11 Pharmacokinetics and related topics

11.1 The three phases of drug action

There are three phases involved in drug action. The first of these is the **pharmaceutical phase**. For an orally administered drug, this includes the disintegration of a pill or capsule in the **gastrointestinal tract** (GIT), the release of the drug, and its dissolution. The pharmaceutical phase is followed by the **pharmacokinetic phase**, which includes absorption from the GIT into the blood supply, and the various factors that affect a drug's survival and progress as it travels to its molecular target. The final **pharmacodynamic phase** involves the mechanism by which a drug interacts with its molecular target and the resulting pharmacological effect.

In previous chapters, we have focused on drug targets and drug design, where the emphasis is on the pharmacodynamic aspects of drug action, for example optimizing the binding interactions of a drug with its target. However, the compound with the best binding interactions for a target is not necessarily the best drug to use in medicine. This is because a drug has to reach its target in the first place if it is to be effective. Therefore, when carrying out a drug design programme, it is important to study pharmacokinetics alongside pharmacodynamics. The four main topics to consider in pharmacokinetics are: absorption, distribution, metabolism, and excretion (often abbreviated to ADME).

11.2 A typical journey for an orally active drug

The preferred method of drug administration is the oral route, and so we shall consider some of the hurdles and hazards faced by such a drug in order to reach its eventual target. When a drug is swallowed, it enters the GIT, which comprises the mouth, throat, stomach, and the upper and lower intestines. A certain amount of the drug may be absorbed through the mucosal membranes of the mouth, but most passes down into the stomach where it encounters gastric juices and hydrochloric acid. These chemicals aid in the digestion of food and will treat a drug in a similar fashion if it is susceptible to breakdown and is not protected within an acid-resistant pill or capsule. For example, the first clinically useful penicillin was broken down in the stomach and had to be administered by injection. Other acid-labile drugs include the **local anaesthetics** and **insulin**. If the drug *does* survive the stomach, it enters the upper intestine where it encounters digestive enzymes that serve to break down food. Assuming the drug survives this attack, it then has to pass through the cells lining the gut wall. This means that it has to pass through a cell membrane on two occasions: first to enter the cell and then to exit it on the other side. Once the drug has passed through the cells of the gut wall, it can enter the blood supply relatively easily, as the cells lining the blood vessels are loose fitting and there are pores through which most drugs can pass. In other words, drugs enter blood vessels by passing between cells, rather than through them.

The drug is now transported in the blood to the body's 'customs office'—the liver. The liver contains enzymes that are ready and waiting to intercept foreign chemicals, and modify them such that they are more easily excreted—a process called drug metabolism (section 11.5). Following this, the drug has to be carried by the blood supply around the body to reach its eventual target, which may require crossing further cell membranes—always assuming that it is neither excreted before it gets there nor diverted to parts of the body where it is not needed.

It can be seen that stringent demands are made on any orally administered drug. It must be stable to both chemical and enzymatic attack. It must also have the correct physicochemical properties to allow it to reach its target in therapeutic concentrations. This includes efficient absorption, effective distribution to target tissues, and an acceptable rate of excretion. We will now look more closely at the various stages.

11.3 Drug absorption

In order to be absorbed efficiently from the GIT, a drug must have the correct balance of water versus fat solubility. On one hand, if the drug is too polar (hydrophilic), it will fail to pass through the fatty cell membranes of the gut wall (section 1.2.1). On the other hand, if the drug is too fatty (hydrophobic), it will be poorly soluble in the gut and will dissolve in fat globules. This means that there will be poor surface contact with the gut wall, resulting in poor absorption.

It is noticeable how many drugs contain an amine functional group. There are good reasons for this. Amines are often involved in a drug's binding interactions with its target. However, they are also an answer to the problem of balancing the dual requirements of water and fat solubility. Amines are weak bases and it is found that many of the most effective drugs contain amine groups having a **pK_a** value in the range 6–8. In other words, they are partially ionized at the slightly acidic and alkaline pHs present in the intestine and blood, respectively, and can easily equilibrate between their ionized and non-ionized forms. This allows them to cross cell membranes in the non-ionized form, while the presence of the ionized form gives the drug good water solubility and permits good binding interactions with its target binding site (Fig. 11.1).

The extent of ionization at a particular pH can be determined by the **Henderson–Hasselbalch equation**:

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

where $[\mathrm{RNH_2}]$ is the concentration of the free base and $[\mathrm{RNH_3^+}]$ is the concentration of the ionized amine. K_a is the equilibrium constant for the equilibrium shown in Fig. 11.1 and the Henderson–Hasselbalch equation can be derived from the equilibrium constant:

$$K_a = \frac{[\mathrm{H^+}][\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

$$\text{Therefore } \mathrm{p}K_a = -\log\frac{[\mathrm{H^+}][\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

$$= -\log[\mathrm{H^+}] - \log\frac{[\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

$$= \mathrm{pH} - \log\frac{[\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

$$\text{Therefore } \mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{RNH_2}]}{[\mathrm{RNH_3^+}]}$$

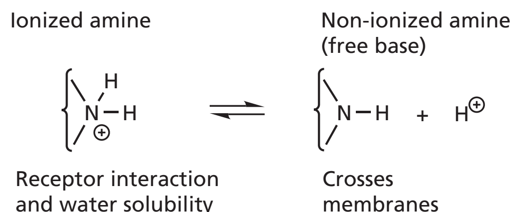


FIGURE 11.1 Equilibrium between the ionized and non-ionized form of an amine.

Note that when the concentrations of the ionized and unionized amines are identical (i.e. when $[\mathrm{RNH_2}] = [\mathrm{RNH_3^+}]$), the ratio ($[\mathrm{RNH_2}]/[\mathrm{RNH_3^+}]$) is 1. As log 1 = 0, the Henderson–Hasselbalch equation will simplify to pH = pK_a. In other words, when the amine is 50% ionized, pH = pK_a. Therefore, drugs with a pK_a of 6–8 are approximately 50% ionized at blood pH (7.4) or the slightly acidic pH of the intestines.

The hydrophilic/hydrophobic character of the drug is the crucial factor affecting absorption through the gut wall; in theory, the molecular weight of the drug should be irrelevant. For example, **ciclosporin** is successfully absorbed through cell membranes although it has a molecular weight of about 1200. In practice, however, larger molecules tend to be poorly absorbed. As a rule of thumb, orally absorbed drugs tend to obey what is known as **Lipinski's rule of five**. The rule of five was derived from an analysis of compounds from the World Drugs Index database aimed at identifying features that were important in making a drug orally active. It was found that the factors concerned involved numbers that are multiples of five:

- a molecular weight less than 500;
- no more than 5 hydrogen bond donor (HBD) groups;
- no more than 10 hydrogen bond acceptor groups;
- a calculated **log *P*** value less than +5 (log *P* is a measure of a drug's hydrophobicity—section 14.1).

The rule of five has been an extremely useful rule of thumb for many years, but it is neither quantitative nor foolproof. For example, orally active drugs, such as **atorvastatin**, **rosuvastatin**, **ciclosporin**, and **vinorelbine**, do not obey the rule of five. It has also been demonstrated that a high molecular weight does not in itself cause poor oral bioavailability. One of the reasons that the molecular weight appears to be important is that larger molecules invariably have too many functional groups capable of forming hydrogen bonds. Another source of debate concerns the calculation of the number of hydrogen bond acceptors (HBAs). In Lipinski's original paper, the number of HBAs corresponded to the total number of oxygen

and nitrogen atoms present in a structure. This was done for simplicity's sake, but most medicinal chemists would discount weak HBAs, such as amide nitrogens (see also section 1.3.2 and Appendix 7). Therefore, it is better to view Lipinski's rules as a set of guidelines rather than rules. Lipinski himself stated that a compound was likely to be orally active as long as it did not break more than one of his 'rules'.

Further research has been carried out to find guidelines that are independent of molecular weight. Work carried out by Veber et al. (2002) demonstrated the rather surprising finding that molecular flexibility plays an important role in oral bioavailability; the more flexible the molecule, the less likely it is to be orally active. In order to measure flexibility, one can count the number of freely rotatable bonds that result in significantly different conformations. Bonds to simple substituents, such as methyl or alcohol groups, are not included in this analysis as their rotation does not result in significantly different conformations.

Veber's studies also demonstrated that the polar surface area of the molecule could be used as a factor instead of the number of hydrogen bonding groups. These findings led to the following parameters for predicting acceptable oral activity. Either:

- a polar surface area ≤140 Å and ≤10 rotatable bonds

or

- ≤12 HBDs and acceptors in total and ≤10 rotatable bonds.

Some researchers set the limit of rotatable bonds to ≤7 as analysis shows a marked improvement in oral bioavailability for such molecules.

These rules are independent of molecular weight and open the way to studying larger structures that have been 'shelved' up to now. Unfortunately, structures that have a molecular weight of larger than 500 are quite likely to have more than 10 rotatable bonds. However, the new rules suggest that rigidifying the structures to reduce the number of rotatable bonds would be beneficial. Rigidification tactics are described in section 13.3.9 as a strategy to improve a drug's pharmacodynamic properties, but these same tactics could also be used to improve pharmacokinetic properties. Appendix 9 provides information on MWt, log P, HBDs, HBAs, rotatable bonds, and polar surface area for drugs covered in this text.

Polar drugs that break the above rules are usually poorly absorbed and have to be administered by injection. Nevertheless, some highly polar drugs *are* absorbed from the digestive system as they are able to 'hijack' **transport proteins** present in the membranes of cells lining the gut wall (sections 2.7.2 and 10.1). These transport proteins normally transport the highly polar building blocks required for various biosynthetic pathways (e.g. amino acids and nucleic acid bases) across cell membranes. If the drug bears a structural resemblance to one of these building blocks then it, too, may be smuggled across. For example, **levodopa** is transported by the transport protein for the amino acid phenylalanine, while **fluorouracil** is transported by transport proteins for the nucleic acid bases thymine and uracil. The antihypertensive agent **lisinopril** is transported by transport proteins for dipeptides. The anticancer agent **methotrexate** and the antibiotic **erythromycin** are also absorbed by means of transport proteins.

Other highly polar drugs can be absorbed into the blood supply if they have a low molecular weight (less than 200), as they can then pass through small pores between the cells lining the gut wall.

Occasionally, polar drugs with high molecular weight can cross the cells of the gut wall without actually passing through the membrane. This involves a process known as **pinocytosis**, where the drug is engulfed by the cell membrane and a membrane-bound **vesicle** is pinched off to carry the drug across the cell (Fig. 11.2). The vesicle then fuses with the membrane to release the drug on the other side of the cell.

Sometimes, drugs are deliberately designed to be highly polar so that they are *not* absorbed from the GIT. These are usually antibacterial agents targeted against gut infections. Making them highly polar ensures that the drug reaches the site of infection in higher concentration (Box 19.2).

Finally, it should be noted that the absorption of some drugs can be affected adversely by interactions with food or other drugs in the gut (section 11.7.1).

Other drug administration routes may involve an absorption process. This is discussed in section 11.7.

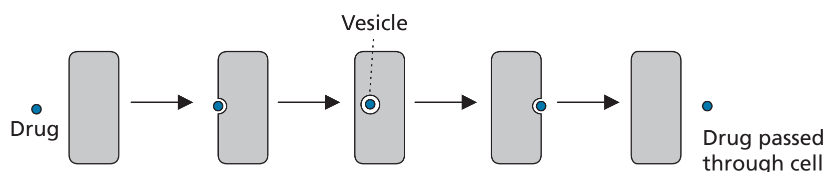


FIGURE 11.2 Pinocytosis.

11.4 Drug distribution

Once a drug has been absorbed it is rapidly distributed around the blood supply, then distributed more slowly to the various tissues and organs. The rate and extent of distribution depends on various factors, including the physical properties of the drug itself.

11.4.1 Distribution around the blood supply

The vessels carrying blood around the body are called **arteries**, **veins**, and **capillaries**. The heart is the pump that drives the blood through these vessels. The major artery carrying blood from the heart is called the **aorta** and, as it moves further from the heart, it divides into smaller and smaller arteries—similar to the limbs and branches radiating from the trunk of a tree. Eventually, the blood vessels divide to such an extent that they become extremely narrow—equivalent to the twigs of a tree. These blood vessels are called capillaries and it is from them that oxygen, nutrients, and drugs can escape in order to reach the tissues and organs of the body. At the same time, waste products, such as cell breakdown products and carbon dioxide, are transferred from the tissues into the capillaries to be carried away and disposed of. The capillaries now start uniting into bigger and bigger vessels, resulting in the formation of veins which return the blood to the heart.

Once a drug has been absorbed into the blood supply, it is rapidly and evenly distributed throughout the blood supply within a minute—the time taken for the blood volume to complete one circulation. However, this does not mean that the drug is evenly distributed around the body—the blood supply is richer to some areas of the body than to others.

11.4.2 Distribution to tissues

Drugs do not stay confined to the blood supply. If they did, they would be of little use as their targets are the cells of various organs and tissues. The drug has to leave the blood supply in order to reach those targets. The body has an estimated 10 billion capillaries with a total surface area of 200 m^2. They probe every part of the body, such that no cell is more than 20–30 μm away from a capillary. Each capillary is very narrow—not much wider than the red blood cells that pass through it. Its walls are made up of a thin, single layer of cells packed tightly together. However, there are pores between the cells which are 90–150 Å in diameter—large enough to allow most drug-sized molecules to pass though, but not large enough to allow the **plasma proteins** present in blood to escape. Therefore, drugs do not have to cross cell membranes in order to leave the blood system, and can be freely and rapidly distributed into the aqueous fluid surrounding the various tissues and organs of the body. Having said that, some drugs bind to plasma proteins in the blood. As the plasma proteins cannot leave the capillaries, the proportion of drug bound to these proteins is also confined to the capillaries and cannot reach its target.

11.4.3 Distribution to cells

Once a drug has reached the tissues, it can immediately be effective if its target site is a receptor situated in a cell membrane. However, there are many drugs that have to enter the individual cells of tissues in order to reach their target. These include local anaesthetics, enzyme inhibitors, and drugs which act on nucleic acids or intracellular receptors. Such drugs must be hydrophobic enough to pass through the cell membrane unless they are smuggled through by carrier proteins or taken in by pinocytosis.

11.4.4 Other distribution factors

The concentration levels of free drug circulating in the blood supply rapidly fall away after administration as a result of the distribution patterns described above. But, there are other factors at work. Drugs that are excessively hydrophobic are often absorbed into fatty tissues and removed from the blood supply. This fat solubility can lead to problems. For example, obese patients undergoing surgery require a larger than normal volume of general anaesthetic because the gases used are particularly fat soluble. Unfortunately, once surgery is over and the patient has regained consciousness, the anaesthetics stored in the fat tissues will be released and may render the patient unconscious again. **Barbiturates** were once seen as potential intravenous anaesthetics which could replace the anaesthetic gases. Unfortunately, they, too, are fat soluble and it is extremely difficult to estimate a sustained safe dosage. The initial dose can be estimated to allow for the amount of barbiturate taken up by fat cells, but further doses lead eventually to saturation of the fat depot and result in a sudden, and perhaps, fatal increase of barbiturate levels in the blood supply.

Ionized drugs may be bound to various macromolecules and also removed from the blood supply. Drugs may also be bound reversibly to blood plasma proteins such as **albumin**, thus lowering the level of free drug. Therefore, only a small proportion of the drug that has been administered may actually reach the desired target.

11.4.5 Blood–brain barrier

The **blood–brain barrier** is an important barrier that drugs have to negotiate if they are to enter the brain. The blood capillaries feeding the brain are lined with tight-fitting cells

which do not contain pores (unlike capillaries elsewhere in the body). Moreover, the capillaries are coated with a fatty layer formed from nearby cells, providing an extra fatty barrier through which drugs have to cross. Therefore, drugs entering the brain have to dissolve through the cell membranes of the capillaries and also through the fatty cells coating the capillaries. As a result, polar drugs, such as **penicillin**, do not enter the brain easily.

The existence of the blood–brain barrier makes it possible to design drugs that will act at various parts of the body (e.g. the heart) and have no activity in the brain, thus reducing any central nervous system (CNS) side effects. This is done by increasing the polarity of the drug such that it does not cross the blood–brain barrier. However, drugs that are intended to act in the brain must be designed such that they *are* able to cross the blood–brain barrier. This means that they must have a minimum number of polar groups or have these groups masked temporarily (see prodrugs; section 14.6). Having said that, some polar drugs can cross the blood–brain barrier with the aid of carrier proteins, while others (e.g. **insulin)** can cross by the process of pinocytosis described previously. The ability to cross the blood–brain barrier has an important bearing on the analgesic activity of opioids (section 24.5). Research is also being carried out to find ways of increasing the permeability of the blood–brain barrier using techniques such as ultrasound or drugs such as **sildenafil.**

11.4.6 Placental barrier

The placental membranes separate a mother's blood from the blood of her fetus. The mother's blood provides the fetus with essential nutrients and carries away waste products, but these chemicals must pass through the **placental barrier**. As food and waste products can pass through the placental barrier, it is perfectly feasible for drugs to pass through as well. Drugs such as **alcohol**, **nicotine**, and **cocaine** can all pass into the fetal blood supply. Fat-soluble drugs will cross the barrier most easily, and drugs such as **barbiturates** will reach the same levels in fetal blood as in maternal blood. Such levels may have unpredictable effects on fetal development. They may also prove hazardous once the baby is born. Drugs and other toxins can be removed from fetal blood by the maternal blood and detoxified. Once the baby is born, it may have the same levels of drugs in its blood as the mother, but it does not have the same ability to detoxify or eliminate them. As a result, drugs will have a longer lifetime and may have fatal effects.

11.4.7 Drug–drug interactions

Drugs such as **warfarin** and **methotrexate** are bound to albumin and plasma proteins in the blood, and are unavailable to interact with their targets. When another drug is taken which can compete for plasma protein binding (e.g. **sulphonamides**), then a certain percentage of previously bound drug is released, increasing the concentration of the drug and its effect.

KEY POINTS

- Pharmacodynamics is the study of how drugs interact with a molecular target to produce a pharmacological effect, whereas pharmacokinetics is the study of how a drug reaches its target in the body and how it is affected on that journey.
- The four main issues in pharmacokinetics are: absorption, distribution, metabolism, and excretion.
- Orally taken drugs have to be chemically stable to survive the acidic conditions of the stomach, and metabolically stable to survive digestive and metabolic enzymes.
- Orally taken drugs must be sufficiently polar to dissolve in the GIT and blood supply, but sufficiently fatty to pass through cell membranes.
- Most orally taken drugs obey Lipinski's rule of five and have no more than seven rotatable bonds.
- Highly polar drugs can be orally active if they are small enough to pass between the cells of the gut wall, are recognized by carrier proteins, or are taken across the gut wall by pinocytosis.
- Distribution around the blood supply is rapid. Distribution to the interstitial fluid surrounding tissues and organs is rapid if the drug is not bound to plasma proteins.
- Some drugs have to enter cells in order to reach their target.
- A certain percentage of a drug may be absorbed into fatty tissue and/or bound to macromolecules.
- Drugs entering the CNS have to cross the blood–brain barrier. Polar drugs are unable to cross this barrier unless they make use of carrier proteins or are taken across by pinocytosis.
- Some drugs cross the placental barrier into the fetus and may harm development or prove toxic in newborn babies.

11.5 Drug metabolism

When drugs enter the body, they are subject to attack from a range of metabolic enzymes. The role of these enzymes is to degrade or modify the foreign structure, such that it can be more easily excreted. As a result, most drugs undergo some form of metabolic reaction, resulting in structures known as **metabolites**. Very often, these metabolites lose the activity of the original drug, but, in some cases, they may retain a certain level of activity. In exceptional cases, the metabolite may even be more active than the parent drug. Some metabolites can possess a

different activity from the parent drugs, resulting in side effects or toxicity. A knowledge of drug metabolism and its possible consequences can aid the medicinal chemist in designing new drugs which do not form unacceptable metabolites. Equally, it is possible to take advantage of drug metabolism to activate drugs in the body. This is known as a prodrug strategy (see section 14.6). It is now a requirement to identify all the metabolites of a new drug before it can be approved. The structure and stereochemistry of each metabolite has to be determined and the metabolite must be tested for biological activity (section 15.1.2).

11.5.1 Phase I and phase II metabolism

The body treats drugs as foreign substances and has methods of getting rid of such chemical invaders. If the drug is polar, it will be quickly excreted by the kidneys (section 11.6). However, non-polar drugs are not easily excreted and the purpose of drug metabolism is to convert such compounds into more polar molecules that *can* be easily excreted.

Non-specific enzymes (particularly **cytochrome P450 enzymes** in the liver) are able to add polar functional groups to a wide variety of drugs. Once the polar functional group has been added, the overall drug is more polar and water soluble, and is more likely to be excreted when it passes through the kidneys. An alternative set of enzymatic reactions can reveal masked polar functional groups which might already be present in a drug. For example, there are enzymes which can demethylate a methyl ether to reveal a more polar hydroxyl group. Once again, the more polar product (metabolite) is excreted more efficiently.

These reactions are classed as phase I reactions and generally involve oxidation, reduction, and hydrolysis (see Figs 11.3–11.9). Most of these reactions occur in the liver, but some (such as the hydrolysis of esters and amides) can also occur in the gut wall, blood plasma, and other tissues. Some of the structures most prone to oxidation are *N*-methyl groups, aromatic rings, the terminal positions of alkyl chains, and the least hindered positions of alicyclic rings. Nitro, azo, and carbonyl groups are prone to reduction by **reductases**, while amides and esters are prone to hydrolysis by peptidases and esterases respectively. For many drugs, two or more metabolic reactions might occur, resulting in different metabolites; other drugs may not be metabolized at all. A knowledge of the metabolic reactions that are possible for different functional groups allows the medicinal chemist to predict the likely metabolic products for any given drug, but only drug metabolism studies will establish whether these metabolites are really formed.

Drug metabolism has important implications when it comes to using chiral drugs, especially if the drug is to be used as a racemate. The enzymes involved in catalysing metabolic reactions will often distinguish between the two enantiomers of a chiral drug, such that one enantiomer undergoes different metabolic reactions from the other. As a result, both enantiomers of a chiral drug have to be tested separately to see what metabolites are formed. In practice, it is usually preferable to use a single enantiomer in medicine or design the drug such that it is not asymmetric (section 13.3.8).

A series of metabolic reactions classed as phase II reactions also occur, mainly in the liver (see Figs 11.10–11.16). Most of these reactions are **conjugation reactions**, whereby a polar molecule is attached to a suitable polar 'handle' that is already present on the drug or has been introduced by a phase I reaction. The resulting conjugate has greatly increased polarity, thus increasing its excretion rate in urine or bile even further.

Both phase I and phase II reactions can be species-specific, which has implications for *in vivo* metabolic studies. In other words, the metabolites formed in an experimental animal may not necessarily be those formed in humans. A good knowledge of how metabolic reactions differ from species to species is important in determining which test animals are relevant for drug metabolism tests. Both sets of reactions can also be regioselective and stereoselective. This means that metabolic enzymes can distinguish between identical functional groups or alkyl groups located at different parts of the molecule (regioselectivity), as well as between different stereoisomers of chiral molecules (stereoselectivity).

11.5.2 Phase I transformations catalysed by cytochrome P450 enzymes

The enzymes that constitute the cytochrome P450 family are the most important metabolic enzymes and are located in liver cells. They are **haemoproteins** (containing haem and iron) and they catalyse a reaction that splits molecular oxygen, such that one of the oxygen atoms is introduced into the drug and the other ends up in water (Fig. 11.3). As a result, they belong to a general class of enzymes called the **monooxygenases**.

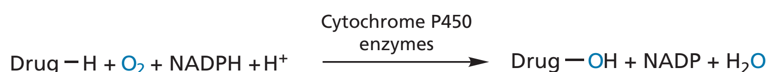


FIGURE 11.3 Oxidation by cytochrome P450 enzymes.

There are at least 33 different cytochrome P450 (CYP) enzymes, grouped into four main families: CYP1–CYP4. Within each family there are various subfamilies designated by a letter, and each enzyme within that subfamily is designated by a number. For example, CYP3A4 is enzyme 4 in the subfamily A of the main family 3. Most drugs in current use are metabolized by five primary CYP enzymes (CYP3A, CYP2D6, CYP2C9, CYP1A2, and CYP2E1). The isozyme CYP3A4 is particularly important in drug metabolism and is responsible for the metabolism of most drugs. The reactions catalysed by cytochrome P450 enzymes are shown in Figs 11.4 and 11.5, and can involve the oxidation of carbon, nitrogen, phosphorus, sulphur, and other atoms.

Oxidation of carbon atoms can occur if the carbon atom is either exposed (i.e. easily accessible to the enzyme) or activated (Fig. 11.4). For example, methyl substituents on the carbon skeleton of a drug are often easily accessible and are oxidized to form alcohols, which may be oxidized further to carboxylic acids. In the case of longer-chain substituents, the terminal carbon and the penultimate carbon are the most exposed carbons in the chain, and are both susceptible to oxidation. If an aliphatic ring is present, the most exposed region is the part most likely to be oxidized.

Activated carbon atoms next to an sp^2 carbon centre (i.e. allylic or benzylic positions) or an sp carbon centre (i.e. a propynylic position) are more likely to be oxidized than exposed carbon atoms (Fig. 11.4). Carbon atoms which are alpha to a heteroatom are also activated and prone to oxidation. In this case, hydroxylation results in an unstable metabolite that is immediately hydrolysed

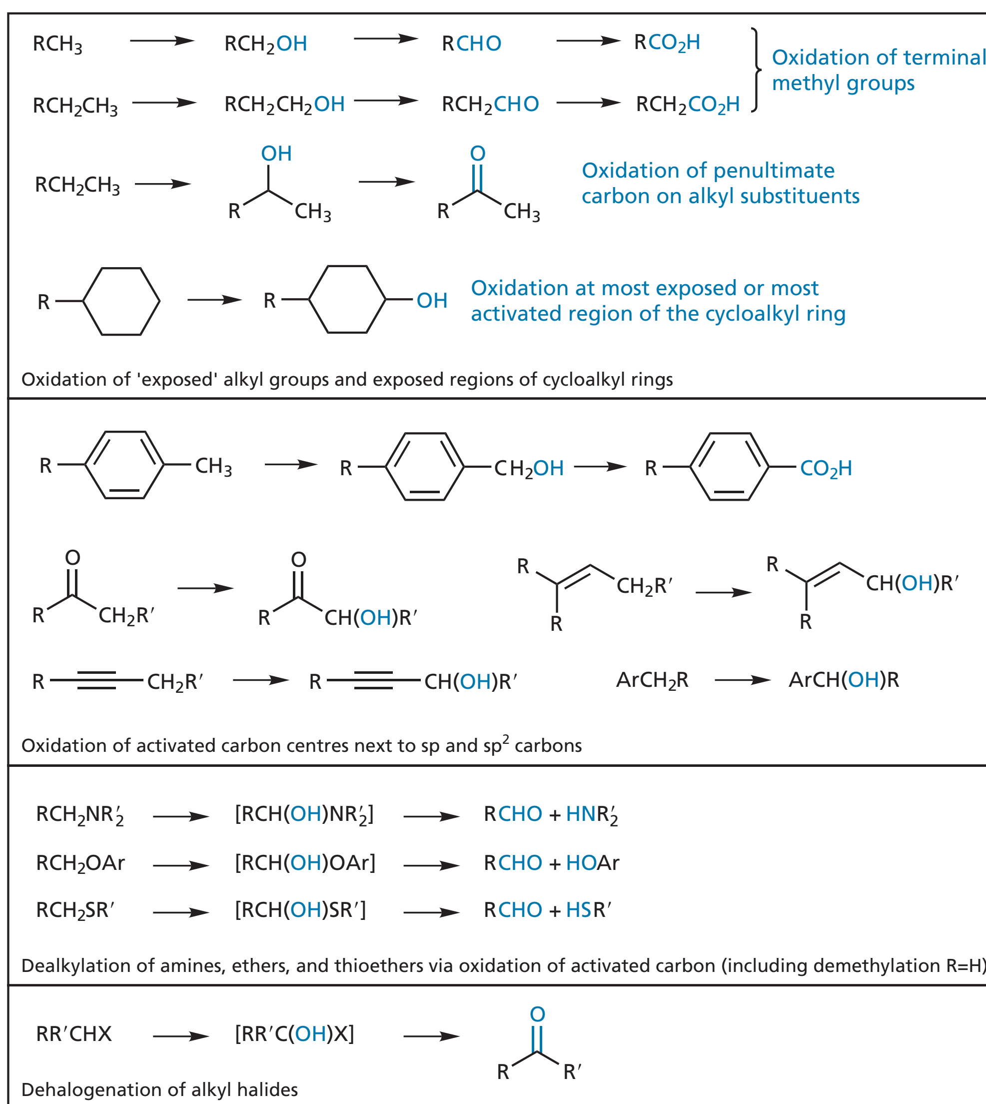
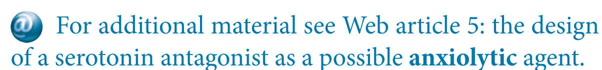


FIGURE 11.4 Oxidative reactions catalysed by cytochrome P450 enzymes on saturated carbon centres.

resulting in the dealkylation of amines, ethers, and thioethers, or the dehalogenation of alkyl halides. The aldehydes which are formed from these reactions generally undergo further oxidation to carboxylic acids by aldehyde dehydrogenases (section 11.5.4). Tertiary amines are found to be more reactive to oxidative dealkylation than secondary amines because of their greater basicity, while *O*-demethylation of aromatic ethers is faster than *O*-dealkylation of larger alkyl groups. *O*-Demethylation is important to the analgesic activity of codeine (section 24.5).

Cytochrome P450 enzymes can catalyse the oxidation of unsaturated sp^2 and sp carbon centres present in alkenes, alkynes, and aromatic rings (Fig. 11.5). In the case of alkenes, a reactive epoxide is formed which is deactivated by the enzyme **epoxide hydrolase** to form a diol. In some cases, the epoxide may evade the enzyme. If this happens, it can act as an alkylating agent and react with nucleophilic groups present in proteins or nucleic acids, leading to toxicity. The oxidation of an aromatic ring results in a similarly reactive epoxide intermediate which can have several possible fates. It may undergo a rearrangement reaction involving a hydride transfer to form a phenol, normally at the *para* position. Alternatively, it may be deactivated by epoxide hydrolase to form a diol or react with **glutathione *S*-transferase** to form a conjugate (section 11.5.5). If the epoxide intermediate evades these enzymes it may act as an alkylating agent and prove toxic. Electron-rich aromatic rings are likely to be epoxidized more quickly than those with electron-withdrawing substituents—this has consequences for drug design.

Tertiary amines are oxidized to *N*-oxides as long as the alkyl groups are not sterically demanding. Primary and secondary amines are also oxidized to *N*-oxides, but these are rapidly converted to hydroxylamines and beyond. Aromatic primary amines are also oxidized in stages to aromatic nitro groups—a process which is related to the toxicity of aromatic amines, as highly electrophilic intermediates are formed which can alkylate proteins or nucleic acids. Aromatic primary amines can also be methylated in a phase II reaction (section 11.5.5) to a secondary amine which can then undergo phase I oxidation to produce formaldehyde and primary hydroxylamines. Primary and secondary amides can be oxidized to hydroxylamides. These functional groups have also been linked with toxicity and carcinogenicity. Thiols can be oxidized to disulphides. There is evidence that thiols can be methylated to methyl sulphides, which are then oxidized to sulphides and sulphones.


For additional material see Web article 5: the design of a serotonin antagonist as a possible **anxiolytic** agent.

11.5.3 Phase I transformations catalysed by flavin-containing monooxygenases

Another group of metabolic enzymes present in the endoplasmic reticulum of liver cells consists of the **flavin-containing monooxygenases**. These enzymes are chiefly responsible for metabolic reactions involving oxidation at nucleophilic nitrogen, sulphur, and phosphorus atoms, rather than at carbon atoms. Several examples are given in Fig. 11.6. Many of these reactions are also catalysed by cytochrome P450 enzymes.

