25 Antiulcer agents

25.1 Peptic ulcers

25.1.1 Definition

Peptic ulcers are localized erosions of the mucous membranes of the stomach or duodenum. The pain associated with ulcers is caused by irritation of exposed surfaces by the stomach acids. Before the appearance of effective antiulcer drugs in the 1960s, ulcer sufferers often suffered intense pain for many years, and if left untreated, the ulcer could result in severe bleeding and even death. For example, the film star Rudolph Valentino died from a perforated ulcer in 1926 at the age of 31.

25.1.2 Causes

The causes of ulcers have been disputed. Stress, alcohol and diet have been considered important factors, but there is no clear evidence for this. Scientific evidence indicates that the two main culprits are the use of non-steroidal anti-inflammatories (NSAIDS) or the presence of a bacterium called *Helicobacter pylori*. As far as the NSAIDS are concerned, agents such as **aspirin** inhibit the enzyme **cyclooxygenase 1 (COX-1)**. This enzyme is responsible for the synthesis of prostaglandins that inhibit acid secretion and protect the gastric mucosa. Once an ulcer has erupted, the presence of gastric acid aggravates the problem and delays recovery.

25.1.3 Treatment

Antiulcer therapy has been a huge money spinner for the pharmaceutical industry with drugs such as **cimetidine**, **ranitidine**, and the proton pump inhibitors (PPIs). None of these drugs were available until the 1960s, however, and it is perhaps hard for us now to appreciate how dangerous ulcers could be before that. In the early 1960s, the conventional treatment was to try to neutralize gastric acid in the stomach by administering antacids. These were bases such as sodium bicarbonate or calcium carbonate. The dose levels required for neutralization were large and caused unpleasant side effects. Relief was only temporary, and patients were often advised to stick to rigid diets such as strained porridge and steamed fish. Ultimately, the only answer to severe ulcers was a surgical operation to remove part of the stomach.

The first effective antiulcer agents were the H_2 histamine antagonists, which appeared in the 1960s. These were followed in the 1980s by the PPIs. The discovery of *H. pylori* then led to the use of antibacterial agents in antiulcer therapy. The current approach for treating ulcers caused by *H. pylori* is to use a combination of drugs, which includes a PPI such as **omeprazole,** and two antibiotics such as **amoxicillin** and **metronidazole**.

25.1.4 Gastric acid release

Gastric juices consist of digestive enzymes and hydrochloric acid designed to break down food. Hydrochloric acid is secreted from parietal cells, and the stomach secretes a layer of mucus to protect itself from its own gastric juices. Bicarbonate ions are also released and are trapped in the mucus to create a pH gradient within the mucus layer.

The H_2 antagonists and PPIs both work by reducing the amount of gastric acid released into the stomach by the parietal cells lining the stomach wall (Fig. 25.1). These parietal cells are innervated with nerves (not shown on the diagram) from the autonomic nervous system (sections 22.1–22.2). When the autonomic nervous system is stimulated, a signal is sent to the parietal cells culminating in the release of the neurotransmitter **acetylcholine** at the nerve termini. Acetylcholine activates the cholinergic receptors of the parietal cells leading to the release of gastric acid into the stomach. The trigger for this process is provided by the sight, smell, or even the thought, of food. Thus, gastric acid is released before food has even entered the stomach.

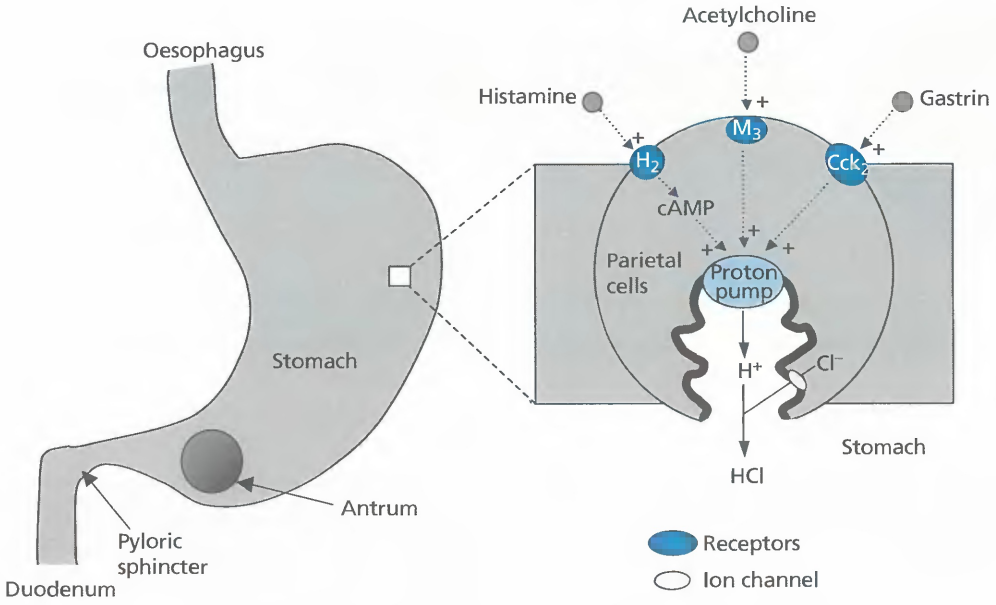


FIGURE 25.1 Factors influencing the release of gastric acid.

Nerve signals also stimulate a region of the stomach called the antrum which contains hormone-producing cells known as G cells. The hormone released is a peptide called **gastrin** (Fig. 25.2) which is also released when food is present in the stomach. The gastrin moves into the blood supply and travels to the parietal cells, further stimulating the release of gastric acid. Release of gastric acid should therefore be inhibited by antagonists blocking either the cholinergic receptor or the receptor for gastrin.

Agents which block the cholinergic receptor are known as anticholinergic drugs (section 22.11). These agents certainly block the cholinergic receptor in parietal cells and inhibit release of gastric acid. Unfortunately, they also inhibit acetylcholine receptors at other parts of the body and cause unwanted side effects.

The local hormone **histamine** also stimulates the release of gastric acid by interacting with a specific type of histamine receptor called the H_2 receptor. Thus, histamine antagonists have proved to be important antiulcer drugs although they have now largely been superseded by the PPIs, which block the mechanism by which hydrochloric acid is released from parietal cells.

25.2 H_2 Antagonists

The first breakthrough in antiulcer therapy came with the design of the H_2 antagonist **cimetidine** (Tagamet) (Fig. 25.32), produced by the company Smith Kline and French (SKF). The cimetidine programme started in 1964, and was one of the early examples of rational drug design. Up until that time, many of the successes in medicinal chemistry involved the fortuitous discovery of useful pharmaceutical agents from natural sources, and the study of analogues often synthesized on a trial-and-error basis. Although this approach yielded a large range of medicinal compounds, it was wasteful in terms of the time and effort involved. Nowadays, the emphasis is on rational drug design using the tools of X-ray crystallography, molecular modelling, and genetic engineering (chapters 13 and 17). Unfortunately, such tools were not available in the 1960s and the story of cimetidine is a good example of how to carry out rational drug design when the target has not been identified or isolated.

The remarkable aspect of the cimetidine story is that at the onset of the project there were no lead compounds

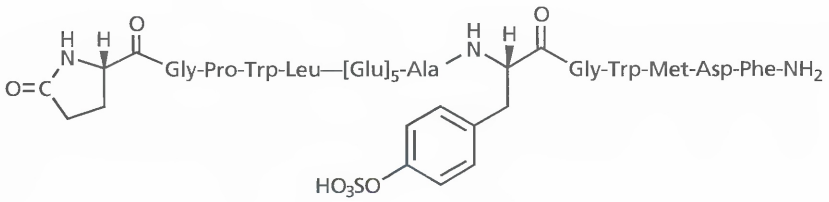


FIGURE 25.2 Gastrin.

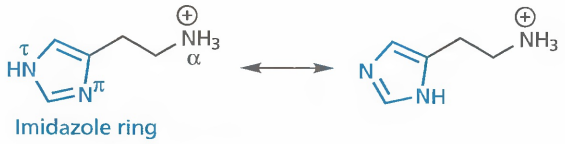


FIGURE 25.3 Histamine.

and it was not even known if the necessary histamine receptor existed! In 1964, the best hope of achieving an antiulcer agent appeared to be in finding a drug which would block the hormone gastrin. Several research teams were active in this field, but the research team at SKF decided to follow a different tack altogether.

It was known experimentally that histamine (Fig. 25.3) stimulated gastric acid release *in vitro*, so the SKF team proposed that an antihistamine agent might be effective in treating ulcers. At the time, this was a highly speculative proposal as it was by no means certain that histamine played any significant role *in vivo*. Many workers at the time discounted the importance of histamine, especially when it was found that conventional antihistamines failed to inhibit gastric acid release. This suggested the absence of histamine receptors in the parietal cells. The fact that histamine had a stimulatory effect was explained away by suggesting that histamine coincidentally switched on the gastrin or cholinergic receptors. Even if a histamine receptor was present, opponents argued that blocking it would have little effect since the receptors for acetylcholine and gastrin would remain unaffected and could still be activated by their respective messengers. Initiating a project which had no known target and no known lead compound was unprecedented, and represented a massive risk. Indeed, for a long time little progress was made and it is said that company accountants demanded that the project be terminated. It says much for the scientists involved that they stuck to their guns and eventually confounded their critics. Why did the SKF team persevere in their search for an effective antihistamine? What was their reasoning? Before answering that, let us look at histamine itself and the antihistamines available at that time.

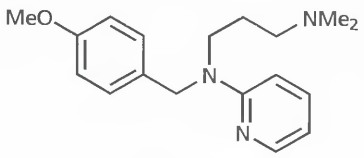
25.2.1 Histamine and histamine receptors

Histamine is made up of an imidazole ring which can exist in two tautomeric forms as shown in Fig. 25.3. Attached to the imidazole ring is a two-carbon chain with a terminal α-amino group. The pK_a of this amino group is 9.80, which means that at a plasma pH of 7.4, the side chain of histamine is 99.6% ionized. The pK_a of the imidazole ring is 5.74 and so the ring is mostly un-ionized at pH 7.4 (Fig. 25.4). Note that the lower the pK_a value, the more acidic the proton. It is also useful to remember that 50% ionization takes place when the pH is the same value as the pK_a (section 11.2).

Whenever cell damage occurs, histamine is released and stimulates the dilatation and increased permeability of small blood vessels. This allows defensive cells such as white blood cells to be released from the blood supply into an area of tissue damage and to combat any potential infection. Unfortunately, the release of histamine can also be a problem. Allergic reactions and irritations are caused by release of histamine when it is not really needed.

The early antihistamine drugs were therefore designed to treat conditions such as hay fever, rashes, insect bites, or asthma. Two examples of these early antihistamines are **mepyramine** and **diphenhydramine** ('Benadryl') (Fig. 25.5), neither of which has any effect on gastric acid release.

Bearing this in mind, why did the SKF team persevere with the antihistamine approach? The main reason was the fact that conventional antihistamines failed to inhibit all the then known actions of histamine. For example, they failed to fully inhibit the dilatation of blood vessels induced by histamine. The SKF



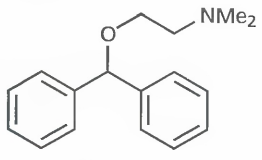


FIGURE 25.5 Early antihistamines.

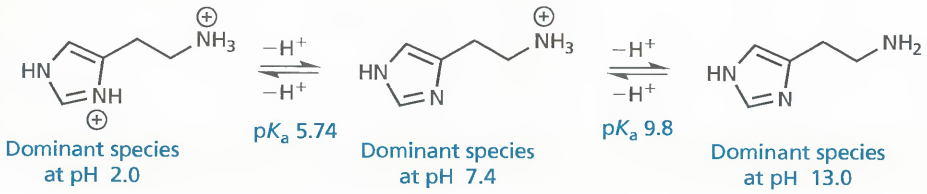


FIGURE 25.4 Ionization of histamine.

scientists therefore proposed that there might be two different types of histamine receptor, analogous to the two types of cholinergic receptor mentioned in chapter 22. Histamine—the natural messenger—would switch both on equally effectively and would not distinguish between them, whereas suitably designed antagonists might be capable of making that distinction. By implication, this meant that the conventional antihistamines known in the early 1960s were already selective in inhibiting the histamine receptors involved in the inflammation process (classified as H_1 receptors), rather than the proposed histamine receptors responsible for gastric acid secretion (classified as H_2 receptors).

It was an interesting theory, but the fact remained that there was no known antagonist for the proposed H_2 receptors. Until such a compound was found, it could not be certain that the H_2 receptors even existed.

25.2.2 Searching for a lead

25.2.2.1 Histamine

The SKF team obviously had a problem. They had a theory but no lead compound. How could they make a start?

Their answer was to start from histamine itself. If the hypothetical H_2 receptor existed, then histamine must bind to it. The task then was to vary the structure of histamine in such a way that it would bind as an antagonist rather than an agonist.

This meant exploring how histamine itself bound to its receptors. Structure–activity relationship (SAR) studies on histamine and histamine analogues revealed that the binding requirements for histamine to the H_1 receptors were as follows:

- The side chain had to have a positively charged nitrogen atom with at least one attached proton. Quaternary ammonium salts that lacked such a proton were extremely weak in activity.
- There had to be a flexible chain between the above cation and a heteroaromatic ring.
- The heteroaromatic ring did not have to be imidazole, but it did have to contain a nitrogen atom with a lone pair of electrons, *ortho* to the side chain.

For the proposed H_2 receptor, SAR studies were carried out experimentally by determining whether histamine analogues could stimulate gastric acid release in stomach tissue. The essential SAR requirements were the same as for the H_1 receptor except that the heteroaromatic ring had to contain an amidine unit (HN–CH–N:).

The results are summarized in Fig. 25.6 and appear to show that the terminal α-amino group is involved in a binding interaction with both types of receptor via ionic and/or hydrogen bonding, while the nitrogen atom(s) in the heteroaromatic ring interacts via hydrogen bonding, as shown in Fig. 25.7.

25.2.2.2 N^{α}-Guanylhistamine

Having gained a knowledge of the SAR for histamine, the task was now to design a molecule that would be recognized by the proposed H_2 receptor, but would not activate it. In other words, an agonist had to be converted to an antagonist. This meant altering the way in which the molecule bound to the receptor.

Pictorially, one can imagine histamine fitting into its binding site and stabilizing a change in shape which

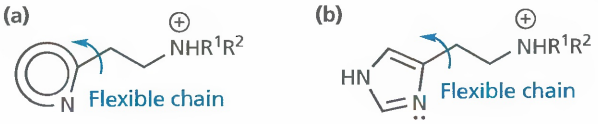


FIGURE 25.6 Summary of structure–activity relationship (SAR) results: (**a**) SAR for agonists at the H_1 receptor; (**b**) SAR for agonists at the proposed H_2 receptor.

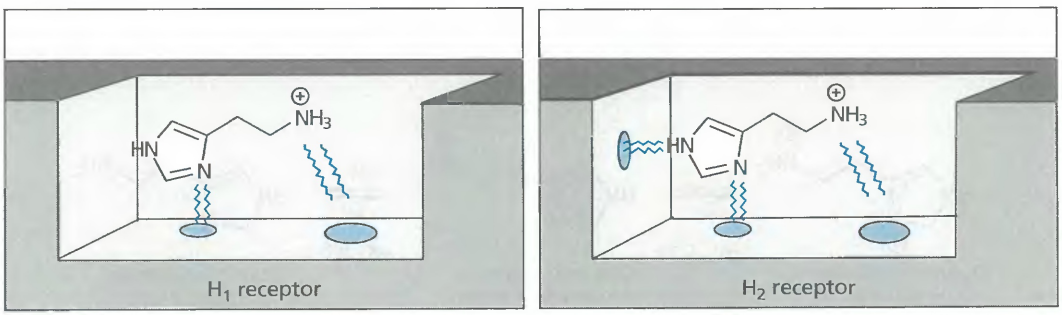


FIGURE 25.7 Binding interactions for the H_1 receptor and the proposed H_2 receptor.

'switches on' the receptor (Fig. 25.8). An antagonist can often be found by adding a functional group which binds to an extra binding region in the binding site and prevents the change in shape required for activation.

This was one of several strategies tried out by the SKF workers. To begin with, the structural differences between agonists and antagonists in other areas of medicinal chemistry were identified and similar alterations were tried on histamine. Analogues were tested to see whether they stimulated or blocked gastric acid release, the assumption being that an H_2 receptor would be responsible for such an effect.

Fusing an aromatic ring on to noradrenaline had been a successful tactic used in the design of adrenergic antagonists (see section 23.11.3). This same tactic was tried with histamine to give analogues such as the one shown in Fig. 25.9, but none of these compounds proved to be an antagonist.

Another approach which had been used successfully in the development of anticholinergic agents (section 22.11.2) had been the addition of non-polar, hydrophobic substituents. Similar substituents were attached to various locations of the histamine skeleton but none proved to be antagonists.

Nevertheless, there was one interesting result which proved relevant to later studies. It was discovered that **4-methylhistamine** (Fig 25.10) was a highly selective H_2 agonist. In other words, it stimulated gastric acid release in the test assay, but had weak activity for all the other actions of histamine. How could this be?

4-Methylhistamine (like 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. In particular, conformation I in Fig. 25.10 is not favoured as a result of a large steric interaction between the 4-methyl group and the side chain. This means that the 4-methyl group is acting as a conformational blocker (section 13.3.10). The selectivity observed suggests that 4-methylhistamine (and by inference histamine) has to adopt two different conformations in order to fit the H_1 and putative H_2 receptor. Since 4-methylhistamine is more active at the hypothetical H_2 receptor, it implies that conformation II is required for the H_2 receptor and conformation I is required for the H_1 receptor.

