused unpleasant side effects. It was thought that a better approach might be the inhibition of gastric acid at its source. Gastric acid (HCl) is released from cells known as parietals in the stomach (Fig. 7.10).



Scheme 7.6: Synthesis of loratadine and desloratadine.

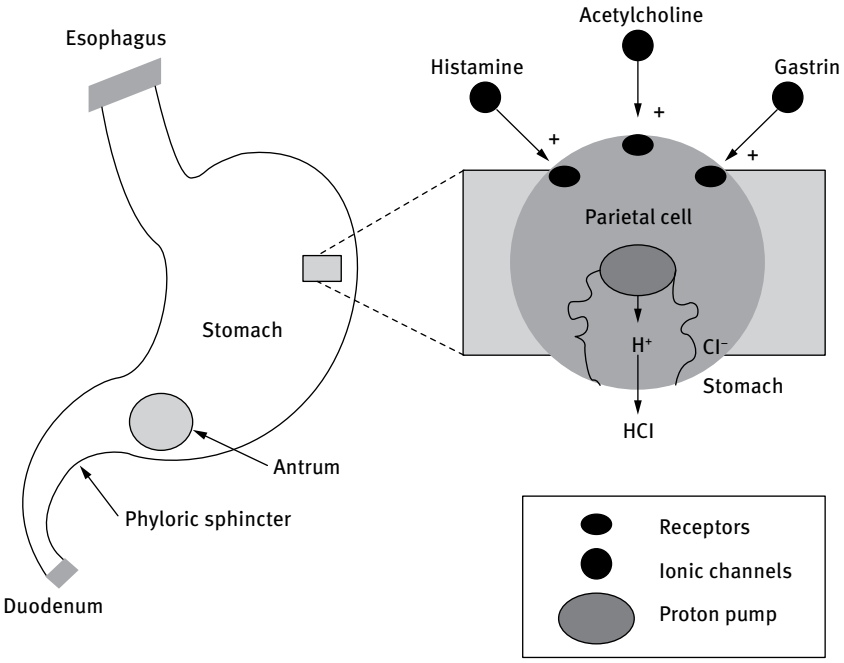


Fig. 7.10: Stomach.

7.3.1 Beginnings: ulcer therapy in 1964

Parietal cells are innervated with the nerves of the ANS. When the ANS is stimulated, a signal is sent to the parietal cells culminating in the release of AcC at the ends of the nerves. The AcA crosses the gap between the nerve and the parietal cell and activates the cholinergic receptors of the parietal cells, which motivates the release of gastric acid in the stomach. This process is triggered by the sight, smell and even the thought of food.

Nerve signals also stimulate a region of the stomach known as the antrum that contains G cells, which release a hormone called gastrin (peptide). This is released when food is present in the stomach. Gastrin passes into the bloodstream and travels to the parietal cells stimulating the release of gastric acid. Therefore, the release of gastric acid should be inhibited by antagonists which block either the AcC receptor (anticholinergic drugs) or the gastrin receptor. SK&F decided to follow another approach.

Based on the knowledge that histamine can also stimulate the release of grastric acid, it was suggested that antihistamises could effectively treat ulcers. In the 1960s, this was a highly speculative proposal. Although histamine had been shown experimentally to stimulate the release of gastric acid, it was not certain that it played an important role in vivo. Many researchers dismissed the importance of histamine, espe-

cially when it was found that conventional antihistamines failed to inhibit the release of gastric acid.

Histamine contains an imidazole ring that may exist in two tautomeric forms (Fig. 7.11).

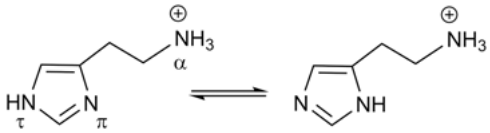


Fig. 7.11: Histamine.

The pK_a of the amino group is 9.80, which means that at the plasma pH (7.4) the side chain of histamine is ionized at 99.6%. The pK_a of the imidazole ring is 5.74 and thus the ring is fundamentally nonionized at pH 7.4.

7.3.2 Two histaminic receptors

Two types of histamine receptors have been proposed, with histamine, the natural messenger, activating both and not distinguishing between them. However, properly designed antagonists should be able to distinguish between them. This means that the conventional antihistamines known in the 1960s were already selective and were able to inhibit the histamine receptors involved in the inflammatory process (now known as H_1 receptors), while being unable to inhibit histamine receptors responsible for secretion (now known as H_2 receptors).

7.3.3 Looking for a leader: histamine

The goal in this context was to vary the structure of the histamine in such a way that it was recognized by the receptor, while joining in such a way that it would act as an antagonist rather than as an agonist.

SAR studies on histamine analogues revealed that the requirements for histamine to bind to H_1 receptors and to the proposed H_2 receptors were slightly different. At the H_1 receptor, the essential requirements are as follows:

- The side chain must have a positively charged nitrogen atom with at least one proton. Quaternary ammonium salts are not active.
- There must be a flexible chain between the cation and the heteroaromatic ring.
- The heteroaromatic ring does not have to be an imidazole, but it must have a nitrogen atom with a pair of electrons at the position adjacent to the side chain.

Requirements for H_2 receptors are the same as for H_1, except that the heteroaromatic ring must have an amidine unit (-HN-CH=N-) (Fig. 7.12).

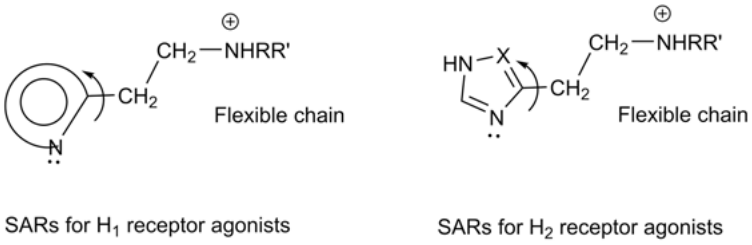


Fig. 7.12: Summary of SARs.

From these results, it appears that the terminal amino group is involved in an ionic interaction with both types of receptors, whereas nitrogen atom(s) of the heteroaromatic ring are bonded *via* hydrogen bond (Fig. 7.13).

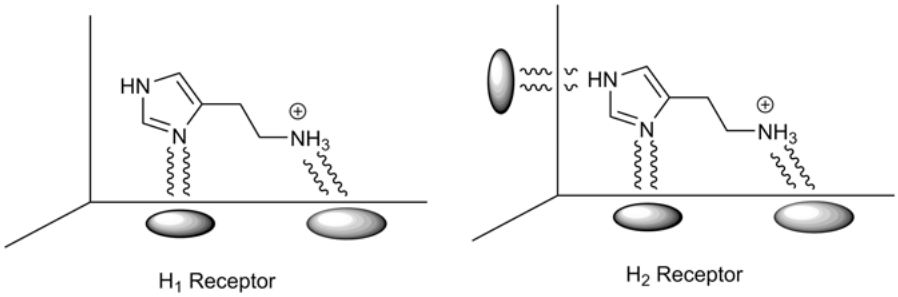


Fig. 7.13: Binding interactions at the H_1 and H_2 receptors.

7.4 Searching for a leader: N^{α}-Guanylhistamine

The strategy employed involved the conversion of an agonist into an antagonist by adding a functional group that would bind to another binding site of the receptor and thereby preventing the form change from taking place for activation (Fig. 7.14).

For example, the fusion of an aromatic ring in NA had been a useful tactic in the design of noradrenaline receptor antagonists, to move from NA to propranolol. The same process was attempted on histamine but none of the resulting compounds proved to be antagonists (Fig. 7.15).

Another approach that had been successfully used in the development of anticholinergic drugs was the addition of nonpolar or hydrophobic substituents. This ap-

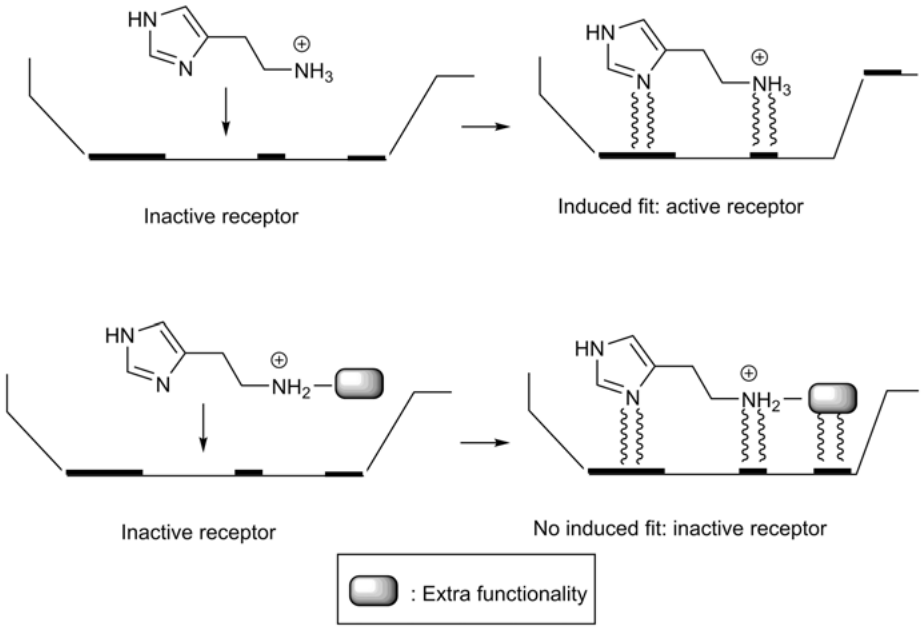


Fig. 7.14: Possible interactions of histamine with its receptor and interactions of an antagonist.

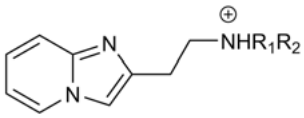


Fig. 7.15: A histamine analogue that did not lead to an antagonist.

proach was used on histamine by means of binding various alkyl and arylalkyl groups at different positions of the histamine backbone. Unfortunately, none of these analogues turned out to be antagonistic. However, an interesting result was obtained that was to be important in later studies. 4(5)-Methylhistamine was found to be a highly selective H_2 agonist showing greater selectivity for H_2 receptors than for H_1 (Fig. 7.16). 4(5)-Methylhistamine (such as histamine) is a highly flexible molecule because of its side chain, but structural studies show that some of its conformations are less stable than others (Fig. 7.17).

In particular, conformation **I** is disfavored due to the steric interaction between the methyl group and the side chain. The selectivity observed suggests that 4(5)-methylhistamine has to adopt two different conformations to bind to the H_1 receptor or to the H_2. Since 4(5)-methylhistamine is more active against the H_2 receptor, it would imply that the required conformation for the H_2 receptor is a stable one (conformation **II**), whereas the conformation required for the H_1 receptor is unstable (conformation **I**).

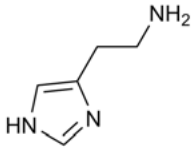
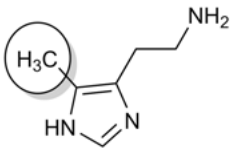
Histamine agonist	H_1 Activity in relation to histamine	H_2 Activity in relation to histamine
	100	100
	0.23	**39.0**

Fig. 7.16: 4(5)-Methylhistamine is a highly selective H_2 agonist showing greater selectivity for H_2 receptors than for H_1

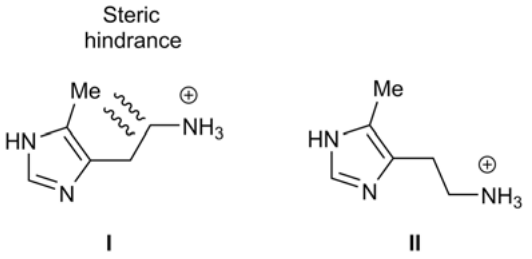


Fig. 7.17: Conformations of 4(5)-methylhistamine: **II** is more stable than **I**.

Despite this interesting result, researchers initially remained far from identifying an H_2 antagonist. More than 200 compounds had been synthesized and none had shown any hint of antagonism. Until then, research had concentrated on finding a hydrophobic binding site on the receptors. From this point onwards, however, the strategy changed to evaluate what would happen should the terminal α-NH_3^+ group be changed by several polar groups. It was reasoned that different polar groups would bind to the same receptor area as α-NH_3^+, but that the geometry of the bond could be sufficiently altered to produce an antagonist. From this study, a breakthrough was achieved with the discovery that N^{α}-guanylhistamine acted very weakly as an antagonist.

Perhaps the guanidino group could act as an antagonist. Several structures were synthesized with the guanidino moiety and without the imidazole moiety, and none showed the desired antagonistic activity demonstrating that both the imidazole ring and the guanidino group were required for antagonist activity.

The structures of *N*-guanylhistamine and histamine were compared. Both structures contain an imidazole ring and a positively charged group bonded by a linker of two carbon atoms. The guanidino group is basic and protonated at pH 7.4, so that this analogue has a similar positive charge to that of histamine (Fig. 7.18).

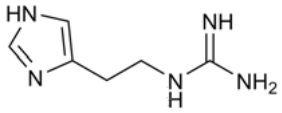


Fig. 7.18: N^{α}-Guanylhistamine.

However, the charge of the guanidino group can be delocalized through the three nitrogen atoms and may potentially be further away from the imidazole ring (Fig. 7.19).

This indicates the possibility that this analogue may be interacting with another receptor-binding site outside of the range of histamine. The histamine cannot reach the antagonist site but the guanidino derivative is able to reach both (Fig. 7.19). Variations were made to synthesize an analogue that only joined the antagonist site. The isothiourea derivative, shown in Fig. 7.20, provided a structure in which the nitrogen atom of the guanidino moiety closest to the imidazole ring was replaced by a sulfur atom. The positive charge of this molecule would now be restricted to the terminal portion of the chain and should interact more strongly with the antagonist binding site.

The antagonist activity was increased, but the compound was still a partial agonist, showing that binding to an agonist site was still possible.

Two analogues were synthesized in which one of the terminal amino groups of the guanidino group was substituted for a methylthio group or a methyl group (Fig. 7.21). Both structures turned out to be partial agonists, but with poorer antagonistic activity.

7.5 The theory of chelation

From the latter two results, it was concluded that both terminal amino groups were required for binding to the antagonist-binding site. It was proposed that the charged guanidino group would be interacting with a carboxylate moiety charged with the receptor via two hydrogen bonds (Fig. 7.22). If any of these terminal amines were absent, the union would be weaker, resulting in a lower level of antagonism.

The chain was extended to three carbon units to see what would happen if the guanidino group was further separated from the imidazole ring. The antagonistic activity increased for the guanidine structure, but unexpectedly decreased for the isothiurea structure. It was therefore proposed that with a two-carbon chain, the hydrogen bond involved the terminal NH_2 groups, but with a chain of three carbon atoms, the H-bond involved a terminal NH_2 group together with the inner NH of the chain (Fig. 7.23).

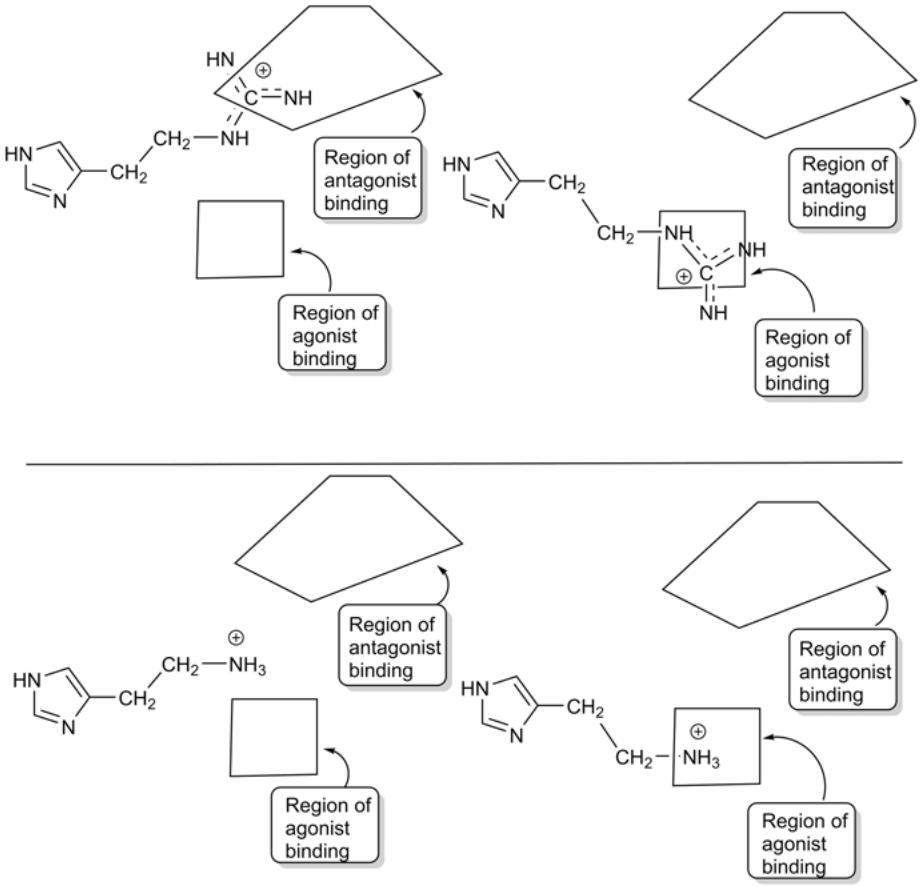


Fig. 7.19: Possible histamine- (agonist only) and N^{α}-guanylhistamine-binding modes.

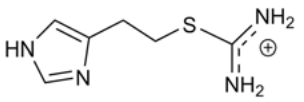


Fig. 7.20: Isothiourea analogue.

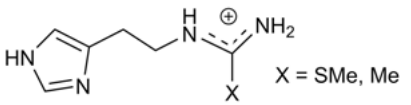


Fig. 7.21: Histamine analogue.

Support was provided for this theory by the finding that replacing one of the terminal NH_2 groups in the guanidine analogue with three carbon atoms did not adversely affect the antagonist activity. This was completely different from the derivative with the bridge of two carbon atoms. These bond interactions are represented in Fig. 7.24.

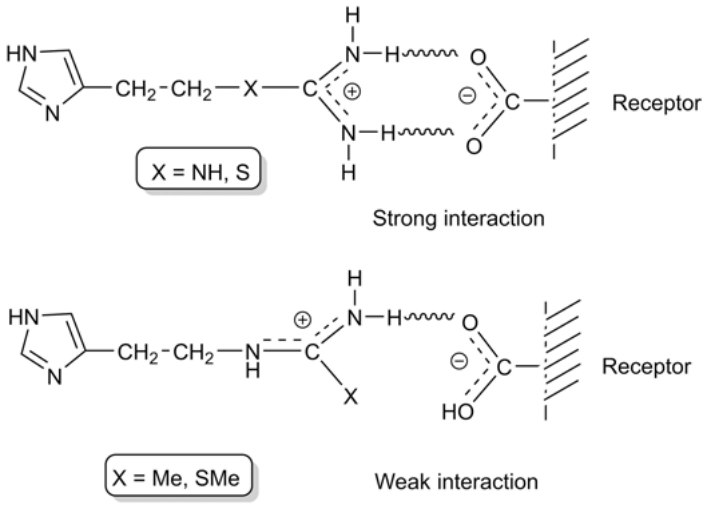


Fig. 7.22: Proposed interaction of the charged guanidino group

7.6 From a partial agonist to an antagonist: Development of burimamide

Is it necessary for the chelating group to be charged? Can a neutral group also chelate with the antagonistic site through hydrogen bonds? The thiourea group was successful: the SK&F 91581 derivative proved to be a weak antagonist with no agonist activity (Fig. 7.25).

Apart from basicity, the properties of the thiourea group are very similar to those of the guanidino group. The thiourea group is neutral. This is due to the group C=S, which exerts a withdrawing-electron effect on the neighboring N, making them non-basic. The finding that a neutral group can be attached to an antagonist site and not to an agonist site could imply that agonist-binding sites involve ionic bonds, while antagonistic effects are based on hydrogen bonds. Further elongation of the chain and the addition of an *N*Me group led to burimamide. These results suggest that chain extension moves the thiourea group closer to the antagonist site and the addition of the NMe group results in increased hydrophobicity (Fig. 7.26).

Burimamide is a highly specific competitive antagonist of histamine at H_2 receptors, and is 100 times more potent than N^{α}-guanylhistamine. Its discovery finally proved the existence of H_2 receptors.

7.7 Development of methiamide

At pH 7.4, it is possible for the imidazole ring to equilibrate between the two tautomeric forms (**III**) and (**IV**) via the protonated intermediate (**V**) (Fig. 7.27).

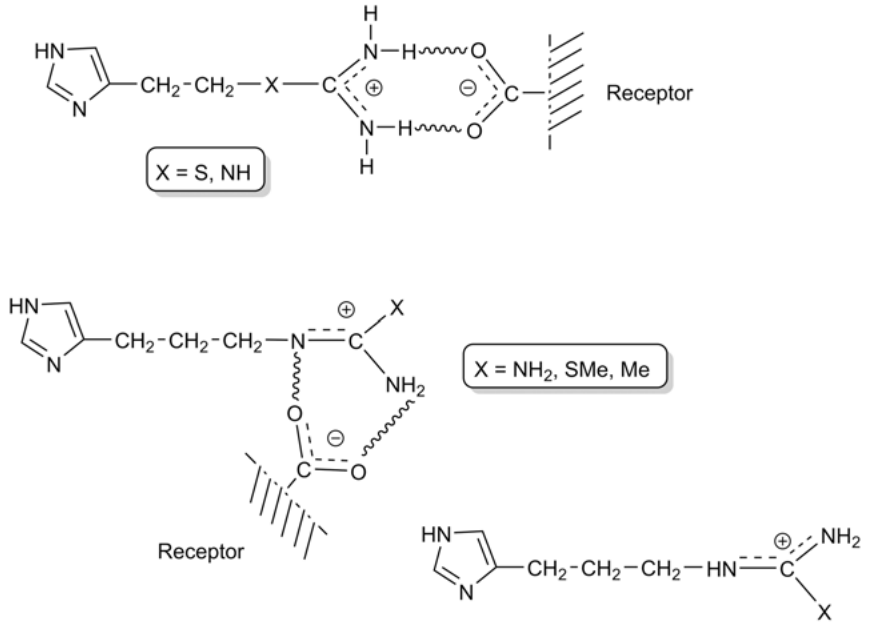


Fig. 7.23: Proposed interaction for analogues with different chain lengths between the terminal functional group and the imidazole ring.

Fig. 7.27 The imidazole ring can be equilibrated between the **III** and **IV** tautomeric forms *via* the protonated intermediate **V**.

According to the hypothesis for receptor binding presented above, the imidazole ring is important for the binding of agonists and antagonists. Therefore, it is reasonable to assume that the preferred imidazole tautomer is the same for both agonists and antagonists. If this is so, then the tautomer preferred for a strong agonist such as histamine should also be the tautomer preferred for a strong antagonist.

In histamine, the exocyclic amino group is protonated at physiological pH, thereby exerting a powerful electron-withdrawing effect on the imidazole ring. This effect -I is more important for the nitrogen closest to the side chain, such that the hydrogen atom on the nitrogen N^{π} will be more acidic than the one that is attached to the N^{τ}. For this reason, the latter tautomer (N^{τ}) is more stable than the π one (N^{π}) in histamine (Fig. 7.28). By contrast, the thiourea group of burimamide exerts a very weak electron-releasing effect and therefore, the N^{π} tautomer is favored.

It is therefore necessary to make the burimamide side chain electron withdrawing rather than electron releasing. This could be achieved by inserting an electronegative atom in the side chain. In other words, an isostere of the methylene group is required.

The sulfur atom is a good isostere of the methylene group, since they similar van der Waals radii and bond angles. The substitution site was chosen based on synthetic reasons (Fig. 7.29).

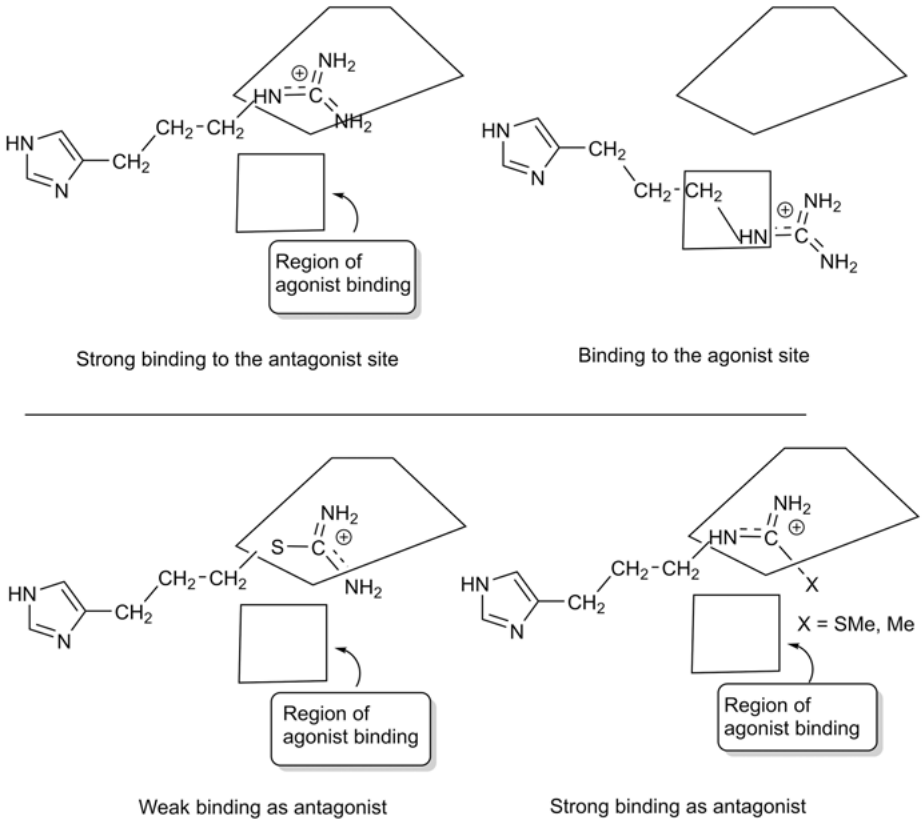


Fig. 7.24: Effect of guanidino group variation on the binding to the antagonist site.

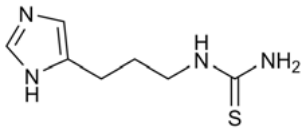


Fig. 7.25: SK&F 91581.

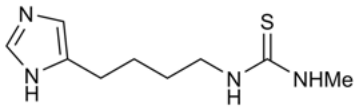


Fig. 7.26: Burimamide.

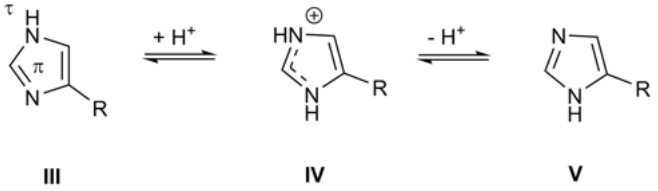


Fig. 7.27: Burimamide.

Since the side chain of thiaburimamide is electron withdrawing, the tau tautomer would also be favored (τ). It was argued that this tautomer could be increased if a group was placed at position five of the ring. At this position, the inductive effect would have a more pronounced impact on the neighboring nitrogen atom (N^{τ}). However, it was important to choose a group that did not negatively interfere with the receptor. The methyl group was chosen as it was known that (4)-methylhistamine was found to be highly selective against the H_2 receptor. Methiamide was obtained, showing the greatest antagonistic activity (Fig. 7.30).

Methiamide is 10 times more potent than burimamide and showed great promise as an antiulcer agent. Unfortunately, a number of patients suffered from kidney problems and granulocytopenia, a disease that leads to a reduction in leukocytes and makes patients more susceptible to infection.

7.8 Cimetidine development

Side effects of methiamide were associated with the thiourea moiety, a group not particularly common in the biochemistry of the human organism. The urea derivative was found to be less active. The guanidine analogue was also less active and it was interesting that this compound had no agonist activity. This contrasts with the guanidine derivative, with three carbon atoms as a linker, which did act as a partial agonist. Thus, the guanidine analogue of Fig. 7.31 was the first example of guanidine with pure antagonistic activity.

One possible explanation is that the four-unit chain extends the guanidino group beyond the agonist binding-site, whereas the 3-unit chain allows binding to both agonist and antagonist sites. The guanidine moiety is nontoxic, since this moiety is present in the natural amino acid arginine. The challenge of increasing its activity remained. It seemed likely that the low levels of activity were due to the fact that the basic guanidine would be ionized at a pH of 7.4. The challenge was to make the guanidine moiety neutral, which is a difficult task considering that guanidine is a strong base.

A literature search revealed a useful study on the ionization of monosubstituted guanidines (Fig. 7.32).

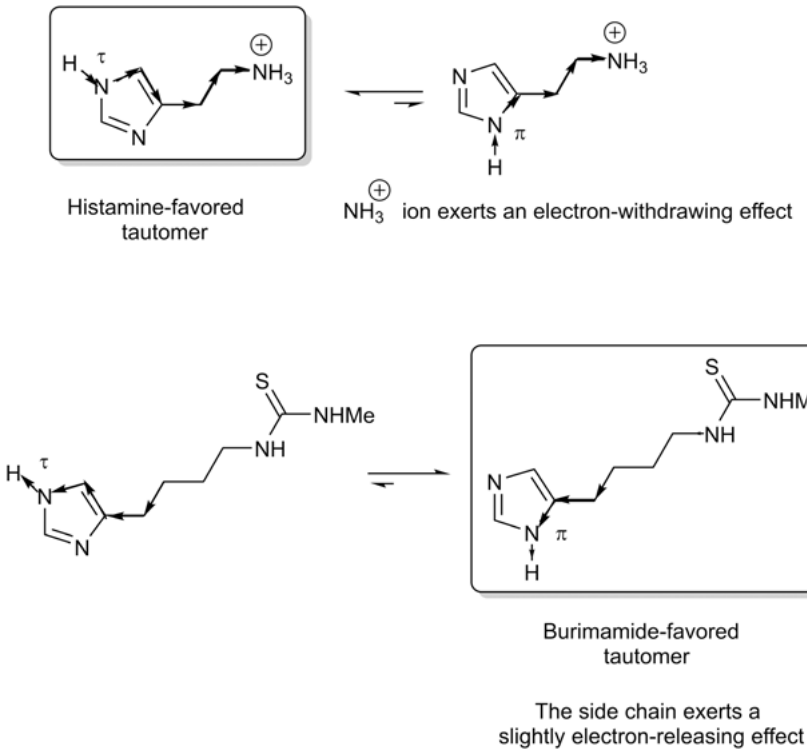


Fig. 7.28: Preferred tautomeric forms of histamine and burimamide.

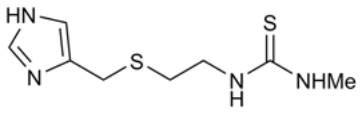


Fig. 7.29: Thiaburimamide.

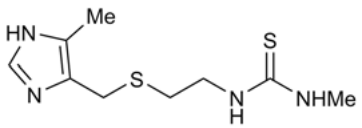


Fig. 7.30: Methiamide.

pK_a was found to be inversely proportional to the electron-withdrawing potency of the substituent (Fig. 7.33).

Thus, the strongly electron-withdrawing substituents make the guanidino group less basic and therefore less ionized. Nitro and cyano are particularly strong electron-withdrawing groups. pK_as of cyanoguanidine and nitroguanidine are 0.4 and 0.9 respectively. Both nitroguanidine and cyanoguanidine analogues of methyamide were

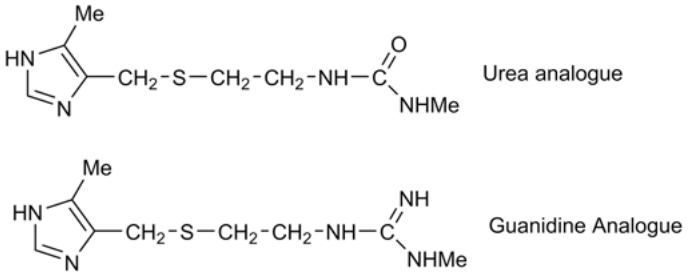


Fig. 7.31: Cimetidine analogues.

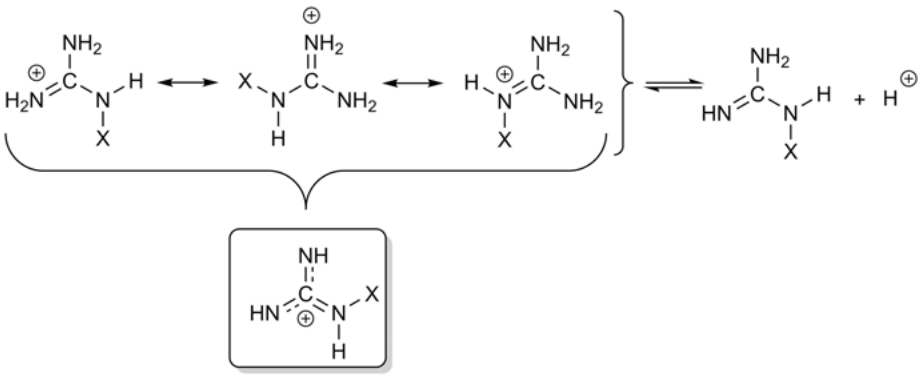


Fig. 7.32: Ionization of monosubstituted guanidines.

synthesized. The first analogue (cimetidine, Fig. 7.34) was the most potent and was chosen for clinical studies.

The fact that methiamide and cimetidine are good H_2 antagonists with similar activities shows that the cyanoguanidine group is a good isostere of the thiourea group. This is the case in spite of the fact that three tautomeric forms are possible for the guanidino group compared to the unique one of the thiourea group (Fig. 7.35).
In fact, this is more apparent than real, since the *N*-cyano imino tautomer (**II**) is the most important form, and justifies why cyanoguanidino has a great structural similarity to thiourea.

7.8.1 Cimetidine metabolism

Cimetidine is metabolically stable and excreted essentially unchanged.

The only metabolites that have been identified occur due to the oxidation of the sulfur bridge or the oxidation of the methyl group of the imidazole ring (Fig. 7.36).

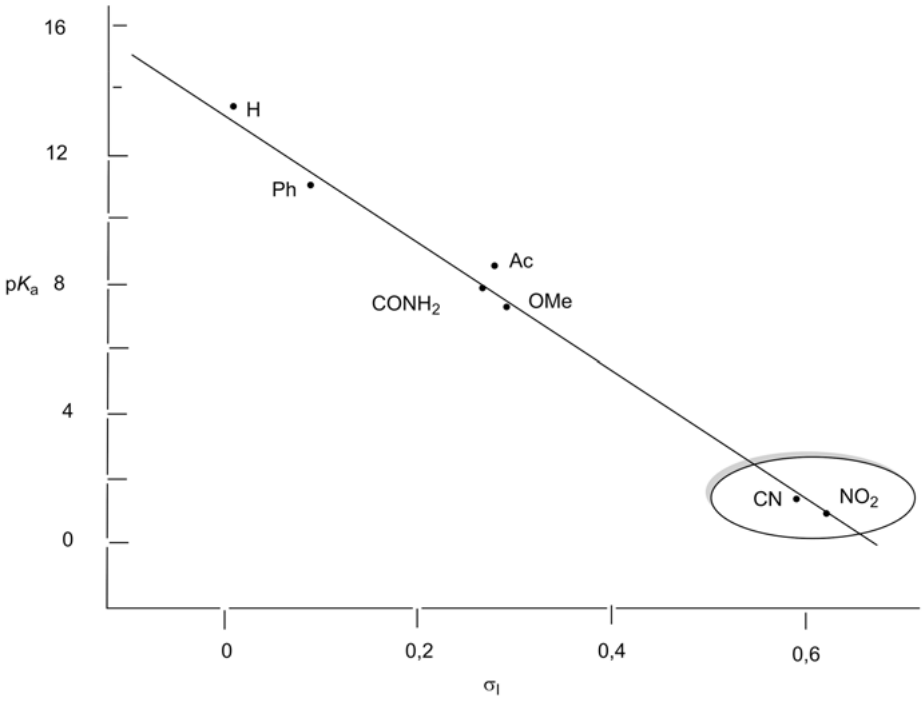


Fig. 7.33: pK_a values *versus* the inductive constant (σ_I) of substituents X in Fig. 7.32.

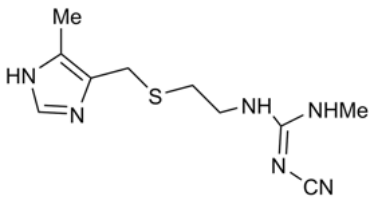


Fig. 7.34: Cimetidine.

7.8.2 Cimetidine synthesis

The preparation of the imidazole ring of cimetidine, shown in Scheme 7.7, is achieved by condensing methyl 2-chloroacetylacetate with formamide (Bredereck reaction), followed by the selective reduction of the ester group to hydroxymethyl, with liquid Na/NH_3.

The preparation of the cimetidine chain occurs in three stages: (1) heating the imidazole with cysteamine in isopropanol yields the 2-aminoethylthiomethyl derivative; (2) subsequent nucleophilic exchange with *N*-cyanoimido-*S*,*S*-dimethylthiocarbonate and (3) treating the resulting intermediate with an excess of methylamine to displace the mercaptan group (Scheme 7.8).

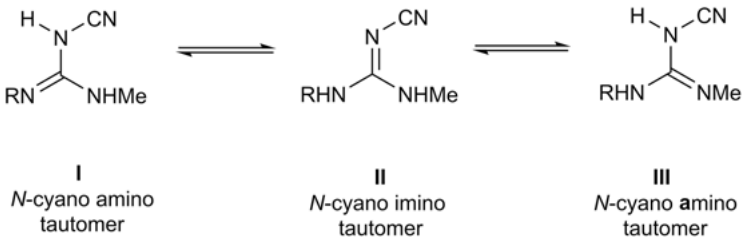


Fig. 7.35: Three tautomeric forms of the guanidine residue.

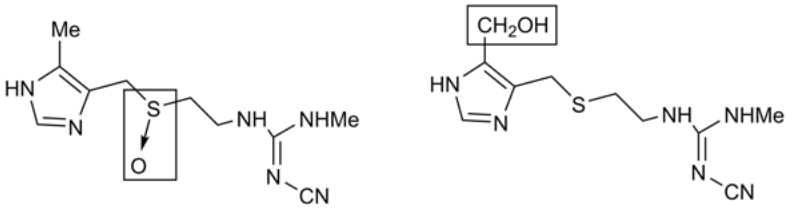


Fig. 7.36: Cimetidine metabolism.

7.8.3 Conformational isomers of cimetidine

To date, the theory favored for the binding of cimetidine to the H_2 receptor has been that of a bidentate hydrogen interaction. However, for this type of chelation to be carried out, the guanidino group of cimetidine would have to adopt a *Z,Z* conformation.