11.5.4 Phase I transformations catalysed by other enzymes

There are several oxidative enzymes in various tissues around the body that are involved in the metabolism of endogenous compounds, but can also play a role in drug metabolism (Fig. 11.7). For example, **monoamine oxidases** are involved in the deamination of catecholamines (section 23.5), but have been observed to oxidize some drugs. Other important oxidative enzymes include alcohol dehydrogenases and aldehyde dehydrogenases. The aldehydes formed by the action of alcohol dehydrogenases on primary alcohols are usually not observed as they are converted to carboxylic acids by aldehyde dehydrogenases.

Reductive phase I reactions are less common than oxidative reactions, but reductions of aldehyde, ketone, azo, and nitro functional groups have been observed in specific drugs (Fig. 11.8). Many of the oxidation reactions described for heteroatoms in Figs 11.5–11.7 are reversible and are catalysed by reductase enzymes. Cytochrome P450 enzymes are involved in catalysing some of these reactions. Remember: enzymes can catalyse a reaction in both directions, depending on the nature of the substrate. So, although cytochrome P450 enzymes are predominantly oxidative enzymes, it is possible for them to catalyse some reductions.

The hydrolysis of esters and amides is a common metabolic reaction, catalysed by **esterases** and **peptidases** respectively (Fig. 11.9). These enzymes are present in various organs of the body, including the liver. Amides tend to be hydrolysed more slowly than esters. The presence of electron-withdrawing groups can increase the susceptibility of both amides and esters to hydrolysis.

11.5.5 Phase II transformations

Most phase II reactions are **conjugation reactions** catalysed by transferase enzymes. The resulting conjugates are usually inactive, but there are exceptions to this rule.

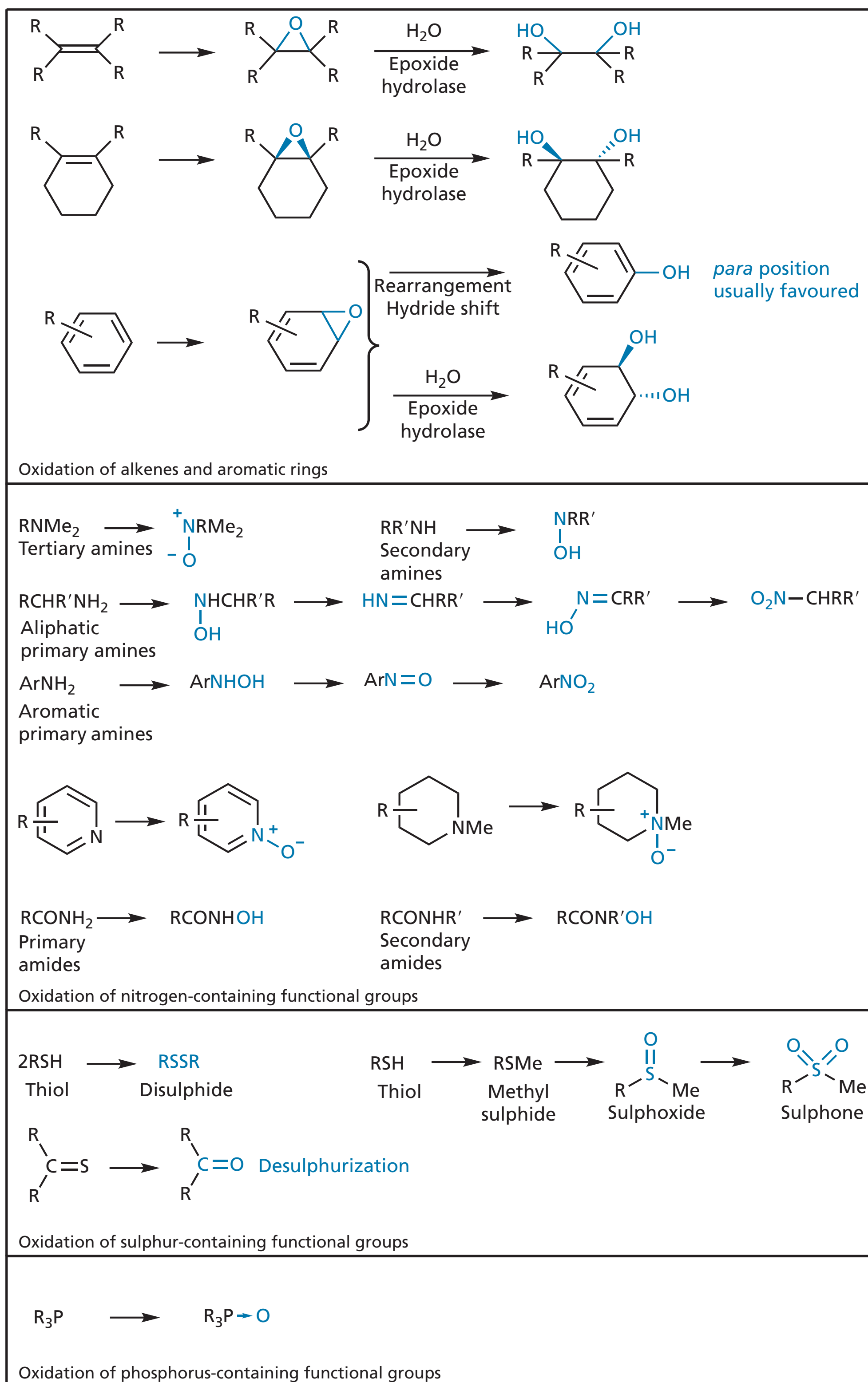


FIGURE 11.5 Oxidative reactions catalysed by cytochrome P450 enzymes on heteroatoms and unsaturated carbon centres.

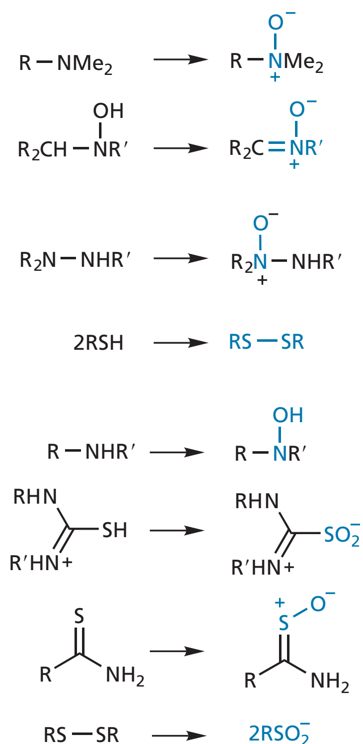


FIGURE 11.6 Phase I reactions catalysed by flavin monooxygenases.

Glucuronic acid conjugation is the most common of these reactions. Phenols, alcohols, hydroxylamines, and carboxylic acids form ***O*-glucuronides** by reaction with **UDFP-glucuronate** such that a highly polar glucuronic acid molecule is attached to the drug (Fig. 11.10). The resulting conjugate is excreted in the urine, but may also be excreted in the bile if the molecular weight is over 300.

A variety of other functional groups, such as sulphonamides, amides, amines, and thiols (Fig. 11.11) can react to form *N*- or *S*-glucuronides. *C*-glucuronides are also possible in situations where there is an activated carbon centre next to carbonyl groups.

Another form of conjugation is sulphate conjugation (Fig. 11.12). This is less common than glucuronation and is restricted mainly to phenols, alcohols, arylamines, and *N*-hydroxy compounds. The reaction is catalysed by **sulphotransferases** using the cofactor **3′-phosphoadenosine 5′-phosphosulfate** as the sulphate source. Primary and secondary amines, secondary alcohols, and phenols form stable conjugates, whereas primary alcohols form reactive sulphates, which can act as toxic alkylating agents. Aromatic hydroxylamines and hydroxylamides also form unstable sulphate conjugates that can be toxic.

Drugs bearing a carboxylic acid group can become conjugated to amino acids by the formation of a peptide link. In most animals, glycine conjugates are generally formed, but L-glutamine is the most common amino acid used for conjugation in primates. The carboxylic acid present in the drug is first activated by formation of a coenzyme A thioester which is then linked to the amino acid (Fig. 11.13).

Electrophilic functional groups, such as epoxides, alkyl halides, sulphonates, disulphides, and radical species, can react with the nucleophilic thiol group of the tripeptide **glutathione** to give glutathione conjugates which can be subsequently transformed to **mercapturic acids** (Fig. 11.14). The glutathione conjugation reaction can take place in most cells, especially those in the liver and kidney, and is catalysed by **glutathione transferase**. This conjugation reaction is important in detoxifying potentially dangerous environmental toxins or electrophilic alkylating agents formed by phase I reactions (Fig. 11.15). Glutathione conjugates are often excreted in the bile, but are more usually converted to mercapturic acid conjugates before excretion.

Not all phase II reactions result in increased polarity. Methylation and acetylation are important phase II reactions which usually *decrease* the polarity of the drug (Fig. 11.16). An important exception is the methylation of pyridine rings, which leads to polar quaternary salts. The functional groups that are susceptible to methylation are phenols, amines, and thiols. Primary amines are also susceptible to acetylation. The enzyme cofactors involved in contributing the methyl group or acetyl group are ***S*-adenosyl methionine** and **acetyl SCoA** respectively. Several methyltransferase enzymes are involved in the methylation reactions. The most important enzyme for *O*-methylations is **catechol *O*-methyltransferase**, which preferentially methylates the *meta* position of catechols (section 23.5). It should be pointed out, however, that

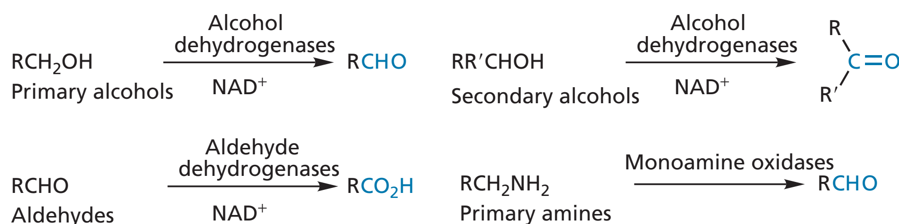


FIGURE 11.7 Phase I oxidative reactions catalysed by miscellaneous enzymes.

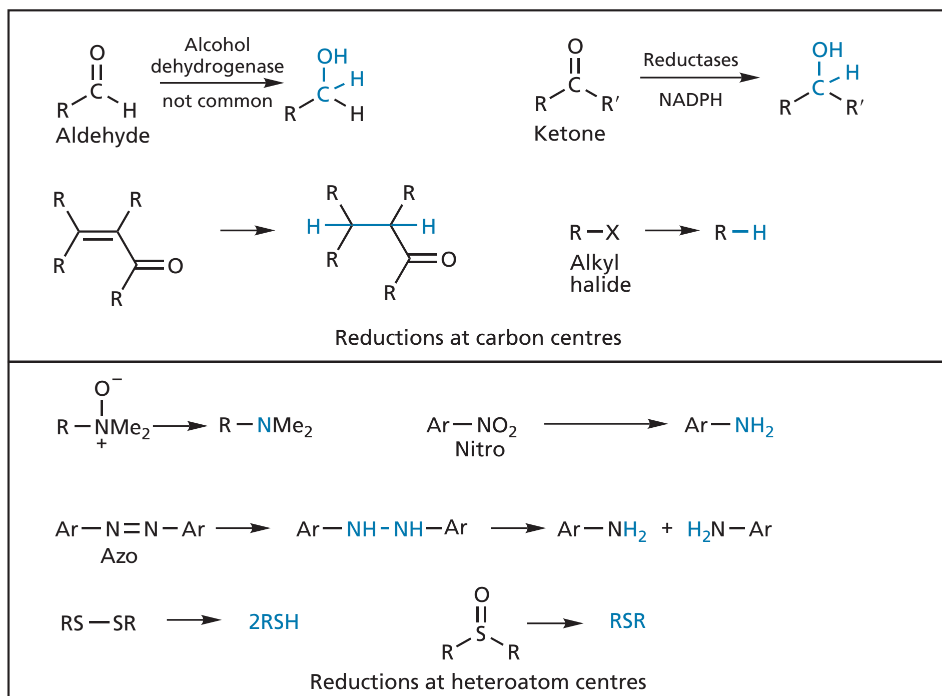


FIGURE 11.8 Phase I reductive reactions.

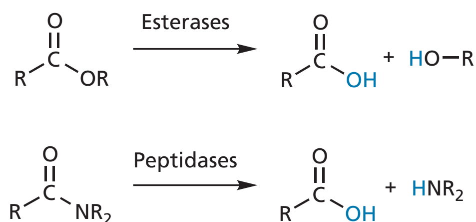


FIGURE 11.9 Hydrolysis of esters and amides.

methylation occurs less frequently than other conjugation reactions and is more important in biosynthetic pathways or the metabolism of endogenous compounds.

It is possible for drugs bearing carboxylic acids to become conjugated with **cholesterol**. Cholesterol conjugates can also be formed with drugs bearing an ester group by means of a transesterification reaction. Some drugs with an alcohol functional group form conjugates with fatty acids by means of an ester link.

11.5.6 Metabolic stability

Ideally, a drug should be resistant to drug metabolism because the production of metabolites complicates drug therapy (see Box 11.1). For example, the metabolites formed will usually have different properties from the original drug. In some cases, activity may be lost. In others, the metabolite may prove to be toxic. For example, the metabolites of **paracetamol** cause liver toxicity, and the carcinogenic properties of some polycyclic hydrocarbons are due to the formation of epoxides.

Another problem arises from the fact that the activity of metabolic enzymes varies from individual to individual. This is especially true of the cytochrome P450 enzymes, with at least a 10-fold variability for the most important isoform, CYP3A4. Individuals may even lack particular isoforms. For example, 8% of Americans lack the CYP2D6 isoform, which means that drugs normally metabolized by this enzyme can rise to toxic levels.

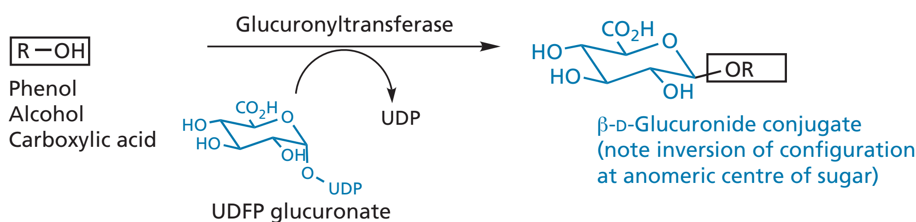


FIGURE 11.10 Glucuronidation of alcohols, phenols, and carboxylic acids.

BOX 11.1 Metabolism of an antiviral agent

Indinavir is an antiviral agent used in the treatment of HIV and is prone to metabolism, resulting in seven different metabolites (Fig. 1). Studies have shown that the CYP3A subfamily of cytochrome P450 enzymes is responsible for six of these metabolites. The metabolites concerned arise from *N*-dealkylation of the piperazine ring, *N*-oxidation of the pyridine ring, *para*-hydroxylation of the phenyl ring, and hydroxylation of the indane ring. The seventh metabolite is a glucuronide conjugate of the pyridine ring. All these reactions occur individually to produce five separate metabolites. The remaining two metabolites arise from two or more metabolic reactions taking place on the same molecule.

The major metabolites are those resulting from dealkylation. As a result, research has been carried out to try and design indinavir analogues that are resistant to this reaction. For example, structures having two methyl substituents on the activated carbon next to pyridine have been effective in blocking dealkylation (Fig. 2).

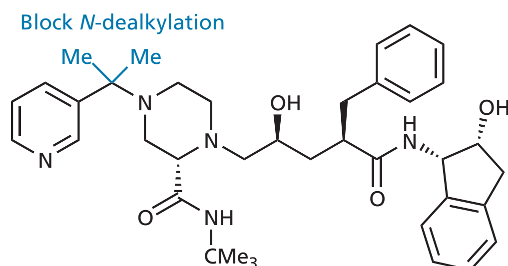


FIGURE 2 Analogue of indinavir resistant to *N*-dealkylation.

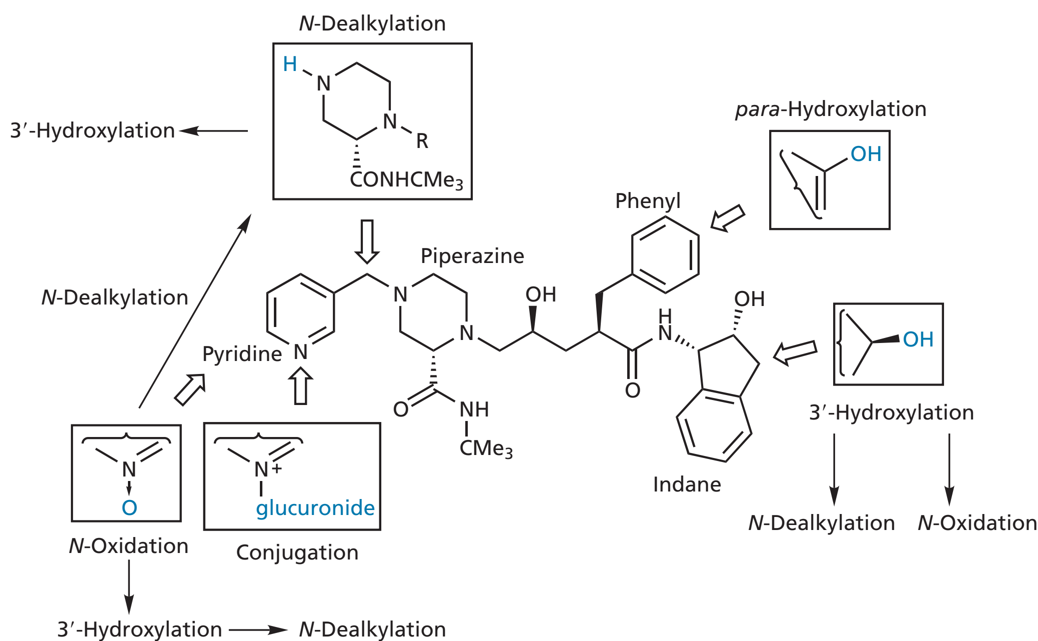


FIGURE 1 Metabolism of indinavir.

Examples of drugs that are normally metabolized by this isozyme are **desipramine, haloperidol**, and **tramadol.** Some prodrugs require metabolism by CYP2D6 in order to be effective. For example, the analgesic effects of **codeine** are due to its metabolism by CYP2D6 to morphine. Therefore, codeine is ineffective in patients lacking this isozyme. The profile of these enzymes in different patients can vary, resulting in a difference in the way a drug is metabolized. As a result, the amount of drug that can be administered safely also varies.

Differences across populations can be quite significant, resulting in different countries having different recommended dose levels for particular drugs. For example, the rate at which the antibacterial agent **isoniazid** is

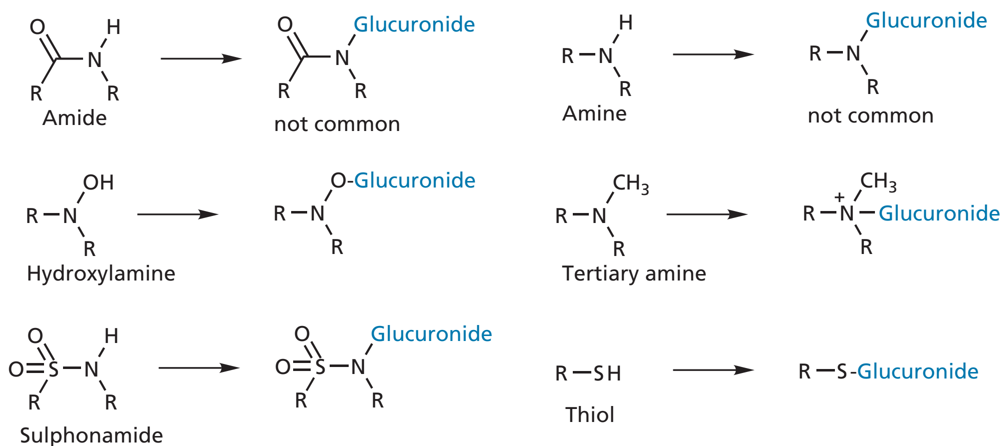


FIGURE 11.11 Glucuronidation of miscellaneous functional groups.

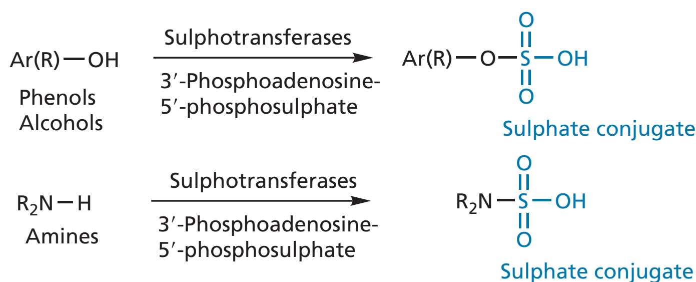


FIGURE 11.12 Examples of sulphoconjugation phase II reactions.

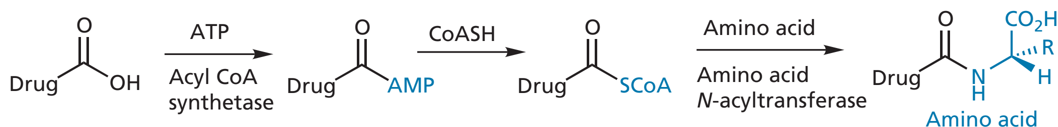


FIGURE 11.13 Formation of amino acid conjugates.

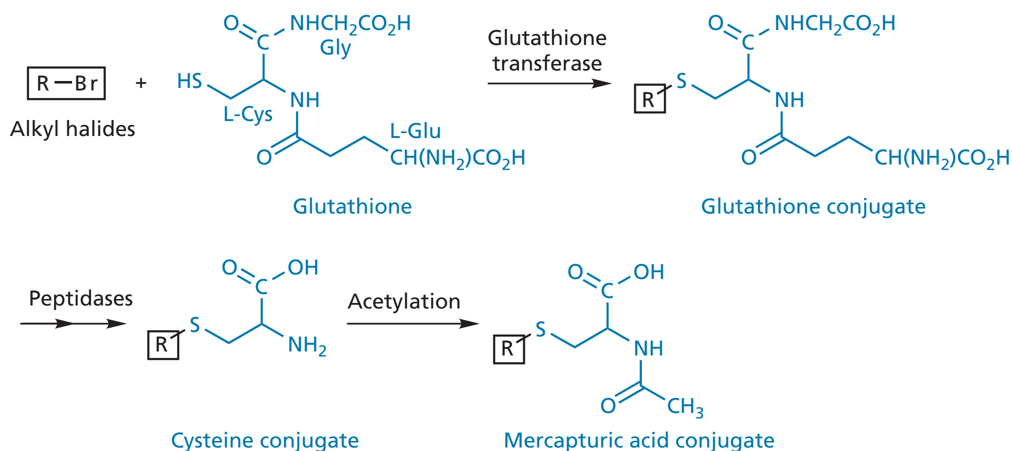


FIGURE 11.14 Formation of glutathione and mercapturic acid conjugates from an alkyl halide.

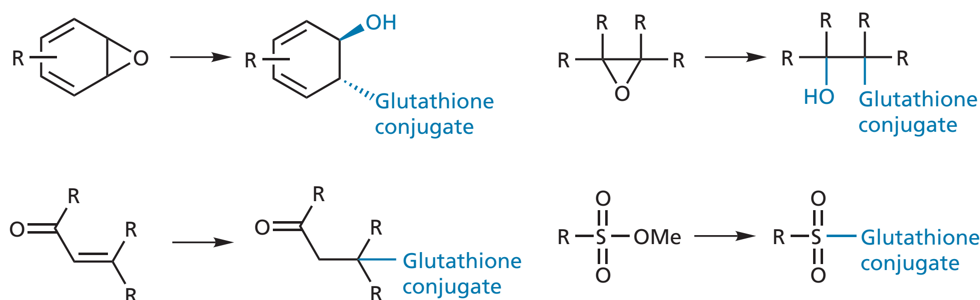


FIGURE 11.15 Formation of glutathione conjugates (Glu–Cys–Gly) with electrophilic groups.

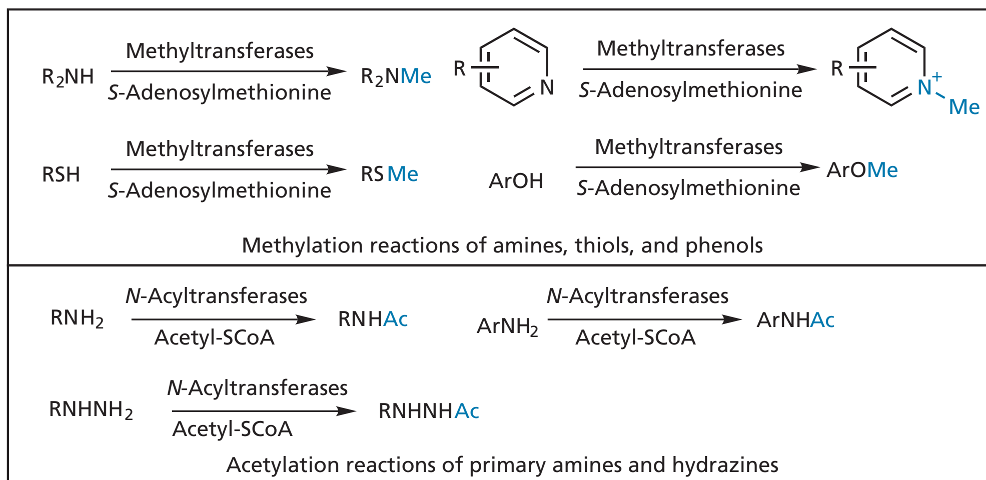


FIGURE 11.16 Methylation and acetylation.

acetylated and deactivated varies among populations. Asian populations acylate the drug at a fast rate, whereas 45–65% of Europeans and North Americans have a slow rate of acylation. **Pharmacogenomics** is the study of genetic variations between individuals and the effect that has on individual responses to drugs. In the future, it is possible that 'fingerprints' of an individual's genome may allow better prediction of which drugs would be suitable for that individual and which drugs might produce unacceptable side effects—an example of **personalized medicine**. This, in turn, may avoid drugs having to be withdrawn from the market as a result of rare toxic side effects.

Another complication involving drug metabolism and drug therapy relates to the fact that cytochrome P450 activity can be affected by other chemicals. For example, certain foods have an influence. Brussels sprouts and cigarette smoke enhance activity, whereas grapefruit juice inhibits activity. This can have a significant effect on the activity of drugs metabolized by cytochrome P450 enzymes. For example, the immunosuppressant drug **ciclosporin** and the dihydropyridine hypotensive agents are more efficient when taken with grapefruit juice, as their metabolism is reduced. However, serious toxic effects can arise if the antihistamine agent **terfenadine** is taken with grapefruit juice. Terfenadine is actually a prodrug and is metabolized to the active agent **fexofenadine** (Fig. 11.17). If metabolism is inhibited by grapefruit juice, terfenadine persists in the body and can cause serious cardiac toxicity. As a result, fexofenadine itself is now favoured over terfenadine and is marketed as **Allegra**.

Certain drugs are also capable of inhibiting or promoting cytochrome P450 enzymes, leading to a phenomenon known as **drug–drug interactions** where the presence of one drug affects the activity of another. For example, several antibiotics can act as cytochrome P450 inhibitors and will slow the metabolism of drugs metabolized by these enzymes. Other examples are the drug–drug interactions that occur between the anticoagulant **warfarin** and the barbiturate **phenobarbital** (Fig. 11.17), or between warfarin and the anti-ulcer drug **cimetidine** (section 25.2.7.3).

Phenobarbital stimulates cytochrome P450 enzymes and accelerates the metabolism of warfarin, making it less effective. In contrast, cimetidine inhibits cytochrome P450 enzymes, thus slowing the metabolism of warfarin.

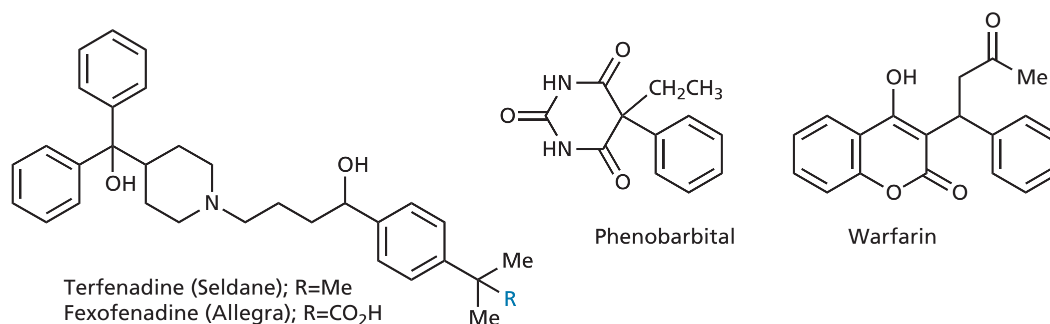


FIGURE 11.17 Drugs which are metabolized by cytochrome P450 enzymes or affect the activity of cytochrome P450 enzymes.

Such drug–drug interactions affect the plasma levels of warfarin and could cause serious problems if the levels move outwith the normal therapeutic range.

Herbal medicine is not immune from this problem either. **St. John's wort** is a popular remedy used for mild-to-moderate depression. However, it promotes the activity of cytochrome P450 enzymes and decreases the effectiveness of contraceptives and warfarin.

Because of the problems caused by cytochrome P450 activation or inhibition, new drugs are usually tested to check whether they have any effect on cytochrome P450 activity, or are, themselves, metabolized by these enzymes. Indeed, an important goal in many projects is to ensure that such properties are lacking.

Drugs can be defined as hard or soft with respect to their metabolic susceptibility. In this context, **hard drugs** are those that are resistant to metabolism and remain unchanged in the body. **Soft drugs** are designed to have a predictable, controlled metabolism where they are inactivated to non-toxic metabolites and excreted. A group is normally incorporated which is susceptible to metabolism, but will ensure that the drug survives for a sufficiently long period to achieve what it is meant to do before it is metabolized and excreted. Drugs such as these are also called **antedrugs**.

11.5.7 The first pass effect

Drugs that are taken orally pass directly to the liver once they enter the blood supply. Here, they are exposed to drug metabolism before they are distributed around the rest of the body, and so a certain percentage of the drug is transformed before it has the chance to reach its target. This is known as the **first pass effect**. Drugs that are administered in a different fashion (e.g. injection or inhalation) avoid the first pass effect and are distributed around the body before reaching the liver. Indeed, a certain proportion of the drug may not pass through the liver at all, but may be taken up in other tissues and organs en route.

11.6 Drug excretion

Drugs and their metabolites can be excreted from the body by a number of routes. Volatile or gaseous drugs are excreted through the lungs. Such drugs pass out of the capillaries that line the air sacs (**alveoli**) of the lungs, then diffuse through the cell membranes of the alveoli into the air sacs, from where they are exhaled. Gaseous **general anaesthetics** are excreted in this way and move down a concentration gradient from the blood supply into the lungs. They are also administered through the lungs, in which case the concentration gradient is in the opposite direction and the gas moves from the lungs to the blood supply.

The **bile duct** travels from the liver to the intestines and carries a greenish fluid called **bile** which contains bile acids and salts that are important to the digestion process. A small number of drugs are diverted from the blood supply back into the intestines by this route. As this happens from the liver, any drug eliminated in this way has not been distributed round the body. Therefore, the amount of drug distributed is less than that absorbed. However, once the drug has entered the intestine, it can be reabsorbed, so it has another chance.

It is possible for as much as 10–15% of a drug to be lost through the skin in sweat. Drugs can also be excreted through saliva and breast milk, but these are minor excretion routes compared with the kidneys. There are concerns, however, that mothers may be passing on drugs such as **nicotine** to their baby through breast milk.