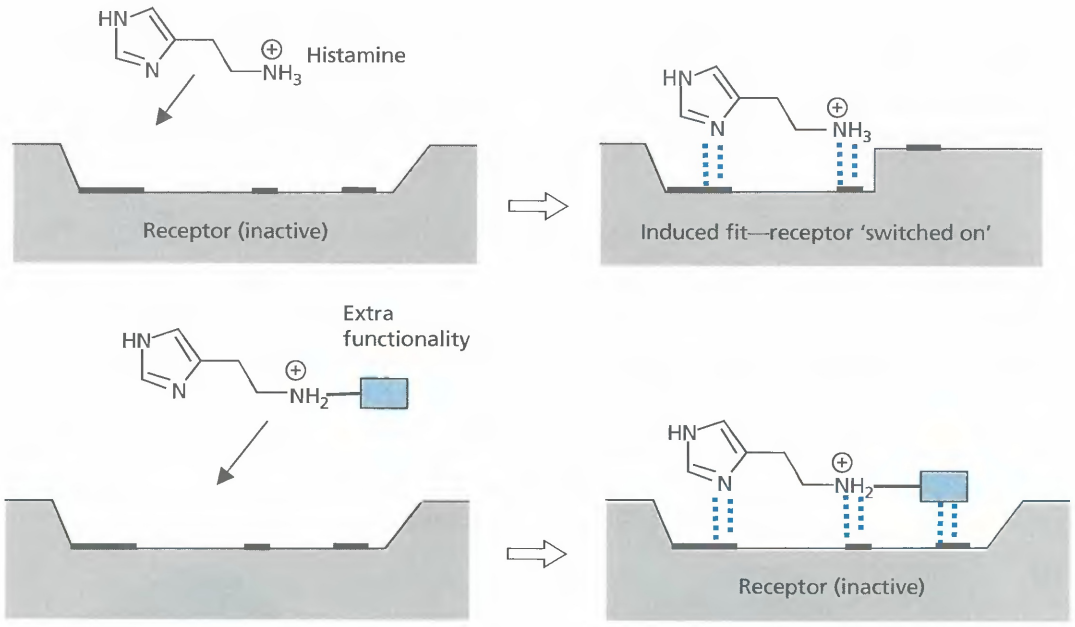


FIGURE 25.8 Possible receptor interactions of histamine and an antagonist.

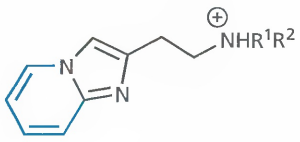


FIGURE 25.9 Histamine analogue—not an antagonist.

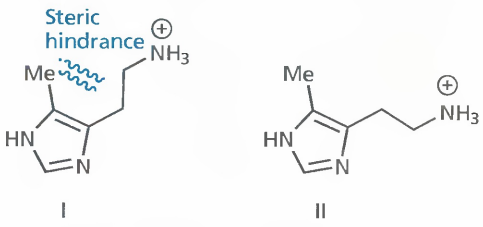


FIGURE 25.10 4-Methylhistamine.

Despite this interesting result, the SKF workers were no closer to an H_2 antagonist. 200 compounds had been synthesized and not one had shown a hint of being an antagonist. Research up until this stage had concentrated on adding hydrophobic groups to search for an additional hydrophobic binding region in the proposed receptor binding site. Now the focus switched to study the effect of varying polar groups on the molecule. In particular, the terminal α-NH_3^+ group was replaced by different polar functional groups, the reasoning being that such groups could bond to the same binding region as the NH_3^+ group, but that the geometry of bonding might be altered sufficiently to produce an antagonist. This led to the first crucial breakthrough, with the discovery that N^{α}-guanylhistamine (Fig. 25.11) was a weak antagonist of gastric acid release.

This structure had in fact been synthesized earlier in the project, but had not been recognized as an antagonist. This is not too surprising since it acts as an agonist! It was not until later pharmacological studies were carried out that it was realized that N^{α}-guanylhistamine was also acting as an antagonist. In other words, it was a partial agonist (section 8.4). N^{α}-Guanylhistamine activates the H_2 receptor, but not to the same extent as histamine. As a result, the amount of gastric acid released is lower. More importantly, as long as N^{α}-Guanylhistamine is bound to the receptor, it prevents histamine from binding and thus prevents complete receptor activation. This was the first indication of any sort of antagonism to histamine, but still did not prove the existence of the H_2 receptor.

The question now arose as to which parts of the N^{α}-guanylhistamine skeleton were really necessary for this effect. Various guanidine structures were synthesized that lacked the imidazole ring, but none had the desired antagonist activity, demonstrating that both the imidazole ring and the guanidine group were required.

The structures of N^{α}-guanylhistamine and histamine were now compared. Both structures contain an imidazole ring and a positively charged group linked by a two-carbon bridge. The guanidine group is basic and protonated at pH 7.4, so the analogue has a positive charge similar to histamine. However, the charge on the guanidine group can be spread around a planar arrangement of three nitrogens and can be further away from the imidazole ring (Fig. 25.11). This leads to the possibility that the analogue could be interacting with an extra polar binding region on the receptor which is 'out of reach' of histamine. This is demonstrated in Figs. 25.12 and 25.13. Two alternative binding regions might be available for the cationic group—an agonist region where binding leads to activation of the receptor, and an antagonist region where binding does not activate the receptor. In Fig. 25.13, histamine is only able to reach the agonist region, whereas the analogue with its extended functionality is capable of reaching either region (Fig. 25.12).

If most of the analogue molecules bind to the agonist region and the remainder bind to the antagonist

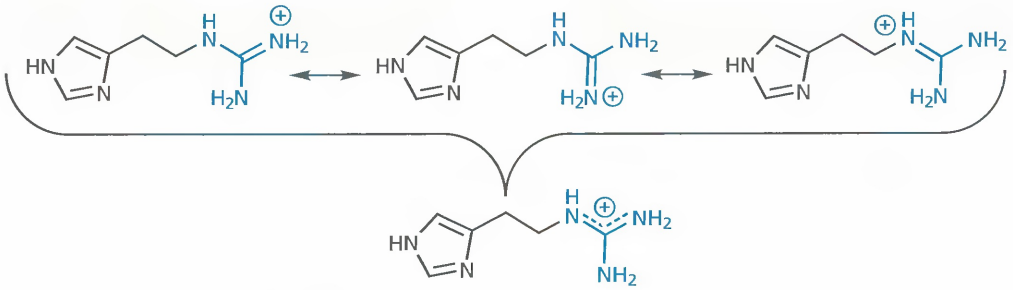


FIGURE 25.11 N^{α}-Guanylhistamine.

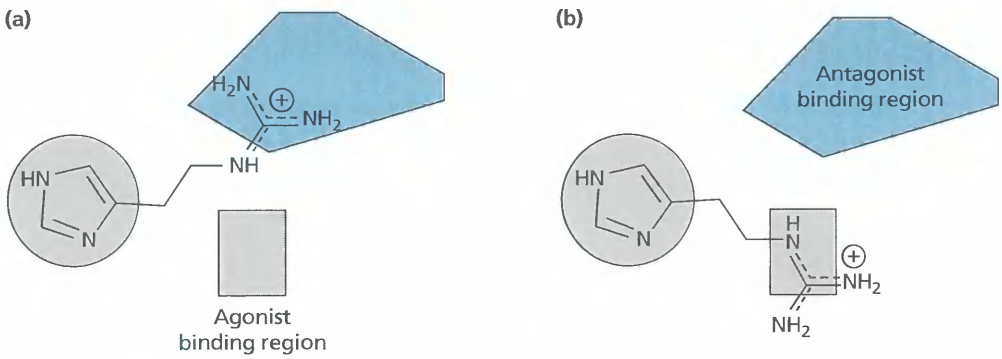


FIGURE 25.12 Possible binding modes for N^{α}-guanylhistamine as **(a)** an antagonist and **(b)** an agonist.

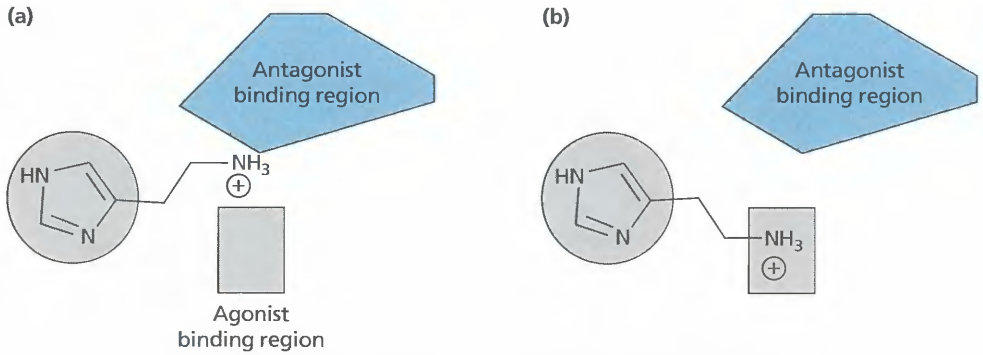


FIGURE 25.13 Binding of histamine: (**a**) no binding to the antagonist binding region, (**b**) binding to the agonist binding region.

region, then this could explain the partial agonist activity. Regardless of the mode of binding, histamine would be prevented from binding and an antagonism would be observed due to the fraction of N^{α}-guanylhistamine bound to the antagonist region.

25.2.3 Developing the lead: a chelation bonding theory

The task was now to find an analogue which would bind to the antagonist region only. The isothiourea (**a** in Fig. 25.14) was synthesized since the positive charge would be restricted to the terminal portion of the chain and should interact more strongly with the more distant antagonist binding region. Antagonist activity did increase, but the compound was still a partial agonist, showing that binding was still possible to the agonist region.

Two other analogues were synthesized, where one of the terminal amino groups in the guanidine group was replaced by a methylthio group or a methyl group (**b** in Fig. 25.14). Both these structures were partial agonists, but with poorer antagonist activity.

From these results, it was concluded that both terminal amino groups were required for binding to the antagonist binding site. It was proposed that the charged guanidine group was interacting with a charged carboxylate residue on the receptor via two hydrogen bonds (Fig. 25.15). If either of these terminal amino groups were absent, then binding would be weaker, resulting in a lower level of antagonism.

The chain was now extended from a two-carbon unit to a three-carbon unit to see what would happen if the guanidine group was moved further away from the imidazole ring. The antagonist activity increased for the guanidine structure (Fig. 25.16), but strangely

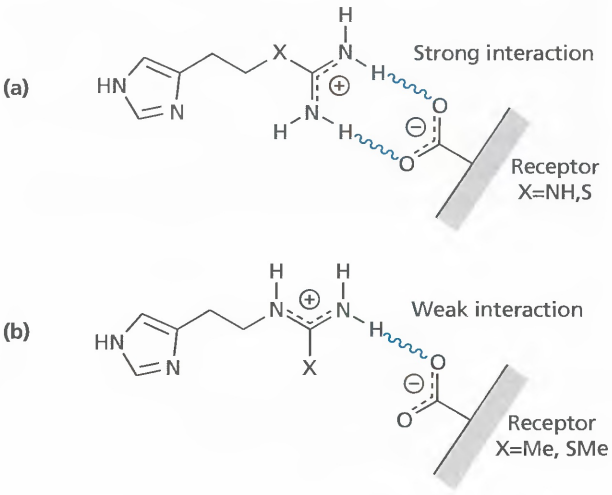


FIGURE 25.15 Proposed hydrogen bonding interactions for (**a**) a structure with two terminal amino groups and (**b**) an analogue with one terminal amino group.

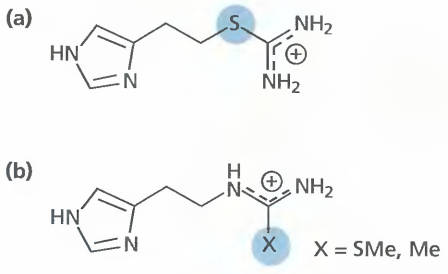


FIGURE 25.14 (**a**) An isothiourea. (**b**) Other analogues.

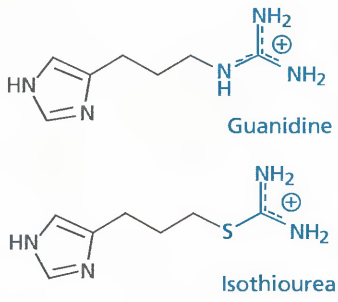


FIGURE 25.16 Guanidine and isothiourea structures with a 3-C linker.

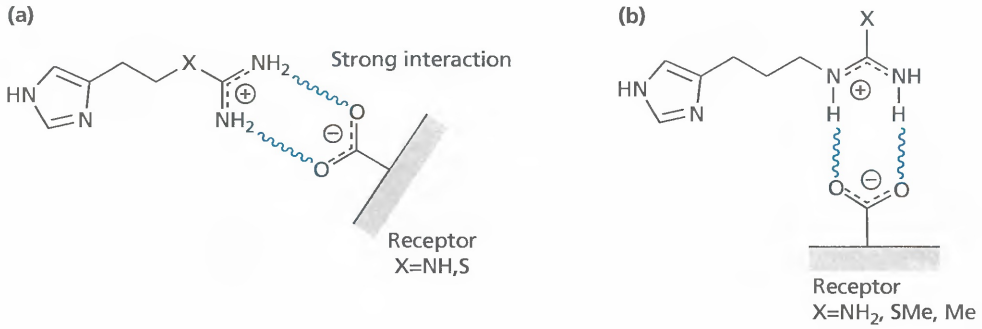


FIGURE 25.17 Proposed binding interactions for analogues of different chain length. (**a**) H-bonding involving two terminal amino groups for the three-atom chain. (**b**) H-bonding involving a terminal and internal amino group for a four-atom chain.

enough, decreased for the isothiourea structure (Fig. 25.16). It was therefore proposed that with a chain length of two carbon units, hydrogen bonding to the receptor involved the terminal NH_2 groups, but with a chain length of three carbon units, hydrogen bonding to the same carboxylate group involved one terminal NH_2 group along with the NH group within the chain (Fig. 25.17). Support for this theory was provided by the fact that replacing one of the terminal NH_2 groups in the guanidine analogue with SMe or Me (Fig. 25.18) did not adversely affect antagonist activity. This was completely different from the results obtained when similar changes were carried out on the C_2 bridged compound.

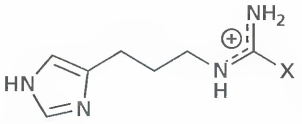


FIGURE 25.18 Guanidine analogue with X = SMe or Me.

These bonding interactions are represented pictorially in Figs. 25.19 and 25.20.

25.2.4 From partial agonist to antagonist: the development of burimamide

The problem was now to completely remove the agonist activity to get a pure antagonist. This meant designing a structure which would differentiate between the agonist and antagonist binding regions. At first sight this looks impossible, as both regions appear to involve the same type of bonding. Histamine's activity as an agonist depends on the imidazole ring and the charged amino function, with the two groups taking part in hydrogen and ionic bonding, respectively. The antagonist activity of the partial agonists described so far also appears to depend on a hydrogen bonding imidazole ring and an ionic bonding guanidine group.

Fortunately, a distinction can be made between the charged groups.

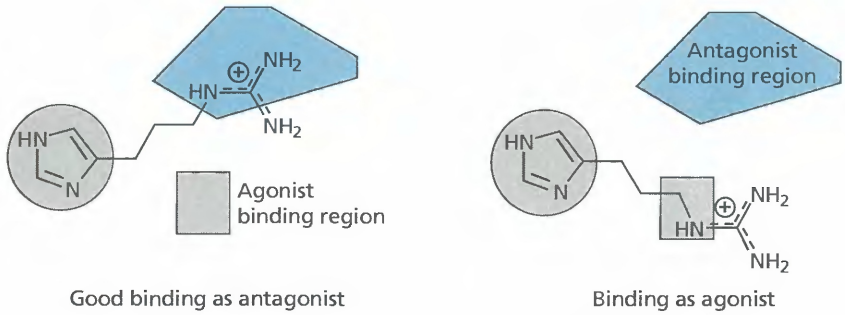


FIGURE 25.19 Proposed binding interactions for the 3-C bridged guanidine analogue.

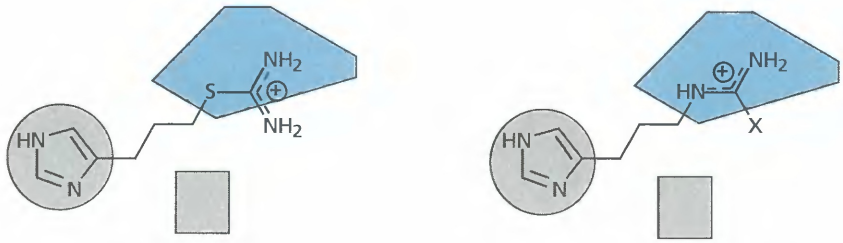


FIGURE 25.20 Proposed binding effect at the antagonist region if the guanidine group is modified.

The structures which show antagonist activity are all capable of forming a chelated bonding structure, as shown in Fig. 25.17. This interaction involves two hydrogen bonds between two charged species, but is it really necessary for the chelating group to be charged? Could a neutral group also chelate to the antagonist region by hydrogen bonding alone? If so, it might be possible to distinguish between the agonist and antagonist regions, especially since ionic bonding appears mandatory for the agonist region.

It was therefore decided to see what would happen if the strongly basic guanidine group was replaced by a neutral group, capable of interacting with the receptor by two hydrogen bonds. There are many such groups, but the SKF workers limited the options by adhering to a principle which they followed throughout their research programme. Whenever they wished to alter a specific physical or chemical property, they strove to ensure that other properties were changed as little as possible. Only in this way could they rationalize any observed improvement in activity. Thus, it was necessary to ensure that the new group was similar to guanidine in terms of size, shape, and hydrophobicity.

Several functional groups were tried, but success was ultimately achieved by using a thiourea group to give **SKF 91581** (Fig. 25.21). The thiourea group is neutral at physiological pH because the C=S group has an electron withdrawing effect on the neighbouring nitrogens, making them non-basic and more like amide nitrogens. Apart from basicity, the properties of the thiourea group are very similar to the guanidine group. Both groups are planar, similar in size, and can take part in hydrogen bonding. This means that the alteration in biological activity can reasonably be attributed to the differences in basicity between the two groups.

SKF 91581 proved to be a weak antagonist with no agonist activity, establishing that the agonist binding region involves ionic bonding, whereas the antagonist region involves hydrogen bonding.