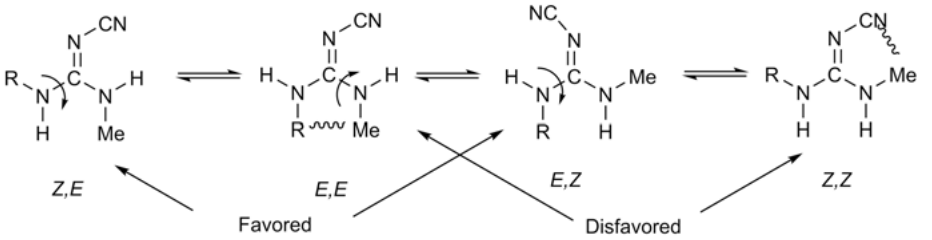
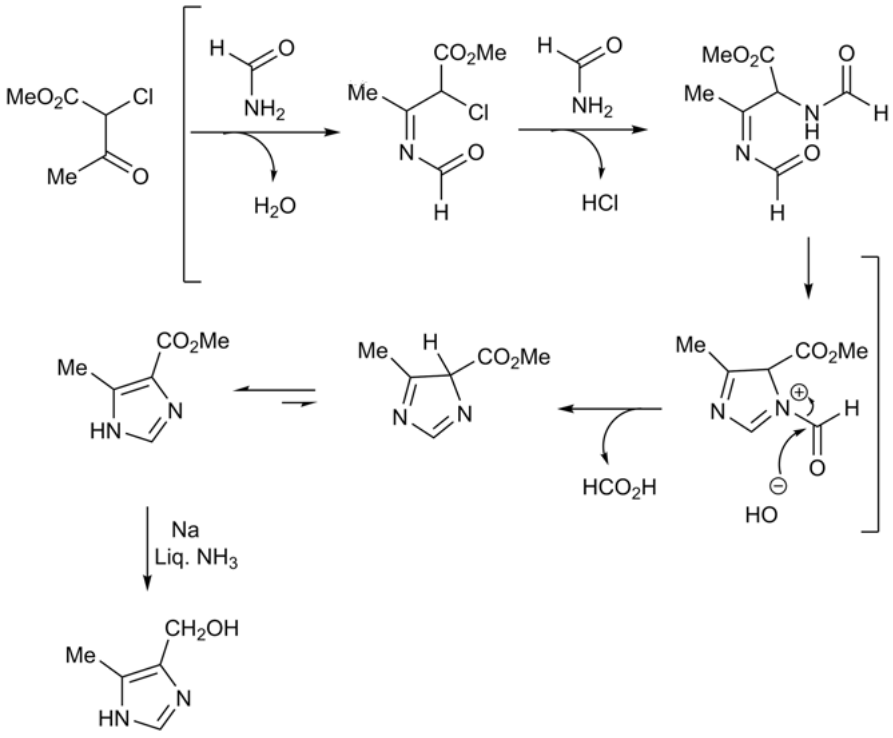


Fig. 7.37: Conformations of the guanidino group of cimetidine.

Yet, X-ray and NMR studies show that cimetidine exists as an equilibrium mixture of *E,Z* and *Z,E* conformations (Fig. 7.37). One possibility is that the guanidine unit is bound by hydrogen bonding to two distinct sites and not to a single carboxylate group (Fig. 7.38).

To prove this, a strategy to reduce the number of conformations was employed, incorporating part of the guanidine structure into a ring (Fig. 7.39).



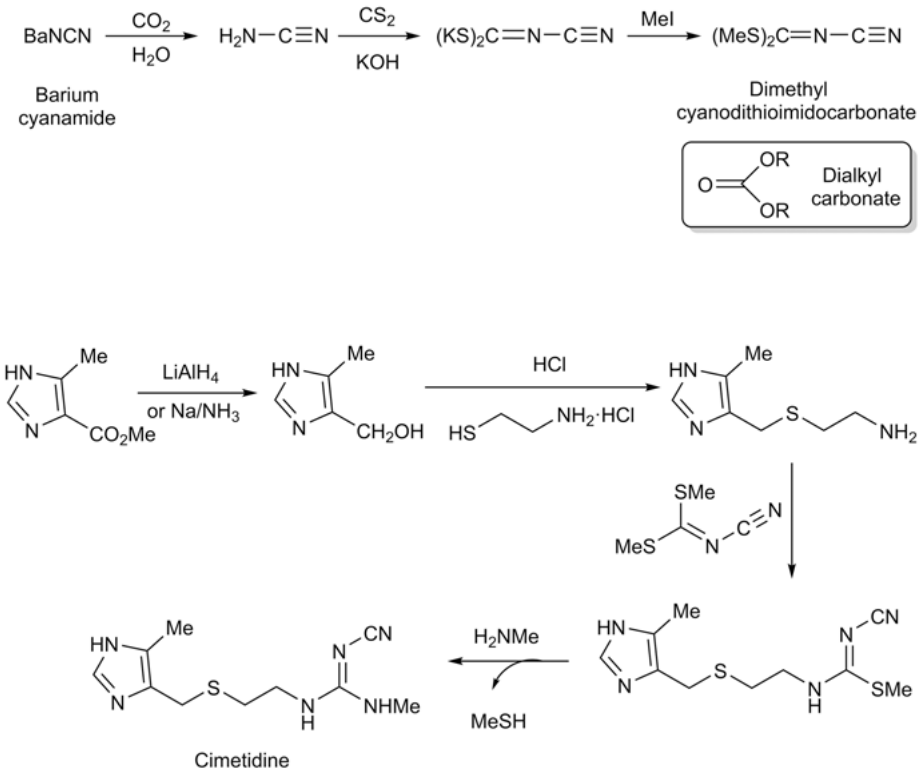
Scheme 7.7: Synthesis of 4-hydroxymethyl-5-methylimidazole.

The isocytosine ring has also been used to limit the number of conformations available (Fig. 7.40).

7.8.4 Desolvation

The guanidino and thiourea groups are polar and hydrophilic. This implies that they are probably solvated (i.e., surrounded by a "water layer"). Before the hydrogen bond is established, the water layer has to be removed. The more solvated the group is, the more difficult the interaction with the receptor becomes. If desolvation is a factor influencing biological activity, a reduction of the polar group solvation should increase activity. One way to achieve this would be to increase the hydrophobic character of the terminus group of the molecule.

A study was carried out on a set of cimetidine analogues containing different flat aminal systems (Z) in order to investigate whether there was any relationship between the antagonistic activity and the hydrophobic character of the aminalic systems (Fig. 7.41).



Scheme 7.8: Synthesis of cimetidine.

This study showed that antagonistic activity was proportional to the hydrophobicity of the aminalic unit Z and supported the theory of desolvation (Eq. (7.1) and Fig. 7.42):

$$\log(1/C) = 2.0 \log \pi + 7.4 \tag{7.1}$$

An important finding is that the nitroketeneaminalic group behaved as an outlier (Fig. 7.42). The reasoning for this will be considered next.

7.8.5 Development of the ketenaminal (or diaminonitroethylene) group

It was decided to see what would happen if the polar imine nitrogen of cimetidine was replaced by a nonpolar carbon atom. This was expected to give rise to the ketenaminalic group. Unfortunately, ketenaminals are more likely to exist as their amidine tautomers, unless a strong electronegative group (e.g., NO_2) is attached to the C atom (Fig. 7.43).

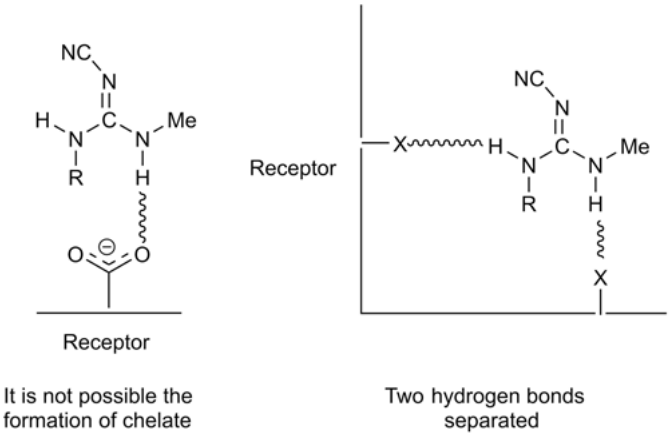


Fig. 7.38: Alternative theory for the binding of cimetidine to the agonist site.

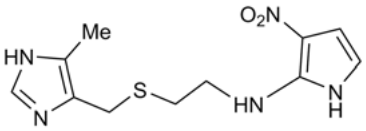


Fig. 7.39: Nitropyrrol derivative of cimetidine.

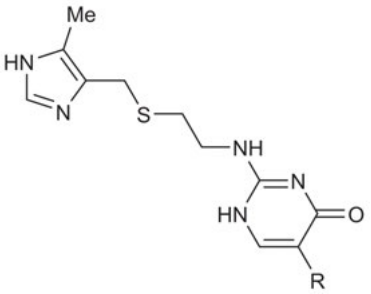


Fig. 7.40: An isocytosine ring incorporated into a moiety present in cimetidine.

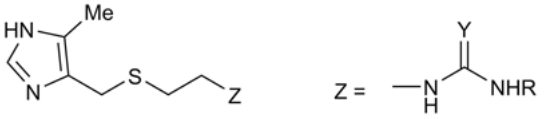


Fig. 7.41: Analogues of cimetidine with flat aminal systems (Z).

The compound with the above moiety was more active than one might think. This indicates the presence of another variable influencing its biological activity and further studies focused on the orientation of the dipole moment (Fig. 7.44).

In Fig. 7.44 the orientation of the dipole moment is defined by the φ angle between the dipole moment and the N-R bond. Compounds with the cyanoguanidino,

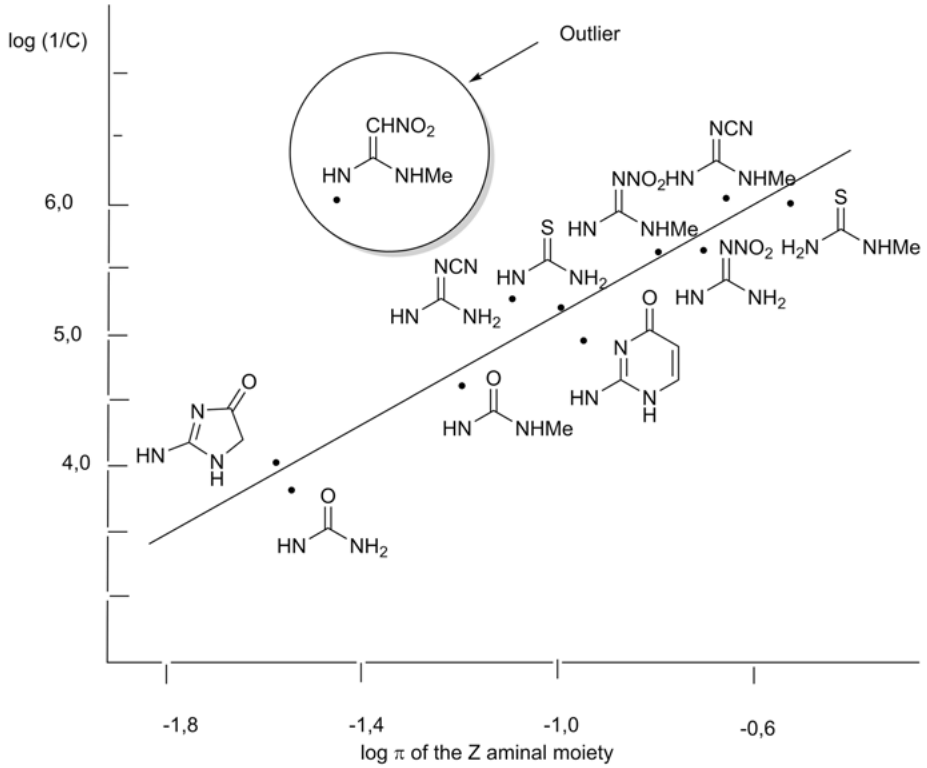


Fig. 7.42: The antagonist activity is proportional to the hydrophobicity of the Z-aminalic moiety.

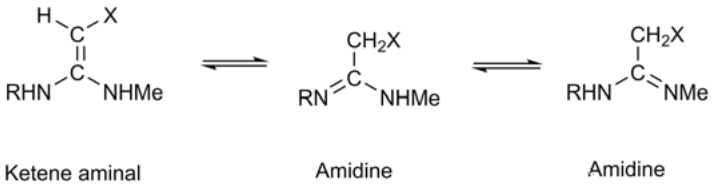


Fig. 7.43: Tautomeric equilibrium.

nitroketenaminal and nitropyrrole groups have a high antagonistic activity and have orientations of the dipole moments of 13°, 33° and 27°, respectively. The isocitosine and imidazolinone groups give rise to a lower activity and have orientations of 2° and -6°, respectively. The strength of the dipole moment (μ) does not seem to be crucial.

A dipole-dipole interaction takes place when the drug approaches the binding site. The dipoles line up, orientating the drug, and a good interaction with the binding site occurs if the binding groups are positioned correctly in relation to the binding regions. This produces a good activity (Fig. 7.45).

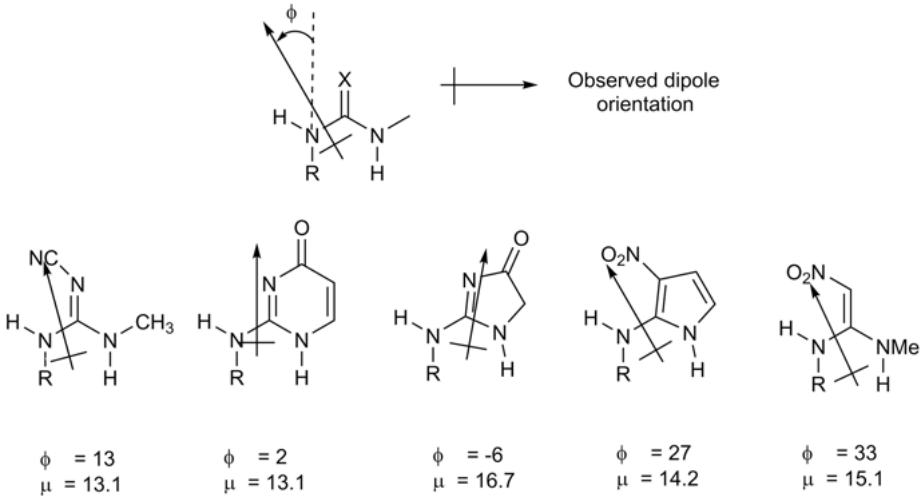


Fig. 7.44: Dipole moments of several antagonistic groups.

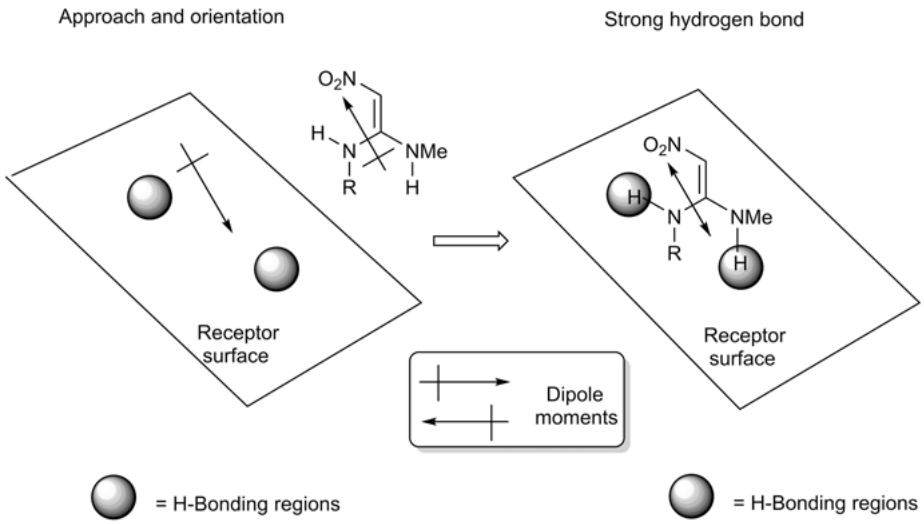


Fig. 7.45: Orientation effects on the receptor active site.

This directs the drug in a specific way before the hydrogen bond takes place and determines the strength of the hydrogen bond to be established. If the dipole moment is correctly oriented as in the ketenaminal analogue, the group will be correctly positioned for a strong hydrogen bond and will lead to high activity.

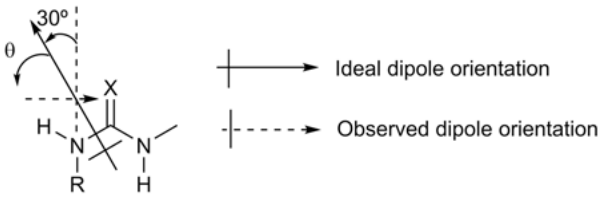


Fig. 7.46: Ideal and observed orientations of the dipole.

QSAR studies were performed to determine the φ optimal angle for the activity. This gave an optimal angle of 30° (Fig. 7.46). A correlation was obtained between the orientation of the distribution coefficient and the activity (Eq. 7.2):

$$\log(1/C) = 9.12 \cos \varphi + 0.6 \log \pi - 2.71 \tag{7.2}$$

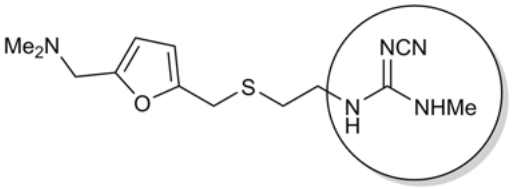
The term cos φ shows that activity decreases if the orientation of φ separates from the ideal value of 30°. With this ideal angle, φ is 0° and cos 0°= 1, whereby the biological activity is maximal. The nitroketenaminal group did not give rise to a more powerful cimetidine analogue, but we will see it again in ranitidine.

7.9 Variation of the imidazole ring and the cyanoguanidine moiety of cimetidine: ranitidine

Glaxo showed that the imidazole could be replaced by a furan with a substituent containing a nitrogen atom and thus, molecule **7.1** was obtained (Fig. 7.47). Nevertheless, its variable melting point and low crystallinity made its pharmaceutical development difficult and the subsequent change of the cyanoguanidino group of **7.1** by the nitroketenaminal group gave rise to ranitidine.
Ranitidine has fewer side effects than cimetidine, lasts longer and is 4–5 times more active. The SAR results are as follows:

- The nitroketenaminal group is optimal for activity, but can be replaced by other π-planar systems capable of forming hydrogen bonds.
- The activity would decrease if a sulfur atom was placed next to the ring.
- Replacing the furan with more hydrophobic rings, such as phenyl or thiophene, reduces activity.
- Disubstitution at 2.5 is the best model for the furan ring.
- Substitution of a methyl group at C-3 of the furan ring eliminates the activity, while the equivalent substitution in the imidazole series increases it.

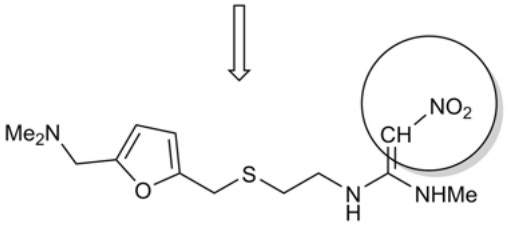
These results imply that cimetidine and ranitidine do not interact in the same way with the H_2 receptor. This assertion is supported by the finding that the corresponding nitroethyleneaminic group attached to cimetidine leads to a decrease in activity.



7.1

Potency = cimetidine

Its variable melting point and low crystanillity difficulted its pharmaceutical development



Ranitidine
4-5 times more potent than cimetidine

Fig. 7.47: Variation of the imidazole ring and the cyanoguanidine moiety of cimetidine to give ranitidine (Zantac®).

7.10 Summary of cimetidine design

The evolution in the development of cimetidine is summarized in Fig. 7.48. Note the change in the tautomeric forms from burimamide to methiamide. The trade name of Tagamet corresponds to the accumulation of uppercase letters of the phrase "anTAGonist And ciMETidine".

7.11 Comparison between H_1 and H_2 antagonists

At the structural level, the differences between the two types of antagonists are quite remarkable, as shown in Fig. 7.49.

H_1 antihistamines are compounds with high lipophilicity due to terminal aryl groups. This results in greater penetration into the CNS and central side effects. In contrast, H_2 antagonists are polar and hydrophilic molecules, largely due to the high dipole moment of the substituents at the terminal side chain. These substituents

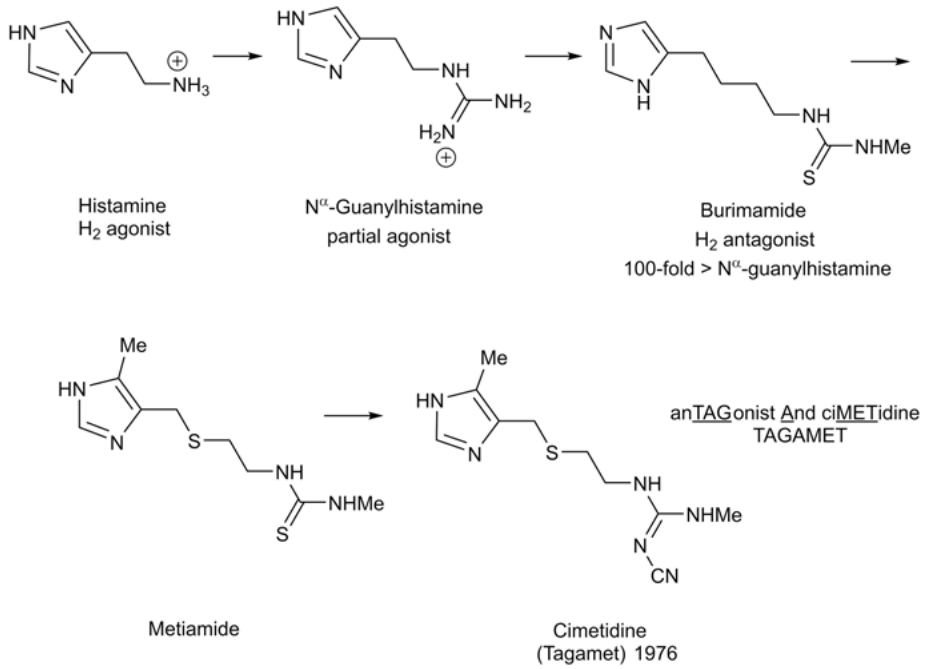


Fig. 7.48: Evolution in the development of cimetidine.

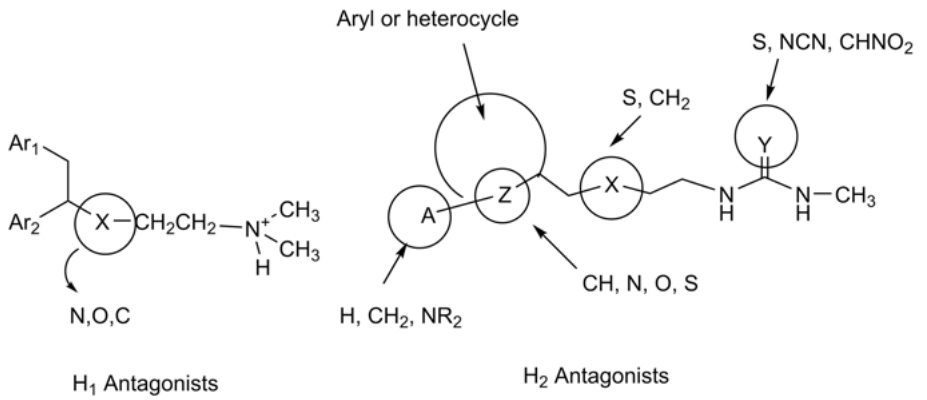


Fig. 7.49: Structural differences between H_1 and H_2 antagonists.

present delocalized and low ionized systems at physiological pH, which explains their limited penetration into the CNS as well as the practical absence of effects at this level.

8 Enzymatic inhibitors I

8.1 Goals

- Foster knowledge of the drugs that act at these levels.
- Foster knowledge of the fundamental role of enzymatic inhibition in the design of new drugs.
- Make the reader aware of the existence of various peptides that play important roles in life.
- Introduce the student to modern antihypertensive drugs.

8.2 Introduction

In general, enzymes are simpler pharmacological targets to study than receptors, since they are easier to purify and their active sites and their catalysis mechanisms are relatively accessible. It is useful to distinguish between two possible situations when it comes to using an enzyme as a target for drug action:

- First, they may be *pharmacodynamic enzyme inhibitors*, which act when the causes or symptoms of a disease are due to an alteration of an enzymatic reaction usually occurring in the healthy organism.
- Second, there are *enzymatic chemotherapeutic inhibitors*, which act when a disease is caused by external agents, generally by bacteria, viruses, fungi or parasites. These drugs can inhibit important enzymes for survival that are not found in the host or, if present, can inhibit their function selectively. Chemotherapeutics are also called antitumor agents that act by inhibiting enzymes for which there are only quantitative differences between normal and tumor cells, whereby selective inhibition is practically impossible, at least in vitro.

8.3 Carbonic anhydrase (CA) inhibitors

Carbonic anhydrase (CA) is an enzyme that catalyzes the formation of carbonic acid from carbon dioxide and water (Fig.8.1):

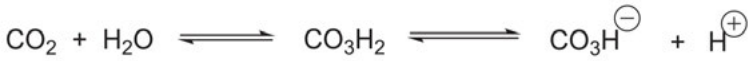


Fig. 8.1: Formations of carbonic acid.

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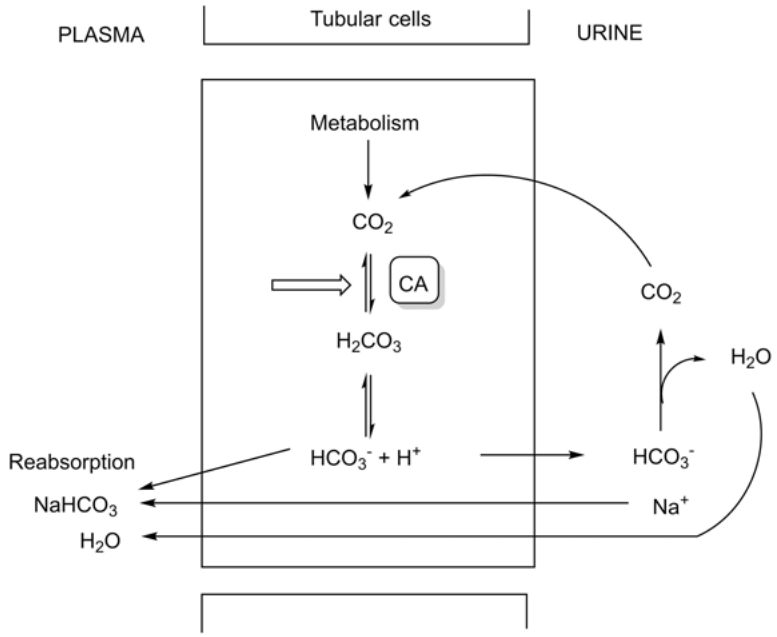


Fig. 8.2: Mechanism of sodium bicarbonate reabsorption by CA.

CA is located in the walls of the proximal kidney tubule cells and its net effect is the reabsorption of sodium bicarbonate together with the osmotic equivalent of water (Fig. 8.2).

It has been observed that diuretic effects of certain sulfonamides are associated with their ability to competitively inhibit CA enzymes. This inhibitory action is most commonly undertaken by in the primary sulfonamides, given their structural analogy with carbonic acid, the natural substrate of the enzyme (Fig. 8.3). A similar interaction with the active center of the enzyme for both types of compounds can be postulated. On the other hand, it is essential that the primary sulfonamide be relatively acidic. This is achieved by introducing electron-withdrawing aromatic systems, such as 1,3,4-thiadiazole, present in various diuretic sulfonamides such as acetazolamide, the first drug of this group (Fig. 8.4).

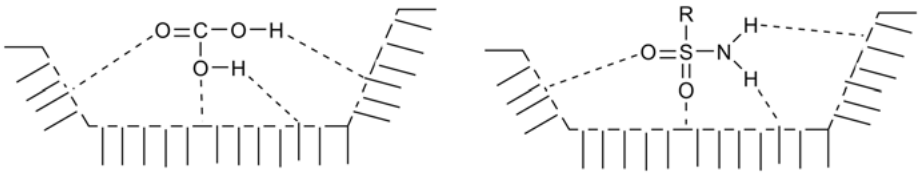


Fig. 8.3: Interaction of a primary sulfonamide and carbonic acid with the active center of CA.

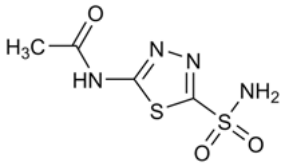


Fig. 8.4: Acetazolamide.

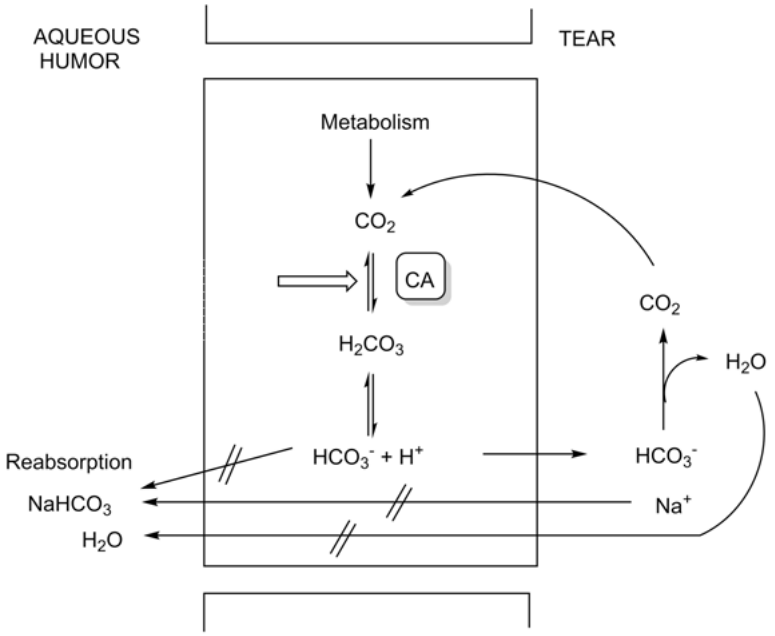
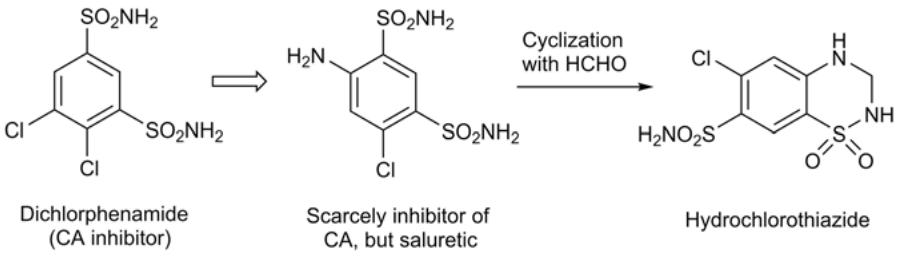


Fig. 8.5: Decreased aqueous humor volume: utility in the treatment of glaucoma.

CA is also found in other tissues where its inhibition may lead to useful therapeutic effects. Thus, since it participates in the formation of aqueous humor, CA inhibitors will lead to a decrease in the reuptake of sodium bicarbonate and water from the tear to the aqueous humor, which is useful in the treatment of glaucoma (Fig. 8.5).

With the prolonged use of diuretic CA inhibitors, urine becomes more alkaline and blood becomes more acidic. When acidosis occurs, CA inhibitors lose efficacy as diuretics and remain ineffective until the body's acid-base balance is restored. For this reason, this class of compounds has a limited utility as diuretics. Today, they are most commonly used in the treatment of glaucoma, in which they inhibit CA in the eye, reduce the rate of aqueous humor formation and consequently reduce intraocular pressure.

Research in the field of diuretic sulfonamides has been very successful in recent years. Through molecular modifications of CA inhibitors, diuretic sulfonamides that are structurally related, but are governed by different mechanisms to those outlined



Scheme 8.1: Structural variation of dichlorphenamide leading to hydrochlorothiazide.

above, have been obtained. This is how so-called thiazides, whose basic nucleus is 1,2,4-benzothiadiazine, emerged.

In the case of hydrochlorothiazide, a cyclic sulfonamide derived from the structural variation of dichlorphenamide, a CA inhibitor is the lead compound (Scheme 8.1).

Thiazides inhibit the active reabsorption of chloride ions in the distal tubule of the kidney and consequently also inhibit the reabsorption of sodium ions. The decrease in the reabsorption of sodium chloride, together with its osmotic equivalent of water, gives rise to the saluretic effect, an increased excretion of sodium and chloride, characteristic of this family of diuretics.

8.4 Renin-angiotensin pathway

The renin-angiotensin system is a complex, highly regulated pathway that is essential in the regulation of blood volume, electrolyte balance and blood pressure.

It consists of two fundamental enzymes, renin and angiotensin-converting enzyme (ACE), whose purpose is to release angiotensin II from its endogenous, angiotensinogenic precursor (Fig. 8.6). Angiotensin II is a potent vasoconstrictor. Angiotensinogen contains 452 amino acids. It is abundant in plasma and is continuously synthesized and secreted by the liver. The most important part of this compound is the Leu^{10}-Val^{11} bond. This bond is broken down by renin and produces the decapeptide angiotensin I. The peptidic Phe^{8}-His^{9} peptidic bond of angiotensin I is cleaved by ACE to produce the octapeptide angiotensin II. Fig. 8.6 shows drugs that are of therapeutic importance (for the treatment of high blood pressure) and act at different levels within the renin-angiotensin pathway, such as captopril, losartan, and aliskiren. The latter was the result of the collaboration of two pharmaceutical companies, Novartis and Speedel. It was approved by the FDA in 2007 for the treatment of primary hypertension. Scientists in these laboratories knew that as a reaction to the chronic use of other antihypertensive substances affecting the renin-angiotensin-aldosterone axis, the organism increased renin production, causing the drugs to lose some of their

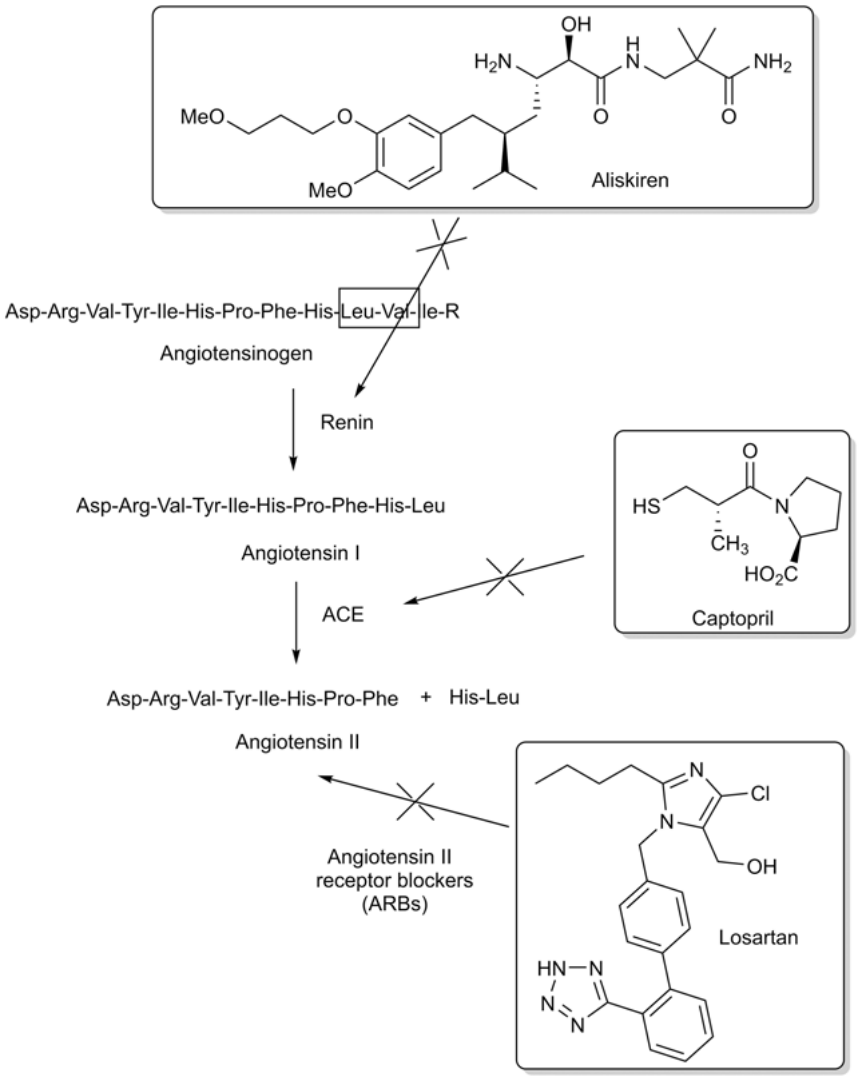


Fig. 8.6: Schematic representation of the renin-angiotensin pathway. The angiotensinogen peptidic bond is highlighted. Drugs of therapeutic importance and acting at various levels within this route are indicated.

effectiveness. It was thought that this would be avoided by directly attacking renin activity. In the following, only the family of "prils" will be studied.

ACE inhibitors, such as captopril and its analogues, have been very successfully used as antihypertensive drugs.

8.4.1 Angiotensin II antagonists. X-ray crystallographic studies

Traditional drug design strategies are usually carried out before the structure of the target is known, so the results obtained are often useful for providing information about the target protein and its drug binding sites. If the protein can be isolated and crystallized, knowledge of its X-ray structure facilitates the entire drug design process, since it can provide information on the structure of the binding site of the protein with the drug. Unfortunately, the X-ray structure of the protein itself does not indicate where the binding site lies, so it is better to obtain the structure of the macromolecule with an inhibitor or antagonist bound to the binding site. The structure can be downloaded (from the corresponding database) into a computer and the complex can be studied to see how the ligand binds to the protein. Once the important functional groups attaching the drug to the binding site have been identified, computer modelling can be used to remove the ligand and add potential drugs to see how they fit together. This also allows for the identification of regions at the binding site that are not occupied by the drug and directs the pharmaceutical chemist to the modifications and additions to the leader compound that may be needed.

Unfortunately, not all enzymes and receptors can be crystallized, especially those that are bound to the membrane. However, structural and mechanistic information on receptors or analogous enzymes can be very useful. For example, ACE is a membrane-bound enzyme, which makes it difficult to isolate and study. It is a member of a group of enzymes called zinc metalloproteinases and catalyzes the hydrolysis of a dipeptide from the terminal part of a decapeptide called angiotensin I to give the octapeptide angiotensin II (Fig. 8.6).

Angiotensin II is an important hormone that causes a constriction of blood vessels, resulting in an increase in blood pressure. Therefore, ACE inhibitors are potential antihypertensives, since they inhibit the production of angiotensin II.