The **kidneys** are the principal route by which drugs and their metabolites are excreted (Fig. 11.18). The kidneys filter the blood of waste chemicals and these chemicals are subsequently removed in the urine. Drugs and their metabolites are excreted by the same mechanism.

Blood enters the kidneys by means of the **renal artery**. This divides into a large number of capillaries, each one of which forms a knotted structure called a **glomerulus**

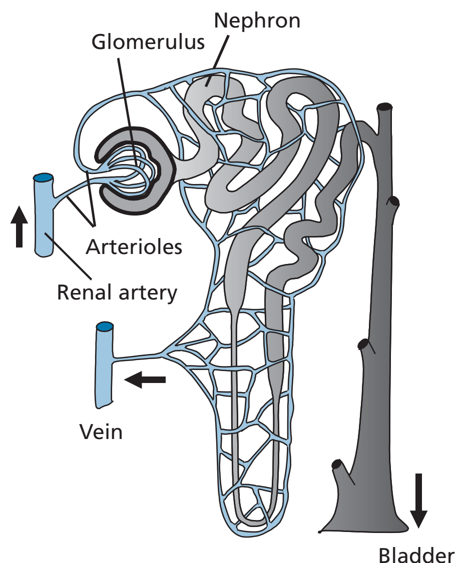


FIGURE 11.18 Excretion by the kidneys.

that fits into the opening of a duct called a **nephron**. The blood entering these glomeruli is under pressure, and so plasma is forced through the pores in the capillary walls into the nephron, carrying with it any drugs and metabolites that might be present. Any compounds that are too big to pass through the pores, such as plasma proteins and red blood cells, remain in the capillaries with the remaining plasma. Note that this is a filtration process, so it does not matter whether the drug is polar or hydrophobic: all drugs and drug metabolites will be passed equally efficiently into the nephron. However, this does not mean that every compound will be *excreted* equally efficiently, because there is more to the process than simple filtration.

The filtered plasma and chemicals now pass through the nephron on their route to the bladder. However, only a small proportion of what starts that journey actually finishes it. This is because the nephron is surrounded by a rich network of blood vessels carrying the filtered blood away from the glomerulus, permitting much of the contents of the nephron to be reabsorbed into the blood supply. Most of the water that was filtered into the nephron is quickly reabsorbed through pores in the nephron cell membrane which are specific for water molecules and bar the passage of ions or other molecules. These pores are made up of protein molecules called **aquaporins**. As water is reabsorbed, drugs and other agents are concentrated in the nephron and a concentration gradient is set up. There is now a driving force for compounds to move back into the blood supply down the concentration gradient. However, this can only happen if the drug is sufficiently hydrophobic to pass through the cell membranes of the nephron. This means that hydrophobic compounds are efficiently reabsorbed back into the blood, whereas polar compounds remain in the nephron and are excreted. This process of excretion explains the importance of drug metabolism to drug excretion. Drug metabolism makes a drug more polar so that it is less likely to be reabsorbed from the nephrons.

Some drugs are actively transported from blood vessels into the nephrons. This process is called **facilitated transport** and is important in the excretion of penicillins (section 19.5.1.9).

KEY POINTS

- Drugs are exposed to enzyme-catalysed reactions which modify their structure. This is called drug metabolism and can take place in various tissues. However, most reactions occur in the liver.
- Orally taken drugs are subject to the first pass effect.
- Drugs administered by methods other than the oral route avoid the first pass effect.
- Phase I metabolic reactions typically involve the addition or exposure of a polar functional group. Cytochrome P450 enzymes present in the liver carry out important phase I oxidation reactions. The types of cytochrome P450 enzymes present vary between individuals, leading to varying rates of drug metabolism.
- The activity of cytochrome P450 enzymes can be affected by food, chemicals, and drugs, resulting in drug–drug interactions and possible side effects.
- Phase II metabolic reactions involve the addition of a highly polar molecule to a functional group. The resulting conjugates are more easily excreted.
- Drug excretion can take place through sweat, exhaled air, or bile, but most excretion takes place through the kidneys.
- The kidneys filter blood such that drugs and their metabolites enter nephrons. Non-polar substances are reabsorbed into the blood supply, but polar substances are retained in the nephrons and excreted in the urine.

11.7 Drug administration

There are a large variety of ways in which drugs can be administered and many of these avoid some of the problems associated with oral administration. The main routes are: oral, sublingual, rectal, epithelial, inhalation, and injection. The method chosen will depend on the target organ and the pharmacokinetics of the drug.

11.7.1 Oral administration

Orally administered drugs are taken by mouth. This is the preferred option for most patients, so there is more chance that the patient will comply with the drug regime and complete the course. However, the oral route places the greatest demands on the chemical and physical properties of the drug, as described earlier in the chapter.

Drugs given orally can be taken as pills, capsules, or solutions. Drugs taken in solution are absorbed more quickly and a certain percentage may even be absorbed through the stomach wall. For example, approximately 25–33% of **alcohol** is absorbed into the blood supply from the stomach; the rest is absorbed from the upper intestine. Drugs taken as pills or capsules are mostly absorbed in the upper intestine. The rate of absorption is partly determined by the rate at which the pills and capsules dissolve. In turn, this depends on such factors as particle size and crystal form. In general, about 75% of an orally administered drug is absorbed into the body within 1–3 hours. Specially designed pills and capsules can remain intact in the stomach to help protect acid-labile drugs from stomach acids. The containers then degrade once they reach the intestine.

Care has to be taken if drugs interact with food. For example, **tetracycline** binds strongly to calcium ions, which inhibits absorption, so foods such as milk should be avoided. Some drugs bind other drugs and prevent absorption. For example, **colestyramine** (used to lower cholesterol levels) binds to **warfarin** and also to the thyroid drug **levothyroxine sodium**, so these drugs should be taken separately.

11.7.2 Absorption through mucous membranes

Some drugs can be absorbed through the mucous membranes of the mouth or nose, thus avoiding the digestive and metabolic enzymes encountered during oral administration. For example, heart patients take **glyceryl trinitrate** (Fig. 11.19) by placing it under the tongue (sublingual administration). The opiate analgesic **fentanyl** (Fig. 11.19) has been given to children in the form of a lollipop and is absorbed through the mucous membranes of the mouth. The Incas absorbed **cocaine** sublingually by chewing coca leaves.

Nasal decongestants are absorbed through the mucous membranes of the nose. Cocaine powder is absorbed in this way when it is sniffed, as is **nicotine** in the form of snuff. Nasal sprays have been used to administer analogues of peptide hormones, such as **antidiuretic hormone**. These drugs would be degraded quickly if taken orally.

Eye drops are used to administer drugs directly to the eye and thus reduce the possibility of side effects elsewhere in the body. For example, the eye condition known as glaucoma is treated in this way. Nevertheless, some absorption into the blood supply can still occur and some asthmatic patients suffer bronchospasms when taking **timolol** eye drops.

11.7.3 Rectal administration

Some drugs are administered rectally as **suppositories**, especially if the patient is unconscious, vomiting, or unable to swallow. However, there are several problems associated with rectal administration: the patient may suffer membrane irritation and, although the extent of drug absorption is efficient, it can be unpredictable. It is not the most popular of methods with patients either!

11.7.4 Topical administration

Topical drugs are those which are applied to the skin. For example, steroids are applied topically to treat local skin irritations. It is also possible for some of the drug to be absorbed through the skin (**transdermal absorption**) and to enter the blood supply, especially if the drug is **lipophilic**. **Nicotine patches** work in this fashion, as do hormone replacement therapies for **estrogen.** Drugs are absorbed by this method at a steady rate and avoid the acidity of the stomach, or the enzymes in the gut or gut wall. Other drugs that have been applied in this way include the analgesic **fentanyl** and the antihypertensive agent **clonidine**. Once applied, the drug is released slowly from the patch and absorbed through the skin into the blood supply over several days. As a result, the level of drug remains relatively constant over that period.

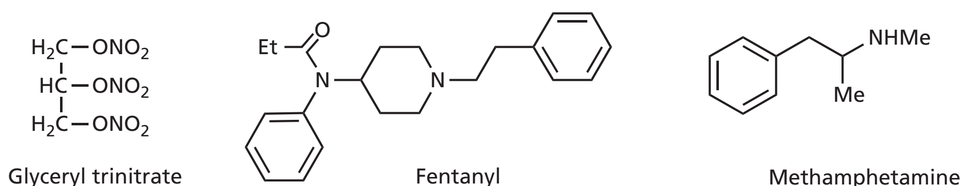


FIGURE 11.19 Glyceryl trinitrate, fentanyl, and methamphetamine.

A technique known as **iontophoresis** is being investigated as a means of topical administration. Two miniature electrode patches are applied to the skin and linked to a reservoir of the drug. A painless pulse of electricity is applied, which has the effect of making the skin more permeable to drug absorption. By timing the electrical pulses correctly, the drug can be administered such that fluctuations in blood levels are kept to a minimum. Similar devices are being investigated which use ultrasound to increase skin permeability.

11.7.5 Inhalation

Drugs administered by inhalation avoid the digestive and metabolic enzymes of the GIT or liver. Once inhaled, the drugs are absorbed through the cell linings of the respiratory tract into the blood supply. Assuming the drug is able to pass through the hydrophobic cell membranes, absorption is rapid and efficient because the blood supply is in close contact with the cell membranes of the lungs. For example, **general anaesthetic gases** are small, highly lipid-soluble molecules which are absorbed almost as fast as they are inhaled.

Non-gaseous drugs can be administered as **aerosols**. This is how anti-asthmatic drugs are administered and it allows them to be delivered to the lungs in far greater quantities than if they were given orally or by injection. In the case of anti-asthmatics, the drug is made sufficiently polar that it is poorly absorbed into the bloodstream. This localizes it in the airways and lowers the possibility of side effects elsewhere in the body (e.g. action on the heart). However, a certain percentage of an inhaled drug is inevitably swallowed and can reach the blood supply by the oral route. This may lead to side effects. For example, tremor is a side effect of the anti-asthmatic **salbutamol** as a result of the drug reaching the blood supply.

Several drugs of abuse are absorbed through inhalation or smoking [e.g. **nicotine**, **cocaine**, **marijuana**, **heroin**, and **methamphetamine** (Fig. 11.19)]. Smoking is a particularly hazardous method of taking drugs. A normal cigarette is like a mini-furnace producing a complex mixture of potentially carcinogenic compounds, especially from the tars present in tobacco. These are not absorbed into the blood supply but coat the lung tissue, leading to long-term problems, such as lung cancer. The tars in cannabis are considerably more dangerous than those in tobacco. If cannabis is to be used in medicine, safer methods of administration are desirable (i.e. inhalers).

11.7.6 Injection

Drugs can be introduced into the body by intravenous, intramuscular, subcutaneous, or intrathecal injection. Injection of a drug produces a much faster response than oral administration because the drug reaches the blood supply more quickly. The levels of drug administered are also more accurate because absorption by the oral route has a level of unpredictability owing to the first pass effect. Injecting a drug, however, is potentially more hazardous. For example, some patients may have an unexpected reaction to a drug and there is little that can be done to reduce the levels once the drug has been injected. Such side effects would be more gradual and treatable if the drug was given orally. Furthermore, sterile techniques are essential when giving injections to avoid the risks of bacterial infection, or of transmitting hepatitis or AIDS from a previous patient. Finally, there is a greater risk of receiving an overdose when injecting a drug.

The **intravenous** route involves injecting a solution of the drug directly into a vein. This method of administration is not particularly popular with patients, but it is a highly effective method of administering drugs in accurate doses and it is the fastest of the injection methods. However, it is also the most hazardous method of injection. As its effects are rapid, the onset of any serious side effects or allergies is also rapid. It is important, therefore, to administer the drug as slowly as possible and to monitor the patient closely. An intravenous drip allows the drug to be administered in a controlled manner, such that there is a steady level of drug in the system. The local anaesthetic **lidocaine** is given by intravenous injection. Drugs that are dissolved in oily liquids cannot be given by intravenous injection as this may result in the formation of blood clots.

The **intramuscular** route involves injecting drugs directly into muscle, usually in the arm, thigh, or buttocks. Drugs administered in this way do not pass round the body as rapidly as they would if given by intravenous injection, but they are still absorbed faster than by oral administration. The rate of absorption depends on various factors, such as the diffusion of the drug, blood supply to the muscle, the solubility of the drug, and the volume of the injection. Local blood flow can be reduced by adding adrenaline to constrict blood vessels. Diffusion can be slowed by using a poorly absorbed salt, ester, or complex of the drug (see also section 14.6.2). The advantage of slowing down absorption is in prolonging activity. For example, oily suspensions of steroid hormone esters are used to slow absorption. Drugs are often administered by intramuscular injection when they are unsuitable for intravenous injection, and so it is important to avoid injecting into a vein.

Subcutaneous injection involves injecting the drug under the surface of the skin. Absorption depends on factors such as how fast the drug diffuses, the level of blood supply to the skin, and the ability of the drug to enter the blood vessels. Absorption can be slowed by the same methods described for intramuscular injection. Drugs which can act as irritants should not be

administered in this way as they can cause severe pain and may damage local tissues.

Intrathecal injection means that the drug is injected into the spinal cord. Antibacterial agents that do not normally cross the blood–brain barrier are often administered in this way. Intrathecal injections are also used to administer **methotrexate** in the treatment of childhood leukaemia in order to prevent relapse in the CNS.

Intraperitoneal injection involves injecting drugs directly into the abdominal cavity. This is very rarely used in medicine, but it is a method of injecting drugs into animals during preclinical tests.

11.7.7 Implants

Continuous osmotically driven minipumps for **insulin** have been developed which are implanted under the skin. The pumps monitor the level of insulin in the blood and release the hormone as required to keep levels constant. This avoids the problem of large fluctuations in insulin levels associated with regular injections.

Gliadel is a wafer that has been implanted into the brain to administer anticancer drugs directly to brain tumours, thus avoiding the blood–brain barrier.

Polymer-coated, drug-releasing stents have been used to keep blood vessels open after a clot-clearing procedure called angioplasty.

Investigations are underway into the use of implantable microchips which could detect chemical signals in the body and release drugs in response to these signals.

KEY POINTS

- Oral administration is the preferred method of administering drugs, but it is also the most demanding on the drug.
- Drugs administered by methods other than the oral route avoid the first pass effect.
- Drugs can be administered such that they are absorbed through the mucous membranes of the mouth, nose, or eyes.
- Some drugs are administered rectally as suppositories.
- Topically administered drugs are applied to the skin. Some drugs are absorbed through the skin into the blood supply.
- Inhaled drugs are administered as gases or aerosols to act directly on the respiratory system. Some inhaled drugs are absorbed into the blood supply to act systemically.
- Polar drugs that are unable to cross cell membranes are given by injection.
- Injection is the most efficient method of administering a drug, but it is also the most hazardous. Injection can be intravenous, intramuscular, subcutaneous, or intrathecal.
- Implants have been useful in providing controlled drug release such that blood concentrations of the drug remain as level as possible.

11.8 Drug dosing

Because of the number of pharmacokinetic variables involved, it can be difficult to estimate the correct dose regimen for a drug (i.e. the amount of drug used for each dose and the frequency of administration). There are other issues to consider as well. Ideally, the blood levels of any drug should be constant and controlled, but this would require a continuous, intravenous drip, which is clearly impractical for most drugs. Therefore, drugs are usually taken at regular time intervals, and the doses taken are designed to keep the blood levels of drug within a maximum and minimum level such that they are not too high to be toxic, yet not too low to be ineffective. In general, the concentration of free drug in the blood (i.e. not bound to plasma protein) is a good indication of the availability of that drug at its target site. This does not mean that blood concentration levels are the same as the concentration levels at the target site. However, any variations in blood concentration will result in similar fluctuations at the target site. Thus, blood concentration levels can be used to determine therapeutic and safe dosing levels for a drug.

Figure 11.20 shows two dose regimens. Dose regimen A quickly reaches the therapeutic level but continues to rise to a steady state which is toxic. Dose regimen B involves half the amount of drug provided with the same frequency. The time taken to reach the therapeutic level is certainly longer, but the steady state levels of the drug remain between the therapeutic and toxic levels—the **therapeutic window**.

Dose regimens involving regular administration of a drug work well in most cases, especially if the size of each dose is less than 200 mg and doses are taken once or twice a day. However, there are certain situations where timed doses are not suitable. The treatment of diabetes with **insulin** is a case in point. Insulin is normally secreted continuously by the pancreas, so the injection of insulin at timed intervals is unnatural and can lead to a whole range of physiological complications.

Other dosing complications include differences of age, sex, and race. Diet, environment, and altitude also have an influence. Obese people present a particular problem, as it can be very difficult to estimate how much of a drug will be stored in fat tissue and how much will remain free in the blood supply. The precise time when drugs are taken may be important because metabolic reaction rates can vary throughout the day.

Drugs can interact with other drugs. For example, some drugs used for diabetes are bound by plasma protein in the blood supply and are therefore not free to react with their targets. However, drugs such as **aspirin** may displace them from plasma protein, leading to a drug overdose. Aspirin has this same effect on anticoagulants.

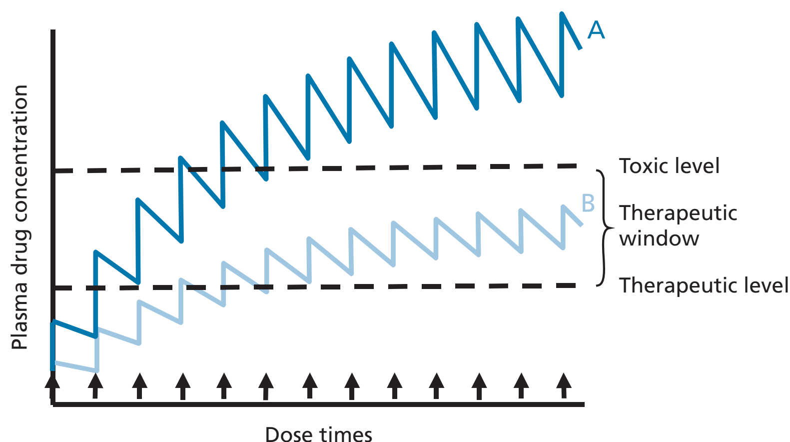


FIGURE 11.20 Dosing regimes.

Problems can also occur if a drug inhibits a metabolic reaction and is taken with a drug normally metabolized by that reaction. The latter is more slowly metabolized than normal, increasing the risk of an overdose. For example, the antidepressant drug **phenelzine** inhibits the metabolism of amines and should not be taken with drugs such as **amphetamines** or **pethidine**. Even amine-rich foods can lead to adverse effects, implying that cheese and wine parties are hardly the way to cheer up a victim of depression. Other examples were described in section 11.5.6.

When one considers all these complications, it is hardly surprising that individual variability to drugs can vary by as much as a factor of 10.

11.8.1 Drug half-life

The half-life ($t_{1/2}$) of a drug is the time taken for the concentration of the drug in blood to fall by half. The removal or elimination of a drug takes place through both excretion and drug metabolism, and is not linear with time. Therefore, drugs can linger in the body for a significant period of time. For example, if a drug has a half-life of 1 hour, then there is 50% of it left after 1 hour. After 2 hours, there is 25% of the original dose left, and after 3 hours, 12.5% remains. It takes 7 hours for the level to fall below 1% of the original dose. Some drugs such as the opioid analgesic **fentanyl**, have short half-lives (45 minutes), whereas others such as **diazepam** (Valium) have a half-life measured in days. In the latter case, recovery from the drug may take a week or more.

11.8.2 Steady state concentration

Drugs are metabolized and eliminated as soon as they are administered, so it is necessary to provide regular doses in order to maintain therapeutic levels in the body. Therefore, it is important to know the half-life of the drug in order to calculate the frequency of dosing required to reach and maintain these levels. In general, the time taken to reach a **steady state concentration** is six times the drug's half-life. For example, the concentration levels of a drug with a half-life of 4 hours, supplied at 4-hourly intervals, is shown in Table 11.1 and Figure 11.21.

Note that there is a fluctuation in level in the period between each dose. The level is at a maximum after each dose and falls to a minimum before the next dose is provided. It is important to ensure that the level does not drop below the therapeutic level but does not rise to such a level that side effects are induced. The time taken to reach steady state concentration is not dependent on the size of the dose, but the blood level achieved at steady state is. Therefore, the levels of drug present at steady state concentration depend on the size of each dose given, as well as the frequency of dosing. During clinical trials, blood samples are taken from patients at regular

TABLE 11.1 Fluctuation of drug concentration levels on regular dosing

Time of dosing (hr)	0	4	8	12	16	20	24
Max. level (μg/ml)	1.0	1.5	1.75	1.87	1.94	1.97	1.98
Min. level (μg/ml)	0.5	0.75	0.87	0.94	0.97	0.98	0.99

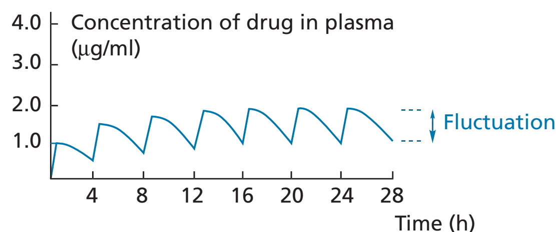


FIGURE 11.21 Graphical representation of fluctuation of drug concentration levels on regular dosing.

time intervals to determine the concentration of the drug in the blood. This helps determine the proper dosing regime in order to get the ideal blood levels.

The **area under the plasma drug concentration curve** (**AUC**) represents the total amount of drug that is available in the blood supply during the dosing regime.

11.8.3 Drug tolerance

With certain drugs, it is found that the effect of the drug diminishes after repeated doses, and it is necessary to increase the size of the dose in order to achieve the same results. This is known as drug tolerance. There are several mechanisms by which drug tolerance can occur. For example, the drug can induce the synthesis of metabolic enzymes which result in increased metabolism of the drug. **Pentobarbital** (Fig. 11.22) is a barbiturate sedative which induces enzymes in this fashion.

Alternatively, the target may adapt to the presence of a drug. Occupancy of a target receptor by an antagonist may induce cellular effects which result in the synthesis of more receptor (section 8.7). As a result, more drug will be needed in the next dose to antagonize all the receptors.

Physical dependence is usually associated with drug tolerance. Physical dependence is a state in which a patient becomes dependent on the drug in order to feel normal. If the drug is withdrawn, uncomfortable **withdrawal symptoms** may arise which can only be alleviated by re-taking the drug. These effects can be explained, in part, by the effects which lead to drug tolerance. For example, if cells have synthesized more receptors to counteract the presence of an antagonist, the removal of the antagonist means that the body will have too many receptors. This results in a 'kickback' effect, where the cell becomes oversensitive to the normal neurotransmitter or hormone—this is what produces withdrawal symptoms. These will continue until the excess receptors have been broken down by normal cellular mechanisms—a process that may take several days or weeks (see also sections 8.6 and 8.7).

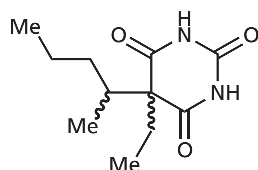


FIGURE 11.22 Pentobarbital.

11.8.4 Bioavailability

Bioavailability refers to how quickly and how much of a particular drug reaches the blood supply once all the problems associated with absorption, distribution, metabolism, and excretion have been taken into account. **Oral bioavailability** (**F**) is the fraction of the ingested dose that survives to reach the blood supply. This is an important property when it comes to designing new drugs and should be considered alongside the pharmacodynamics of the drug (i.e. how effectively the drug interacts with its target).

11.9 Formulation

The way a drug is formulated can avoid some of the problems associated with oral administration. Drugs are normally taken orally as tablets or capsules. A tablet is usually a compressed preparation that contains 5–10% of the drug, 80% of fillers, disintegrants, lubricants, glidants, and binders, and 10% of compounds which ensure easy disintegration, disaggregation, and dissolution of the tablet in the stomach or the intestine—a process which is defined as the **pharmaceutical phase** of drug action. The disintegration time can be modified for a rapid effect or for sustained release. Special coatings can make the tablet resistant to the stomach acids such that it only disintegrates in the duodenum as a result of enzyme action or pH. Pills can also be coated with sugar, varnish, or wax to disguise taste. Some tablets are designed with an osmotically active bi-layer core surrounded by a semi-permeable membrane with one or more laser-drilled pores in it. The osmotic pressure of water entering the tablet pushes the drug through the pores at a constant rate as the tablet moves through the digestive tract. Therefore, the rate of release is independent of varying pH or gastric motility. Several drugs, such as **hydromorphone, albuterol**, and **nifedipine**, have been administered in this way.

A capsule is a gelatinous envelope enclosing the active substance. Capsules can be designed to remain intact for some hours after ingestion in order to delay absorption. They may also contain a mixture of slow- and fast-release particles to produce rapid and sustained absorption in the same dose.

The drug itself needs to dissolve in aqueous solution at a controlled rate. Such factors as particle size and crystal form can significantly affect dissolution. Fast dissolution is not always ideal. For example, slow dissolution rates can prolong the duration of action or avoid initially high plasma levels.

Formulation can also play an important role in preventing drugs being abused. For example, a tablet preparation **(Oxecta)** of the opioid analgesic **oxycodone** was approved in 2011 as an orally active opioid analgesic and includes deterrents to abuse. For example, chemicals are present that prevent the drug being dissolved in solvent and injected. Other chemicals cause a burning sensation in the nose, which discourages drug abusers crushing the tablets and snorting the powder. Finally, other chemicals are present which produce non-toxic, but very unpleasant effects if too many pills are taken orally.

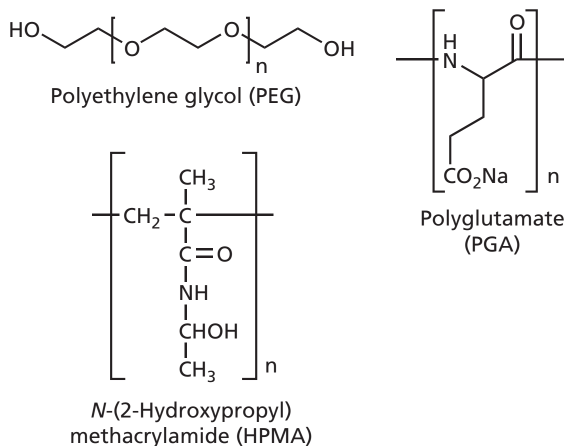


FIGURE 11.23 Synthetic polymers used for polymer–drug conjugates.

11.10 Drug delivery

The various aspects of drug delivery could fill a textbook in itself, so any attempt to cover the topic in a single section is merely tickling the surface, let alone scratching it! However, it is worth appreciating that there are various methods by which drugs can be physically protected from degradation and/or targeted to treat particular diseases, such as cancer and inflammation. One approach is to use a prodrug strategy (section 14.6), which involves chemical modifications to the drug. Another approach covered in this section is the use of water-soluble macromolecules to help the drug reach its target. The macromolecules concerned are many and varied, and include synthetic polymers, proteins, liposomes, and antibodies. The drug itself may be covalently linked to the macromolecule or encapsulated within it. The following are some illustrations of drug delivery systems.

Antibodies were described in section 10.7.2 and have long been seen as a method of targeting drugs to cancer cells. Methods have been devised for linking anticancer drugs to antibodies to form **antibody–drug conjugates** that remain stable on their journey through the body, but release the drug at the target cell. A lot of research has been carried out on these conjugates and this is discussed in detail in section 21.9.2. However, there are problems associated with antibodies. The amount of drug that can be linked to the protein is quite limited and there is the risk of an immune reaction where the body identifies the antibody as foreign and tries to reject it.

A similar approach is to link the drugs to synthetic polymers, such as polyethylene glycol (PEG), polyglutamate, or *N*-(2-hydroxypropyl)methacrylamide (HPMA) to form polymer–drug conjugates (Fig. 11.23). Again, the amount of drug that can be linked is limited, but a variety of anticancer–polymer conjugates are currently undergoing clinical trials. Such conjugates help to protect the lifetime of the drug by decreasing the rates of metabolism and excretion. **Pegaptanib** is a preparation that was approved for treating a vascular disease in the eye and consists of an oligonucleotide drug linked to PEG (section 10.5).

Protein-based polymers are being developed as drug delivery systems for the controlled release of ionized drugs. For example, the cationic drugs **Leu-enkephalin** or **naltrexone** could be delivered using polymers with anionic carboxylate groups. Ionic interactions between the drug and the protein result in folding and assembly of the protein polymer to form a protein–drug complex, and the drug is then released at a slow and constant rate. The amount of drug carried could be predetermined by the density of carboxylate binding sites present and the accessible surface area of the vehicle. The rate of release could be controlled by varying the number of hydrophobic amino acids present. The greater the number of hydrophobic amino acids present, the weaker the affinity between the carboxylate binding groups and the drug. Once the drug is released, the protein carrier would be metabolized like any normal protein.

A physical method of protecting drugs from metabolic enzymes in the bloodstream and allowing a steady slow release of the drug is to encapsulate the drug within small vesicles called **liposomes**, and then inject them into the blood supply (Fig. 11.24). These vesicles or globules consist of a bilayer of fatty phospholipid molecules (similar to a cell membrane) and will travel around the circulation, slowly leaking their contents. Liposomes are known to be concentrated in malignant tumours and this provides a possible method of delivering anti-tumour drugs to these cells. It is also found that liposomes can fuse with

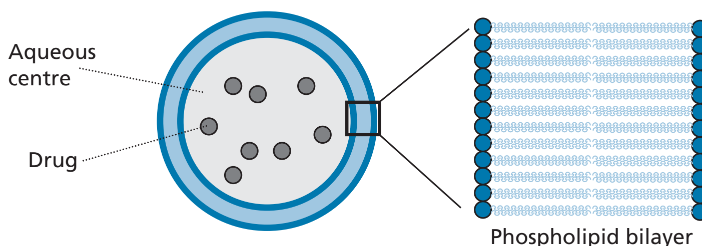


FIGURE 11.24 Liposome containing a drug.

the plasma membranes of a variety of cells, allowing the delivery of drugs or DNA into these cells. As a result, they may be useful for gene therapy. The liposomes can be formed by sonicating a suspension of a phospholipid (e.g. phosphatidylcholine) in an aqueous solution of the drug.

Another future possibility for targeting liposomes is to incorporate antibodies into the liposome surface such that specific tissue antigens are recognized. Liposomes have a high drug-carrying capacity, but it can prove difficult to control the release of drug at the required rate. Slow leakage is a problem if the liposome is carrying a toxic anticancer drug such as **doxorubicin**. The liposomes can also be trapped by the **reticuloendothelial system** (RES) and removed from the blood supply. The RES is a network of cells which can be viewed as a kind of filter. One answer to this problem has been to attach PEG polymers to the liposome (see also section 14.8.2). The tails of the PEG polymers project out from the liposome surface and act as a polar outer shell, which both protects and shields the liposome from destructive enzymes and the RES. This increases its lifetime significantly and reduces leakage of its passenger drug. **DOXIL** is a PEGylated liposome containing doxorubicin which is used successfully in anticancer therapy as a once-monthly infusion.

The use of injectable **microspheres** has been approved for the delivery of human growth hormone. The microspheres containing the drug are made up of a biologically degradable polymer and slowly release the hormone over a four-week period.

A large number of important drugs have to be administered by injection because they are either susceptible to digestive enzymes or cannot cross the gut wall. This includes the ever-growing number of therapeutically useful peptides and proteins being generated by biotechnology companies using recombinant DNA technology. Drug delivery systems which could deliver these drugs orally would prove a huge step forward in medicine. For example, liposomes are currently being studied as possible oral delivery systems. Another approach being investigated currently is to link a therapeutic protein to a hydrophobic polymer such that it is more likely to be absorbed. However, it is important that the conjugate breaks up before the drug enters the blood supply or else it would have to be treated as a new drug and undergo expensive preclinical and clinical trials. **Hexyl-insulin monoconjugate 2** consists of a polymer linked to a lysine residue of insulin. It is currently being investigated as an oral delivery system for **insulin**.