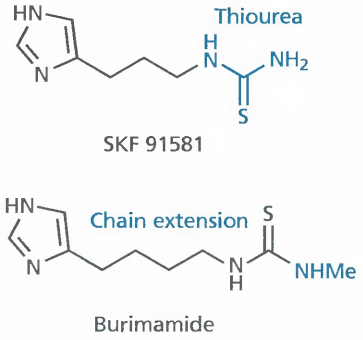


FIGURE 25.21 SKF 91581 and burimamide.

Further chain extension and the addition of an *N*-methyl group led to **burimamide** (Fig. 25.21), which was found to have enhanced activity, suggesting that the thiourea group has been moved closer to the antagonist binding region. The beneficial addition of the *N*-methyl group is due to an increase in hydrophobicity and a possible explanation for this will be described in section 25.2.8.2 (desolvation).

Burimamide is a highly specific competitive histamine antagonist at H_2 receptors, and is 100 times more potent than N^{α}-guanylhistamine in inhibiting gastric acid release induced by histamine. Its discovery gave the SKF researchers far greater evidence for the existence of H_2 receptors.

25.2.5 Development of metiamide

Despite this success, burimamide was not suitable for clinical trials since its activity was still too low for oral administration. Attention was now directed to the imidazole ring of burimamide and, in particular, to its possible tautomeric and protonated forms. It was argued that if one of these forms was preferred for binding with the H_2 receptor, then activity might be enhanced by modifying the burimamide structure to favour that form.

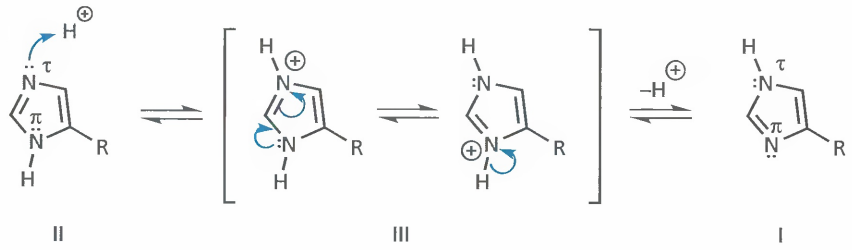


FIGURE 25.22 Imidazole ring can equilibrate between tautomeric forms (I and II) via the protonated intermediate (III).

At pH 7.4, it is possible for the imidazole ring to equilibrate between the two tautomeric forms (I) and (II) via the protonated intermediate (III) (Fig. 25.22). The necessary proton for this process is supplied by water or by an exchangeable proton on a suitable amino acid residue in the binding site. If the exchange is slow, then it is possible that the drug will enter and leave the receptor at a faster rate than the equilibration between the two tautomeric forms. If bonding involves only one of the tautomeric forms or the protonated form, then clearly antagonism would be increased if the structure was varied to prefer that form over the other. Our model hypothesis for receptor binding shows that the imidazole ring is important for the binding of both agonists and antagonists. Therefore, it is reasonable to assume that the preferred imidazole form is the same for both agonists and antagonists. If so, then the preferred form for a strong agonist such as histamine should also be the preferred form for a strong antagonist.

Figure 25.22 shows that the imidazole ring can exist as two un-ionized tautomers and one protonated form. Is the protonated form likely?

We have already seen that the pK_a for the imidazole ring in histamine is 5.74, meaning that the ring is a weak base and mostly un-ionized at physiological pH. The pK_a value for imidazole itself is 6.80 and for the imidazole ring in burimamide it is 7.25, showing that these rings are more basic and more likely to be ionized. Why should this be so?

The explanation is that the side chains have an electronic effect on the imidazole ring that affects the basicity of the ring. A measure of the electronic effect of the side chain can be worked out by the Hammett equation (section 18.2.2):

$$pK_{a(R)} = pK_{a(H)} + \rho\sigma_R$$

where $pK_{a(R)}$ is the pK_a of the imidazole ring bearing a side chain R, $pK_{a(H)}$ is the pK_a of the unsubstituted imidazole ring, ρ is a constant, and σ_R is the Hammett substituent constant for the side chain R.

From the pK_a values, the value of the Hammett substituent constant can be calculated to show whether the side chain R is electron-withdrawing or electron-donating. In burimamide, the side chain is slightly electron-donating (of the same order as a methyl group). Therefore, the imidazole ring in burimamide is more likely to be ionized than in histamine, where the side chain is electron-withdrawing. At pH 7.4, 40% of the imidazole ring in burimamide is ionized, compared to approximately 3% in histamine. This represents quite a difference between the two structures and since the binding of the imidazole ring is important for both antagonist and agonist activity, it suggests that a pK_a value closer to that of histamine might lead to better binding and to better antagonist activity.

It was necessary, therefore, to make the side chain electron-withdrawing rather than electron-donating. This can be done by inserting an electronegative atom into the side chain—preferably one which causes minimum disturbance to the rest of the molecule. In other words, an isostere for a methylene group is required; one which has an electronic effect, but which has approximately the same size and properties as the methylene group.

The first isostere to be tried was a sulfur atom. Sulfur is quite a good isostere for the methylene unit, since both groups have similar van der Waals radii and similar bond angles. On the other hand, the C–S bond is slightly longer than a C–C bond, leading to a slight extension (15%) of the structure.

The methylene group replaced was next but one to the imidazole ring. This site was chosen, not for any strategic reasons, but because a synthetic route was readily available to carry out that particular transformation. As hoped, the resulting compound, **thiaburimamide** (Fig. 25.23), had a significantly lower pK_a of 6.25 and was found to have enhanced antagonistic activity, supporting the theory that the un-ionized form is preferred over the protonated, ionized form.

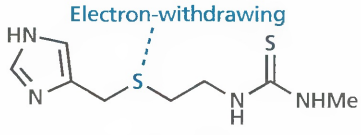


FIGURE 25.23 Thiaburimamide.

Thiaburimamide favours the un-ionized imidazole ring over the ionized ring, but there are two possible un-ionized tautomers. The next question is whether either of these are preferred for receptor binding.

Let us return to histamine. If one of the un-ionized tautomers is preferred over the other, it would be reasonable to assume that the preferred tautomer is the favoured tautomer for receptor binding, since it is more likely to be present. The preferred tautomer for histamine is tautomer I (Fig. 25.22) where Nτ is protonated and Nπ is not. This implies that Nτ in tautomer II is more basic than Nπ in tautomer I. This might not appear to be obvious, but we can rationalize it as follows. If Nτ in tautomer II is more basic than Nπ in tautomer I, it is more likely to become protonated to form the ionized intermediate (III). Moreover, deprotonation of III is more likely to give the weaker base which would be Nπ in tautomer I. Therefore the equilibrium should shift to favour tautomer I.

This is all very well, but why should Nτ (tautomer II) be more basic than Nπ (tautomer I)? The answer lies in the side chain R. The side chain on histamine has a positively charged terminal amino group, which means that the side chain has an electron-withdrawing effect on the imidazole ring. Since this effect is inductive, the strength of the effect will decrease with distance round the ring, which means that the nitrogen atom closest to the side chain (Nπ) experiences a greater electron-withdrawing effect than the one further away (Nτ). As a result, the closer nitrogen (Nπ) is less basic, and is less likely to bond to hydrogen (Fig. 25.24). Since the side chain in thiaburimamide is also electron-withdrawing, then tautomer I will be favoured here as well.

It was now argued that tautomer I could be further enhanced if an electron-*donating* group was placed at position 4 of the imidazole ring. At this position, the inductive effect would be felt most strongly at the neighbouring nitrogen (Nτ), further enhancing its basic character over Nπ. At the same time, it was important to choose a group that would not interfere with the normal receptor binding interactions. For example, a large substituent might be too bulky and prevent the analogue fitting the binding site. A methyl group was chosen because it was known that 4-methylhistamine was an agonist that was highly selective for the H_2 receptor (section 25.2.2.2). This resulted in **metiamide** (Fig. 25.25) which was found to have enhanced antagonist activity, supporting the proposed theory.

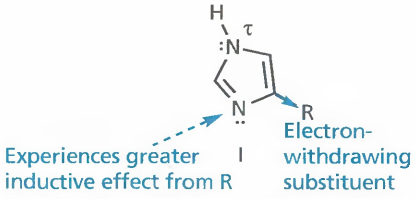


FIGURE 25.24 Inductive effect of the side chain on the imidazole nitrogens.

It is interesting to note that the percentage increase in tautomer I outweighs an undesirable rise in pK_a. By adding an electron-donating methyl group, the pK_a of the imidazole ring rises to 6.80 compared to 6.25 for thiaburimamide. Coincidentally, this is the same pK_a as for imidazole itself, which shows that the electronic effects of the methyl group and the side chain cancel each other out as far as pK_a is concerned. A pK_a of 6.80 means that 20% of metiamide exists as the protonated form (III), but this is still lower than the corresponding 40% for burimamide. More importantly, the effect on activity due to the increase in tautomer (I) outweighs the detrimental effect caused by the increase in the protonated form (III).

4-Methylburimamide (Fig. 25.26) was also synthesized for comparison. Here, the introduction of the 4-methyl group does not lead to an increase in activity. The pK_a is increased to 7.80, resulting in the population of ionized imidazole ring rising to 72%. This demonstrates the importance of rationalizing structural changes. Adding the 4-methyl group to thiaburimamide is advantageous, but adding it to burimamide is not.

The design and synthesis of metiamide followed a rational approach aimed at favouring one specific tautomer. Such a study is known as a **dynamic structure–activity analysis**.

Strangely enough, it has since transpired that the improvement in antagonism may have resulted from

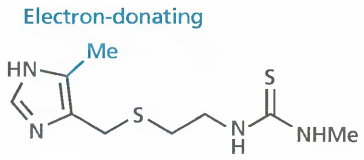
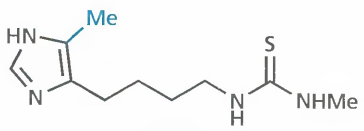
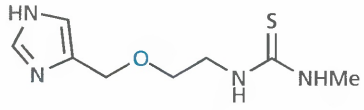


FIGURE 25.25 Metiamide.



4-Methylburimamide



Oxaburimamide

FIGURE 25.26 4-Methylburimamide and oxaburimamide.

conformational effects. X-ray crystallography studies have indicated that the longer thioether linkage in the chain increases the flexibility of the side chain and that the 4-methyl substituent in the imidazole ring may help to orientate the imidazole ring correctly for receptor binding. It is significant that the oxygen analogue **oxaburimamide** (Fig. 25.26) is less potent than burimamide, despite the fact that the electron-withdrawing effect of the oxygen-containing chain on the ring is similar to the sulfur-containing chain. The bond lengths and angles of the ether link are similar to the methylene unit, and in this respect it is a better isostere than sulfur. The oxygen atom is substantially smaller than sulfur. It is also significantly more basic and more hydrophilic than either sulfur or methylene. Oxaburimamide's lower activity might be due to a variety of reasons. For example, the oxygen may not allow the same flexibility permitted by the sulfur atom. Alternatively, the oxygen may be involved in a hydrogen bonding interaction, either with the receptor or with the imidazole ring, resulting in a change in receptor binding interaction. Another possibility is the fact that oxygen is more likely to be solvated than sulfur, and there is an energy penalty involved in desolvating the group before binding.

Metiamide is 10 times more active than burimamide and showed promise as an antiulcer agent. Unfortunately, a number of patients suffered from kidney damage and granulocytopenia—a condition which results in the reduction of circulating white blood cells, and makes patients susceptible to infection. Further developments were now required to find an improved drug without these side effects.

25.2.6 Development of cimetidine

It was proposed that metiamide's side effects were associated with the thiourea group—a group which is not particularly common in the body's biochemistry. Therefore, consideration was given to replacing the thiourea with a group which was similar in property but which would be more acceptable in a biochemical context. The urea analogue (Fig. 25.27) was tried, but found to be less active. The guanidine analogue (Fig. 25.27) was also less active, but it was interesting to note that this compound had no agonist activity. This contrasts with the C_3-bridged guanidine (Fig. 25.16), which is a partial agonist. Therefore, the guanidine analogue (Fig. 25.27) was the first example of a guanidine having pure antagonist activity.

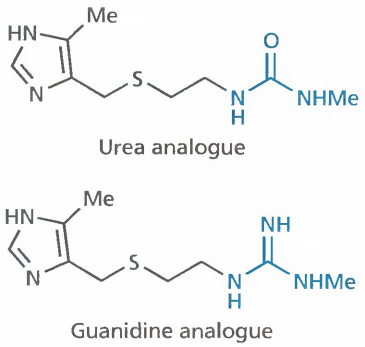


FIGURE 25.27 Urea and guanidine analogues.

One possible explanation for this is that the longer 4 atom chain extends the guanidine binding group beyond the reach of the agonist binding region (Fig. 25.28), whereas the shorter 3 atom chain still allows binding to both agonist and antagonist regions (Fig. 25.29).

The antagonist activity for the guanidine analogue (Fig. 25.27) is weak, but it was decided to look more closely at this compound, as it was thought that the guanidine unit would lack the toxic side effects of the thiourea unit. This is a reasonable assumption since the guanidine unit is present naturally in the amino acid **arginine** (Appendix 1). The problem now was to retain the guanidine unit, but to increase activity. It seemed likely that the low activity was because the basic guanidine group would be essentially fully protonated and ionized at pH 7.4. The problem was how to make this group non-basic—no easy task, considering the fact that guanidine is one of the strongest neutral organic bases in organic chemistry.

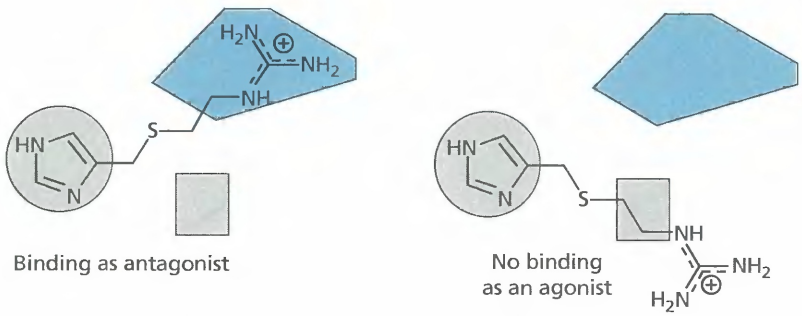


FIGURE 25.28 Binding of the guanidine analogue with a four-atom linker.

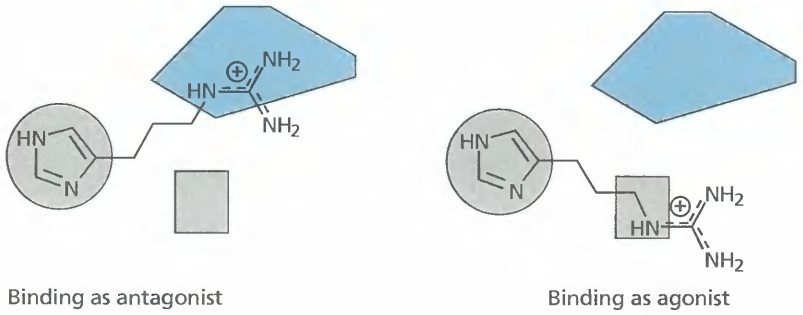


FIGURE 25.29 Binding of the guanidine analogue with a three-atom linker.

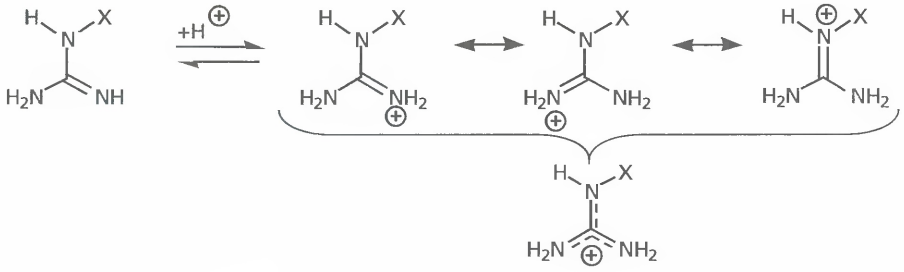


FIGURE 25.30 Ionization of monosubstituted guanidines.

Nevertheless, a search of the literature revealed a useful study on the ionisation of monosubstituted guanidines (Fig. 25.30). A comparison of the pK_a values of these compounds with the inductive substituent constants σ_i (section 18.2.2) for the substituents X gave a straight line as shown in Fig. 25.31, showing that pK_a is inversely proportional to the electron-withdrawing power of the substituent. Thus, strongly electron-withdrawing substituents make the guanidine group less basic and less ionized. The nitro and cyano groups are particularly strong electron-withdrawing groups. The pK_as for cyanoguanidine and nitroguanidine are 0.4 and 0.9 respectively (Fig. 25.31)—similar values to the pK_a for thiourea itself (−1.2).

Both the nitroguanidine and cyanoguanidine analogues of metiamide were synthesized and found to have comparable antagonist activities to metiamide. The cyanoguanidine analogue (**cimetidine**; Fig. 25.32) was the more potent analogue and was chosen for clinical studies. Its synthesis is described in Box 25.1.

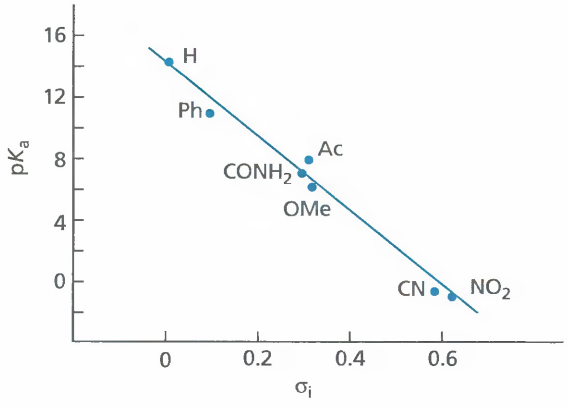


FIGURE 25.31 pK_a versus inductive substituent constants (σ_i).

