14 Drug design: optimizing access to the target

In chapter 13, we looked at drug design strategies aimed at optimizing the binding interactions of a drug with its target. However, the compound with the best binding interactions is not necessarily the best drug to use in medicine. The drug needs to overcome many barriers if it is to reach its target in the body (chapter 11). In this chapter, we shall study design strategies which can be used to counter such barriers and which involve modification of the drug itself. There are other methods of aiding a drug in reaching its target, which include linking the drug to polymers or antibodies, or encapsulating it within a polymeric carrier. These topics are discussed in sections 11.9 and 21.9. In general, the aim is to design drugs that will be absorbed into the blood supply, will reach their target efficiently, be stable enough to survive the journey, and will be eliminated in a reasonable period of time. This all comes under the banner of a drug's pharmacokinetics.

14.1 Optimizing hydrophilic/hydrophobic properties

The relative hydrophilic/hydrophobic properties of a drug are crucial in influencing its solubility, absorption, distribution, metabolism and excretion (ADME). Drugs which are too polar or hydrophilic do not easily cross the cell membranes of the gut wall. One way round this is to inject them, but they cannot be used against intracellular targets since they will not cross cell membranes. They are also likely to have polar functional groups which will make them prone to plasma protein binding, metabolic phase II conjugation reactions and rapid excretion (chapter 11). Very hydrophobic drugs fare no better. If they are administered orally, they are likely to be dissolved in fat globules in the gut and will be poorly absorbed. If they are injected, they are poorly soluble in blood and are likely to be taken up by fat tissue, resulting in low circulating levels.

The hydrophobic character of a drug can be measured experimentally by testing the drug's relative distribution in an *n*-octanol/water mixture. Hydrophobic molecules will prefer to dissolve in the *n*-octanol layer of this two-phase system, whereas hydrophilic molecules will prefer the aqueous layer. The relative distribution is known as the partition coefficient (P) and is obtained from the following equation:

$$P = \frac{\text{Concentration of drug in octanol}}{\text{Concentration of drug in aqueous solution}}$$

Hydrophobic compounds have a high P value, whereas hydrophilic compounds have a low P value. In fact, log P values are normally used as a measure of hydrophobicity. It is also possible to calculate log P values for a given structure using suitable software progams. Such estimates are referred to as **Clog *P*** values to distinguish them from experimentally derived log P values.

Many drugs can exist as an equilibrium between an ionized and an un-ionized form. However, log P measures only the relative distribution of the un-ionized species between water and octanol. The relative distribution of all species (both ionized and un-ionized) is given by log D.

In general, the hydrophilic/hydrophobic balance of a drug can be altered by changing easily accessible substituents. Such changes are particularly open to a quantitative approach known as QSAR (quantitative structure–activity relationships), discussed in chapter 18.

As a postscript, the hydrophilic/hydrophobic properties of a drug are not the only factors that influence drug absorption and oral bioavailability. Molecular flexibility also has an important role in oral bioavailability (section 11.2) and so the tactics of rigidification described in section 13.3.9 can be useful in improving drug absorption.

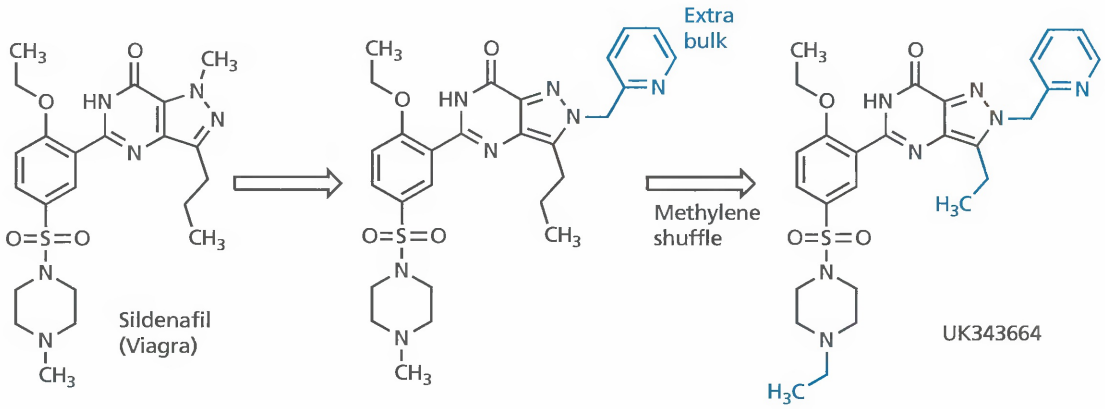


FIGURE 14.1 The development of UK343664 via a methylene shuffle strategy.

14.1.1 Variation of alkyl or acyl substituents to vary polarity

Molecules can be made less polar by masking a polar functional group with an alkyl or acyl group. For example, an alcohol or a phenol can be converted to an ether or ester, a carboxylic acid can be converted to an ester or amide, and primary and secondary amines can be converted to amides or to secondary and tertiary amines. Polarity is decreased not only by masking the polar group, but by the addition of an extra hydrophobic alkyl group—larger alkyl groups having a greater hydrophobic effect. One has to be careful in masking polar groups, though, as they may be important in binding the drug to its target, and masking them may prevent binding. If this is the case, it is often useful to mask the polar group temporarily such that the mask is removed once the drug is absorbed (section 14.6). Alternatively, extra alkyl groups could be added to the carbon skeleton of the molecule, but this usually involves a more involved synthesis.

If the molecule is not sufficiently polar, then the opposite strategy can be used (i.e. replacing large alkyl groups with smaller alkyl groups, or removing them entirely).

Sometimes there is a benefit in increasing the size of one alkyl group and decreasing the size of another. This is called a **methylene shuffle** and has been used to modify the hydrophobicity of compounds. An example of this is in the design of second-generation anti-impotence drugs based on **sildenafil (Viagra).** It was found that adding extra bulk on the right-hand side of the molecule (as drawn in the figure) increased the drug's selectivity to its target. However, this also made the drug too lipophilic. Therefore, a methylene shuffle was carried out to alter a propyl and a methyl group to two ethyl groups. This resulted in reduced lipophilicity and better *in vivo* activity. The compound (**UK 343664**) entered clinical trials (Fig. 14.1).

14.1.2 Varying polar functional groups to vary polarity

A polar functional group could be added to a drug to increase its polarity. For example, the antifungal agent **tioconazole** is only used for skin infections because it is non-polar and is poorly soluble in blood. Introducing a polar hydroxyl group and more polar heterocyclic rings led to the orally active antifungal agent **fluconazole** with improved solubility and enhanced activity against systemic infection (i.e. in the blood supply) (Fig. 14.2).

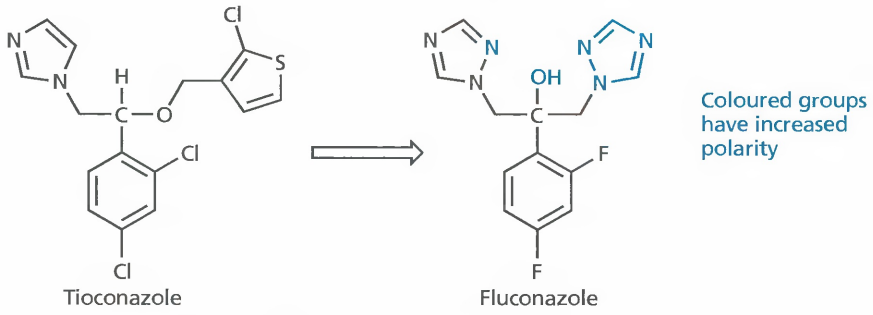


FIGURE 14.2 Increasing polarity in antifungal agents.

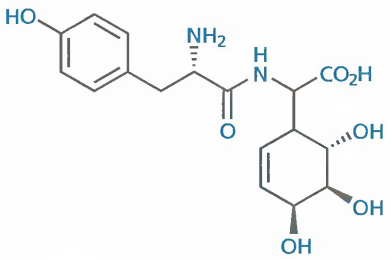


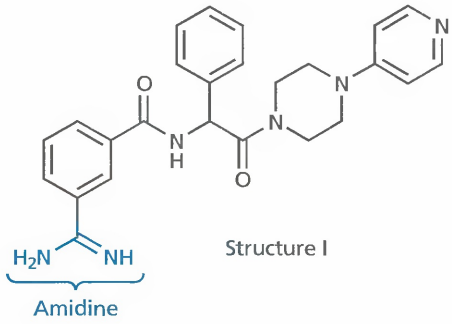
FIGURE 14.3 Excess polarity (coloured) in a drug.

Another example can be found in case study 1. Here, a polar sulfonamide group was added to **rosuvastatin** to make it more hydrophilic and more tissue selective. Finally, nitrogen-containing heterocycles (e.g. morpholine or pyridine) are often added to drugs in order to increase their polarity and water solubility. This is because the nitrogen is basic in character, and it is possible to form water-soluble salts. Examples of this tactic can be seen in the design of **gefitinib** (section 21.6.2.1) and in the design of a thymidylate synthase inhibitor (Case Study 5). If a polar group is added in order to increase water solubility, it is preferable to add it to the molecule in such a way that it is still exposed to surrounding water when the drug is bound to the target binding site. This means that energy does not have to be expended in desolvation (section 1.3.6).

The polarity of an excessively polar drug can be lowered by removing polar functional groups. This strategy has been particularly successful with lead compounds derived from natural sources (e.g. alkaloids or endogenous peptides). It is important, though, not to remove functional groups which are important to the drug's binding interactions with its target. In some cases, a drug may have too many essential polar groups. For example, the antibacterial agent shown in Fig. 14.3 has good *in vitro* activity but poor *in vivo* activity because of the large number of polar groups. Some of these groups can be removed or masked, but most of them are required for activity. As a result, the drug cannot be used clinically.

14.1.3 Variation of *N*-alkyl substituents to vary pK_a

Drugs with a pK_a outside the range 6–9 tend to be too strongly ionized, and are poorly absorbed through cell membranes. The pK_a can often be altered to bring it into the preferred range. For example, this can be done by varying any *N*-alkyl substituents that are present. However, it is sometimes difficult to predict how such variations will affect the pK_a. Extra *N*-alkyl groups or larger *N*-alkyl groups have an increased electron-donating



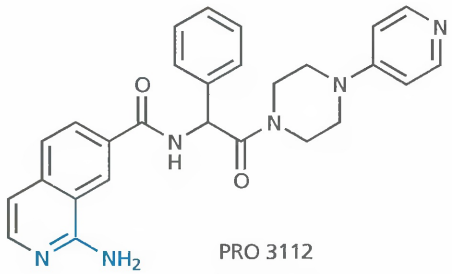


FIGURE 14.4 Varying basicity in antithrombotic agents.

effect which should increase basicity, but increasing the size or number of alkyl groups increases the steric bulk around the nitrogen atom. This hinders water molecules from solvating the ionized form of the base and prevents stabilization of the ion. This in turn decreases the basicity of the amine. Therefore, there are two different effects acting against each other. Nevertheless, varying alkyl substituents is a useful tactic to try.

A variation of this tactic is to 'wrap up' a basic nitrogen within a ring. For example, the benzamidine structure (I in Fig. 14.4) has antithrombotic activity, but the amidine group present is too basic for effective absorption. Incorporating the group into an isoquinoline ring system (**PRO 3112**) reduced basicity and increased absorption.

14.1.4 Variation of aromatic substituents to vary pK_a

The pK_a of an aromatic amine or carboxylic acid can be varied by adding electron-donating or electron-withdrawing substituents to the ring. The position of the substituent relative to the amine or carboxylic acid is important if the substituent interacts with the ring through resonance (section 18.2.2). An illustration of this can be seen in the development of **oxamniquine** (Case Study 4).

14.1.5 Bioisosteres for polar groups

The use of bioisosteres has already been described in section 13.3.7 in the design of compounds with improved

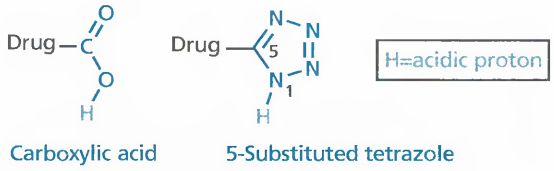


FIGURE 14.5 5-Substituted tetrazole ring as a bioisostere for a carboxylic acid.

target interactions. Bioisosteres have also been used as substitutes for important functional groups which are required for target interactions but which pose pharmacokinetic problems. For example, a carboxylic acid is a highly polar group which can ionize and hinder absorption of any drug containing it. One way of getting round this problem is to mask it as an ester prodrug (section 14.6.1.1). Another strategy is to replace it with a bioisostere which has similar physicochemical properties but which offers some advantage over the original carboxylic acid. Several bio-isosteres have been used for carboxylic acids, but among the most popular is a 5-substituted tetrazole ring (Fig. 14.5). Like carboxylic acids, tetrazoles contain an acidic proton and are ionized at pH 7.4. They are also planar in structure. However, they have an advantage in that the tetrazole anion is 10 times more lipophilic than a carboxylate anion and drug absorption is enhanced as a result (Box 14.1). They are also resistant to many of the metabolic reactions that occur on carboxylic acids. *N*-Acylsulfonamides have also been used as bioisosteres for carboxylic acids (section 13.3.7).