Biologically erodable microspheres have also been designed to stick to the gut wall such that absorption of the drug within the sphere through the gut wall is increased. This has still to be used clinically, but has proved effective in enhancing the absorption of insulin and **plasmid DNA** in test animals. In a similar vein, drugs have been coated with bioadhesive polymers designed to adhere to the gut wall so that the drug has more chance of being absorbed. The use of anhydride polymers has the added advantage that these polymers are capable of crossing the gut wall and entering the bloodstream, taking their passenger drug with them. Emisphere Technologies Inc. have developed derivatives of amino acids and have shown that they can enhance the absorption of specific proteins. It is thought that the amino acid derivatives interact with the protein and make it more lipophilic so that it can cross cell membranes directly.

Drug delivery systems are being investigated which will carry oligonucleotides such as DNA, antisense molecules, and siRNAs (section 9.7.2). For example, nucleic acid–lipid particles are being investigated as a means of delivering oligonucleotides into liver cells. Such particles are designed to have a positive charge on their exterior as this encourages adsorption to the negatively charged cell membranes of target cells. Another method of carrying and delivering oligonucleotides is to incorporate them into viruses that are capable of infecting cells. However, there are risks associated with this approach and there have been instances of fatalities during clinical trials. Therefore, nanotechnology is being used to construct artificial viruses which will do the job more safely. Clinical trials have demonstrated that it is possible to use engineered viruses to target drugs to tumour cells.

Other areas of research include studies of crown ethers, nanoparticles, nanospheres, nanowires, nanomagnets, biofuel cells, hydrogel polymers, and superhydrophobic materials as methods of delivering drugs.

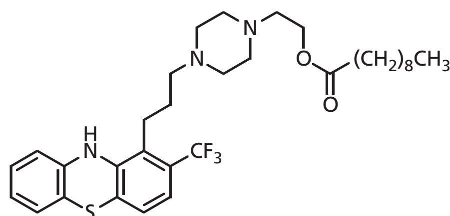
KEY POINTS

- Drugs should be administered at the correct dose levels and frequency to ensure that blood concentrations remain within the therapeutic window.
- The half-life of a drug is the time taken for the blood concentration of the drug to fall by half. A knowledge of the half-life is required to calculate how frequently doses should be given to ensure a steady state concentration.
- Drug tolerance is where the effect of a drug diminishes after repeated doses. In physical dependence a patient becomes dependent on a drug and suffers withdrawal symptoms on stopping the treatment.
- Formulation refers to the method by which drugs are prepared for administration, whether by solution, pill, capsule, liposome, or microsphere. Suitable formulations can protect drugs from particular pharmacokinetic problems.

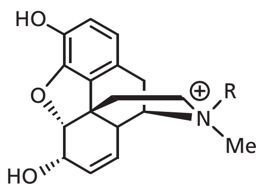
QUESTIONS

1. Benzene used to be a common solvent in organic chemistry, but is no longer used because it is a suspected carcinogen. Benzene undergoes metabolic oxidation by cytochrome P450 enzymes to form an electrophilic epoxide which can alkylate proteins and DNA. Toluene is now used as a solvent in place of benzene. Toluene is also oxidized by cytochrome P450 enzymes, but the metabolite is less toxic and is rapidly excreted. Suggest what the metabolite might be and why the metabolism of toluene is different from that of benzene.

2. The prodrug of the antipsychotic drug **fluphenazine** shown below has a prolonged period of action when it is given by intramuscular injection, but not when it is given by intravenous injection. Suggest why this is the case.



Fluphenazine prodrug



Morphine; R=H
Quaternary salt; R=Me

3. Morphine binds strongly to opioid receptors in the brain to produce analgesia. *In vitro* studies on opioid receptors show that the quaternary salt of morphine also binds strongly. However, the compound is inactive *in vivo* when injected intravenously. Explain this apparent contradiction.

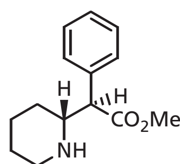
4. The phenol group of morphine is important in binding morphine to opioid receptors and causing analgesia. Codeine has the same structure as morphine, but the phenol group is masked as a methyl ether. As a result, codeine binds poorly to opioid receptors and should show no analgesic activity. However, when it is taken *in vivo*, it shows useful analgesic properties. Explain how this might occur.

5. The pK_a of histamine is 5.74. What is the ratio of ionized to un-ionized histamine (a) at pH 5.74 (b) at pH 7.4?

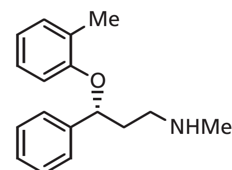
6. A drug contains an ionized carboxylate group and shows good activity against its target in *in vitro* tests. When *in vivo* tests were carried out, the drug showed poor activity when it was administered orally, but good activity when it was administered by intravenous injection. The same drug was converted to an ester, but proved inactive *in vitro*. Despite that, it proved to be active *in vivo* when it was administered orally. Explain these observations.

7. Atomoxetine and methylphenidate are used in the treatment of attention deficit hyperactivity disorder. Suggest possible metabolites for these structures.

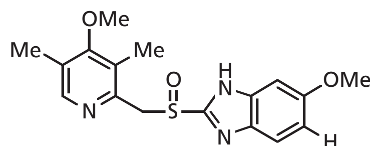
8. Suggest metabolites for the proton pump inhibitor omeprazole.



Methylphenidate



Atomoxetine



Omeprazole

9. A drug has a half-life of 4 hours. How much of the drug remains after 24 hours?

10. Salicylic acid is absorbed more effectively from the stomach than from the intestines, whereas quinine is absorbed more effectively from the intestines than from the stomach. Explain these observations.

FURTHER READING

Duncan, R. (2003) The dawning era of polymer therapeutics. *Nature Reviews Drug Discovery* **2**, 347–360.

Goldberg, M. and Gomez-Orellana, I. (2003) Challenges for the oral delivery of macromolecules. *Nature Reviews Drug Discovery* **2**, 257–258.

Guengerich, F. P. (2002) Cytochrome P450 enzymes in the generation of commercial products. *Nature Reviews Drug Discovery* **1**, 359–366.

King, A. (2011) Breaking through the barrier. *Chemistry World* June, 36–39.

Langer, R. (2003) Where a pill won't reach. *Scientific American* April, 32–39.

LaVan, D. A., Lynn, D. M., and Langer, R. (2002) Moving smaller in drug discovery and delivery. *Nature Reviews Drug Discovery* **1**, 77–84.

Lindpaintner, K. (2002) The impact of pharmacogenetics and pharmacogenomics on drug discovery. *Nature Reviews Drug Discovery* **1**, 463–469.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **23**, 3–25.

Mastrobattista, E., van der Aa, M. A., Hennink, W. E., and Crommelin, D. J. (2006) Artifical viruses: a nanotechnological approach to gene delivery. *Nature Reviews Drug Discovery* **5**, 115–121.

Nicholson, J. K. and Wilson, I. D. (2003) Understanding global systems biology: metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery* **2**, 668–676.

Pardridge, W. M. (2002) Drug and gene targeting to the brain with molecular Trojan horses. *Nature Reviews Drug Discovery* **1**, 131–139.

Roden, D. M. and George, A. L. (2002) The genetic basis of variability in drug responses. *Nature Reviews Drug Discovery* **1**, 37–44.

Roses, A. D. (2002) Genome-based pharmacogenetics and the pharmaceutical industry. *Nature Reviews Drug Discovery* **1**, 541–549.

Rowland, M. and Tozer, T. N. (1980) *Clinical Pharmacokinetics*. Lea and Febiger, Philadelphia.

Saltzman, W. M. and Olbricht, W. L. (2002) Building drug delivery into tissue engineering. *Nature Reviews Drug Discovery* **1**, 177–186.

Sam, A. P. and Tokkens, J. G. (eds) (1996) *Innovations in Drug Delivery: Impact on Pharmacotherapy*. Anselmus Foundation, Hauten.

Stevenson, R. (2003) Going with the flow. *Chemistry in Britain* November, 18–20.

van de Waterbeemd, H., Testa, B., and Folkers, G. (eds) (1997) *Computer-assisted Lead Finding and Optimisation*. Wiley-VCH, New York.

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., and Kopple, K. D. (2002) Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry* **45**, 2615–2623.

Willson, T. M. and Kliewer, S. A. (2002) PXR, CAR and drug metabolism. *Nature Reviews Drug Discovery* **1**, 259–266.

Titles for general further reading are listed on p.763.

CASE STUDY 1
Statins

Statins are an important group of cholesterol-lowering drugs that act as enzyme inhibitors. The market for cholesterol-lowering drugs is the largest in the pharmaceutical sector and is dominated by the statins, with substantial rewards for the companies that produce them. In 2002, **atorvastatin** and **simvastatin** recorded revenues of about $7 bn and $5.3 bn dollars respectively. In this case study, we shall look at how these drugs were discovered and how they interact with their target at the molecular level. Firstly, we shall consider the role of cholesterol in coronary heart disease and how the inhibition of an enzyme can lower cholesterol levels.

CS1.1 Cholesterol and coronary heart disease

Cholesterol is an important constituent of cell membranes and is also the biosynthetic precursor for steroid hormones. It is vital, therefore, to the normal, healthy functioning of cells and can be obtained both from the diet and biosynthesis in cells. Problems arise if too much cholesterol is present in the diet as this can lead to cardiovascular disease.

As cholesterol is a fatty molecule, it cannot dissolve in blood and so it has to be transported round the body by particles known as **low-density lipoprotein (LDL)** or **high-density lipoprotein (HDL)**. LDLs are particles about 22 nm in diameter that have a mass of 3 million Daltons. Each particle contains a lipoprotein of 4536 amino acid residues that encircles a variety of fatty acids, keeping them soluble in the aqueous environment of the blood supply. The particle also contains a polyunsaturated fatty acid called **linoleate**, several phospholipids, and a large number of cholesterol molecules. LDL serves to transport cholesterol and triglycerides from the liver to the peripheral tissues. When a cell requires cholesterol it produces LDL receptors, which are placed in the cell membrane. LDL binds to these receptors and is then endocytosed into the cell where it releases cholesterol into the cytoplasm.

HDLs are lipoprotein particles about 8–11 nm in diameter that carry fatty acids and cholesterol from tissues back to the liver, where they are removed from the blood supply. They are called HDLs because they contain a higher proportion of protein than the LDLs. When they travel round the body, they steadily increase in size as they pick up cholesterol from the tissues.

Mortality from coronary heart disease has been shown to be associated with high levels of LDL or low levels of HDL. Inevitably, LDLs transport cholesterol to the arteries and, if cholesterol is retained there, it can lead to the formation of fatty plaques which narrow the arteries, resulting in an increased risk of atherosclerosis. If a clot forms and blocks an artery supplying blood to heart muscle, it leads to a heart attack. If the clot blocks an artery serving the brain, a stroke results. Thus, lowering the levels of LDL and/or increasing HDL should reduce the risk of heart attacks and strokes. When the statins were first designed, the aim was to lower the levels of cholesterol that were synthesized in the body. The statins certainly do this, but, as we shall see later, it is the subsequent effects on LDL plasma levels that are more important in their protective actions against cardiovascular disease.

CS1.2 The target enzyme

Cholesterol is synthesized within cells, and one way of lowering cholesterol levels in the blood is to block this synthesis. This can be achieved by finding a drug which will inhibit one of the enzymes involved in cholesterol biosynthesis. However, there are more than 30 enzymes involved in the biosynthetic pathway, so how does one decide which of these enzymes is the best target? The choice can be narrowed down by targeting the enzyme that catalyses the rate-limiting step for the overall process, as this provides the most effective inhibition of the biosynthetic pathway. The enzyme catalysing the rate-limiting step is a reductase enzyme called **3-hydroxy-3-methylglutaryl-coenzyme A reductase** (HMGR or HMG-CoA reductase). The reaction involved is the conversion of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate with the aid of NADPH as a cofactor (section 3.5.4) (Fig CS1.1).

HMGR consists of four protein subunits and is one of the most highly regulated enzymes known. The enzyme's activity can be decreased in a number of ways if too much cholesterol is produced. Firstly, high levels of cholesterol within the cell trigger a signal transduction process which activates a protein kinase responsible for

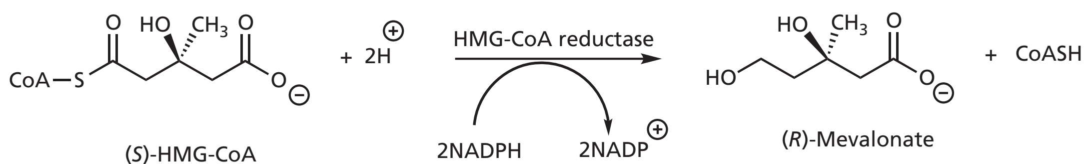


FIGURE CS1.1 Reaction catalysed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR or HMG-CoA reductase).

phosphorylating HMGR and inactivating it. Secondly, the rate at which the enzyme is synthesized through transcription and translation is controlled by intracellular levels of cholesterol. Finally, the rate at which the enzyme is degraded appears to be influenced by cholesterol levels.

There are two active sites present in the tetrameric structure and each one is located between two of the monomers. The portion of the active site which binds the substrate HMG-CoA is predominantly on one monomer, while the portion that binds the cofactor NADPH is situated on a neighbouring monomer.

As far as the reaction pathway is concerned, it involves a reductive cleavage involving two hydride transfers (Fig. CS1.2). The hydride is provided by the cofactor NADPH and so two NADPH molecules are required for each reaction.

The HMGR enzyme is found to be highly flexible in its three-dimensional structure and this has an important role to play in the binding and activity of statins.

Different amino acid residues in the active site have important roles to play in the enzyme-catalysed reaction, either in binding the substrate or in the mechanism of the reaction.

As far as binding goes, a positively charged lysine residue (Lys-735) forms an ionic bond with the negatively charged carboxylate group of HMG-CoA. Other residues, such as Ser-684 and Asp-690 interact with the alcohol group by hydrogen bonding, while Lys-691 is involved in a hydrogen bonding interaction with the carbonyl group (Fig. CS1.3). The coenzyme A moiety is also bound by different interactions into a narrow hydrophobic slot within the active site.

Other amino acids play an important part in the mechanism of the enzyme-catalysed reaction (Fig CS1.4). A histidine residue (His-866) acts as an acid catalyst and provides the proton required by coenzyme A to depart as a leaving group. A special mention needs to be made of the binding role of Lys-691. We have already seen that this forms a hydrogen bond to the substrate, but it also plays a particularly important role in stabilizing the negatively charged oxygen of mevaldyl-CoA through hydrogen bonding and ionic interactions. This not only helps to stabilize the intermediate but also stabilizes the transition state leading to it. Consequently, the activation energy for the first step in the mechanism is lowered, allowing the reaction to occur more easily.

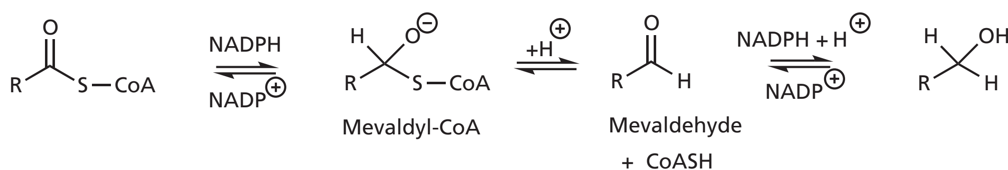


FIGURE CS1.2 Reaction pathway catalysed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR or HMG-CoA reductase).

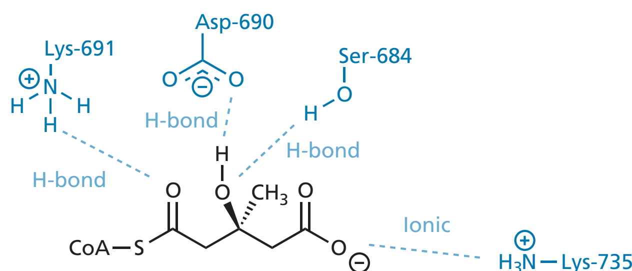


FIGURE CS1.3 Binding interactions for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA).

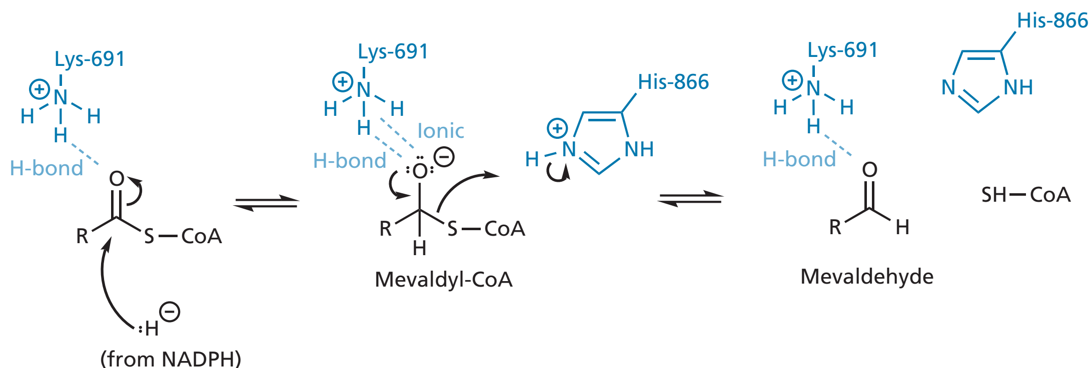


FIGURE CS1.4 Mechanism of the first reduction.

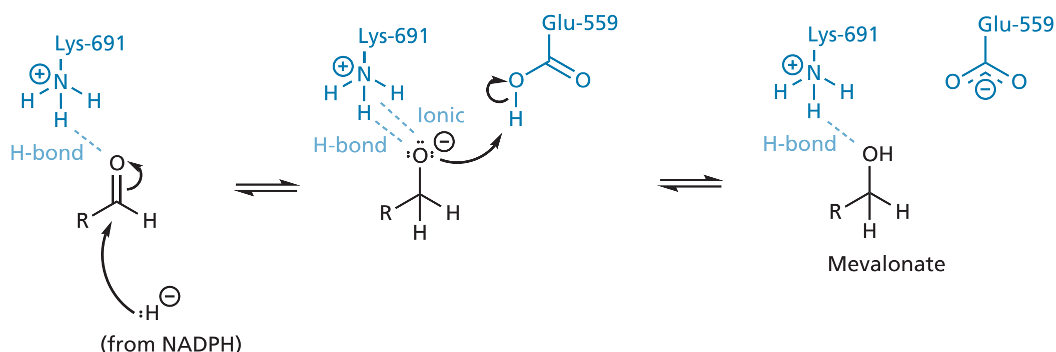


FIGURE CS1.5 Mechanism of the second reduction.

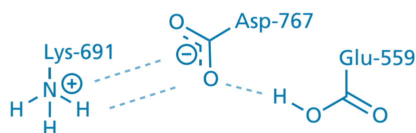


FIGURE CS1.6 Hydrogen bonding network involving Lys-691, Asp-767, and Glu-559.

An uncharged glutamic acid residue (Glu-559) is also involved as an acid catalyst and provides a proton for the final stage where mevaldehyde is reduced to mevalonate (Fig. CS1.5).

It is rather unusual to have an uncharged glutamic acid residue present in an active site. The reason that it is not ionized is that there is a neighbouring aspartate residue (Asp-767) which affects the pK_a of the glutamic acid residue. The aspartate residue also helps to stabilize the ionic form of Lys-691 through the hydrogen bonding network shown in Fig CS1.6.

CS1.3 The discovery of statins

Once the HMGR enzyme was identified as a potential target, researchers set out to find a lead compound that would inhibit it. They started by concentrating their attention on compounds produced by microorganisms. This might appear odd, but the rationale was that microorganisms are constantly involved in chemical warfare with each other, and so a microorganism that produces a chemical that is toxic to another microorganism gains an advantage in the never-ending fight for survival (section 12.4.1.2). It seemed likely that microorganisms lacking HMGR might produce HMGR inhibitors which would be toxic to microbes that require HMGR in order to produce important sterols.

Compactin (Mevastatin) (Fig CS1.7) was the first potent statin to be found that inhibited HMGR, and it can be viewed as the lead compound for this group of drugs. It is a natural product that was isolated from *Penicillium citrinum* in the 1970s, following an investigation of 6000 microbes by the Japanese pharmaceutical scientist Akira Endo. Studies showed that it was a highly potent inhibitor and had a 10,000-fold higher affinity for the enzyme than the natural substrate. Although it entered clinical trials, the drug never reached the market. The reason for this has never been fully revealed, but it is likely that adverse toxic effects were observed during preclinical trials.

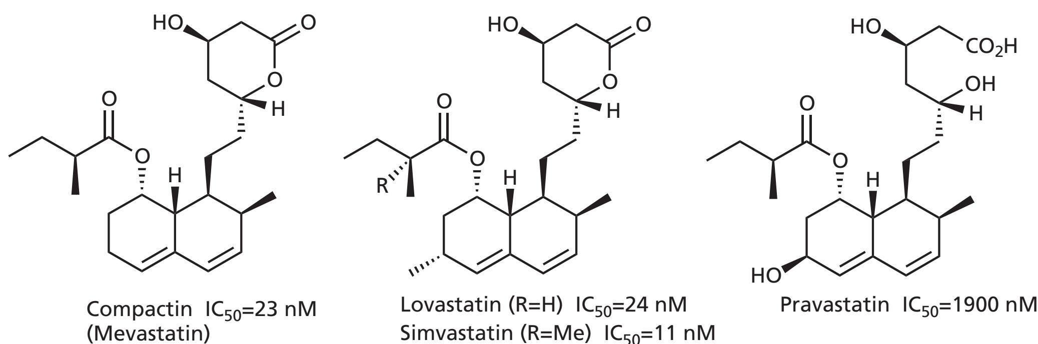


FIGURE CS1.7 Type I statins.

In 1978, Merck isolated a closely related structure called **mevinolin** from the fermentation broth of *Aspergillus terreus*. This was also a potent inhibitor and clinical trials began in 1980. The drug was marketed in 1987 as **lovastatin** (Fig. CS1.7) and it revolutionized the treatment of hypercholesterolaemia (high cholesterol levels).

Other statins soon followed (Fig. CS1.7). **Simvastatin** is a semi-synthetic structure prepared from lovastatin and was first approved in 1988. **Pravastatin** is derived from compactin by biotransformation and reached the market in 1991.

These statins represent the first generation of statins and have been classified as **Type I statins**. They are all derived directly or indirectly from fungal metabolites, and share a similar structure which contains a polar 'head group' and a hydrophobic moiety which includes a bicyclic decalin ring (Fig. CS1.8).

Observant readers will notice that the structures for lovastatin and simvastatin contain a lactone ring, and not the acyclic polar head-group shown in Fig. CS1.8. However, the lactone rings observed in these structures are hydrolysed by enzymes in the body to produce the polar 'head-group', and this structure represents the active drug. Lovastatin and simvastatin are therefore termed as prodrugs (section 14.6).

Although the Type I statins have been extremely effective in lowering cholesterol levels, they do have side effects.

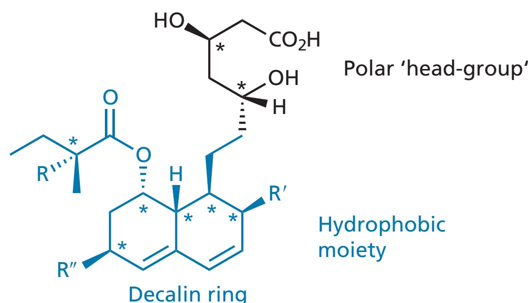


FIGURE CS1.8 General structure for the Type I statins (*represents an asymmetric centre).

They are also difficult to synthesize owing to the number of asymmetric centres associated with the decalin ring, and so further work was carried out to find statins with improved activity and reduced side effects, and which would be easier to synthesize. This resulted in a second generation of statins known as the Type II statins.

CS1.3.1 Type II statins

In contrast to type I statins, type II statins are synthetic structures that contain a different (and larger) hydrophobic moiety from the decalin ring system present in Type I statins (Fig CS1.9). The hydrophobic moieties present in Type II statins may be larger than the decalin system, but they are easier to synthesize as they contain no asymmetric centres (simplification; *see section 13.3.8*). **Fluvastatin** was marketed in 1994, **atorvastatin** in 1997, **cerivastatin** in 1998, and **rosuvastatin** in 2003. The structures share a number of common structural features, and can be viewed as 'me too' or 'me better' drugs (section 12.4.4.1). In 2001, atorvastatin became the biggest selling drug in history. It is the most commonly prescribed statin and has remained the biggest selling drug in the world for several years, bringing in nearly £10 billion in sales for Pfizer during 2010 alone.

Of these structures, cerivastatin is the most hydrophobic, while pravastatin and rosuvastatin are the least hydrophobic. Studies have shown that statins with a lower hydrophobic character are more selective for liver cells, where most cholesterol synthesis takes place, and that such statins have fewer side effects. Side effects are thought to be caused by the inhibition of HMGR in other tissues, particularly muscle cells, where a condition known as **myalgia** can occur. This is a type of muscle pain or weakness that is particularly prevalent among individuals who take statins and who exercise vigorously. A severe form of muscle toxicity is a condition known as **rhabdomyolysis**, which can be fatal. Indeed, cerivastatin was withdrawn in 2001 following a large number of reported cases of rhabdomyolysis, which included 50 fatalities caused by kidney failure.

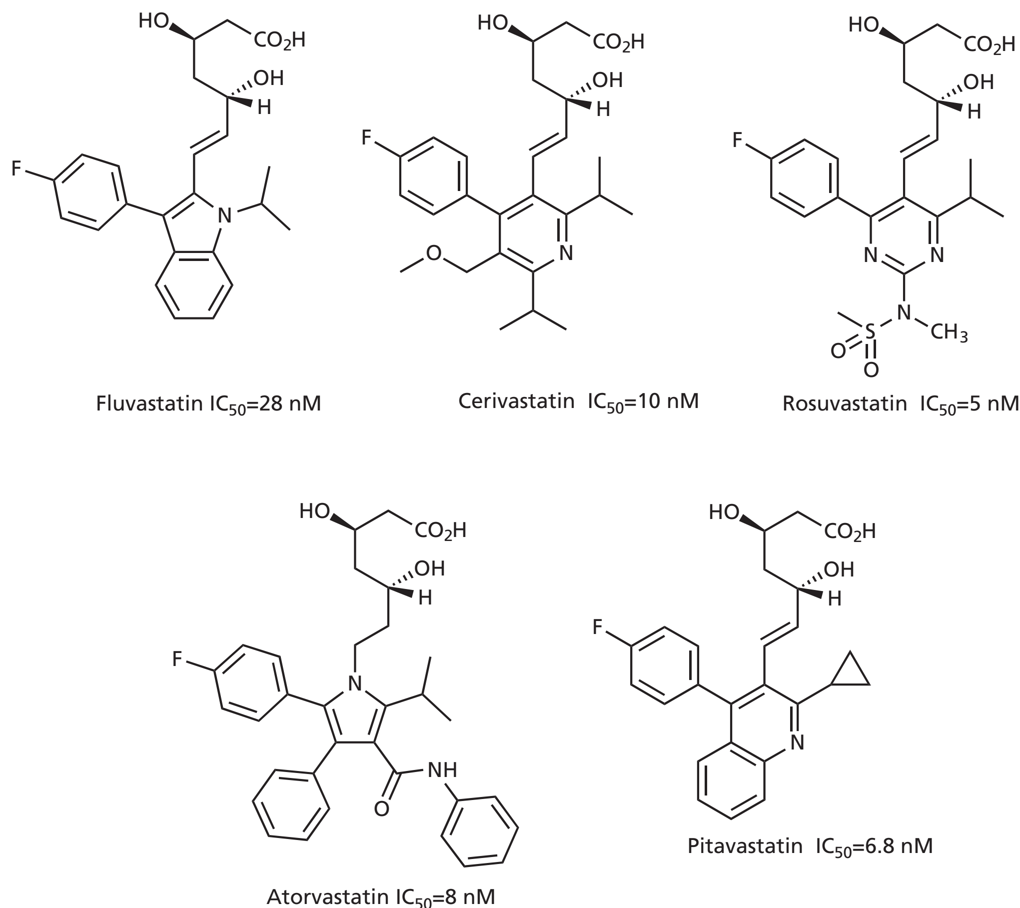


FIGURE CS1.9 Type II statins.

The most potent statin currently available is rosuvastatin. This structure contains a sulphonamide group which was introduced to lower the drug's hydrophobic character. Coincidentally, the introduction of this group resulted in enhanced binding interactions as described later.

The selectivity associated with less hydrophobic statins is a result of the way they access cells. The less hydrophobic statins do not diffuse easily through cell membranes and require transport proteins to reach effective levels within cells (see also sections 2.7.2 and 14.1). Liver cells possess a transport protein which can carry statins across the cell membrane, whereas muscle cells do not.

CS1.4 Mechanism of action for statins—pharmacodynamics

The statins work by acting as competitive inhibitors (section 7.1.1). They mimic the natural substrate and compete with it in order to bind to the active site. Unlike the natural substrate, they do not undergo an enzyme-catalysed reaction and they bind more strongly. How can we explain all of this?

Both the polar head group and the hydrophobic moieties are important to the action of statins. All the statins share the same polar head-group and it is this group which mimics the natural substrate (HMG-SCoA). This can be seen more clearly if we redraw the structure of HMG-SCoA as shown in Fig. CS1.10 and compare it with a general structure for the statins. The head-group of the statins can, therefore, mimic the natural substrate and bind to the active site using the same binding interactions. We now need to explain why statins bind more strongly than the natural substrate and why they are resistant to the enzyme-catalysed reaction.

- Firstly, the statins contain an extra hydrophobic region which can form additional hydrophobic interactions with a hydrophobic binding region present in the enzyme. This allows the statins to bind more strongly.
- Secondly, the statins are resistant to the enzyme-catalysed reaction as the coenzyme A moiety in the

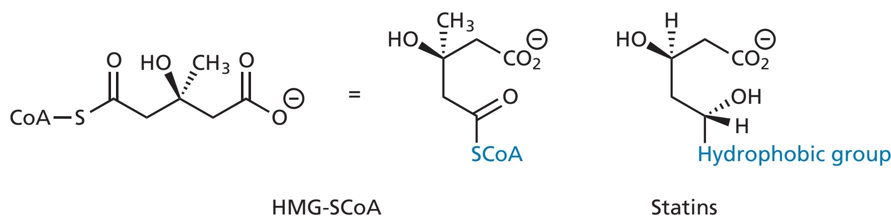


FIGURE CS1.10 Structural comparison of HMG-SCoA with statins.

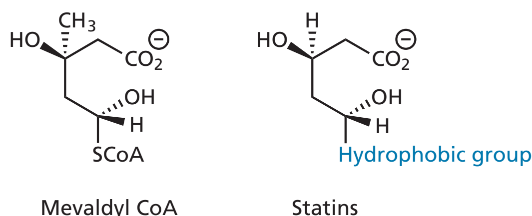


FIGURE CS1.11 Structural comparison of mevaldyl CoA with statins.

substrate (which acts as a leaving group) has been replaced with a hydrophobic group that cannot act as a leaving group.

There is one other interesting feature about the statins. They are actually more similar to the first intermediate in the enzyme-catalysed mechanism—mevaldyl CoA—than to the substrate (Fig. CS1.11). Assuming that mevaldyl CoA is less stable than the substrate, this implies that the statins bear some resemblance to the transition state leading to mevaldyl CoA. Consequently, they would be expected to have a stronger binding interaction than the natural substrate and are likely to be acting as transition-state analogues (section 7.4). We shall now look in more detail at the binding interactions of the statins.

CS1.5 Binding interactions of statins

The binding interactions of the substrate with the enzyme have been studied by X-ray crystallography, as have the binding interactions of the statins* (see also section 13.3.11).