25.2.7 Cimetidine

25.2.7.1 Biological activity

Cimetidine is an antagonist at H_2 receptors and thus inhibits gastric acid release. The drug does not show the toxic side effects observed for metiamide and has been shown

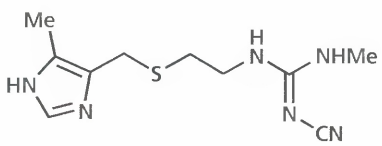
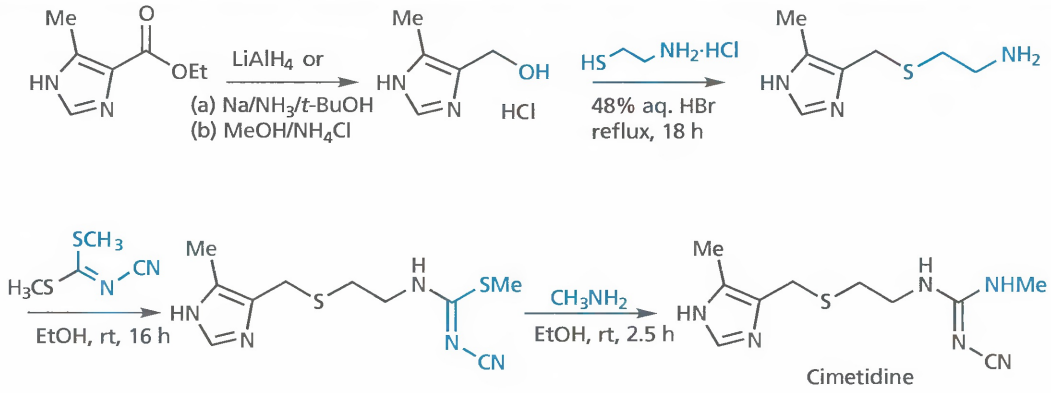


FIGURE 25.32 Cimetidine.

BOX 25.1 Synthesis of cimetidine

The synthesis of cimetidine was originally carried out as a four-step process, where lithium aluminium hydride was used as the reagent for the initial reduction step. Subsequent research revealed that this reduction could be carried out more cheaply and safely using sodium in liquid ammonia, and so this became the method used in the manufacture of cimetidine.



Synthesis of cimetidine.

to be slightly more active. It has also been found to inhibit **pentagastrin** (Fig. 25.33) from stimulating release of gastric acid. Pentagastrin is an analogue of gastrin and the fact that cimetidine inhibits it suggests some relationship between histamine and gastrin in the release of gastric acid.

Cimetidine was first marketed in the UK in 1976 under the trade name of **Tagamet** (derived from an*tag*onist and c*imet*idine). It was the first really effective antiulcer drug, doing away with the need for surgery. For several years, it was the world's biggest selling prescription product, until it was pushed into second place in 1988 by **ranitidine** (section 25.2.9.1).

25.2.7.2 Structure and activity

The finding that metiamide and cimetidine are both good H_2 antagonists of similar activity shows that the cyano-guanidine group is a good bioisostere for the thiourea group. Three tautomeric forms (Fig. 25.34) are possible for the guanidine group with the imino tautomer (II) being the preferred tautomer. This is because the cyano group has a stronger electron-withdrawing effect on the neighbouring nitrogen compared with the two nitrogens further away. As a result, the neighbouring nitrogen is less basic and less likely to be protonated. Moreover, tautomer II has an extra stabilization due to the conjugation of the double bond and the cyano group.

Since tautomer II is favoured, the guanidine group bears a close structural similarity to the thiourea group. Both groups have a planar π electron system with similar geometries (equal C–N distances and angles). They are polar and hydrophilic with high dipole moments and low partition coefficients. They are weakly basic and also weakly acidic such that they are un-ionized at pH 7.4.

(*t*-Boc)N-β-Ala-Trp-Met-Asp-Phe-NH_2

FIGURE 25.33 Pentagastrin.

25.2.7.3 Metabolism

It is important to study the metabolism of a new drug in case the metabolites have biological activity in their own right. Any such activity might lead to undesirable side

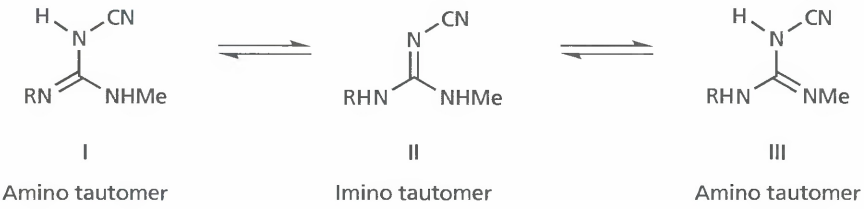


FIGURE 25.34 Three tautomeric forms of guanidine unit.

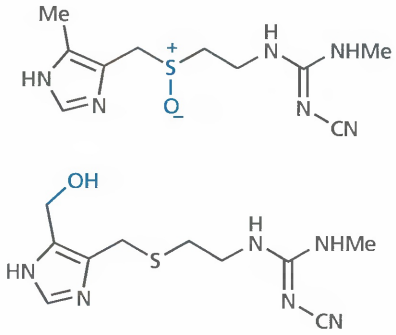


FIGURE 25.35 Metabolites of cimetidine.

effects. Alternatively, a metabolite might have enhanced activity of the type desired and give clues to further development. Cimetidine itself is metabolically stable and is excreted largely unchanged. The only metabolites that have been identified are due to oxidation of the sulfur link or oxidation of the ring methyl group (Fig. 25.35).

It has been found that cimetidine inhibits the cytochrome P-450 enzymes in the liver (section 11.4.2). These enzymes are involved in the metabolism of several clinically important drugs, and inhibition by cimetidine may result in toxic side effects as a result of increased blood levels of these drugs. In particular, caution is required when cimetidine is taken with drugs such as **diazepam, lidocaine, warfarin,** or **theophylline.**

25.2.8 Further studies of cimetidine analogues

25.2.8.1 Conformational isomers

A study of the various stable conformations of the guanidine group in cimetidine led to a rethink of the type of bonding that might be taking place at the antagonist binding region. Up until this point, the favoured theory had been a bidentate hydrogen interaction as shown in the top diagram of Fig. 25.15, where the two hydrogens involved in hydrogen bonding are pointing in the same direction. In order to achieve this kind of bonding, the guanidine group in cimetidine would have to adopt the *Z,Z* conformation shown in Fig. 25.36.

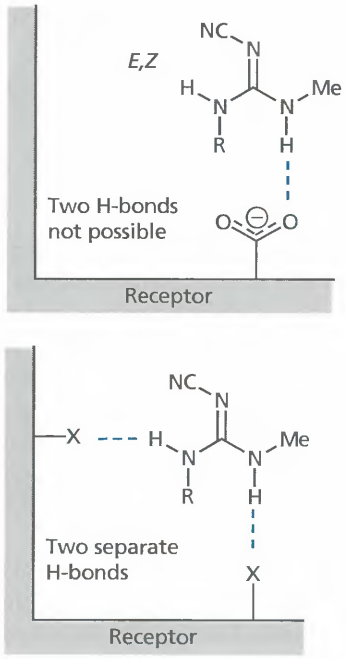


FIGURE 25.37 Alternative theory for cimetidine bonding at the agonist region.

(The Z and E nomenclature is relevant here, as there is double bond character in all the N–C bonds of the guanidine unit.)

However, X-ray and NMR studies have shown that cimetidine exists as an equilibrium mixture of the *E,Z* and *Z,E* conformations. Neither the *Z,Z* nor the *E,E* form is favoured because of steric interactions. If either the *E,Z* or *Z,E* form is the active conformation, then it implies that the chelation type of hydrogen bonding described previously is not taking place. An alternative possibility is that the guanidine unit is hydrogen bonding to two distinct hydrogen bonding regions rather than to a single carboxylate group (Fig. 25.37). Further support for this theory is provided by the weak activity observed for the urea analogue (Fig. 25.27). This compound is known to prefer the *Z,Z* conformation over the *Z,E* or *E,Z* conformations, and would therefore be unable to bind to both hydrogen bonding regions.

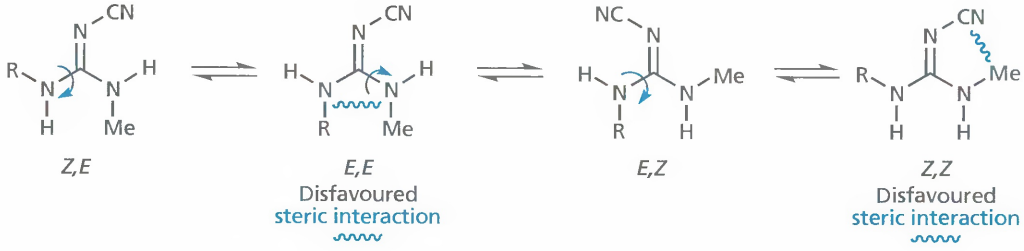


FIGURE 25.36 Conformations of the guanidine group in cimetidine.

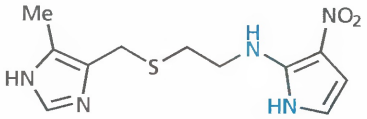


FIGURE 25.38 Nitropyrrole derivative of cimetidine.

If this bonding theory is correct and the active conformation is the *E, Z* or *Z, E* form, restricting the group to adopt one or other of these forms may lead to more active compounds and the identification of the active conformation. This can be achieved by incorporating part of the guanidine unit within a ring—a strategy of rigidification (section 13.3.9). For example, the nitropyrrole derivative (Fig. 25.38) has been shown to be the strongest antagonist in the cimetidine series, implying that the *E, Z* conformation is the active conformation.

The isocytosine ring (Fig. 25.39) has also been used to 'lock' the guanidine group, limiting the number of conformations available. The ring allows for further substitution and development, as described below.

25.2.8.2 Desolvation

The guanidine and thiourea groups, used so successfully in the development of H_2 antagonists, are polar and hydrophilic. This implies that they are likely to be highly solvated and surrounded by a 'water coat'. Before hydrogen bonding can take place to the receptor, this water coat has to be removed, and the more solvated the group is, the more difficult that will be.

One possible reason for the low activity of the urea derivative (Fig. 25.27) has already been described above. Another possible reason could be the fact that the urea group is more hydrophilic than the thiourea or cyanoguanidine groups and is therefore more highly solvated. The energy penalty involved in desolvating the urea group might explain why this analogue has a lower activity than cimetidine, despite having a lower partition coefficient and greater water solubility.

Leading on from this, if the ease of desolvation is a factor in antagonist activity, then reducing the solvation of the polar group should increase activity. One way of achieving this would be to increase the hydrophobic character of the polar binding group.

A study was carried out on a range of cimetidine analogues containing different planar aminal systems (Z) (Fig. 25.40) to see whether there was any relationship between antagonist activity and the hydrophobic character of the aminal system (HZ).

This study showed that antagonist activity was proportional to the hydrophobicity (log *P*) of the aminal unit HZ (Fig. 25.41) and supported the desolvation theory. The relationship could be quantified as follows:

$$\log(\text{activity}) = 2.0\log P + 7.4$$

Further studies on hydrophobicity were carried out by adding hydrophobic substituents to the isocytosine analogue (Fig. 25.39). These studies showed that there was an optimum hydrophobicity for activity corresponding to the equivalent of a butyl or pentyl substituent. A benzyl substituent was particularly good for activity, but proved to have toxic side effects. These could be reduced by adding alkoxy substituents to the aromatic ring and this led to the synthesis of **oxmetidine** (Fig. 25.42) which had enhanced activity over cimetidine. Oxmetidine was considered for clinical use, but was eventually withdrawn as it still retained undesirable side effects.

25.2.8.3 Development of the nitroketeneaminal binding group

As we have seen, antagonist activity increases if the hydrophobicity of the polar binding group is increased. It was therefore decided to see what would happen if the polar imino nitrogen of cimetidine was replaced by a non-polar

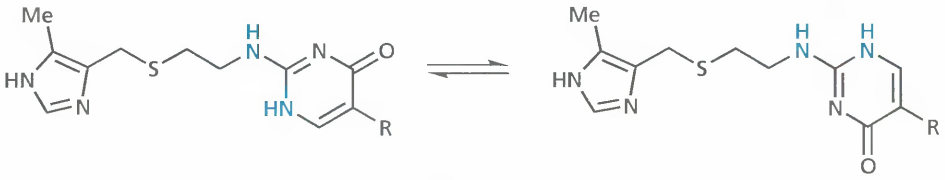


FIGURE 25.39 Isocytosine ring.

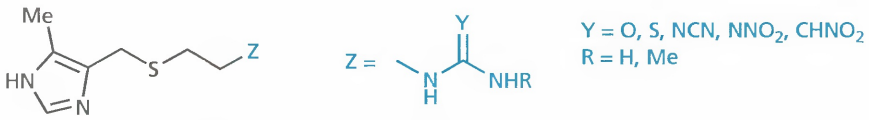


FIGURE 25.40 Cimetidine analogue with planar aminal system (Z).

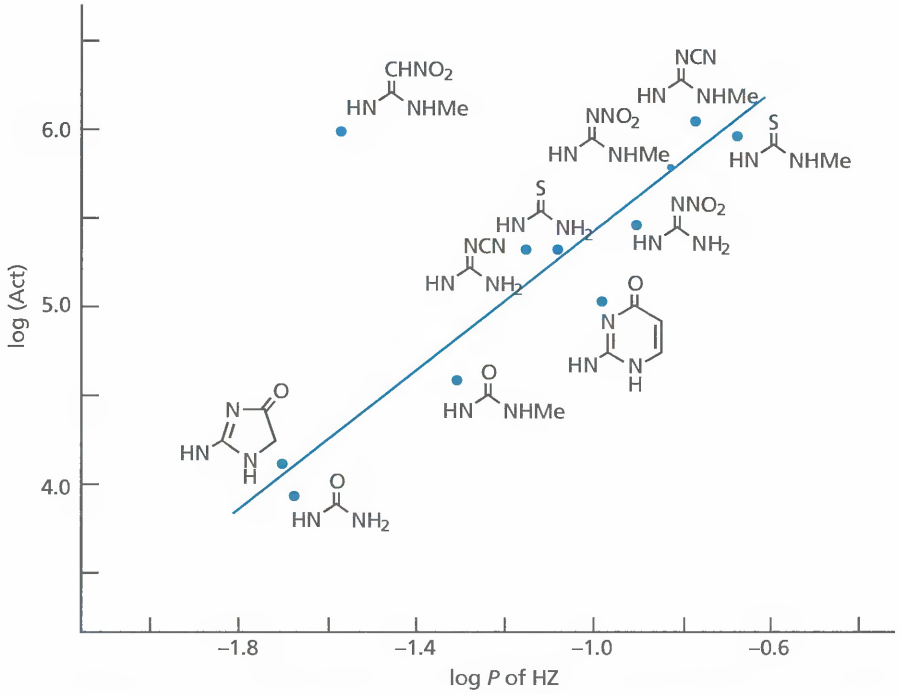


FIGURE 25.41 Antagonist activity is proportional to the hydrophobicity (log *P*) of the aminal unit Z.

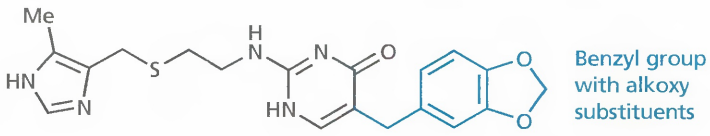


FIGURE 25.42 Oxmetidine.

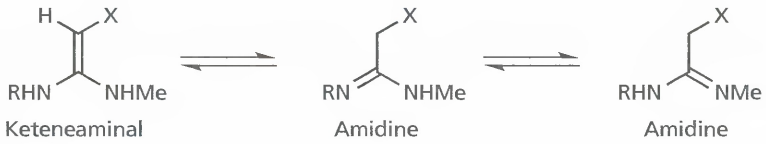


FIGURE 25.43 The keteneaminal group and amidine tautomers.

carbon atom. This would result in a keteneaminal group as shown in Fig. 25.43. Unfortunately, keteneaminals are more likely to exist as their amidine tautomers unless a strongly electronegative group (e.g. NO_2) is attached to the carbon atom.

A nitroketeneaminal group was therefore used to give the structure shown in Fig. 25.44. Surprisingly, there was no great improvement in activity, but when the structure was studied in detail, it was discovered that it was far more hydrophilic then expected. This explained why the activity had not increased, but it highlighted a different puzzle. The compound was *too* active. Based on its hydrophilicity, it should have been a much weaker antagonist (Fig. 25.41).

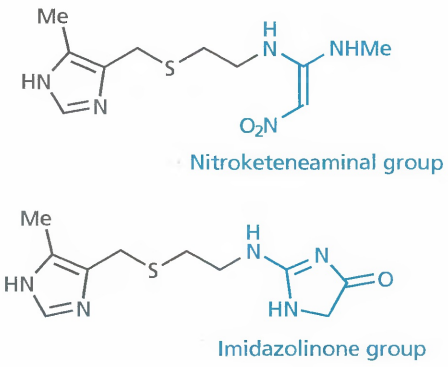


FIGURE 25.44 Cimetidine analogues with unexpected levels of activity.

It was clear that this compound did not fit the pattern followed by previous compounds as its antagonist activity was 30 times higher than predicted. Nor was the nitroketeneaminal the only analogue to deviate from the expected pattern. The imidazolinone analogue (Fig. 25.44), which is relatively hydrophobic, had a much lower activity than would have been predicted from the equation. Findings like these are particularly exciting, as any deviation from the normal pattern suggests that some other factor is at work which may give a clue to future development.

In this case, it was concluded that the polarity of the group might be important in some way. In particular, the orientation of the dipole moment appeared to be crucial. In Fig. 25.45, the orientation of the dipole moment is defined by ϕ—the angle between the dipole moment and the NR bond. The cyanoguanidine, nitroketeneaminal, and nitropyrrole groups all have high antagonist activity and have dipole moment orientations of 13°, 33°, and 27° respectively (Fig. 25.46). The isocytosine and imidazolinone groups have lower activity and have dipole orientations of 2° and –6°, respectively. The strength of the dipole moment (μ) does not appear to be crucial.

Why should the orientation of a dipole moment be important? One possible explanation is as follows. As the drug approaches the receptor, its dipole interacts with a dipole on the receptor surface such that the dipole moments are aligned. This orientates the drug in a specific way before hydrogen bonding takes place and determines how strong the subsequent hydrogen bonding will be (Fig. 25.47). If the dipole moment is correctly orientated as in the keteneaminal analogue, the group is correctly positioned for strong hydrogen bonding and high activity will result. If the orientation is wrong as in the imidazolinone analogue, then the bonding is less efficient and activity is weaker.