14.2 Making drugs more resistant to chemical and enzymatic degradation

There are various strategies that can be used to make drugs more resistant to hydrolysis and drug metabolism, and thus prolong their activity.

14.2.1 Steric shields

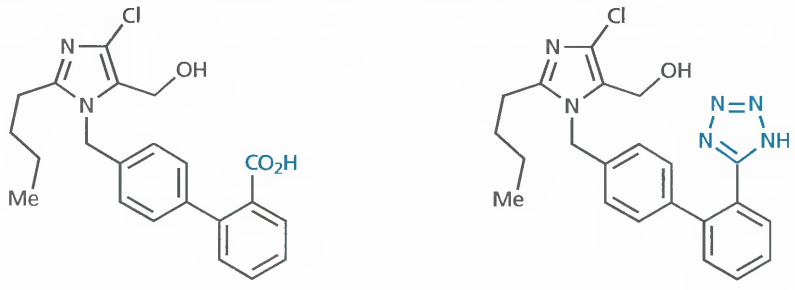
Some functional groups are more susceptible to chemical and enzymatic degradation than others. For example, esters and amides are particularly prone to hydrolysis. A common strategy that is used to protect such groups is to add steric shields, designed to hinder the approach of a nucleophile or an enzyme to the susceptible group. These usually involve the addition of a bulky alkyl group close to the functional group. For example, the *t*-butyl group in the antirheumatic agent **D 1927** serves as a steric shield and blocks hydrolysis of the terminal peptide bond (Fig. 14.6). Steric shields have also been used to protect penicillins from lactamases (section 19.5.1.8), and to prevent drugs interacting with cytochrome P450 enzymes (Case Study 6)—see also section 22.9.1, SCh 226374 (Fig. 21.45) and CGS27023 (Fig. 21.63).

14.2.2 Electronic effects of bioisosteres

Another popular tactic used to protect a labile functional group is to stabilize the group electronically using

BOX 14.1 Use of bioisosteres to increase absorption

The biphenyl structure (Structure I) was shown by Du Pont to inhibit the receptor for angiotensin II, and had potential as an antihypertensive agent. However, the drug had to be injected as it showed poor absorption through the gut wall. Replacing the carboxylic acid with a tetrazole ring led to **losartan,** which was launched in 1994.



Structure I Losartan

FIGURE 1 Development of losartan.

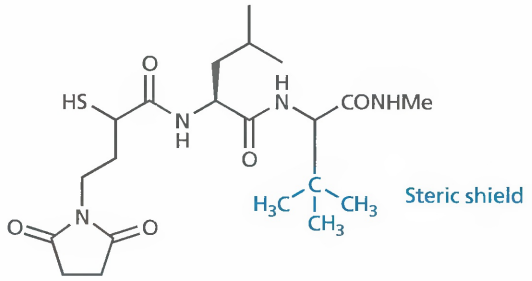


FIGURE 14.6 The use of a steric shield to protect the antirheumatic agent D 1927.

FIGURE 14.7 Isosteric replacement of a methyl group with an amino group.

a bioisostere. A bioisostere is a chemical group used to replace another chemical group within the drug, without affecting the important biological activity. Other features such as the drug's stability may also be improved. Isosteres and non-classic isosteres are frequently used as bioisosteres (sections 13.1.15, 13.3.7, and 14.1.5). For example, replacing the methyl group of an ethanoate ester with NH_2 results in a urethane functional group which is more stable than the original ester (Fig. 14.7). The NH_2 group is the same valency and size as the methyl group and therefore has no steric effect, but it has totally different electronic properties since it can feed electrons into the carboxyl group and stabilize it from hydrolysis. The cholinergic agonist **carbachol** is stabilized in this way (section 22.9.2), as is the cephalosporin **cefoxitin** (section 19.5.2.4).

Alternatively, a labile ester group could be replaced by an amide group (NH replacing O). Amides are more resistant to chemical hydrolysis, due again to the lone pair of the nitrogen feeding its electrons into the carbonyl group and making it less electrophilic.

It is important to realize that bioisosteres are not general and are often specific to a particular field. Replacing an ester with a urethane or an amide may work in one category of drugs but not another. One must also appreciate that bioisosteres are different from isosteres. It is the retention of important biological activity that determines whether a group is a bioisostere, not the valency. Therefore, non-isosteric groups can be used as bioisosteres. For example, a pyrrole ring was used as a bioisostere for an amide bond in the development of the dopamine antagonist **Du 122290** from **sultopride** (section 13.3.7). Similarly, thiazolyl rings were used as bioisosteres for pyridine rings in the development of ritonavir (section 20.7.4.4).

One is not confined to the use of bioisosteres to increase stability. Groups or substituents having an inductive electronic effect have frequently been incorporated into molecules to increase the stability of a labile functional group. For example, electron-withdrawing groups were incorporated into the side chain of penicillins to increase their resistance to acid hydrolysis (section 19.5.1.8). The inductive effects of groups can also determine the ease with which ester prodrugs are hydrolysed (Box 14.3).

14.2.3 Stereoelectronic modifications

Steric hindrance and electronic stabilization have often been used together to stabilize labile groups. For example, **procaine** (Fig. 14.8) is a good local anaesthetic, but it is short-lasting because its ester group is quickly hydrolysed. Changing the ester group to the less reactive amide group reduces chemical hydrolysis. Furthermore, the presence of two *ortho*-methyl groups on the aromatic ring helps to shield the carbonyl group from attack by nucleophiles or enzymes. This results in the longer acting, local anaesthetic **lidocaine**. Since steric and electronic influences are both involved, the modifications are defined as stereoelectronic. Further successful examples of stereoelectronic modification are demonstrated by **oxacillin** (Box 19.5) and **bethanechol** (section 22.9.3).

14.2.4 Metabolic blockers

Some drugs are metabolized by the introduction of polar groups at particular positions in their skeleton. For example, the oral contraceptive **megestrol acetate** is oxidized at position 6 to give a hydroxyl group at that position (Fig. 14.9). The introduction of a polar hydroxyl group allows the formation of polar conjugates which can be quickly eliminated from the system. By introducing a methyl group at position 6, metabolism is blocked and the activity of the drug is prolonged.

On the same lines, a popular method of protecting aromatic rings from metabolism at the *para*-position is to introduce a fluoro substituent. For example, **CGP 52411** (Fig. 14.10) is an enzyme inhibitor which acts on the kinase active site of the epidermal growth factor receptor (section 4.8). It went forward for clinical trials as

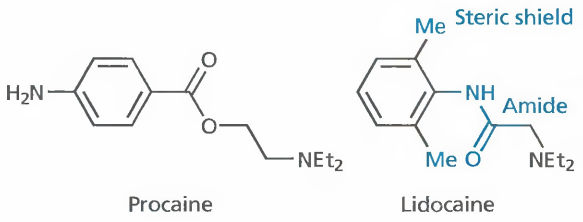


FIGURE 14.8 Stereoelectronic modifications which make lidocaine a longer lasting, local anaesthetic compared with procaine.

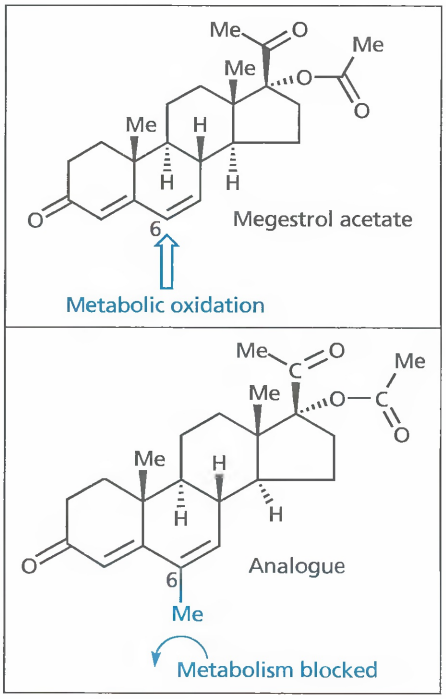


FIGURE 14.9 Metabolic blocking by the addition of a methyl substituent.

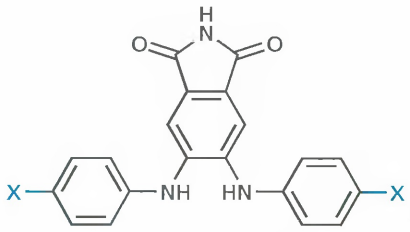


FIGURE 14.10 The use of fluorine substituents as metabolic blockers. X=H, CGP 52411; X=OH, metabolite; X=F, CGP 53353.

an anticancer agent and was found to undergo oxidative metabolism at the *para*-position of the aromatic rings. Fluoro-substituents were successfully added to block this metabolism and the analogue **CGP 53353** was also put forward for clinical trials. This tactic was also applied successfully in the design of **gefitinib** (section 21.6.2.1).

14.2.5 Removal or replacement of susceptible metabolic groups

Certain chemical groups are particularly susceptible to metabolic enzymes. For example, methyl groups on aromatic rings are often oxidized to carboxylic acids (section 11.4.2). These acids can then be quickly eliminated from the body. Other common metabolic reactions include aliphatic and aromatic *C*-hydroxylations, *N*- and

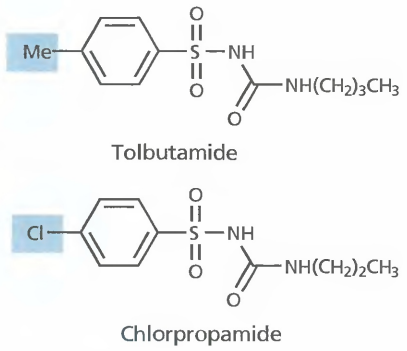


FIGURE 14.11 Replacing metabolically labile groups.

S-oxidations, *O*- and *S*-dealkylations, and deaminations (section 11.4).

Susceptible groups can sometimes be removed or replaced by groups that are stable to oxidation, in order to prolong the lifetime of the drug. For example, the aromatic methyl substituent of the antidiabetic **tolbutamide** was replaced by a chloro substituent to give **chlorpropamide**, which is much longer lasting (Fig. 14.11). This tactic was also used in the design of **gefitinib** (section 21.6.2.1). An alternative strategy which is often tried is to replace the susceptible methyl group with CF_3, CHF_2 or CH_2F. The fluorine atoms alter the oxidation potential of the methyl group and make it more resistant to oxidation.

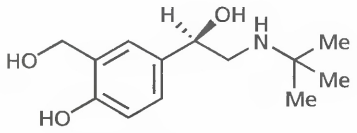
Another example where a susceptible metabolic group is replaced is seen in section 19.5.2.3 where a susceptible ester in cephalosporins is replaced with metabolically stable groups to give **cephaloridine** and **cefalexin**.

14.2.6 Group shifts

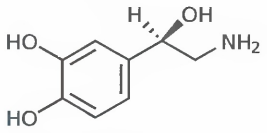
Removing or replacing a metabolically vulnerable group is feasible if the group concerned is not involved in important binding interactions with the binding site. If the group *is* important, then we have to use a different strategy.

There are two possible solutions. We can either mask the vulnerable group on a temporary basis by using a prodrug (section 14.6) or we can try shifting the vulnerable group within the molecular skeleton. The latter tactic was used in the development of **salbutamol** (Fig. 14.12). Salbutamol was introduced in 1969 for the treatment of asthma, and is an analogue of the neurotransmitter **noradrenaline**—a catechol structure containing two *ortho*-phenolic groups.

One of the problems faced by catechol compounds is metabolic methylation of one of the phenolic groups. Since both phenol groups are involved in hydrogen bonds to the receptor, methylation of one of the phenol groups disrupts the hydrogen bonding and makes the compound inactive. For example, the noradrenaline analogue



Salbutamol



Noradrenaline

FIGURE 14.12 Salbutamol and noradrenaline.

(I in Fig. 14.13) has useful anti-asthmatic activity, but the effect is of short duration because the compound is rapidly metabolized to the inactive methyl ether (II in Fig. 14.13).

Removing the OH or replacing it with a methyl group prevents metabolism, but also prevents the important hydrogen bonding interactions with the binding site. So how can this problem be solved? The answer was to move the vulnerable hydroxyl group out from the ring by one carbon unit. This was enough to make the compound unrecognizable to the metabolic enzyme but not to the receptor binding site.

Fortunately, the receptor appears to be quite lenient over the position of this hydrogen bonding group and it is interesting to note that a hydroxyethyl group is also acceptable (Fig. 14.14). Beyond that, activity is lost because the OH group is out of range, or too large to fit. These results demonstrate that it is better to consider a binding region within the receptor binding site as an available volume, rather than imagining it as being fixed at one spot. A drug can then be designed such that the relevant binding group is positioned into any part of that available volume.