The polar head-group of the statins binds in a similar fashion to the substrate, as described previously (Fig. CS1.3). As far as the hydrophobic region is concerned, we might be tempted to think that it would bind to the same region of the active site as coenzyme A. However, studies carried out on the enzyme–substrate complex show that the binding pocket for coenzyme A is narrow and could not possibly accommodate the bulky hydrophobic groups that are present in statins. There is also no other hydrophobic region into which the statins could bind and so they should really be inactive compounds. The fact that they do bind to the enzyme reflects a marked flexibility that is inherent to the enzyme.

Let us return to look more closely at how the substrate binds to HMGR. When the substrate binds, an alpha-helical section of the protein folds over the active site, shielding it from water and creating a narrow hydrophobic binding region for the coenzyme A portion of the substrate. When a statin binds, the enzyme alters shape in a different manner. Movement of flexible *C*-terminal alpha helices exposes a shallow, but different, hydrophobic binding region next to the active site that can accommodate the hydrophobic moiety present in the statin. Thus, the statins are effective inhibitors because they can take advantage of the enzyme's flexibility and essentially create their own binding site.

Comparing the binding interactions of type I and type II statins with the enzyme, it is found that the methylethyl group in type II statins binds to the same part of the shallow hydrophobic region as the decalin ring of type I statins. Type II statins have additional interactions which include van der Waals interactions with the hydrophobic side chains of amino acids, such as leucine, valine, and alanine. A particularly important interaction involves the fluorophenyl group of type II statins and an arginine residue in the binding region (Fig. CS1.12). Firstly, there is a polar interaction between this residue and the fluoro substituent. Secondly, the planar guanidinium group of the residue is stacked over the phenyl ring allowing additional interactions.

Atorvastatin and rosuvastatin can form an extra hydrogen bonding interaction with the enzyme that does not occur with other statins. This involves a serine residue which acts as hydrogen bond donor to the carbonyl oxygen atom of atorvastatin (Fig. CS1.12) or to the sulphone oxygen of rosuvastatin.

* These studies were actually carried out using the catalytic portion of the HMGR enzyme rather than the whole enzyme.

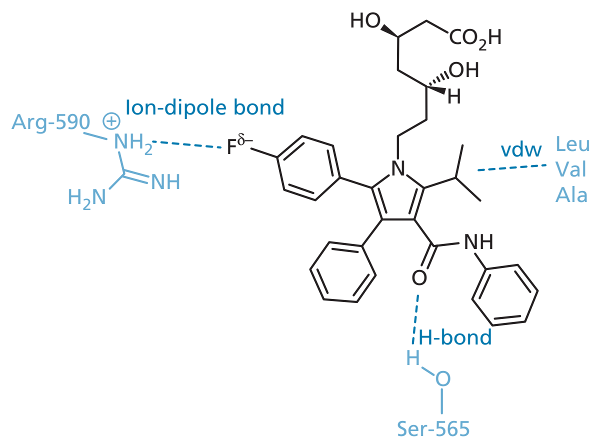


FIGURE CS1.12 Binding interactions for the hydrophobic moiety of atorvastatin with HMGR (3-hydroxy-3-methylglutaryl-coenzyme A reductase).

Rosuvastatin is unique among the statins in having an extra binding interaction between the sulphone group of the drug and Arg-568, making it the most strongly bound statin.

CS1.6 Other mechanisms of action for statins

The action of statins is not purely down to inhibition of HMGR. Certainly, inhibition causes a decrease in the levels of mevalonate and cholesterol, but this, in turn, leads to an up-regulation in the transcription and translation processes leading to new HMGR which should counteract the inhibition. The fact that statins are still effective is a result of other factors. In particular, the lowering of cholesterol levels in liver cells causes an increase in the synthesis of hepatic LDL receptors which are then incorporated into the cell membrane. These receptors are responsible for clearing LDL cholesterol from the plasma and it is this that is crucial to the effectiveness of the statins.

CS1.7 Other targets for cholesterol-lowering drugs

We mentioned earlier that over 30 enzymes are involved in the biosynthesis of cholesterol. Early attempts to find cholesterol-lowering drugs studied the inhibition of enzymes catalysing the later steps in the biosynthetic pathway. There is sense in this, because inhibiting an enzyme late on in a biosynthesis is likely to have a more selective action. In other words, levels of the final product are lowered without affecting the biosynthesis of other compounds which share part of the same biosynthetic pathway. Although the inhibitors were effective, it led to an accumulation of unused substrate which proved insoluble and toxic. When HMG-CoA reductase is inhibited, the substrate is water soluble and easily metabolized. Therefore, it does not build up to toxic levels.

Statins have also been used in combination with drugs that target proteins not directly involved in cholesterol biosynthesis. For example, **Vytorin** is a preparation which includes **simvastatin** and a cholesterol absorption inhibitor called **ezetimibe** (Fig. CS1.13). The latter lowers the levels of cholesterol absorbed from the gastrointestinal tract.

Another approach currently being studied is to inhibit the **cholesteryl ester transfer protein** (CETP). This is a plasma protein that aids the transfer of triglcyerides and cholesterol between LDLs and HDLs. Studies on CETP inhibitors indicate that these can raise HDL levels and lower LDL levels in the presence of statins. **Anacetrapib** (Fig. CS1.13) is one such inhibitor undergoing clinical trials.

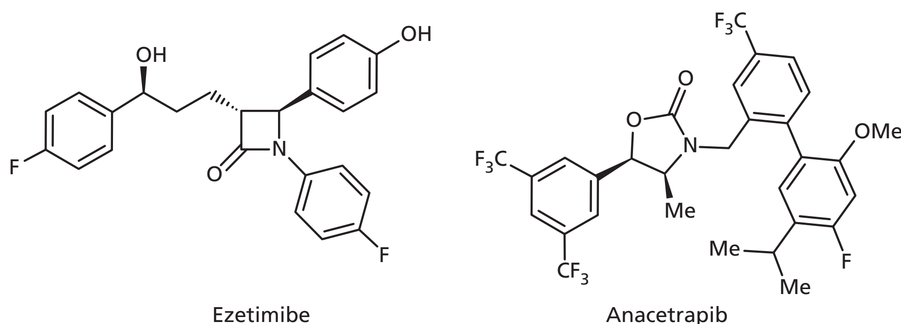


FIGURE CS1.13 Ezetimibe and anacetrapib.

FURTHER READING

Bottorff, M. and Hansten, P. (2000) Long-term safety of hepatic hydroxymethyl glutaryl coenzyme A reductase inhibitors. *Archives of Internal Medicine* **160**, 2273–2280.

Istvan, E. (2003) Statin inhibition of HMG-CoA reductase: a 3-dimensional view. *Atherosclerosis Supplements* **4**, 3–8.

Istvan, E. S. and Deseinhofer, J. (2001) Structural mechanisms for statin inhibition of HMG-CoA reductase. *Science* **292**, 1160–1164.

Istvan, E. S., Palnitkar, M., Buchanan, S., K., and Deisenhofer, J. (2000) Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. *The EMBO Journal* **19**, 819–830.

Jain, K. S., Kathiravan, M. K., Somani, R. S., and Shishoo, C. J. (2007) The biology and chemistry of hyperlipidemia. *Bioorganic and Medicinal Chemistry* **15**, 4674–4699.

Tobert, J. A. (2003) Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery* **2**, 517–526.

12 Drug discovery: finding a lead

In this chapter, we shall look at what happens when a pharmaceutical company or university research group initiates a new medicinal chemistry project through to the identification of a lead compound.

12.1 Choosing a disease

How does a pharmaceutical company decide which disease to target when designing a new drug? Clearly, it would make sense to concentrate on diseases where there is a need for new drugs. However, pharmaceutical companies have to consider economic factors, as well as medical ones. A huge investment has to be made in the research and development of a new drug. Therefore, companies must ensure that they get a good financial return for their investment. As a result, research projects tend to focus on diseases that are important in the developed world because this is the market best able to afford new drugs. A great deal of research is carried out on ailments such as migraine, depression, ulcers, obesity, flu, cancer, and cardiovascular disease. Less is carried out on the tropical diseases of the developing world. Only when such diseases start to make an impact on Western society do the pharmaceutical companies sit up and take notice. For example, there has been a noticeable increase in antimalarial research as a result of the increase in tourism to more exotic countries and the spread of malaria into the southern states of the USA (see also Case study 3). Moreover, pharmaceutical companies are becoming more involved in partnerships with governments and philanthropic organizations, such as the **Wellcome Trust**, the **Bill and Melinda Gates Foundation**, and **Medicines for Malaria Venture** in order to study diseases such as tuberculosis, malaria, and dengue.

Choosing which disease to tackle is usually a matter for a company's market strategists. The science becomes important at the next stage.

12.2 Choosing a drug target

12.2.1 Drug targets

Once a therapeutic area has been identified, the next stage is to identify a suitable drug target (e.g. receptor, enzyme, or nucleic acid). An understanding of which biomacromolecules are involved in a particular disease state is clearly important (see Box 12.1). This allows the medicinal research team to identify whether agonists or antagonists should be designed for a particular receptor or whether inhibitors should be designed for a particular enzyme. For example, agonists of serotonin receptors are useful for the treatment of migraine, while antagonists of dopamine receptors are useful as antidepressants. Sometimes it is not known for certain whether a particular target will be suitable or not. For example, **tricyclic antidepressants**, such as **desipramine** (Fig. 12.1), are known to inhibit the uptake of the neurotransmitter **noradrenaline** from nerve synapses by inhibiting the carrier protein for noradrenaline (section 23.12.4). However, these drugs also inhibit uptake of a separate neurotransmitter called **serotonin**, and the possibility arose that inhibiting serotonin uptake might also be beneficial. A search for **selective serotonin uptake inhibitors** was initiated, which led to the discovery of the best-selling antidepressant drug **fluoxetine** (**Prozac**) (Fig. 12.1), but when this project was initiated it was not known for certain whether serotonin uptake inhibitors would be effective or not.

12.2.2 Discovering drug targets

If a drug or a poison produces a biological effect, there must be a molecular target for that agent in the body. In the past, the discovery of drug targets depended on finding the drug first. Many early drugs, such as the analgesic **morphine**, are natural products derived from plants and just happen to interact with a molecular target in the human body. As this involves coincidence

BOX 12.1 Recently discovered targets: the caspases

The **caspases** are examples of recently discovered enzymes which may prove useful as drug targets**.** They are a family of protease enzymes that catalyse the hydrolysis of important cellular proteins, and which have been found to play a role in inflammation and cell death. Cell death is a natural occurrence in the body, and cells are regularly recycled. Therefore, caspases should not necessarily be seen as 'bad' or 'undesirable' enzymes. Without them, cells could be more prone to unregulated growth, resulting in diseases such as cancer.

The caspases catalyse the hydrolysis of particular target proteins such as those involved in DNA repair and the regulation of **cell cycles**. By understanding how these enzymes operate, there is the possibility of producing new therapies for a variety of diseases. For example, agents which promote the activity of caspases and lead to more rapid cell death might be useful in the treatment of diseases such as cancer, autoimmune disease, and viral infections. For example, **carboplatin** is an anticancer agent that promotes caspase activity. Alternatively, agents which inhibit caspases and reduce the prevalence of cell death could provide novel treatments for trauma, neurodegenerative disease, and strokes. It is already known that the active site of caspases contains two amino acids that are crucial to the mechanism of hydrolysis—cysteine, which acts as a nucleophile, and histidine, which acts as an acid–base catalyst. The mechanism is similar to that used by acetylcholinesterase (section 22.12.3.2).

Caspases recognize aspartate groups within protein substrates and cleave the peptide link next to the aspartate group. Selective inhibitors have been developed which include aspartate or a mimic of it, but it remains to be seen whether such inhibitors have a clinical role.

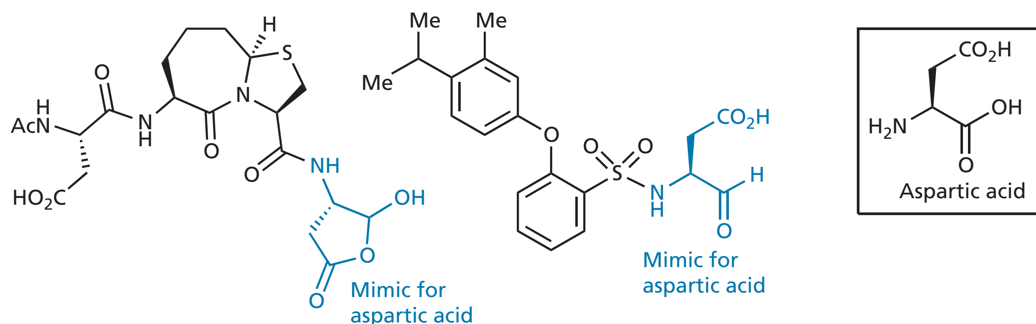


FIGURE 1 Selective caspase inhibitors.

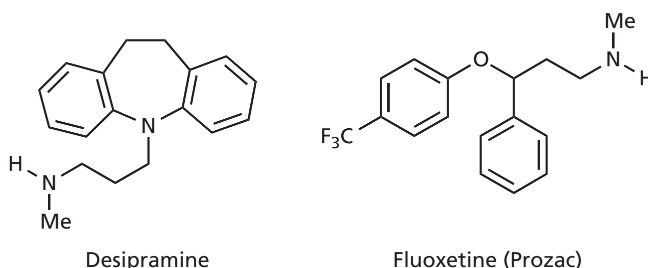


FIGURE 12.1 Antidepressant drugs.

more than design, the detection of drug targets was very much a hit and miss affair. Later, the body's own chemical messengers started to be discovered and pointed the finger at further targets. For example, since the 1970s a variety of peptides and proteins have been discovered which act as the body's own analgesics (enkephalins and endorphins). Another example is the rather surprising discovery that nitric oxide acts as a chemical messenger (Box 3.1 and section 22.3.2). Despite this, relatively few of the body's messengers were identified, either because they were present in such small quantity or because they were too short-lived to be isolated. Indeed, many chemical messengers still remain undiscovered today. This, in turn, means that many of the body's potential drug

targets remain hidden. Or at least it did! The advances in genomics and proteomics have changed all that. The various genome projects which have mapped the DNA of humans and other life forms, along with the newer field of proteomics (section 2.6), are revealing an ever increasing number of new proteins which are potential drug targets for the future. These targets have managed to stay hidden for so long that their natural chemical messengers are also unknown, and, for the first time, medicinal chemistry is faced with new targets, but with no lead compounds to interact with them. Such targets have been defined as **orphan receptors**. The challenge is now to find a chemical that will interact with each of these targets in order to find out what their function is and whether they will be suitable as drug targets. This was one of the main driving forces behind the development of **combinatorial** and **parallel synthesis** (Chapter 16).

12.2.3 Target specificity and selectivity between species

Target specificity and selectivity is a crucial factor in modern medicinal chemistry research. The more selective a drug is for its target, the less chance there is that it will interact with different targets and have undesirable side effects.

In the field of antimicrobial agents, the best targets to choose are those that are unique to the microbe and are not present in humans. For example, **penicillin** targets an enzyme involved in bacterial cell wall biosynthesis. Mammalian cells do not have a cell wall, so this enzyme is absent in human cells and penicillin has few side effects (section 19.5). In a similar vein, sulphonamides inhibit a bacterial enzyme not present in human cells (section 19.4.1.5), and several agents used to treat AIDS inhibit an enzyme called **retroviral reverse transcriptase**, which is unique to the infectious agent HIV (section 20.7.3).

Other cellular features that are unique to microorganisms could also be targeted. For example, the microorganisms which cause sleeping sickness in Africa are propelled by means of a tail-like structure called a **flagellum**. This feature is not present in mammalian cells, so designing drugs that bind to the proteins making up the flagellum and prevent it from working could be potentially useful in treating that disease.

Having said all that, it is still possible to design drugs against targets which are present both in humans and microbes, as long as the drugs show selectivity against the microbial target. Fortunately, this is perfectly feasible. An enzyme which catalyses a reaction in a bacterial cell differs significantly from the equivalent enzyme in a human cell. The enzymes may have been derived from an ancient common ancestor, but several million years of evolution have resulted in significant structural differences. For example, the antifungal agent **fluconazole** (Fig. 12.2) inhibits a fungal demethylase enzyme involved in steroid biosynthesis. This enzyme is also present in humans, but the structural differences between the two enzymes are significant enough that the antifungal agent is highly selective for the fungal enzyme. Other examples of bacterial or viral enzymes which are sufficiently different from their human equivalents are **dihydrofolate reductase** (section 19.4.2) and **viral DNA polymerase** (section 20.6.1).

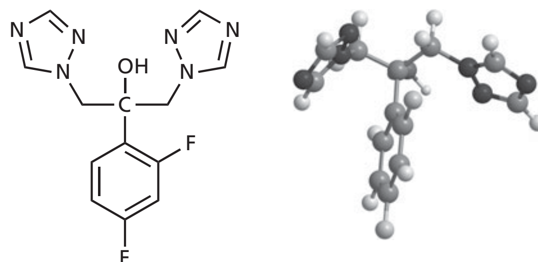


FIGURE 12.2 Fluconazole.

12.2.4 Target specificity and selectivity within the body

Selectivity is also important for drugs acting on targets within the body. Enzyme inhibitors should only inhibit the target enzyme and not some other enzyme. Receptor agonists/antagonists should, ideally, interact with a specific kind of receptor (e.g. the adrenergic receptor) rather than a variety of different receptors. However, nowadays, medicinal chemists aim for even higher standards of target selectivity. Ideally, enzyme inhibitors should show selectivity between the various isozymes of an enzyme (isozymes are the structural variants of an enzyme that result from different amino acid sequences or quaternary structure—section 3.7). For example, there are three different isoforms of **nitric oxide synthase** (NOS)—the enzyme responsible for generating the chemical messenger **nitric oxide** (Box 3.1). Selective inhibitors for one of these isoforms (nNOS) could potentially be useful in treating cerebral palsy and other neurodegenerative diseases.

Receptor agonists and antagonists should not only show selectivity for a particular receptor (e.g. an adrenergic receptor) or even a particular receptor type (e.g. the β-adrenergic receptor), but also for a particular receptor subtype (e.g. the β_2-adrenergic receptor). One of the current areas of research is to find antipsychotic agents with fewer side effects. Traditional antipsychotic agents act as antagonists of dopamine receptors. However, it has been found that there are five dopamine receptor subtypes

and that traditional antipsychotic agents antagonize two of these (D_3 and D_2). There is good evidence that the D_2 receptor is responsible for the undesirable Parkinsonian-type side effects of current drugs and so research is now underway to find a selective D_3 antagonist.

12.2.5 Targeting drugs to specific organs and tissues

Targeting drugs against specific receptor subtypes often allows drugs to be targeted to specific organs or to specific areas of the brain. This is because the various receptor subtypes are not distributed uniformly around the body, but are often concentrated in particular tissues. For example, the β-adrenergic receptors in the heart are predominantly β_1, whereas those in the lungs are β_2. This makes it feasible to design drugs that will work on the lungs with a minimal side effect on the heart, and vice versa.

Attaining subtype selectivity is particularly important for drugs that are intended to mimic neurotransmitters. Neurotransmitters are released close to their target receptors and, once they have passed on their message, they are quickly deactivated and do not have the opportunity to 'switch on' more distant receptors. Therefore, only those receptors which are fed by 'live' nerves are switched on.

In many diseases there is a 'transmission fault' to a particular tissue or in a particular region of the brain. For example, in Parkinson's disease, **dopamine** transmission is deficient in certain regions of the brain, although it is functioning normally elsewhere. A drug could be given to mimic dopamine in the brain. However, such a drug acts like a hormone rather than as a neurotransmitter because it has to travel round the body in order to reach its target. This means that the drug could potentially 'switch on' all the dopamine receptors around the body and not just the ones that are suffering the dopamine deficit. Such drugs would have a large number of side effects, so it is important to make the drug as selective as possible for the particular type or subtype of dopamine receptor affected in the brain. This would target the drug more effectively to the affected area and reduce side effects elsewhere in the body.

12.2.6 Pitfalls

A word of caution! It is possible to identify whether a particular enzyme or receptor plays a role in a particular ailment. However, the body is a highly complex system. For any given function, there are usually several messengers, receptors, and enzymes involved in the process. For example, there is no one simple cause for hypertension (high blood pressure). This is illustrated by the variety of receptors and enzymes which can be targeted in its treatment. These include β_1-adrenoceptors, calcium ion channels, angiotensin-converting enzyme (ACE), potassium ion channels, and angiotensin II receptors.

As a result, more than one target may need to be addressed for a particular ailment (Box 12.2). For example, most of the current therapies for asthma involve a

BOX 12.2 Pitfalls in choosing particular targets

Drugs are designed to interact with a particular target because that target is believed to be important to a particular disease process. Occasionally, though, a particular target may not be so important to a disease as was first thought. For example, the dopamine D_2 receptor was thought to be involved in causing nausea. Therefore, the D_2 receptor antagonist **metoclopramide** was developed as an anti-emetic agent. However, it was found that more potent D_2 antagonists were less effective, implying that a different receptor might be more important in producing nausea. Metoclopramide also antagonizes the 5-hydroxytryptamine ($5HT_3$) receptor so antagonists for this receptor were studied, which led to the development of the antiemetic drugs **granisetron** and **ondansetron**.

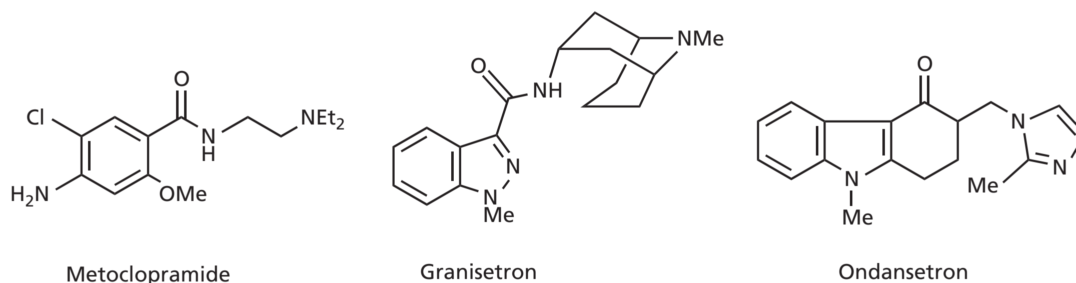


FIGURE 1 Anti-emetic agents.

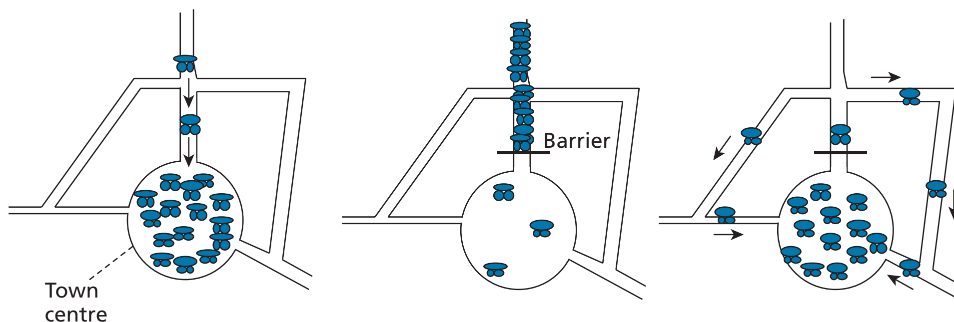


FIGURE 12.3 Avoiding the jam.

combination of a **bronchodilator** (β_2-agonist) and an anti-inflammatory agent, such as a corticosteroid.

Sometimes, drugs designed against a specific target become less effective over time. Because cells have a highly complex system of signalling mechanisms, it is possible that the blockade of one part of that system could be bypassed. This could be compared to blocking the main road into town to try and prevent congestion in the town centre. To begin with, the policy works, but, in a day or two, commuters discover alternative routes, and congestion in the centre becomes as bad as ever (Fig. 12.3).

12.2.7 Multi-target drugs

In certain diseases and afflictions, there can be an advantage in 'hitting' a number of different targets selectively, as this can be more beneficial than hitting just one. Combination therapy is normally used to achieve this by administering two or more drugs showing selectivity against the different targets. This is particularly the case in the treatment of cancer (Chapter 21) and HIV infection (Box 20.2). However, combination therapies are also used in a variety of other situations (sections 19.4.2.1 and 19.5.4). The disadvantage of combination therapies is the number of different medications and the associated dose regimens. Therefore, there are benefits in designing a single drug that can act selectively at different targets in a controlled manner—a **multi-target-directed ligand**. Many research projects now set out to discover new drugs with a defined profile of activity against a range of specific targets. For example, a research team may set out to find a drug that has agonist activity for one receptor subtype and antagonist activity at another. A further requirement may be that the drug neither inhibits metabolic enzymes (section 11.5) nor acts on targets that can lead to toxicity (Box 12.3). A current area of research is in designing dual-action drugs to treat depression (section 23.12.4 and Case study 7). Dual and triple action drugs are also

BOX 12.3 Early tests for potential toxicity

In vivo and *in vitro tests* are often carried out at an early stage to find out whether lead compounds or candidate drugs are likely to have certain types of toxicity. One such test is to see whether compounds inhibit HERG potassium ion channels in the heart. HERG stands for the gene that codes for this protein, the so called **Human Ether-a-go-go Related Gene**! Who makes up these names? Several promising drugs have had to be withdrawn at a very late stage in their development because they were found to inhibit the HERG potassium ion channels. Inhibition can result in disruption in the normal rhythm of the heart, leading to fibrillation, heart failure, and death. The gastric agent **cisapride** (Fig. 1) and the antihistamine **terfenadine** (section 11.5.6) both had to be withdrawn from the market because of this problem. A large variety of other structures have been found to have this unwanted effect and so tests to detect this property are best done as early as possible in order to remove this property as part of the drug optimization process.

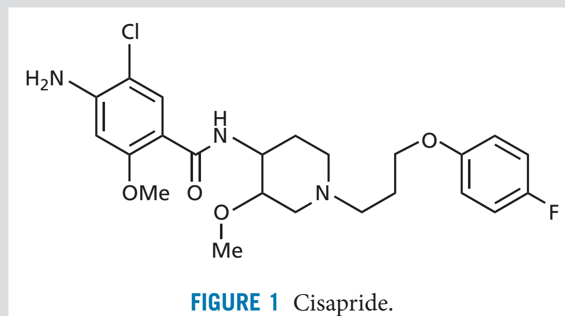


FIGURE 1 Cisapride.

(Continued)

BOX 12.3 Early tests for potential toxicity (*Continued*)

The **Ames test** is another early test that is worth carrying out in order to detect potential mutagenicity or carcinogenicity in new compounds. It involves the use of a mutated bacterial strain of *Salmonella typhimurium* that can only grow in a medium containing histidine, because the organism lacks the ability to synthesize histidine. The test involves growing the mutant strain in a medium that contains a small amount of histidine, as well as the test compound. As there is only a small amount of histidine present, the mutant bacteria will soon stop growing and dividing. However, some of the mutant bacteria will 'back mutate' to the original wild-type strain. These cells are now able to synthesize their own histidine and will keep growing. The bacterial colonies that are present on the plate are subcultured onto plates lacking histidine to detect the presence of the wild-type strains, allowing a measure of the mutation rate. Any mutagenic or carcinogenic drug that is present in the original medium will increase the mutation rate, relative to a reference culture containing no drug.

Many research groups now concentrate on 'taming' Ames and HERG liabilities at an early stage of drug development. For example, structure I (Fig. 2) is an antagonist for the **melanin-concentrating hormone receptor** (MCH-R)—a receptor that has been identified as an important target for novel anti-obesity drugs. Unfortunately, structure I blocks HERG ion channels and has Ames liability (i.e. it has mutagenic properties). A library of analogues was prepared by parallel synthesis (Chapter 16), which identified structure II as a potent antagonist having no Ames liability. Further work led to structure III, which lacked the Ames liability and has a greatly reduced capacity to block HERG ion channels.

Another example where studies were carried out to avoid interactions with the HERG ion channels was in the development of the antiviral agent maraviroc (section 20.7.5).

Microbioassay tests are also been developed to test for drug toxicity. These involve the use of microfluidic systems on microchips. Cells from different organs are grown in microchannels on the microchip and then tiny volumes of drug solution are passed through the microchip to see what effect they have.

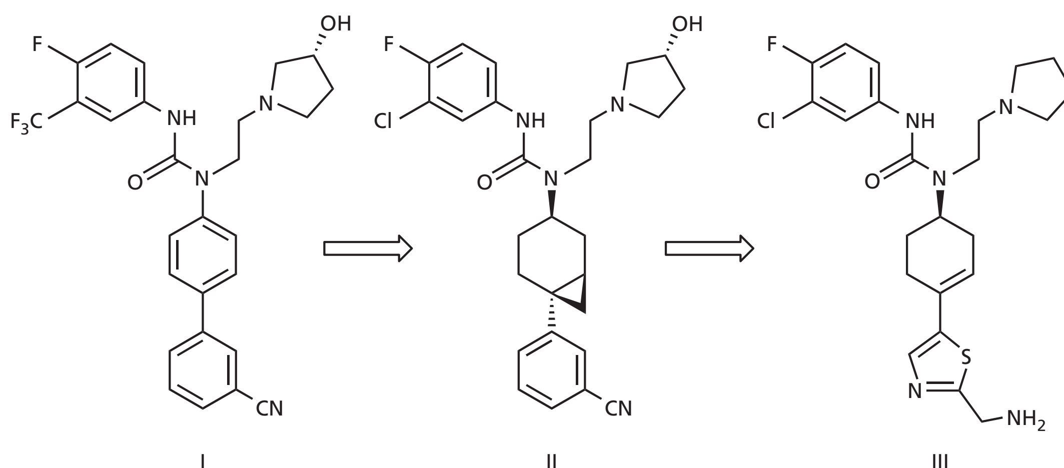


FIGURE 2 The development of agents to remove undesirable properties.

being studied for the treatment of Alzheimer's disease (section 22.15).

A less selective example is **olanzapine** (Fig. 12.4). This drug binds to more than a dozen receptors for serotonin, dopamine, muscarine, noradrenaline, and histamine. This kind of profile would normally be unacceptable, but olanzapine has been highly effective in the treatment of schizophrenia, probably because it blocks both serotonin and dopamine receptors. Drugs which interact with a large range of targets are called **promiscuous ligands** or **dirty drugs**. Such drugs can act as lead compounds for

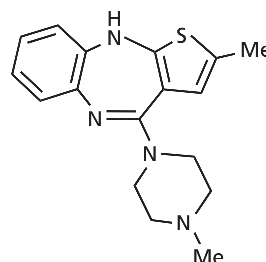


FIGURE 12.4 Olanzapine.

the development of more selective multi-targeted ligands (see also section 22.15.3).

12.3 Identifying a bioassay

12.3.1 Choice of bioassay

Choosing the right bioassay or test system is crucial to the success of a drug research programme. The test should be simple, quick, and relevant, as there are usually a large number of compounds to be analysed. Human testing is not possible at such an early stage, so the test has to be done *in vitro* (i.e. on isolated cells, tissues, enzymes, or receptors) or *in vivo* (on animals). In general, *in vitro* tests are preferred over *in vivo* tests because they are cheaper, easier to carry out, less controversial and they can be automated. However, *in vivo* tests are often needed to check whether drugs have the desired pharmacological activity and also to monitor their pharmacokinetic properties. In modern medicinal chemistry, a variety of tests are usually carried out both *in vitro* and *in vivo* to determine not only whether the candidate drugs are acting at the desired target, but also whether they have activity at other undesired targets (Box 12.3). The direction taken by projects is then determined by finding drugs that have the best balance of good activity at the desired target and minimal activity at other targets. In this way, there is less likelihood of millions of dollars being wasted developing a drug that will either fail clinical trials or be withdrawn from the market with all the associated litigation that might be involved—a '**fail fast, fail cheap**' strategy.