QSAR studies (chapter 18) were carried out to determine what the optimum angle ϕ should be for activity. This resulted in an ideal angle for ϕ of 30°. A correlation was worked out between the dipole moment orientation, partition coefficient, and activity as follows:

$$\log A = 9.12 \cos\theta + 0.6 \log P - 2.71$$
$$(n = 13, r = 0.91, s = 0.41)$$

where A is the antagonist activity, P is the partition coefficient, and θ is the deviation in angle of the dipole moment from the ideal orientation of 30° (Fig. 25.48).

The equation shows that activity increases with increasing hydrophobicity (P). The cos θ term shows that activity drops if the orientation of the dipole moment varies from the ideal angle of 30°. At the ideal angle, θ is 0° and cos θ is 1. If the orientation of the dipole moment deviates from 30°, then cos θ will be less than 1 and will lower the calculated activity. The nitroketeneaminal group did not result in a more powerful cimetidine analogue, but we shall see it again in ranitidine (section 25.2.9.1).

25.2.9 Further H_2 antagonists

25.2.9.1 Ranitidine

Further studies on cimetidine analogues showed that the imidazole ring could be replaced by other nitrogen-containing heterocyclic rings. Glaxo moved one step further, however, and replaced the imidazole ring with a furan ring bearing a nitrogen-containing substituent. This led to the introduction of ranitidine (Zantac) (Fig. 25.49). Ranitidine has fewer side effects than cimetidine, lasts longer, and is 10 times more active. SAR results for ranitidine include the following:

- The nitroketeneaminal group is optimum for activity, but can be replaced by other planar π systems capable of hydrogen bonding.
- Replacing the sulfur atom with a methylene atom leads to a drop in activity.
- Placing the sulfur next to the ring lowers activity.

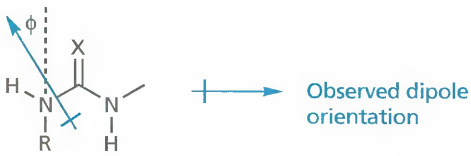


FIGURE 25.45 Orientation of dipole moment.

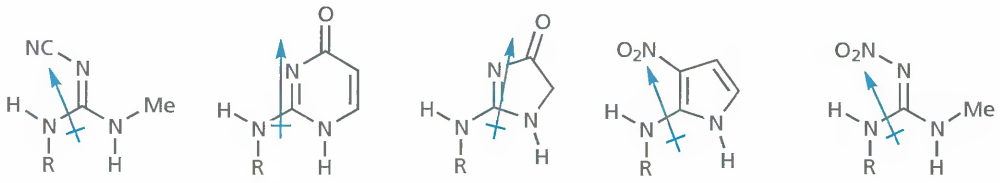


FIGURE 25.46 Dipole moments of various antagonistic groups.

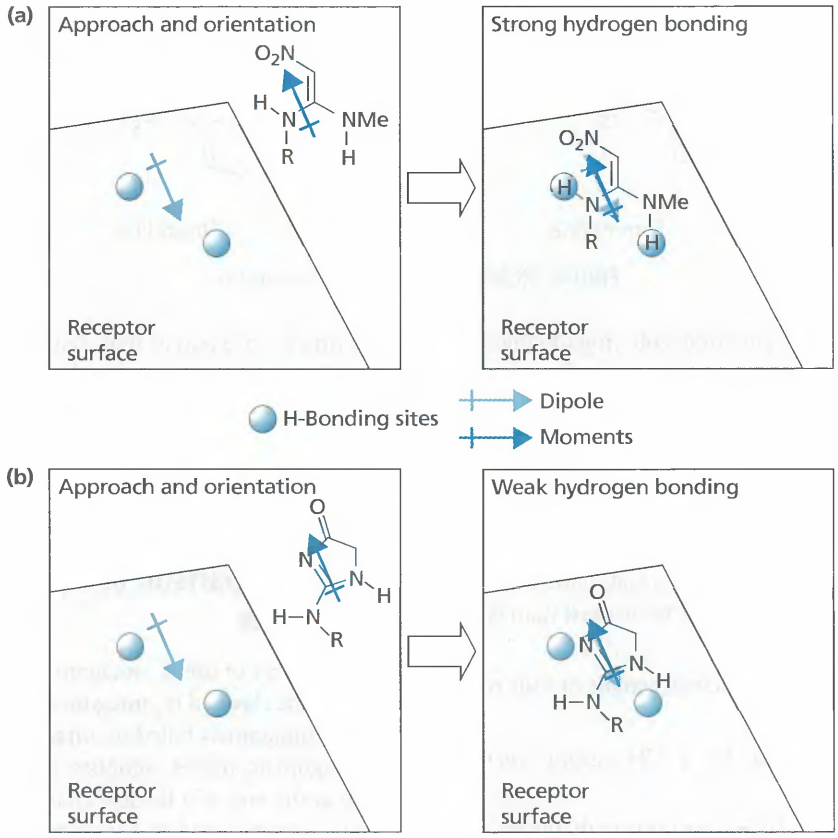


FIGURE 25.47 Dipole–dipole interactions and their effects on orientation and receptor binding. (**a**) Strong binding of the nitroketeneaminal group. (**b**) Weak binding of the imidazolinone group.

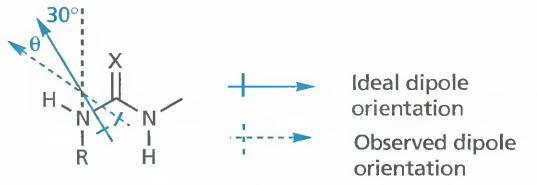


FIGURE 25.48 Definition of the angle θ.

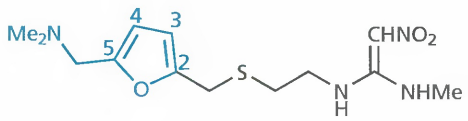


FIGURE 25.49 Ranitidine.

- Replacing the furan ring with more hydrophobic rings such as phenyl or thiophene reduces activity.
- 2,5-Disubstitution is the best substitution pattern for the furan ring.
- Substitution on the dimethylamino group can be varied, showing that the basicity and hydrophobicity of this group are not crucial to activity.
- Methyl substitution at carbon-3 of the furan ring eliminates activity, whereas the equivalent substitution in the imidazole series increases activity.
- Methyl substitution at carbon-4 of the furan ring increases activity.

The last two results imply that the heterocyclic rings for cimetidine and ranitidine are not interacting in the same way with the H_2 receptor. This is supported by the fact that a corresponding dimethylaminomethylene group attached to cimetidine leads to a drop in activity. Ranitidine was introduced to the market in 1981, and by 1988 it had taken over from cimetidine as the world's biggest selling prescription drug. Over a 10 year period, it earned Glaxo profits of around £4 billion ($7 billion), and at one time was earning profits of £4 million ($7 million) per day.

25.2.9.2 Famotidine and nizatidine

During 1985–87, two new antiulcer drugs were introduced to the market—famotidine and nizatidine (Fig. 25.50).

Famotidine (Pepcid) is 30 times more active than cimetidine *in vitro*. The side chain contains a sulfonylamidine

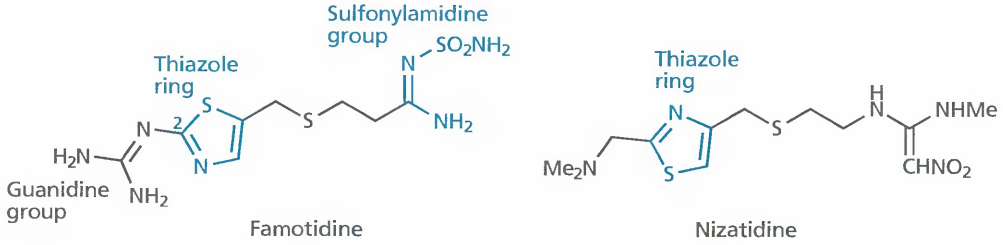


FIGURE 25.50 Famotidine and nizatidine.

group, and the heterocyclic imidazole ring of cimetidine has been replaced by a 2-guanidinothiazole ring. SAR studies gave the following results:

- The sulfonylamidine binding group is not essential and can be replaced by a variety of structures as long as they are planar, have a dipole moment, and are capable of interacting with the receptor by hydrogen bonding. A low pK_a is not essential, which allows a larger variety of planar groups to be used than is possible for cimetidine.
- Activity is optimum for a chain length of four or five units.
- Replacement of sulfur by a CH_2 group *increases* activity.
- Modification of the chain is possible with, for example, inclusion of an aromatic ring.
- A methyl substituent on the heterocyclic ring, *ortho* to the chain leads to a drop in activity (unlike the cimetidine series).
- Three of the four hydrogens in the two guanidine NH_2 groups are required for activity.

There are several results here which are markedly different from cimetidine, implying that famotidine and cimetidine are not interacting in the same way with the H_2 receptor. Further evidence for this is the fact that replacing the guanidine of cimetidine analogues with a sulfonylamidine group leads to very low activity.

Nizatidine (Fig. 25.50) was introduced into the UK in 1987 by the Lilly Corporation and is equipotent with ranitidine. The furan ring in ranitidine is replaced by a thiazole ring.

25.2.9.3 H_2 Antagonists with prolonged activity

Glaxo carried out further development on ranitidine by placing the oxygen of the furan ring exocyclic to a phenyl ring and replacing the dimethylamino group with a piperidine ring to give a series of novel structures (I in Fig. 25.51). The most promising of these compounds were **lamitidine** and **loxtidine** (Fig. 25.51), which were 5–10 times more potent than ranitidine and three times longer lasting. Unfortunately, these compounds showed toxicity in long-term animal studies, with the possibility that they caused gastric cancer, so they were subsequently withdrawn from clinical study. The relevance of these studies has been disputed.

25.2.10 Comparison of H_1 and H_2 antagonists

The structures of the H_2 antagonists are markedly different from the classical H_1 antagonists, so it is not surprising that H_1 antagonists failed to antagonize the H_2 receptor. H_1 antagonists, like H_1 agonists, possess an ionic amino group at the end of a flexible chain. Unlike the agonists, they possess two aryl or heteroaryl rings in place of the imidazole ring (Fig. 25.52). Because of the aryl rings, H_1 antagonists are hydrophobic molecules having high partition coefficients.

In contrast, H_2 antagonists are polar, hydrophilic molecules having high dipole moments and low partition coefficients. At the end of the flexible chain they have a polar, π electron system which is amphoteric and un-ionized at pH 7.4. This binding group appears to be the key feature leading to antagonism of H_2 receptors (Fig. 25.52). The heterocycle generally contains a nitrogen atom or, in the case of furan or phenyl, a nitrogen-containing side chain. The hydrophilic character of H_2 antagonists helps

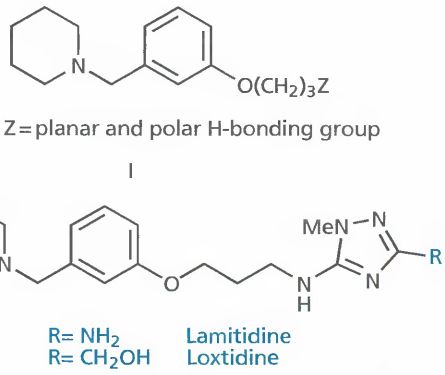


FIGURE 25.51 Long-lasting antiulcer agents.

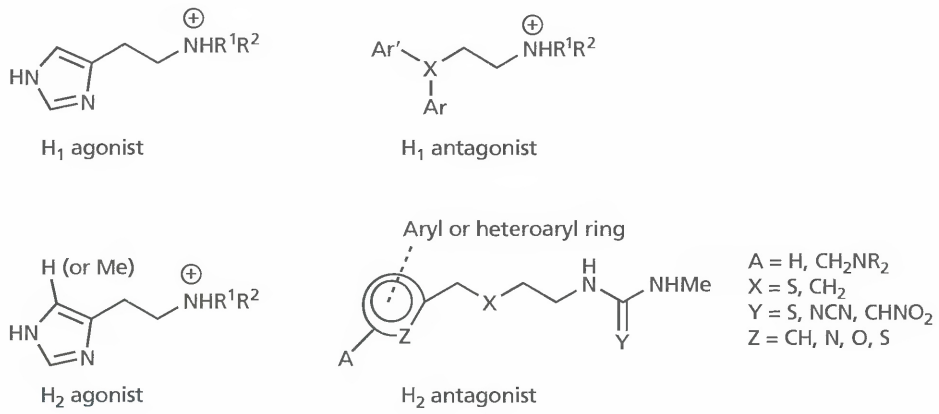


FIGURE 25.52 Comparison of H_1 agonists, H_1 antagonists, H_2 agonists and H_2 antagonists.

to explain why H_2 antagonists are less likely to have the central nervous system side effects often associated with H_1 antagonists.

25.2.11 H_2 Receptors and H_2 antagonists

H_2 receptors are present in a variety of organs and tissues, but their main role is in acid secretion. As a result, H_2 antagonists are remarkably safe and mostly free of side effects. The four most used agents on the market are cimetidine, ranitidine, famotidine, and nizatidine. They inhibit all aspects of gastric secretion and are rapidly absorbed from the gastrointestinal tract with half-lives of 1–2 hours. About 80% of ulcers are healed after 4–6 weeks. Attention must be given to possible drug interactions when using cimetidine, because of inhibition of drug metabolism (section 25.2.7.3). The other three H_2 antagonists mentioned do not inhibit the P450 cytochrome oxidase system and are less prone to such interactions.

KEY POINTS

- Peptic ulcers are localized erosions of the mucous membranes which occur in the stomach and duodenum. The hydrochloric acid present in gastric juices results in increased irritation and so drugs which lower the amount of hydrochloric acid released act as antiulcer agents. Such agents relieve the symptoms rather than the cause.
- The chemical messengers histamine, acetylcholine and gastrin stimulate the release of hydrochloric acid from stomach parietal cells by acting on their respective receptors.
- H_2 antagonists are antiulcer drugs that act on H_2 receptors present on parietal cells and reduce the amount of acid released.
- The design of H_2 antagonists was based on the natural agonist histamine as a lead compound. Chain extension accessed an antagonist-binding region, and the replacement of an ionized terminal group with a polar, un-ionized group capable of hydrogen bonding led to pure antagonists.
- The design of improved H_2 antagonists was aided by dynamic structure–activity analysis, where changes were made to favour one tautomer over another.
- The orientation of dipole moments between a drug and its binding site plays a role in the binding and activity of H_2 antagonists. Desolvation of polar groups also has an important effect on binding affinity.

25.3 Proton pump inhibitors

Although the H_2 antagonists have been remarkably successful in the treatment of ulcers, they have been largely superseded by the PPIs. These work by irreversibly inhibiting an enzyme complex called the proton pump, and have been found to be superior to the H_2 antagonists. They are used on their own to treat ulcers that are caused by NSAIDs, and in combination with antibacterial agents to treat ulcers caused by the bacterium *H. pylori* (section 25.4).

25.3.1 Parietal cells and the proton pump

When the parietal cells are actively secreting hydrochloric acid into the stomach, they form invaginations called **canaliculi** (Fig. 25.53). Each canaliculus can be viewed as a sheltered channel or inlet that flows into the overall 'ocean' of the stomach lumen. Being a channel, it is not part of the cell but it penetrates 'inland' and increases the amount of 'coastline' (surface area) available to the cell, across which it can release its hydrochloric acid. The protons required for the hydrochloric acid are generated from water and carbon dioxide, catalysed by an enzyme called **carbonic anhydrase** (Fig. 25.54). Once the protons have

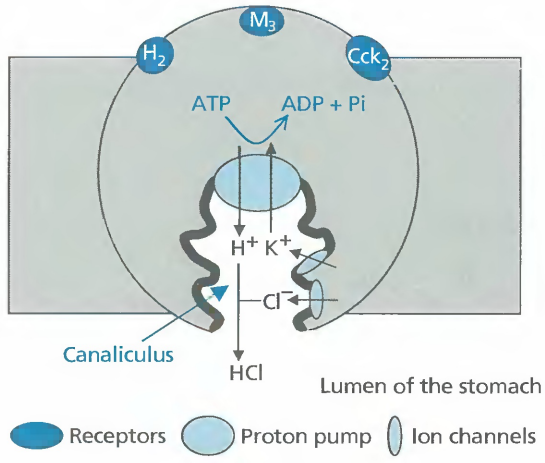


FIGURE 25.53 Role of the proton pump in secreting HCl.

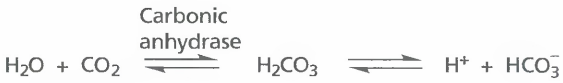


FIGURE 25.54 Enzyme-catalysed generation of protons in the parietal cell.

been generated, they have to be exported out of the cell rather than stored. There are two reasons for this. First, a build-up of acid within the cell would prove harmful to the cell. Second, the enzyme-catalysed reaction which generates the protons is reversible, and so a build-up of protons within the cell would encourage the reverse reaction and slow the production down. The export of protons from the parietal cell is achieved by an enzyme complex called the proton pump or H^+/K^+-ATPase.

The proton pump is only present in the canalicular membranes of parietal cells and is crucial to the mechanism by which hydrochloric acid is released into the stomach. It is called an H^+/K^+-ATPase because it pumps protons out of the cell into the canaliculus at the same time as it pumps potassium ions back in. Energy is required for this process, as both the protons and the potassium ions are being moved against their concentration gradients. In fact, the ratio of protons inside the cell to protons in the canaliculus is 1 to 10^6! The energy required to carry out this exchange is obtained by the hydrolysis of ATP (Fig. 25.55)—hence the term ATPase.

The pump is not responsible for the efflux of chloride ions; these depart the cell through separate chloride ion channels. This outflow closely matches the efflux of protons, such that a chloride ion is released for every proton that is pumped out. As a result, hydrochloric acid is formed in the canaliculus rather than inside the parietal cell.

As each chloride ion departs the cell, it is accompanied by a potassium ion which flows through its own ion channel. No energy is required for this outflow, since the potassium ion is flowing down a concentration gradient. The potassium ion acts as a counterion for the chloride, and once it is in the canaliculus, it is pumped back into the cell by the proton pump as described previously. Consequently, potassium ions undergo a cyclic movement in and out of the cell.