Although the enzyme ECA could not be isolated, the design of ACE inhibitors was aided by studies of the structure and mechanism of action of another zinc metallopro-

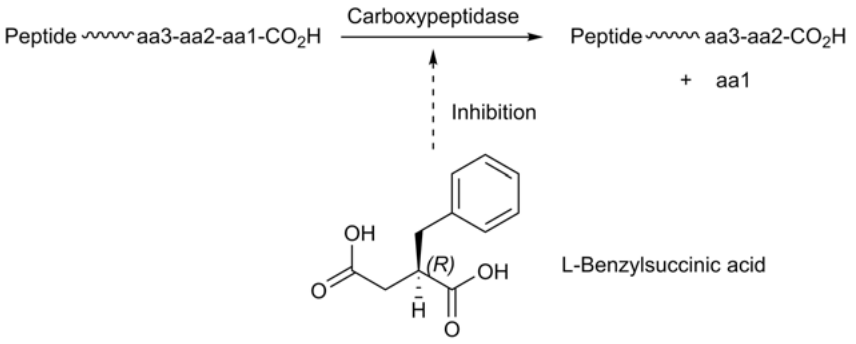


Fig. 8.7: Hydrolysis of the terminal amino acid of a peptide chain by the carboxypeptidase enzyme.

teinase, called carboxypeptidase. The carboxypeptidase enzyme breaks the terminal amino acid of a peptide chain, and is inhibited by L-benzylsuccinic acid (Fig. 8.7).

The carboxypeptidase active center contains a charged arginine (Arg^{145}) and a zinc ion, which are crucial for binding the peptide substrate. The peptide is bound in such a way that the terminal carboxylic acid binds ionically to the arginine, while the carbonyl group of the terminal peptide bond interacts with the zinc ion. There is also a pocket called S1', which can accept the side chain of the terminal amino acid (Phe in the example). Hydrolysis of the terminal peptide then takes place (Fig. 8.8).

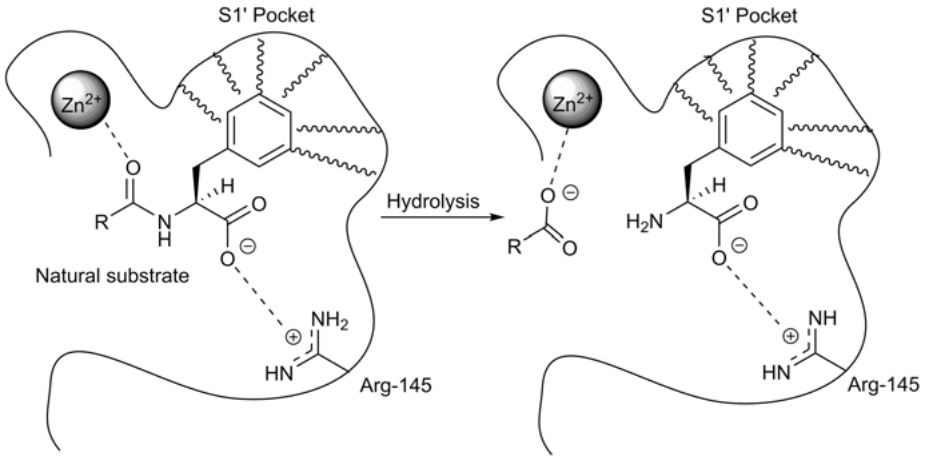


Fig. 8.8: Interactions of a substrate bound to the binding site of the active center of carboxypeptidase.

The design of carboxypeptidase inhibitors such as L-benzylsuccinic acid was based on the hydrolysis products arising from this enzymatic reaction. The benzyl group was included to occupy pocket S1', while the adjacent carboxylate anion needed to be present to form an ionic interaction with Arg^{145}. The second carboxylate is present to act as a zinc ion ligand, mimicking the carboxylate ion of the hydrolysis product. L-Benzylsuccinic acid binds to the active site as shown in Fig. 8.9. However, a hydrolysis of L-benzylsuccinic acid is not possible, since there is no peptide bond, so the enzyme is inhibited and the compound remains bound.

Knowledge of the above mechanism and inhibition facilitated the design of ACE inhibitors. First, it was assumed that the active site contains the same zinc ion and arginine group. The ECA breaks a dipeptide unit from the peptide chain instead of an amino acid and therefore these groups (Zn^{2+} and the arginine residue) should probably be further away from each other. Therefore, an inhibitor analogous to benzylsuccinic acid should be an amino acid with the succinyl moiety. Succinyl proline was

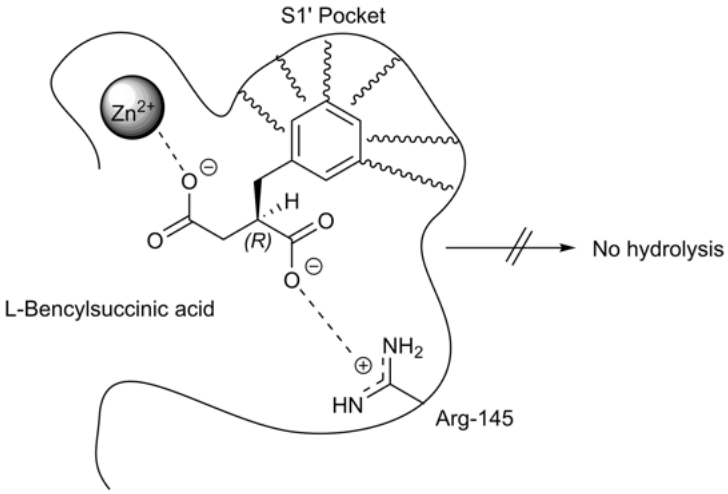
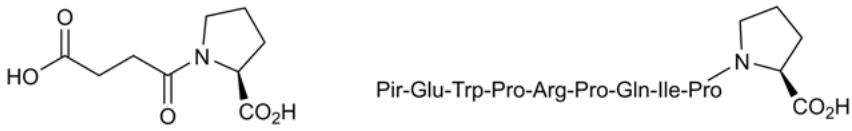


Fig. 8.9: Inhibition by L-benzylsuccinic acid (*R*-enantiomer).



Succinyl proline: IC_{50} = 628 μM

Teprotide: IC_{50} = 0.9 μM

Fig. 8.10: Inhibitors of angiotensin-converting enzyme (ACE).

chosen, since proline is present in the terminal part of the known ACE inhibitor, teprotide (Fig. 8.10).

Succinyl proline actually inhibited ECA and was proposed as a prototype, since both carboxylate groups are ionized, one interacting with the arginine residue and the other with the zinc ion. However, it was reasoned that there should be more pockets available to accommodate the side chains of amino acids (pockets S1 and S1'). The extension strategy was used to find a group that would fit into pocket S1' and increase activity.

A methyl group fitted perfectly into the pocket and led to increased activity. The next step was to see if there was a better group than the carboxylate ion interacting with zinc, and the thiol group was found to lead to an increase in activity. Through this process, captopril became the first commercially available nonpeptidic ACE inhibitor (Fig. 8.11).

The next step was to use the strategy of extension with the aim of finding a group that would completely fill the pocket S1. This time, a derivative of glutaryl proline was

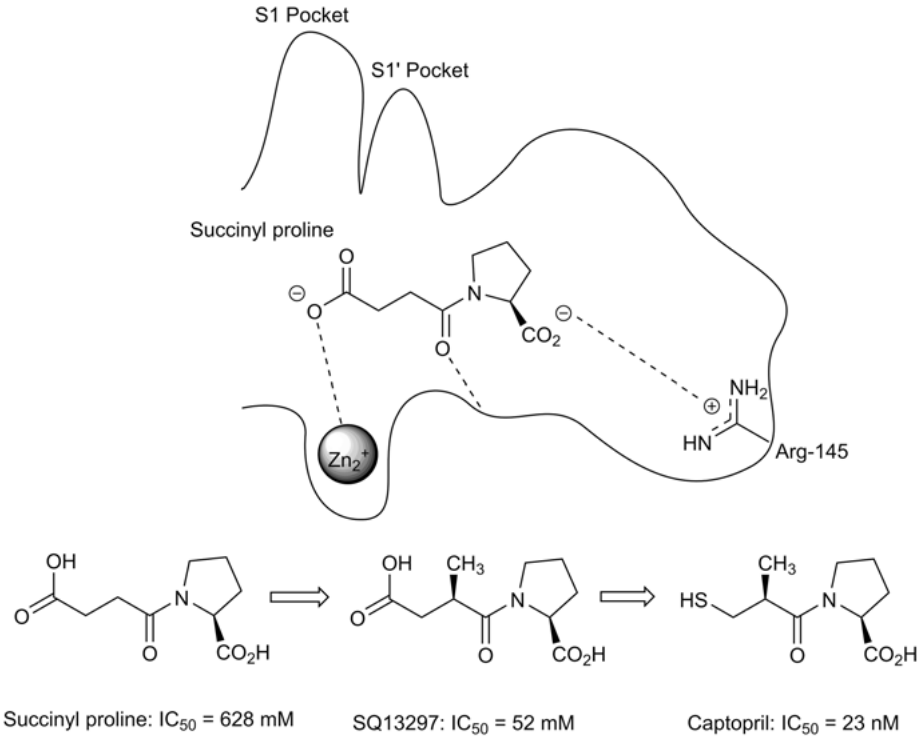


Fig. 8.11: Interactions of succinyl proline with the active site of the enzyme angiotensin-converting enzyme (ACE). Development of captopril.

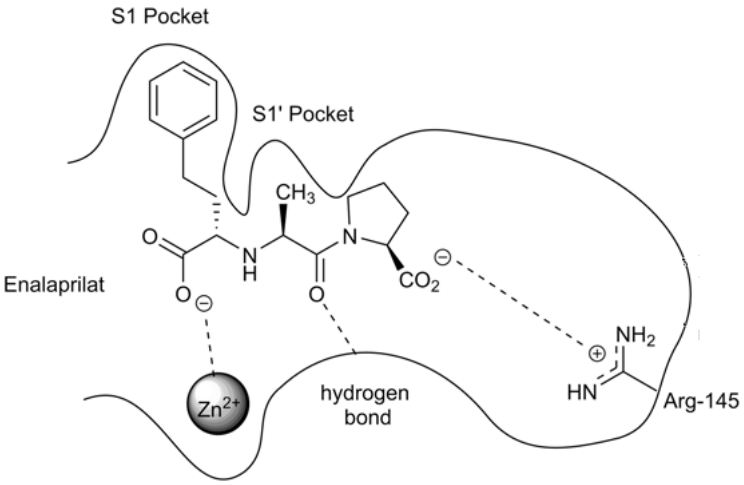
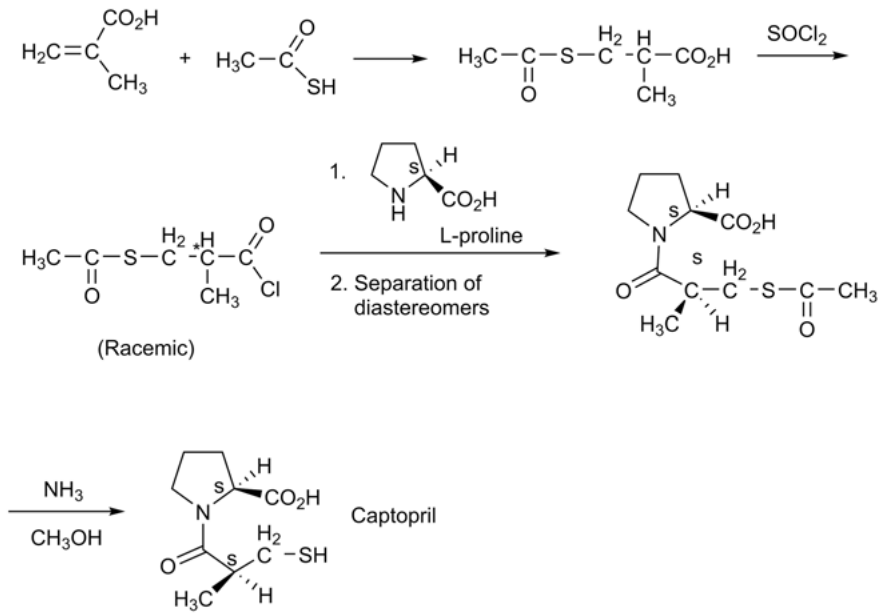


Fig. 8.12: Enalaprilat.



Scheme 8.2: Captopril synthesis.

used instead of succinyl proline, resulting in a new ACE inhibitor, called enalaprilat (Fig. 8.12).

The antihypertensive captopril, which contains a pyrrolidine heterocycle, has two stereogenic carbon atoms and therefore, its synthesis must take into account the stereochemical aspects. To obtain the pure active (*S*,*S*) diastereoisomer, L-proline is used and after proceeding to the corresponding *N*-acylation with the appropriate acid chloride, the mixture of diastereoisomers (*S*,*S*, active and *S*,*R*, inactive) was separated. The deprotection of the acetyl group bound to sulfur with NH_3/CH_3OH gives rise to captopril (Scheme 8.2).

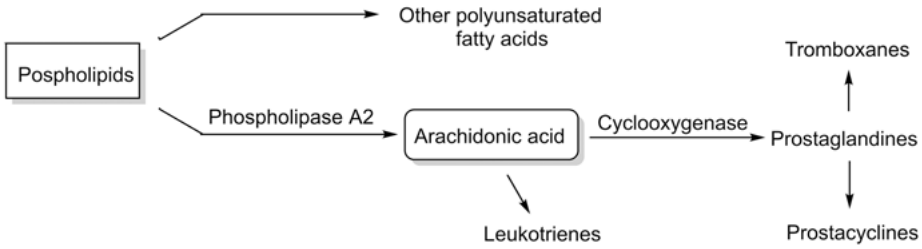
9 Enzimatic inhibitors II

9.1 Goals

- To know the isoforms of cyclooxygenase and its competitive inhibition.
- To know the therapeutic arsenal referred to as NSAIDs.
- To discuss the validity of coxibs as specific anti-inflammatory agents.

9.2 Introduction

Arachidonic acid is a polyunsaturated fatty acid with 20 carbon atoms that comes from the hydrolysis of the structural phospholipids of the cell membrane in a process catalyzed by phospholipase A2. Although arachidonic acid usually comes from the diet, it can also be biosynthesized from linoleic acid, an unsaturated fatty acid with 18 carbon atoms. From a biosynthetic point of view, arachidonic acid constitutes the point of origin for two independent enzyme pathways: (1) the cyclooxygenase pathway, which leads to the biosynthesis of prostaglandins, thromboxanes and prostacyclins and (2) the lipoxygenase pathway, which gives rise to leukotrienes (Scheme 9.1).



Scheme 9.1: Origin and metabolism of arachidonic acid.

The above biochemical pathways are structurally complex and we will reflect only on the biosynthesis of prostaglandins PGG_1 and PGH_1 in Scheme 9.2.

The Fe^{IV} oxo-radical species abstracts a hydrogen atom (XH) from the Tyr-385 residue of the COX enzyme. The radical thus formed abstracts the hydrogen atom of the *pro-S* C-13 of arachidonic acid (or its 5,6-dihydro derivative), resulting in a highly resonance-stabilized allylic radical. The addition of radical oxygen followed by cyclization and the addition of another oxygen molecule gives rise to prostaglandin G (PGG_2 or PGG_1, depending on whether or not a double bond is present). The importance of arachidonic acid metabolites as target molecules in the design of therapeutic agents lies in their intervention in the control and regulation of a great diversity of

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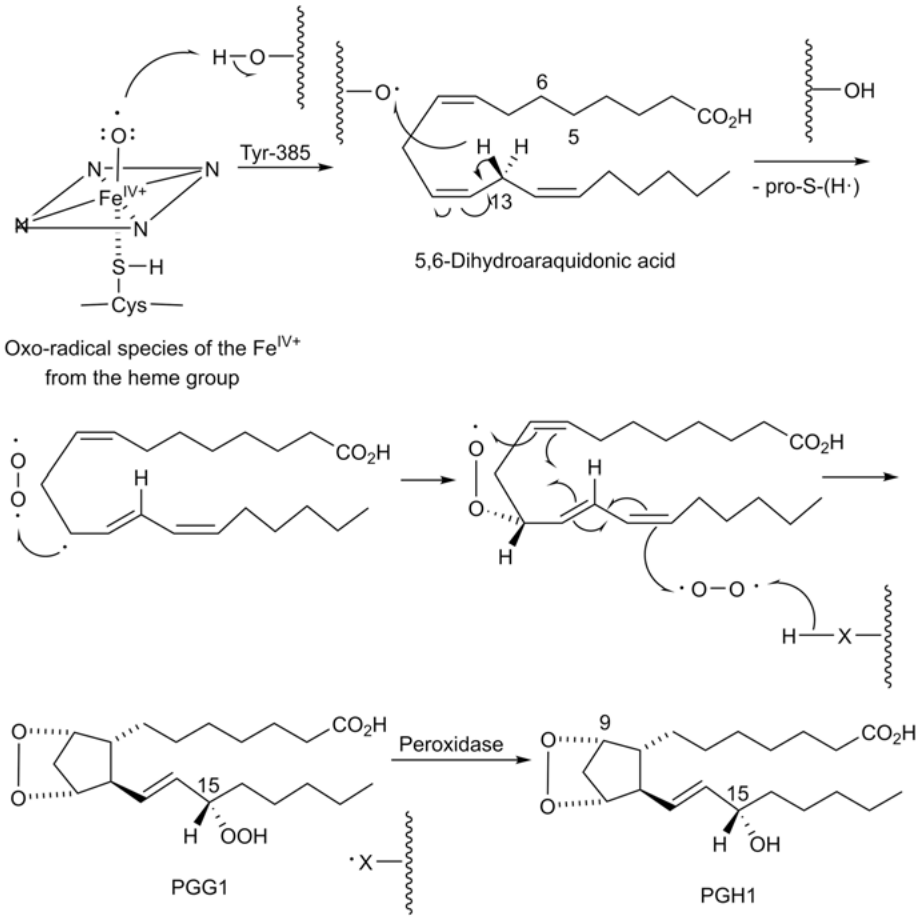


Scheme 9.2: Catalytic mechanism of prostaglandins PGG_1 and PGH_1.

physiological processes. These include inflammation, platelet aggregation, vascular tone, gastric secretion and anaphylactic processes. Although the therapeutic potential of arachidonic acid metabolites is very broad, their use as drugs is relatively limited so far. This is due to the low yields of the extraction processes from natural sources and their low chemical stability. Moreover, their total synthesis or semisynthesis requires long and complex processes with low overall yields.

There are at least three forms of the cyclooxygenase enzyme: COX-1, COX-2 and COX-3. The former is responsible for the production of the prostaglandins involved in the maintenance of the tissues lining the stomach, while the latter is induced by cytokines and is responsible for the increased production of prostaglandins in the inflammatory processes associated with pain and fever. Many nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, inhibit both COX-1 and COX-2 forms and

thus cause irritation and even stomach ulcers. [*Cytokines are substances that modify cell behavior or activity and include growth factors as well as rejuvenation proteins. Due to their small size (they are peptides or small proteins), they have a good level of topical penetration. Additionally, they raise the cellular population that is lost with the passage of the years and increase the level of replacement of the epidermal cells, whose loss contributes to an aged and tired appearance of the epidermis. They regulate cellular activity, coordinate defense processes and stimulate rejuvenation.*]

In 2002, a third form of COX, COX-3, was identified, but its exact function has not yet been accurately determined. The inhibition of this isoform may be related to the antipyretic effect of many NSAIDs, including paracetamol (acetaminophen). COX-3 has been identified only in experimental animals and never in humans, and it has been suggested that there are analgesic NSAIDs in the central nervous system, such as paracetamol that have antipyretic and analgesic effects but no anti-inflammatory response.

The structures of both COX-1 and COX-2 were dilucilated by X-ray and they are very similar. The active sites of the human COX enzymes are located outside the cytoplasmic membrane and are made up of long and narrow hydrophobic channels, in which an arginine residue (Arg120) interacts with the carboxylic acid group of the traditional NSAIDs. There are only two minor differences: isoleucine at positions 434 and 523 of COX-1 is exchanged for valine in COX-2. The smaller size of Val in COX-2 allows an inhibitor to access a lateral pocket, away from the main channel, while the larger size of Ile inCOX-1 sterically blocks the access of the inhibitor. The space available in the COX-2 joining pocket is 20% to 25% larger than in the same COX-1 space. Another difference between COX-1 and COX-2 is that the latter lacks a sequence of 17 amino acids in its *N*-terminal, but at the same time contains a sequence of 18 amino acids in the C-terminal. These differences in amino acid sequences cause a disparity in the numbering systems of the two isoforms so that the aspirin-acetylated residue in COX-1 is numbered as Ser^{530}, whereas the serine residue that is acetylated in COX-2 is Ser^{516}.

COX-1 appears to be more specific than COX-2 for fatty acids. While selective COX-2 inhibitors do not bind to Arg120, COX-1 inhibitors do bind to it by means of their –COOH group of arachidonic acid and their carboxylic acid group. A schematic representation of the active centers of COX-1 and COX-2 is depicted in Fig. 9.1.

As previously mentioned, Ser^{530} residue, to which aspirin (an analgesic, anti-inflammatory, antipyretic and antithrombotic drug) is bound, leads to cyclooxygenase inactivation through the formation of a covalent bond (Scheme 9.3).

NSAIDs are competitive inhibitors of cyclooxygenase, which generally have an acidic function conferring a pKa of 4-6 (carboxylic acid), 9-10 (sulfonamide) or 8-10 (phenolic enol).

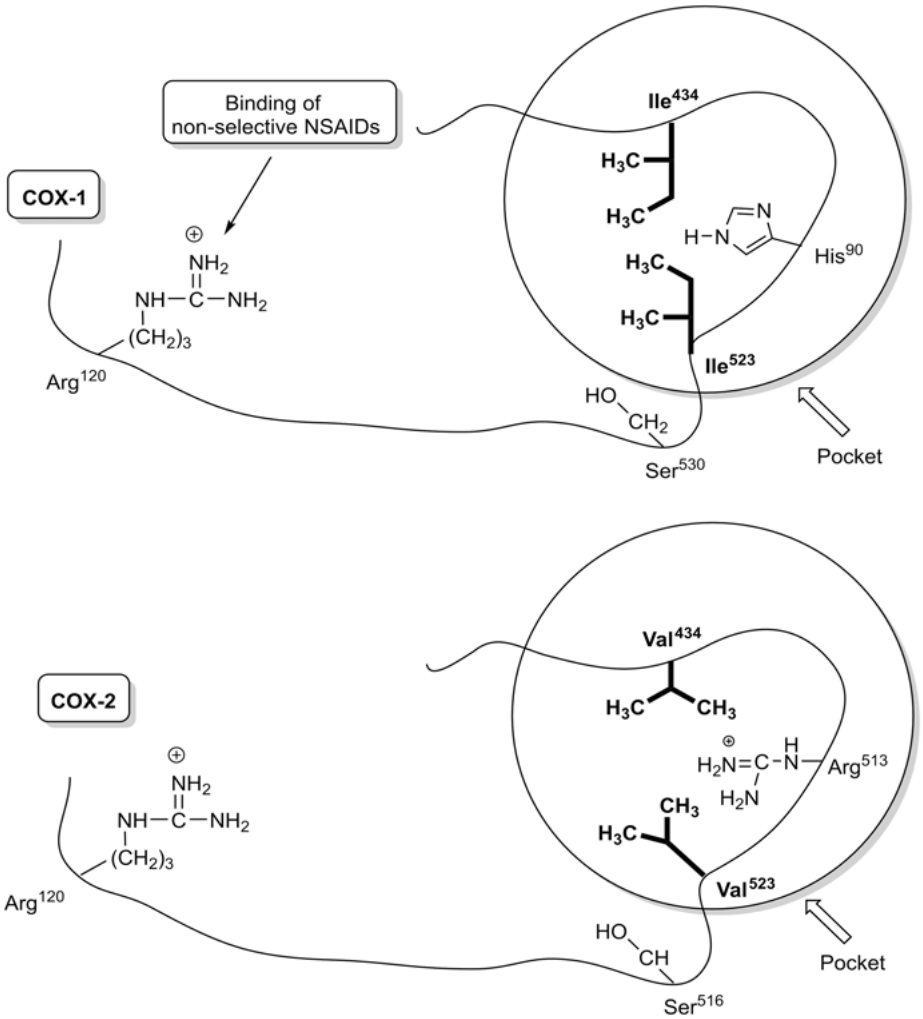
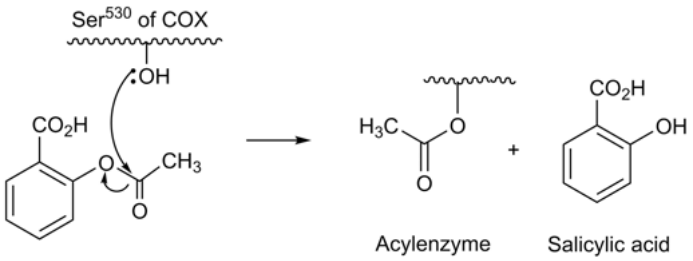


Fig. 9.1: A schematic representation of the active centers of COX-1 and COX-2.

9.3 Classification of nonsteroidal anti-inflammatory drugs (NSAIDs)

1. Arylalkanoic acids
   - Arylacetic acids (fenacs)
   - Arylpropionic acids (profens)
2. *N*-Arylanthranilic acids (fenamates)
3. Enols (oxicams)
4. Coxibs

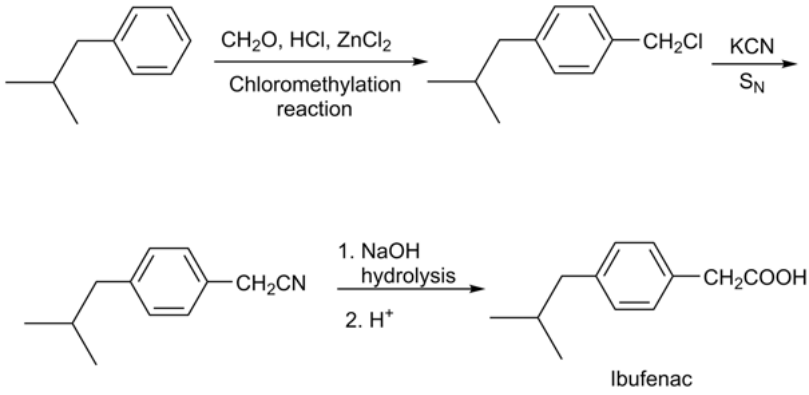


Scheme 9.3: Inhibition of cyclooxygenases by aspirin.

NSAIDs comprise a group of structurally diverse compounds that exhibit similar physiological effects. The name NSAID is misleading, since these drugs exhibit various degrees of analgesic, antipyretic, as well as anti-inflammatory activities. They are widely prescribed to relieve inflammation associated with diseases such as arthritis and for relief from weak to moderate pain such as headache.

9.3.1 Arylacetic acids or "fenacs"

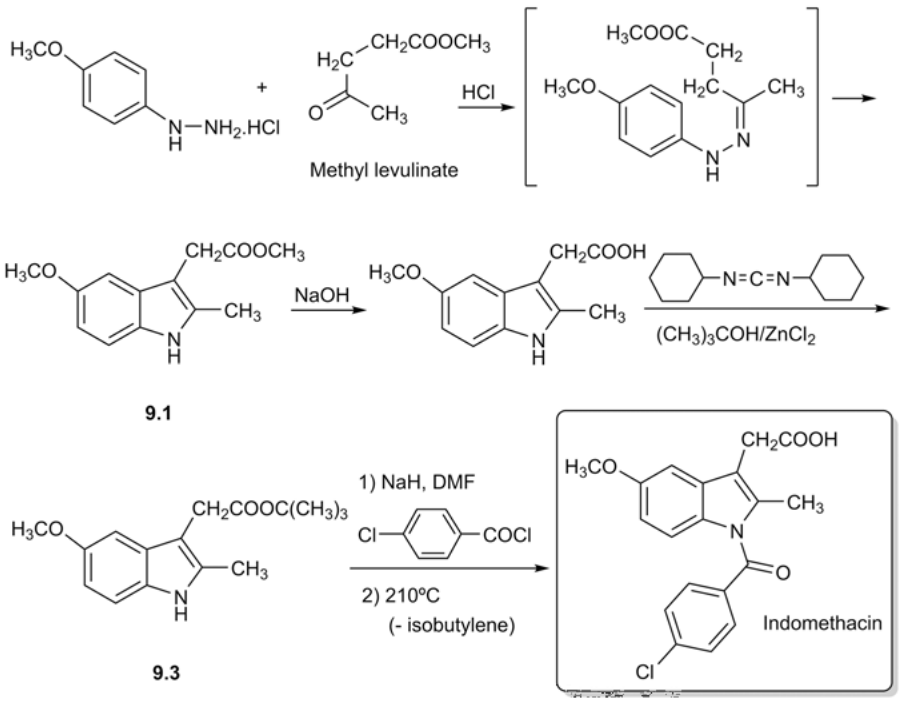
The prototype of this category of NSAIDs is ibufenac. This drug was soon replaced by its α-methylated congener, ibuprofen, which was synthesized in the same laboratory (Boots, UK) (Scheme 9.4).



Scheme 9.4: Ibufenac synthesis.

The first compound introduced in therapeutics as a nonsteroidal anti-inflammatory was indomethacin. It was prepared by Fischer synthesis from 4-methoxyphenylhydrazine hydrochloride and methyl levulinate (or methyl 4-oxopentanoate) to give

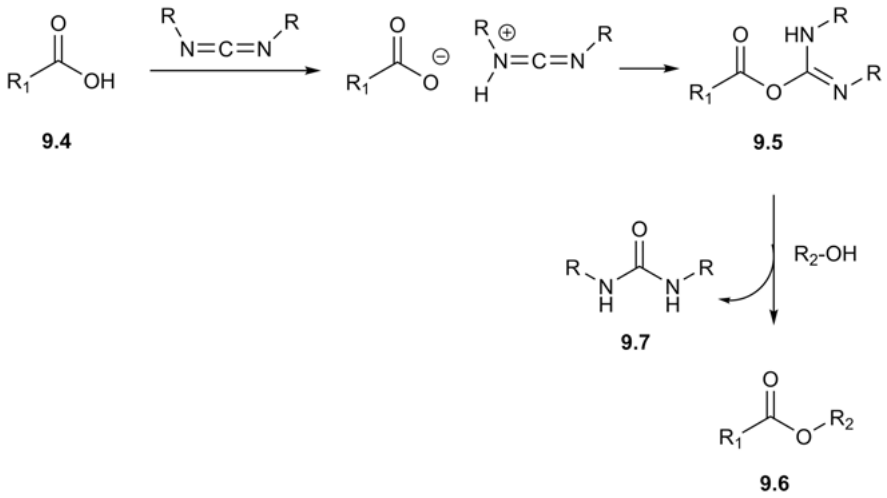
methyl 2-methyl-5-methoxy-3-indoleacetate, which was subsequently saponified and then converted into the *tert*-butyl ester in the presence of dicyclohexylcarbodiimide (DCC). The indole obtained is acylated with *p*-chlorobenzoyl chloride and the *tert*-butyl ester is subsequently deprotected by pyrolysis to yield indomethacin. (Scheme 9.5).



Scheme 9.5: Synthesis of indomethacin.

The synthesis of indomethacin can pose the following two questions: (a) Why is it necessary to use DCC in the esterification process of **9.2** with *tert*-butanol to yield **9.3**? (b) Why is the conversion of **9.1** into **9.3** necessary, given that both are esters? The mechanism of DCC in the neutral medium esterification process is shown in Scheme 9.6.

In synthetic organic chemistry, compounds containing the carbodiimide functionality are dehydrating agents and are frequently used to activate carboxylic acids for the formation of amides or esters. Acid **9.4** will react with the carbodiimide to yield the key intermediate *O*-acylisourea **9.5**, which may be considered a carboxylic ester with an activated leaving group. The *O*-acylisourea will react with alcohols to produce the desired ester **9.6** and the symmetrical dialkylated urea **9.7** (Scheme 9.6). This neutral esterification is essential because the alcohol used is *tert*-butanol, which is very prone to undergoing an elimination process to yield isobutylene.



Scheme 9.6: Mechanism of DCC during the esterification process.

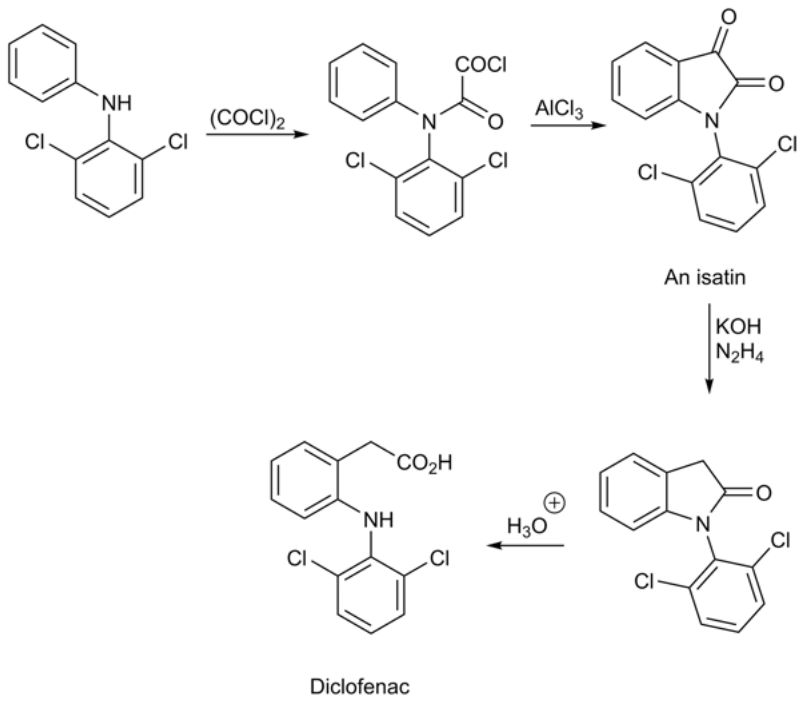
In relation to question (b), other possible methods of deprotection, different from the pyrolysis processes such as acidic or basic hydrolysis, are practically not feasible because they lead to the rupture of the 1-acylindole bond. This is the reason for converting the methyl into the *t*-buty ester (Scheme 9.5).

Both the potency and the duration of action are increased by the addition of appropriate substituents to the benzene ring. The routes of preparation for such compounds differ markedly from those used to prepare ibufenac.

The synthesis of some of the most popular NSAIDs, such as diclofenac, begins with the careful acylation of 2,6-dichlorodiphenylamine with one equivalent of oxalyl chloride (Scheme 9.7). Ring closure by Friedel–Crafts acylation leads to an isatin (isatin is 1*H*-indole-2,3-dione). The two carbonyl groups of isatin differ in that one is an amide, while the one that is closest to benzene is a ketone. The treatment of isatin with hydrazine and potassium hydroxide under Wolf–Kischner conditions effects the reduction of the ketone and the final product is a lactam. Finally, the hydrolysis of the amide yields diclofenac.

9.3.2 Arylpropionic acids or “profens”

Further work on arylacetic acids by researchers at Boots revealed that the incorporation of a methyl group in the side chain increased the potency of ibufenac. The resulting NSAID, ibuprofen, found widespread use, since it is superior to aspirin, which had been the mainstay drug for the same indications for decades, in terms of potency and tolerability.

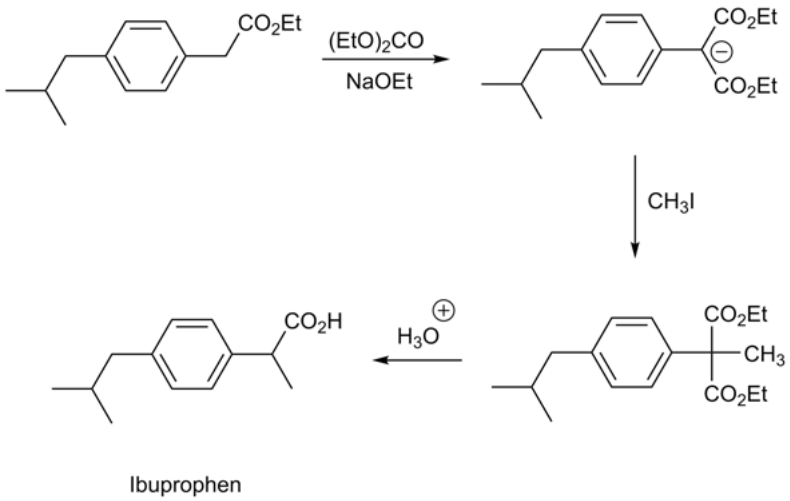


Scheme 9.7: Synthesis of diclofenac.

The reaction of the ethyl ester of ibufenac with diethyl carbonate in the presence of sodium ethoxide leads to the malonate carbanion. This compound is not isolated, but is treated directly with methyl iodide to yield the alkylation product. The malonate is then hydrolyzed into monoalkylated malonic acid, which spontaneously decarboxylates to yield ibuprofen (Scheme 9.8).

9.3.3 Naproxen

Naproxen is the name of (*S*)-2-(6-methoxy-2-naphthyl)propanoic acid. It was shown in an *in vivo* assay that naproxen is 28 times more active as an anti-inflammatory than its (R) enantiomer and 3 times more active than racemic. The resolved material for these studies was obtained by crystallization of the pair of diastereoisomeric salts formed from the racemic propanoic acid derivative and cinchonidine (Fig. 9.2).



Scheme 9.8: Synthesis of ibuprofen.

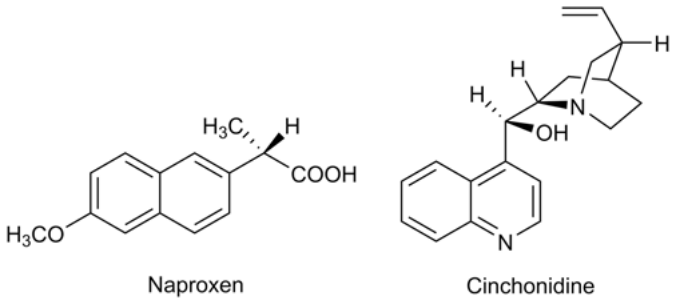


Fig. 9.2: Naproxen and the resolving agent for the racemic propanoic acid derivative.

9.3.4 *N*-Arylanthranilic (fenamic) acids

The class of anthranilic acids within NSAIDs is the result of the application of the concept of bioisosterism to the design of drugs, because fenamic acids are nitrogenated isosteres of salicylic acid. In addition, they may be considered structural analogues of diclofenac. Mefenamic acid was introduced in the USA in 1967 as an analgesic and this continues to be its first indication, despite showing a modest anti-inflammatory activity (Fig. 9.3). Meclofenamic acid has a higher anti-inflammatory activity than mefenamic acid.