Shifting an important binding but metabolically susceptible group worked for salbutamol, but one cannot guarantee that the same tactic will always be successful. Shifting the group may make the molecule unrecognizable both to its target and to the metabolic enzyme.

14.2.7 Ring variation and ring substituents

Certain ring systems may be susceptible to metabolism, and so varying the ring might improve metabolic stability. This can be done by adding a nitrogen into

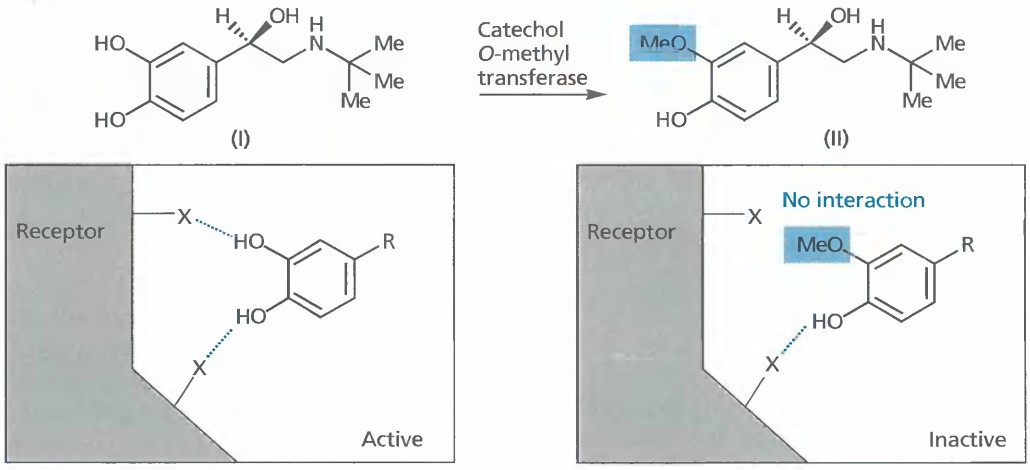


FIGURE 14.13 Metabolic methylation of a noradrenaline analogue. X denotes an electronegative atom.

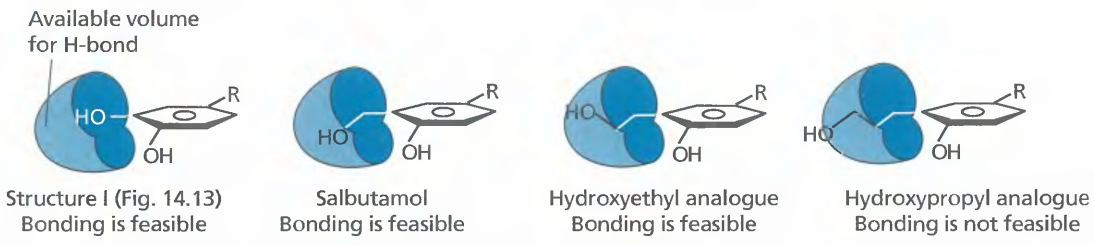


FIGURE 14.14 Viewing a binding region as an available volume.

the ring to lower the electron density of the ring system. For example, the imidazole ring of the antifungal agent **tioconazole** mentioned previously is susceptible to metabolism, but replacement with a 1,2,4-triazole ring as in **fluconazole** results in improved stability (Fig. 14.2).

Electron-rich aromatic rings such as phenyl groups are particularly prone to oxidative metabolism, but can be stabilized by replacing them with nitrogen-containing heterocyclic rings such as pyridine or pyrimidine. Alternatively, electron-withdrawing substituents could be added to the aromatic ring to lower the electron density. Examples of this kind of strategy are given in Case Study 6.

Ring variation can also help to stabilize metabolically susceptible aromatic or heteroaromatic methyl substituents. Such substituents could be replaced with more stable substituents as described in section 14.2.5, but sometimes the methyl substituent has to be retained for good activity. In such cases, introducing a nitrogen atom into the aromatic/heteroaromatic ring can be beneficial, since lowering the electron density in the ring also helps to make the methyl substituent more resistant to metabolism. For example, F13640 is undergoing phase II clinical trials as an analgesic (Fig. 14.15). The methyl substituent on the pyridine ring is susceptible to oxidation and is converted to a carboxylic acid which is inactive. The methyl group plays an important binding role and has to be present. Therefore, the pyridine ring was changed to a pyrimidine ring resulting in a compound (F15599) that has increased metabolic stability without affecting binding affinity.

14.3 Making drugs less resistant to drug metabolism

So far, we have looked at how the activity of drugs can be prolonged by inhibiting their metabolism. However, a drug that is extremely stable to metabolism and is very slowly excreted can pose just as many problems as one that is susceptible to metabolism. It is usually desirable to have a drug that does what it is meant to do, then stops doing it within a reasonable time. If not, the effects of the drug could last too long and cause toxicity and lingering side effects. Therefore, designing drugs with decreased chemical and metabolic stability can sometimes be useful.

14.3.1 Introducing metabolically susceptible groups

Introducing groups that are susceptible to metabolism is a good way of shortening the lifetime of a drug (Box 14.2). For example, a methyl group was introduced to the anti-arthritic agent **L 787257** to shorten its lifetime. The methyl group of L 791456 was metabolically oxidized to a polar alcohol as well as to a carboxylic acid (Fig. 14.16).

Another example is the analgesic **remifentanil** (section 24.6.3.4), where ester groups were incorporated to make it a short lasting agent.

14.3.2 Self-destruct drugs

A self-destruct drug is one which is chemically stable under one set of conditions but becomes unstable and spontaneously degrades under another set of conditions. The advantage of a self-destruct drug is that inactivation does not depend on the activity of metabolic enzymes, which could vary from patient to patient. The best example of a self-destruct drug is the neuromuscular blocking agent **atracurium**, which is stable at acid pH but self-destructs when it meets the slightly alkaline conditions of the blood (section 22.12.2.4). This means that the drug has a short duration of action, allowing anaesthetists to control its blood levels during surgery by providing it as a continuous intravenous drip.

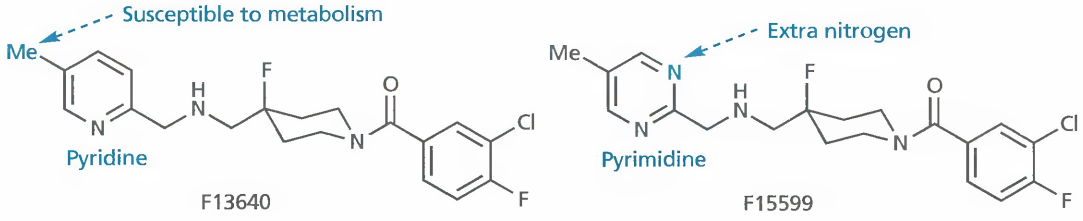
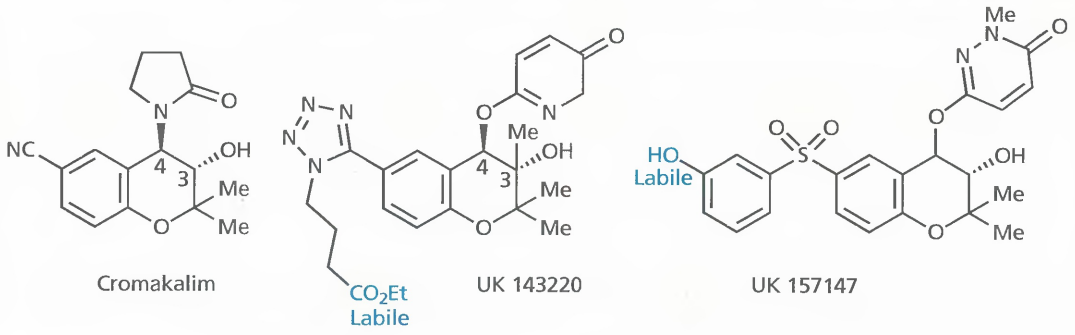


FIGURE 14.15 Stabilizing an aromatic or heteroaromatic methyl substituent by adding a nitrogen to the ring.

BOX 14.2 Shortening the lifetime of a drug

Anti-asthmatic drugs are usually taken by inhalation to reduce the chances of side effects elsewhere in the body. However, a significant amount is swallowed and can be absorbed into the blood supply from the gastrointestinal tract. Therefore, it is desirable to have an anti-asthmatic drug which is potent and stable in the lungs but which is rapidly metabolized in the blood supply. **Cromakalim** has useful anti-asthmatic properties but has cardiovascular side effects if it gets into the blood supply. Structures **UK 143220** and **UK 157147** were developed from cromakalim so that they would be quickly metabolized. UK 143220 contains an ester which is quickly hydrolysed by esterases in the blood to produce an inactive carboxylic acid, while UK 157147 contains a phenol group which is quickly conjugated by metabolic conjugation enzymes and eliminated. Both these compounds were considered as clinical candidates.



Metabolically labile analogues of cromakalim.

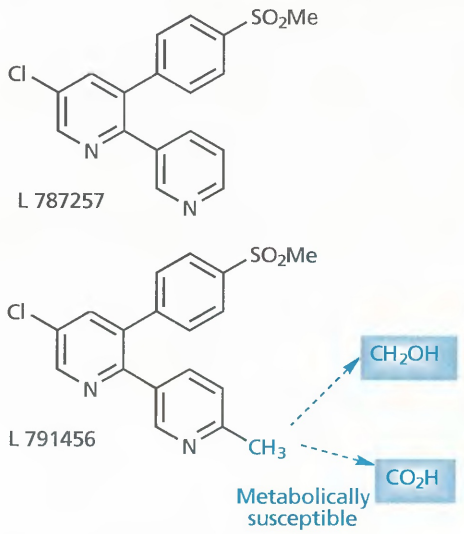


FIGURE 14.16 Adding a metabolically labile methyl group to shorten a drug's lifetime.

KEY POINTS

- The polarity or pK_a of a lead compound can be altered by varying alkyl substituents or functional groups, allowing the drug to be absorbed more easily.
- Drugs can be made more resistant to metabolism by introducing steric shields to protect susceptible functional groups. It may also be possible to modify the functional group itself to make it more stable. When both tactics are used together, this is termed a stereoelectronic modification.
- Metabolically stable groups can be added to block metabolism at certain positions.
- Groups that are susceptible to metabolism may be modified or removed to prolong activity, as long as the group is not required for drug–target interactions.
- Metabolically susceptible groups that are necessary for drug–target interactions can be shifted in order to make them unrecognizable by metabolic enzymes, as long as they are still recognizable to the target.
- Varying a heterocyclic ring in the lead compound can sometimes improve metabolic stability.
- Drugs that are slowly metabolized may linger too long in the body and cause side effects.
- Groups that are susceptible to metabolic or chemical change can be incorporated to reduce a drug's lifetime.

14.4 Targeting drugs

One of the major goals in drug design is to find ways of targeting drugs to the exact locations in the body where they are most needed. The principle of targeting drugs

can be traced back to Paul Ehrlich who developed antimicrobial drugs that were selectively toxic for microbial cells over human cells. Drugs can also be made more selective to distinguish between different targets within the body, as discussed in chapter 13. Here, we discuss other tactics related to the targeting of drugs.

14.4.1 Targeting tumour cells: 'search and destroy' drugs

A major goal in cancer chemotherapy is to target drugs efficiently against tumour cells rather than normal cells. One method of achieving this is to design drugs which make use of specific molecular transport systems. The idea is to attach the active drug to an important 'building block' molecule that is needed in large amounts by the rapidly dividing tumour cells. This could be an amino acid or a nucleic acid base (e.g. uracil mustard; section 21.2.3.1). Of course, normal cells require these building blocks as well, but tumour cells often grow more quickly than normal cells and require the building blocks more urgently. Therefore, the uptake is greater in tumour cells.

A more recent idea has been to attach the active drug (or a poison such as **ricin**) to **monoclonal antibodies**, which can recognize antigens unique to the tumour cell. Once the antibody binds to the antigen, the drug or poison is released to kill the cell. The difficulties in this approach include the identification of suitable antigens and the production of antibodies in significant quantity. Nevertheless, the approach has great promise for the future and is covered in more detail in section 21.9.2. Another tactic which has been used to target anticancer drugs is to administer an enzyme–antibody conjugate where a suitable enzyme is chosen to activate an anticancer prodrug and the antibody is chosen to direct the enzyme to the tumour. This is a strategy known as ADEPT and is covered in more detail in section 21.9.3. Other targeting strategies include ADAPT and GDEPT covered in sections 21.9.4 and 21.9.5 respectively. Antibodies are also being studied as a means of targeting viruses (section 20.10.5).

14.4.2 Targeting gastrointestinal infections

If a drug is to be targeted against an infection of the gastrointestinal tract, it must be prevented from being absorbed into the blood supply. This can easily be done by using a fully ionized drug which is incapable of crossing cell membranes. For example, highly ionized sulfonamides are used against gastrointestinal infections because they are incapable of crossing the gut wall (Box 19.2).