25.3.2 Proton pump inhibitors

There are four PPIs in clinical use: **omeprazole, lansoprazole, pantoprazole,** and **rabeprazole** (Fig. 25.56). The *S*-enantiomer of omeprazole has also been recently approved. All the PPIs have a pyridyl methylsulfinyl benzamidazole skeleton and act as prodrugs, as they are activated when they reach the acidic canaliculi of parietal cells. Once activated, they bind irreversibly to exposed cysteine residues of the proton pump and 'block' the pump, preventing further release of hydrochloric acid.

There is a big strategic advantage in inhibiting the proton pump rather than blocking histamine or cholinergic receptors. For example, H_2 antagonists block the histamine receptors and in doing so block the stimulatory effect of histamine, but this does not block the receptors for acetylcholine or gastrin, and so it is still possible for the parietal cell to be activated towards secretion. The proton pump is 'downstream' of all these targets and operates the final stage of hydrochloric acid release. Blocking it prevents the release of hydrochloric acid regardless of what mechanisms are involved in stimulating hydrochloric acid secretion.

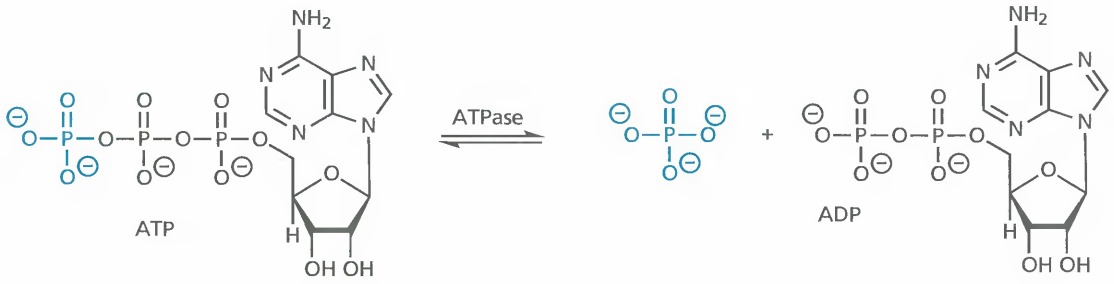


FIGURE 25.55 Enzyme-catalysed hydrolysis of ATP.

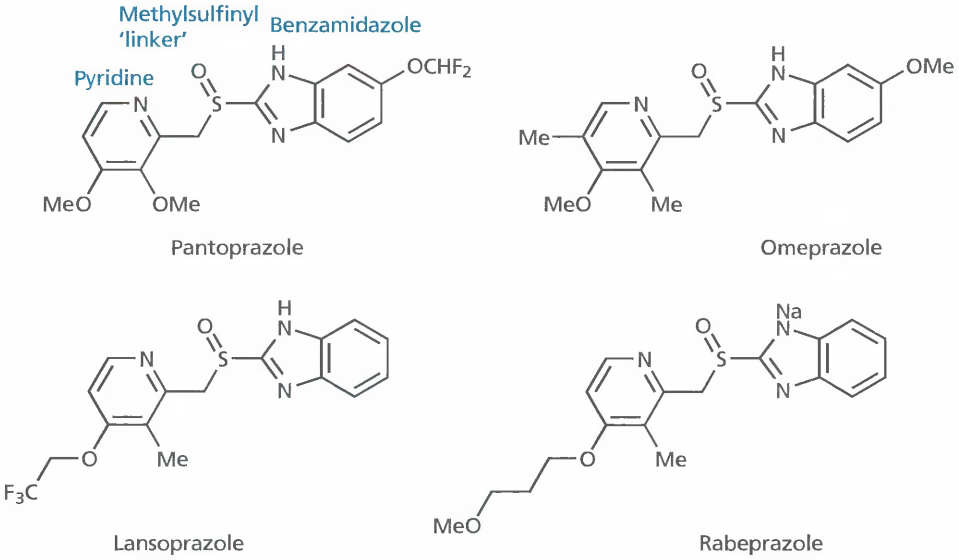


FIGURE 25.56 Proton pump inhibitors (PPIs).

25.3.3 Mechanism of inhibition

The PPIs are weak bases having a pK_a of about 4.0. As a result, they are free bases at blood pH (7.4) and are only ionized in strongly acidic environments where the pH is less than 4. These are conditions found only in the secretory canaliculus of the parietal cell, where the pH is 2 or less. The drugs are taken orally and are absorbed into the blood supply where they are carried round the body as fairly innocuous passengers until they reach the parietal cells. Since they are un-ionized weak bases at this stage, and are also lipophilic in nature, they are able to cross the cell membrane of the parietal cell into the strongly acidic conditions of the canaliculi. Here, the drugs undergo a personality change and become particularly vicious! The canaliculus is highly acidic, so the drug becomes protonated. The consequences of this are twofold:

- The ionized drug is too polar to cross back into the cell through the cell membrane. This leads to a 1000-fold accumulation of the drug in the canaliculi where it is intended to act.
- Protonation triggers an acid-catalysed conversion, as shown in Fig. 25.57, which activates the drug.

Protonation takes place on the benzimidazole ring of the drug. The nitrogen of the pyridine ring then acts as a nucleophile and uses its lone pair of electrons to form a bond to the electron-deficient 2-carbon of the benzimidazole ring to form a spiro structure. By doing so, the aromatic character of the imidazole portion of the ring is lost and so there is a high tendency for this ring to re-aromatize. This can be achieved by a lone pair of electrons from nitrogen reforming the double bond and cleaving the S–C bond to form a sulfenic acid. Sulfenic acids are highly reactive to nucleophiles and so a rapid reaction takes place involving an intramolecular attack by the NH group of the benzimidazole on the sulfenic acid to displace the hydroxyl group. A cationic, tetracyclic pyridinium sulfenamide is formed which acts as an irreversible enzyme inhibitor (Fig. 25.57). It does so by forming a covalent bond to an accessible cysteine residue on the proton pump. There are three such accessible cysteine residues (Cys-813, Cys-892, and Cys-821) and it has been found that the specific cysteine residues attacked depend on which PPI is involved. For example, omeprazole binds to two of the accessible cysteine residues (Cys-813 and Cys-892), lansoprazole binds to all three, and pantoprazole only binds to one (either Cys-813 or Cys-822). Cys-813 is the only cysteine residue which appears to be bound by all the PPIs.

As acid conditions are required to activate the PPIs, they are most active when parietal cells are actively secreting hydrochloric acid, and show little activity when the parietal cells are in a resting state. Since a covalent disulfide bond has been formed between the inhibitor and the proton pump, inhibition is irreversible and so PPIs have a long duration of action. The duration depends on how quickly new pumps are generated by the cell.

PPIs also have very few side effects because of their selectivity of action. This can be put down to several factors.

- The target enzyme (H^+/K^+-ATPase) is only present in parietal cells.
- The canaliculi of the parietal cells are the only compartments in the body which have such a low pH (1–2).

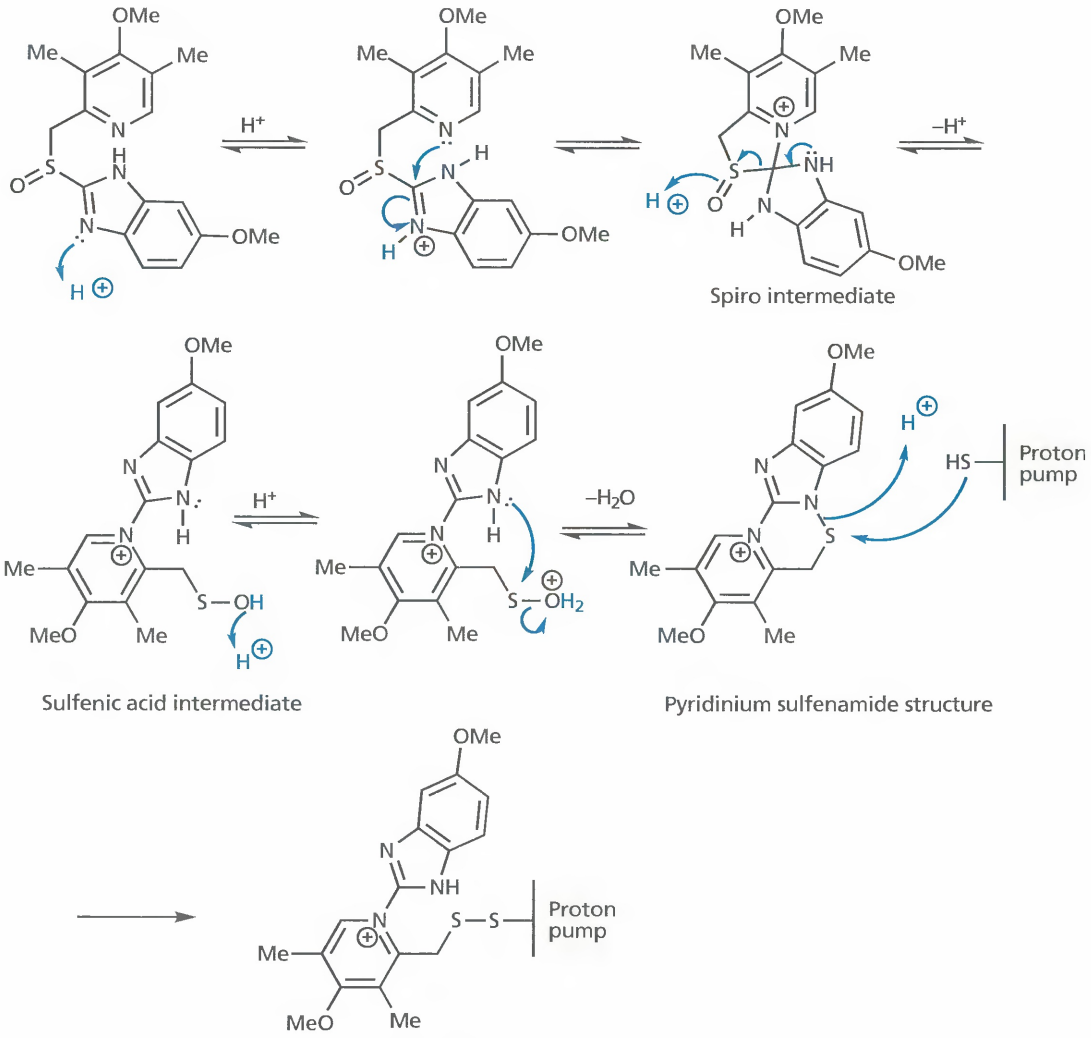


FIGURE 25.57 Mechanism of inhibition by proton pump inhibitors.

- The drug is concentrated at the target site due to protonation and is unable to return to the parietal cell or to the general circulation.
- The drug is rapidly activated close to the target.
- Once activated, the drug reacts rapidly with the target.
- The drug is inactive at neutral pH.

25.3.4 Metabolism of proton pump inhibitors

PPIs are metabolized by cytochrome P450 enzymes, particularly ***S*-mephenytoin hydroxylase** (CYP2C19) and **nifedipine hydroxylase** (CYP3A4). As a result of genetic variations, about 3% of white people of European descent are slow metabolizers of PPIs. Pantoprazole, in contrast to omeprazole and lansoprazole, is also metabolized by the conjugating enzyme **sulfotransferase.**

25.3.5 Design of omeprazole and esomeprazole

Omeprazole was the first PPI to reach the market in 1988 and was marketed as **Losec.** In 1996, it became the biggest selling pharmaceutical ever. The story of how omeprazole was developed can be traced back to the 1970s. The lead compound for the project was a thiourea structure (CMN 131 in Fig. 25.58). This had originally been investigated as an antiviral drug, but general pharmacological tests showed that it could inhibit acid secretion. Unfortunately, toxicology tests showed that the compound was toxic to the liver and this was put down to the presence

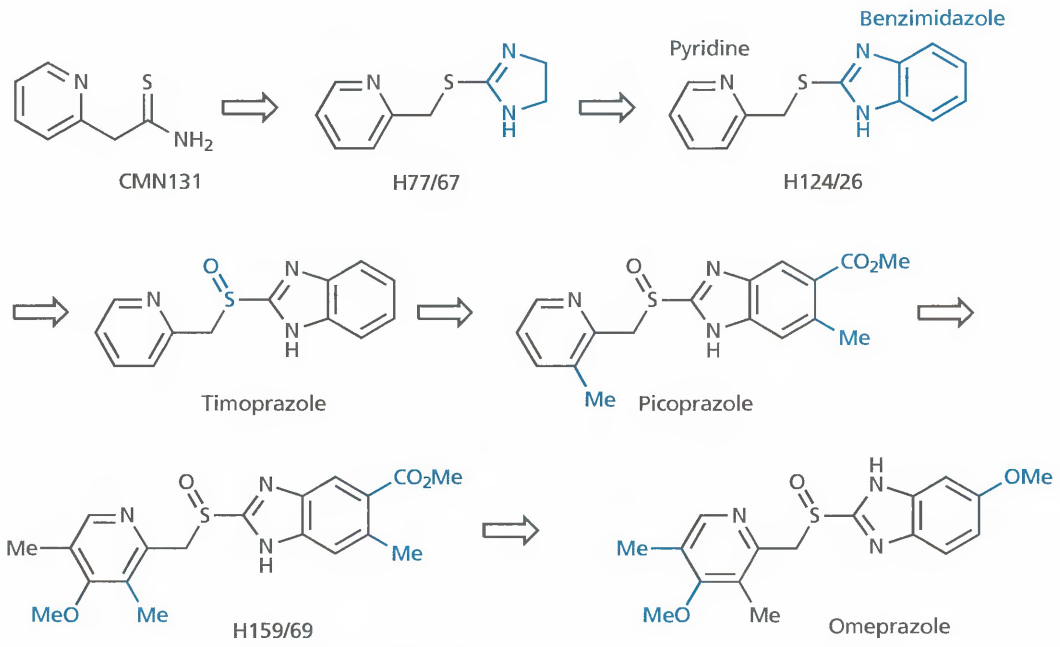


FIGURE 25.58 Development of omeprazole.

of the thioamide group. Various analogues were made to try to modify or disguise this group, which included incorporating a thiourea skeleton within a ring. This led eventually to the discovery of H 77/67, which was also found to inhibit acid secretion. A variety of analogues having the general structure (heterocycle–X–Y–heterocycle) were synthesized, which demonstrated that the pyridine ring and the bridging CH_2–S group already present in H 77/67 were optimal for activity. However, activity was increased by replacing the imidazole ring of H 77/67 with a benzimidazole group to give H 124/26. At this stage, drug metabolism studies revealed that a sulfoxide metabolite of H 124/26 was formed *in vivo* and this structure was more active than the original structure. The metabolite was called **timoprazole** and was the first example of a pyridinylmethylsulfinyl benzimidazole structure. It went forward for preclinical trials, but toxicological studies revealed that it inhibited iodine uptake by the thyroid gland and so it could not go on to clinical trials.

Analogues were now synthesized to find a structure which retained the antisecretory properties but did not inhibit iodine uptake. Eventually, it was found that the two effects could be separated by placing suitable substituents on the two heterocyclic rings. This led to **picoprazole** which showed potent antisecretory properties over a long period without the toxic side effect on the thyroid. Animal toxicology studies showed no other toxic effects and the drug went forward for clinical trials, where it was found to be the most effective antisecretory compound so far tested in humans. At this point (1977), the proton pump was discovered and this was identified as the target for picoprazole. Further development was carried out with the aim of getting a more potent drug by varying the substituents on the pyridine ring.

It was discovered that substituents which increased the basicity of the pyridine ring were good for activity. This fits in with the mechanism of activation (Fig. 25.57) where the nitrogen of the pyridine ring acts as a nucleophile. In order to increase the nucleophilicity of the pyridine ring, a methoxy group was placed at the *para* position relative to the nitrogen, and two methyl groups were placed at the *meta* positions. The latter have an inductive effect which is electron-donating and increases the electron density of the ring. The methoxy substituent was added at the *para* position so that electron density could be increased on the pyridine nitrogen by the resonance mechanism shown in Fig. 25.59.

It is noticeable that all the PPIs shown in Fig. 25.56 have an alkoxy substituent at the *para* position of the pyridine. The position of the substituent is important. If the substituent were at the *meta* position, none of the possible resonance structures would place the negative charge on the nitrogen atom (Fig. 25.60). Finally, if the methoxy substituent was at the *ortho* position it would be likely to have a bad steric effect and hinder the mechanism.

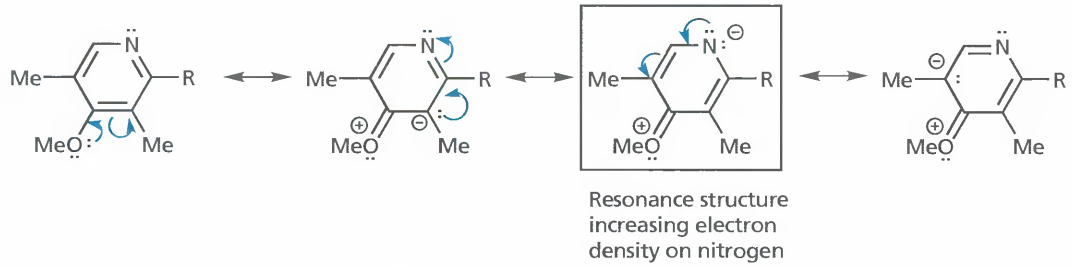


FIGURE 25.59 Influence of the methoxy substituent.

FIGURE 25.60 Possible resonance structures for methoxy substitution at the *meta* position.

The introduction of two methyl groups and a methoxy group led to H 159/69 (Fig. 25.58), which was extremely potent but chemically too labile. Further analogues were synthesized where substituents round the benzimidazole ring were varied in order to get the right balance of potency, chemical stability, and synthetic accessibility. Finally, omeprazole was identified as the structure having the best balance of these properties.

Omeprazole was launched in 1988 and became the world's highest-selling drug, earning its makers vast profits. For example, world wide sales in 2000 were $6.2 billion (£3.6 billion). The patents on omeprazole ran out in Europe in 1999 and in the USA in 2001, but its makers (Astra) had already started a programme to find an even better compound. In particular, they were looking for a compound with better bioavailability.