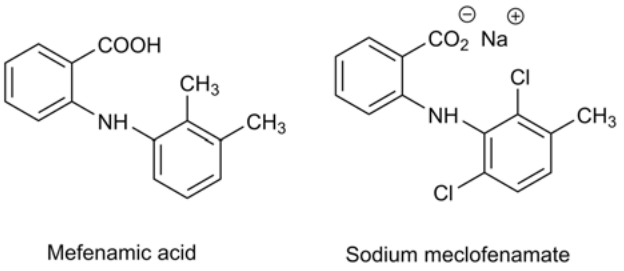
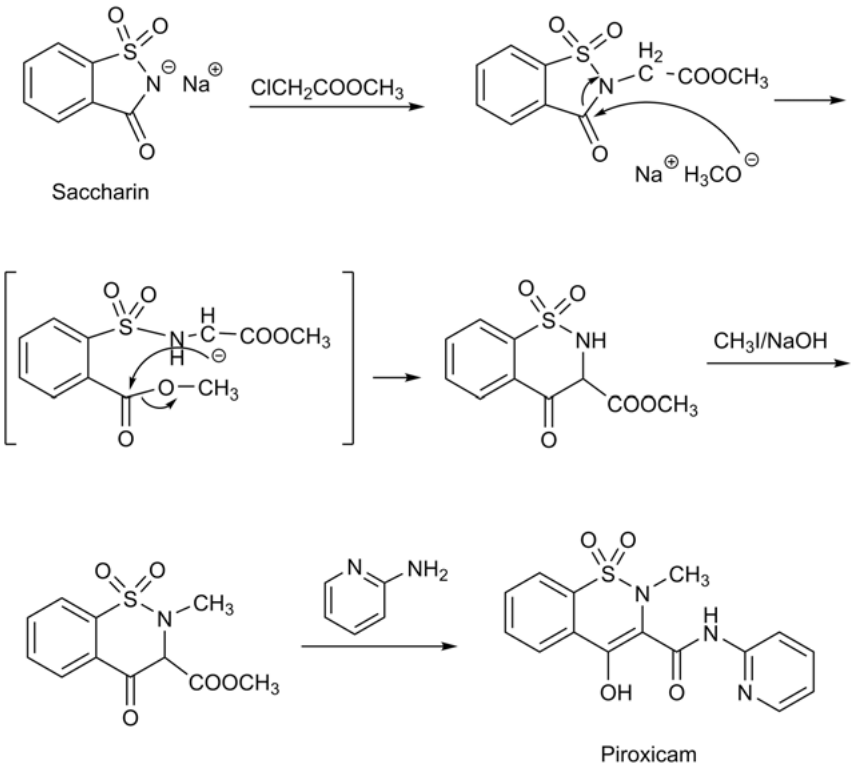


Fig. 9.3: Structures of various *N*-arylanthranilic (fenamic) acids.



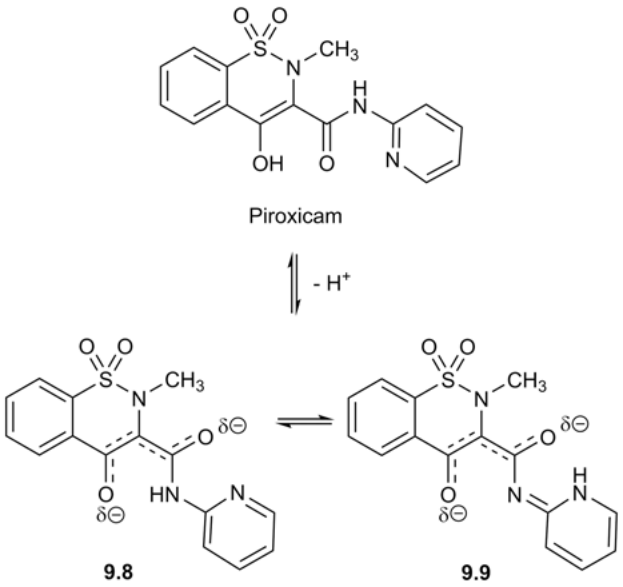
Scheme 9.9: Synthesis of piroxicam.

9.3.5 Enols (oxicams)

An example of a drug with a benzothiazine structure is piroxicam (Scheme 9.9).

It is an NSAID whose synthesis is performed from saccharin by reacting it with methyl chloroacetate, expanding the dihydroisothiazole ring to dihydro-1,2-thiazine with sodium methoxide in dimethylsulfoxide, followed by methylation with methyl iodide and treatment with 2-aminopyridine (Scheme 9.9).

Oxicams are acidic compounds with pK_a values ranging from 4 to 6. *N*-Heterocyclic carboxamides are more acidic than the corresponding *N*-aryl carboxamides and this higher acidity can be attributed to the stabilization of the enolate anion by the pyridinium nitrogen atom, as illustrated with tautomer **9.8,** and further stabilization by tautomer **9.9** (Scheme 9.10).



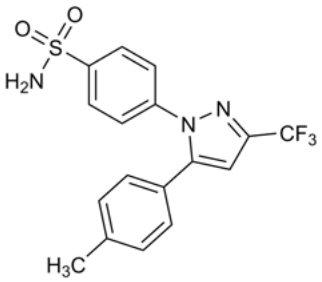
Scheme 9.10: Stabilization of the piroxicam enolate.

9.3.6 COX-2 selective inhibitors: Coxibs

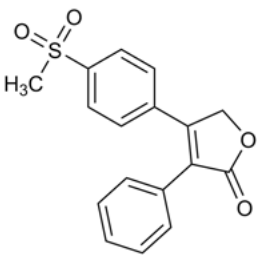
There are new drugs that selectively inhibit COX-2, constituting the group of coxibs, hypothetically less toxic, whose first representatives were celecoxib and rofecoxib (Fig. 9.4).

Coxibs bind selectively to the allosteric site of the COX-2 isoform of swollen tissue. The allosteric center of COX-1 is more sterically restricted than that of COX-2, which prevents coxibs from having access to the binding residues of the former isoform. In celecoxib, the anionic form of the sulfonamide group helps to bind to the allosteric site of COX-2 through an ionic interaction with an Arg residue. Many coxibs are diaryl-heteroaromatic compounds, whereby the two aromatic rings of these structures increase the affinity for COX-2. Syntheses of celecoxib and rofecoxib are shown in Chapter 8 of Volume 1 of this series.

Data from major clinical trials indicate that although these drugs would be associated with a lower frequency of gastrointestinal disorders, this would be at the expense



Celocoxib
CELEBREX (Pfizer)



Rofecoxib
VIOXX (Merck)

Fig. 9.4: Celecoxib and rofecoxib.

of a greater frequency of other serious side effects, particularly those related to thrombotic events. This would imply that the use of these drugs for the moment should be limited to very specific indications and not as usual alternatives to the "classic" drugs.

10 Design of drugs acting on transport through biological membranes

10.1 Goals

- Foster knowledge of the different ion channels and types of transport that regulate them.
- Foster knowledge of the biological function associated with each channel and the design of the associated drugs.
- Foster knowledge of drugs such as local anesthetics, antiarrhythmics and others.

10.2 Design of drugs that act on transport through cell membranes

Ion channels are pore-forming membrane proteins that provide the cell with selective permeability to various ions. We have seen the general mechanism of transmission by which an endogenous ligand is capable of activating the opening or closing of an ion channel. These types of channels constitute the so-called ligand-gated ion channels. Examples are the nicotinic receptors of acetylcholine or the GABA receptor-linked chloride channels.

Here we will consider some families of drugs whose modes of action consist of the modulation of certain ion channels that, unlike those considered until now, are regulated by differences in potential rather than specific ligands. This occurs, for instance, when the arrival of a nerve impulse changes the membrane potential and thereby regulates the opening or closing of ion channels. These channels are given the generic name of voltage-dependent channels and in general, they result in faster membrane potential changes than those caused by the activation of ligand-gated channels.

10.3 Voltage-gated sodium channels

Voltage-gated sodium channels are membrane glycoproteins that regulate the permeability of the cell against sodium ions. In the resting state, sodium channels remain closed and impermeable to the passage of ions, while the arrival of a nerve impulse causes their opening and the flow of sodium ions into the cell. This process, together with the flow of potassium and chloride ions through specific channels, constitutes the biochemical basis of the action potential and transmission of the nerve impulse through the axon of the nervous fiber.

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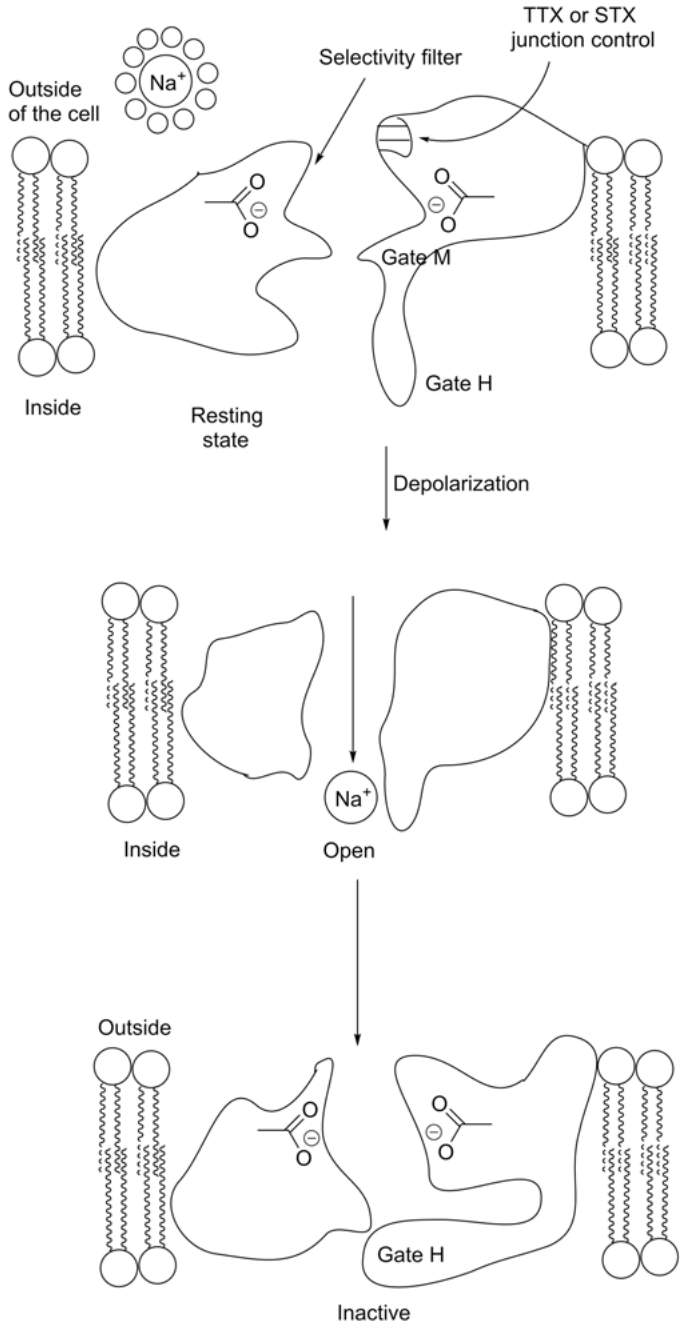


Fig. 10.1: Scheme of a sodium channel.

The filter consists of a pore, slightly larger in diameter than the desolvated cation, consisting of an inner zone rich in strongly negatively charged amino acid residues. Its function is to diminish the free energy required for the process of the desolvation of the cation, a condition that is indispensable for the passage of the ion through the channel. The alignment of negative charges along the filter allows for the easy desolvation of the cation, which is thus stabilized in its passage through this highly selective zone.

Voltage-gated Na^+ channels have three main conformational states: closed, open and inactivated. Forward/back transitions between these states are correspondingly referred to as activation/deactivation (between open and closed, respectively), inactivation/reactivation (between inactivated and open, respectively), and recovery from inactivation/closed-state inactivation (between inactivated and closed, respectively). Closed and inactivated states are ion impermeable (Fig. 10.1).

Before an action potential occurs, the axonal membrane is at its normal resting potential and Na^+ channels are in their deactivated state, blocked on the extracellular side by their activation gates (M-gates). In response to an electric current (in this case, an action potential), the activation gates open, allowing positively charged Na^+ ions to flow into the neuron through the channels and causing the voltage across the neuronal membrane to increase. Because the voltage across the membrane is initially negative, as its voltage increases *to* and *past* zero, it depolarizes. This increase in voltage constitutes the rising phase of an action potential.

At the peak of the action potential, when enough Na^+ has entered the neuron and the potential of the membrane has risen enough, the Na^+ channels inactivate by closing their inactivation gate (H-gate). The inactivation gate acts as a "plug". Closure of the inactivation gate causes Na^+ flow through the channel to stop, which in turn causes the membrane potential to stop rising. With its inactivation gate closed, the channel is inactivated. As the Na^+ channel is no longer contributing to the membrane potential, the potential decreases back to its resting potential as the neuron repolarizes and subsequently hyperpolarizes itself. This decrease in voltage constitutes the falling phase of the action potential.

When the membrane voltage becomes low enough, the inactivation gate reopens and the activation gate closes in a process called deinactivation. With the activation gate closed and the inactivation gate open, the Na^+ channel is once again in its deactivated state, and is ready to participate in another action potential (Fig. 10.1).

Although under physiological conditions, the opening and closing of the channels are regulated by changes in the membrane potential, some compounds capable of blocking the channels are known. The neurotoxins tetrodotoxin (TTX) and saxitoxin (STX) are examples of such compounds (Fig. 10.2).

Tetrodotoxin is found in certain species of fish in Asia and often leads to food poisoning. Various constituents of the marine phytoplankton produce saxitoxin. In certain environmental conditions, their multiplication is abnormally high, resulting in so-called "red tides". The consumption of these algae by mollusks, the accumulation

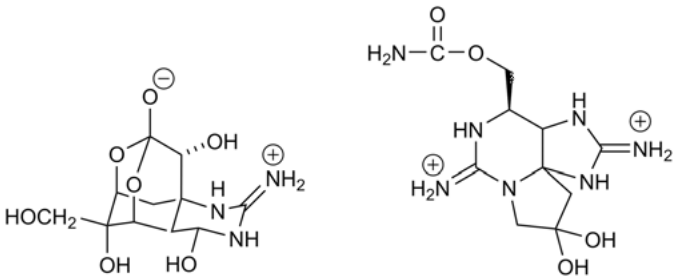


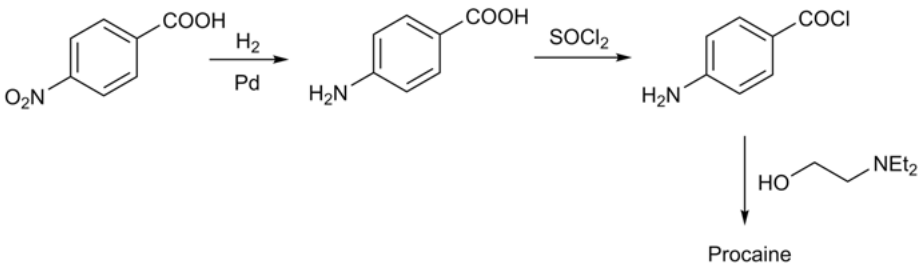
Fig. 10.2: Sodium channel blockers.

of the algae in them and their subsequent consumption by man can lead to serious intoxication. Both TTX and STX give rise to a blockage of sodium channels through an interaction of the strongly cationic guanidinium residues with a complementary zone on the outside of the channels.

10.3.1 Local anesthetics

Local anesthetics are compounds that decrease the excitability of cells as a result of blocking potential-dependent sodium channels. From a structural point of view, these drugs originate from the cocaine alkaloid, which has central stimulant effects. In order to alleviate these adverse effects, a number of cocaine analogues were synthesized based on the disjunctive structural variation processes with the aim of determining the pharmacophore for local anesthetic activity (anesthesiophoric group, Fig. 10.3).

Procaine was the first local anesthetic synthesized to be devoid of the side effects of cocaine and represented a prototype in the design of more effective local anesthetics. Its synthesis can be carried out from *p*-nitrobenzoic acid by a reduction to aminobenzoic acid, a conversion to acid chloride and further treatment with 2-(diethylamino) ethanol (Schema 10.1).



Scheme 10.1: Synthesis of procaine.

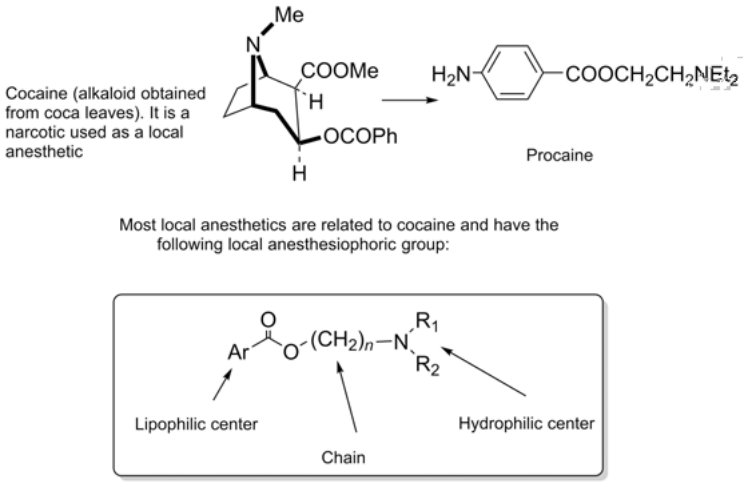


Fig. 10.3: Structural relationship between cocaine and procaine: Local anesthesiophoric group.

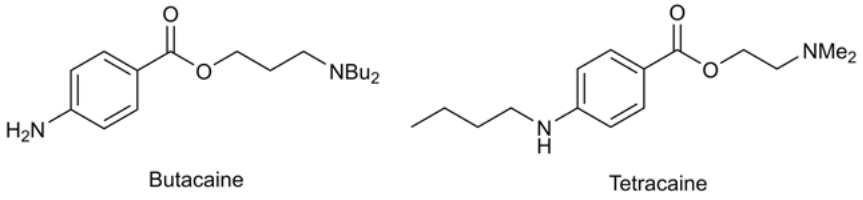
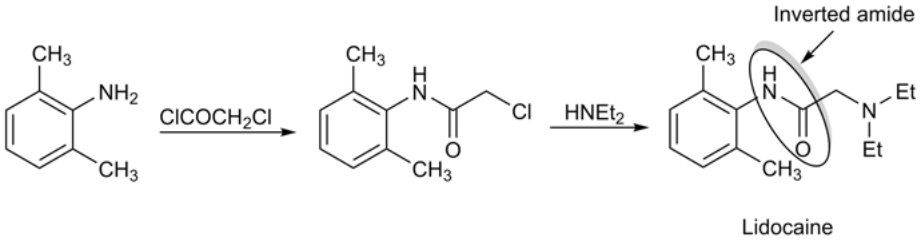


Fig. 10.4: Local anesthetics with longer durations of action than procaine.



Scheme 10.2: Synthesis of lidocaine.

One of the main limitations of procaine is its relatively short duration of action. Longer durations of action and the possibility of topical application are achieved with an increase in the chain length between O and N and with an increase in lipophilicity around N. Examples are butacaine and tetracaine (Fig. 10.4).

Another derivative is lidocaine (Scheme 10.2), the prototype of amide-derived local anesthetics. Lidocaine and its structural analogues are compounds with greater resistance to hydrolysis than the corresponding esters, which leads to anesthetics with longer durations of action.

10.3.1.1 Structure-activity relationships in local anesthetics

Most local anesthetics can be represented by the general formula in Fig. 10.5.

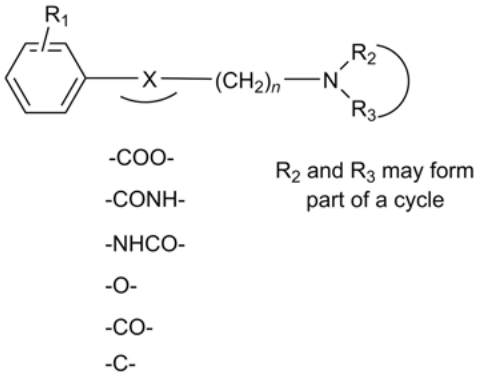


Fig. 10.5: Structure-activity relationships in local anesthetics.

The aromatic ring consists of a phenyl group substituted with electron-releasing groups at the *ortho* and/or *para* positions. Regarding X, although various functional groups have been described at this position, the most interesting ones are esters and amides. However, local anesthetics derived from carbamates, amidines, ketones and ethers are also known and $-NR_2R_3$ is a secondary or tertiary amine, separated by a linear chain of two or three carbon atoms from the electronegative center. These structural requirements have much in common with those of other groups of drugs, mainly anticholinergics and H_1 antihistamines, which explains side effects shown by many of the local anesthetics of this type.

In the series of esters (procaine analogues), the presence of electron-releasing groups is essential due to the resonance effect on the aromatic ring. This allows the structure of these compounds to be a resonance hybrid between **10.1** and **10.2**. Similarly, in the series of amides (lidocaine analogues) the participation of two resonance forms **10.3** and **10.4** can be expected, without the need for an intervention of the aromatic ring substituents (Fig. 10.6).

Although none of the resonant forms alone represents the actual structure of these compounds, it appears that the greater the "zwitterionic" character of the structure, the greater the affinity of the compound will be for the ion channel. An indirect proof of this is that the insertion of a methylene group between the aro-

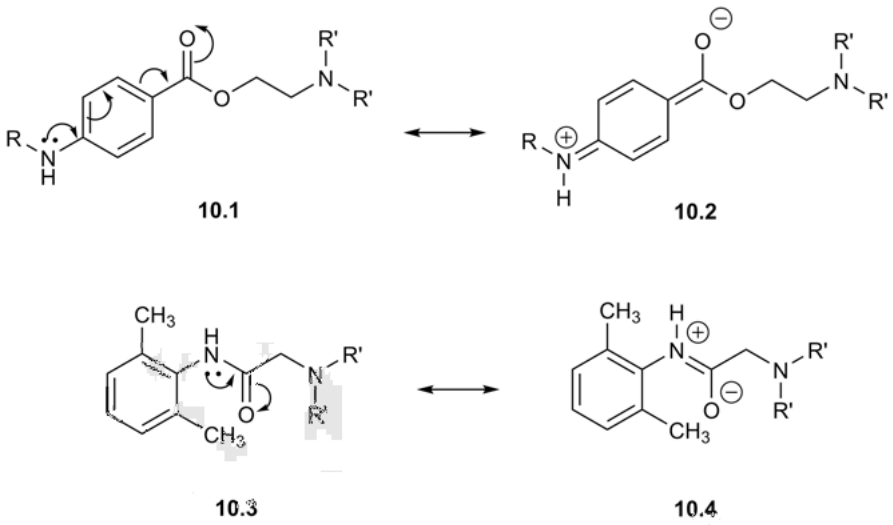


Fig. 10.6: Resonance structures of local anesthetics structurally related to procaine and lidocaine.

matic ring and the carbonyl group in procaine prevents the participation of the resonance form **10.2**, leading to a compound devoid of local anesthetic activity. In addition, local anesthetic activity is directly related to the overall lipophilicity of the molecule.

10.3.1.2 Physicochemical properties and mode of action

As mentioned above, sodium channels are composed of membrane glycoproteins that regulate the permeability of sodium versus the sodium cation. The mode of action for local anesthetics involves their binding with an area close to the ion channel filter.

In 1984, Hille and colleagues proposed a theory to explain the activity of local anesthetics. The active species is the ionized form, which binds to the receptor zone next to the sodium ion selective filter. These compounds can reach the receptor directly from the outside of the membrane (via *a*) or from the inside (via *b*) after passing through the lipid membrane (Fig. 10.7). The activity of local anesthetics depends on an adequate balance between the lipophilicity of the molecule and its degree of ionization.

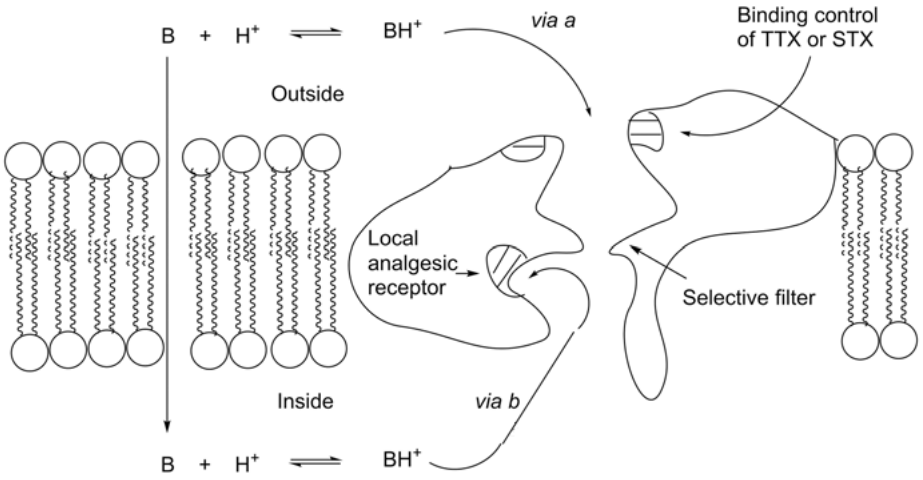


Fig. 10.7: The sodium channel model suggested by Hille, which shows a hydrophilic (indicated by *b*) and a hydrophobic path (indicated by *a*), by which local anesthetics can reach their binding sites.

10.4 Voltage-dependent calcium channels

Calcium ions play a role in the control of a great diversity of processes, including the contraction of the cardiac fiber. At this level, the function of the calcium ion can be modulated by the design of selective channel-blocking drugs that regulate its flow through the cell membrane.

From the therapeutic point of view, the design of calcium channel blockers has acquired great importance for their use as antiarrhythmic drugs for their ability to regulate the rhythm of cardiac contractions, as hypotensors for their ability to relax cardiac muscle and the smooth fibers of blood vessels, and as antianginals for their ability to counteract the coronary ischemia associated with angina.

10.4.1 Calcium channel blockers: Structural families

Blockers of the calcium ion channels are grouped into four structural families (Fig. 10.8):

1. 1,4-Dihydropyridines, whose prototype is nifedipine.
2. Phenylakylamines, represented by verapamil.
3. Benzothiazepines, whose most representative drug is diltiazem.
4. Diphenylmethylalkylamines, represented by prenylamine.

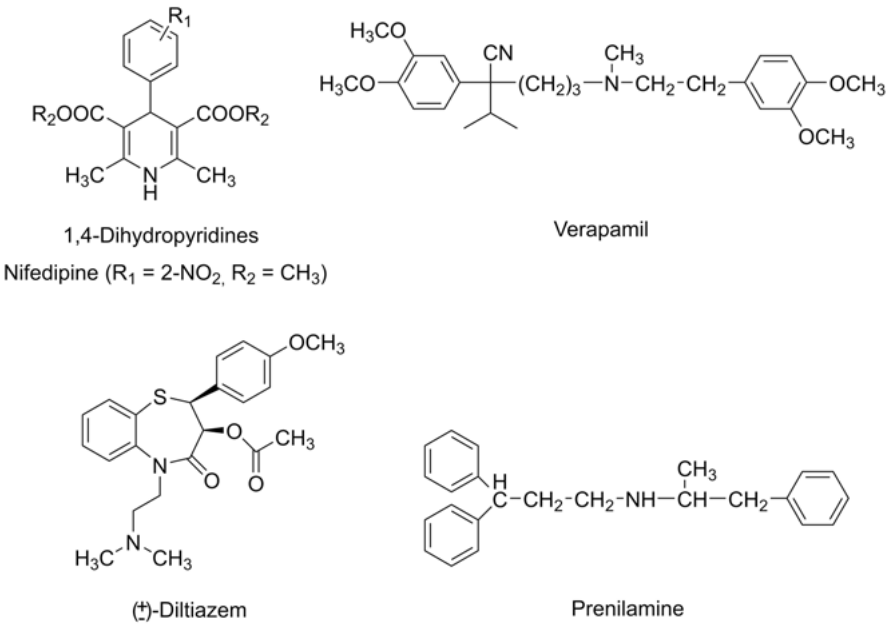


Fig. 10.8: Structural families of calcium channel blockers.

10.4.1.1 1,4-Dihydropyridines

The presence of esters at positions 3 and 5 of the dihydropyridine system and the need for the 1-position nitrogen atom to be unsubstituted are indispensable conditions for the hypotensive activity of 1,4-dihydropyridines.

Although many 1,4-dihydropyridines are achiral because they contain a plane of symmetry, some of them are not. In most cases, a remarkable stereoselectivity is observed, some presenting a high eudismic ratio (ER), as observed in nivaldipine (ER = 100) (Fig. 10.9).

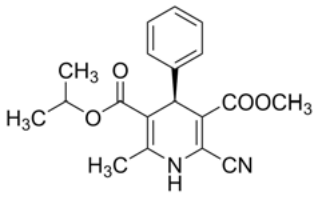
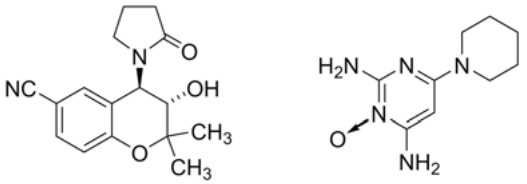


Fig. 10.9: Nivaldipine.

In other cases, however, the differences between enantiomers translate into different effects on calcium channels. One enantiomer can behave as an antagonist (channel blocker) and another as an agonist (channel activator).

10.4.1.2 Agents that act as activators of K^+

The discovery of selective activators of K^+ channels has led to compounds used as vasodilators for their ability to cause a hyperpolarization of the smooth fiber of the vessels. Some compounds of this type have been described, among which minoxidil is one of the most used. However, curiously, much of its therapeutic use is due to its side effect as an anti-alopecia drug (Fig. 10.10).



Cromakalim Minoxidil

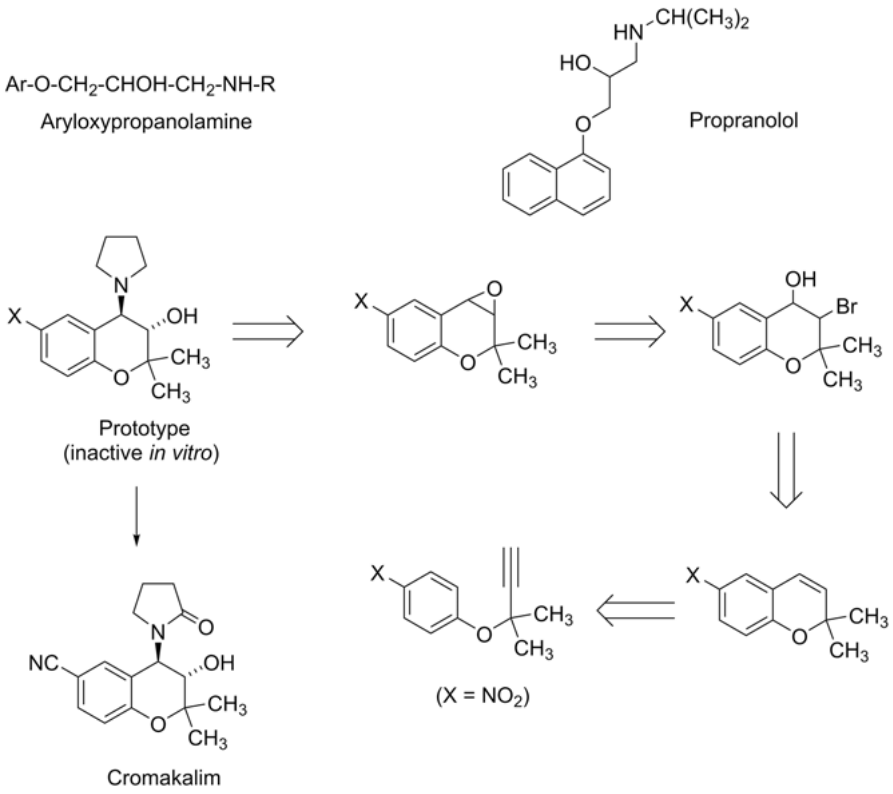
Fig. 10.10: Cromakalim and minoxidil.

Cromakalim (Fig. 10.10) arose from studies related to the antihypertensive activity of β-adrenergic blockers. These drugs have the general structure of aryloxypropanolamines with great conformational freedom in the propanolamine moiety, with propranolol being the most representative.

In light of assertions that its antihypertensive effects may not derive from the beta-adrenergic receptor blockade, restrictions of the conformation of the chain were considered and cyclic analogues were prepared, which were unable to bind to the beta-adrenergic receptor, but did retain the antihypertensive activity. Its synthesis was planned by means of the cyclization of the 1,1-dimethyl-2-propynyl-*p*-nitrophenyl ether, the subsequent formation of bromohydrin, transformation of bromohydrin to epoxide and opening of the epoxide with amines. This led to an antihypertensive prototype that did not block the β-adrenergic receptor. This manipulation revealed the need for a *gem*-dimethyl group at position 2 and an electron-withdrawing group (the best was the cyano group) at position 6. The best amine was not isopropylamine, but pyrrolidine. This compound was assumed to be a prodrug, as it was not active in vitro. Since oxidation at position α in relation to amino groups is a very common metabolic reaction, the analogue derived from pyrrolidone was synthesized (cromakalim, Scheme 10.3).

10.4.2 H^+/K^+-ATPase inhibitors: Antiulcer drugs

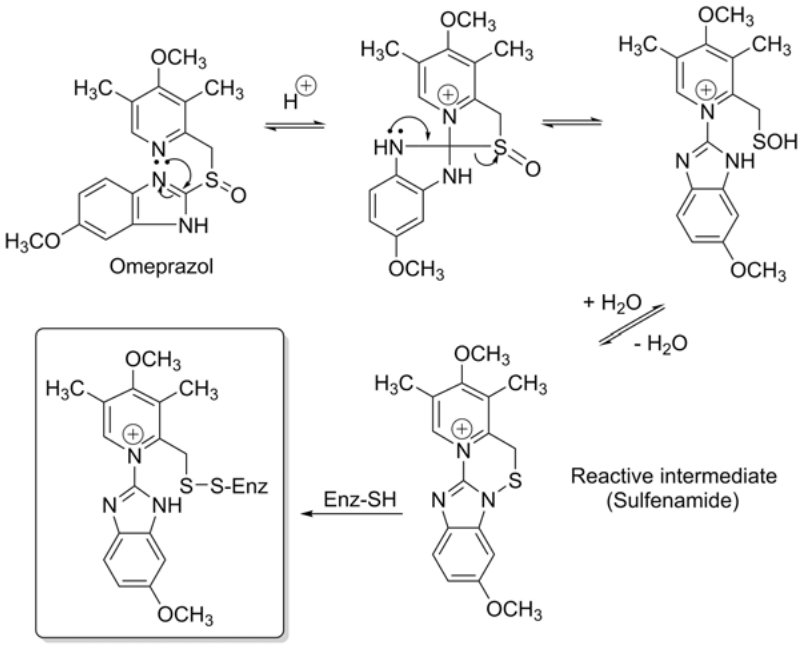
The enzyme H^+/K^+-ATPase, which catalyzes the exchange of a proton for a K^+ ion, is located in the membranes of gastric mucosal parietal cells specialized in the secretion of hydrochloric acid. Although there are several mechanisms by which acid secretion can be modulated (for example with H_2 antihistaminics), the H^+/K^+ pump inhibitors



Scheme 10.3: Evolution in the design of the cromakalim.

lead to a decrease in gastric acidity induced by any type of stimulation (histaminergic, cholinergic or gastrinergic), so they are used in the treatment of peptic ulcers.

Omeprazole is a synthetic compound designed as an irreversible inhibitor of the H^+/K^+-ATPase enzyme. The mechanism of action requires its previous activation in the gastric parietal cells, so it should be considered a prodrug because of its basic weak character, protonates in the parietal cells, in which omeprazole accumulates. The protonation of the nitrogen of the benzimidazole ring is accompanied by the addition of the pyridine ring nitrogen to yield a spiro intermediate, which, through a rearrangement process, converts the sulfoxide group to sulfenic acid. The sulfenamide derived by dehydration is a thiol active species, which forms a disulfide-type covalent bond with cysteine residues of the H^+/K^+-ATPase enzyme (Scheme 10.4).



Scheme 10.4: Metabolic activation of omeprazole.

11 Enzymatic inhibition: Inhibitors of the biosinthesis of the cellular wall

11.1 Goals

- Foster knowledge of the main penicillin antibiotics.
- Foster knowledge of the main cephalosporin antibiotics.
- Provide an introduction to semisynthetic drugs.

11.2 Antibiotics

An antibiotic is a compound produced by microorganisms capable of inhibiting the growth of other microorganisms. Antibiotics with the β-lactam structure block, through the formation of a covalent bond, inhibit the biosynthesis in the bacterium of a polymer called peptidoglycan, a structural constituent of the bacterial wall. When this wall is weakened, the cell undergoes lysis, which is exposes it to the defenses of the host organism. Since mammalian cells lack walls, β-lactam antibiotics are selective chemotherapeutics of very low toxicity to mammals.

11.3 Penicillins

Although the use of penicillin is very extensive, its structure was the subject of bitter discussions and it was not until 1945 that Dorothy Hodgkins established its structure by X-ray analysis (Fig. 11.1).

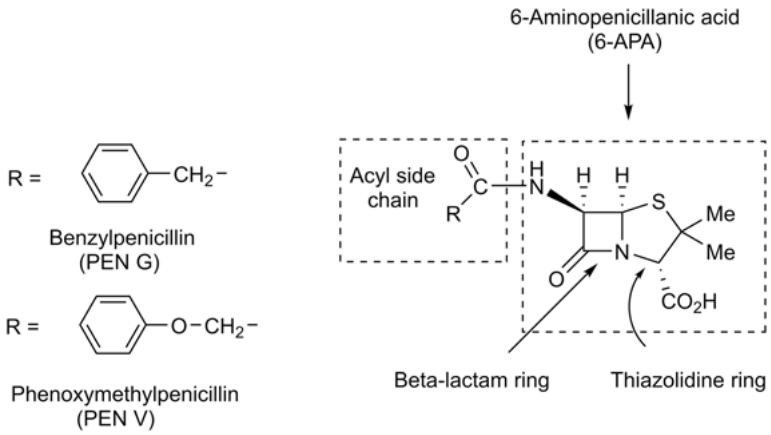


Fig. 11.1: Structure of penicillins.

https://doi.org/10.1515/9783110528527-012

11.3.1 Structure of penicillins

Penicillin contains a highly unstable bicyclic system that contains a β-lactam four-membered ring fused to a five-membered thiazolidine ring. The skeleton of the molecule suggests that it derives from the amino acids cysteine and valine. The overall shape of the molecule is a semi-open book (Fig. 11.2).