14.4.3 Targeting peripheral regions rather than the central nervous system

It is often possible to target drugs such that they act peripherally and not in the central nervous system. By increasing the polarity of drugs, they are less likely to cross the blood–brain barrier (section 11.3.5) and this means they are less likely to have central nervous system side effects. Achieving selectivity for the central nervous system over the peripheral regions of the body is not so straightforward.

14.5 Reducing toxicity

It is often found that a drug fails clinical trials because of toxic side effects. This may be due to toxic metabolites, in which case the drug should be made more resistant to metabolism as described earlier (section 14.2). It is also worth checking to see whether there are any functional groups present which are particularly prone to producing toxic metabolites. For example, it is known that functional groups such as aromatic nitro groups, aromatic amines, bromoarenes, hydrazines, hydroxylamines, or polyhalogenated groups are often metabolized to toxic products (see section 11.4 for typical metabolic reactions).

Side effects might also be reduced or eliminated by varying apparently harmless substituents. For example, the halogen substituents of the antifungal agent **UK 47265** were varied in order to find a compound that was less toxic to the liver. This led to the successful antifungal agent **fluconazole** (Fig. 14.17).

Varying the position of substituents can sometimes reduce or eliminate side effects. For example, the dopamine antagonist **SB 269652** inhibits cytochrome P450 enzymes as a side effect. Placing the cyano group at a different position prevented this inhibition (Fig. 14.18).

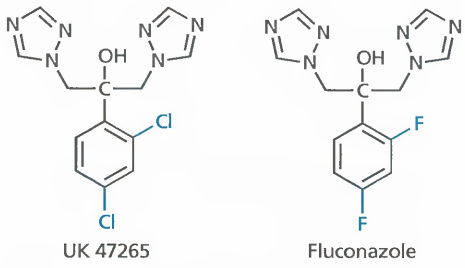


FIGURE 14.17 Varying aromatic substituents to reduce toxicity.

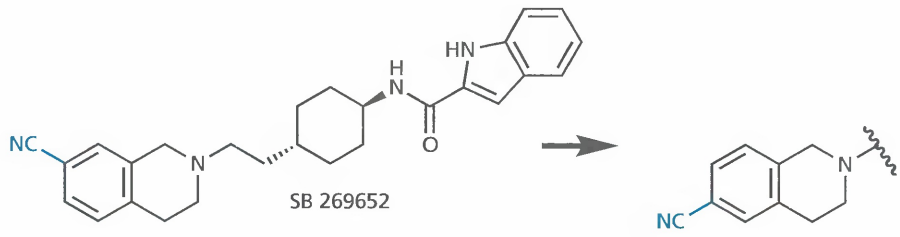


FIGURE 14.18 Varying substituent positions to reduce side effects.

KEY POINTS

- Strategies designed to target drugs to particular cells or tissues are likely to lead to safer drugs with fewer side effects.
- Drugs can be linked to amino acids or nucleic acid bases to target them against fast-growing and rapidly dividing cells.
- Drugs can be targeted to the gastrointestinal tract by making them ionized or highly polar such that they cannot cross the gut wall.
- The central nervous system side effects of peripherally acting drugs can be eliminated by making the drugs more polar so that they do not cross the blood–brain barrier.
- Drugs with toxic side effects can sometimes be made less toxic by varying the nature or position of substituents, or by preventing their metabolism to a toxic metabolite.

14.6 Prodrugs

Prodrugs are compounds which are inactive in themselves, but which are converted in the body to the active drug. They have been useful in tackling problems such as acid sensitivity, poor membrane permeability, drug toxicity, bad taste, and short duration of action. Usually, a metabolic enzyme is involved in converting the prodrug to the active drug, and so a good knowledge of drug metabolism and the enzymes involved allows the medicinal chemist to design a suitable prodrug which turns drug metabolism into an advantage rather than a problem. Prodrugs have been designed to be activated by a variety of metabolic enzymes. Ester prodrugs which are hydrolysed by esterase enzymes are particularly common, but prodrugs have also been designed which are activated by *N*-demethylation, decarboxylation, and the hydrolysis of amides and phosphates. Not all prodrugs are activated by metabolic enzymes, however. For example, photodynamic therapy involves the use of an external light source to activate prodrugs. When designing prodrugs, it is important to ensure that the prodrug is effectively converted to the active drug once it has been absorbed into the blood supply, but it is also important to ensure that any groups that are cleaved from the molecule are non-toxic.

14.6.1 Prodrugs to improve membrane permeability

14.6.1.1 Esters as prodrugs

Prodrugs have proved very useful in temporarily masking an 'awkward' functional group which is important to target binding, but which hinders the drug from crossing the cell membranes of the gut wall. For example, a carboxylic acid functional group may have an important role to play in binding a drug to its binding site via ionic or hydrogen bonding. However, the very fact that it is an ionizable group may prevent it from crossing a fatty cell membrane. The answer is to protect the acid function as an ester. The less polar ester can cross fatty cell membranes, and once it is in the bloodstream it is hydrolysed back to the free acid by esterases in the blood. Examples of ester prodrugs used to aid membrane permeability include **enalapril** which is the prodrug for the antihypertensive agent **enalaprilate** (Fig. 14.19, and Case Study 2), and **pivampicillin,** which is a penicillin prodrug (Box 19.7).

Not all esters are hydrolysed equally efficiently, and a range of esters may need to be tried to find the best one (Box 14.3). It is possible to make esters more susceptible to hydrolysis by introducing electron-withdrawing groups to the alcohol moiety (e.g. OCH_2CF_3, OCH_2CO_2R,

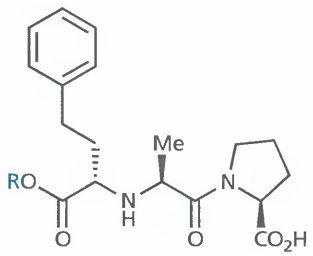


FIGURE 14.19 Enalapril (R=Et); Enalaprilate (R=H).

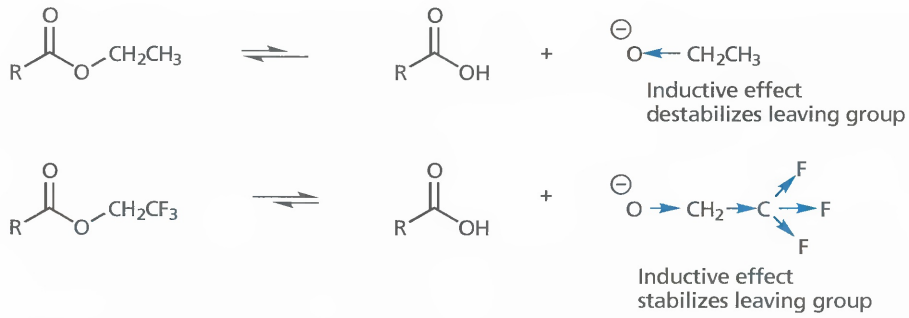
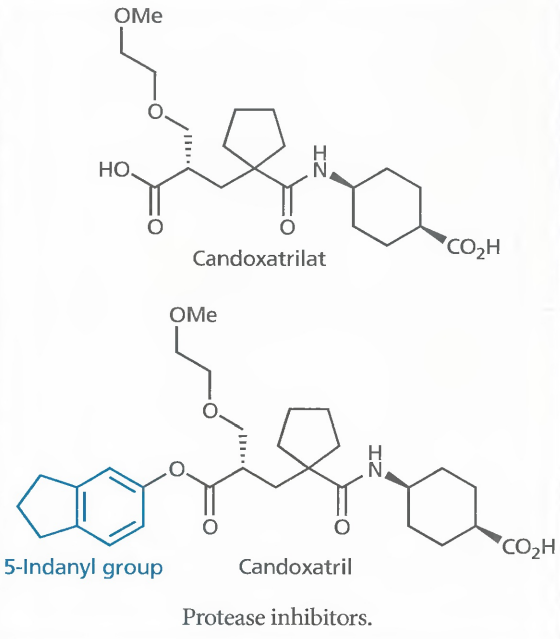


FIGURE 14.20 Inductive effects on the stability of leaving groups.

BOX 14.3 Varying esters in prodrugs

The protease inhibitor **candoxatrilat** has to be given intravenously because it is too polar to be absorbed from the gastrointestinal tract. Different esters were tried as prodrugs to get round this problem. It was found that an ethyl ester was absorbed but was inefficiently hydrolysed. A more activated ester was required, and a 5-indanyl ester proved to be the best. The 5-indanol released on hydrolysis is non-toxic.



Protease inhibitors.

14.6.1.2 *N*-Methylated prodrugs

N-Demethylation is a common metabolic reaction in the liver, so polar amines can be *N*-methylated to reduce polarity and improve membrane permeability. Several hypnotics and antiepileptics take advantage of this reaction, for example **hexobarbitone** (Fig. 14.21).

14.6.1.3 Trojan horse approach for transport proteins

Another way round the problem of membrane permeability is to design a prodrug which can take advantage of transport proteins (section 2.7.2) in the cell membrane, such as the ones responsible for carrying amino acids into a cell. A well-known example of such a prodrug is **levodopa** (Fig. 14.22). Levodopa is a prodrug for the neurotransmitter **dopamine** and has been used in the treatment of Parkinson's disease—a condition due primarily to a deficiency of that neurotransmitter in the brain. Dopamine itself cannot be used, since it is too polar to cross the blood–brain barrier. Levodopa is even more polar and seems an unlikely prodrug, but it is also an amino acid

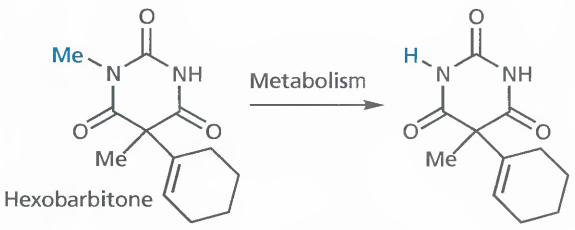


FIGURE 14.21 *N*-Demethylation of hexobarbitone.

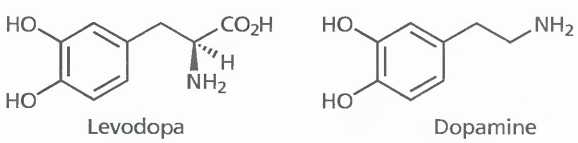


FIGURE 14.22 Levodopa and dopamine.

$OCONR_2$, OAr). The inductive effect of these groups aids the hydrolytic mechanism by making the alcohol a better leaving group (Fig. 14.20). Care has to be taken, however, not to make the ester too reactive in case it becomes chemically unstable and is hydrolysed before it reaches the blood supply.

and so it is recognized by the transport proteins for amino acids which carry it across the cell membrane. Once in the brain, a decarboxylase enzyme removes the acid group and generates dopamine (Fig. 14.23).

14.6.2 Prodrugs to prolong drug activity

Sometimes prodrugs are designed to be converted slowly to the active drug, thus prolonging a drug's activity. For example, **6-mercaptopurine** (Fig. 14.24) suppresses the body's immune response and is therefore useful in protecting donor grafts. Unfortunately, the drug tends to be eliminated from the body too quickly. The prodrug **azathioprine** has the advantage that it is slowly converted to 6-mercaptopurine by being attacked by glutathione, allowing a more sustained activity. The rate of conversion can be altered, depending on the electron-withdrawing ability of the heterocyclic group. The greater the electron-withdrawing power, the faster the breakdown. The NO_2 group is therefore present to ensure an efficient conversion to 6-mercaptopurine, since it is strongly electron-withdrawing on the heterocyclic ring.

There is a belief that the well-known sedatives **Valium** (Fig. 14.25) and **Librium** might be prodrugs and are active because they are metabolized by *N*-demethylation to **nordazepams**. Nordazepam itself has been used as a sedative, but loses activity quite quickly as a result of metabolism and excretion. **Valium,** if it is a prodrug for nordazepam, demonstrates again how a prodrug can be used to lead to a more sustained action.

Another approach to maintaining a sustained level of drug over long periods is to deliberately associate a very lipophilic group to the drug. This means that most of the drug is stored in fat tissue, and if the lipophilic group is only slowly removed, the drug is steadily and slowly released into the bloodstream. The antimalarial agent cycloguanil pamoate (Fig. 14.26) is one such agent. The active drug is bound ionically to an anion with a large lipophilic group.