Substitution was varied on both the pyridine and benzimidazole rings, but the best compound was eventually found to be the *S*-enantiomer of omeprazole—**esomeprazole (Nexium;** Fig. 25.61). At first sight, it may not be evident that omeprazole has an asymmetric centre. In fact, the sulfur atom is an asymmetric centre as it has a lone pair of electrons and is tetrahedral. Unlike the nitrogen atoms of amines, sulfur atoms do not undergo pyramidal inversion and so it is possible to isolate both enantiomers.

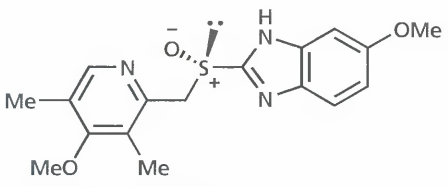


FIGURE 25.61 Esomeprazole.

The *S*-enantiomer of omeprazole was found to be superior to the *R*-enantiomer in terms of its pharmacokinetic profile, and was launched as esomeprazole in Europe in 2000 and in the USA in 2001. The story of esomeprazole is an example of **chiral switching** (section 15.2.1) where a racemic drug is replaced on the market with a single enantiomer. There is no difference between the two enantiomers as far as the mechanism of action is concerned, but because of its better pharmacokinetic profile, it is possible to use double the dose levels of esomeprazole compared to omeprazole, resulting in greater activity. Esomeprazole is metabolized mainly by CYP2C19 in the liver, to form the hydroxy and desmethyl metabolites shown in Fig. 25.62. However, it undergoes less hydroxylation than the *R*-isomer and has a lower clearance rate. A sulfone metabolite is formed by CYP3A4. Due to differences in metabolism, higher plasma levels of the *S*-enantiomer are achieved compared to the *R*-enantiomer. The synthesis of omeprazole and esomeprazole is described in Box 25.2.

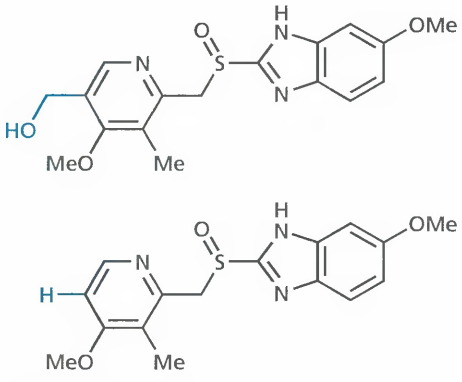


FIGURE 25.62 Metabolites of esomeprazole.

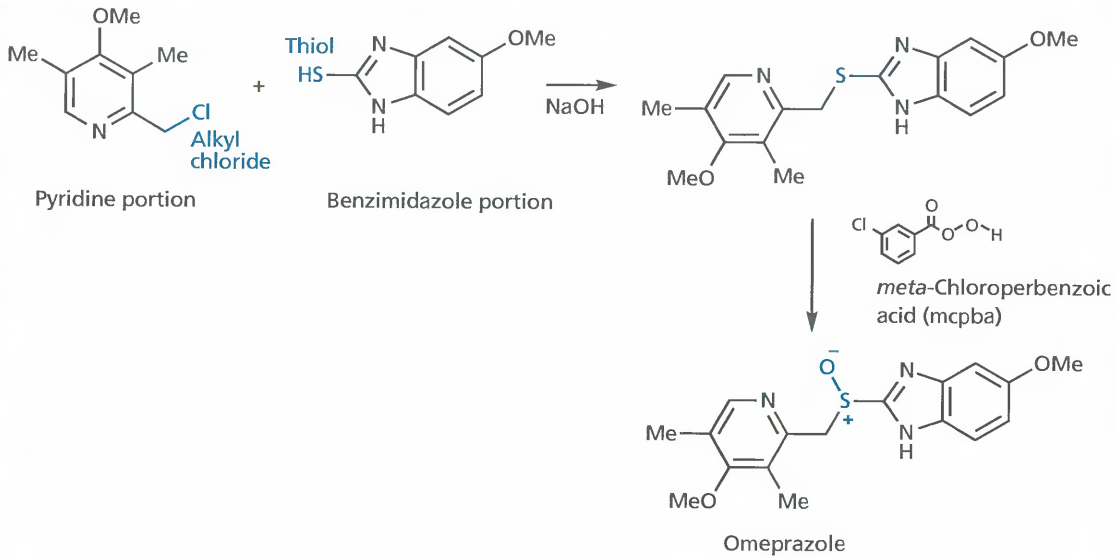
BOX 25.2 Synthesis of omeprazole and esomeprazole

The synthesis of omeprazole appears relatively simple, involving the linkage of the two halves of the molecule through a nucleophilic substitution reaction. The benzimidazole half of the molecule has a thiol substituent which is treated with sodium hydroxide to give a thiolate. On reaction with the chloromethylpyridine, the thiolate group displaces the chloride ion to link up the two halves of the molecule. Subsequent oxidation of the sulfur atom with *meta*-chloroperbenzoic acid gives omeprazole. What is not obvious from the scheme is the effort required to synthesize the required chloromethylpyridine started material. This is not the sort of molecule that is easily bought off the shelf and its synthesis involves six steps.

The same route can be used for the synthesis of esomeprazole (the *S*-enantiomer of omeprazole) by employing asymmetric conditions for the final sulfoxidation step. Early attempts to carry out this reaction involved the Sharpless reagent formed from $Ti(O\text{-}iPr)_4$, the oxidizing agent cumene hydroperoxide ($Ph(CH_3)_2OOH$, and the chiral auxiliary (*S,S*)-diethyl tartrate. Although sulfoxidation took place, it required almost stoichiometric quantities of the titanium reagent, and there was little enantioselectivity. The reaction conditions were modified in three ways to improve enantioselectivity to over 94% enantiomeric excess, and which required less of the titanium reagent (4–30 mol%).

- Formation of the titanium complex was carried out in the presence of the sulfide starting material.
- The solution of the titanium complex was equilibrated at an elevated temperature for a prolonged time period.
- The oxidation was carried out in the presence of an amine such as *N,N*-diisopropylethylamine. The role of the amine is not fully understood, but it may participate in the titanium complex.

The enantiomeric excess can be enhanced further by preparing a metal salt of the crude product and carrying out a crystallization, which boosts the enantiomeric excess to more than 99.5%.



Synthesis of omeprazole.

25.3.6 Other proton pump inhibitors

The other PPIs shown in Fig. 25.56 retain the pyridinylmethylsulfinyl benzimidazole structure of omeprazole. They also share the alkoxy substituent at the *para* position of the pyridine ring. Variation has been limited to the other substituents present on the heterocyclic rings. These play a role in determining the lipophilic character of the drug as well as its stability. As far as the latter is concerned, there has to be a balance between the drug being sufficiently stable and un-ionized at neutral pH to reach its target unchanged, and its ability to undergo rapid acid-induced conversion into the active sulfenamide when it reaches the target. Stability to mild acid is important to avoid activation in other cellular compartments such as lysosomes and chromaffin granules. Drugs which undergo the acid-induced conversion extremely easily are more active, but they are less stable and are

more likely to undergo transformation in the blood supply before they reach their target. Drugs which are too stable are less reactive under acid conditions and react slower with the target.

The various PPIs all work by the same mechanism, but have slightly different properties. For example, pantoprazole is chemically more stable than omeprazole or lansoprazole under neutral to mildly acidic conditions (3.5–7.4), but it is a weaker, irreversible inhibitor under strong acid conditions. Rabeprazole is the least stable at neutral pH and is the most active inhibitor.

KEY POINTS

- The proton pump is responsible for pumping protons out of the parietal cell in exchange for potassium ions which are pumped in. The process involves the movement of protons against a concentration gradient, so it requires energy, which is provided by the hydrolysis of ATP.
- PPIs prevent the proton pump from functioning. They offer a strategic advantage over H_2 antagonists since they act on the final stage of hydrochloric acid release.
- PPIs are prodrugs that are activated by the acidic conditions found in the canaliculi of parietal cells. They undergo an acid-catalysed rearrangement to form a reactive tetracyclic pyridinium sulfenamide, which acts as an irreversible inhibitor. Reaction takes place with accessible cysteine residues on the proton pump to form a covalent disulfide bond between cysteine and the drug.
- PPIs need to be reactive enough to undergo acid-catalysed interconversion in the canaliculi of parietal cells, but stable enough to survive their journey through the bloodstream.

25.4 *Helicobacter pylori* and the use of antibacterial agents

One of the problems relating to antiulcer therapy both with the H_2 antagonists and the PPIs is the high rate of ulcer recurrence once the therapy is finished. The reappearance of ulcers has been attributed to the presence of a microorganism called *Helicobacter pylori*, which is naturally present in the stomachs of many people, and can cause inflammation of the stomach wall. As a result, patients who are found to have *H. pylori* are currently given a combination of three drugs—a PPI to reduce gastric acid secretion, and two antibacterial agents (such as nitroimidazole, clarithromycin, amoxicillin or tetracycline) to eradicate the organism.

It was once considered unthinkable that a bacterium could survive the acid conditions of the stomach. In

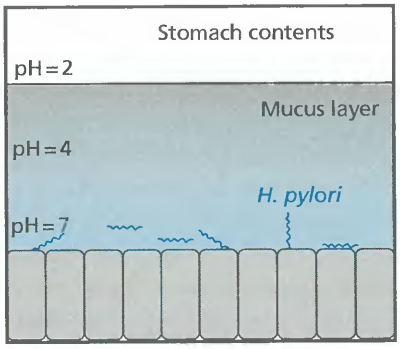


FIGURE 25.63 *Helicobacter pylori* attached to stomach cells.

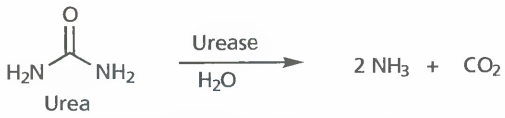


FIGURE 25.64 Action of urease.

1979, however, it was shown that *H. pylori* can do just that. The organism is able to attach to a sugar molecule on the surface of the cells that line the stomach wall, and use the mucus layer which protects the stomach wall from gastric juices as its own protection. Since there is a pH gradient across the mucus layer, the organisms can survive at the surface of the mucus cells where the pH is closer to neutral (Fig. 25.63). *H. pylori* is a spiral, curved bacterium which is highly motile and grows best in oxygen concentrations of 5% matching those of the mucus layer. The bacterium also produces large amounts of the enzyme **urease** which catalyses the hydrolysis of urea to ammonia and carbon dioxide, thus neutralizing any acid in the local environment (Fig. 25.64). The organisms can contribute to the formation of stomach ulcers, as they secrete proteins and toxins that interact with the stomach's epithelial cells, leading to inflammation and cell damage. It is also thought that the organism increases the risk of gastric cancers.

25.4.1 Treatment

As mentioned earlier, *H. pylori* is treated with a triple therapy of a PPI and at least two antibacterial agents. A PPI is administered because the antibiotics used work best at higher pH levels than those normally present in the stomach. The combination of omeprazole, amoxicillin, and metronidazole is frequently used, but combinations involving other antibacterial agents such as clarithromycin and tetracycline are also possible. Bismuth chelate (bismuth subcitrate and tripotassium

dicitratobismuthate) is present in some combination therapies. This preparation has a toxic effect on *H. pylori* and may help to prevent adherence to the mucosa. Other protective properties include an enhancement of local prostaglandin synthesis, a coating of the ulcer base, and an adsorption of pepsin.

Combination therapy has been shown to eradicate *H. pylori* in over 90% of duodenal ulcers and significantly reduce ulcer recurrence. Similar treatment is recommended for *H-pylori* related stomach ulcers.

Eradication of *H. pylori* can be difficult because of the emergence of resistant strains and the difficulty in delivering the antibacterial agents at the required therapeutic concentration. *H. pylori* can also assume a resting coccoid form that is more resistant to therapy.

It has been found that PPIs have an inherent anti-*H. pylori* action and it has been suggested that they inhibit urease, possibly by linking to exposed cysteine residues. However, the PPIs also inhibit strains of *H. pylori* which do not have urease, so this is not the full story. This antibacterial activity is sufficient to suppress the organism but not eradicate it, so traditional antibacterial agents are still required.

Research has been carried out into the design of drugs which act as sugar decoys to prevent *H. pylori* binding with stomach cells in the first place.

25.5 Traditional and herbal medicines

Several herbal remedies have been used for the treatment of ulcers.

Liquorice has been reported to have a variety of medicinal properties and has been used as a medicine for several thousand years. It is reported to have antiulcer properties and this has been attributed to a component called **glycrrhetinic acid**—the aglycone of **glycyrrhizin. Carbenoxolone** is a derivative of glycrrhetinic acid and has been used in ulcer therapy. It is thought to have a mucosal protective role by increasing mucus production, and has some antibacterial action against *H. pylori*.

Silymarin is a mixture of compounds (mainly silibinin, silichristin and siliianin) obtained from the fruit of the milk thistle (*Silybum marianum*) and has antiulcer activity. It has been shown to reduce histamine concentrations in rats.

Extracts from the neem tree (*Azadirachta indica*) have been used extensively in India as a medicine for a variety of ailments. The aqueous extract of the neem bark has been reported to have antiulcer effects. Possible mechanisms include proton pump inhibition or antioxidant effects in the scavenging of OH radicals.

Other herbal medicines include comfrey and marshmallow.

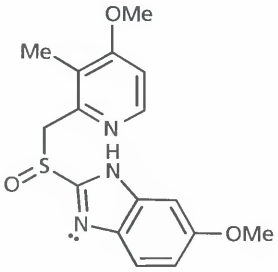
KEY POINTS

- *H. pylori* is a bacterium that is responsible for many ulcers. It can survive at the surface of mucus cells and produce proteins and toxins that damage epithelial cells.
- Ulcers that are caused by *H. pylori* are treated with a combination of drugs, which include a PPI and at least two antibiotics.
- Several traditional and herbal remedies are used in the treatment of ulcers.

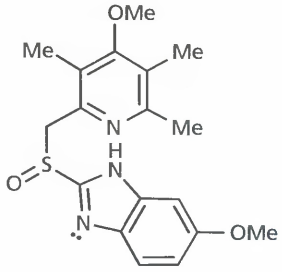
QUESTIONS

1. Omeprazole is administered orally as a galenic formulation to protect it from being activated during its journey through the acidic contents of the stomach. Once it is released in the intestines, it is absorbed into the blood supply and carried to the parietal cells where it crosses the cell membrane into the canaliculi and is activated. Since the canaliculi lead directly into the lumen of the stomach, why is omeprazole not orally administered directly to the stomach?
2. In the development of omeprazole, the methoxy and methyl groups were added to the pyridine ring to increase the pK_a. Subsequently, it was found that analogue (I) with only one of the methyl groups had a higher pK_a than omeprazole. Suggest why this might be the case.
3. Suggest whether you think structure (I) would be a better PPI than omeprazole.
4. The acid-catalysed activation of PPIs requires pyridine to be nucleophilic, which is why two methyl groups and a methoxy group are present in omeprazole. Suggest whether the addition of an extra methyl group (structure II) would lead to a more potent PPI.

5. The phenol (III) is a very difficult compound to synthesize and is unstable at neutral pH. Suggest why this might be the case.



I



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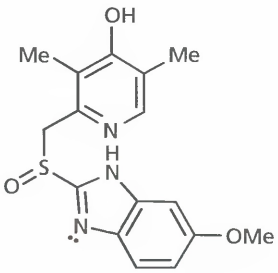

III

6. Suggest what types of metabolite might be possible from omeprazole.

7. One of the metabolic reactions that takes place on cimetidine is oxidation of the methyl substituent on the imidazole ring (Fig. 25.35). A common strategy to prevent such a metabolic reaction occurring is to replace a susceptible methyl group with a chloro substituent. Why is a chloro substituent commonly used for this purpose? Do you think an analogue of cimetidine with a 4-chloro substituent would be an improvement over cimetidine itself?

8. The acidic contents of the stomach encourage the digestion of food and the destruction of cells. Why are the cells lining the stomach not digested in that case?

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Titles for general further reading are listed on p. 725.

CASE STUDY 7
Current research into antidepressant agents

Introduction

> "I am worn out with grief;
> every night my bed is damp from my weeping;
> my pillow is soaked with tears.
> I can hardly see;
> my eyes are so swollen
> from the weeping caused by my enemies."
>
> Psalm 6, verses 6 & 7

Major depression is a common ailment that affects up to 10% percentage of the population. It is estimated that 18 million people suffer from it in the USA, and 340 million worldwide. The World Health Organization believes that by the year 2020, depression could be the second leading ailment in the world after heart disease. Depression is common in the elderly, and it is estimated that 21% of women and 13% of men will suffer major depression at some point in their lives. Symptoms include misery, apathy, pessimism, low self-esteem, feelings of guilt, inability to concentrate or work, loss of libido, poor sleep patterns, loss of motivation and loss of appetite. Sufferers of long-term depression are more prone to other diseases and their lifespan can be shortened.

The causes of depression are many and varied. Some are genetically predisposed to depression, but in many cases, a stressful life-changing event precipitates the condition. Such events include losing employment, divorce, bereavement, rejection, victimization, false accusation, and slander. Often, the sufferer has no control or redress over what has taken place, and the sense of helplessness and hopelessness that results exacerbates the situation.

Those suffering severe depression describe each day as a living nightmare. The same distressing thoughts whirl round in their minds pulling them deeper and deeper into a bottomless pyschological whirlpool from which there seems to be no escape. Each day is an ordeal to be endured, and for some it can be too much. Some turn to alcohol or illicit drugs for a temporary oblivion. A few turn to suicide for a permanent oblivion. Those who have never suffered depression have no concept of what is involved. Telling the sufferer to 'snap out of it' or 'pull yourself together' is worse than useless.