12.3.2 *In vitro* tests

In vitro tests do not involve live animals. Instead, specific tissues, cells, or enzymes are used. Enzyme inhibitors can be tested on the pure enzyme in solution. In the past, it could be a major problem to isolate and purify sufficient enzyme to test, but, nowadays, genetic engineering can be used to incorporate the gene for a particular enzyme into fast-growing cells, such as yeast or bacteria. These then produce the enzyme in larger quantities, making isolation easier. For example, **HIV protease** (section 20.7.4.1) has been cloned and expressed in the bacterium *Escherichia coli*. A variety of experiments can be carried out on this enzyme to determine whether an enzyme inhibitor is competitive or non-competitive, and to determine IC_{50} values (section 7.8).

Receptor agonists and antagonists can be tested on isolated tissues or cells which express the target receptor on their surface. Sometimes these tissues can be used to test drugs for physiological effects. For example, bronchodilator activity can be tested by observing how well compounds inhibit contraction of isolated tracheal smooth muscle. Alternatively, the affinity of drugs for receptors (how strongly they bind) can be measured by **radioligand studies** (section 8.9). Many *in vitro* tests have been designed by genetic engineering where the gene coding for a specific receptor is identified, cloned, and expressed in fast-dividing cells, such as bacterial, yeast, or tumour cells. For example, **Chinese Hamster Ovarian cells** (CHO cells) are commonly used for this purpose, as they express a large amount of the cloned receptor on their cell surface. *In vitro* studies on whole cells are useful because there are none of the complications of *in vivo* studies, where the drug has to survive metabolic enzymes or cross barriers, such as the gut wall. The environment surrounding the cells can be easily controlled, and both intracellular and intercellular events can be monitored, allowing measurement of efficacy and potency (section 8.9). Primary cell cultures (i.e. cells that have not been modified) can be produced from embryonic tissues; transformed cell lines are derived from tumour tissue. Cells grown in this fashion are all identical.

Antibacterial drugs are tested *in vitro* by measuring how effectively they inhibit or kill bacterial cells in culture. It may seem strange to describe this as an *in vitro* test, as bacterial cells are living microorganisms. However, *in vivo* antibacterial tests are defined as those that are carried out on animals or humans to test whether antibacterial agents combat infection.

In vitro tests are also used to test for the pharmacokinetic properties of compounds. For example, the **Caco-2 cell monolayer absorption** model is used to assess how well a drug is likely to be absorbed from the gastrointestinal tract. Microsomes and hepatocytes extracted from liver cells contain cytochrome P450 enzymes, and can be used to assess the likely metabolism of drug candidates, as well as identifying possible **drug–drug interactions**. Another *in vitro assay* using artificial membranes has been developed as a simple and rapid measure of how effectively drugs will cross the blood–brain barrier.

12.3.3 *In vivo* tests

In vivo tests on animals often involve inducing a clinical condition in the animal to produce observable symptoms. The animal is then treated to see whether the drug alleviates the problem by eliminating the observable symptoms. For example, the development of non-steroidal inflammatory drugs was carried out by inducing inflammation on test animals, then testing drugs to see whether they relieved the inflammation.

Transgenic animals are often used in *in vivo* testing. These are animals whose genetic code has been altered. For example, it is possible to replace some mouse genes

with human genes. The mouse produces the human receptor or enzyme and this allows *in vivo* testing against that target. Alternatively, the mouse's genes could be altered such that the animal becomes susceptible to a particular disease (e.g. breast cancer). Drugs can then be tested to see how well they prevent that disease.

There are several problems associated with *in vivo* testing. It is slow and expensive, and it also causes animal suffering. There are the many problems of pharmacokinetics (Chapter 11), and so the results obtained may be misleading and difficult to rationalize if *in vivo* tests are carried out in isolation. For example, how can one tell whether a negative result is due to the drug failing to bind to its target or not reaching the target in the first place? Thus, *in vitro* tests are usually carried out first to determine whether a drug interacts with its target, and *in vivo* tests are then carried out to test pharmacokinetic properties.

Certain *in vivo* tests might turn out to be invalid. It is possible that the observed symptoms might be caused by a different physiological mechanism than the one intended. For example, many promising anti-ulcer drugs which proved effective in animal testing were ineffective in clinical trials. Finally, different results may be obtained in different animal species. For example, **penicillin methyl ester prodrugs** (Box 19.7) are hydrolysed in mice or rats to produce active penicillins, but are not hydrolysed in rabbit, dogs, or humans. Another example involves **thalidomide**, which is teratogenic in rabbits and humans, but has no such effect in mice.

Despite these issues, *in vivo* testing is still crucial in identifying the particular problems that might be associated with using a drug *in vivo* and which cannot be picked up by *in vitro* tests.

12.3.4 Test validity

Sometimes the validity of testing procedures is easy and clear-cut. For example, an antibacterial agent can be tested *in vitro* by measuring how effectively it kills bacterial cells. A local anaesthetic can be tested *in vitro* on how well it blocks **action potentials** in isolated nerve tissue. In other cases, the testing procedure is more difficult. For example, how do you test a new antipsychotic drug? There is no animal model for this condition and so a simple *in vivo* test is not possible. One way round this problem is to propose which receptor or receptors might be involved in a medical condition, and to carry out *in vitro* tests against these in the expectation that the drug will have the desired activity when it comes to clinical trials. One problem with this approach is that it is not always clear-cut whether a specific receptor or enzyme is as important as one might think to the targeted disease (see Box 12.2).

12.3.5 High-throughput screening

Robotics and the miniaturization of *in vitro* tests on genetically modified cells has led to a process called **high-throughput screening (HTS)**, which is particularly effective in identifying potential new lead compounds. This involves the automated testing of large numbers of compounds versus a large number of targets; typically, several thousand compounds can be tested at once in 30–50 biochemical tests. It is important that the test should produce an easily measurable effect which can be detected and measured automatically. This effect could be cell growth, an enzyme-catalysed reaction which produces a colour change, or displacement of radioactively labelled ligands from receptors.

Receptor antagonists can be studied using modified cells which contain the target receptor in their cell membrane. Detection is possible by observing how effectively the test compounds inhibit the binding of a radiolabelled ligand. Another approach is to use yeast cells which have been modified such that activation of a target receptor results in the activation of an enzyme which, when supplied with a suitable substrate, catalyses the release of a dye. This produces an easily identifiable colour change.

In general, positive hits are compounds which have an activity in the range 30 μM–1 nM. Unfortunately, HTS can generate many false-positive hits, and there is a high failure rate between the number of hits, and those compounds which are eventually identified as authentic lead compounds. One of the main causes of false hits is what are known as **promiscuous inhibitors**. These are agents which appear to inhibit a range of different target proteins and show very poor selectivity. It is believed that agents working in this manner come together in solution to form molecular aggregates which adsorb target proteins onto their surface, resulting in the inhibition observed. The effect is more pronounced if mixtures of compounds are being tested in solution, such as those prepared by combinatorial syntheses. This kind of inhibition is of no use to drug design and it is important to eliminate these agents early on as potential lead compounds, such that time is not wasted resynthesizing and investigating them. One way of finding out whether promiscuous inhibition is taking place is to add a detergent to the test solution. This reverses and prevents the phenomenon.

Other false hits include agents which are chemically reactive and carry out a chemical reaction with the target protein, such as the alkylation or acylation of a susceptible nucleophilic group. This results in an irreversible inhibition of the protein as the agent becomes covalently linked to the target. Although there are important drugs which act as irreversible inhibitors, the emphasis in HTS is to find reversible inhibitors which interact with their targets through intermolecular binding interactions. For that

reason, known alkylating or acylating agents should not be included in HTS, or, if they are, they should not be considered as potential lead compounds. Examples of reactive groups include alkyl halides, acid chlorides, epoxides, aldehydes, α-chloroketones, and trifluoromethyl ketones.

12.3.6 Screening by nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy is an analytical tool which has been used for many years to determine the molecular structure of compounds. More recently, it has been used to detect whether a compound binds to a protein target. In NMR spectroscopy, a compound is radiated with a short pulse of energy which excites the nuclei of specific atoms, such as hydrogen, carbon, or nitrogen. Once the pulse of radiation has stopped, the excited nuclei slowly relax back to the ground state, giving off energy as they do so. The time taken by different nuclei to give off this energy is called the **relaxation time**, and this varies depending on the environment or position of each atom in the molecule. Therefore, a different signal will be obtained for each atom in the molecule and a spectrum is obtained which can be used to determine the structure.

The size of the molecule also plays an important role in the length of the relaxation time. Small molecules, such as drugs, have long relaxation times, whereas large molecules, such as proteins, have short relaxation times. Therefore, it is possible to delay the measurement of energy emission such that only small molecules are detected. This is the key to the detection of binding interactions between a protein and a test compound.

First of all, the NMR spectrum of the drug is taken, then the protein is added and the spectrum is re-run, introducing a delay in the measurement such that the protein signals are not detected. If the drug fails to bind to the protein, then its NMR spectrum will still be detected. If the drug binds to the protein, it essentially becomes part of the protein. As a result, its nuclei will have a shorter relaxation time and no NMR spectrum will be detected.

This screening method can also be applied to a mixture of compounds arising from a natural extract or from a combinatorial synthesis. If any of the compounds present bind to the protein, its relaxation time is shortened and so signals due to that compound will disappear from the spectrum. This will show that a component of the mixture is active and determine whether it is worthwhile separating the mixture or not.

There are several advantages in using NMR as a detection system:

- it is possible to screen 1000 small-molecular-weight compounds a day with one machine;
- the method can detect weak binding which would be missed by conventional screening methods;
- it can identify the binding of small molecules to different regions of the binding site (section 12.4.10);
- it is complimentary to HTS—the latter may give false-positive results, but these can be checked by NMR to ensure that the compounds concerned are binding in the correct binding site (section 12.4.10);
- the identification of small molecules which bind weakly to part of the binding site allows the possibility of using them as building blocks for the construction of larger molecules that bind more strongly (section 12.4.10);
- screening can be done on a new protein without needing to know its function.

Disadvantages include the need to purify the protein and to obtain it in a significant quantity (at least 200 mg).

12.3.7 Affinity screening

A nice method of screening mixtures of compounds for active constituents is to take advantage of the binding affinity of compounds for the target. This not only detects the presence of such agents, but picks them out from the mixture. For example, the vancomycin family of antibacterial agents has a strong binding affinity for the dipeptide D-Ala-D-Ala (section 19.5.5.2). D-Ala-D-Ala was linked to sepharose resin, and the resin was mixed with extracts from various microbes which were known to have antibacterial activity. If an extract lost antibacterial activity as a result of this operation, it indicated that active compounds had bound to the resin. The resin could then be filtered off and, by changing the pH, the compounds could be released from the resin for identification.

12.3.8 Surface plasmon resonance

Surface plasmon resonance (SPR) is an optical method of detecting when a ligand binds to its target. The procedure is patented by Pharmacia Biosensor as **BIAcore** and makes use of a dextran-coated, gold-surfaced glass chip (Fig. 12.5). A ligand that is known to bind to the target is immobilized by linking it covalently to the dextran matrix, which is in a flow of buffer solution. Monochromatic, plane-polarized light is shone at an angle of incidence (α) from below the glass plate and is reflected back at the interface between the dense gold-coated glass and the less dense buffer solution. However, a component of the light called the **evanescent wave** penetrates a distance of about one wavelength into the buffer/dextran matrix. Normally, all of the light including the evanescent wave is reflected back, but if the gold film is very thin (a fraction of the evanescent wavelength)

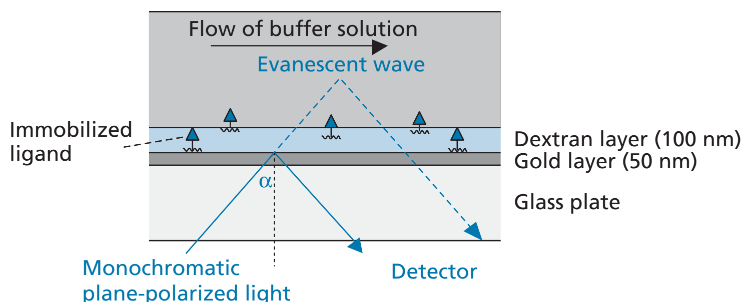


FIGURE 12.5 Surface plasmon resonance. The word evanescent means 'passing out of sight'.

and the angle of incidence is exactly right, the evanescent wave interacts with free oscillating electrons called **plasmons** in the metal film. This is the SPR. Energy from the incident light is then lost to the gold film. As a result, there is a decrease in the reflected light intensity, which can be measured.

The angle of incidence when SPR occurs depends crucially on the refractive index of the buffer solution close to the metal film surface. This means that if the refractive index of the buffer changes, the angle of incidence at which SPR takes place also changes.

If the macromolecular target for the immobilized ligand is now introduced into the buffer flow, some of it will be bound by the immobilized ligand. This leads to a change of refractive index in the buffer solution close to the metal-coated surface, which can be detected by measuring the change in the angle of incidence required to get SPR. The technique allows the detection of ligand–target binding, and can also be used to measure rate and equilibrium binding constants.

Suppose, now, we want to test whether a novel compound is binding to the target. This can be tested by introducing the novel compound into the buffer flow along with the target. If the test compound *does* bind to the target, less target will be available to bind to the immobilized ligands, so there will be a different change in both the refractive index and the angle of incidence.

12.3.9 Scintillation proximity assay

Scintillation proximity assay (SPA) is a visual method of detecting whether a ligand binds to a target. It involves the immobilization of the target by linking it covalently to beads which are coated with a scintillant. A solution of a known ligand labelled with iodine-125 is then added to the beads. When the labelled ligand binds to the immobilized target, the ^{125}I acts as an energy donor and the scintillant-coated beads act as an energy acceptor, resulting in an emission of light that can be detected. In order to find out whether a novel compound interacts with the target, the compound is added to the solution of the labelled ligand and the mixture is added to the beads. Successful binding by the novel compound will mean that less of the labelled ligand will bind, resulting in a reduction in the emission of light.

12.3.10 Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) is a technique that is used to determine the thermodynamic properties of binding between a drug and its protein target—the binding affinity and enthalpy change, in particular. Two identical glass cells are used which are filled with buffer solution. One of the cells acts as the reference cell, while the other acts as the sample cell and contains the protein target in solution. The reference cell is heated slightly to a constant temperature. The sample cell is heated to the same temperature through an automatic feedback system, whereby any temperature difference between the two cells is detected and power is applied to the sample cell to equalize the temperature. Once the apparatus has stabilized, a constant level of power is used to maintain the two cells at the same constant temperature.

The drug is now added to the sample cell and binds to the protein target. If the binding interaction is exothermic, heat energy is generated within the sample cell and so less external power is needed to maintain the cell temperature. If the interaction is endothermic, the opposite holds true and more external power has to be applied to maintain the temperature. The external power required to maintain the temperature of the sample cell is measured with respect to time, with power 'spikes' occurring every time the drug is injected into the cell. Measurement of these spikes allows the determination of the thermodynamic properties of binding.

12.3.11 Virtual screening

Virtual screening involves the use of computer programs to assess whether known compounds are likely to be lead compounds for a particular target. There is no guarantee that 'positive hits' from a virtual screening will, in fact, be active, and the compounds still have to be screened

experimentally, but the results from a virtual screening can be used to make experimental screening methods more efficient. In other words, if there are several thousand compounds available for testing, virtual screening can be used to identify those compounds which are most likely to be active, and so those are the structures which would be given priority for actual screening. Virtual screening can involve a search for pharmacophores known to be required for activity, or docking the compounds into target binding sites (sections 17.11–13).

KEY POINTS

- Pharmaceutical companies tend to concentrate on developing drugs for diseases which are prevalent in developed countries and aim to produce compounds with better properties than existing drugs.
- A molecular target is chosen which is believed to influence a particular disease when affected by a drug. The greater the selectivity that can be achieved, the less chance of side effects.
- A suitable bioassay must be devised which will demonstrate whether a drug has activity against a particular target. Bioassays can be carried out *in vitro* or *in vivo*, and usually a combination of tests is used.
- HTS involves the miniaturization and automation of *in vitro* tests such that a large number of tests can be carried out in a short period of time.
- Compounds can be tested for their affinity to a macromolecular target by NMR spectroscopy. The relaxation times of ligands bound to a macromolecule are shorter than when they are unbound.
- SPR, SPA, and ITC are three visual methods of detecting whether ligands bind to macromolecular targets.
- Virtual screening can be used to identify compounds most likely to be active in experimental screening.

12.4 Finding a lead compound

Once a target and a testing system have been chosen, the next stage is to find a lead compound—a compound which shows the desired pharmacological activity. The level of activity may not be very great and there may be undesirable side effects, but the lead compound provides a start for the drug design and development process. There are various ways in which a lead compound might be discovered as described in the following sections.

12.4.1 Screening of natural products

Natural products are a rich source of biologically active compounds. Many of today's medicines are either obtained directly from a natural source or were developed from a lead compound originally obtained from a natural source. Usually, the natural source has some form of biological activity, and the compound responsible for that activity is known as the **active principle**. Such a structure can act as a lead compound. Most biologically active natural products are **secondary metabolites** with quite complex structures and several chiral centres. This has an advantage in that they are extremely novel compounds. Unfortunately, this complexity also makes their synthesis difficult and the compounds usually have to be extracted from their natural source—a slow, expensive, and inefficient process. As a result, there is usually an advantage in designing simpler analogues (section 13.3.8).

Many natural products have radically new chemical structures which no chemist would dream of synthesizing. For example, the antimalarial drug **artemisinin** (Fig. 12.6) is a natural product with an extremely unstable looking trioxane ring—one of the most unlikely structures to have appeared in recent years (see also Case Study 3).

The study of medicines derived from natural sources is known as **pharmacognosy**, and includes both crude extracts and purified active principles.

12.4.1.1 The plant kingdom

Plants have always been a rich source of lead compounds (e.g. **morphine, cocaine, digitalis, quinine, tubocurarine**, **nicotine**, and **muscarine**). Many of these lead compounds are useful drugs in themselves (e.g. morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anaesthetics developed from cocaine). Plants still remain a promising source of new drugs and will continue to be so. Clinically useful drugs which have recently been isolated from plants include the anticancer agent **paclitaxel (Taxol)** from the yew tree, the antimalarial agent artemisinin from a Chinese plant (Fig. 12.6), and the Alzheimer's drug **galantamine** from daffodils (section 22.15.1).

Plants provide a bank of rich, complex, and highly varied structures which are unlikely to be discovered from other sources. Furthermore, evolution has already carried out a screening process that favours compounds which provide plants with an 'edge' when it comes to survival. For example, biologically potent compounds can deter animals or insects from eating the plants that contain them. Considering the debt medicinal chemistry owes to the natural world, it is sobering to think that very few plants have been fully studied and the vast majority have not been studied at all. The rainforests of the world are particularly rich in plant species which have still to be discovered, let alone studied. Who knows how many exciting new lead compounds

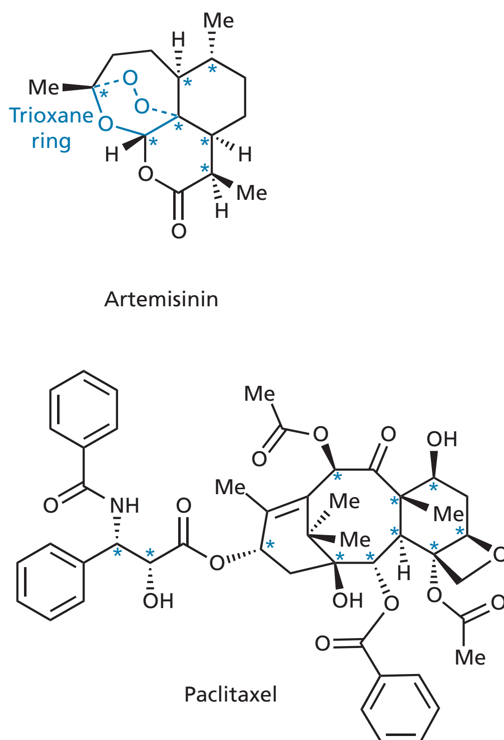


FIGURE 12.6 Plant natural products as drugs (the asterisks indicate chiral centres).

await discovery for the fight against cancer, AIDS, or any of the other myriad of human afflictions? This is one reason why the destruction of rain forests and other ecosystems is so tragic; once these ecosystems are destroyed, unique plant species are lost to medicine for ever. For example, **silphion**—a plant that was cultivated near Cyrene in North Africa and was famed as a contraceptive agent in ancient Greece—is now extinct. It is certain that many more useful plants have become extinct without medicine ever being aware of them.

12.4.1.2 Microorganisms

Microorganisms such as bacteria and fungi have also provided rich pickings for drugs and lead compounds. These organisms produce a large variety of antimicrobial agents which have evolved to give their hosts an advantage over their competitors in the microbiological world. The screening of microorganisms became highly popular after the discovery of **penicillin**. Soil and water samples were collected from all round the world in order to study new fungal or bacterial strains, leading to an impressive arsenal of antibacterial agents, such as the **cephalosporins**, **tetracyclines**, **aminoglycosides**, **rifamycins**, **chloramphenicol**, and **vancomycin** (Chapter 19). Although most of the drugs derived from microorganisms are used in antibacterial therapy, some microbial metabolites have provided lead compounds in other fields of medicine. For example, **asperlicin**—isolated from *Aspergillus alliaceus*—is a novel antagonist of a peptide hormone called **cholecystokinin** (CCK), which is involved in the control of appetite. CCK also acts as a neurotransmitter in the brain and is thought to be involved in panic attacks. Analogues of asperlicin may, therefore, have potential in treating anxiety (see also Box 13.2).

Other examples include the fungal metabolite **lovastatin**, which was the first of the clinically useful statins found to lower cholesterol levels (Case study 1), and another fungal metabolite called **ciclosporin** (Fig. 12.7), which is used to suppress the immune response after organ transplants. **Lipstatin** (Fig. 12.7) is a natural product which was isolated from *Streptomyces toxytricini*. It inhibits pancreatic lipase and was the lead compound for the anti-obesity compound **orlistat** (Box 7.2). Finally, a fungal metabolite called **rasfonin** (isolated from a fungus in New Zealand) promotes cell death (apoptosis) in cancer cells, but not normal cells. It represents a promising lead compound for novel anticancer agents.

12.4.1.3 Marine sources

In recent years, there has been great interest in finding lead compounds from marine sources. Coral, sponges, fish, and marine microorganisms have a wealth of biologically potent chemicals with interesting inflammatory, antiviral, and anticancer activity. For example,

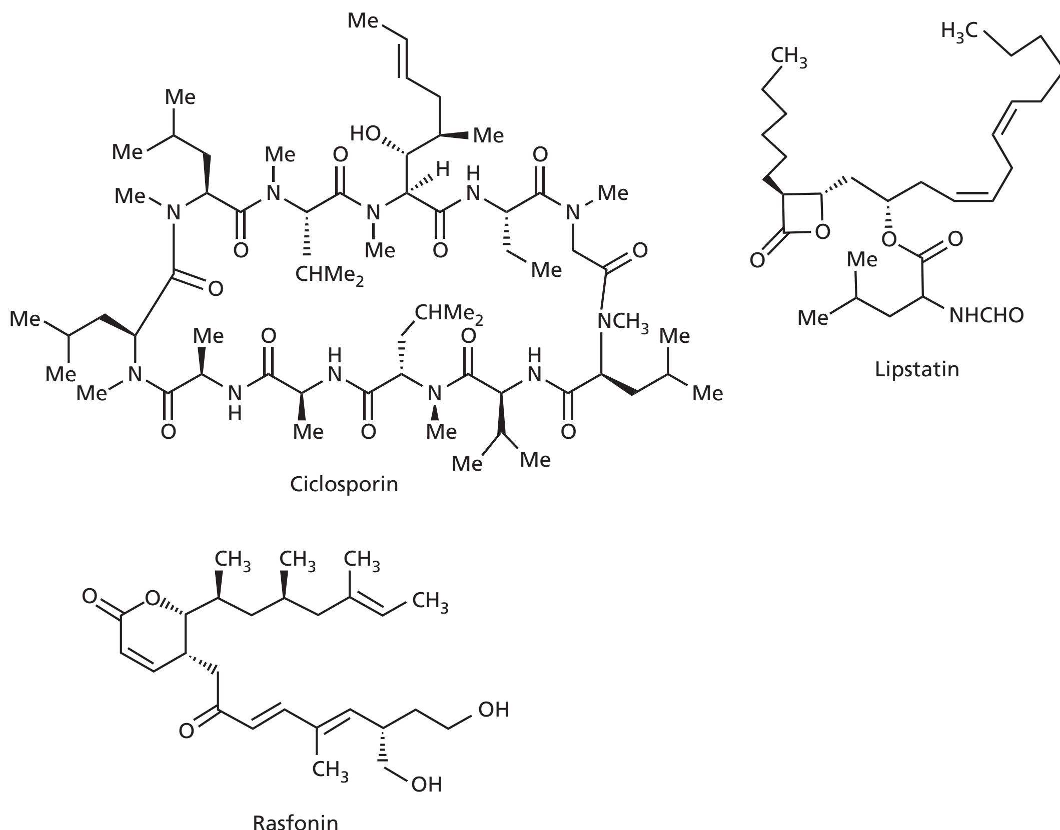


FIGURE 12.7 Lead compounds from microbiological sources.

curacin A (Fig. 12.8) is obtained from a marine cyanobacterium, and shows potent antitumour activity. Other antitumour agents derived from marine sources include **eleutherobin**, **bryostatins**, **dolastatins**, **cephalostatins**, and **halichondrin B** (sections 21.5.2 and 21.8.2). In 2010, a simplified analogue of halichondrin B was approved for the treatment of breast cancer.

12.4.1.4 Animal sources

Animals can sometimes be a source of new lead compounds. For example, a series of antibiotic polypeptides known as the **magainins** were extracted from the skin of the African clawed frog *Xenopus laevis*. These agents protect the frog from infection and may provide clues to the development of novel antibacterial and antifungal agents in human medicine. Another example is a potent analgesic compound called **epibatidine** (Fig. 12.9), obtained from the skin extracts of the Ecuadorian poison frog.

12.4.1.5 Venoms and toxins

Venoms and toxins from animals, plants, snakes, spiders, scorpions, insects, and microorganisms are extremely potent because they often have very specific interactions with a macromolecular target in the body. As a result, they have proved important tools in studying receptors, ion channels, and enzymes. Many of these toxins are polypeptides (e.g. **α-bungarotoxin** from cobras). However,

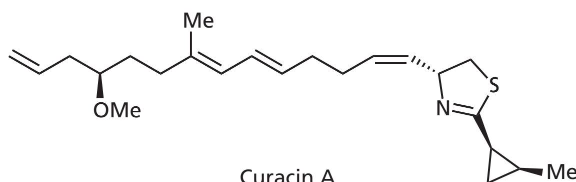


FIGURE 12.8 Curacin A.

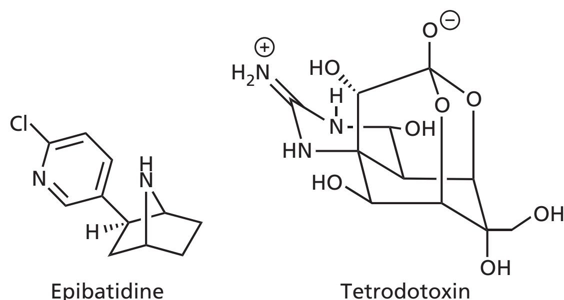


FIGURE 12.9 Natural products as drugs.

non-peptide toxins such as **tetrodotoxin** from the puffer fish (Fig. 12.9) are also extremely potent.

Venoms and toxins have been used as lead compounds in the development of novel drugs. For example, **teprotide**, a peptide isolated from the venom of the Brazilian viper, was a lead compound for the development of the antihypertensive agents **cilazapril** and **captopril** (Case study 2).

The neurotoxins from *Clostridium botulinum* are responsible for serious food poisoning (**botulism**), but they have a clinical use as well. They can be injected into specific muscles (such as those controlling the eyelid) to prevent muscle spasm. These toxins prevent cholinergic transmission (Chapter 22) and could well prove a lead for the development of novel anticholinergic drugs.

Finally, **conotoxin** is a peptide toxin derived from the marine cone snail, and has very powerful analgesic properties in humans. A synthetic form of conotoxin called **ziconotide** was approved in 2004 for the treatment of chronic pain.

12.4.2 Medical folklore

In the past, ancient civilizations depended greatly on local flora and fauna for their survival. They would experiment with various berries, leaves, and roots to find out what effects they had. As a result, many brews were claimed by the local healer or shaman to have some medicinal use. More often than not, these concoctions were useless or downright dangerous, and, if they worked at all, it was because the patient willed them to work—a **placebo effect**. However, some of these extracts may, indeed, have a real and beneficial effect, and a study of medical folklore can give clues as to which plants might be worth studying in more detail. **Rhubarb** root has been used as a purgative for many centuries. In China, it was called 'The General' because of its 'galloping charge'! The most significant chemicals in rhubarb root are anthraquinones, which were used as the lead compounds in the design of the laxative **dantron** (Fig. 12.10).

The ancient records of Chinese medicine also provided the clue to the novel antimalarial drug **artemisinin** mentioned in section 12.4.1 (see also Case study 3). The therapeutic properties of the opium poppy (active principle **morphine**) were known in Ancient Egypt, as were those of the *Solanaceae* plants in ancient Greece (active principles **atropine** and **hyoscine**; section 22.9.2). The snakeroot plant was well regarded in India (active principle **reserpine**; Fig. 12.10), and herbalists in medieval England used extracts from the willow tree (active principle **salicin**; Fig. 12.10) and foxglove (active principle **digitalis**—a mixture of compounds such as digitoxin, digitonin, and digitalin). The Aztec and Mayan cultures of South America used extracts from a variety of bushes and trees, including the ipecacuanha root (active principle **emetine**; Fig. 12.10), coca bush (active principle **cocaine**), and cinchona bark (active principle **quinine**).

12.4.3 Screening synthetic compound 'libraries'

The thousands of compounds which have been synthesized by the pharmaceutical companies over the years are another source of lead compounds. The vast majority of these compounds have never made the market place, but they have been stored in compound 'libraries' and are still available for testing. Pharmaceutical companies often screen their library of compounds whenever they study a new target. However, it has to be said that the vast majority of these compounds are merely variations on a theme, for example 1000 or so different penicillin structures. This reduces the chances of finding a novel lead compound.

Pharmaceutical companies often try to diversify their range of structures by purchasing novel compounds

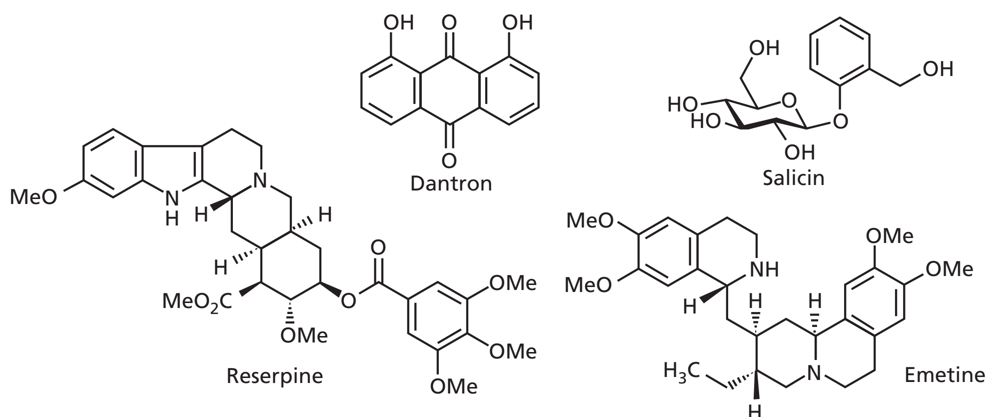


FIGURE 12.10 Active compounds resulting from studies of herbs and potions.

prepared by research groups elsewhere—a useful source of revenue for hard-pressed university departments! These compounds may never have been synthesized with medicinal chemistry in mind and may be intermediates in a purely synthetic research project, but there is always the chance that they may have useful biological activity.