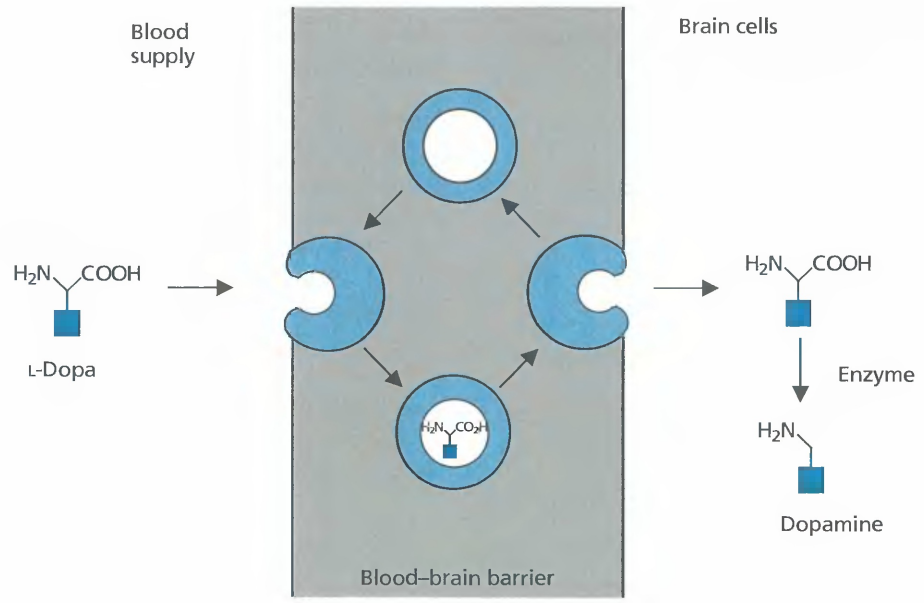


FIGURE 14.23 Transport of levodopa across the blood–brain barrier.

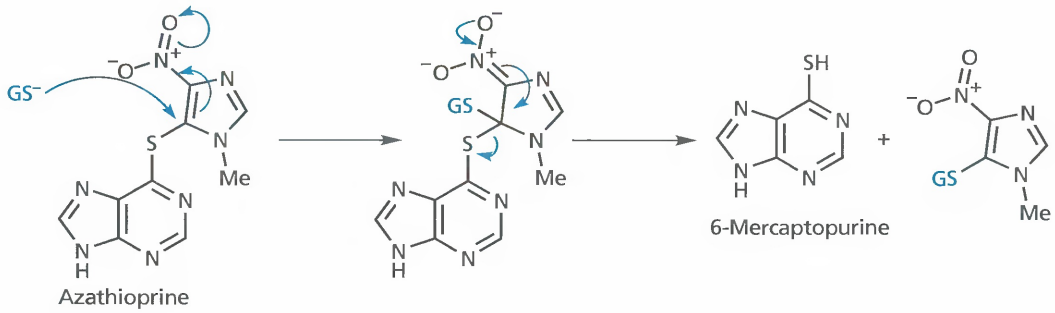


FIGURE 14.24 Azathioprine acts as a prodrug for 6-mercaptopurine (GS = glutathione).

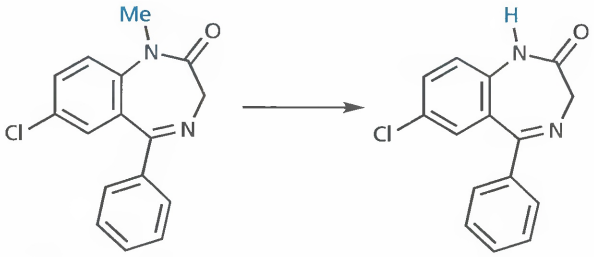


FIGURE 14.25 Valium (diazepam) as a possible prodrug for nordazepam.

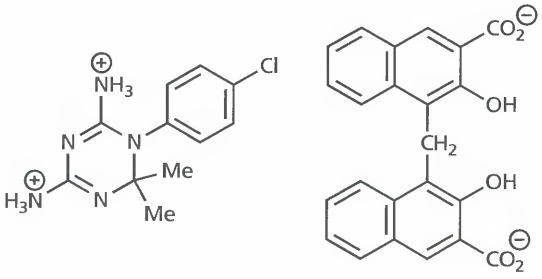


FIGURE 14.26 Cycloguanil pamoate.

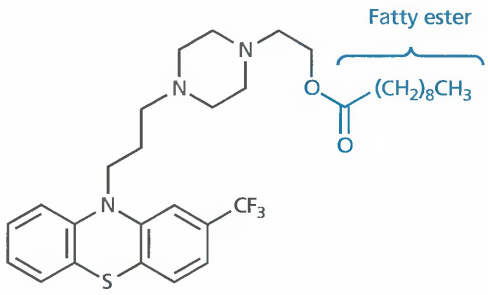


FIGURE 14.27 Fluphenazine decanoate.

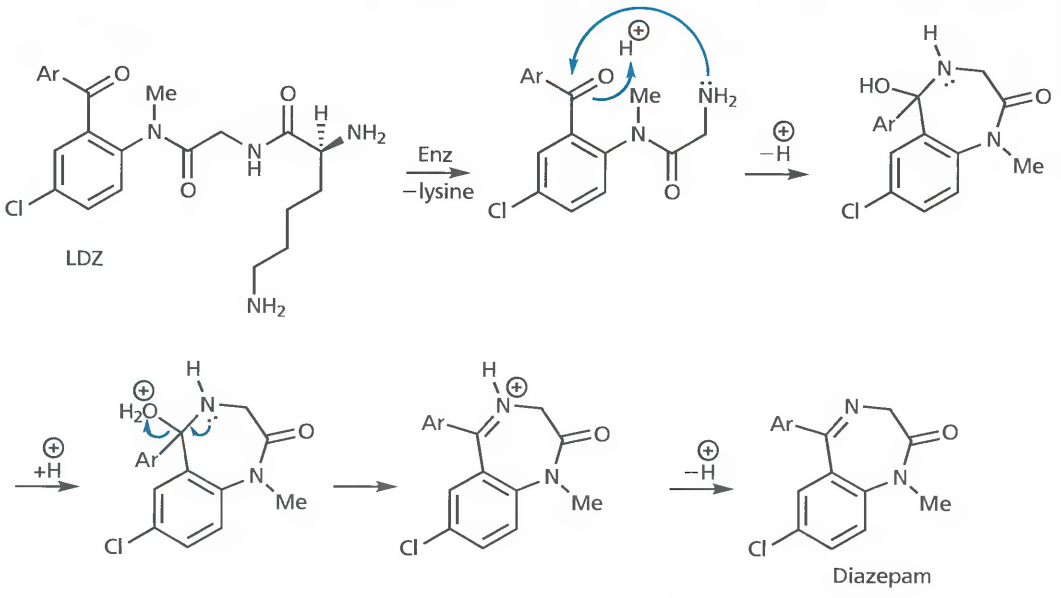
Similarly, lipophilic esters of the antipsychotic drug **fluphenazine** are used to prolong its action. The prodrug is given by intramuscular injection and slowly diffuses from fat tissue into the blood supply where it is rapidly hydrolysed (Fig 14.27).

14.6.3 Prodrugs masking drug toxicity and side effects

Prodrugs can be used to mask the side effects and toxicity of drugs (Box 14.4). For example, **salicylic acid** is a good painkiller, but causes gastric bleeding due to the

BOX 14.4 Prodrugs masking toxicity and side effects

LDZ is an example of a diazepam prodrug that avoids the drowsiness side effects associated with **diazepam.** These side effects are associated with the high initial plasma levels of diazepam following administration. The use of a prodrug avoids this problem. An aminopeptidase enzyme hydrolyses the prodrug to release a non-toxic lysine moiety, and the resulting amine spontaneously cyclizes to the diazepam (as shown below).



LDZ as a diazepam prodrug.

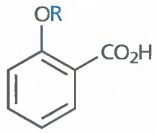


FIGURE 14.28 Aspirin (R=CH_3CO) and salicylic acid (R=H).

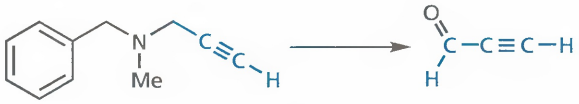


FIGURE 14.29 Pargylene as a prodrug for propiolaldehyde.

free phenolic group. This is overcome by masking the phenol as an ester (**aspirin**) (Fig. 14.28). The ester is later hydrolysed to free the active drug.

Prodrugs can be used to give a slow release of drugs that would be too toxic to give directly. **Propiolaldehyde** is useful in the aversion therapy of alcohol, but is not used itself because it is an irritant. The prodrug **pargylene** can be converted to propiolaldehyde by enzymes in the liver (Fig. 14.29).

Cyclophosphamide is a successful, non-toxic prodrug which can be safely taken orally. Once absorbed, it is metabolized in the liver to a toxic alkylating agent which is useful in the treatment of cancer (section 21.2.3.1).

Many important antiviral drugs such as **aciclovir** and **penciclovir** are non-toxic prodrugs which show selective toxicity towards virally infected cells. This is because they are converted to toxic triphosphates by a viral enzyme which is only present in infected cells (sections 9.5 and 20.6.1). In a similar vein, the antischistosomal agent oxamniquine is converted to an alkylating agent by an enzyme which is only present in the parasite (Case Study 4).

14.6.4 Prodrugs to lower water solubility

Some drugs have a revolting taste! One way to avoid this problem is to reduce their water solubility so they do not dissolve on the tongue. For example, the bitter taste of the antibiotic **chloramphenicol** can be avoided by using the palmitate ester (Fig. 14.30). This is more hydrophobic because of the masked alcohol and the long chain fatty group that forms the ester. It does not dissolve easily on the tongue and is quickly hydrolysed once swallowed.

14.6.5 Prodrugs to improve water solubility

Prodrugs have been used to increase the water solubility of drugs (Box 14.5). This is particularly useful for

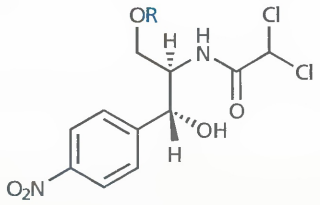
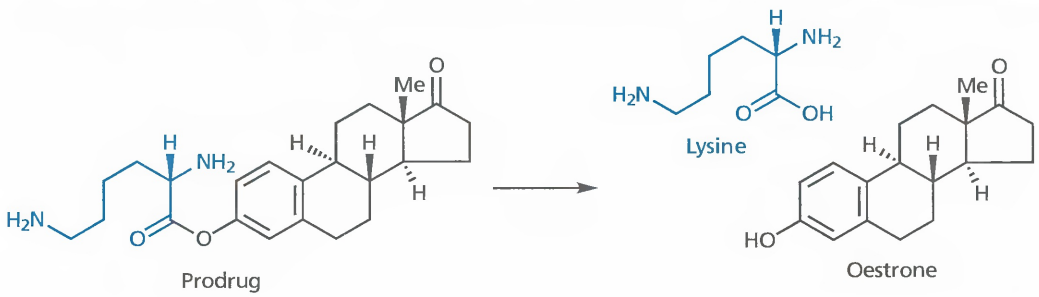


FIGURE 14.30 Chloramphenicol (R=H) and chloramphenicol prodrugs; R=$CO(CH_2)_{14}CH_3$, chloramphenicol palmitate; R=$CO(CH_2)_2CO_2H$, chloramphenicol succinate.

BOX 14.5 Prodrugs to improve water solubility

Polar prodrugs have been used to improve the absorption of non-polar drugs from the gut. Drugs have to have some water solubility if they are to be absorbed, otherwise they dissolve in fatty globules and fail to interact effectively with the gut wall. The steroid **oestrone** is one such drug. By using a lysine ester prodrug, water solubility and absorption is increased. Hydrolysis of the prodrug releases the active drug, and the amino acid lysine as a non-toxic by-product.



The lysine ester of oestrone to improve water solubility and absorption.

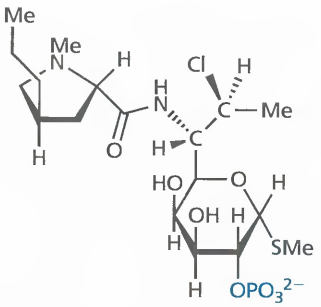


FIGURE 14.31 Clindamycin phosphate.

drugs which are given intravenously, as it means that higher concentrations and smaller volumes can be used. For example, the succinate ester of **chloramphenicol** (Fig. 14.30) increases the latter's water solubility due to the extra carboxylic acid that is present. Once the ester is hydrolysed, chloramphenicol is released along with succinic acid which is naturally present in the body.

Prodrugs designed to increase water solubility have proved useful in preventing the pain associated with some injections, which is caused by the poor solubility of the drug at the site of injection. For example, the antibacterial agent **clindamycin** is painful when injected, but using a phosphate ester prodrug improves solubility because of the ionic phosphate group, and thus prevents the pain (Fig. 14.31).

14.6.6 Prodrugs used in the targeting of drugs

Methenamine (Fig. 14.32) is a stable, inactive compound when the pH is more than 5. At a more acidic pH, however, the compound spontaneously degrades to generate formaldehyde, which has antibacterial properties. This is useful in the treatment of urinary tract infections. The normal pH of blood is slightly alkaline (7.4) and so methenamine passes round the body unchanged. However, once it is excreted into the infected urinary tract, it encounters urine which is acidic as a result of certain bacterial infections. Consequently, methenamine degrades to generate formaldehyde just where it is needed.

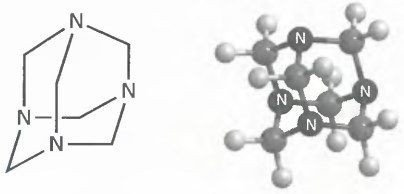


FIGURE 14.32 Methenamine.