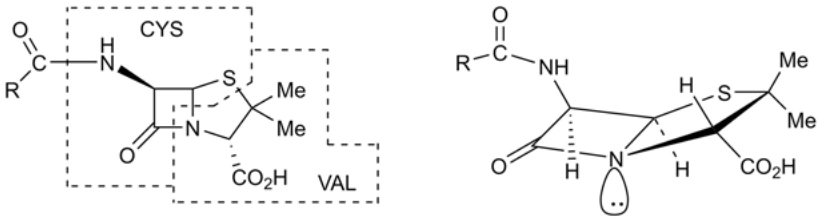


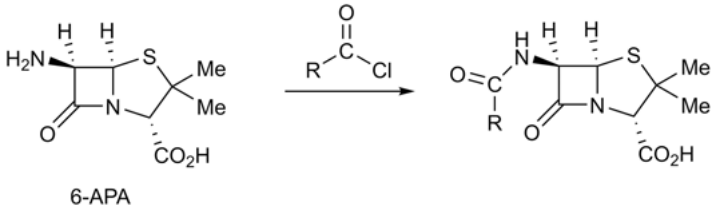
Fig. 11.2: Biosynthetic precursors and three-dimensional structure of penicillins.

The product formed in the fermentation process leading to penicillins can be controlled to some extent by the addition of precursors to the culture medium → biosynthetic penicillins. The most important limitation is that these precursors have to be derived from monosubstituted acetic acids, which give rise to the formation of penicillins with a group R-CH_2-CO-NH- at position 6β. The most outstanding examples of antibiotics obtained by fermentation in the presence of precursors are penicillins G, V and O (benzyl-, phenoxymethyl- and allylthiomethyl-penicillins, respectively). The penicillin studied at the University of Oxford was designated by penicillin F (from Fleming) and that of the Northern Laboratories by the following letter of the alphabet, G. The other biosynthetic penicillins were assigned letters alluding to some of their characteristics. Thus, penicillin V was named this way because it is the fifth in the series and O received this designation for its smell reminiscent of onion. The names G, V and O are still valid for biosynthetic penicillins, whose clinical value justifies their production on a large scale.

11.3.2 Various penicillins

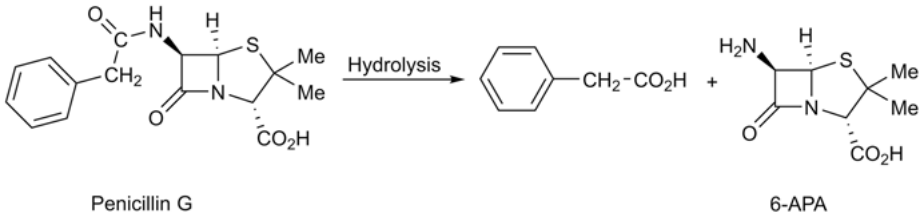
In 1957, Sheehan synthesized penicillin V in a multistep process with an overall yield of 1%. This procedure is not competitive from an economic point of view. Between years 1958–1960, Beechams succeeded in isolating a biosynthetic intermediate from penicillin, which was in turn one of the intermediates of Sheehan's multistep synthesis. The compound was 6-aminopenicillanic acid (6-APA) and allowed for the preparation of an enormous number of analogues through a semisynthetic procedure. In other words, fermentation yielded 6-APA which was synthetically treated to yield penicillin

analogues. This was done by acylating 6-APA with a wide range of acid chlorides (Scheme 11.1).



Scheme 11.1: Acylation reaction of 6-APA.

However, 6-APA is currently produced by hydrolyzing penicillin G or V using the enzyme penicillinase or by chemical methods (Scheme 11.2). These are more effective than fermentation.



Scheme 11.2: 6-APA is obtained by hydrolysis from penicillin G.

11.3.3 Properties of penicillin G

- Active against Gram-positive bacilli (e.g., staphylococci, meningitis, and gonorrhea) and many (but not all) gram-negative cocci.
- Nontoxic. This point is very important. Penicillins are among the safest drugs used in medicine.
- Not active against a wide range of bacteria.
- Ineffective when taken orally. Penicillin G can only be administered parenterally. It is ineffective orally as it degrades in the acidic conditions of the stomach.
- Sensitive to all known β-lactamases. These are enzymes that produce bacteria resistant to penicillin and that catalyze the degradation of penicillins.
- Some people suffer from allergic reactions.

Therefore, the most serious problems with penicillin G are its acid sensitivity, sensitivity to β-lactamases and a narrow spectrum of activity. In order to solve these problems,

SAR studies are needed to establish which important characteristics for their activity should be kept in the analogues to be prepared.

11.3.4 Structure-activity relationships of penicillins

The results are as follows (Fig. 11.3):

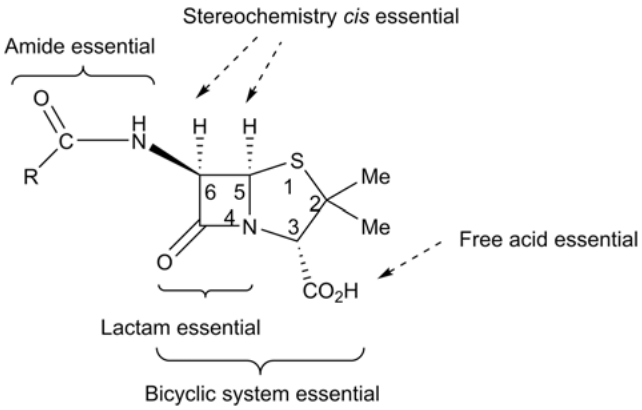


Fig. 11.3: Structure-activity relationships of penicillins.

Therefore, the only variations that can be carried out affect the acylamino chain side.

11.3.5 Sensitivity of penicillin G to acids

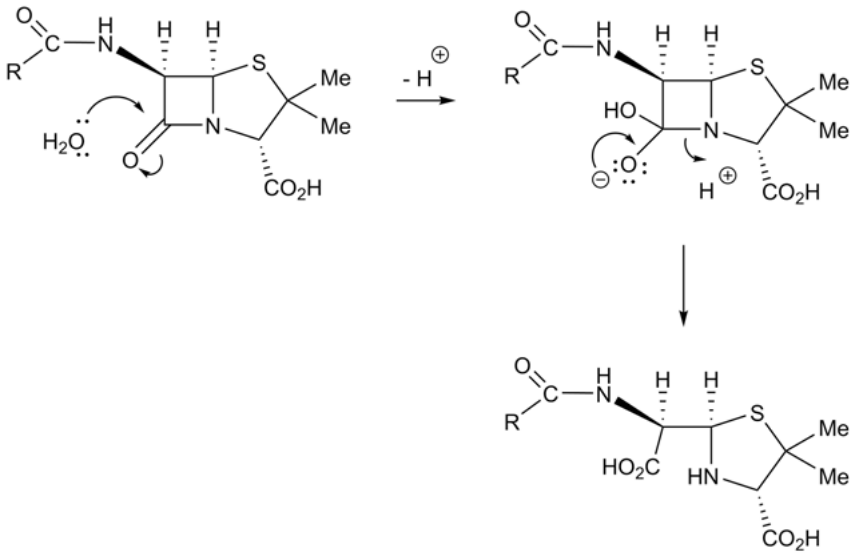
There are three reasons:

11.3.5.1 Ring strain

The bicyclic system of penicillin G suffers from large angular and torsional strains. Therefore, the acid-catalyzed opening alleviates these tensions by opening the stressed ring, which is the β-lactam system (Scheme 11.3).

11.3.5.2 The highly reactive carbonyl group of the β-lactam system

The carbonyl group of the β-lactam ring is very sensitive to nucleophiles and therefore does not behave as the component of a tertiary amide, which is normally quite resistant to nucleophilic attack. This difference in reactivity of the carbonyl group is due to the fact that stabilization in the tertiary amide is possible, but impossible in the lactam ring. The β-lactam nitrogen is unable to supply its electron pair to the car-



Scheme 11.3: Opening of the β-lactam ring under acidic conditions.

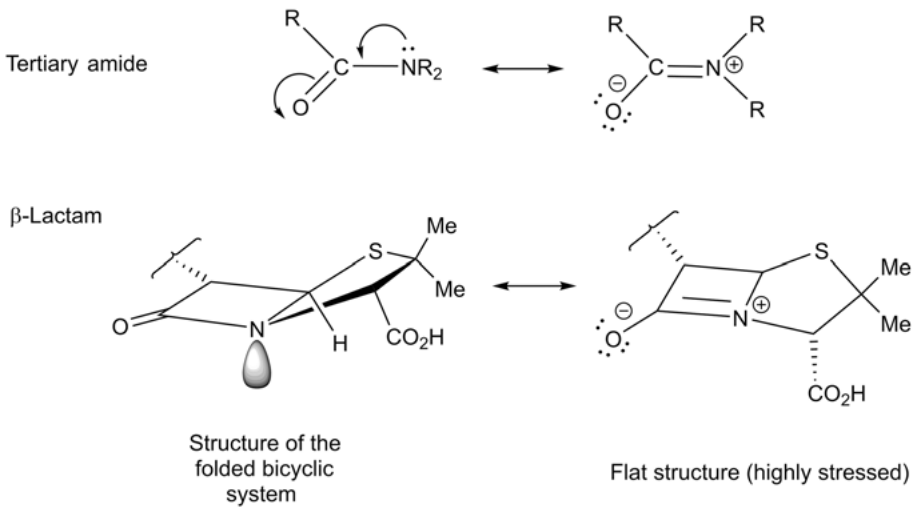
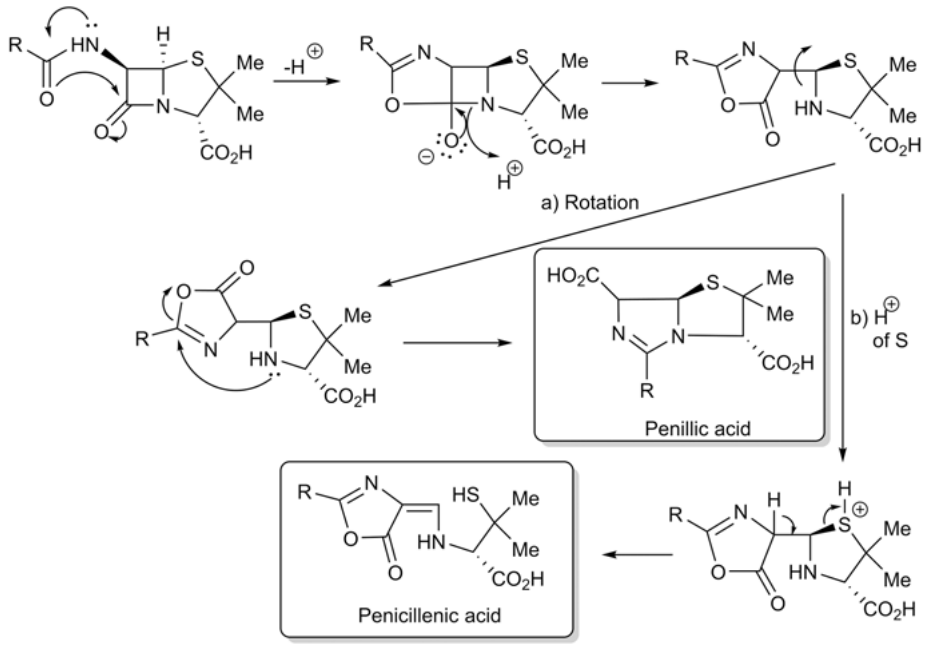


Fig. 11.4: Comparison between a tertiary amide and the carbonyl group of the β-lactam ring.

bonyl group, since it would be necessary for the bicyclic rings to adopt a flat, extremely strained arrangement. The electron pair is accordingly located on the N atom and the carbonyl group is much more electrophilic than would be expected for a tertiary amide (Fig. 11.4).



Scheme 11.4: Influence of the acyl side chain on the sensitivity of penicillins to acids.

11.3.5.3 Influence of the acylic lateral chain (neighboring-group participation)

The acyl group may participate in the β-lactam ring opening mechanism. In this way, penicillin G undergoes a mechanism of self-destruction (Scheme 11.4).

11.3.5.4 Facing the problem of acid sensitivity

Of the three factors that influence the sensitivity of penicillin G to acids, the last can be dealt with: decreased participation of the neighboring group to hinder the attack of the carbonyl group of the acyl against the β-lactam ring. If a good electron-withdrawing group were placed in the carbonyl group, then the inductive effect would withdraw electrons from the carbonyl oxygen and thus reduce its reactivity as a nucleophile (Fig. 11.5).

Penicillin V has an oxygen atom in the lateral acrylic chain with the consequent electron-withdrawing effect. This molecule has a better stability in the acid medium than penicillin G. Therefore, it can be administered orally. However, penicillin V is sensitive to penicillinase and is slightly less active than penicillin G.

The most interesting modifications are shown in Fig. 11.6.

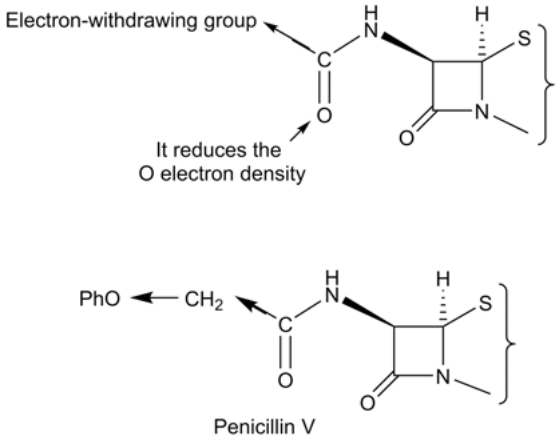


Fig. 11.5: Reduction of neighboring group participation by means of an electron-withdrawing group.

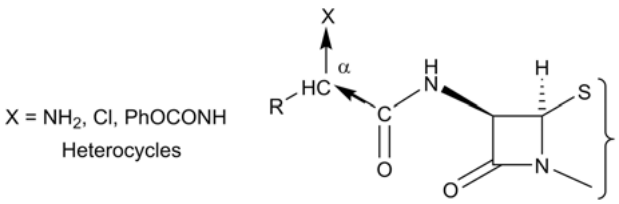
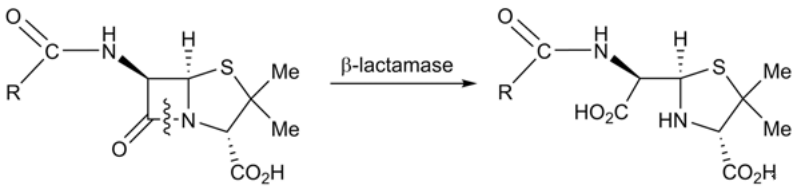


Fig. 11.6: Amino, chlorine and phenoxycarboxamido groups reduce the neighboring-group participation.

11.3.6 Penicillins sensitive to β-lactamases

β-Lactamases are enzymes produced by penicillin-resistant bacteria that catalyze the breakdown of the four-membered ring (Scheme 11.5):



Scheme 11.5: Action of β-lactamases on the four-membered ring of penicillins.

The β-lactamase problem became critical in the 1960s when the uncontrolled use of penicillin G led to an alarming increase in infections caused by *Staphylococcus aureus*. Eighty percent of all *S. aureus* infections in hospitals were due to strains of penicillin-resistant bacteria.

11.3.7 Facing the problem of β-lactamase sensitivity

The strategy is to block the access of penicillinase to penicillin and this is achieved by placing a bulky group in the side chain that would act as a "protective shield" (Fig. 11.7).

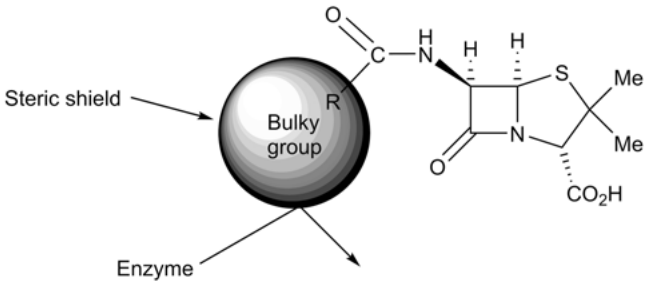


Fig. 11.7: Use of a steric shield to block the access of β-lactamase to the four-membered ring of penicillins.

Methicillin was the first semisynthetic penicillin that was not affected by penicillinase and was developed to combat the *S. aureus* problem (Fig. 11.8).

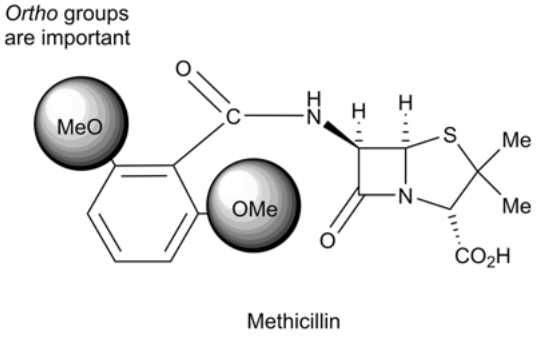


Fig. 11.8: Use of the steric shield to block the access of β-lactamase to the four-membered ring of methicillin.

Despite this, methicillin is not the ideal drug. It is sensitive to acids and has to be administered parenterally, because it does not have an electron-withdrawing group in the side chain. A more in-depth study solved the problem by incorporating a five-membered heterocyclic ring into the side chain with the intention of creating an electron-withdrawing group and a protective shield (Fig. 11.9).

These compounds (oxacillin, cloxacillin and flucloxacillin) are resistant to acids and penicillinase and are useful against *S. aureus* infections.

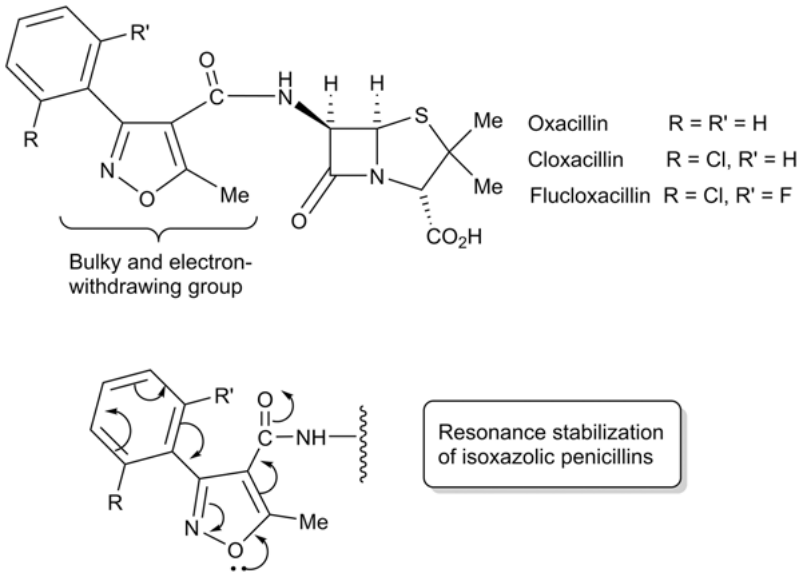


Fig. 11.9: Oxacillin, cloxacillin and flucloxacillin.

11.3.8 Resistance to penicillins

Bacterial strains show differing susceptibilities to penicillin. Some species, such as streptococci, are very vulnerable, while bacteria such as *Pseudomonas aeruginosa* are particularly resistant. Other species, such as *Staphylococcus aureus*, although initially vulnerable, gained resistance when exposed to penicillin for a certain period of time. There are several reasons that may explain these differences in susceptibility (Fig. 11.10).

11.3.8.1 Permeability barrier

If penicillin has to inhibit the enzyme transpeptidase, it has to reach the outermost part of the bacterial cell membrane where the enzyme is located. Therefore, penicillin has to traverse the cell walls of Gram-positive and Gram-negative bacteria. The cell wall is much thicker in Gram-positive bacteria than in Gram-negative bacteria, so one might think that penicillin would be more effective against Gram-negative bacteria. However, it is not so. Although the cell wall is a strong and rigid structure, it is also highly porous in such a way that small molecules such as penicillin can move through it without difficulty.

Gram-positive bacteria do not have this outer coating and this is the reason why penicillin G has good activity against these organisms. Gram-negative bacteria have a liposaccharide membrane that surrounds the cell wall, which is impermeable to water

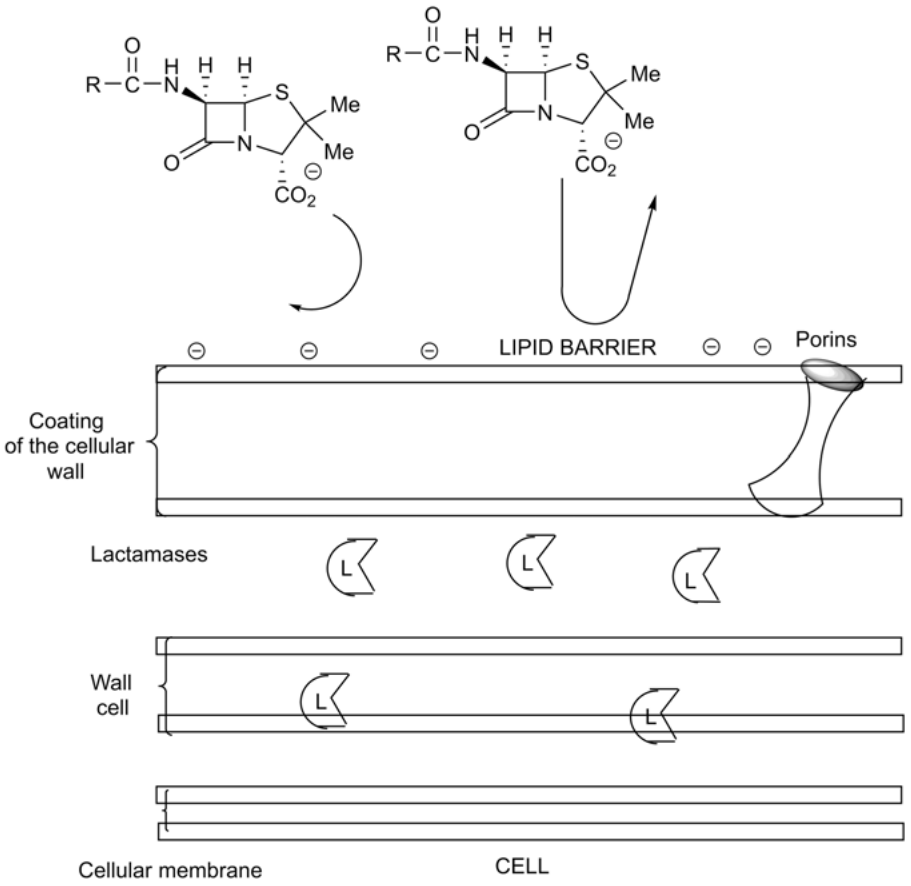


Fig. 11.10: Outside surface of a Gram-negative bacterial cell.

and to polar molecules such as penicillin (Fig. 11.10). This may explain why Gram-negative bacteria are generally resistant, but not why some Gram-negative bacteria are sensitive and others are not.

The answer lies in protein structures called porins, which are located in the outer lining. They act as pores through which water and essential nutrients can pass to reach the interior of the cell. The fact that penicillin can or cannot pass through the outer coating depends on both the structure of the pores and the characteristics of penicillin (i.e., its size, structure and charge). In general, drugs are less likely to pass through porins if they are large, have a negative charge and are hydrophobic. Conversely, a small hydrophilic drug that may exist as a double ion ("zwitterion" in German) will cross the pore rapidly. Therefore, porins play a crucial role in controlling the amount of penicillin capable of reaching the space between the lining of the cell wall and the cell membrane.

Penicillin has a free carboxylic acid that would be repelled by the first type of coating. In addition, the lipidic portion of this coating may act as a barrier against polar penicillins.

11.3.8.2 High levels of the enzyme transpeptidase

The enzyme transpeptidase is attacked by penicillin. In some Gram-negative bacteria, a large amount of transpeptidase is produced, and penicillin is unable to inactivate all the molecules of the present enzyme.

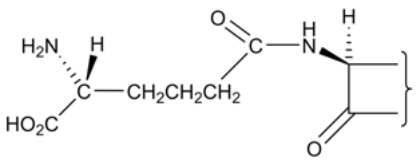
11.3.8.3 Presence of β-lactamases

They are located between the cell wall and the outer lining.

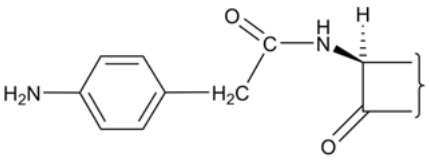
11.3.9 Addressing the narrow-spectrum problem

A large number of derivatives have been prepared and the results are as follows:

- Hydrophobic groups in the side chain (e.g., penicillin G) favor activity against Gram-positive bacteria, but give rise to poor activity against Gram-negative bacteris.
- Side chain hydrophilic groups do not have any effect on Gram-positive activity (e.g., penicillin T) or cause a reduction in activity (e.g., penicillin N) (Fig. 11.11). However, they lead to an increase in the activity against Gram-negative bacteria.
- The increase in the Gram-negative activity is greater if the hydrophilic group (e.g., $-NH_2$, -OH, $-CO_2H$) is located at the Cα in relation to the carbonyl group in the side chain (Fig. 11.11).



Penicillin N



Penicillin T

Antibacterial activities against penicillin G

Gram +	Gram -	Gram +	Gram -
1%	Higher	Same	2-4 times greater

Fig. 11.11: Antibacterial activities of penicillins N and T.

Penicillins that exhibit activity against both Gram-positive and -negative bacteria are known as broad-spectrum antibiotics. There are two classes, and both have a polar hydrophilic group in the α-position with respect to the carbonyl group of the lateral acrylic chain. In the first type, the hydrophilic group is an amino function, as in ampicillin and amoxicillin (Fig 11.12):

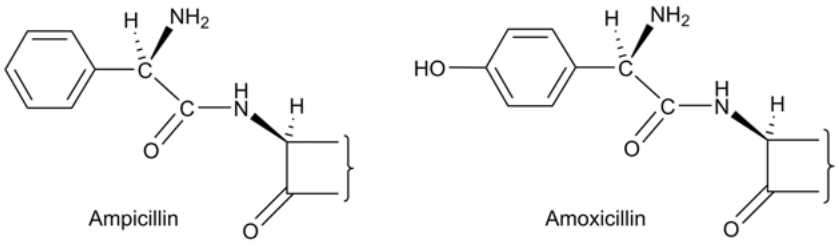


Fig. 11.12: Broad-spectrum antibiotics.

Both suffer from poor absorption through the intestine, due to the dipole nature of the molecule, since both have a free amino group and another carboxylic acid one. This problem can be solved by using a prodrug in which one of the polar groups is masked. This group is removed metabolically, once the prodrug has been absorbed through the walls of the intestine (Fig.11.13).

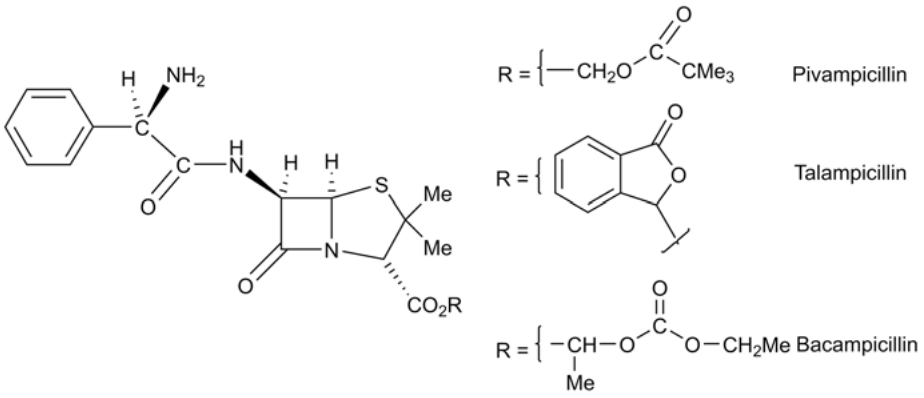


Fig. 11.13: Ampicillin prodrugs.

Why do not use a simple methyl ester? The reason is that the methyl esters of penicillins are not metabolized in man. Perhaps the size of the penicillin skeleton, being so close to the functional group, prevents the attack of esterases. Fortunately, acyloxymethyl esters have been found to be susceptible to nonspecific esterases. These

"extended" esters contain a second ester group beyond the penicillin nucleus and are therefore more prone to attack. The hydrolysis products are unstable and decompose spontaneously to release the free carboxylic acid (Fig. 11.14).

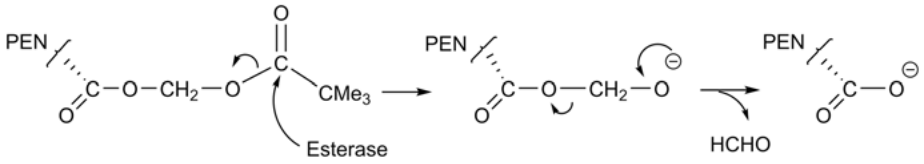


Fig. 11.14: Breakdown of penicillin prodrugs with acyloxymethyl esters.

The second type includes the group of carboxypenicillins: among them, carbenicillin (Fig. 11.15) is the first example of this class of compounds and is active against a greater range of Gram-negative bacteria than ampicillin. It is resistant against most penicillinases and is also active against *Pseudomonas aeruginosa*. This is an "opportunistic" pathogen that attacks debilitated patients. This organism is usually found in the body but is controlled by self-defense mechanisms.

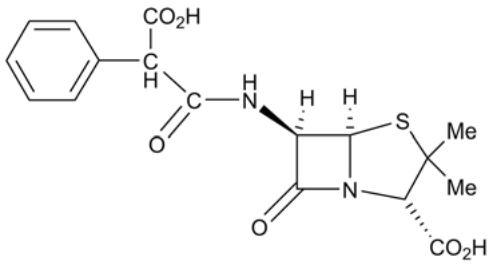


Fig. 11.15: Carbenicillin.

11.4 Cephalosporins

The first known cephalosporin was cephalosporin C (Fig. 11.16), isolated in 1948 from a fungus obtained from fecal waters on the island of Sardinia.

A study of the cephalosporin skeleton reveals that it can be derived from the same biosynthetic precursors as penicillin, i.e., cysteine and valine (Fig 11.17).

The transpeptidase, responsible for the formation of the peptidoglycan that gives robustness to the wall of the bacterium, attacks the carbonyl group of the β-lactam ring in the form sketched in Fig. 11.18.

The bicyclic system of cephalosporins is not as unstable as that of penicillins. However, the presence of the enamine moiety in the six-membered cycle of the cephalosporins causes the electron pair of the nitrogen atom on one of the bridge-

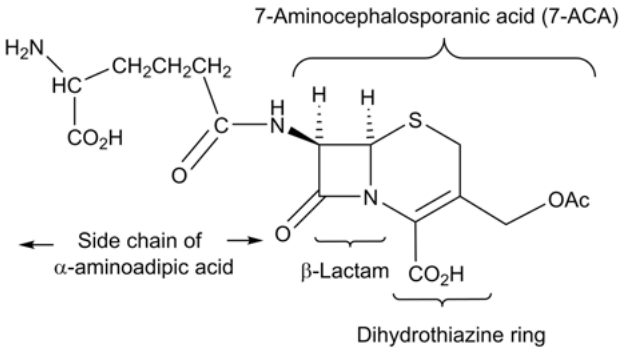


Fig. 11.16: Cephalosporin C.

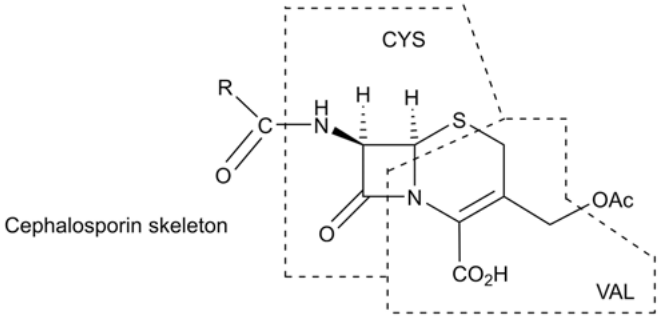


Fig. 11.17: Biosynthetic precursors of cephalosporins.

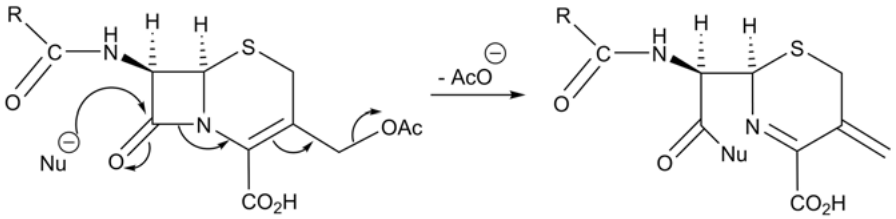


Fig. 11.18: Attack of a nucleophile on the four-membered ring of cephalosporins.

heads of the spiro system to "divide" between delocalization with the carbonyl group (as it would in a classical amide) and the enaminic double bond. This is even more favored by the presence of a good leaving group (such as the acetoxy group). The lactam group does not behave as a classical amide group, showing reactivity closer to that of an acid chloride, thus demonstrating the antibiotic properties of cephalosporins.

Cephalosporins (Δ^3-cephalosporins, taking into account that S is number 1, and the spiro is 5, as locants) and penicillins are structurally related by the fact that the β-lactam ring is fused to different rings. The position of the double bond on

Δ^3-cephalosporins is very important, since Δ^2-cephalosporins, regardless of the nature of the side chain, are not antibacterial agents. In contrast, Δ^2-penicillins are highly active against Gram-positive and Gram-negative bacteria (Fig. 11.19).

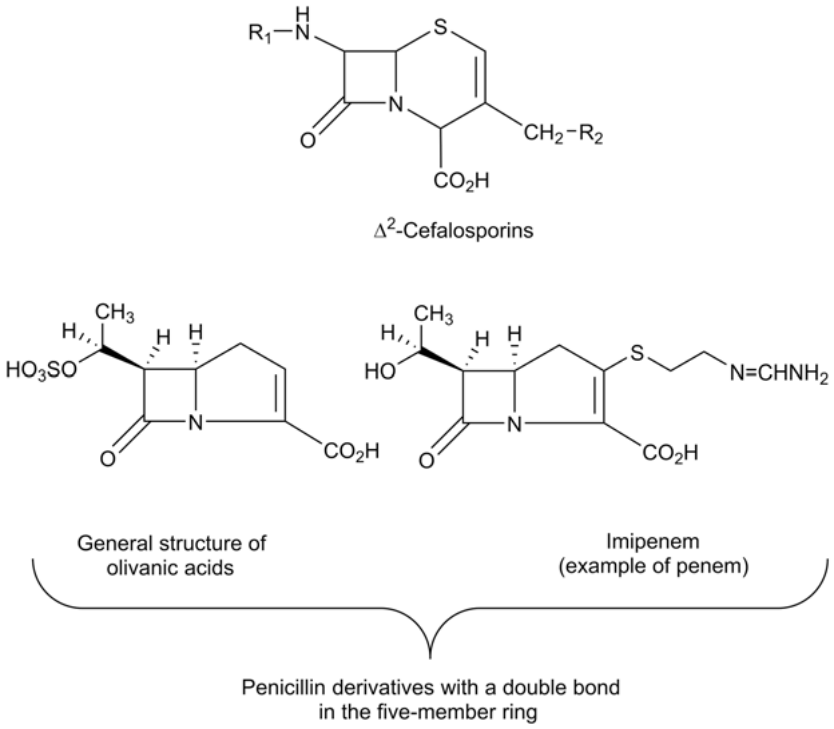


Fig. 11.19: Various structures related to cephalosporins.

Cephalosporin C itself has been used in the treatment of urinary tract infections since it has been found to be concentrated in the urine and survives the body's hydrolytic enzymes. Fermentation in the presence of precursors is not useful for obtaining cephalosporins modified in acyl, since in all cases the same derivative is formed, i.e. cephalosporin C with the D-aminoadipoyl group in 7β.

11.4.1 SARs of cephalosporin C

- The β-lactam ring is essential.
- A free carboxylic group is needed at position 2.
- The bicyclic system is essential.
- Stereochemistry of the side chain and rings is important.

Consequently, there are only a limited number of sites where modifications can be made (Fig. 11.20).

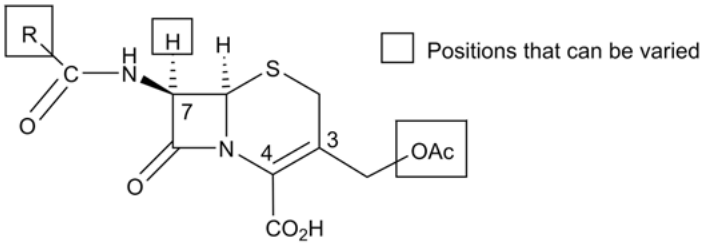
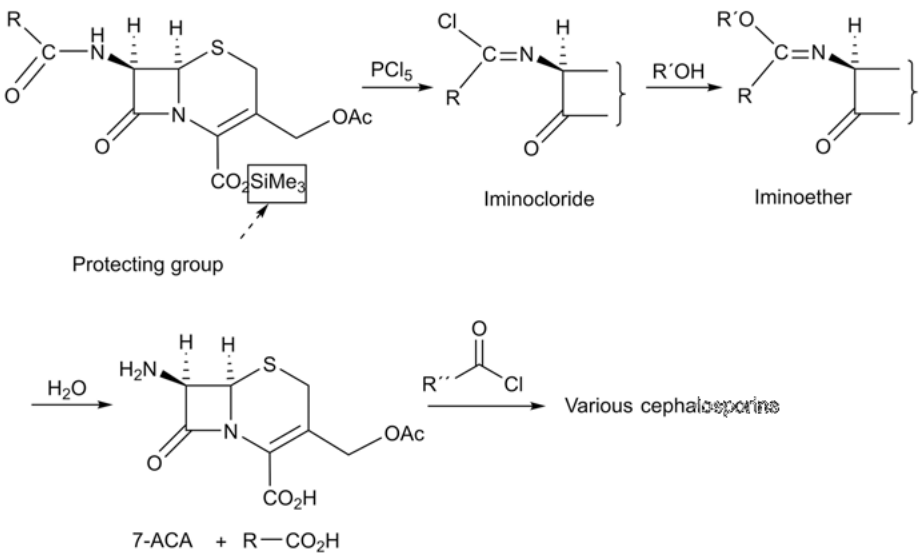


Fig. 11.20: Positions for possible modifications in cephalosporin C. The squares indicate the positions that can be varied.

11.4.2 Cephalosporin C analogues by variation of the 7-acylamine side chain

It is not possible to obtain 7-aminocephalosporanic acid (7-ACA) by fermentation nor by enzymatic hydrolysis of cephalosporin C, so the semisynthetic approach would not be possible in a manner analogous to the preparation of penicillins from 6-APA. The correct approach is shown in Scheme 11.6.



Scheme 11.6: Synthesis of 7-ACA, and cephalosporin analogues.

The strategy takes advantage of the β-lactam nitrogen being unable to share its electron pair with the neighboring carbonyl group. The first step requires the formation of a double bond between the side chain nitrogen and the carbonyl group. This is only possible for the secondary amide group since the ring constraints prevent the β-lactam nitrogen from forming a double bond with the β-lactam ring. An interesting analogue is cephalothin (Fig. 11.21).