It can also be worth testing synthetic intermediates. For example, a series of thiosemicarbazones was synthesized and tested as antitubercular agents in the 1950s. This included **isonicotinaldehyde thiosemicarbazone**, the synthesis of which involved the hydrazide structure **isoniazid** (Fig. 12.11) as a synthetic intermediate. It was found subsequently that isoniazid had greater activity than the target structure. Similarly, a series of **quinoline-3-carboxamide** intermediates (Fig. 12.11) were found to have antiviral activity.

12.4.4 Existing drugs

12.4.4.1 'Me too' and 'me better' drugs

Many companies use established drugs from their competitors as lead compounds in order to design a drug that gives them a foothold in the same market area. The aim is to modify the structure sufficiently such that it avoids patent restrictions, retains activity, and, ideally, has improved therapeutic properties. For example, the antihypertensive drug **captopril** was used as a lead compound by various companies to produce their own antihypertensive agents (Fig. 12.12, see also Case study 2).

Although often disparaged as 'me too' drugs, they can often offer improvements over the original drug ('me better' drugs). For example, modern penicillins are more selective, more potent, and more stable than the original penicillins. Newer statins that lower cholesterol levels also have improved properties over older ones (Case study 1). It should also be noted that it is not unusual for companies to be working on similar looking structures for a particular disease at the same time. The first of these drugs to reach the market gets all the kudos, but it is rather unfair to call the drugs that follow it as 'me too' drugs, as they were designed and developed independently.

12.4.4.2 Enhancing a side effect

An existing drug usually has a minor property or an undesirable side effect which could be of use in another area of medicine. As such, the drug could act as a lead compound on the basis of its side effects. The aim would then be to enhance the desired side effect and to eliminate the major biological activity. This has been described as the SOSA approach—**selective optimization of side activities**. Choosing a known drug as the lead compound for a side effect has the advantage that the compound is already 'drug-like' and it should be more feasible to develop a clinically useful drug with the required pharmacodynamic and pharmacokinetic properties. Many of the 'hits' obtained from HTS do not have a 'drug-like' structure and it may require far more effort to optimize them. Indeed, it has been argued that modifications of known drug structures should provide lead compounds in several areas of medicinal chemistry. Many research groups are now screening compounds that are either in clinical use or have reached late-stage clinical trials to see whether they have side effects that would make them suitable lead compounds. The John Hopkins Clinical Compound Library is one such source of these compounds.

For example, most sulphonamides have been used as antibacterial agents. However, some sulphonamides with antibacterial activity could not be used clinically because they had convulsive side effects brought on by **hypoglycaemia** (lowered glucose levels in the blood). Clearly, this is an undesirable side effect for an antibacterial agent, but the ability to lower blood glucose levels would be useful in the treatment of diabetes. Therefore, structural alterations were made to the sulphonamides concerned in order to eliminate the antibacterial activity

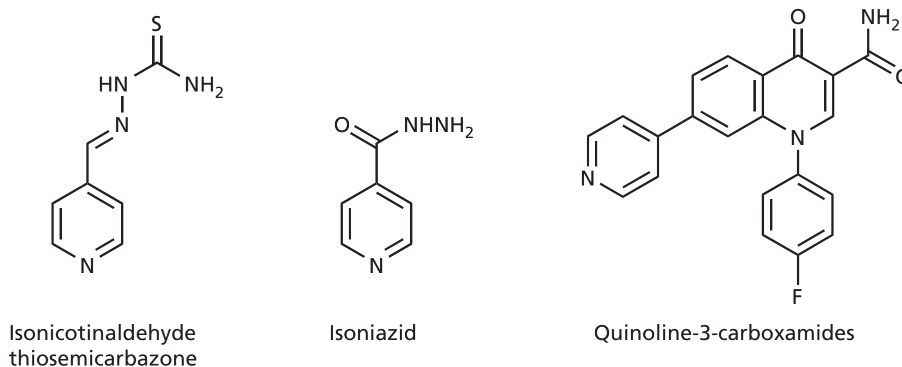


FIGURE 12.11 Pharmaceutically active compounds discovered from synthetic intermediates.

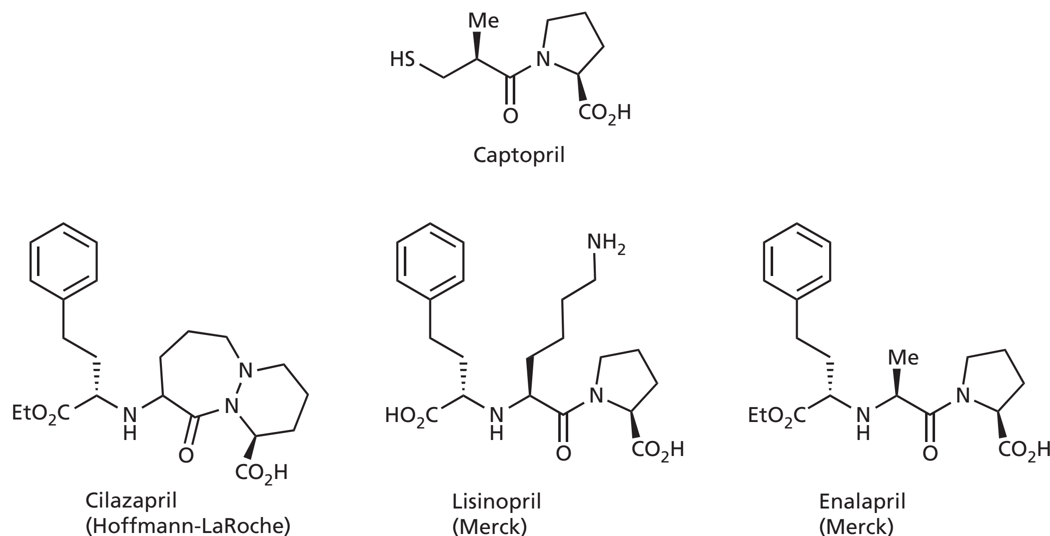


FIGURE 12.12 Captopril and 'me too' drugs.

and to enhance the hypoglycaemic activity. This led to the antidiabetic agent **tolbutamide** (Fig. 12.13). Another example was the discovery that the anticoagulant **warfarin** is also a weak inhibitor of a viral enzyme that is important in the life cycle of HIV. Warfarin was used as the lead compound in the development of an anti-HIV drug called **tipranavir** (section 20.7.4.10).

In some cases, the side effect may be strong enough that the drug can be used without modification. For example, the anti-impotence drug **sildenafil** (Viagra) (Fig. 12.13) was originally designed as a vasodilator to treat angina and hypertension. During clinical trials, it was found that it acted as a vasodilator more effectively in the penis than in the heart, resulting in increased erectile function. The drug is now used to treat erectile dysfunction and sexual impotence. Another example is the antidepressant drug **bupropion**. Patients taking this drug reported that it helped them give up smoking, and so the drug is now marketed as an antismoking aid (Zyban) (section 23.12.4). **Astemizole** (Fig. 12.13) is a medication used in the treatment of allergy, but has been found to be a potent antimalarial agent.

The moral of the story is that a drug used in one field of medicinal chemistry could be the lead compound in another field (Box 12.4). Furthermore, one can fall into the trap of thinking that a structural group of compounds all have the same type of biological activity. The sulphonamides are generally thought of as antibacterial agents, but we have seen that they can also have other properties.

12.4.5 Starting from the natural ligand or modulator

12.4.5.1 Natural ligands for receptors

The natural ligand of a target receptor has sometimes been used as the lead compound. The natural ligands **adrenaline** and **noradrenaline** were the starting points

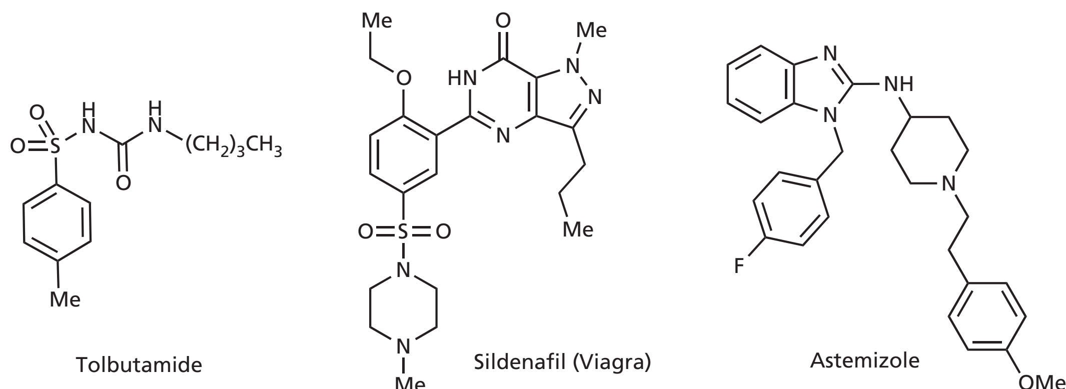


FIGURE 12.13 Tolbutamide and sildenafil (Viagra).

BOX 12.4 Selective optimization of side activities (SOSA)

Several drugs have been developed by enhancing the side effect of another drug (Fig. 1). **Chlorpromazine** is used as a neuroleptic agent in psychiatry, but was developed from the antihistamine agent **promethazine**. This might appear an odd thing to do, but it is known that promethazine has sedative side effects, and so medicinal chemists modified the structure to enhance the sedative effects at the expense of antihistamine activity. Similarly, the development of sulphonamide diuretics such as **chlorothiazide** arose from the observation that **sulphanilamide** has a diuretic effect in large doses (owing to its action on an enzyme called **carbonic anhydrase**).

Sometimes, slight changes to a structure can result in significant changes in pharmacological activity. For example, **minaprine** (Fig. 2) is an antidepressant agent that acts as a serotonin agonist. Adding a phenolic substituent resulted in **4-hydroxyminaprine**, which is a potent dopamine agonist, whereas adding a cyano substituent gave **bazinaprine**, which is a potent inhibitor of the enzyme **monoamine oxidase-A**. Minaprine also binds weakly to muscarine receptors, and modifications were successfully carried out to give structure I, having potent activity for the muscarinic receptor, and negligible activity for dopamine and serotonin receptors. Minaprine also has weak affinity for the cholinesterase enzyme and modifications led to structure II with over 1000-fold increased affinity.

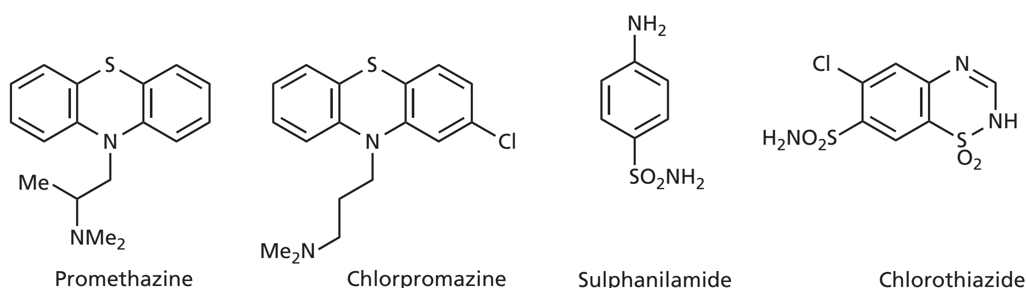


FIGURE 1 Drugs developed by enhancing a side effect.

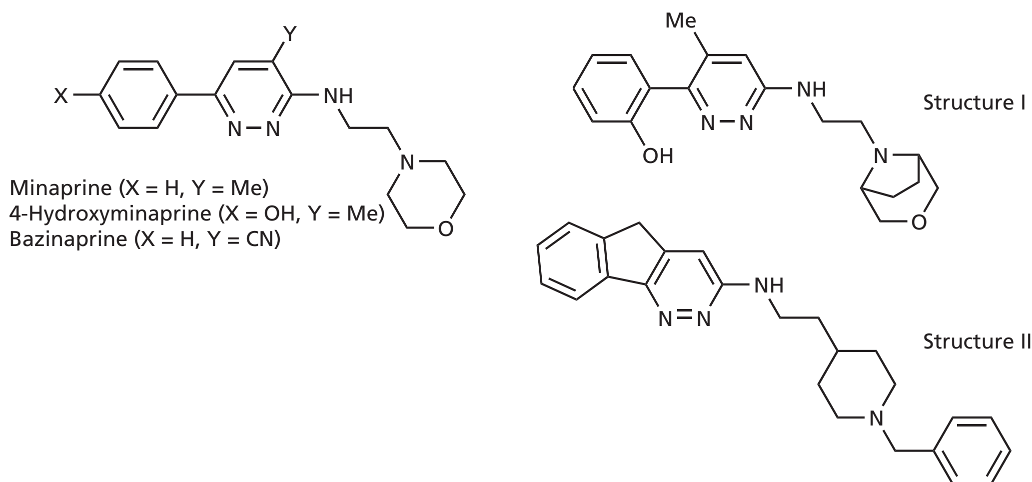


FIGURE 2 Structures with different pharmacological properties derived from the lead compound minaprine.

for the development of adrenergic β-agonists, such as **salbutamol**, **dobutamine**, and **xamoterol** (section 23.10), and 5-hydroxytryptamine (5-HT) was the starting point for the development of the 5-HT_1 agonist **sumatriptan** (Fig. 12.14).

The natural ligand of a receptor can also be used as the lead compound in the design of an antagonist. For example, **histamine** was used as the original lead compound in the development of the H_2 histamine antagonist **cimetidine** (section 25.2). Turning an agonist into

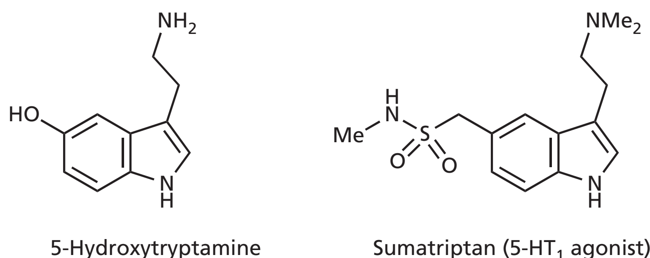


FIGURE 12.14 5-Hydroxytryptamine and sumatriptan.

an antagonist is frequently achieved by adding extra binding groups to the lead structure. Other examples include the development of the adrenergic antagonist **pronethalol** (section 23.11.3.1), the H_2 antagonist **burimamide** (section 25.2), and the 5-HT_3 antagonists **ondansetron** and **granisetron** (Box 12.2).

Sometimes the natural ligand for a receptor is not known (an **orphan receptor**) and the search for it can be a major project in itself. If the search is successful, however, it opens up a brand-new area of drug design (see Box 12.5). For example, the identification of the opioid receptors for **morphine** led to a search for endogenous opioids (natural body painkillers), which eventually led to the discovery of **endorphins** and **enkephalins**, and their use as lead compounds (section 24.8).

12.4.5.2 Natural substrates for enzymes

The natural substrate for an enzyme can be used as the lead compound in the design of an enzyme inhibitor. For example, **enkephalins** have been used as lead compounds for the design of enkephalinase inhibitors. **Enkephalinases** are enzymes which metabolize enkephalins, and their inhibition should prolong the activity of enkephalins (section 24.8.4).

The natural substrate for HIV protease was used as the lead compound for the development of the first protease inhibitor used to treat HIV (section 20.7.4). Other examples of substrates being used as lead compounds for inhibitors include the substrates for farnesyl transferase (section 21.6.1), matrix metalloproteinase (section 21.7.1), and 17β-hydroxysteroid dehydrogenase type 1.

For additional material see Web article 1: steroids as novel anticancer agents.

12.4.5.3 Enzyme products as lead compounds

It should be remembered that enzymes catalyse a reaction in both directions, and so the product of an enzyme-catalysed reaction can also be used as a lead compound for an enzyme inhibitor. For example, the design of the carboxypeptidase inhibitor **L-benzylsuccinic acid** was based on the products arising from the **carboxypeptidase**-catalysed hydrolysis of peptides (see Case study 2).

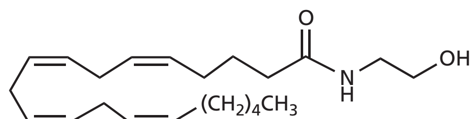
12.4.5.4 Natural modulators as lead compounds

Many receptors and enzymes are under allosteric control (sections 3.6 and 8.3.2). The natural or endogenous chemicals that exert this control (modulators) could also serve as lead compounds.

In some cases, a modulator for an enzyme or receptor is suspected but has not yet been found. For example, the **benzodiazepines** are synthetic compounds that modulate the receptor for **γ-aminobutyric acid** (GABA) by binding to an allosteric binding site. The natural modulators for this allosteric site were not known at the time benzodiazepines were synthesized, but endogenous peptides called **endozepines** have since been discovered which bind to the same allosteric binding site, and which may serve as lead compounds for novel drugs having the same activity as the benzodiazepines.

BOX 12.5 Natural ligands as lead compounds

The discovery of **cannabinoid** receptors in the early 1990s led to the discovery of two endogenous cannabinoid messengers—**arachidonylethanolamine** (**anandamide**) and **2-arachidonyl glycerol**. These have now been used as lead compounds for developing agents that will interact with cannabinoid receptors. Such agents may prove useful in suppressing nausea during chemotherapy or in stimulating appetite in patients with AIDS.



Anandamide.

12.4.6 Combinatorial and parallel synthesis

The growing number of potentially new drug targets arising from genomic and proteomic projects has meant that there is an urgent need to find new lead compounds to interact with them. Unfortunately, the traditional sources of lead compounds have not managed to keep pace and, in the last decade or so, research groups have invested greatly in combinatorial and parallel synthesis in order to tackle this problem. Combinatorial synthesis is an automated solid-phase procedure aimed at producing as many different structures as possible in as short a time as possible. The reactions are carried out on very small scale, often in a way that will produce mixtures of compounds in each reaction vial. In a sense, combinatorial synthesis aims to mimic what plants do, i.e. produce a pool of chemicals, one of which may prove to be a useful lead compound. Combinatorial synthesis has developed so swiftly that it is almost a branch of chemistry in itself and a separate chapter is devoted to it (Chapter 16). Parallel synthesis involves the small-scale synthesis of large numbers of compounds at the same time using specialist miniaturized equipment. The synthesis can be carried out in solution or solid phase, and each reaction vial contains a distinct product (Chapter 16). Nowadays, parallel synthesis is generally preferred over combinatorial synthesis in order to produce smaller, more focused compound libraries.

12.4.7 Computer-aided design of lead compounds

A detailed knowledge of a target binding site aids significantly in the design of novel lead compounds intended to bind with that target. In cases where enzymes or receptors can be crystallized, it is possible to determine the structure of the protein and its binding site by **X-ray crystallography**. Molecular modelling software programs can then be used to study the binding site and to design molecules which will fit and bind to the site—*de novo* drug design (section 17.15).

In some cases, the enzyme or receptor cannot be crystallized and so X-ray crystallography cannot be carried out. However, if the structure of an analogous protein has been determined, this can be used as the basis for generating a computer model of the protein. This is covered in more detail in section 17.14. NMR spectroscopy has also been effective in determining the structure of proteins and can be applied to proteins that cannot be studied by X-ray crystallography.

12.4.8 Serendipity and the prepared mind

Frequently, lead compounds are found as a result of serendipity (i.e. chance). However, it still needs someone with an inquisitive nature or a prepared mind to recognize the significance of chance discoveries and to take advantage of these events. The discovery of **cisplatin** (section 9.3.4) and **penicillin** (section 19.5.1.1) are two such examples, but there are many more (see Box 12.6).

Sometimes, the research carried out to improve a drug can have unexpected and beneficial spin offs. For example, **propranolol** (Fig. 12.15) and its analogues are effective β-blockers (antagonists of β-adrenergic receptors) (section 23.11.3). However, they are also lipophilic, which means that they can cross the blood–brain barrier and cause central nervous system (CNS) side effects. To counteract this, more hydrophilic analogues were designed by decreasing the size of the aromatic ring system and adding a hydrophilic amide group. One of the compounds made was **practolol**. As expected, this compound had fewer CNS side effects, but, more importantly, it was found to be a selective antagonist for

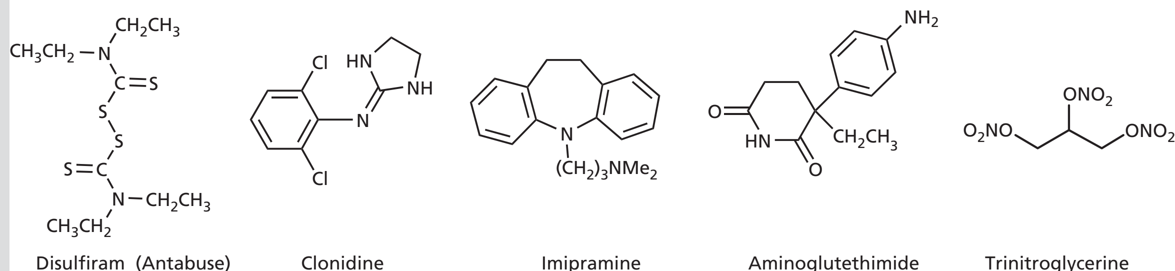
BOX 12.6 Examples of serendipity

During World War II, a US ship carrying **mustard gas** exploded in an Italian harbour. It was observed that many of the survivors who had inhaled the gas lost their natural defences against microbes. Further study showed that their white blood cells had been destroyed. It is perhaps hard to see how a drug that weakens the immune system could be useful. However, there is one disease where this *is* the case—leukemia. Leukemia is a form of cancer which results in the excess proliferation of white blood cells, so a drug that kills these cells is potentially useful. As a result, a series of mustard-like drugs were developed based on the structure of the original mustard gas (sections 9.3.1 and 21.2.3.1).

Another example involved the explosives industry, where it was quite common for workers to suffer severe headaches. These headaches resulted from dilatation of blood vessels in the brain caused by handling **trinitroglycerine**. Once again, it is hard to see how such a drug could be useful. Certainly, the dilatation of blood vessels in the brain may not be particularly beneficial, but dilating the blood vessels in the heart is useful in cardiovascular medicine. As a result, trinitroglycerine (or **glyceryl trinitrate** as it is called in medical circles) is used as a spray or sublingual tablet for the prophylaxis and treatment of angina. The agent acts as a prodrug for the generation of **nitric oxide**, which causes vasodilation.

(Continued)

BOX 12.6 Examples of serendipity (*Continued*)



Drugs discovered by serendipity.

Workers in the rubber industry found that they often acquired a distaste for **alcohol**! This was caused by an antioxidant used in the rubber manufacturing process which found its way into workers' bodies and prevented the normal oxidation of alcohol in the liver. As a result, there was a build up of **acetaldehyde**, which was so unpleasant that workers preferred not to drink. The antioxidant became the lead compound for the development of **disulfiram** (**Antabuse**), which is used for the treatment of chronic alcoholism.

The following are further examples of lead compounds arising as a result of serendipity:

- **clonidine** was originally designed to be a nasal vasoconstrictor to be used in nasal drops and shaving soaps. Clinical trials revealed that it caused a marked fall in blood pressure, and so it became an important antihypertensive instead;
- **imipramine** was synthesized as an analogue of chlorpromazine (Box 12.4) and was initially to be used as an antipsychotic. However, it was found to alleviate depression and this led to the development of a series of compounds classified as the **tricyclic antidepressants** (section 23.12.4);
- **aminoglutethimide** was prepared as a potential anti-epileptic drug, but is now used as an anticancer agent (section 21.4.5);
- The anti-impotence drug **sildenafil (Viagra)** (Fig. 12.13) was discovered by chance from a project aimed at developing a new heart drug;
- **isoniazid** (Fig. 12.11) was developed originally as an anti-tuberculosis agent. Patients taking it proved remarkably cheerful and this led to the drug becoming the lead compound for a series of antidepressant drugs known as the **monoamine oxidase inhibitors** (MAOIs) (section 23.12.5);
- **chlorpromazine** (Box 12.4) was synthesized as an antihistamine for possible use in preventing surgical shock, and was found to make patients relaxed and unconcerned. This led to the drug being tested in people with manic depression, where it was found to have tranquillizing effects. As a result, it was marketed as the first of the neuroleptic drugs (major tranquillizers) used for schizophrenia;
- **ciclosporin A** (Fig. 12.7) suppresses the immune system and is used during organ and bone marrow transplants to prevent the immune response rejecting the donor organs. The compound was isolated from a soil sample as part of a study aimed at finding new antibiotics. Fortunately, the compounds were more generally screened and the immunosuppressant properties of ciclosporin A were identified;
- in a similar vein, the anticancer alkaloids **vincristine** and **vinblastine** (section 10.2.2) were discovered by chance when searching for compounds that could lower blood sugar levels. Vincristine is used in the treatment of Hodgkin's disease.

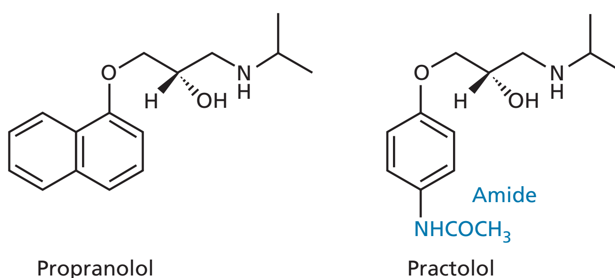


FIGURE 12.15 Propranolol and practolol.

the β-receptors of the heart over β-receptors in other organs—a result that was highly desirable, but not the one that was being looked for at the time.

Frequently, new lead compounds have arisen from research projects carried out in a totally different field of medicinal chemistry. This emphasizes the importance of keeping an open mind, especially when testing for biological activity. For example, we have already described the development of the antidiabetic drug **tolbutamide** (section 12.4.4.2), based on the observation that some antibacterial sulphonamides could lower blood glucose levels.

12.4.9 Computerized searching of structural databases

New lead compounds can be found by carrying out computerized searches of structural databases. In order to carry out such a search, it is necessary to know the desired **pharmacophore** (sections 13.2 & 17.11). Alternatively, docking experiments can be carried out if the structure of the target binding site is known (section 17.12). This type of database searching is also known as **database mining** and is described in section 17.13.

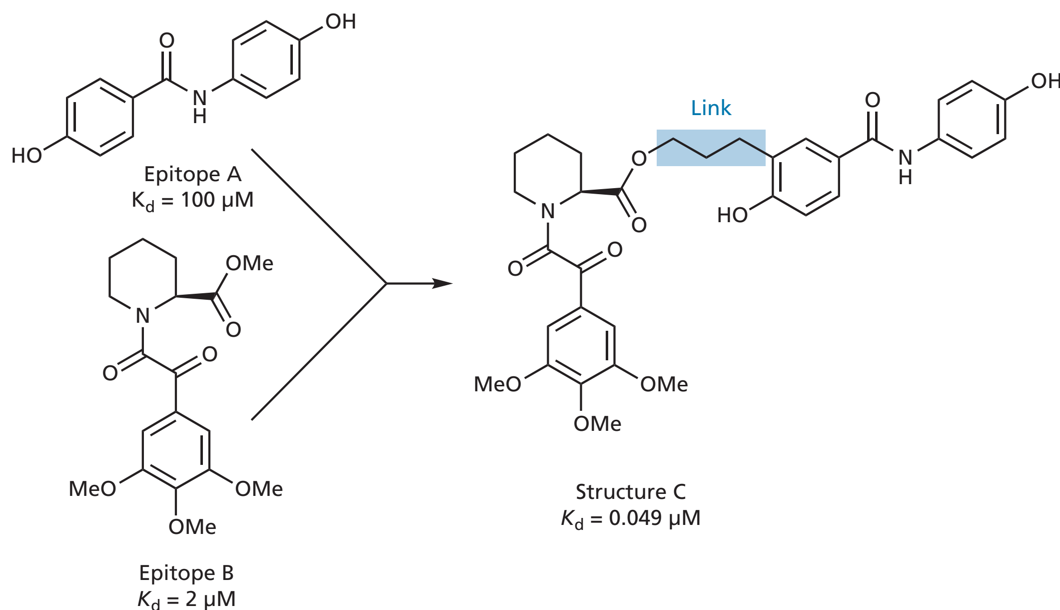
12.4.10 Fragment-based lead discovery

So far, we have described methods by which a lead compound can be discovered from a natural or synthetic source, but all these methods rely on an active compound being present. Unfortunately, there is no guarantee that this will be the case. Recently, NMR spectroscopy has been used to *design* a lead compound rather than to discover one (see Box 12.7). In essence, the method sets out to find small molecules (**epitopes**), which will bind to specific, but different, regions of a protein's binding site. These molecules will have no activity in themselves as they only bind to one part of the binding site, but if a larger molecule is designed which links these epitopes together, then a lead compound may be created which *is* active and which binds to the whole of the binding site (Fig. 12.16).

Lead discovery by NMR is also known as **structure–activity relationships** (SAR) by NMR and can be applied to proteins of known structure which are labelled with

BOX 12.7 The use of NMR spectroscopy in finding lead compounds

NMR spectroscopy was used in the design of high-affinity ligands for the FK506 binding protein—a protein involved in the suppression of the immune response. Two optimized epitopes (A and B) were discovered, which bound to different regions of the binding site. Structure C was then synthesized, where the two epitopes were linked by a propyl link. This compound had higher affinity than either of the individual epitopes and represents a lead compound for further development.



Design of a ligand for the FK506 binding protein (K_d is defined in section 8.9).

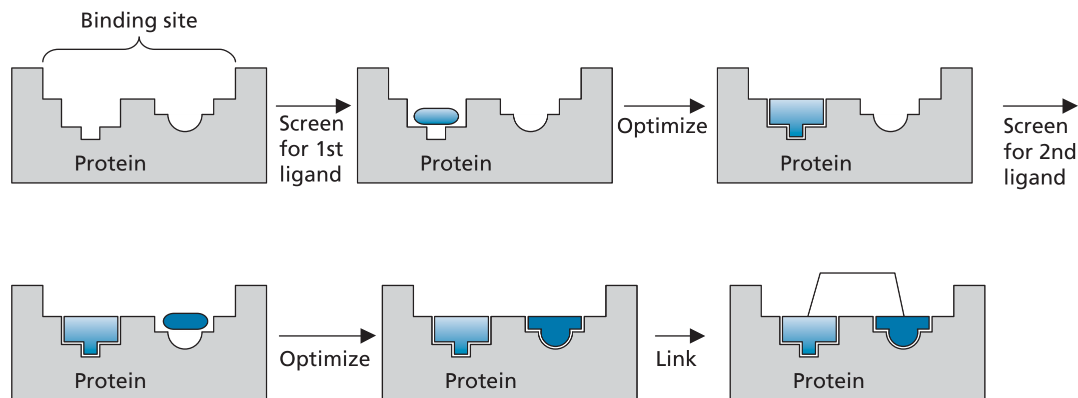


FIGURE 12.16 Epitope mapping.

^{15}N or ^{13}C, such that each amide bond in the protein has an identifiable peak.

A range of low molecular weight compounds is screened to see whether any of them bind to a specific region of the binding site. Binding can be detected by observing a shift in any of the amide signals, which will not only show that binding is taking place, but will also reveal which part of the binding site is occupied. Once a compound (or ligand) has been found that binds to one region of the binding site, the process can be repeated to find another ligand that will bind to a different region. This is usually done in the presence of the first ligand to ensure that the second ligand does, in fact, bind to a distinct region.

Once two ligands (or epitopes) have been identified, the structure of each can be optimized to find the best ligand for each of the binding regions. Then, a molecule can be designed where the two ligands are linked together.