Prodrugs have also been used to target sulfonamides against intestinal infections (Box 19.2). Other examples of prodrugs used to target infections are the antischistosomal drug oxamniquine (case study 4) and the antiviral drugs described in sections 9.5 and 20.6.1.

The targeting of prodrugs to tumour cells by antibody-related strategies was mentioned in section 14.4.1 and is described in more detail in section 21.9. Antibody-drug conjugates can also be viewed as prodrugs and are described in that section.

14.6.7 Prodrugs to increase chemical stability

The antibacterial agent **ampicillin** decomposes in concentrated aqueous solution as a result of intramolecular attack of the side chain amino group on the lactam ring. **Hetacillin** (Fig 14.33) is a prodrug which locks up the offending nitrogen in a ring and prevents this reaction. Once the prodrug has been administered, hetacillin slowly decomposes to release ampicillin and acetone.

14.6.8 Prodrugs activated by external influence (sleeping agents)

Conventional prodrugs are inactive compounds which are normally metabolized in the body to the active form. A variation of the prodrug approach is the concept of a 'sleeping agent'. This is an inactive compound which is only converted to the active drug by some form of external influence. The best example of this approach is the use

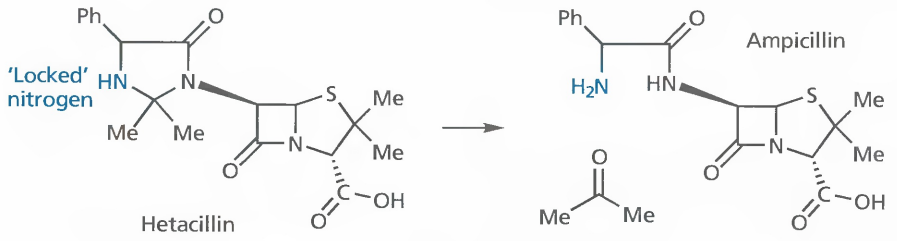


FIGURE 14.33 Hetacillin and ampicillin.

of photosensitizing agents such as porphyrins or chlorins in cancer treatment—a strategy known as photodynamic therapy. Given intravenously, these agents accumulate within cells and have some selectivity for tumour cells. By themselves, the agents have little effect, but if the cancer cells are irradiated with light, the porphyrins are converted to an excited state and react with molecular oxygen to produce highly toxic singlet oxygen. This is covered in section 21.10.

KEY POINTS

- Prodrugs are inactive compounds that are converted to active drugs in the body—usually by drug metabolism.
- Esters are commonly used as prodrugs to make a drug less polar, allowing it to cross cell membranes more easily. The nature of the ester can be altered to vary the rate of hydrolysis.
- Introducing a metabolically susceptible *N*-methyl group can sometimes be advantageous in reducing polarity.
- Prodrugs with a similarity to important biosynthetic building blocks may be capable of crossing cell membranes with the aid of transport proteins.
- The activity of a drug can be prolonged by using a prodrug, which is converted slowly to the active drug.
- The toxic nature of a drug can be reduced by using a prodrug, which is slowly converted to the active compound, preferably at the site of action.
- Prodrugs that contain metabolically susceptible polar groups are useful in improving water solubility. They are particularly useful for drugs that have to be injected, or for drugs that are too hydrophobic for effective absorption from the gut.
- Prodrugs that are susceptible to pH or chemical degradation can be effective in targeting drugs or increasing stability in solution prior to injection.
- Prodrugs that are activated by light are the basis for photodynamic therapy.

14.7 Drug alliances

Some drugs are found to affect the activity or pharmacokinetic properties of other drugs, and this can be put to good use. The following are some examples.

14.7.1 'Sentry' drugs

In this approach, a second drug is administered with the principal drug in order to guard or assist it. Usually, the second drug inhibits an enzyme that metabolizes the principal drug. For example, **clavulanic acid** inhibits the enzyme β-**lactamase** and is therefore able to protect penicillins from that particular enzyme (section 19.5.4.1).

The antiviral drug **Kaletra** used in the treatment of AIDS is a combination of two drugs called **ritonavir** and **lopinavir.** Although the former has antiviral activity, it is principally present to protect lopinavir. Lopinavir is metabolized by the metabolic cytochrome P450 enzyme (CYP3A4). Ritonavir is a strong inhibitor of this enzyme and so the metabolism of lopinavir is decreased allowing lower doses to be used for therapeutic plasma levels (section 20.7.4.4).

Another example is to be found in the drug therapy of Parkinson's disease. The use of **levodopa** as a prodrug for **dopamine** has already been described (section 14.6.1.3). To be effective, however, large doses of levodopa (3–8 g per day) are required, and over a period of time these dose levels lead to side effects such as nausea and vomiting. Levodopa is susceptible to the enzyme **dopa decarboxylase** and as a result, much of the levodopa administered is decarboxylated to dopamine before it reaches the central nervous system (Fig. 14.34). This build-up of dopamine in the peripheral blood supply leads to the observed nausea and vomiting.

The drug **carbidopa** has been used successfully as an inhibitor of dopa decarboxylase and allows smaller doses of levodopa to be used. Furthermore, since it is a highly polar compound containing two phenolic groups, a hydrazine moiety, and an acidic group, it is unable to cross the blood-brain barrier, and so cannot prevent the conversion of levodopa to dopamine in the brain. Carbidopa is marketed as a mixture with levodopa and is called co-careldopa.

Several important peptides and proteins could be used as drugs if it were not for the fact that they are quickly broken down by **protease** enzymes. One way round this problem is to inhibit the protease enzymes. **Candoxatril** (Box 14.3) is a protease inhibitor which has some potential in this respect and is under clinical evaluation.

Finally, the action of penicillins can be prolonged if they are administered alongside probenecid (section 19.5.1.9).

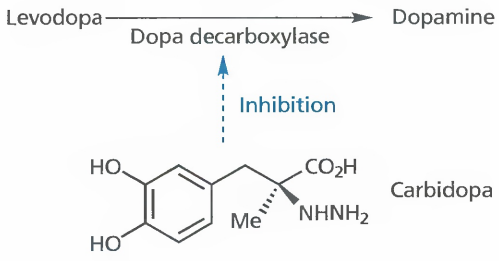


FIGURE 14.34 Inhibition of levodopa decarboxylation.

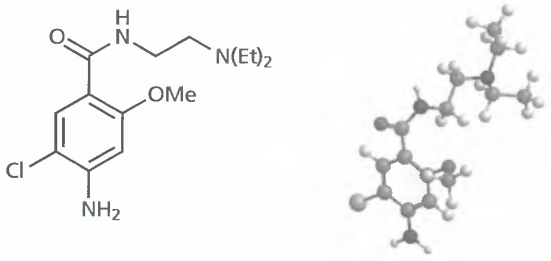


FIGURE 14.35 Metoclopramide.

14.7.2 Localizing a drug's area of activity

Adrenaline is an example of a drug which has been used to localize the area of activity for another drug. When injected with the local anaesthetic **procaine,** adrenaline constricts the blood vessels in the vicinity of the injection and so prevents procaine being rapidly removed from the area by the blood supply.

14.7.3 Increasing absorption

Metoclopramide (Fig. 14.35) is administered alongside analgesics in the treatment of migraine. Its function is to increase gastric motility, leading to faster absorption of the analgesic and quicker pain relief.

KEY POINTS

- A sentry drug is a drug that is administered alongside another drug to enhance the latter's activity.
- Many sentry drugs protect their partner drug by inhibiting an enzyme that acts on the latter.
- Sentry drugs have also been used to localize the site of action of local anaesthetics, and to increase the absorption of drugs from the gastrointestinal tract.

14.8 Endogenous compounds as drugs

Endogenous compounds are molecules which occur naturally in the body. Many of these could be extremely useful in medicine. For example, the body's hormones are natural chemical messengers, so why not use them as medicines instead of synthetic drugs that are foreign to the body? In this section we look at important molecules such as neurotransmitters, hormones, peptides, proteins, and antibodies, and see how feasible it is to use them as drugs.

14.8.1 Neurotransmitters

Many non-peptide neurotransmitters are simple molecules that can easily be prepared in the laboratory, so why are these not used commonly as drugs? For example, if there is a shortage of dopamine in the brain, why not administer more dopamine to make up the balance?

Unfortunately, this is not possible for a number of reasons. Many neurotransmitters are not stable enough to survive the acid of the stomach, and would have to be injected. Even if they were injected, there is little chance that they would survive to reach their target receptors. The body has efficient mechanisms which inactivate neurotransmitters as soon as they have passed on their message from nerve to target cell. Therefore, any neurotransmitter injected into the blood supply would be swiftly inactivated by enzymes or taken up by cells via transport proteins. Even if they were not inactivated or removed, they would be poor drugs indeed, leading to many undesirable side effects. For example, the shortage of neurotransmitter may only be at one small area in the brain; the situation may be normal elsewhere. If we gave the natural neurotransmitter, how would we stop it producing an overdose of transmitter at these other sites? Of course this is a problem with all drugs, but it has been discovered that the receptors for a specific neurotransmitter are not all identical. There are different types and subtypes of a particular receptor, and their distribution around the body is not uniform. One subtype of receptor may be common in one tissue, whereas a different subtype is common in another tissue. The medicinal chemist can design synthetic drugs which take advantage of that difference, ignoring receptor subtypes which the natural neurotransmitter would not. In this respect, the medicinal chemist has actually improved on nature.

We cannot even assume that the body's own neurotransmitters are perfectly safe, and free from the horrors of tolerance and addiction associated with drugs such as **heroin**. It is quite possible to be addicted to one's own neurotransmitters and hormones. Some people are addicted to exercise, and are compelled to exercise long hours each day in order to feel good. The very process of exercise leads to the release of hormones and neurotransmitters which can produce a 'high', and this drives susceptible people to exercise more and more. If they stop exercising, they suffer withdrawal symptoms such as deep depression. The same phenomenon probably drives mountaineers into attempting feats which they know might well lead to their death. The thrill of danger produces hormones and neurotransmitters which in turn produce a 'high'. This may also explain why some individuals choose to become mercenaries and risk their lives travelling the globe in search of wars to fight.

To conclude, many of the body's own neurotransmitters are known and can be easily synthesized, but they cannot be effectively used as medicines.

14.8.2 Natural hormones, peptides and proteins as drugs

Unlike neurotransmitters, natural hormones have potential in drug therapy as they normally circulate round the body and behave like drugs. Indeed, **adrenaline** is commonly used in medicine to treat (among other things) severe allergic reactions (section 23.10.1). Most hormones are peptides and proteins, and some naturally occurring peptide and protein hormones are already used in medicine. These include **insulin**, **calcitonin**, **erythropoietin**, **human growth factor**, **interferons**, and **colony stimulating factors**.

The availability of many protein hormones owes a great deal to genetic engineering (section 6.4). It is extremely tedious and expensive to obtain substantial quantities of these proteins by other means. For example, isolating and purifying a hormone from blood samples is impractical because of the tiny quantities of hormone present. It is far more practical to use **recombinant DNA techniques**, whereby the human genes for the protein are cloned and then incorporated into the DNA of fast-growing bacterial, yeast, or mammalian cells. These cells then produce sufficient quantity of the protein.

Using these techniques, it is also possible to produce 'cut down' versions of important body proteins and polypeptides which can also be used therapeutically. For example, **teriparatide** is a polypeptide which has been approved for the treatment of osteoporosis and which was produced by recombinant DNA technology using a genetically modified strain of the bacterium *Escherichia coli*. It consists of 34 amino acids that represent the *N*-terminal end of human parathyroid hormone (consisting of 84 amino acids). Another recombinant protein that has been approved is **etanercept**, which is used for the treatment of rheumatoid arthritis. More than 80 polypeptide drugs have reached the market as a result of the biotechnology revolution with more to come. A recent addition is **abatacept** which was approved in 2005 for the treatment of rheumatoid arthritis. This disease is caused by T-cells binding and interacting with susceptible cells to cause cell damage and inflammation. The binding process involves a protein–protein interaction between a T-cell protein and a protein in the membrane of the susceptible cell. Abatacept is an agent which mimics the T-cell protein and binds to the susceptible cell before the T-cell does, thus preventing the damage and inflammation that would result from such an interaction. Abatacept was prepared by taking the extracellular portion of the T-cell protein and linking it to part of an antibody. Therefore, it is classed as a **fusion protein**.

Many endogenous peptides and proteins have proved ineffective though. This is because peptides and proteins suffer serious drawbacks such as susceptibility to digestive and metabolic enzymes, poor absorption from the gut, and rapid clearance from the body. Furthermore, proteins are large molecules which could possibly induce an adverse immunological response. This involves the body producing antibodies against the proteins, resulting in serious side effects.

Solutions to some of these problems are appearing, though. It has been found that linking the polymer **polyethylene glycol** (PEG) to a protein can increase the latter's solubility and stability as well as decreasing the likelihood of an immune response (Fig. 14.36). PEGylation, as it is called, also prevents the removal of small proteins from the blood supply by the kidneys or the reticuloendothelial system. The increased size of the PEGylated protein means that it is not filtered into the kidney nephrons and remains in the blood supply.