The monoamine hypothesis

The pharmacological processes that cause depression are still an area of a research, but the accepted current theory proposes that a deficit of monoamine neurotransmitters in certain parts of the brain causes the condition. This is known as the **monoamine** or **monoaminergic hypothesis**. The principle neurotransmitters believed to be involved are **dopamine, noradrenaline** and **serotonin** (also known as 5-hydroxytryptamine, 5-HT). There are various lines of evidence that support this. For example, the antihypertensive agent **reserpine** lowers monoamine levels in the brain and is known to have caused depression in patients who took it. Moreover, the clinically important antidepressant agents are known to increase monoamine levels by a variety of mechanisms. However, there are anomalies that indicate that there is more to the story than an increase in monamine levels. For example, **amphetamine** and **cocaine** are agents that increase noradrenaline and serotonin transmission, but are ineffective antidepressant agents. There is also evidence that a wide range of endogenous hormones and neurotransmitters play a role in depression, (i.e. substance P, corticotrophin-releasing factor, arginine vasopressin, neuropeptide Y, melanin-concentrating hormone, acetylcholine, glutamic acid, gamma-aminobutyric acid, glucocorticoids, cytokines, enkephalins, and anandamide). Nevertheless, most clinically useful agents in use today are responsible for raising monoamine levels.

Current antidepressant agents

First-generation antidepressants were introduced about 50 years ago, and include the **monoamine oxidase inhibitors** (MAOIs), which are discussed in section 23.12.5, and the **tricyclic antidepressants** (TCAs) which are described in section 23.12.4. Unfortunately, these drugs have low target selectivity, and have many side effects.

Second-generation antidepressants were introduced in the 1980s and are represented by agents known as **selective serotonin reuptake inhibitors** (SSRIs) (Box 10.1). These represented a major step forward in treatment since they are more selective and have fewer side effects.

However, like the TCAs and MAOIs, they have a slow onset of action, and it can take 2–6 weeks before patients feel any benefit. Another problem with their use is their negative effect on the libido.

Third-generation antidepressant agents include **selective noradrenaline reuptake inhibitors** (SNRIs) (section 23.12.4), and dual action **serotonin and noradrenaline reuptake inhibitors** (SNRIs) (section 23.12.4).

Current areas of research

Currently, there is research into novel agents designed to interact with the following targets:

- transport proteins for dopamine, serotonin and noradrenaline
- adrenergic receptors such as the α_2-adrenoceptor
- serotonin receptors such as the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors.

Dual action agents that act on two of the above targets are of particular interest, for example, agents that:

- block the reuptake of both noradrenaline and serotonin
- block α_2-adrenoceptors (section 23.12.6), and activate 5-HT receptors
- block serotonin reuptake, and are antagonists for the 5-HT_{1A} receptor. (The 5-HT_{1A} receptor is an autoreceptor present on the presynaptic neurons that release serotonin. When activated, this receptor inhibits the release of serotonin from the neuron, and so an antagonist should counteract this effect.)
- block serotonin reuptake, and act as antagonists for the 5-HT_{2A} receptor. This receptor is responsible for sexual dysfunction side effect associated with SSRIs.

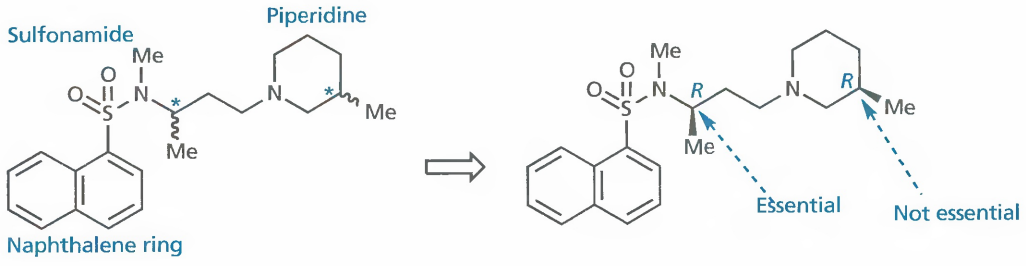
In this case study, we shall look at a research project aimed at discovering antagonists for the 5-HT_7 receptor.

Antagonists for the 5-HT_7 receptor

There are seven main types of serotonin receptors (5-HT_1 – 5-HT_7) and several subtypes of these. The 5-HT_7 receptor is the most recent serotonin receptor to be discovered and appears to play an important role in psychiatric disorders such as depression. It has been shown that antagonists of this receptor have an antidepressant activity in animal studies, although the mechanism by which this takes place is currently unclear. At first sight, it may seem odd that a serotonin antagonist should have an antidepressant activity, since antidepressant activity is normally associated with increased serotonin levels and increased activation of serotonin receptors. However, it should be borne in mind that different receptors for the same neurotransmitter serve different purposes and some act as autoreceptors to provide a negative feedback control for neurotransmitter release. For example, the α_2-adrenergic receptor is a presynaptic autoreceptor which has the effect of inhibiting noradrenaline release (sections 23.6.3 and 23.12.6). It is conceivable that activation of 5-HT_7 receptors might lead to a drop in serotonin levels by a similar manner. Therefore, an antagonist that is selective for this receptor over other serotonin receptors could be advantageous.

Workers at SmithKline Beecham carried out high-throughput screening of their compound bank for structures having affinity for the 5-HT_7 receptor, and identified the sulfonamide (I; Fig. CS7.1) as a lead compound with slight selectivity. The structure has two asymmetric centres and was tested as a mixture of the two possible diastereomers. Since there are two enantiomers for each diastereomer, this means that there are four possible stereoisomers (*R,R; S,S; .R,S* and *S,R*) All four stereoisomers were tested separately and the *R,R* isomer (II) was found to have the best affinity.

The affinity for the *R,S*-diastereomer was still 6.2, which indicated that the stereochemistry of the asymmetric centre in the piperidine ring was not essential for activity. Therefore, it was decided to remove this asymmetric



I; pK_i 7.2

II; pK_i 6.9

FIGURE CS7.1 Identification of a lead compound.

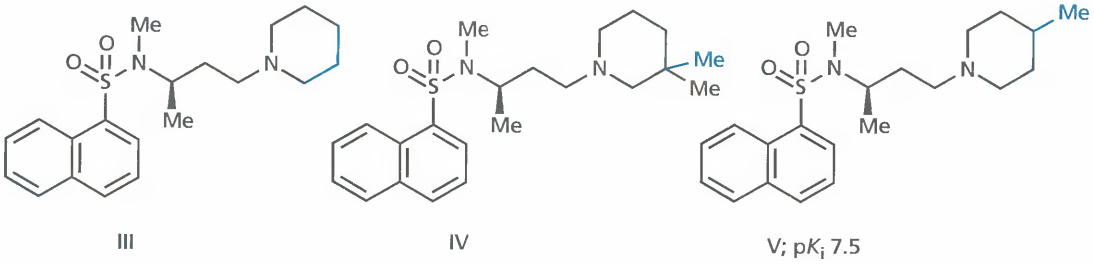


FIGURE CS7.2 Methods of removing the asymmetric centre in the piperidine ring.

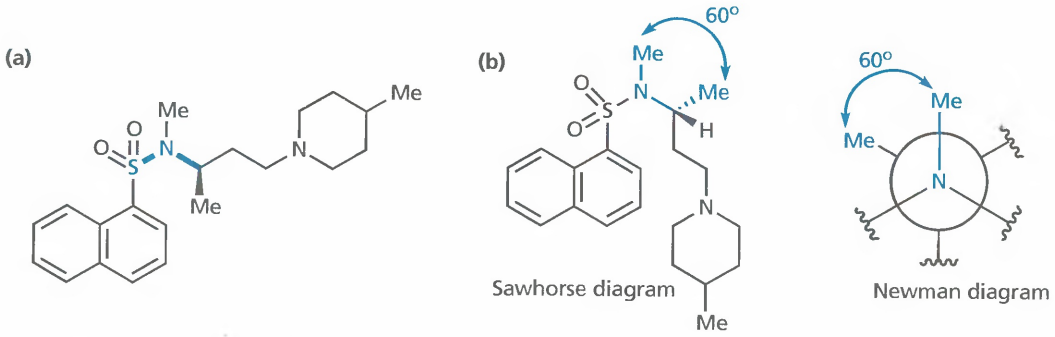


FIGURE CS7.3 Conformational analysis shows that **(a)** the bonds shown in blue have restricted rotation, and **(b)** there is a stable conformation having a torsion angle of 60°.

centre as this would simplify the synthesis of analogues (*simplification; section 13.3.8*). Otherwise, it would be necessary to separate and purify diastereomers for each analogue produced. The obvious way of removing the asymmetric centre was to remove the methyl substituent, but the resulting structure III (Fig. CS7.2) had no affinity. This indicated the importance of the methyl group, and suggested that it might be interacting with a hydrophobic pocket in the binding site. Another method of removing the asymmetric centre was to add a second methyl substituent at the same position, but the resulting structure IV had no affinity either, suggesting that the second methyl group might be bad for steric reasons. The problem was eventually solved by shifting the methyl group to position 4 of the piperidine ring, which not only removed the asymmetric centre but improved affinity (*simplification and group shift; sections 13.3.8 and 14.2.6*).

A conformational analysis of the flexible chain linking the two ring systems was now carried out (*section 17.8*). This revealed that all the bonds are relatively free to rotate apart from the bonds shown in bold (Fig. CS7.3). Concentrating on conformations involving these bonds, an energy minimum was found when the two methyl substituents are gauche with respect to each other, corresponding to a dihedral angle of 60°.

Since the gauche conformation is an energy minimum, it represents a stable conformation and the molecule will spend a greater amount of time in this conformation than in others. Therefore, there is a possibility that this might correspond to the active conformation (*section 13.2*). If this is the case, locking the molecule into this conformation should increase binding affinity (*rigidification; section 13.3.9*).

Rigidification can be carried out by introducing a ring which incorporates both methyl groups and the connecting bonds; for example structures VI and VII where the ring is 6-membered and 5-membered respectively (Fig. CS7.4 and CS7.5). Before synthesizing these struc-

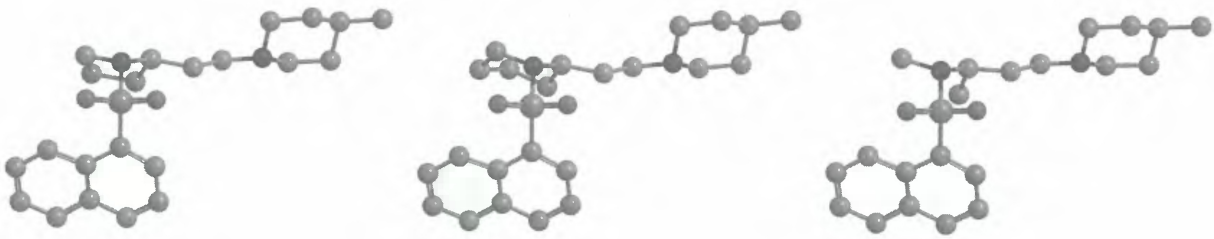


FIGURE CS7.4 Three-dimensional representations of the lead compound and rigidified analogues.

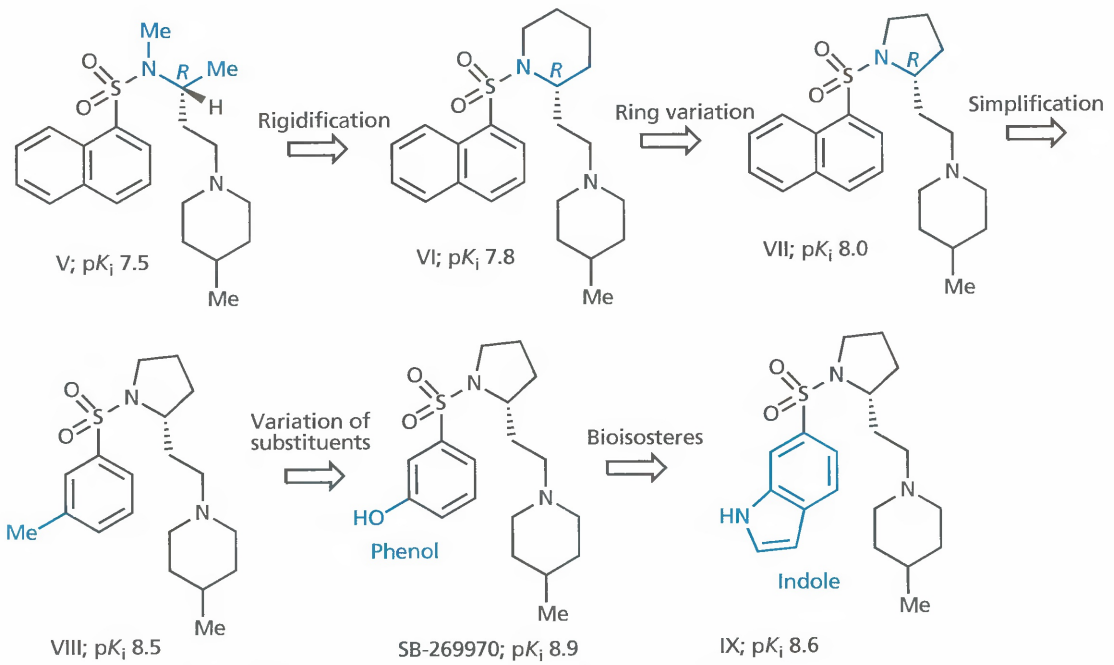


FIGURE CS7.5 The development process from lead compound to structure IX.

tures, docking experiments were carried out using a 5-HT_7 receptor homology model (*sections 17.12 and 17.14.1*). These predicted that the *R*-enantiomer of structure VI would have greater binding affinity than the *S*-enantiomer. Both enantiomers were duly synthesized, and the *R*-enantiomer had a 25-fold better affinity as predicted. It also had slightly better affinity than structure V. Structure VII containing the five membered ring was then synthesized (*ring contraction; section 13.3.4*), which resulted in an increase in affinity.

The naphthalene ring system is not essential for activity and it was possible to replace it with a single aromatic ring to give structure VIII (*simplification or ring variation; sections 13.3.8 and 13.3.5*). A number of different aromatic substituents were tested at different positions (*variation of aromatic substituents; section 13.3.1.2*) and it was found that a phenolic group was best for activity giving SB 269970. It is possible that this group is participating in a hydrogen bonding interaction with the binding site, since a methoxy substituent has less affinity. This was confirmed by docking the structure into the model binding site and identifying a possible hydrogen bonding interaction.

The selectivity of SB 269970 was tested against various receptors, and it was found to have greater than 250-fold selectivity over 13 other receptors, as well as a 50-fold selectivity over 5-HT_{5A}. Further testing with a commercial screening package (Cerep) showed that it had a 100-fold selectivity over a total of 50 other receptors, enzymes or ion channels. The compound has been shown to be an inverse agonist (section 8.5)

Since SB 269970 contains a phenolic group, it is prone to phase II conjugation reactions (*section 11.4.5*) which leads to rapid excretion. The phenolic group is involved in an important binding interaction, and so rather than removing it entirely, it was replaced with a metabolically stable bioisostere (*sections 13.3.7 and 14.1.5*), which would still be capable of forming the important hydrogen bond. This was achieved by fusing a five-membered heterocycle onto the aromatic ring such that an NH group would be placed at the same position as the original phenol. Various heterocycles were tried with an indole ring system being the best (structure IX, Fig. CS7.5).

Unfortunately, the compound was rapidly cleared from the blood and had zero bioavailability when tested in rats, and so attention now turned to the methyl substituent on the piperidine ring, as this was also likely to be susceptible to metabolism (*section 11.4.2*). Molecular modelling showed that it might be possible to replace the methyl group with a substituent that would extend into a large hydrophobic pocket close by. It was decided to try a substituent containing an aromatic ring. This would not only remove the susceptible methyl group, but offer the possibility of increased binding with the hydrophobic pocket (*extension; section 13.3.2*). Various substituents were tried and a fluorobenzoyl substituent was one of the best (structure X; Fig. CS7.6). Unfortunately, structure X had increased affinity for the α_{1B}

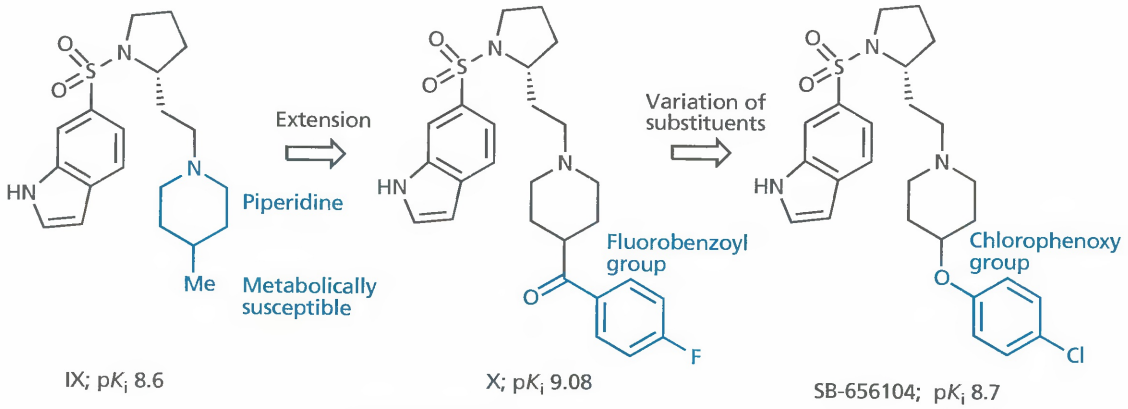


FIGURE CS7.6 Development of SB 656104.

adrenoceptor as well as the $5HT_7$ receptor. Variation of the substituents (*section 13.3.1.2*) at either end of the aromatic ring was now tried and the chlorophenoxy group resulted in much better selectivity (SB 656104; Fig. CS7.6). Although binding affinity for the $5HT_7$ receptor had dropped, the structure had the best balance of properties. Crucially it lasted far longer than SB 269970 in the blood supply and had an oral bioavailability of 16%. This compound was taken forward as the basis for further studies.

FURTHER READING

Forbes, I. T., *et al.* (2002) SB-656104-A: A novel 5-HT_7 receptor antagonist with improved in vivo properties. *Bioorganic and Medicinal Chemistry Letters*, **12**, 3341–3344.

Leopoldo, M. (2004) Serotonin 7 receptors (5-HT_7Rs) and their ligands. *Current Medicinal Chemistry*, **11**, 629–661.

Pacher, P., and Kecskemeti, V. (2004) Trends in the development of new antidepressants. Is there a light at the end of the tunnel? *Current Medicinal Chemistry*, **11**, 925–943.