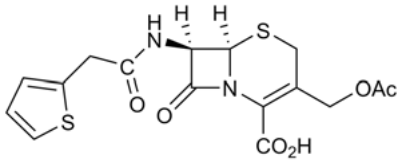
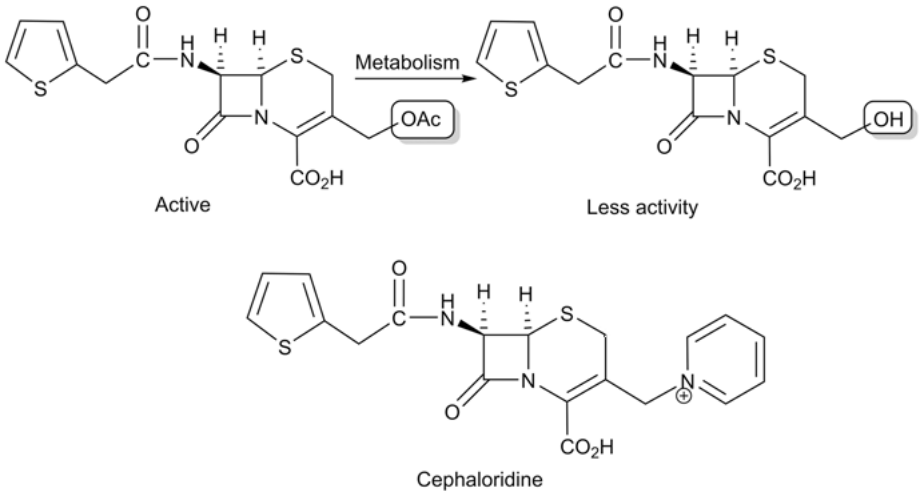


Fig. 11.21: Cephalothin.

11.4.3 Cephalosporin C analogues by variation of the 3-acetoxymethyl side chain

The loss of the 3-acetyl group leaves the alcohol group free and results in a loss of activity. This hydrolysis occurs metabolically and therefore it would be useful to block this process, prolonging the activity of cephalosporins. An example is cephaloridine, which contains a pyridinium residue instead of the acetoxy group (Scheme 11.7).



Scheme 11.7: Metabolic hydrolysis of cephalothin. Structure of cephaloridine.

A second example is cephalexin, which has no substitution at the 3-position. It is one of the few cephalosporins that is absorbed through the intestine and can be taken orally (Fig. 11.22).

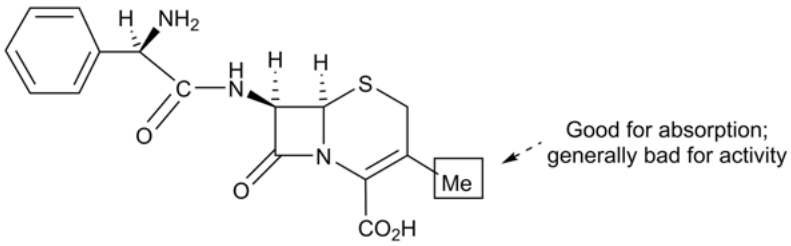
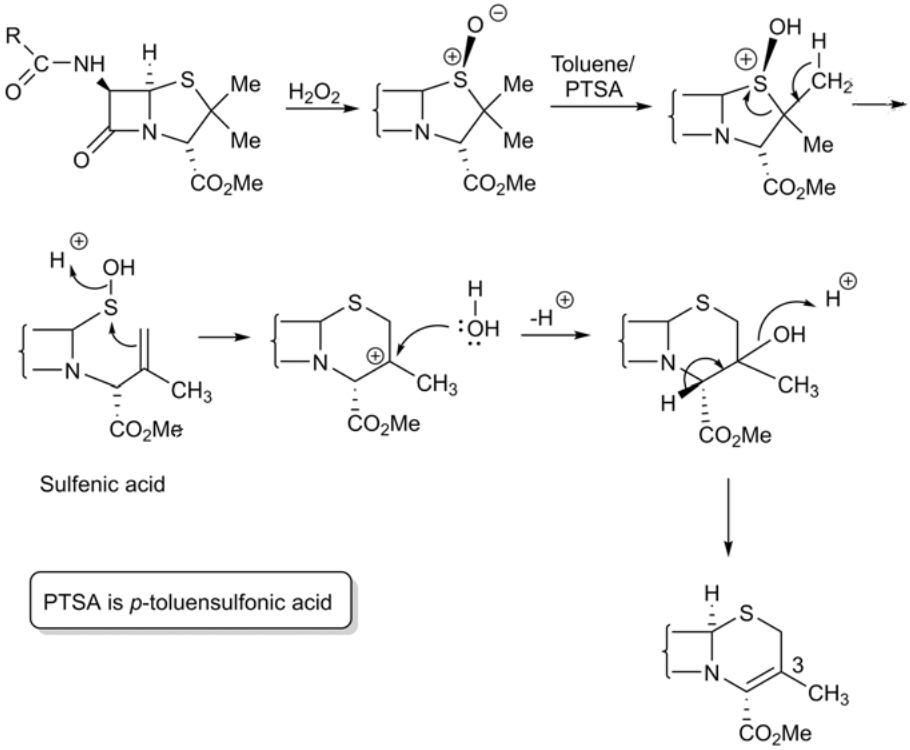


Fig. 11.22: Cephalexin.

11.4.4 Synthesis of 3-methylated cephalosporins

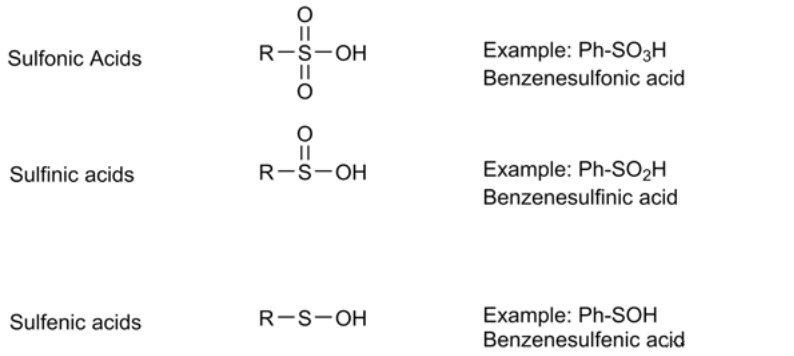
For the synthesis of these derivatives, it is better to start from the penicillin nucleus, and to carry out an expansion of the thiazolidine ring to the dihydrothiazine ring (Scheme 11.8).



Scheme 11.8: Synthesis of 3-methylated cephalosporins from a penicillin.

11.4.5 Disclaimer

Acids derived from sulfur are of three types, as specified in Scheme 11.9.



Scheme 11.9: Sulfur acids.

11.4.6 Summary of the properties of cephalosporins

– Injectable cephalosporins of clinical use have high activity against a large number of Gram-positive and Gram-negative organisms, including *staphylococci* that are resistant to penicillins.

– Most cephalosporins are poorly absorbed through the intestinal wall.
– In general, cephalosporins have lower activity than penicillins, but a better interval.

Introducing a “steric shield” into the cephalosporins leads to inactive compounds. The next event was the discovery that cephalosporins substituted at the 7-position are active. Introducing a 7-methoxy group gave rise to a group of compounds known as cephamycins (Fig. 11.23).

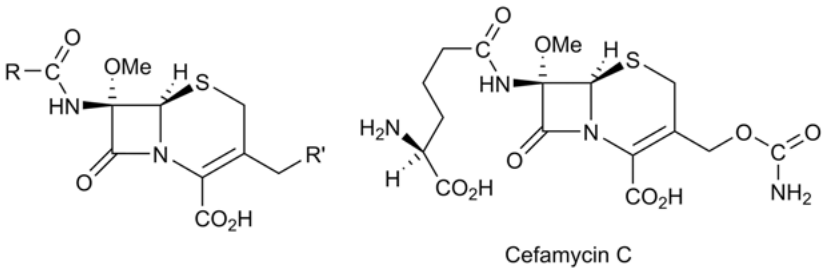


Fig. 11.23: Cefamycin and analogues derivatives.

11.5 Clavulanic acid (Beechams, 1976)

Clavulanic acid (Fig. 11.24) has no antibiotic activity. However, it is a powerful irreversible inhibitor of most β-lactamases, and is currently used with traditional penicillins such as amoxicillin to give Augmentin® (amoxicillin and clavulanate). This decreases the amount of amoxicillin and also increases the spectrum of activity. Fig. 11.24 shows the essential requirements for β-lactamase activity of clavulanic acid.

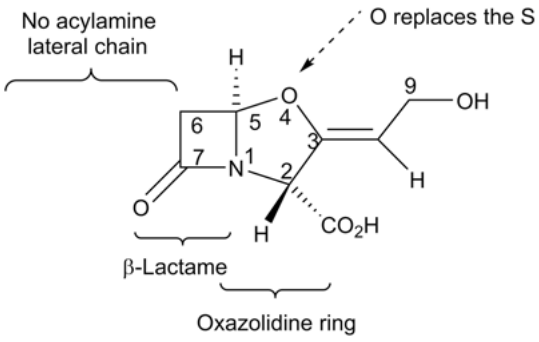


Fig. 11.24: Clavulanic acid.

Clavulanic acid is a suicide substrate. It is an inhibitor of most β-lactamases, so it is used in combination with nonresistant penicillins, such as amoxicillin. The drug fits into the active site of β-lactamase, and the β-lactam ring is opened by a serine moiety. This acyl-enzyme intermediate reacts with another enzymatic nucleophilic group (NH_2) to irreversibly bind the drug to the enzyme. The mechanism requires the loss or gain of protons in several stages, so that the histidine present in the active site is able to act as a donor/proton acceptor (Fig. 11.25).

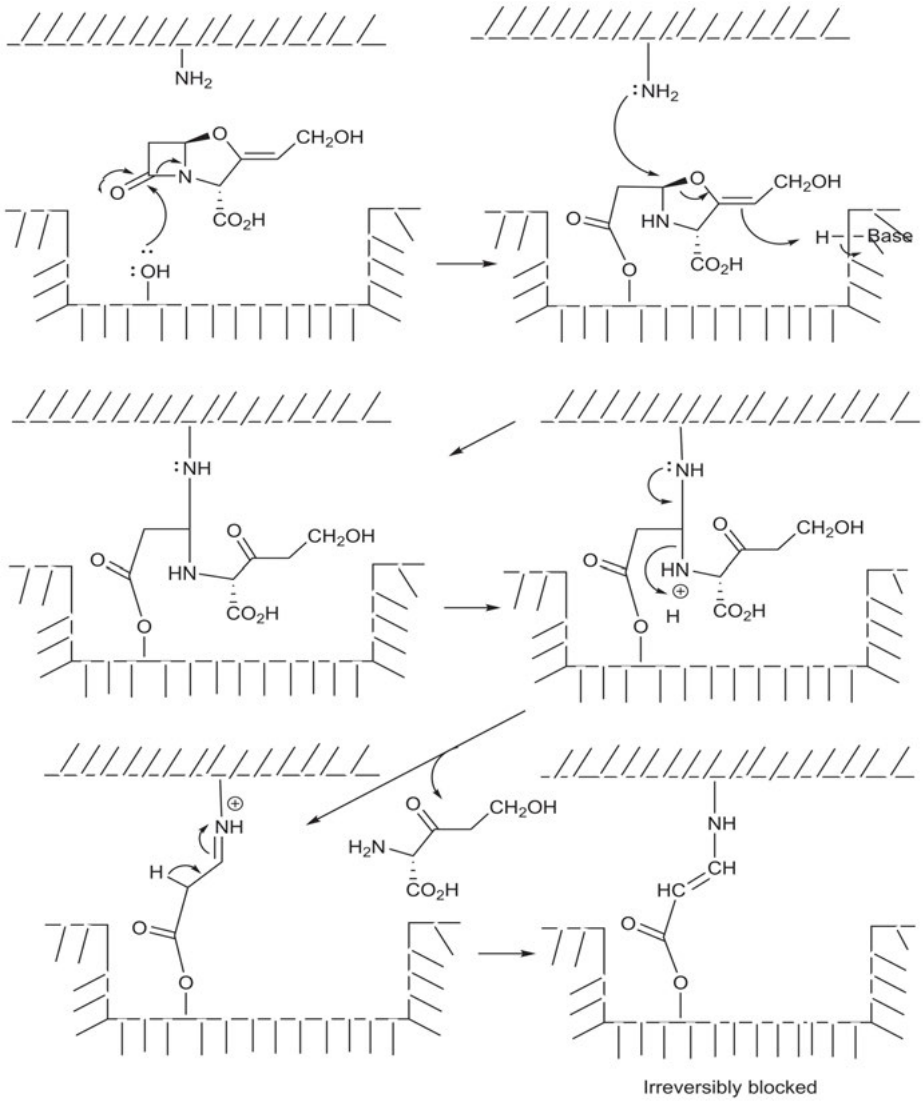


Fig. 11.25: Mechanism of action of clavulanic acid as a suicide inhibitor of β-lactamases.

11.6 Mechanism of action of penicillins and cephalosporins

The wall of the bacteria has a peptidoglycan structure. It is formed by both peptide units and sugars. The wall structure consists of a parallel series of sugar residues containing two types of sugars [*N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG)]. Peptide chains bind to NAM sugars and in the final phase of cell wall biosynthesis, these peptide chains bind together by replacing the D-alanine from one chain with the glycine of the other one (Fig. 11.26).

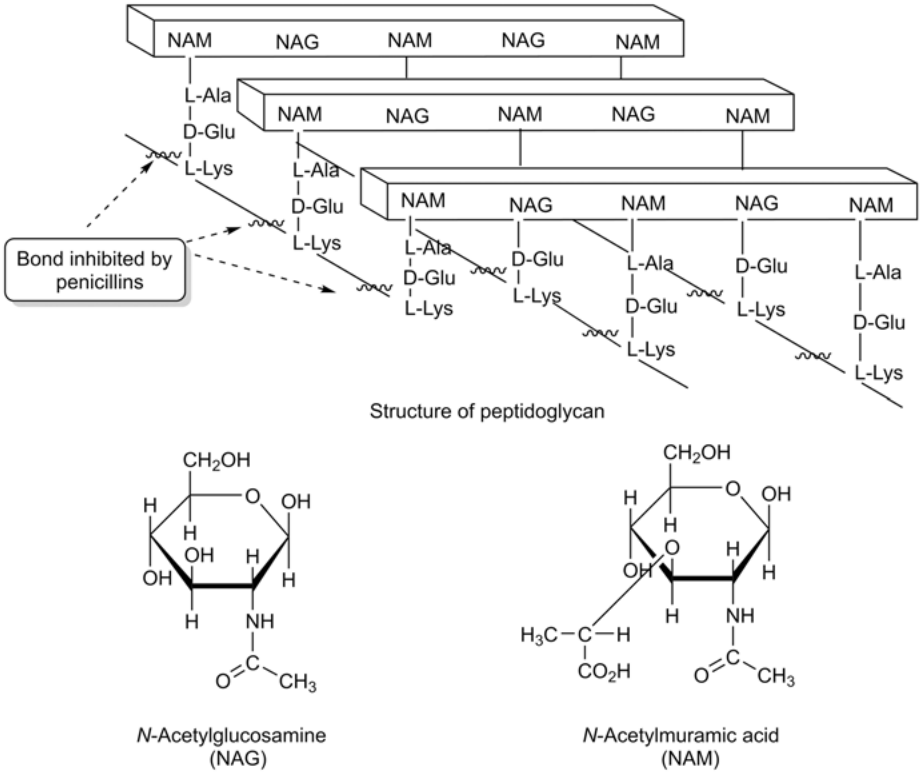


Fig. 11.26: Structure of the bacterial peptidoglycan of the cell wall. *N*-Acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM).

It is this final crosslinking reaction that is inhibited by penicillins and cephalosporins, so that the cell wall is not consistent because the cell wall framework is not meshed together. The enzyme responsible for this crosslinking reaction is known as transpeptidase (Fig. 11.27).

It has been proposed that penicillin exhibits a conformation which is similar to the conformation of the transition state D-Ala-D-Ala, the portion of the chain of amino

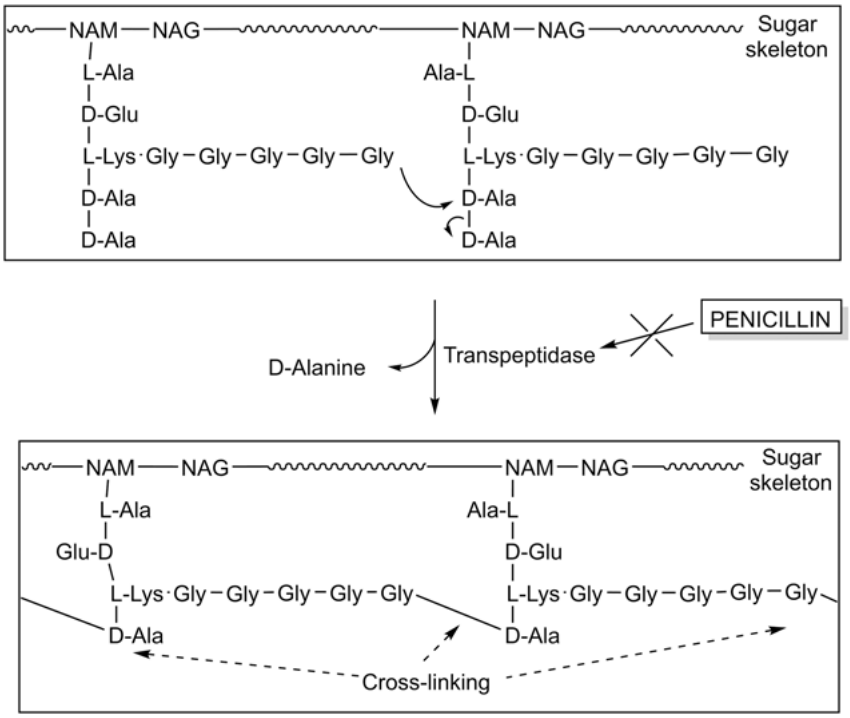


Fig. 11.27: Crosslinking of the bacterial cell wall which is inhibited by penicillins.

acids involved in the crosslinking reaction. The enzyme can attack the β-lactam ring and open it. However, since penicillin is cyclic, the molecule is not divided into two and remains attached to the active site. The following hydrolysis of the acyl group does not occur, presumably because the glycine is unable to reach the active site due to the steric hindrance caused by penicillin (Fig. 11.28).

However, there are some doubts regarding this theory, since there are two abnormalities. For example, 6-methylpenicillin (Fig. 11.29) is a very close analogue of D-Ala-D-Ala. It should be fixed better to the active center and lead to increased activity. However, it shows lower activity.

An alternative proposition is that penicillin does not bind to the active site, but does so in a place close to it. In this way, the structure of penicillin overlaps the active center and prevents the reagents from gaining access: the umbrella effect. If a nucleophilic group (not necessarily in the active center) attacks the β-lactam ring, penicillin remains irreversibly bound, permanently blocking the active site (Fig. 11.30).

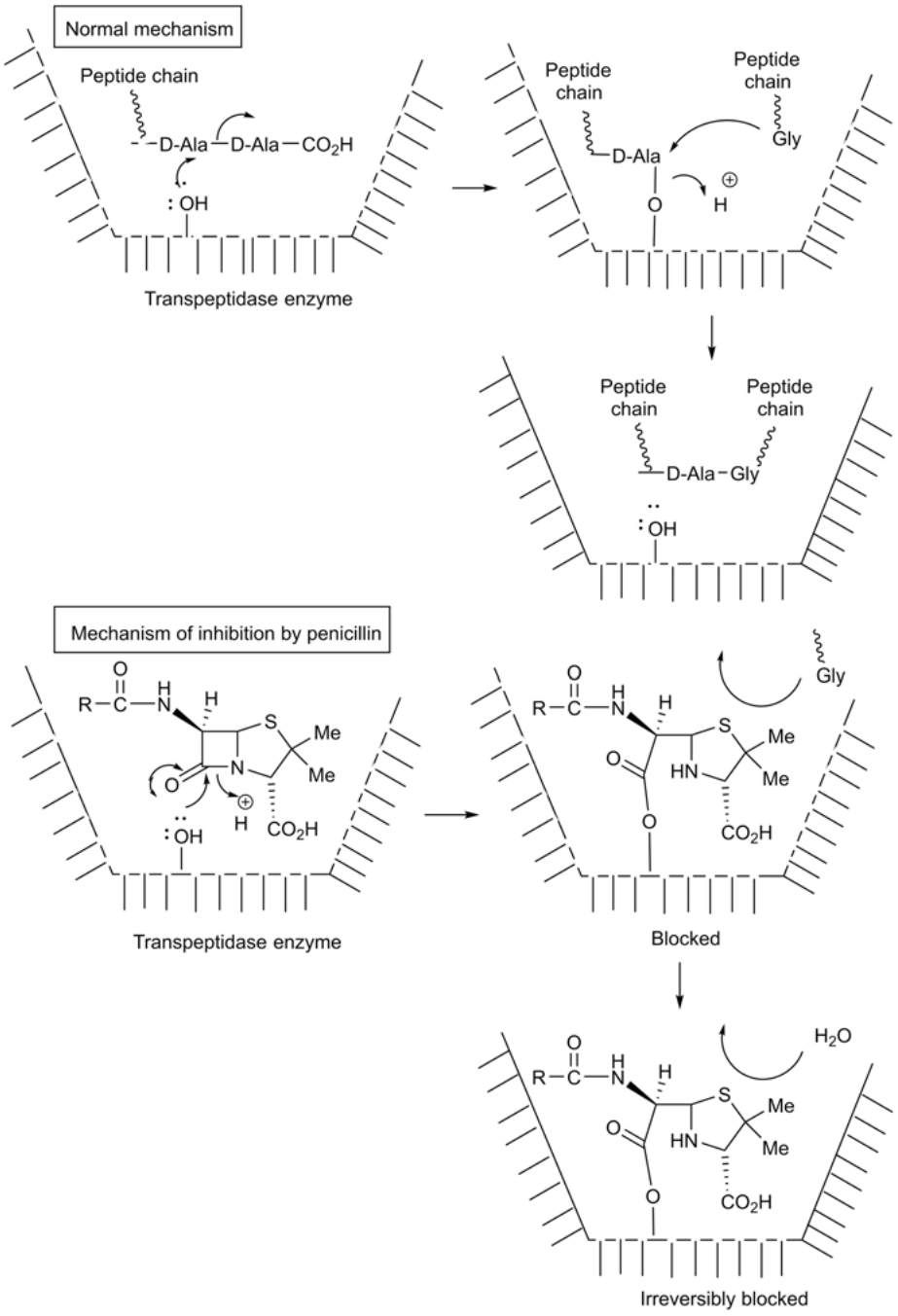


Fig. 11.28: Mechanism of transpeptidase crosslinking and inhibition by penicillin.

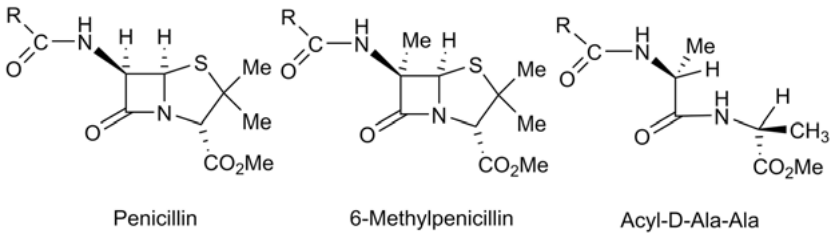


Fig. 11.29: Analogy between 6-methylpenicillin and Acyl-D-Ala-Ala.

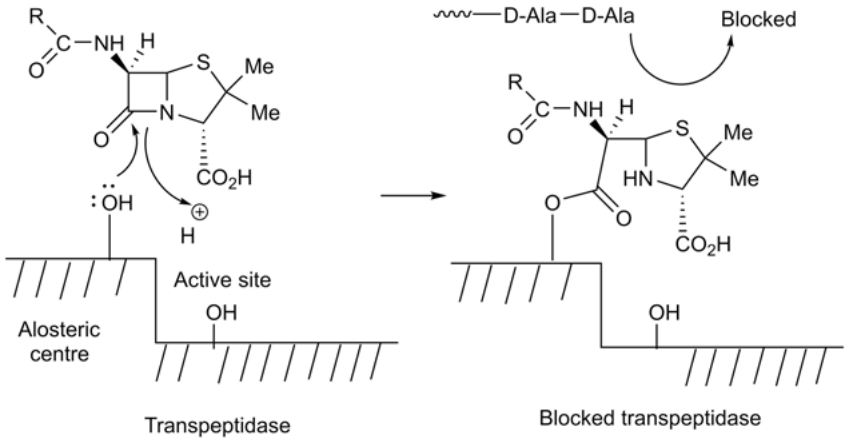


Fig. 11.30: Inhibitory character of penicillins in the crosslinking reaction produced by transpeptidase.

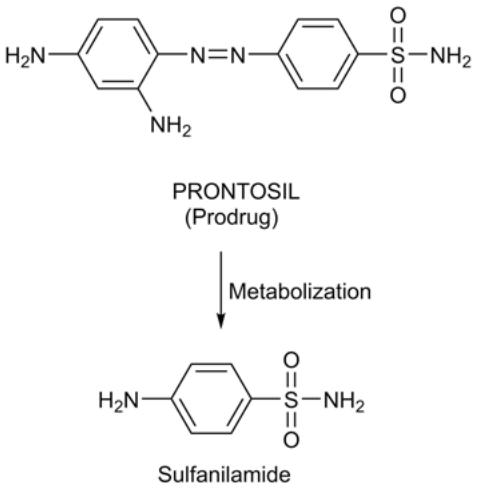
12 Enzymatic inhibition: Other antibacterial agents

12.1 Goals

- Foster knowledge of the therapeutic antibacterial arsenal belonging to other fields and their mechanisms of action.
- Recognize the need for these antibacterials and their semisynthetic compounds.

12.2 Introduction

The best example of antibacterial agents are sulfonamides (sometimes called sulfas), acting as antimetabolites. The history of sulfonamides began in 1935 when it was discovered that a red dye called prontosil had antibacterial properties in vivo. The dye was found to be metabolized by the bacteria in the host's small intestine, and degraded to give a product called sulfanilamide. This compound is the true antibacterial agent. Thus, prontosil was the first example of a prodrug (Scheme 12.1).



Scheme 12.1: Metabolization of prontosil.

Despite its undeniable benefits, sulfas have been ineffective against infections caused by *Salmonella Typhi* (they are combated with chloramphenicol and third-generation cephalosporins), the organism responsible for typhoid fevers. Other problems are the products of metabolization, since toxic products are frequently obtained. This led to sulfonamides being replaced by penicillins.

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12.3 SARs

- The *p*-amino group is essential for activity and does not have to be substituted.
- Both the aromatic ring and the sulfonamide functional group are required.
- Nitrogen of the sulfonamides has to be secondary. R is the only possible place that can be varied in sulfonamides.

12.4 Sulfanilamide analogues

R can be varied by incorporating a wide variety of heterocyclic or aromatic structures (Fig. 12.1), which affect the extent to which the drug binds to plasma proteins. This in fact controls the blood levels, so it may be a short- or long-lasting drug. In this way, a drug that binds strongly to plasma proteins will be released slowly into the bloodstream and will be a long-lasting compound.

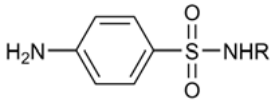


Fig. 12.1: Sulfonamide analogues.

The change in the nature of the R group has also helped to reduce the toxicity of some sulfonamides. The primary amino group of the sulfonamides is acetylated in the body and the resulting amides exhibit reduced solubility, which can lead to toxic effects. For example, the metabolite formed from sulfathiazole (one of the first sulfonamides) is very sparingly soluble and can be fatal if it blocks the tubules of the kidney (Fig. 12.2).

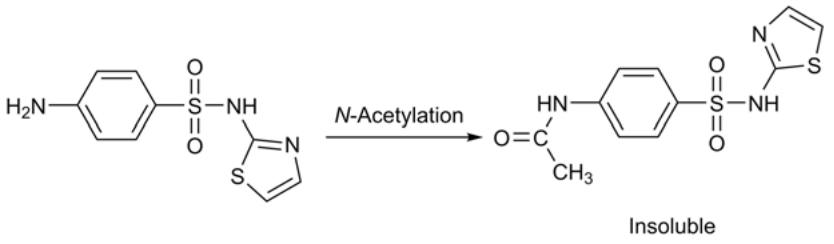


Fig. 12.2: *N*-Acetylation of sulfathiazole.

It was discovered that the problem of solubility can be solved by replacing the thiazole ring in the sulfatiazole with a pyrimidine ring, to give the sulfadiazine. The reason for the solubility lies in the acidity of the proton of the NH group of the sulfonamide moiety. In sulfatiazol, this proton is not very acidic (high pK_a). Therefore, sulfathi-

azole and its metabolite are fundamentally not ionized at the pH of the blood. The substitution of the thiazole ring for a stronger electron-withdrawing ring, such as the pyrimidine ring, increases the acidity of the NH group proton by stabilizing the resulting anion. Therefore, sulfadiazine and its metabolite are ionized to the pH of the blood (Fig. 12.3). They are accordingly more soluble and less toxic.

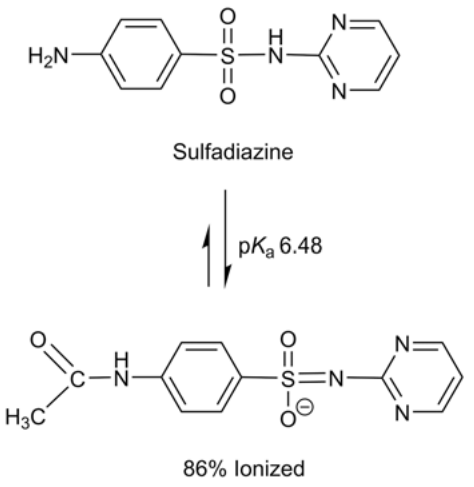


Fig. 12.3: Sulfadiazine and its metabolite are fundamentally ionized to the pH of the blood.

Sulfadiazine was found to also be more active than sulphathiazole and ultimately it has been substituted in therapy.

12.5 Applications of sulfonamides

Prior to the appearance of penicillins, sulfonamides were the drugs of choice in the treatment of infectious diseases. There has been a resurgence of interest in sulfonamides since the long-term sulfonamides have been developed. An example of this generation is sulfamethoxine (Fig. 12.4).

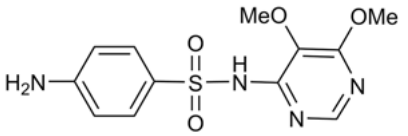


Fig. 12.4: Sulfamethoxine.

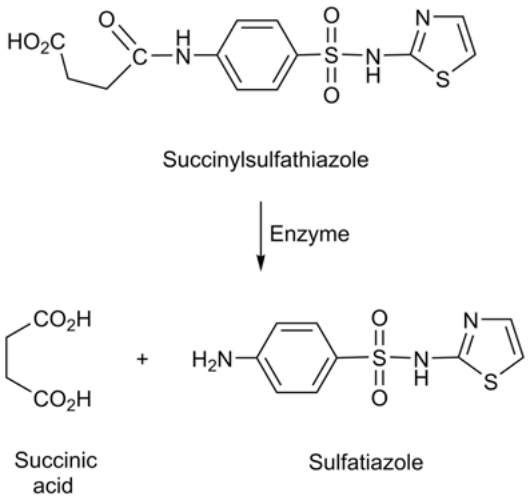
Pneumocystis carinii infection is the most frequent and one of the most serious infections affecting AIDS patients. It causes severe bronchopneumonia, which often has a

fatal evolution. The recommended drug is trimethoprim-sulfamethoxazole (the combination is called co-trimoxazole).

Sulfas have the following applications in medicine:

- Treatment of urinary tract infections.
- Treatment of infections of the intestine.
- Ophthalmic eye drops (this subject was studied within carbonic acid inhibitors in Chapter 8).
- Prophylaxis of respiratory infections in HIV-infected patients.

Sulfonamides have been particularly useful against infections of the gut and this site of action can be fixed by the use of prodrugs. For example, succinyl sulfathiazole is a prodrug of sulfathiazole. The succinyl group converts the basic sulfatiazole into an acid whereby the prodrug ionizes under the mildly alkaline conditions of the intestine. It is not absorbed accordingly into the bloodstream and is retained in the intestine. Slow enzymatic hydrolysis of the succinyl group liberates the active sulfatiazole (Scheme 12.2).



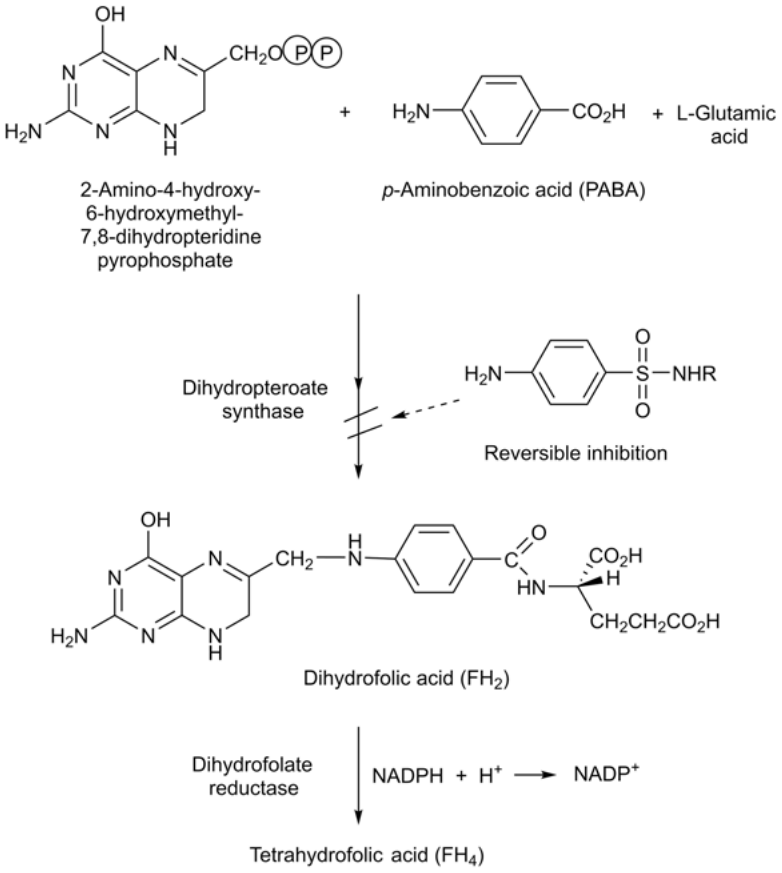
Scheme 12.2: Enzymatic hydrolysis of succinylsulfathiazole.

12.6 Mechanism of action

Sulfonamides act as competitive enzyme inhibitors and block the biosynthesis of tetrahydrofolic acid (THF) in bacterial cells. They do this by inhibiting the enzyme responsible for binding the different components of folic acid. The consequences of this are disastrous for the cell. Under normal conditions, folic acid is the precursor of

tetrahydrofolic, a compound that is crucial for the biochemistry of the cell, as it acts as a carrier of one carbon atom, necessary for many biosynthetic pathways. If THF is not synthesized, any biosynthetic pathway that requires fragments of one carbon atom is disrupted. The biosynthesis of the nucleic acids is interrupted accordingly, and this fact leads to the cessation of cell growth and division (Scheme 12.3).

It should be pointed out that sulfonamides do not kill bacterial cells: they prevent them from dividing and multiplying. Antibacterial agents that inhibit cell growth are classified as bacteriostatic, whereas those that kill bacterial cells (e.g., penicillins) are classified as bactericidal.



Scheme 12.3: Mechanism of inhibition of sulfamides.

Sulfonamides act as inhibitors mimicking PABA (acronym for *p*-aminobenzoic acid), one of the normal constituents of folic acid. The sulfonamide molecule is quite similar in structure to the PABA, so the enzyme is deceived, and accepts it in its active center

(Fig. 12.5). As a result, folic acid is no longer synthesized. Since folic acid is essential for cell growth, division of the cell ceases.

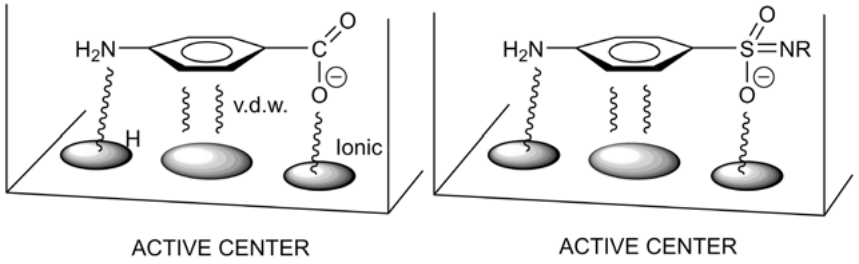


Fig. 12.5: Sulfonamide (by mimetism) prevents PABA from binding to the enzyme.

Folic acid is also vital for the survival of human cells. Consequently, why do sulfonamides not affect human cells as well? The answer lies in the fact that human cells cannot synthesize folic acid. Human cells acquire folic acid through the diet. Folic acid passes through the cell membrane by means of a transport protein. It is known that bacterial cells are unable to assimilate folic acid because they lack the necessary transport protein that is required to transport it through the cell membrane. Therefore, they are forced to synthesize folic acid.

To summarize, the success of sulfonamides is due to two metabolic differences between mammalian and bacterial cells: (a) bacteria have an enzyme that is not present in mammalian cells; (b) bacteria lack the transport protein that would allow for folic acid to be supplied from outside the cell.

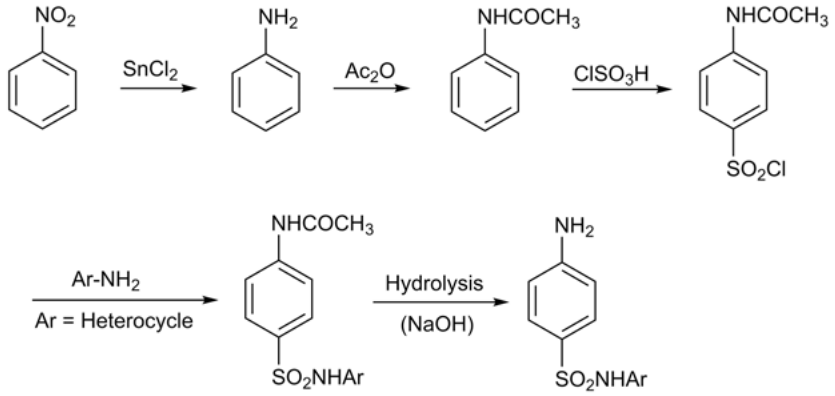
12.7 Synthesis of sulfonamides

Their syntheses are carried out from the aniline, which after protection allows the sulfonation in *para*. The greater ease of hydrolysis of the carboxamides against the sulfonamides is used for the final deprotection (Scheme 12.4).