The PEG molecules surrounding the protein can be viewed as a kind of hydrophilic, polymeric shield which both protects and disguises the protein. The PEG polymer has the added advantage that it shows little toxicity. The enzymes **L-asparaginase** and **adenosine deaminase** have been treated in this way to give protein–PEG conjugates called **pegaspargase** and **pegademase,** which have been used for the treatment of leukaemia and SCID syndrome respectively. (SCID, or severe combined immunodeficiency disease, is an immunological defect associated with a lack of adenosine deaminase.) The conjugates have longer plasma half-lives than the enzymes alone and are less likely to produce an immune response. **Interferon** has similarly been PEGylated to give a preparation called **peginterferon α2b**, which is used for the treatment of hepatitis C.

Pegvisomant is the PEGylated form of **human growth hormone antagonist** and is used for the treatment of a condition known as acromegaly which results in abnormal

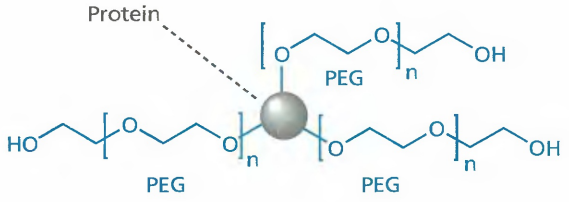


FIGURE 14.36 PEGylated protein.

enlargement of the skull, jaw, hands, and feet due to the excessive production of growth hormone.

Pegfilgrastim is the PEGylated form of **filgrastim** and is used as an anticancer agent. (Filgrastim is recombinant human granulocyte-colony stimulating factor).

PEGylation has also been used to protect liposomes for drug delivery (section 11.9).

14.8.3 Antibodies as drugs

Biotechnology companies are producing an ever increasing number of antibodies and antibody-based drugs with the aid of genetic engineering and monoclonal antibody technology.

Because antibodies can recognize the chemical signature of a particular cell, they have great potential in targeting the immune response against cancer cells. Alternatively, they could be used to carry drugs or poisons to cancer cells (sections 14.4.1 and 21.9). Antibodies that recognize a particular antigen are generated by exposing a mouse to the antigen so that the mouse produces the desired antibodies (known as murine antibodies). However, the antibodies themselves are not isolated. Antibodies are produced by cells called B lymphocytes, and it is a mixture of B lymphocytes that is isolated from the mouse. The next task is to find the B lymphocyte responsible for producing the desired antibody. This is done by fusing the mixture with immortal (cancerous) human B lymphocytes to produce cells called **hybridomas**. These are then separated and cultured. The culture that produces the desired antibody can then be identified by its ability to bind to the antigen, and is then used to produce antibody on a large scale. Since all the cells in this culture are identical, the antibodies produced are also identical and are called **monoclonal antibodies**.

There was great excitement when this technology appeared in the 1980s, and an expectation that antibodies would be the magic bullet to tackle many diseases. Unfortunately, the early antibodies failed to reach the clinic because they triggered an immune response in patients which resulted in antibodies being generated against the antibodies! In hindsight, this is not surprising; the antibodies were mouselike in character and were identified as 'foreign' by the human immune system, resulting in the production of human anti-mouse antibodies (the **HAMA response**).

In order to tackle this problem, **chimeric antibodies** have been produced which are part human (66%) and part mouse in origin, to make them less 'foreign'. Genetic engineering has also been used to generate 'humanized' antibodies which are 90% human in nature. In another approach, genetic engineering has been used to insert the human genes responsible for antibodies into mice such that the mice (transgenic mice) produce human antibodies rather than murine antibodies when they are exposed to the antigen. As a result of these efforts, 10 antibodies had reached the clinic in 2002 and were being used as immune suppressants, antiviral agents (section 20.10.5), and anticancer agents (section 21.9.1). Many others are in the pipeline. **Omalizumab** is an example of a recombinant humanized monoclonal antibody which targets **immunoglobulin E** (IgE) and was approved in 2003 for the treatment of allergic asthmatic disease. It is known that exposure to allergens results in increased levels of IgE, which triggers the release of many of the chemicals responsible for the symptoms of asthma. Omalizumab works by binding to IgE and thus preventing it from acting in this way.

Another example is **adalimumab**, which was launched in 2003 and is the first fully humanized antibody to be approved. It is used for the treatment of rheumatoid arthritis and works by binding to an inflammatory molecule called a cytokine, specifically one called tumour-necrosis factor (TNF-α). Molecules such as these are endogenous chemicals but are overproduced in arthritis, leading to chronic inflammation. By binding to the cytokine, the antibody prevents it interacting with its receptor. The antibody can also tag cells that are producing the chemical messenger leading to the cell's destruction by the body's immune system. **Infliximab** is another monoclonal antibody that targets TNF-α, but this is a chimeric monoclonal antibody and there is greater chance of the body developing an immune response against it during long-term use.

Work on the large-scale production of antibodies has also been continuing. They have traditionally been produced using hybridoma cells in bioreactors, but more recently, companies have been looking at the possibility of using transgenic animals so that the antibodies can be collected in milk. Another possibility is to harvest transgenic plants which produce the antibody in their leaves or seeds.

A different approach to try and prevent antibodies producing an immune response has been to treat them with PEG (section 14.8.2). Unfortunately, this tends to be counterproductive, as it prevents the antibody acting out its role as a targeting molecule. However, controlling the PEGylation such that it only occurs on the thiol group of cysteine residues could be beneficial, as it would limit the number of PEG molecules attached and make it more likely that the antibody remains functional.

14.9 Peptides and peptidomimetics in drug design

Endogenous peptides and proteins serve as highly important lead compounds for the design of novel drugs. Current examples include renin inhibitors (section 7.4), protease inhibitors (section 20.7.4), LHRH agonists (section 21.4.2), matrix metalloproteinase inhibitors (section 21.7.1), and enkephalin analogues (section 24.8.2). Peptides will continue to be important lead compounds, as many of the new targets in medicinal chemistry, such as the protein kinases, involve proteins as substrates. Consequently, drugs which are designed from these lead compounds are commonly peptide-like in nature. The pharmacokinetic properties of these 'first-generation' drugs are often unsatisfactory and so various strategies have been developed to disguise the peptide nature of these drugs and to design an orally active molecule that is more stable to digestive and metabolic enzymes, and is more easily absorbed, so that it attains an acceptable level in the blood supply. Such analogues are known as **peptidomimetics**, and the fraction of an orally administered peptidomimetic that reaches the blood supply is known as its **bioavailability**.

14.9.1 Peptidomimetics

One approach that is used to increase bioavailability is to replace a chemically or enzymatically susceptible peptide bond with a functional group that is more stable to hydrolytic attack by peptidase enzymes, or binds less readily to these enzymes. For example, a peptide bond might be replaced by an alkene (Fig. 14.37). If the compound retains activity, then the alkene represents a **bioisostere** for the peptide link. An alkene has the advantage that it mimics the double bond nature of a peptide bond and is not a substrate for peptidases. However, the peptide bonds in lead compounds are often involved in hydrogen bond interactions with the target binding site, where the NH acts as a hydrogen bond donor and the carbonyl C=O acts as a hydrogen bond acceptor. Replacing both of these groups may result in a significant drop in binding strength. Therefore, an alternative approach might be to replace the amide with a ketone or an amine such that only one possible interaction is lost. The problem now is that the double bond nature of the original amide group is lost, resulting in greater chain flexibility and a possible drop in binding affinity (section 13.3.9). A thioamide group is another option. This group retains the planar shape of the amide, and the NH moiety can still act as a hydrogen bond donor. The sulfur is a poor hydrogen bond acceptor, but this could be advantageous if the original carbonyl oxygen forms a hydrogen bond to the active site of peptidase enzymes.

A different approach is to retain the amide but to protect it or disguise it. One strategy that has been used successfully is to methylate the nitrogen of the amide group. The methyl group may help to protect the amide from hydrolysis by acting as a steric shield, or prevent an important hydrogen bonding interaction taking place between the NH of the original amide and the active site of the peptidase enzyme which would normally hydrolyse it.

A second strategy is to replace an L-amino acid with the corresponding D-enantiomer (Fig. 14.38). Such a move alters the relative orientation of the side chain with respect to the rest of the molecule and can make the molecule unrecognizable to digestive or metabolic enzymes, especially if the side chain is involved in binding interactions. The drawback to this strategy is that the resulting peptidomimetic may become unrecognizable to the desired target as well.

A third strategy is to replace natural amino acid residues with unnatural ones. This is a tactic that has

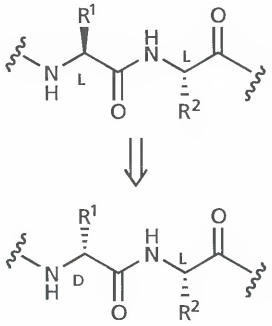


FIGURE 14.38 Replacing an L-amino acid with a D-amino acid. The common L-amino acids have the *R*-configuration except for L-cysteine which has the *S*-configuration.

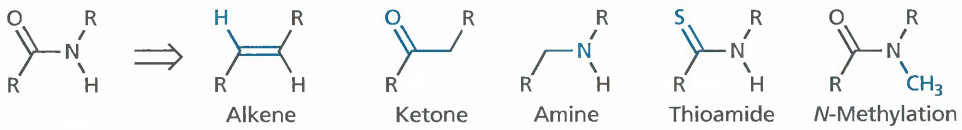


FIGURE 14.37 Examples of functional groups that might be used to replace a peptide bond.

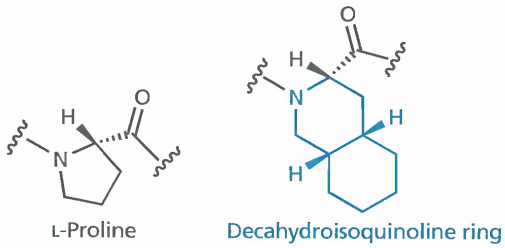


FIGURE 14.39 Replacing a natural residue with an unnatural one.

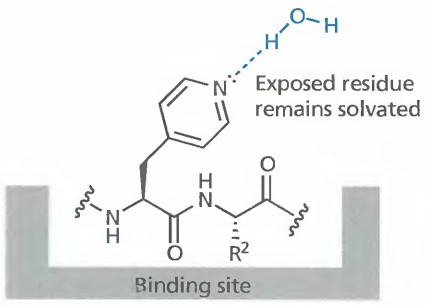


FIGURE 14.41 Altering exposed residues to increase water solubility.

worked successfully in structure-based drug design where the binding interactions of the peptidomimetic and a protein target are studied by X-ray crystallography and molecular modelling. The idea is to identify binding subsites in the target binding site into which various amino acid residues fit and bind. The residues are then replaced by groups which are designed to fit the subsites better, but which are not found on natural amino acids. This increases the binding affinity of the peptidomimetic to the target binding site, and at the same time, makes it unrecognizable to digestive and metabolic enzymes. For example, the lead compound for the antiviral drug **saquinavir** contained an L-proline residue that occupied a hydrophobic subsite of a viral protease enzyme. The proline residue was replaced by a decahydroisoquinoline ring which filled the hydrophobic subsite more fully, resulting in better binding interactions (Fig. 14.39) (see also section 20.7.4.3).

It is even possible to design extended groups which fill two different subsites (Fig. 14.40). This means that the peptidomimetic can be pruned to a smaller molecule. The resulting decrease in molecular weight often leads to better absorption (sections 20.7.4.6 and 20.7.4.7).

Peptidomimetics are often lipophilic in nature, and this can pose a problem because poor water solubility may result in poor oral absorption. Water solubility can be increased by increasing the polarity of residues. For example, an aromatic ring could be replaced by a pyridine ring. However, it is important that this group is not involved in any binding interactions with the target and remains exposed to the surrounding water medium when the peptidomimetic is bound (Fig. 14.41). Otherwise it would have to be desolvated and this would carry an energy penalty which would result in a decreased binding affinity.

Another potential problem with peptidomimetics is that they can often be flexible molecules with a large number of freely rotatable bonds. Flexibility has been shown to be detrimental to oral bioavailability (section 11.2), and so rigidification tactics (section 13.3.9) may well be beneficial.

The structure-based design of protease inhibitors and matrix metalloproteinase inhibitors is described in sections 20.7.4 and 21.7.1 respectively, and illustrates many of the principles described above.

Finally, there is current research into designing structures which mimic particular features of protein secondary structure such as α-helices, β-sheets and β-turns (section 10.5). The goal here is to design molecules with substituents that will mimic the substituents of amino acids, and to use a stable molecular scaffold that will position the substituents in the same relative positions as amino acid residues in common protein features. This might be useful in designing peptidomimetics that mimic peptide neurotransmitters or peptide hormones. For example, it is found that such messengers adopt a

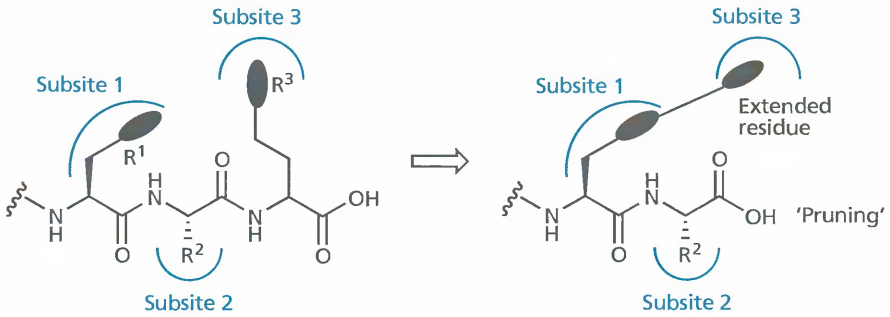


FIGURE 14.40 Extended residues.

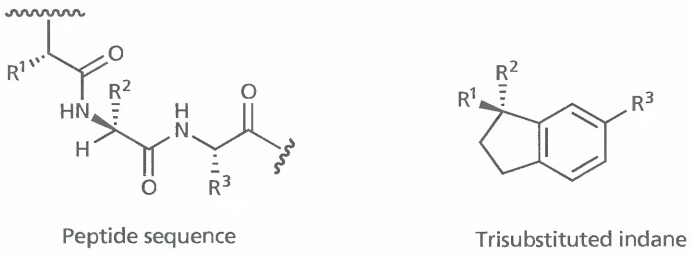


FIGURE 14.42 Trisubstituted indanes as a peptidomimetic for a tripeptide sequence in an α-helix.

helical conformation when they bind to their receptor. 1,1,6-Trisubstituted indanes have been designed to mimic three consecutive amino acid residues in an α-helix (Fig. 14.42).

14.9.2 Peptide drugs

As stated above, there is often a reluctance to use peptides as drugs because of the many pharmacokinetic difficulties that can be encountered, but this does not mean that peptide drugs have no role to play in medicinal chemistry. For example, the immunosuppressant **ciclosporin** can be administered orally (section 11.2). Another important peptide drug is **goserelin** (Fig. 14.43), which is administered as a subcutaneous implant and is used against breast and prostate cancers, earning $700 million dollars a year for its maker. In 2003, **enfuvirtide** (Fuzeon) was approved as the first of a new class of anti-HIV drugs (section 20.7.5). It is a polypeptide of 36 amino acids which is injected subcutaneously and offers another weapon in the combination therapies used against HIV. **Teriparatide,** which was mentioned in section 14.8.2, is also administered by subcutaneous injection. Peptide drugs can be useful if one chooses the right disease and method of administration.

14.10 Oligonucleotides as drugs

Oligonucleotides are being studied as antisense drugs and aptamers. The rationale and therapeutic potential of these agents are described in sections 9.7.2 and 10.5. However, there are disadvantages to the use of oligonucleotides as drugs, as they are rapidly degraded by enzymes called **nucleases.** They are also large and highly charged, and are not easily absorbed through cell membranes. Attempts to stabilize these molecules, and to reduce their polarity have involved modifying the phosphate linkages in the sugar–phosphate backbone. For example, phosphorothioates and methylphosphonates have been extensively studied, and oligonucleotides containing these linkages show promise as therapeutic agents (Fig. 14.44). An antisense oligonucleotide with such a modified backbone has been approved as an antiviral drug (section 20.6.3). Alterations to the sugar moiety have also been tried. For example, placing a methyl group at position 2′, or using the α-anomer of a deoxyribose sugar, increases resistance to nucleases. Bases have also been modified to improve and increase the number of hydrogen bonding interactions with target nucleic acids.

The biopharmaceutical company Genta has developed an antisense drug called **oblimersen** which consists

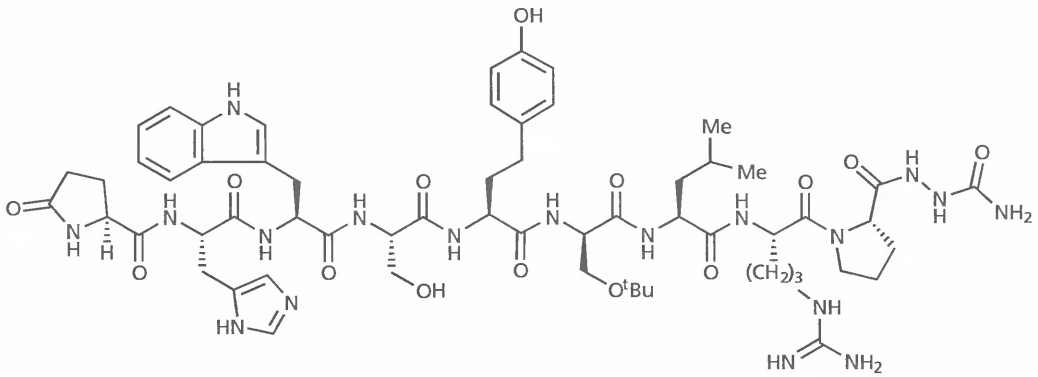


FIGURE 14.43 Goserelin (Zoladex).

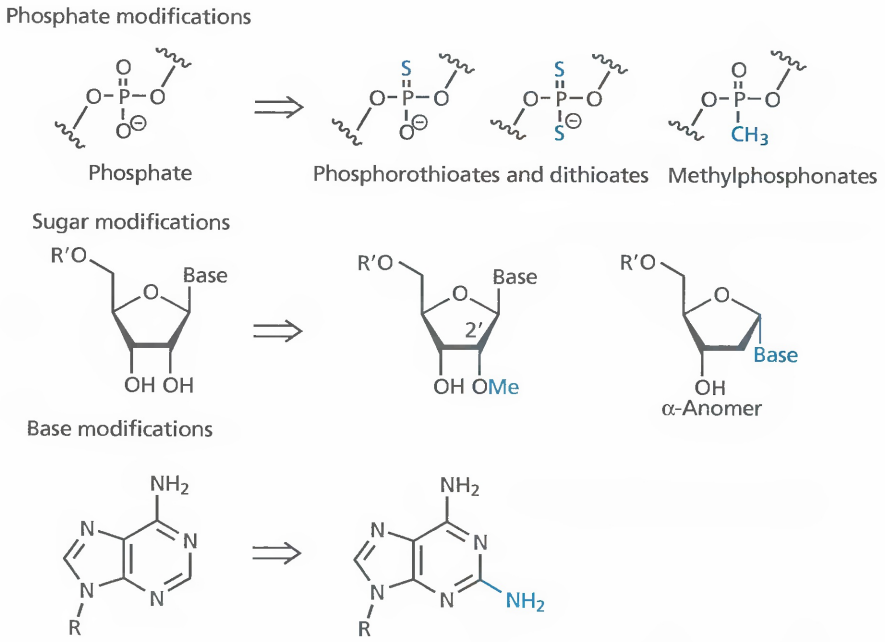


FIGURE 14.44 Modifications on oligonucleotides.

of 18 deoxynucleotides linked by a phosphorothioate backbone. It binds to the initiation codon of the messenger RNA molecule carrying the genetic instructions for Bcl-2. Bcl-2 is a protein which suppresses cell death (apoptosis), and so suppressing its synthesis will increase the chances of apoptosis taking place when chemotherapy or radiotherapy is being used for the treatent of cancer. The drug is currently undergoing Phase III clinical trials in combination with the anticancer drugs **docetaxel** and **irenotecan.**

Phosphorothioate oligonucleotides are also being investigated which will target the genetic instructions for Raf and PKCγ, two proteins which are involved in signal transduction pathways. These too have potential as anticancer drugs.

KEY POINTS

- Neurotransmitters are not effective as drugs as they have a short lifetime in the body, and have poor selectivity for the various types and subtypes of a particular target.
- Hormones are more suitable as drugs and several are used clinically. Others are susceptible to digestive or metabolic enzymes, and show poor absorption when taken orally. Adverse immune reactions are possible.
- Peptides and proteins generally suffer from poor absorption or metabolic susceptibility. Peptidomimetics are compounds that are derived from peptide lead compounds, but have been altered to disguise their peptide character.
- Many of the body's hormones are peptides and proteins and can be produced by recombinant DNA techniques. However, there are several disadvantages in using such compounds as drugs.
- Antibodies are proteins which are important to the body's immune response and which can identify foreign cells or macromolecules, marking them for destruction. They have been used therapeutically and can also be used to carry drugs to specific targets.
- Oligonucleotides are susceptible to metabolic degradation, but can be stabilized by modifying the sugar–phosphate backbone so that it is no longer recognized by relevant enzymes.
- Antisense molecules have been designed to inhibit the mRNA molecules that code for the proteins that suppress cell death.

QUESTIONS

1. Suggest a mechanism by which methenamine (Fig. 14.32) is converted to formaldehyde under acid conditions.

2. Suggest a mechanism by which ampicillin (Fig. 14.33) decomposes in concentrated solution.

3. Carbidopa (Fig. 14.34) protects levodopa from decarboxylation in the peripheral blood supply, but is too polar to cross the blood–brain barrier into the central nervous system. Carbidopa is reasonably similar in structure to levodopa so why can it not mimic levodopa and cross the blood–brain barrier by means of a transport protein?

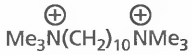
4. Acetylcholine (Fig. 4.3) is a neurotransmitter that is susceptible to chemical and enzymatic hydrolysis. Suggest strategies that could be used to stabilize the ester group of acetylcholine and show the sort of analogues that might have better stability.

5. Decamethonium is a neuromuscular blocking agent that requires both positively charged nitrogen groups to be active. Unfortunately, it is slowly metabolized and lasts too long in the body. Suggest analogues that might be expected to be metabolized more quickly and lead to inactive metabolites.

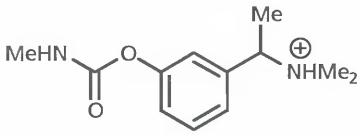
6. Miotine has been used in the treatment of a muscle-wasting disease, but there are side effects because a certain amount of the drug enters the brain. Suggest how one might modify the structure of miotine to eliminate this side effect.

7. The oral bioavailability of the antiviral drug aciclovir is only 15–30%. Suggest why this may be the case, and how one might increase the bioavailability of this drug.

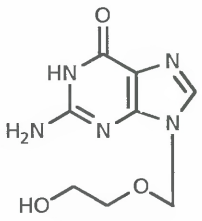
8. The tetrapeptide H–Cys–Val–Phe–Met–OH is susceptible to metabolism by an aminopeptidase enzyme, which hydrolyses the peptide bond between cysteine and valine. In order to prevent this, an analogue lacking the carbonyl group in blue was prepared. A solution of this compound was found to be chemically unstable and the amino acid methionine was identified as being present in the solution. What was the rationale for preparing this analogue, and why did it prove unstable?



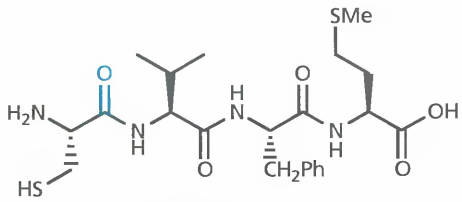
Decamethonium



Miotine

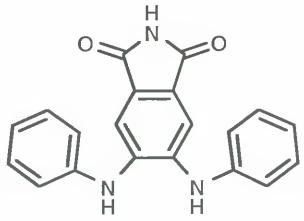


Aciclovir



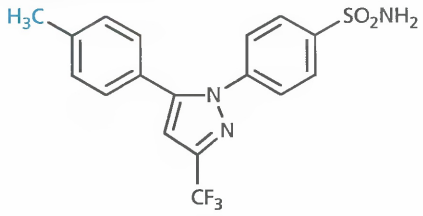
H-Cys-Val-Phe-Met-OH

9. CGP 52411 is a useful inhibitor of a protein kinase enzyme. Studies on structure-activity relationships demonstrate that substituents on the aromatic rings such as Cl, Me, or OH are bad for activity. Drug metabolism studies show that *para*-hydroxylation occurs to produce inactive metabolites. How would you modify the structure to protect it from metabolism?

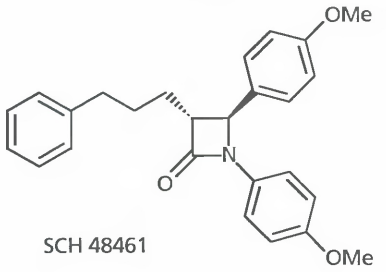


CGP52411

Methyl substituent



Celecoxib



SCH 48461

10. Celecoxib is a COX-2 inhibitor and contains a methyl substituent on the phenyl ring. It is known that inhibitory activity increases if this methyl substituent is not present, or if it is replaced with a chloro substituent. However, neither of these analogues were used clinically. Why not?

11. SCH 48461 has been found to lower cholesterol levels by inhibiting cholesterol absorption. Unfortunately, it is susceptible to metabolism. Identify the likely metabolic reactions which this molecule might undergo, and what modifications could be made to reduce metabolic susceptibility.

FURTHER READING

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