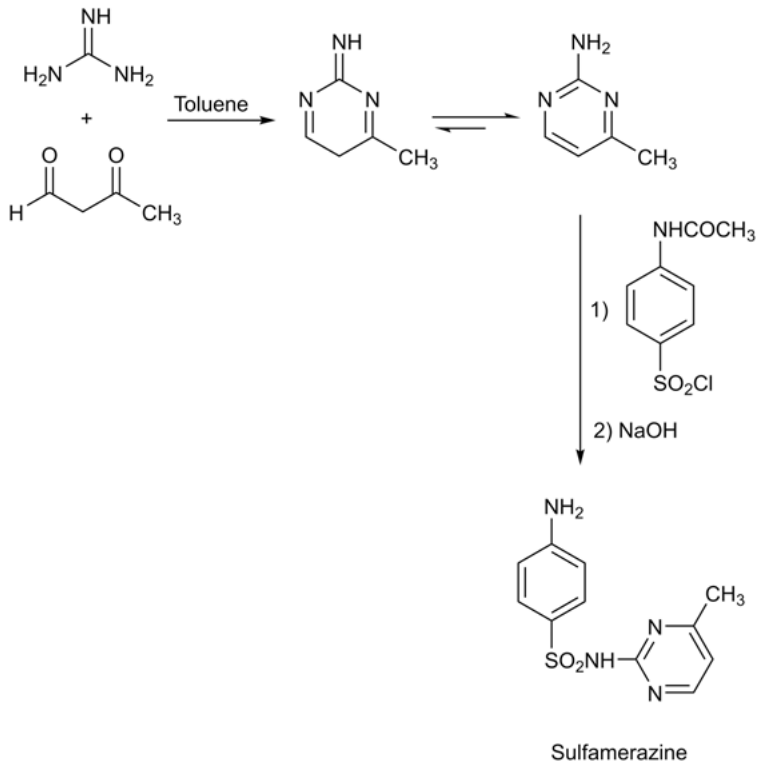
The ArNH- group is obtained by standard procedures of heterocyclic chemistry. For example, let us look at the synthesis of sulfamerazine (Scheme 12.5).

12.8 Examples of other antimetabolites

There are other antimetabolites for clinical use: trimethoprim and a group of compounds known as sulfones (Fig. 12.6).



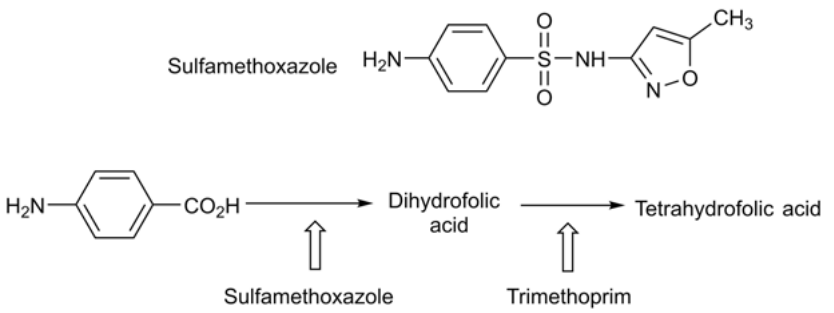
Scheme 12.4: General synthesis for sulfonamides.



Scheme 12.5: Synthesis of sulfamerazine.

12.8.1 Trimethoprim

Trimethoprim is a diaminopyrimidine, which is an orally active antimalarial and antibacterial agent. Unlike sulfonamides, it acts against dihydrofolate reductase, the



Scheme 12.6: Locations where sulfamethoxazole and trimethoprim inhibitors work

enzyme that carries out the conversion of dihydrofolic acid to tetrahydrofolic acid. However, the overall effect is the same as that of the sulfonamides: inhibition of both DNA synthesis and cell growth.

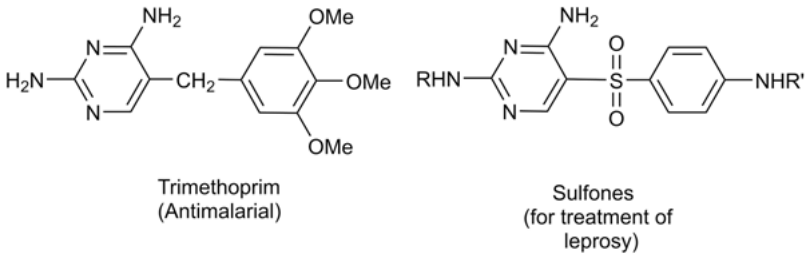


Fig. 12.6: Examples of antimetabolites for medical use.

Trimethoprim is often given along with sulfamethoxazole. The latter inhibits the incorporation of PABA into folic acid, whereas the former inhibits dihydrofolate reductase. This is a very effective method to inhibit a biosynthetic pathway and has the advantage that the doses of both drugs can be reduced. This approach has been described as "sequential blocking" (Scheme 12.6).

12.9 Antibacterial agents affecting protein synthesis

Examples of such agents are rifamycins that act against RNA, and the aminoglycosides, tetracyclines and chloramphenicol, which act against ribosomes.

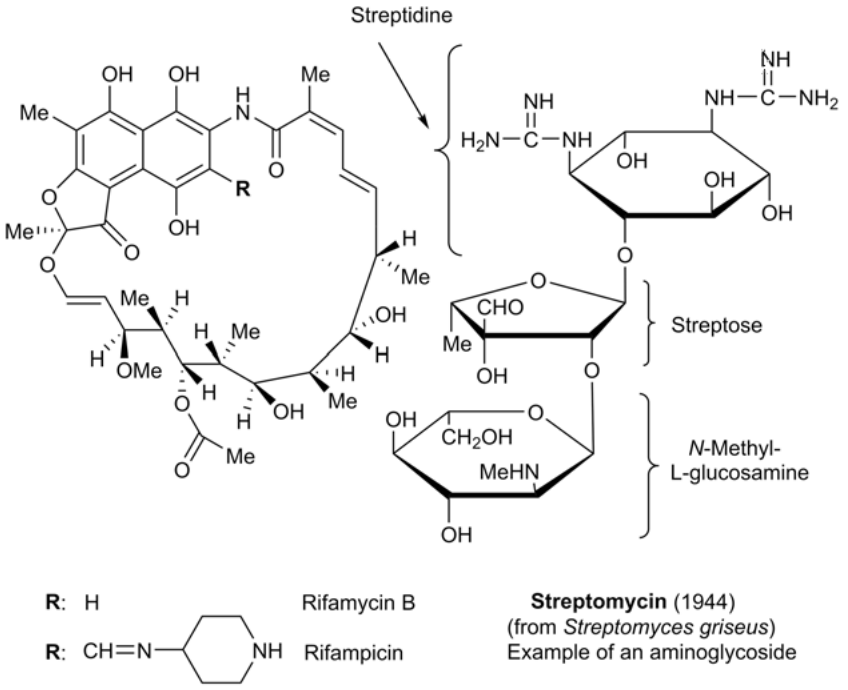


Fig. 12.7: Rifamycins and streptomycin.

12.9.1 Rifamycins

Rifampicin is a semisynthetic rifamycin prepared from rifamycin B, an antibiotic isolated from *Streptomyces mediterranei*. It inhibits Gram-positive bacteria and its mechanism of action operates by noncovalent binding to RNA polymerase and, consequently, by inhibition of RNA synthesis. However, DNA-dependent RNA polymerases are not affected in eukaryotic cells, since the drug binds to a peptide chain not present in the mammalian RNA polymerase. Consequently, it is highly selective. The drug is used in the treatment of tuberculosis and Staphylococcus infections that are resistant to penicillin. Unfortunately, it is very expensive. The naphthalene ring and several of the hydroxyl groups are essential for the activity (Fig. 12.7).

12.9.2 Aminoglycosides

Streptomycin (from *Streptomyces griseus*, 1944) is an important example of aminoglycosides (Fig. 12.7). Streptomycin was the next most important antibiotic to be discovered after penicillin and proved to be an effective antibiotic against tuberculosis. The drug acts by inhibiting protein synthesis. It binds to the 30S ribosomal subunit

and prevents the growth of the peptide chain as well as preventing the recognition of the triplet codon in the mRNA (triplets that encode amino acids are called codons). Aminoglycosides act quickly but cause problems in the ear and kidney if dosages are not carefully controlled.

12.9.3 Tetracyclines

Tetracyclines have a broad spectrum of activity and are the most prescribed antibiotics after penicillins. They are also capable of attacking the malaria parasite. One of the best known tetracyclines is chlortetracycline (Aureomycin®) (Fig. 12.8), which was discovered in 1948. It is a broad-spectrum antibiotic active against Gram-positive and Gram-negative bacteria. It unfortunately kills the intestinal flora that is responsible for the preparation of vitamin K, a vitamin necessary for the coagulation process. There are three main types of vitamin K: K1 is the most efficient of the three, and is found in abundance in fruits and vegetables. Vitamin K2, on the other hand, is of animal origin, is synthesized within the human organism itself by intestinal bacteria. Vitamin K3 is a synthetic variety of vitamin K, developed in the laboratory, and whose use is recommended only under medical prescription.

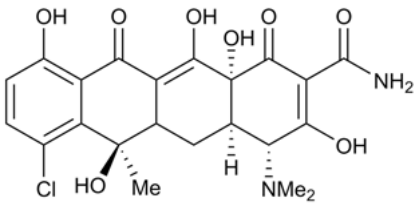


Fig. 12.8: Aureomycin®, as an example of a tetracycline.

12.9.4 Chloramphenicol

Chloramphenicol is an antibiotic that was obtained for the first time from a soil bacterium of the actinomycetales family, *Streptomyces venezuelae*. It is currently produced by synthesis. It has two stereocenters, but only the *R,R* isomer is the active form (Fig. 12.9).

Chloramphenicol binds to the 50S subunit of ribosomes and appears to act by inhibiting the movement of ribosomes along the mRNA, probably by inhibition of the peptidyl transferase reaction, by which the peptide chain is increased. Chloramphenicol, a drug effective against a broad spectrum of microorganisms, especially staphylococci, is limited to very serious infections such as typhoid fever, due to its serious side effects (bone marrow damage, including aplastic anemia) in humans.

The synthesis of chloramphenicol begins with the aldol condensation of the benzaldehyde with 2-nitroethanol to give a mixture of the four enantiomers of the nitro-

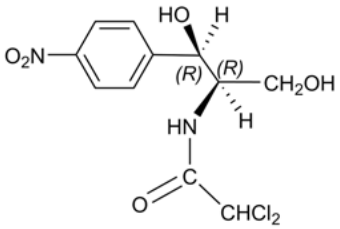
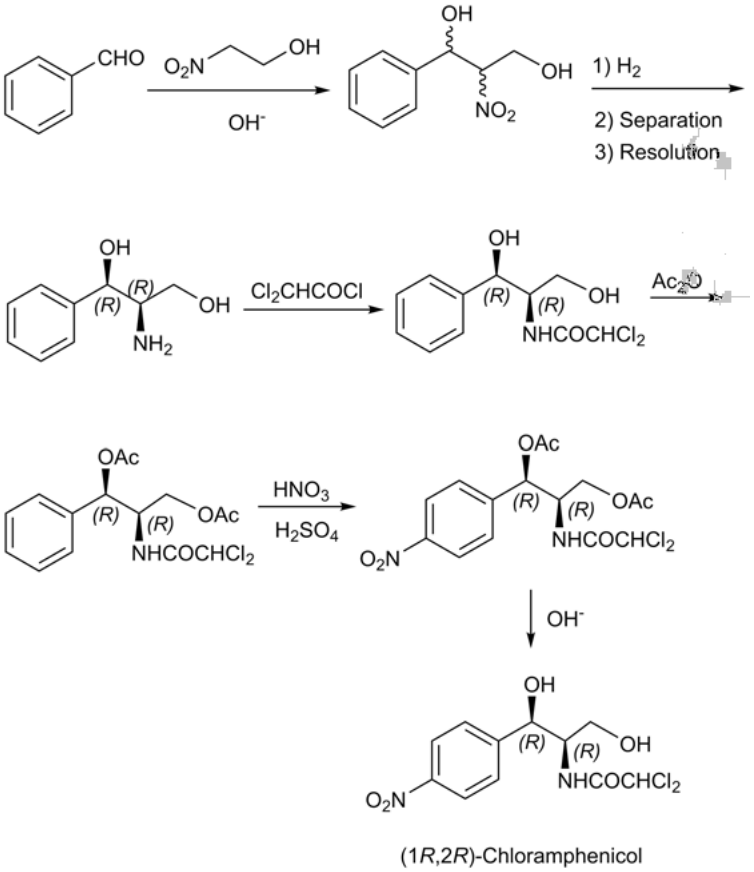


Fig. 12.9: Chloramphenicol.



Scheme 12.7: Synthesis of chloramphenicol.

propanediol, which are catalytically reduced to aminodiols. *threo* isomer is crystallized out and resolved as a diastereomeric salt to give the enantiomer of the desired D-(-)-*threo* configuration. Further acylation with dichloroacetyl chloride, followed by protection of the hydroxyl groups with acetic anhydride, allows nitration of the aromatic ring. Finally, saponification leads to the active enantiomer (Scheme 12.7).

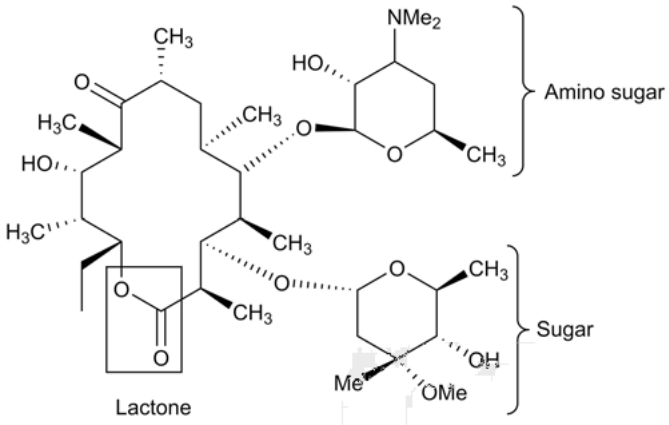


Fig. 12.10: Erythromycin.

12.9.5 Erythromycin

Erythromycin, an antibiotic belonging to the macrolide family, is very effective against infections produced by Gram-positive cocci (Fig. 12.10). It is used to treat various bacterial infections of the respiratory tract, urinary tract, ear and skin infections, gonorrhea, syphilis, rheumatic fever, whooping cough and diphtheria.

It acts by interfering with the production of proteins that bacteria need to multiply, thus halting the growth of bacteria and the spread of infection. Erythromycin has an antibacterial activity very similar to penicillins and is used as an antibiotic alternative in patients who are allergic to penicillins. It may have a bactericidal or bacteriostatic action, depending on the microorganism and the concentration of the drug. It interferes with the formation of essential proteins in the invasive bacteria, which prevents their multiplication and growth.

12.9.6 Aminoacridines

Aminacridridines, such as proflavin, are topical antibacterial agents used during World War II for the treatment of superficial wounds. Proflavin is intercalated between the double DNA helix, inhibiting transcription and replication (Fig. 12.11).

Acridine is a flat, weakly basic molecule, but when the amino group is substituted at the 3, 6 or 9 positions, strong bases are obtained as a result of the resonance that delocalizes the positive charge of cation **12.1** (Scheme 12.8).

Albert's studies in 1939, involving a considerable number of acridine derivatives and various species of bacteria, revealed that only those capable of high ionization at physiological pH were active as antibacterials. Consequently, proflavin and 9-aminoacridine were widely used as antiseptics.

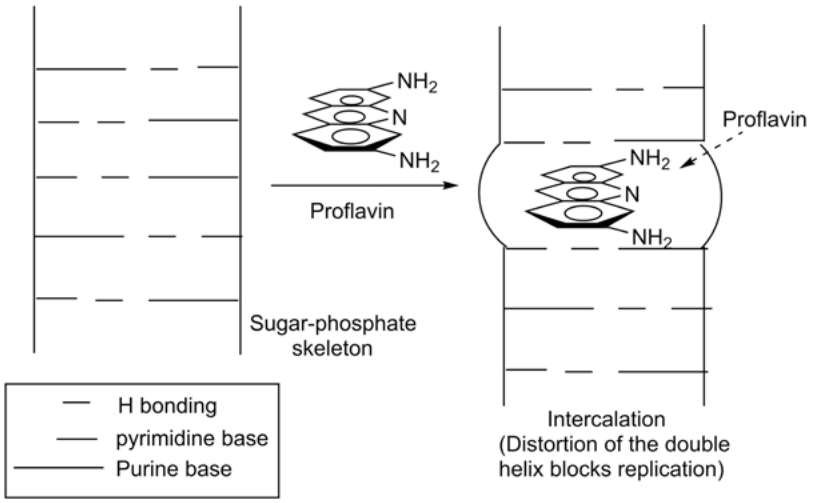
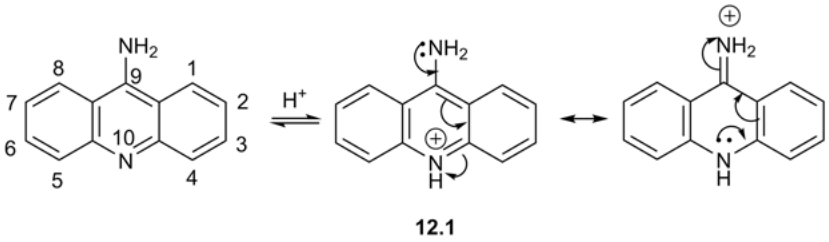


Fig. 12.11: Intercalation of proflavin in DNA.



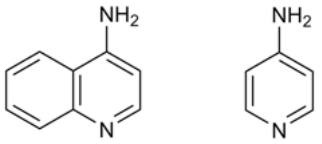
12.1

Scheme 12.8: Resonance forms of the 9-aminoacridinium cation.

A second clue about the mode of action of the aminoacridines was provided by the discovery that a minimal area of planarity for antibacterial activity was essential. When compounds **12.2** and **12.3** (Fig. 12.12), representing successive removal of one or two benzene rings from aminoacridine, were examined, antibacterial activity was lost even when high ionization, i.e., significance of ionic resonance hybrids, was maintained.

The loss of antibacterial activity on decreasing the planar surface was reaffirmed by the reduced activity of 1,2,3,4-tetrahydro-9-acridine. The acridine flat ring is intercalated in the DNA due to the following two factors:

(a) By the van der Waals forces that are established between the purine (e.g., adenine) and pyrimidine rings (e.g., thymine), since acridine is a π-electron deficient system, whereas both adenine and thymine are π-electron excedent systems. A charge-transfer complex can therefore be established between the two types of heterocyclic aromatic systems.



12.2 12.3

Fig. 12.12: 4-Aminoquinoline and 4-aminopyridine.

(b) In addition, the acridine ring thus binding in this way is in the ideal arrangement for its two positively charged nitrogens to establish ionic interactions with two phosphate groups of the double-stranded DNA structure.

The interaction of proflavin with DNA increases the viscosity coefficient and decreases the sedimentation coefficient of the complex in solution. These changes are attributed among others to increased stiffness of the double helix. X-ray studies indicate that an aminoacridine molecule stacks parallel to base pairs in a 1:3 ratio.

12.9.7 1,8-Naphthyridine and fluoroquinolones

Quinolones and fluoroquinolones are modern drugs within the antibacterial therapeutic arsenal (Fig. 12.13). They are particularly interesting for the treatment of both urinary tract infections and infections that are resistant to classical antibacterial agents.

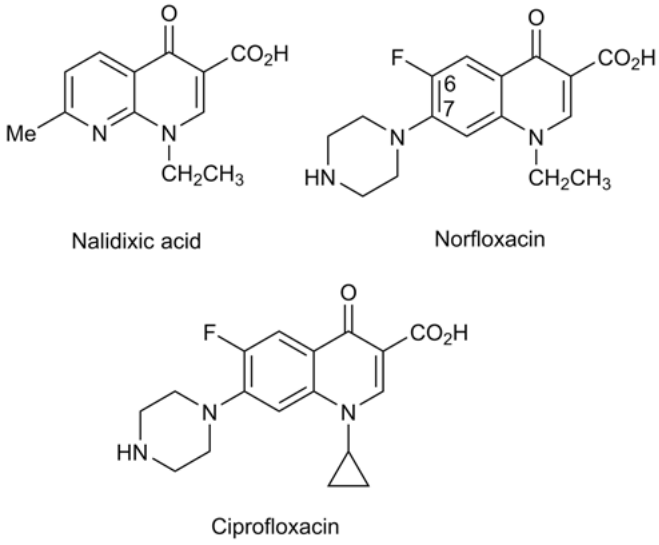


Fig. 12.13: 1,8-Naphthyridine and fluoroquinolones.

Nalidixic acid is active against Gram-negative bacteria and is useful as a therapeutic agent against infections of the urinary tract. It can be administered orally, but bacteria can develop resistance.

A great advancement was achieved when a fluorine atom at 6-position and a piperazino moiety at the 7-position of the heteroaromatic skeleton were introduced. These modifications led to norfloxacin, which has high activity against Gram-negative and Gram-positive bacteria. It is also active against the highly resistant *P. aeruginosa*.

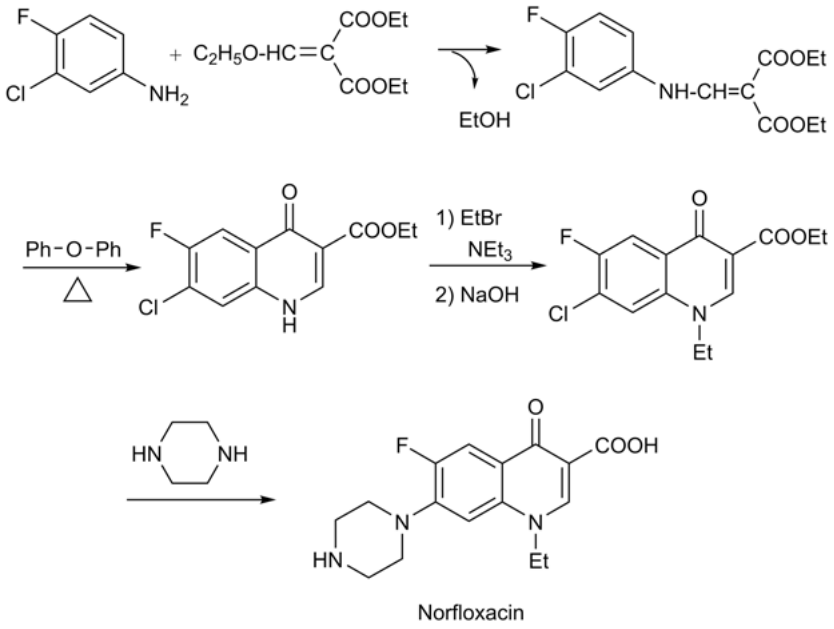
An extra modification led to ciprofloxacin, which is considered the most widely available antibacterial agent on the market. They are the only antibacterial agents that exert their bactericidal activity by binding to bacterial topoisomerases and inhibiting them, although this would not be the only mechanism of action. Topoisomerases are enzymes that control the supercoiling and unwinding of bacterial DNA. Supercoiling allows the long molecule of DNA to pack inside a bacterial cell. This structure must be unwound to allow different functions such as replication, transcription and DNA repair. Inhibiting the activity of these enzymes prevents the bacterial cell from producing the proteins necessary for its repair, growth and reproduction. Prolonged inhibition would thus lead to the death of the cell. There are four types of topoisomerases. The quinolones can act on DNA-gyrase (also called topoisomerase type II) and topoisomerase type IV. They do not act on topoisomerases I and III.

Norfloxacin is obtained by condensation of 4-fluoro-3-chloroaniline and diethyl 2-ethoxymethylenemalonate with elimination of ethanol, followed by heating in a high-boiling point solvent such as diphenyl ether, to give the quinolone. Subsequently the nitrogen is alkylated, the ester saponified and the chlorine replaced by the piperazine ring to give the desired product. This reaction is possible because the chlorine atom is *para* with respect to an electron-withdrawing carbonyl group (Scheme 12.9).

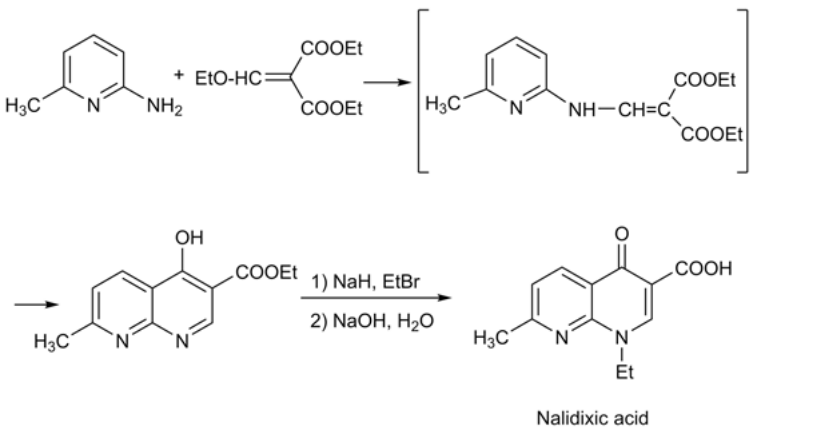
Nalidixic acid is synthesized by a similar route, but starting from 2-amino-6-methylpyridine. In this case, the benzene ring of norfloxacin has been replaced by its isostere pyridine (Scheme 12.10).

The synthesis of ciprofloxacin is outlined in Scheme 12.11. Bayer synthesis of ciprofloxacin uses 2,4-dichloro-5-fluorobenzoyl chloride (**12.4**) as the starting material. With the aid of magnesium ethoxide, condensation of the acid chloride **12.4** with diethyl malonate yields ketone **12.5**, which is then decarboxylated using *p*-toluenesulfonic acid to form ethyl 2,4-dichloro-5-fluorobenzoylacetate (**12.6**).

A Dieckman-type condensation of **12.6** with the ethyl orthoformate is carried out in acetic anhydride, which refluxes to yield the ethyl acrylate **12.7**. When **12.7** is treated with cyclopropylamine in ethanol, an Michael addition is produced, followed by the subsequent expulsion of the ethoxy group to give enamine **12.8** with the stereochemistry shown in Scheme 12.10. Under the influence of a base such as K_2CO_3, NaH or KH, **12.8** then undergoes an intramolecular S_NAr to produce quinolone **12.9**. Hydrolysis of the ethyl ester group of **12.9** is carried out using a catalytic amount of concentrated sulfuric acid in a 1:1 mixture of acetic acid/H_2O. Finally, a chemioselective S_NAr reaction

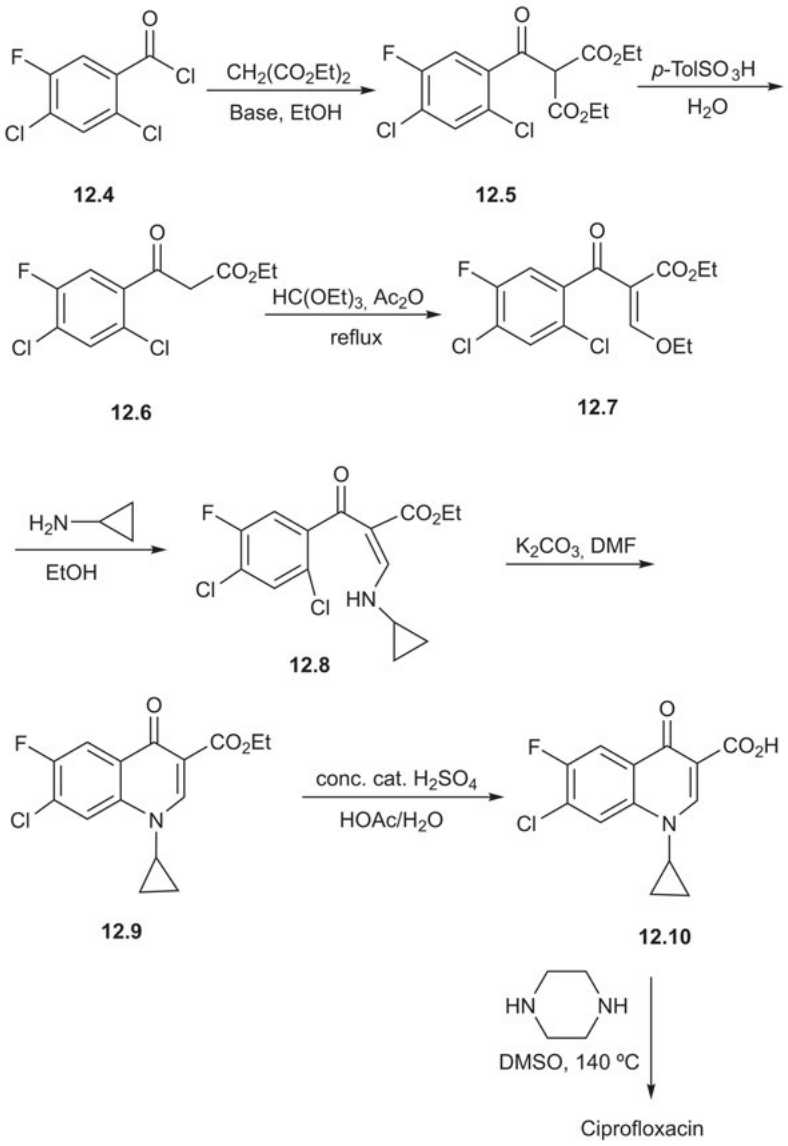


Scheme 12.9: Synthesis of norfloxacin.



Scheme 12.10: Synthesis of nalidixic acid.

takes place between **12.10** and piperazine to give ciprofloxacin. Chemoselectivity is the result of the "activating" effect of the carbonyl group at *para* position (Scheme 12.11).



Scheme 12.11: Synthesis of ciprofloxacin.

13 Enzymatic inhibition: Inhibitors of biosinthesis of nitrogenated bases

13.1 Goals

- Knowledge of the preponderant role of some enzymes in various pathologies.
- Knowledge of the various ways of inhibiting enzymes and/or coenzymes.
- Use of some inhibitors as antitumor and antiviral agents.

13.2 Introduction

In this chapter we will first discuss the anticancer drugs that fall within the category of drugs that interfere with DNA synthesis. However, other categories of drugs such as those that interact directly with DNA (alkylating agents, metal complexes that bind to DNA, derivatives causing DNA degradation and antisense agents) will not be treated because they are not enzymatic inhibitors. A more modern approach, such as tyrosine kinase inhibitors, will be discussed later. Finally, antivirals will also be treated briefly.

13.3 Nucleic acids

Cells contain two types of nucleic acids: RNA and DNA. These complex structures are essential in the biosynthesis of proteins. DNA is also the genetic material of cells.

Smooth degradation of nucleic acids produces a mixture of acids known as nucleotides: purine and pyrimidine bases, a phosphate and a pentose moiety (Schemes 13.1 and 13.2). Phosphate group can be selectively removed by careful hydrolysis and the nucleotide is converted into a nucleoside. In a nucleotide, the C-1 of the sugar is bound to the *N*-1 of the pyrimidine or the *N*-9 atoms of the purine. The phosphoric acid forms an ester with the C-5' of the sugar (Fig. 13.1).

13.4 Thymidylate synthase inhibitors

Rapidly dividing cells require an abundant supply of deoxythymidylate (dTMP) to synthesize their DNA. The susceptibility of these cells to inhibiting the synthesis of dTMP has been exploited in cancer chemotherapy. Uracil is not a component of DNA. Instead, DNA contains thymine, the methylated analogue of uracil. Deoxyuridylate (dUMP) is methylated to deoxythymidylate (dTMP) by means of thymidylate synthase. The methyl donor of this reaction is N^5,N^{10}-methylenetetrahydrofolate. In this

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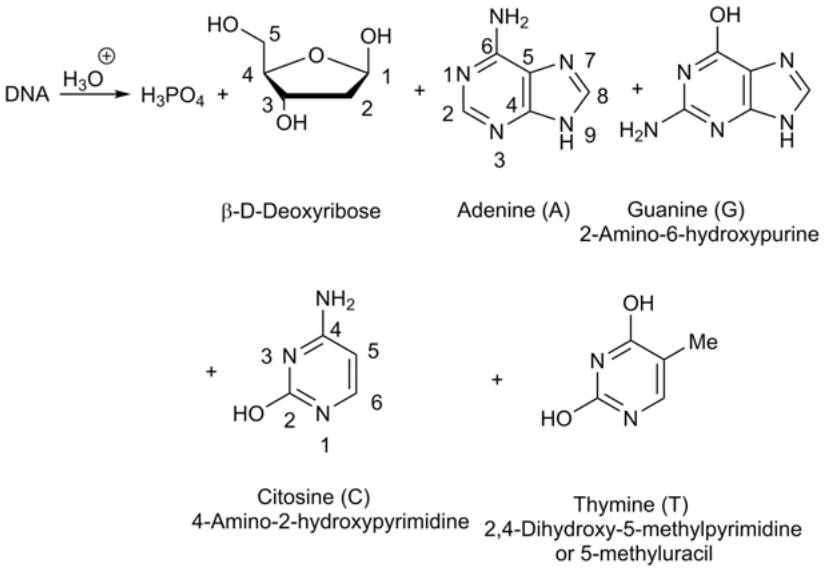
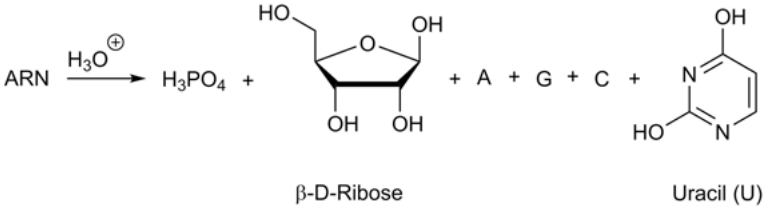


Scheme 13.1: DNA degradation.



Scheme 13.2: RNA degradation.

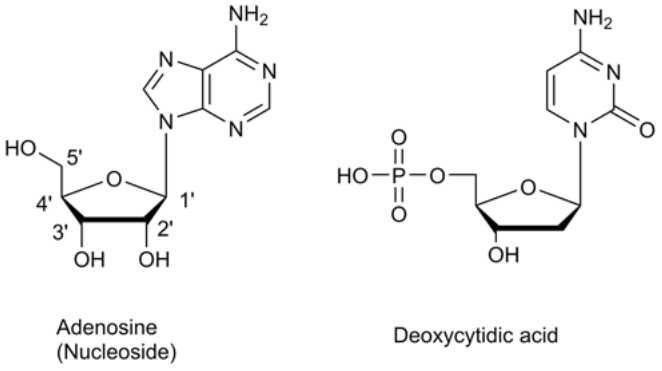
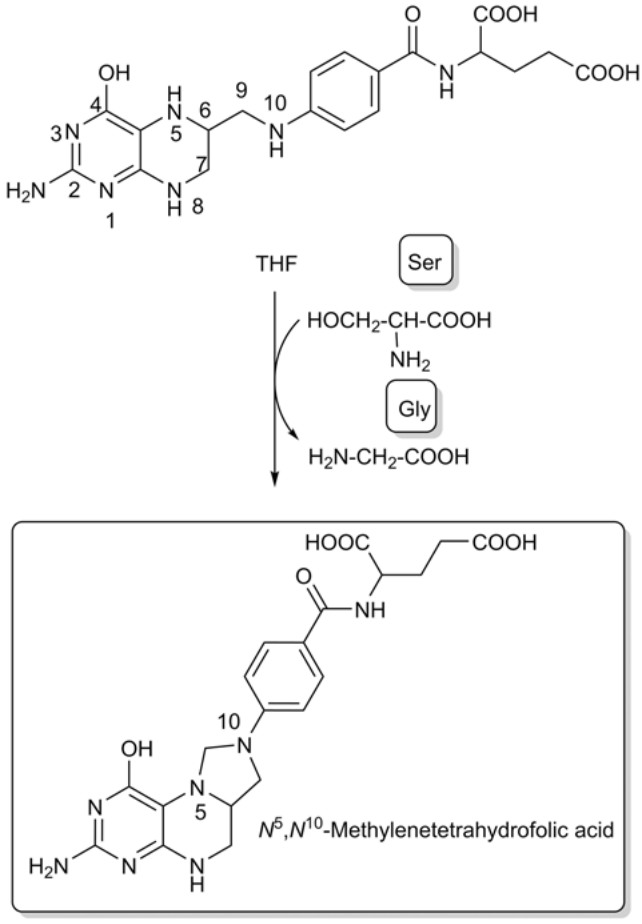


Fig. 13.1: Examples of a nucleoside and a nucleotide.

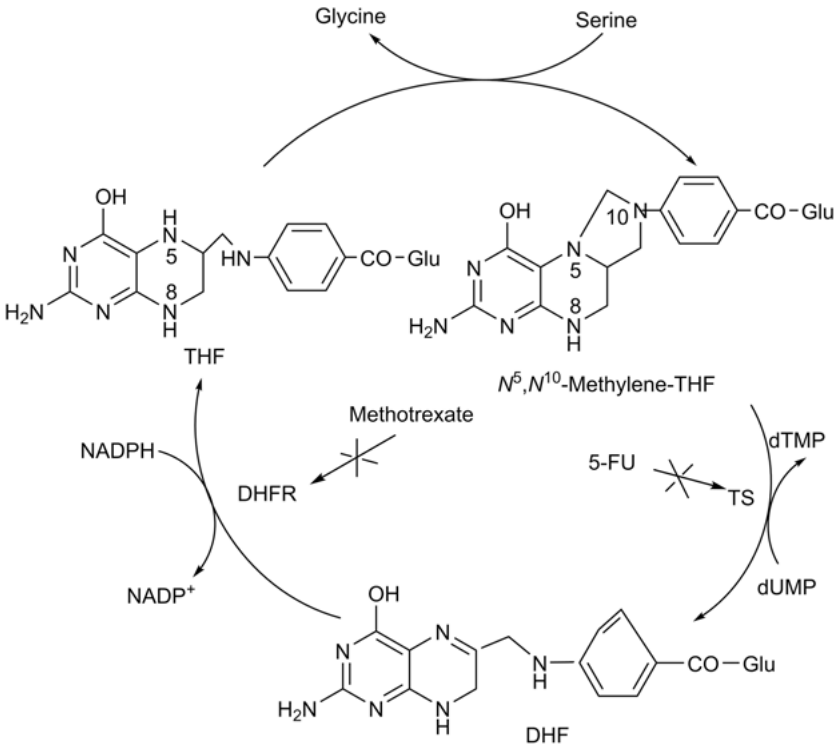
reaction tetrahydrofolate is oxidized to dihydrofolate. On the other hand, transfers of monocarbon fragments occur at the level of tetrahydrofolate and not of dihydrofolate. Therefore, tetrahydrofolate should be regenerated. This is achieved by means of dihydrofolate reductase, which uses NADPH as reducing agent. The target enzymes are dihydrofolate reductase and thymidylate synthase.

13.4.1 Tetrahydrofolic acid

THF is a very versatile carrier of active units of one carbon atom. The monocarbon fragment is attached to the *N*-5 and *N*-10 atoms of THF and comes from the methylene group of the serine (Scheme 13.3).



Scheme 13.3: Formation of N^5,N^{10}-methylenetetrahydrofolic acid (N^5,N^{10}-methylene-THF).



Scheme 13.4: Use of methotrexate and 5-FU as anticancer drugs.

Methotrexate has an indirect effect on thymidylate synthase by decreasing the amount of the required cofactor N^5,N^{10}-methylene-THF.

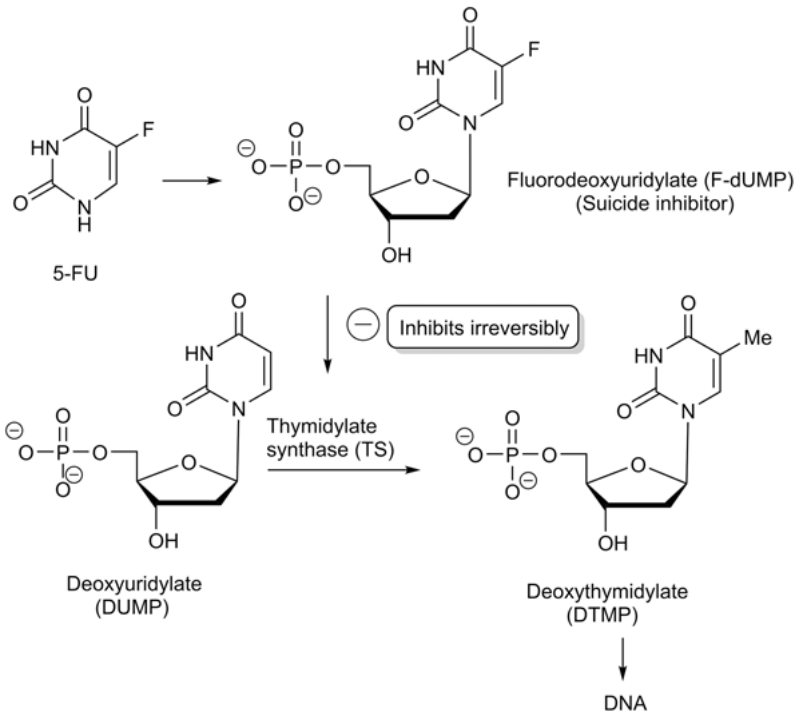
5-Fluorouracil (5-FU, Scheme 13.4) is an anticancer drug that directly inhibits this enzyme.

13.4.2 5-Fluorouracil (5-FU)

5-FU, a clinically useful anticancer drug, is converted in vivo into fluorodeoxyuridylate. It acts as an anticancer prodrug of a suicide substrate. 5-FU transforms in the organism into the fluorinated analogue of 2'-deoxyuridine (F-dUMP) (Scheme 13.5). Under normal conditions, DNA biosynthesis occurs from dTMP.

The mechanism of inhibition of thymidylate synthase is as follows:

dUMP is combined with the enzyme and cofactor (Schemes 13.6 and 13.7). Tetrahydrofolate has formed a covalent bond with the uracil fragment via the methylene fragment, which is subsequently transferred to uracil. This would be the normal mechanism.

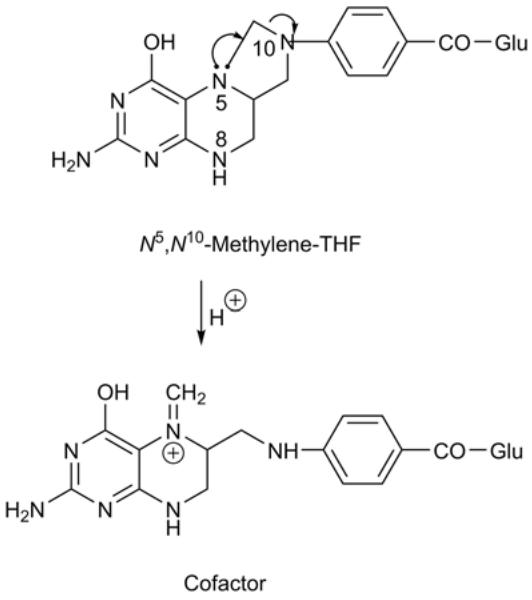


Scheme 13.5: Biosynthesis of dTMP, and inhibition of thymidylate synthase by FU-dUMP.

Scheme 13.7 shows the mechanism of thymine nucleotide formation of uracil nucleotide.

Under normal conditions, a proton is lost from position 5 of uracil (see Scheme 3.7). However, 5-FU has a fluorine atom in this position instead of a hydrogen atom. It is not possible for any further reaction to proceed, as this would require fluorine to leave as a positive ion (F^+). A fluorine atom is too electronegative, since its usual behavior is to produce the fluorine (F^-) anion. As a result, the 5-FU backbone remains covalently and irreversibly attached to the active site of the enzyme. Synthesis of thymidine is terminated, which halts DNA synthesis (Fig. 13.2). Consequently, replication and cell division are blocked.

5-FU is administered intravenously for the palliative treatment of colorectal, breast, stomach and pancreatic cancers. Patients are treated for four consecutive days, followed by a treatment of odd days up to a maximum of 12 days. Although up to 20% of the dose is excreted unchanged in the urine, the majority undergoes hepatic catabolism via a series of enzymes including dihydropyrimidine dehydrogenase (DPD) (Scheme 13.8). Patients who are genetically deficient in this enzyme (~5% of the population) will experience a greater effect of this drug and are at additional risks, unless the doses are adequately adjusted.



Scheme 13.6: Cofactor obtained from N^5,N^{10}-methylene-THF.

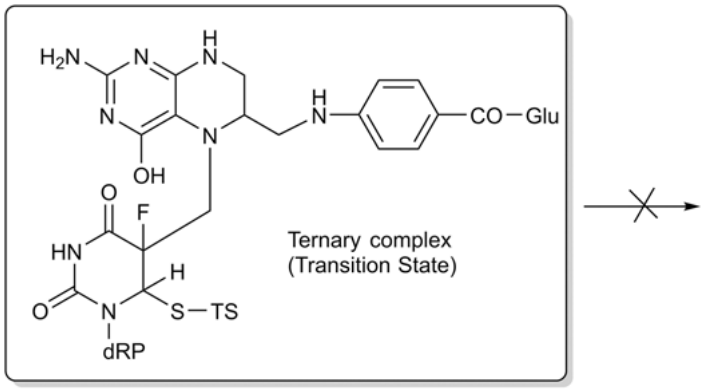
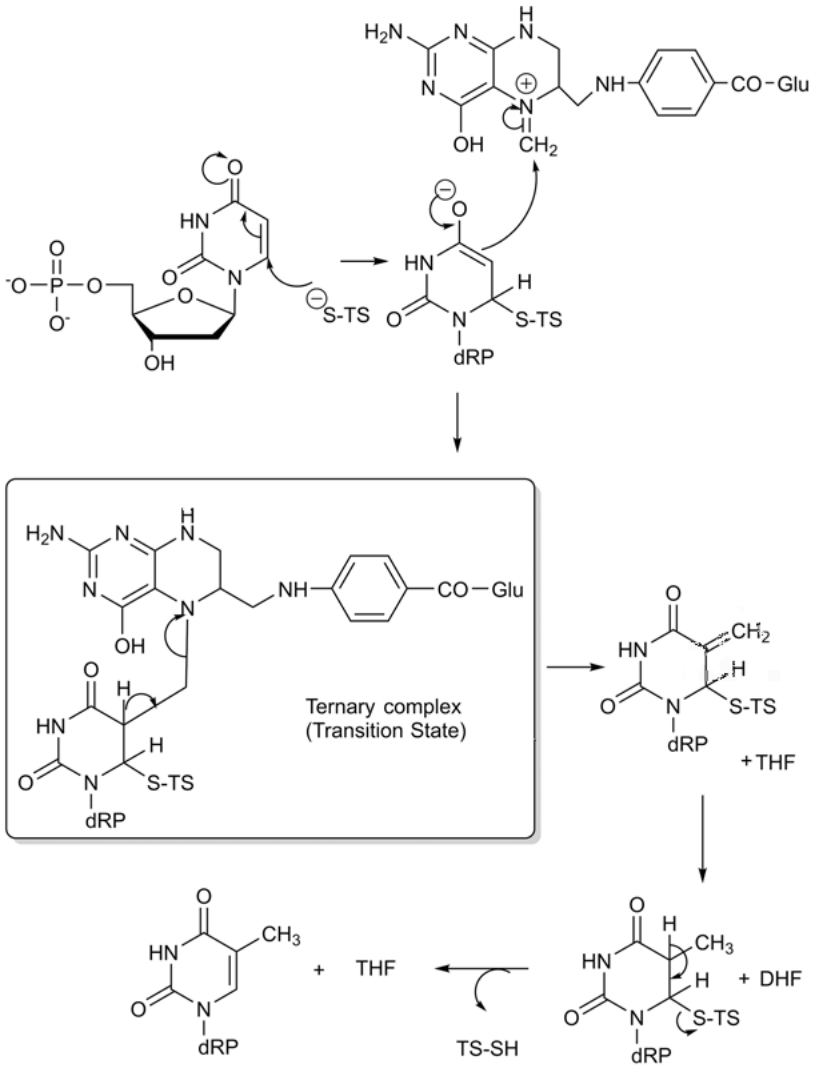


Fig. 13.2: A ternary complex formed between THF, thymidylate synthase and 5-FU mononucleotide.

The most important toxicities are spinal cord depression, stomatitis, esophagopharyngitis, and ulcerations of the gastrointestinal tract. Nausea and vomiting are also common. Scheme 13.8 shows the metabolism of 5-FU.

Although capecitabine is a cytidine carbamate, the drug is actually a prodrug of F-dUMP (Scheme 13.9).

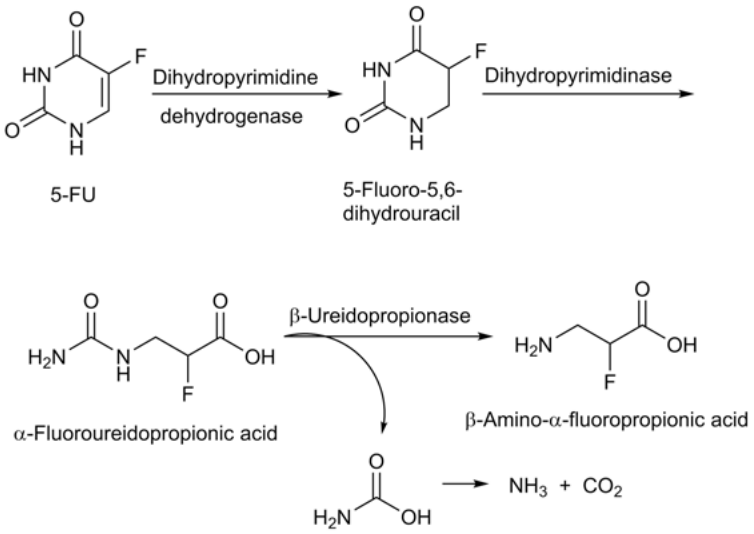
Administered orally it is metabolized to 5-FU, which is converted into the previously described active fluorinated deoxyribonucleotide. Thymidine phosphorylase,



Scheme 13.7: Mechanism of formation of thymine nucleotide from uracil nucleotide. TS is thymidilate synthase.

an enzyme involved in its biotransformation, is much more active in tumors than in healthy tissues, which improves the selective generation of 5-FU in tumors. Active drug levels in the tumor may be 3.5 times greater than in surrounding tissues, which translates into fewer side effects compared to 5-FU therapy.

Capecitabine is indicated as first-line therapy in patients with colorectal cancer. It is also used alone or in combination with docetaxel (Taxotere®) in patients with metastatic breast cancer who have experienced a disease progression or recurrence af-



Scheme 13.8: Metabolism of 5-FU.

ter treatment with anthracycline. Paclitaxel (Taxol®) and its semisynthetic analogue docetaxel are important anticancer agents that inhibit depolymerization of tubulin. Paclitaxel was isolated from yew bark and was identified in 1971, following a screening program conducted by the US National Cancer Institute. The term taxoid is generally used for paclitaxel and its derivatives (Fig. 13.3).

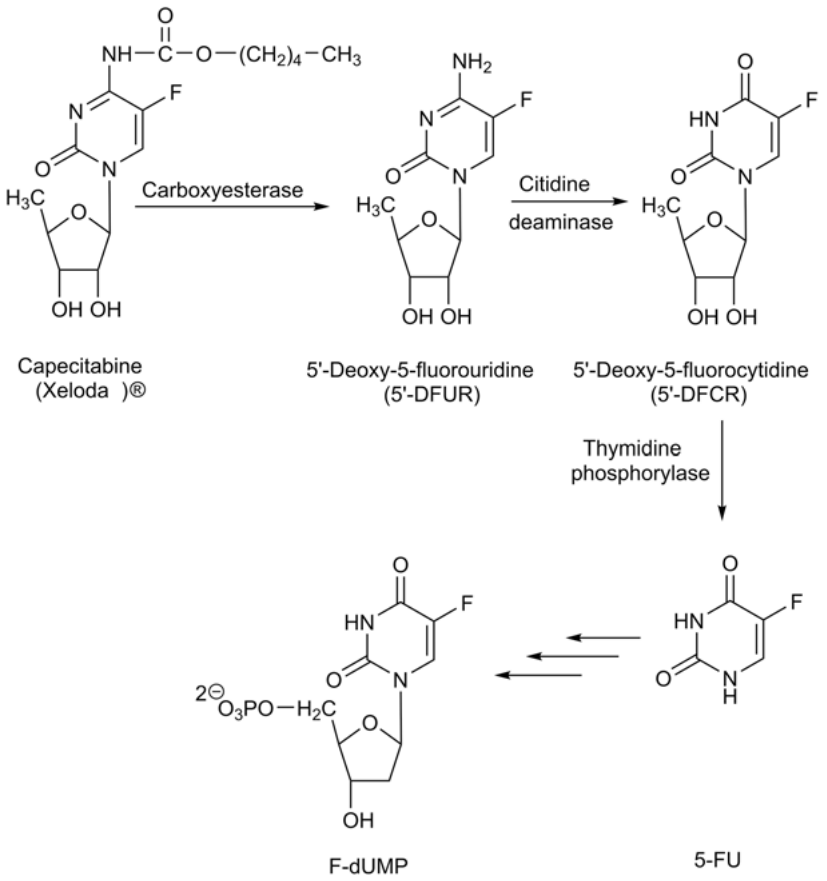
13.5 DHFR inhibitors

Aminopteridine and methotrexate (Fig. 13.4) are competitive inhibitors that bind to the active site of DHFR, between 3,000 to 100,000 times more strongly than the natural substrate, by formation of hydrogen bonds between the portion of 2,4-diaminopteridine, protonated at physiological pH , and the anionic groups of the active site of the enzyme.

Methotrexate is given orally for the treatment of cancers of the breast, head and neck, and several lung cancers.

13.6 Tyrosine kinase inhibitors

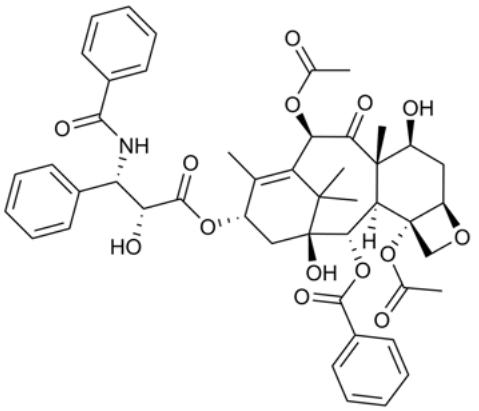
Today it is a widely accepted fact that "there is a growing need for new targets for the development of anticancer drugs, in addition to DNA." Traditionally anticancer drugs have been directed towards inhibition of both DNA synthesis and intracellular



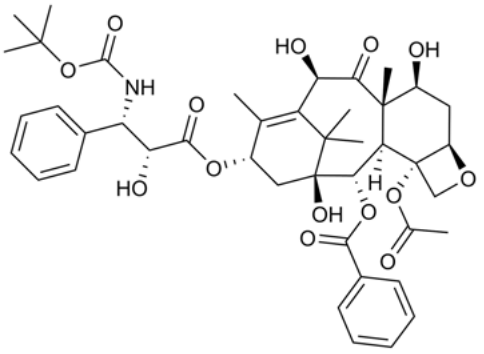
Scheme 13.9: Activation of capecitabine.

organelles that are required for proper segregation of chromosomes during mitosis. Regardless of the mechanism used by such compounds, it ultimately appears that drugs stimulate the self-destructive (apoptotic) pathways within the tumor. Unfortunately, such cytotoxic approaches appear to be limited by the degree of efficacy that can induce cell death and the degree of selectivity between tumoral and normal cells, especially in organs requiring rapid cell proliferation.

A new approach is to use signaling pathways that mediate the effects of growth factors and oncogenes on cell proliferation as molecular targets for the development of anticancer drugs. An oncogene is any gene encoding a protein capable of transforming cultured cells or inducing cancer in animals. Oncogenes are usually derived from normal genes, either by mutation or by incorrect regulation. Such approaches could simply attenuate the effect of these aberrant signaling pathways and thus prevent tumors from continuing to grow, in which case such approximations could be

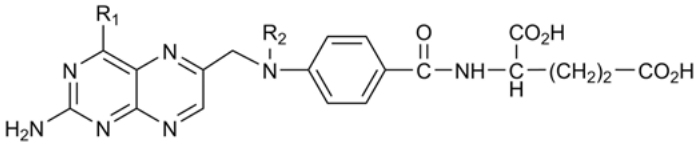


Paclitaxel (Taxol®)



Docetaxel (Taxotere®)

Fig. 13.3: Paclitaxel (Taxol®) and docetaxel (Taxotere®).



R_1 = OH, R_2 = H: Folic acid
R_1 = NH_2, R_2 = H: Aminopterin
R_1 = NH_2, R_2 = Me: Methotrexate (antineoplastic)

Fig. 13.4: Folic acid, aminopterin and methotrexate.

considered *cytostatic*. Alternatively, they could induce cell death of tumors, in which case they would be *cytotoxic* approaches. The belief that kinases frequently act as oncogenes explains the interest in phosphorylation and dephosphorylation involved in signal transduction.

The receptors bound to kinases represent a superfamily of receptors that activate enzymes directly and do not require G protein. Tyrosine kinase receptors are important biological targets for novel anticancer drugs. In these structures, the protein involved plays the dual role of receptor and enzyme. Receptor protein is embedded within the cell membrane, with part of its structure exposed to the outside and another part inside the cell.

The outer surface contains the binding site of the chemical messenger, while the inside of the cytoplasm has an active site, which is closed in the resting state. When a chemical messenger binds to the receptor, there is a change in the conformation of the protein, which opens the active center, allowing the protein to act as an enzyme within the cell. The catalyzed reaction is phosphorylation, whereby tyrosine residues from a substrate of the protein are phosphorylated. The enzyme that catalyzes phosphorylation reactions is known as a kinase, and the protein is known as receptor tyrosine kinase. ATP is required as cofactor to provide the necessary phosphate groups.

A large number of polypeptide hormones, growth factors and cytokines activate receptors bound to kinases. Loss of function of these receptors can lead to the development of defects or hormonal resistance. Moreover, their overexpression can lead to malignant growth.

13.6.1 Structure of tyrosine kinase receptors

The basic structure of a tyrosine kinase receptor consists of a single extracellular region (the *N*-terminal chain) that contains the binding site for the chemical messenger, a hydrophobic region that traverses the membrane as an α-helix of seven transmembrane domains, and a C-terminal chain on the inside of the cell membrane (Fig. 13.5). The C-terminal region contains the catalytic binding site. Examples of tyrosine kinase receptors include the insulin receptor, and the receptors for various cytokines and growth factors.

13.6.2 Mechanisms of activation of the EGF receptor tyrosine kinase

A specific example of a tyrosine kinase receptor is the hormone receptor called epidermal growth factor (EGF). EGF is a bivalent ligand that can bind to two receptors at the same time. This leads to dimerization of the receptor as well as activation of enzymatic activity. The dimerization process is important because the active sites of each dimeric receptor half catalyze the phosphorylation of accessible tyrosine residues in the other

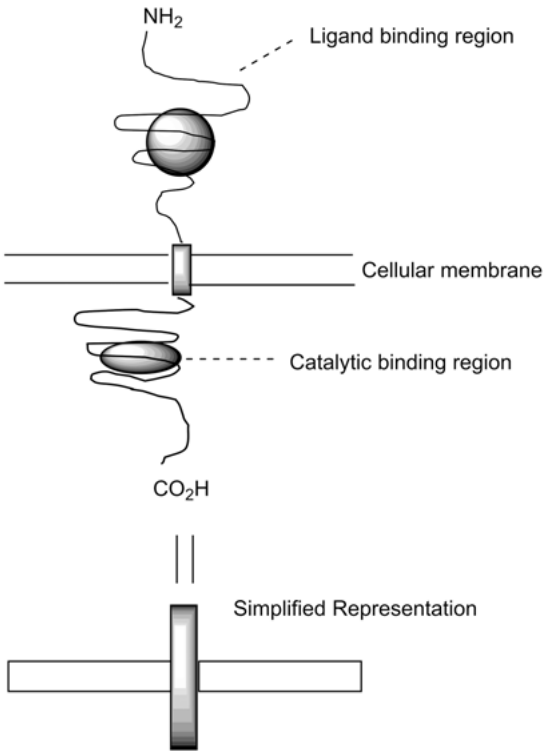


Fig. 13.5: Structure of tyrosine kinase receptors.

half (Fig. 13.6). If dimerization did not take place, phosphorylation would not take place. The important fact is that an external chemical messenger manages to transmit its message to the inside of the cell without being altered or without having to enter the cell.

13.6.3 Example of a kinase inhibitor for clinical use (gefitinib)

There are several agents that have been studied as inhibitors of EGF kinases and one of the first used in clinics is gefitinib (Iressa®) for the treatment of chronic myeloid leukemia. Gefitinib was developed by Astra Zeneca and belongs to the 4-anilinoquinazolines group (Fig. 13.7).

It was developed from a potent inhibitor (structure **13.1** in Fig. 13.8), which has important characteristics previously identified by means of SAR studies: a secondary amine, electron-releasing substituents at positions 6 and 7, and a small lipophilic substituent in the aromatic ring. The structure has an interesting activity in vitro, but its activity in vivo is diminished by the fact that it is rapidly metabolized by cy-

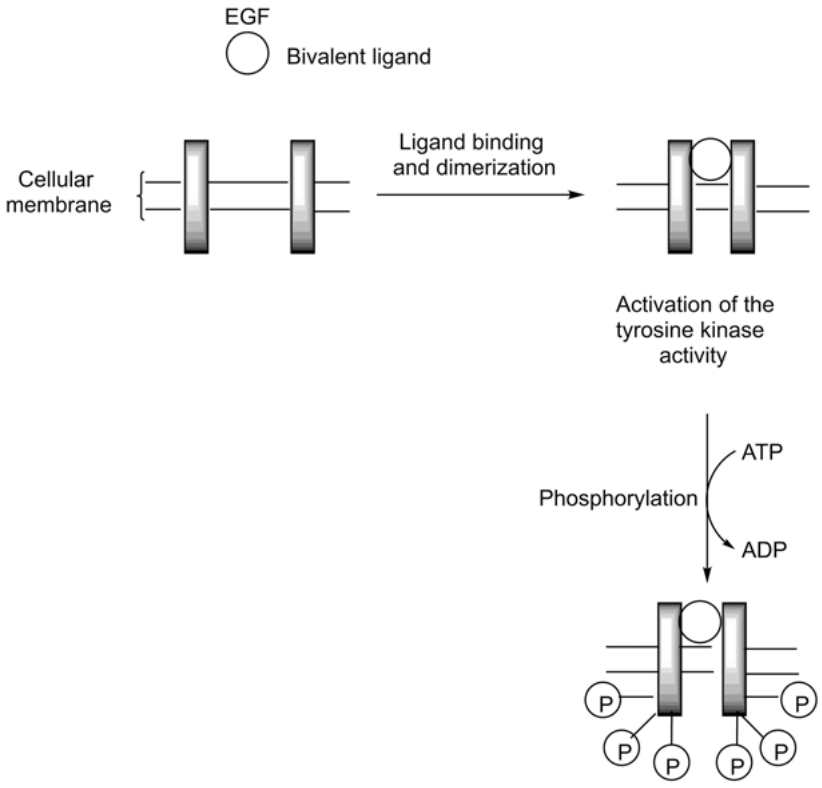


Fig. 13.6: Mechanism of activation of the epidermal growth factor receptor (EGF).

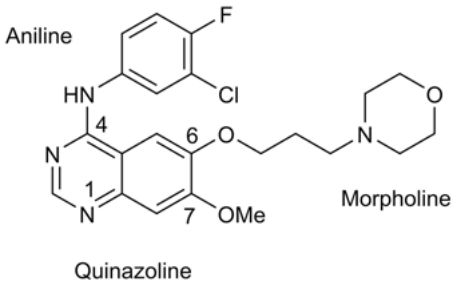


Fig. 13.7: Gefitinib (Iressa®).

tochrome P450 to give two metabolites. Oxidation of the aromatic methyl group gives rise to metabolite **13.2**, and oxidation at *para* position of the aromatic ring gives rise to metabolite **13.3**. Both positions were known to be vulnerable to oxidative metabolism. Therefore, it was decided to modify the structure so that both metabolic routes were blocked.

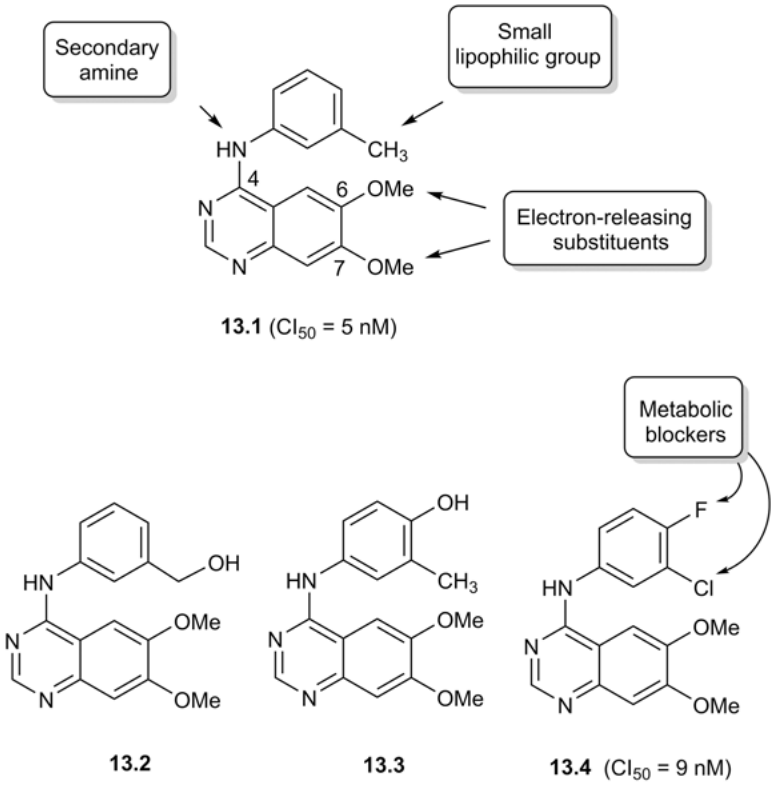


Fig. 13.8: Design of metabolically stable analogues of structure **13.1**.

A chlorine atom replaced the methyl group (**13.4** of Fig. 13.8). This halogen can be seen as a bioisostere of the methyl group as it has similar size and lipophilicity, but has the advantage that it is resistant to oxidation. In addition, a fluorine atom was chosen to block the oxidation of the *para* position of the benzene ring. The fluorine atom has essentially the same size as the hydrogen atom, so there are few risks of the existence of some steric effect as a result of its introduction. Although the resulting compound was less active in vitro as enzyme inhibitor, it nevertheless showed a better in vivo activity since it was resistant to metabolism.

Further modifications were made to optimize the pharmacokinetic properties of the drug. Several alkoxy groups were tried at 6-position and finally, the discovery of gefitinib was completed. It contains a morpholine ring, which is often introduced to increase solubility in water. Because the morpholine moiety has a nitrogen atom, it is possible to protonate and form soluble drug salts (e.g., hydrochloride or succinate).

13.7 Antivirals

Antiviral drugs are useful in the treatment of viral diseases when there is no effective vaccine, or when infection has already occurred. The life cycle of a virus implies that most of the time it is in a host cell in the human body, and is protected from the immune system and circulating drugs. Because it uses the biochemical mechanisms of host cells to multiply, the number of potential targets for antiviral drugs is more limited than that for invading microorganisms in general. Therefore, the search for effective antiviral drugs has proved to be a more difficult challenge than the search for antibacterial drugs. In fact, the first antiviral drugs appeared relatively late, during the 1960s.

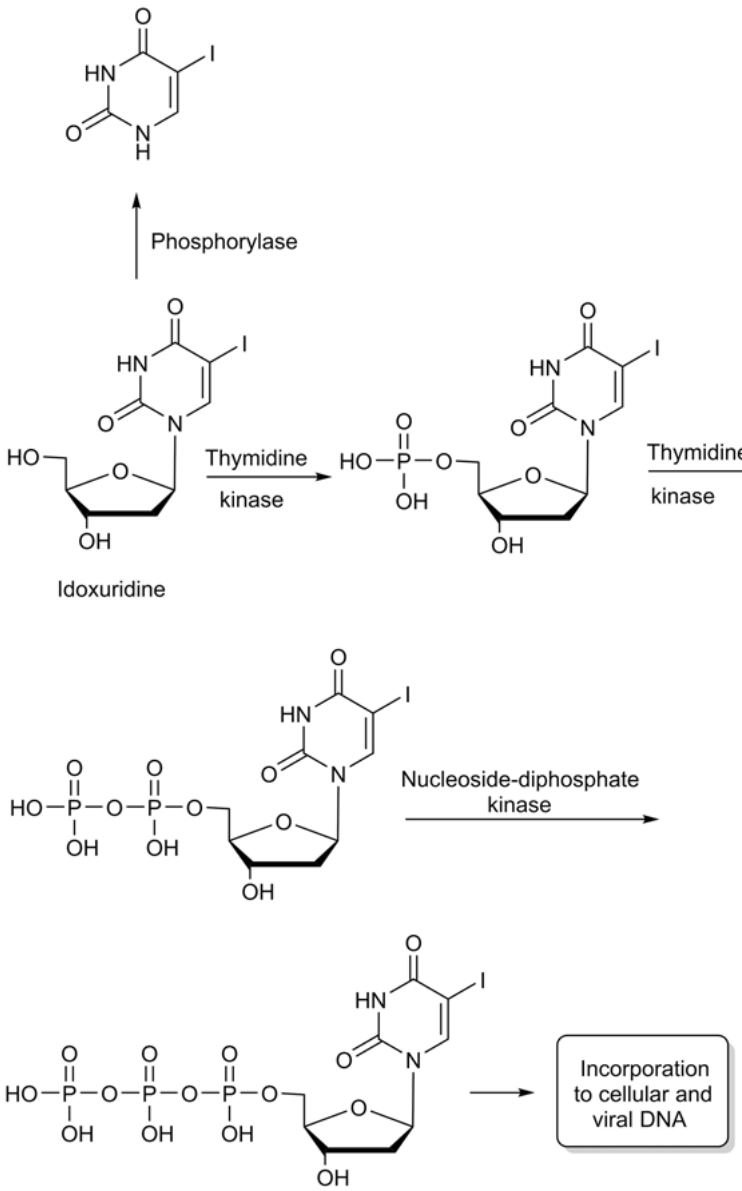
13.7.1 Antiviral inhibitors of DNA polymerases and other enzymes

Many chemotherapeutics used as antitumor or antiviral drugs are derived from structures analogous to the pyrimidine or purine bases present in nucleic acids that inhibit DNA polymerases or other enzymes that affect their normal functioning. Polymerases are enzymes that catalyze the nucleophilic attack of a 3'-OH group from a deoxyribose moiety of the growing polymer to the triphosphate group at the 5'-O position of a deoxynucleotide, which is incorporated according to the pairing between nitrogenous bases in the DNA template, with the withdrawal of the pyrophosphate anion. Their inhibitors are often added to polymeric structures of fraudulent nucleic acids, as they possess one or more units differentiated from normal bases. They can also act as chain terminators.

Among the antivirals, 2'-deoxynucleosides of pyrimidines, such as idoxuridine (5-iodo-2'-deoxyuridine, Scheme 13.10), have been developed which, when transformed into mono-, di- and finally tri-phosphate, compete with thymidine and their phosphates for the phosphorylating enzymes thymidine and thymidylate kinases, thus preventing the use of thymidine. However, its antiviral action is primarily due to its incorporation into the viral DNA, rather than to thymidine. Idoxuridine is used in ocular viral infections, such as herpetic keratitis, and in the treatment of herpes zoster, by application of a solution in DMSO to the lesions. Its toxicity prevents its use at a general level.

In most antivirals that have a nucleoside structure, the base or sugar has been modified. 3'-Azido-3'-deoxythymidine (AZT or zidovudine) exhibits a strong resemblance to thymidine (Fig. 13.9).

AZT is an inhibitor of the reverse transcriptase (RT) enzyme, which was the first step in the chemotherapy against HIV-1, the AIDS retrovirus. It is about 100–300-fold less active as a mammalian DNA polymerase inhibitor. Hydroxy at 3'-position has been replaced by an azido, and its bioactivation requires transformation to triphosphate.



Scheme 13.10: Metabolic changes of idoxuridine.

Enzyme thymidine kinase is crucial in the anabolism of pyrimidine 2'-deoxynucleosides, as it catalyzes its conversion to 5'-monophosphates. Some herpes simplex viruses (HSV-1 and HSV-2) and varicella zoster viruses (VZV) encode their own thymidine kinases, and it has been observed that the introduction of a substituent at C-5

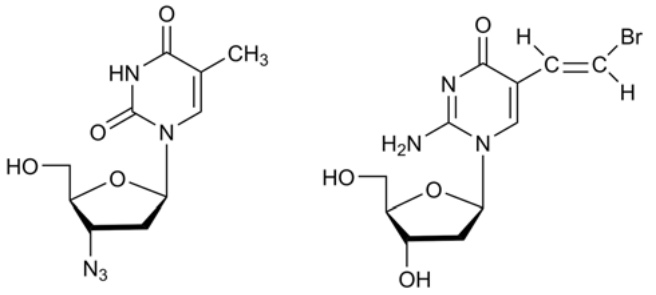


Fig. 13.9: Structure of some pyrimidine nucleosides used as antivirals.

produces an increased selectivity for them with the possibility of obtaining less toxic antiviral drugs. An example is 5-(*E*)-(2-bromovinyl)-2'-deoxyuridine (BVDU), Fig. 13.9, which is superior to other agents in the topical treatment of HSV-1 eye infections. The bioactivation of this prodrug and its incorporation into DNA take place only in virus-infected cells.

Another successful modification has been to replace the sugar moiety by an acyclic chain: Acyclovir is used, orally, intravenously and topically in herpes keratitis and cold sores. Acyclovir has led to numerous analogues such as ganciclovir, whose use is restricted to infections caused by cytomegalovirus in immunocompromised patients (Fig. 13.10).

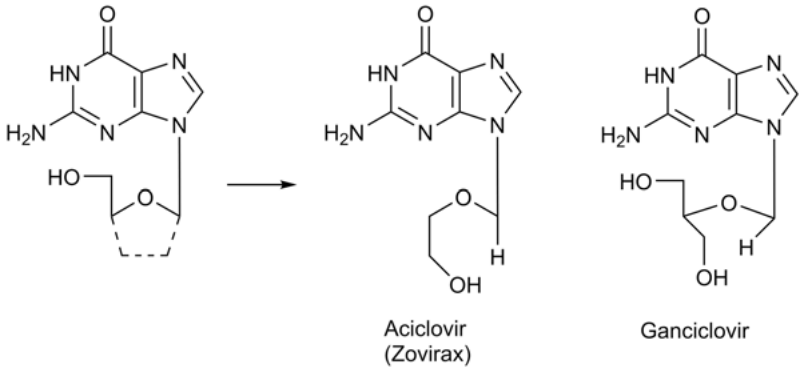


Fig. 13.10: Acyclovir and analogues.

Among other nucleosides in which the sugar has been modified, vidarabine (ara-A, 9-β-D-arabinofuranosyladenine, Fig. 13.11), an antiviral having a multiple mechanism of action, has been found. It phosphorylates first to monophosphate and then

to di- and tri-phosphate, which inhibits DNA polymerases, thus explaining its action against virus DNA. It can also be incorporated into both the host cell and the virus DNA. It has been used in the treatment of HSV-1 encephalitis and shingles in immunocompromised patients. 1β-D-Arabinofuranosylcytosine, termed cytarabine or ara-C, is used in acute leukemias or as an antiviral (Fig. 13.11).

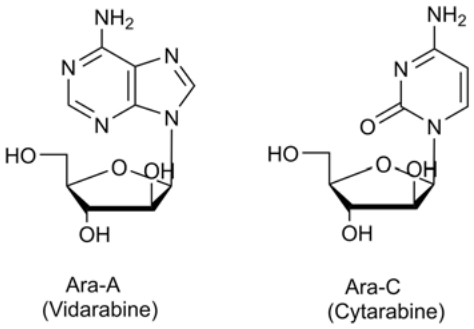


Fig. 13.11: Ara-A and Ara-C.

Cytarabine is the 2'-epimer of cytosine (it has a D-arabinose residue instead of D-ribose). It interferes with DNA synthesis after transformation to monophosphate and then to triphosphate (ara-CTP), which by analogy with deoxycytidine triphosphate, competitively inhibits DNA polymerases by incorporating its structure (cytarabine) into the DNA and RNA strands and disrupting their elongation steps.

Nucleosides whose sugar moiety has the L (non-natural) configuration have also been studied, and can therefore be considered as enantiomers of the natural nucleosides. Although it was believed that they would not be recognized by the corresponding enzymes, it seems that this is not veracious. Thus, the L-isomer of 2',3'-dideoxycytidine (Fig. 13.12), although less active than the D-isomer against HIV virus, is less toxic.

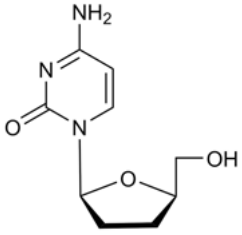


Fig. 13.12: Example of a nucleoside that has antiviral action, with the L non-natural configuration (L-2',3'-dideoxycytidine).

Index

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