There are several advantages to this approach. As the individual ligands are optimized for each region of the binding site, a lot of synthetic effort is spared. It is much easier to synthesize a series of small molecular weight compounds to optimize the interaction with specific parts of the binding site, than it is to synthesize a range of larger molecules to fit the overall binding site. A high level of diversity is also possible, as various combinations of fragments could be used. A further advantage is that it is more likely to find epitopes that will bind to a particular region of a binding site, than to find a lead compound that will bind to the overall binding site. Moreover, fragments are more likely to be efficient binders, having a high binding energy per unit molecular mass. Finally, some studies have demonstrated a 'super-additivity' effect where the binding affinity of the two linked fragments is much greater than one might have expected from the binding affinities of the two independent fragments.

The method described above involves the linking of fragments. Another strategy is to 'grow' a lead compound from a single fragment—a process called **fragment evolution**. This involves the identification of a single fragment that binds to part of the binding site, then finding larger and larger molecules which contain that fragment, but which also bind to other parts of the binding site.

A third strategy is known as **fragment self-assembly** and is a form of dynamic combinatorial chemistry (section 16.6.3). Fragments are chosen that can bind to different regions of the binding site, then react with each other to form a linked molecule *in situ*. This could be a reversible reaction as described in section 16.6.3. Alternatively, the two fragments can be designed to undergo an irreversible linking reaction when they bind to the binding site. This has been called '**click chemistry** *in situ*' (see Box 12.8).

NMR spectroscopy is not the only method of carrying out fragment-based lead discovery. It is also possible to identify fragments that bind to target proteins using the techniques of X-ray crystallography, *in vitro* bioassays, and mass spectrometry. X-ray crystallography, like NMR, provides information about how the fragment binds to the binding site and does so in far greater detail. However, it can be quite difficult obtaining crystals of protein–fragment complexes because of the low affinity of the fragments. Recently, a screening method called **CrystalLEAD** has been developed which can quickly screen large numbers of compounds and detect ligands by monitoring changes in the electron density map of protein–fragment complexes, relative to the unbound protein.

Finally, it is possible to use fragment-based strategies as a method of optimizing lead compounds that may have been obtained by other means. The strategy is to identify distinct fragments within the lead compound and then to optimize these fragments by the procedures already described. Once the ideal fragments have been identified, the full structure is synthesized incorporating the optimized fragments. This can be a much quicker method of optimization than synthesizing analogues of the larger lead compound.

For additional material see Web article 17: Fragment-based drug discovery

BOX 12.8 Click chemistry *in situ*

A femtomolar inhibitor for the acetylcholinesterase enzyme was obtained by fragment self-assembly within the active site of the enzyme. One of the molecular fragments contained an azide group while the other contained an alkyne group. In the presence of the enzyme, both fragments were bound to the active site and were positioned close enough to each other for an irreversible 1,3 dipolar cycloaddition to take place, forming the inhibitor *in situ* (Fig. 1). This type of reaction has been called 'click chemistry *in situ*'.

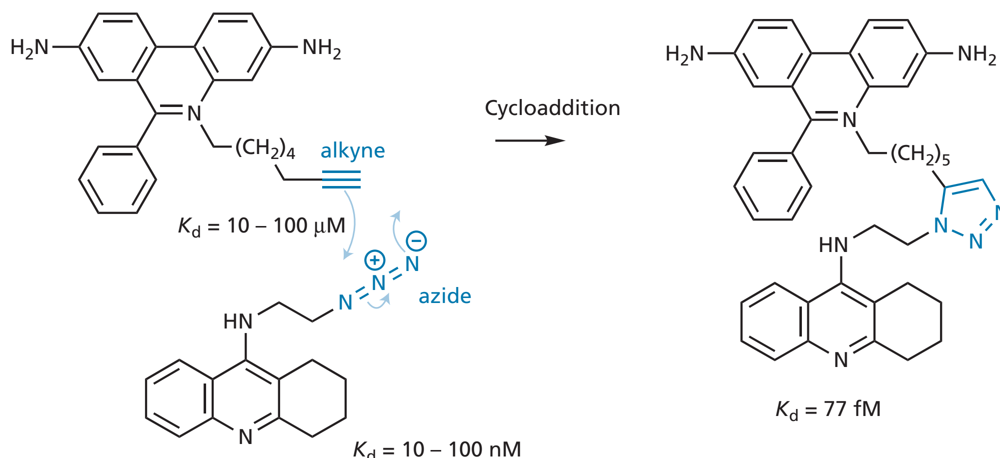


FIGURE 1 'Click' chemistry by means of a cycloaddition reaction (K_d is defined in section 8.9).

12.4.11 Properties of lead compounds

Some of the lead compounds that have been isolated from natural sources have sufficient activity to be used directly in medicine without serious side effects, for example morphine, quinine, and paclitaxel. However, most lead compounds have low activity and/or unacceptable side effects, which means that a significant amount of structural modification is required (see Chapters 13 and 14). If the aim of the research is to develop an orally active compound, certain properties of the lead compound should be taken into account. Most orally active drugs obey the rules laid down in Lipinski's Rule of Five or Veber's parameters (section 11.3). A study of known orally active drugs and the lead compounds from which they were derived demonstrated that the equivalent rules for a lead compound should be more stringent. This is because the structure of the lead compound almost certainly has to be modified and increased, both in terms of size and hydrophobicity. The suggested properties for a lead compound are that it should have a molecular weight of 100–350 amu and a Clog*P* value of 1–3. (Clog*P* is a measure of how hydrophobic a compound is; section 14.1.) In general, there is an average increase in molecular weight of 80 amu and an increase of 1 in Clog*P* when going from a lead compound to the final drug. Studies also show that a lead compound generally has fewer aromatic rings and hydrogen bond acceptors compared with the final drug. Such considerations can be taken into account when deciding which lead compound to use for a research project if several such structures are available. Another approach in making this decision is to calculate the **binding** or **ligand 'efficiency'** of each potential lead compound. This can be done by dividing the free energy of binding for each molecule by the number of non-hydrogen atoms present in the structure. The better the ligand efficiency, the lower the molecular weight of the final optimized structure is likely to be. Moreover, if you have a choice of lead compounds, the most suitable one is not necessarily the most potent.

For fragment-based lead discovery (section 12.4.10), a rule of three has been suggested for the fragments used:

- a molecular weight less than 300;
- no more than three hydrogen bond donors;
- no more than three hydrogen bond acceptors;
- cLog$P$ = 3;
- no more than three rotatable bonds;
- a polar surface area = 60 Å^{2}.

12.5 Isolation and purification

If the lead compound (or **active principle**) is present in a mixture of compounds from a natural source or a combinatorial synthesis (Chapter 16), it has to be isolated and purified. The ease with which the active principle can be isolated and purified depends very much on the structure, stability, and quantity of the compound. For example, Fleming recognized the antibiotic qualities of **penicillin** and its remarkable non-toxic nature to humans, but he disregarded it as a clinically useful drug because he was unable to purify it. He could isolate it in aqueous solution, but whenever he tried to remove the water, the drug was destroyed. It was not until the development of new experimental procedures, such as freeze-drying and chromatography, that the successful isolation and purification of penicillin and other natural products became feasible. A detailed description of the experimental techniques involved in the isolation and purification of compounds is outwith the scope of this textbook, and can be obtained from textbooks covering the practical aspects of chemistry.

12.6 Structure determination

It is sometimes hard for present-day chemists to appreciate how difficult structure determinations were before the days of NMR and infrared spectroscopy. A novel structure, which may now take a week's work to determine, would have provided two or three decades of work in the past. For example, the microanalysis of **cholesterol** was carried out in 1888 to get its molecular formula, but its chemical structure was not fully established until an X-ray crystallographic study was carried out in 1932.

In the past, structures had to be degraded to simpler compounds, which were further degraded to recognizable fragments. From these scraps of evidence, a possible structure was proposed, but the only sure way of proving the proposal was to synthesize the structure and to compare its chemical and physical properties with those of the natural compound.

Today, structure determination is a relatively straightforward process and it is only when the natural product is obtained in minute quantities that a full synthesis is required to establish its structure. The most useful analytical techniques are **X-ray crystallography** and **NMR spectroscopy**. The former technique comes closest to giving a 'snapshot' of the molecule, but requires a suitable crystal of the sample. The latter technique is used more commonly, as it can be carried out on any sample, whether it be a solid, oil, or liquid. There are a large variety of different NMR experiments that can be used to establish the structure of quite complex molecules. These include various two-dimensional NMR experiments which involve a comparison of signals from different types of nuclei in the molecule (e.g. carbon and hydrogen). Such experiments allow the chemist to build up a picture of the molecule atom by atom and bond by bond.

In cases where there is not enough sample for an NMR analysis, mass spectrometry can be helpful. The fragmentation pattern can give useful clues about the structure, but it does not prove the structure. A full synthesis is still required as final proof.

12.7 Herbal medicine

We have described how useful drugs and lead compounds can be isolated from natural sources, so where does this place herbal medicine? Are there any advantages or disadvantages in using herbal medicines instead of the drugs developed from their active principles? There are no simple answers to this. Herbal medicines contain a large variety of different compounds—several of which may have biological activity—so there is a significant risk of side effects and even toxicity. The active principle is also present in a small quantity, so the herbal medicine may be expected to be less active than the pure compound. Herbal medicines such as St. John's wort can also interact with prescribed medicines (section 11.5.6) and, in general, there is a lack of regulation or control over their use. Another example is **Ginko**, which is often used to treat memory problems. However, it also has anticoagulant properties and should not be used alongside other drugs having similar properties, for example warfarin, aspirin, or ibuprofen. Having said all that, several of the issues identified above may actually be advantageous. If the herbal extract contains the active principle in small quantities, there is an inbuilt safety limit to the dose levels received. Different compounds within the extract may also have roles to play in the medicinal properties of the plant and enhance the effect of the active principle—a phenomenon known as **synergy.** Alternatively, some plant extracts have a wide variety of different active principles which act together to produce a beneficial effect. The **aloe plant** (the 'wand of heaven') is an example of this. It is a cactus-like plant found in the deserts of Africa and Arizona, and has long been revered for its curative properties. Supporters of herbal medicine have proposed the use of aloe preparations to treat burns, irritable bowel syndrome, rheumatoid arthritis, asthma, chronic leg ulcers, itching, eczema, psoriasis, and acne, thus avoiding the undesirable side effects of long-term steroid use. The preparations are claimed to contain analgesic, anti-inflammatory, antimicrobial, and many other agents, which all contribute to the overall effect. Trying to isolate

each active principle would detract from this. However, critics have stated that many of the beneficial effects claimed for aloe preparations have not been proven and that although the effects may be useful in some ailments, they are not very effective.

KEY POINTS

- A lead compound is a structure which shows a useful pharmacological activity and can act as the starting point for drug design.
- Natural products are a rich source of lead compounds. The agent responsible for the biological activity of a natural extract is known as the active principle.
- Lead compounds have been isolated from plants, trees, microorganisms, animals, venoms, and toxins. A study of medical folklore indicates plants and herbs which may contain novel lead compounds.
- Lead compounds can be found by screening synthetic compounds obtained from combinatorial syntheses and other sources.
- Existing drugs can be used as lead compounds for the design of novel structures in the same therapeutic area. Alternatively, the side effects of an existing drug can be enhanced to design novel drugs in a different therapeutic area.
- The natural ligand, substrate, product, or modulator for a particular target can act as a lead compound.
- The ability to crystallize a molecular target allows the use of X-ray crystallography and molecular modelling to design lead compounds which will fit the relevant binding site.
- Serendipity has played a role in the discovery of new lead compounds.
- A knowledge of an existing drug's pharmacophore allows the computerized searching of structural databases to identify possible new lead compounds which share that pharmacophore. Docking experiments are also used to identify potential lead compounds.
- NMR spectroscopy can be used to identify whether small molecules (epitopes) bind to specific regions of a binding site. Epitopes can be optimized then linked together to give a lead compound.
- If a lead compound is present in a natural extract or a combinatorial synthetic mixture, it has to be isolated and purified such that its structure can be determined. X-ray crystallography and NMR spectroscopy are particularly important in structure determination.
- Herbal medicines contain different active principles that may combine to produce a beneficial effect. However, toxic side effects and adverse interactions may occur when taken in combination with prescribed medicines.

QUESTIONS

1. What is meant by target specificity and selectivity? Why is it important?
2. What are the advantages and disadvantages of natural products as lead compounds?
3. Fungi have been a richer source of antibacterial agents than bacteria. Suggest why this might be so.
4. Scuba divers and snorkellers are advised not to touch coral. Why do you think this might be? Why might it be of interest to medicinal chemists?
5. You are employed as a medicinal chemist and have been asked to initiate a research programme aimed at finding a drug which will prevent a novel tyrosine kinase receptor from functioning. There are no known lead compounds that have this property. What approaches can you make to establish a lead compound? (Consult section 4.8 to find out more about protein kinase receptors.)
6. A study was set up to look for agents that would inhibit the kinase active site of the epidermal growth factor receptor (section 4.8). Three assay methods were used: an assay carried out on a genetically engineered form of the protein that was water-soluble and contained the kinase active site; a cell assay that measured total tyrosine phosphorylation in the presence of epidermal growth factor; and an *in vivo* study on mice that had tumours grafted onto their backs. How do you think these assays were carried out to measure the effect of an inhibitor? Why do you think three assays were necessary? What sort of information did they provide?

FURTHER READING

Abad-Zapatero, C. and Metz, J. T. (2005) Ligand efficiency indices as guideposts for drug discovery. *Drug Discovery Today* **10**, 464–469.

Bleicher, K. H., Böhm, H. J., Müller, K., and Alanine Al. (2003) Hit and lead generation: beyond high-throughput screening. *Nature Reviews Drug Discovery* **2**, 369–378.

Blundell, T. L., Jhoti, H., and Abell, C. (2002) High-throughput crystallography for lead discovery in drug design. *Nature Reviews Drug Discovery* **1**, 45–54.

Bolognesi, M. L., Matera, R., Minarini, A., Rosini, M., and Melchiorre, C. (2009) Alzheimer's disease: new approaches to drug discovery. *Current Opinion in Chemical Biology* **13**, 303–308.

Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., and Melchiorre, C. (2008) Multi-target-directed ligands to combat neurodegenerative diseases. *Journal of Medicinal Chemistry* **51**, 347–372.

Clardy, J. and Walsh, C. (2004) Lessons from natural molecules. *Nature* **432**, 829–837.

Di, L., Kerns, E. H., Fan, K., McConnell, O. J., and Carter, G. T. (2003) High throughput artificial membrane permeability assay for blood–brain barrier, *European Journal of Medicinal Chemistry* **38**: 223–232.

Engel, L. W. and Straus, S. E. (2002) Development of therapeutics: opportunities within complementary and alternative medicine. *Nature Reviews Drug Discovery* **1**, 229–237.

Gershell, L. J. and Atkins, J. H. (2003) A brief history of novel drug discovery technologies. *Nature Reviews Drug Discovery* **2**, 321–327.

Honma, T. (2003) Recent advances in *de novo* design strategy for practical lead identification. *Medicinal Research Reviews* **23**, 606–632.

Hopkins, A. L. and Groom, C. R. (2002) The druggable genome. *Nature Reviews Drug Discovery* **1**, 727–730.

Lewis, R. J. and Garcia, M. L. (2003) Therapeutic potential of venom peptides. *Nature Reviews Drug Discovery* **2**, 790–802.

Lindsay, M. A. (2003) Target discovery. *Nature Reviews Drug Discovery* **2**, 831–838.

Lipinski, C. and Hopkins, A. (2004) Navigating chemical space for biology and medicine. *Nature* **432**, 855–861.

Lowe, D. (2009) In the pipeline. *Chemistry World* Nov, 20.

Megget, K. (2011) Of mice and men. *Chemistry World* April, 42–45.

O'Shannessy, D. J., Brigham-Burke, M., Soneson, K. K., Hensley, P., and Brooks, I. (1993) Determination of rate and equilibrium binding constants for macromolecular interactions using surface plasmon resonance: use of nonlinear least squares analysis methods. *Analytical Biochemistry* **212**, 457–468.

Pellecchia, M., Sem, D. S., and Wuthrich, K. (2002) NMR in drug discovery. *Nature Reviews Drug Discovery* **1**, 211–219.

Perks, B. (2011) Extreme potential. *Chemistry World* June, 48–51.

Phillipson, J. D. (2007) Phytochemistry and pharmacognosy. *Phytochemistry* **68**, 2960–2972.

Rees, D. C., Congreve, M., Murray, C. W., and Carr, R. (2004) Fragment-based lead discovery. *Nature Reviews Drug Discovery* **3**, 660–672.

Rishton, G. B. (2003) Nonleadlikeness and leadlikeness in biochemical screening. *Discovering Drugs Today* **8**, 86–96.

Sauter, G., Simon, R., and Hillan, K. (2003) Tissue microarrays in drug discovery. *Nature Reviews Drug Discovery* **2**, 962–972.

Shuker, S. B., Hajduk, P. J., Meadows, R. P., and Fesik, S. W. (1996) Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**, 1531–1534.

Srivastava, A. S., Negi, A. S., Kumar, J. K., Gupta, M. M., and Khanuja, S.P. (2005) Plant-based anticancer molecules. *Bioorganic Medicinal Chemistry* **13**, 5892–5908.

Stockwell, B. R. (2004) Exploring biology with small organic molecules. *Nature* **432**, 846–854.

Su, J., McKittrick, B. A., Burnett, D. A., Clader, J. W., Greenlee, W. J., Hawes, B. E., *et al.* (2007) SAR study of bicyclo[4.1.0]heptanes as melanin-concentrating hormone receptor R1 antagonists: taming hERG. *Bioorganic and Medicinal Chemistry* **15**, 5369–5385.

Walters, W. P. and Namchuk, M. (2003) Designing screen: how to make your hits a hit. *Nature Reviews Drug Discovery* **2**, 259–266.

Wermuth, C. G. (2006) Selective optimization of side activities: the SOSA approach. *Drug Discovery Today* **11**, 160–164.

Titles for general further reading are listed on p. 763.

For additional material see Web article 17: fragment based drug discovery.

13 Drug design: optimizing target interactions

In Chapter 12, we looked at the various methods of discovering a lead compound. Once it *has* been discovered, the lead compound can be used as the starting point for drug design. There are various aims in drug design. The eventual drug should have a good selectivity and level of activity for its target, and have minimal side effects. It should be easily synthesized and chemically stable. Finally, it should be non-toxic and have acceptable pharmacokinetic properties. In this chapter, we concentrate on design strategies that can be used to optimize the interaction of the drug with its target in order to produce the desired pharmacological effect; in other words, its pharmacodynamic properties. In Chapter 14, we look at the design strategies that can improve the drug's ability to reach its target and have an acceptable lifetime, i.e. its **pharmacokinetic** properties. Although these topics are in separate chapters, it would be wrong to think that they are tackled separately during drug optimization. For example, it would be foolish to spend months or years perfecting a drug that interacts perfectly with its target, but has no chance of reaching that target because of adverse pharmacokinetic properties. Pharmacodynamics and pharmacokinetics should have equal priority in influencing drug design strategies and determining which analogues are synthesized.

13.1 Structure–activity relationships

Once the structure of a lead compound is known, the medicinal chemist moves on to study its structure–activity relationships (SAR). The aim is to identify those parts of the molecule that are important to biological activity and those that are not. If it is possible to crystallize the target with the lead compound bound to the binding site, the crystal structure of the complex could be solved by X-ray crystallography, then studied with molecular modelling software to identify important binding interactions. However, this is not possible if the target structure has not been identified or cannot be crystallized. It is then necessary to revert to the traditional method of synthesizing a selected number of compounds that vary slightly from the original structure, then studying what effect that has on the biological activity.

One can imagine the drug as a chemical knight entering the body in order to make battle with an affliction. The drug is armed with a variety of weapons and armour, but it may not be obvious which weapons are important to the drug's activity or which armour is essential to its survival. We can only find this out by removing some of the weapons and armour to see if the drug is still effective. The weapons and armour involved are the various structural features in the drug that can either act as binding groups with the target binding site (section 1.3), or assist and protect the drug on its journey through the body (Chapter 14). Recognizing functional groups and the sort of intermolecular bonds that they can form is important in understanding how a drug might bind to its target.

Let us imagine that we have isolated a natural product with the structure shown in Fig. 13.1. We shall name it glipine. There are a variety of functional groups present in the structure and the diagram shows the potential binding interactions that are possible with a target binding site.

It is unlikely that all of these interactions take place, so we have to identify those that do. By synthesizing analogues (such as the examples shown in Fig. 13.2) where one particular functional group of the molecule is removed or altered, it is possible to find out which groups are essential and which are not. This involves testing all the analogues for biological activity and comparing them with the original compound. If an analogue shows a significantly lowered activity, then the group that has been modified must have been important. If the activity remains similar, then the group is not essential.

The ease with which this task is carried out depends on how easily we can synthesize the necessary analogues. It may be possible to modify some lead compounds

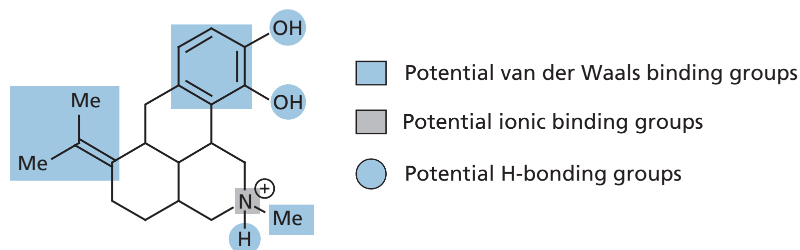


FIGURE 13.1 Glipine.

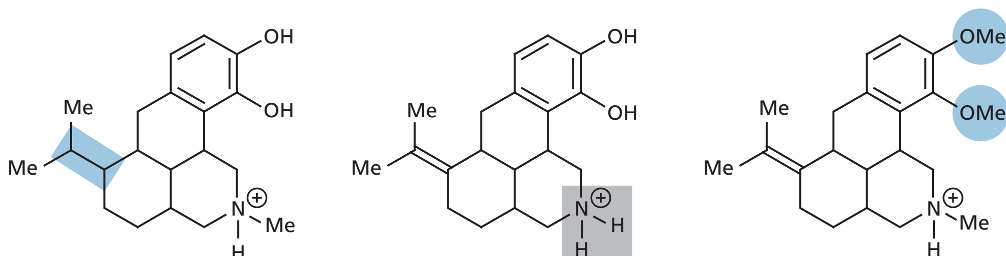


FIGURE 13.2 Modifications of glipine.

directly to the required analogues, whereas the analogues of other lead compounds may best be prepared by total synthesis. Let us consider the binding interactions that are possible for different functional groups and the analogues that could be synthesized to establish whether they are involved in binding or not (see also section 1.3 and Appendix 7)

13.1.1 Binding role of alcohols and phenols

Alcohols and phenols are functional groups which are commonly present in drugs and are often involved in hydrogen bonding. The oxygen can act as a hydrogen bond acceptor, and the hydrogen can act as a hydrogen bond donor (Fig. 13.3). The directional preference for hydrogen bonding is indicated by the arrows in the figure, but it is important to realize that slight deviations are possible (section 1.3.2). One, or all, of these interactions may be important in binding the drug to the binding site. Synthesizing a methyl ether or an ester analogue would be relevant in testing this, as it is highly likely that the hydrogen bonding would be disrupted in either analogue. Let us consider the methyl ether first.

There are two reasons why the ether might hinder or prevent the hydrogen bonding of the original alcohol or phenol. The obvious explanation is that the proton of the original hydroxyl group is involved as a hydrogen bond donor and, by removing it, the hydrogen bond is lost (Frames 1 and 2 in Fig. 13.4). However, suppose the oxygen atom is acting as a hydrogen bond acceptor (Frame 3, Fig. 13.4)? The oxygen is still present in the ether analogue, so could it still take part in hydrogen bonding? Well, it may, but possibly not to the same extent. The extra bulk of the methyl group should hinder the close approach that was previously attainable and is likely to disrupt hydrogen bonding (Frame 4, Fig. 13.4). The hydrogen bonding may not be completely prevented, but we could reasonably expect it to be weakened.

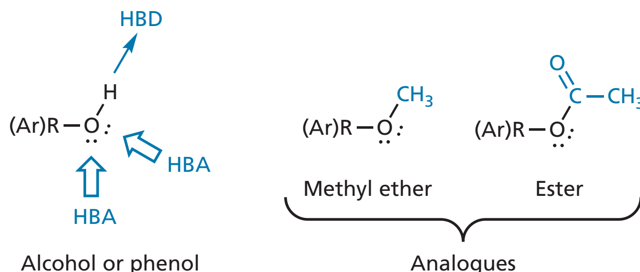


FIGURE 13.3 Possible hydrogen bonding interactions for an alcohol or phenol.

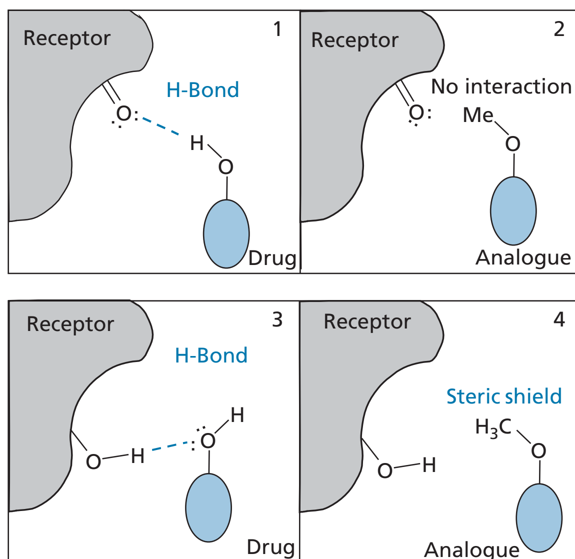


FIGURE 13.4 Possible hydrogen bond interactions for an alcohol/phenol in comparison with an ether analogue.

An ester analogue cannot act as a hydrogen bond donor either. There is still the possibility of it acting as a hydrogen bond acceptor, but the extra bulk of the acyl group is even greater than the methyl group of the ether, and this, too, should hinder the original hydrogen bonding interaction. There is also a difference between the electronic properties of an ester and an alcohol. The carboxyl group has a weak pull on the electrons from the neighbouring oxygen, giving the resonance structure shown in Fig. 13.5. Because the lone pair is involved in such an interaction, it will be less effective as a hydrogen bond acceptor. Of course, one could then argue that the carbonyl oxygen is potentially a more effective hydrogen bond acceptor; however, it is in a different position relative to the rest of the molecule and may be poorly positioned to form an effective hydrogen bond interaction with the target binding region.

It is relatively easy to acetylate alcohols and phenols to their corresponding esters, and this was one of the early reactions that was carried out on natural products such as morphine (sections 24.3 and 24.5). Alcohols and phenols can also be converted easily to ethers.

In this section, we considered the OH group of alcohols and phenols. It should be remembered that the OH group of a phenol is linked to an aromatic ring, which can also be involved in intermolecular interactions (section 13.1.2).

13.1.2 Binding role of aromatic rings

Aromatic rings are planar, hydrophobic structures, commonly involved in van der Waals interactions with flat hydrophobic regions of the binding site. An analogue containing a cyclohexane ring in place of the aromatic ring is less likely to bind so well, as the ring is no longer flat. The axial protons can interact weakly, but they also serve as buffers to keep the rest of the cyclohexane ring at a distance (Fig. 13.6). The binding region for the aromatic ring may also be a narrow slot rather than a planar surface. In that scenario, the cyclohexane ring would be incapable of fitting into it, because it is a bulkier structure.

Although there are methods of converting aromatic rings to cyclohexane rings, they are unlikely to be successful

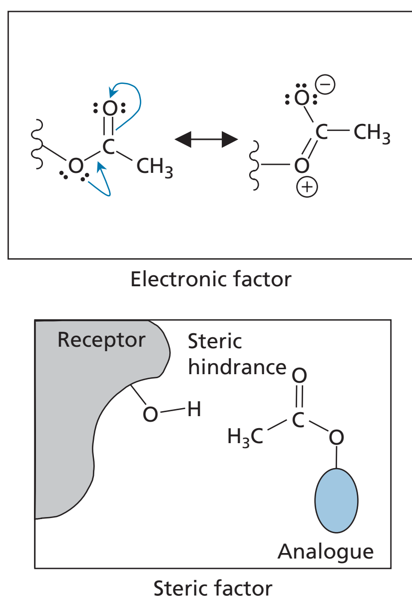


FIGURE 13.5 Factors by which an ester group can disrupt the hydrogen bonding of the original hydroxyl group.

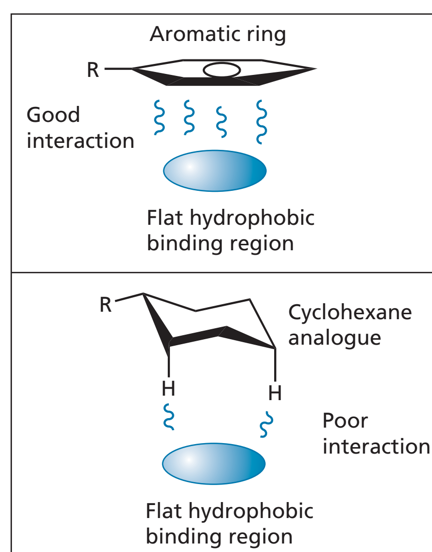


FIGURE 13.6 Binding comparison of an aromatic ring with a cyclohexyl ring.

with most lead compounds, and so such analogues would normally be prepared using a full synthesis.

Aromatic rings could also interact with an aminium or quaternary ammonium ion through induced dipole interactions or hydrogen bonding (sections 1.3.4 and 1.3.2). Such interactions would not be possible for the cyclohexyl analogue.

13.1.3 Binding role of alkenes

Like aromatic rings, alkenes are planar and hydrophobic so they too can interact with hydrophobic regions of the binding site through van der Waals interactions. The activity of the equivalent saturated analogue would be worth testing, as the saturated alkyl region is bulkier and cannot approach the relevant region of the binding site so closely (Fig. 13.7). Alkenes are generally easier to reduce than aromatic rings, so it may be possible to prepare the saturated analogue directly from the lead compound.

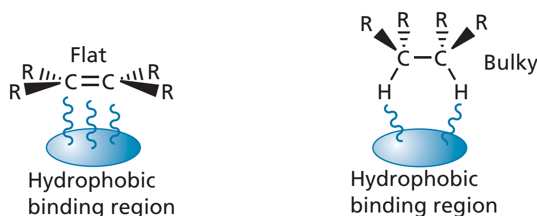


FIGURE 13.7 Binding comparison of an alkene with an alkane.

13.1.4 The binding role of ketones and aldehydes

A ketone group is not uncommon in many of the structures studied in medicinal chemistry. It is a planar group that can interact with a binding site through hydrogen bonding where the carbonyl oxygen acts as a hydrogen bond acceptor (Fig. 13.8). Two such interactions are possible, as two lone pairs of electrons are available on the carbonyl oxygen. The lone pairs are in sp^2-hybridized orbitals which are in the same plane as the functional group. The carbonyl group also has a significant dipole moment and so a dipole–dipole interaction with the binding site is also possible.

It is relatively easy to reduce a ketone to an alcohol and it may be possible to carry out this reaction directly on the lead compound. This significantly changes the geometry of the functional group from planar to tetrahedral. Such an alteration in geometry may well weaken any existing hydrogen bonding interactions and will certainly weaken any dipole–dipole interactions, as both the magnitude and orientation of the dipole moment will be altered (Fig. 13.9). If it was suspected that the oxygen present in the alcohol analogue might still be acting as a hydrogen bond acceptor, then the ether or ester analogues could be studied as described above. Reactions are available that can reduce a ketone completely to an alkane and remove the oxygen, but they are unlikely to be practical for many of the lead compounds studied in medicinal chemistry.

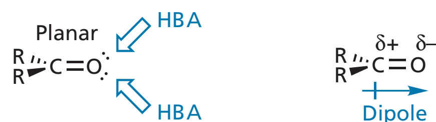


FIGURE 13.8 Binding interactions that are possible for a carbonyl group.

Aldehydes are less common in drugs because they are more reactive and are susceptible to metabolic oxidation to carboxylic acids. However, they could interact in the same way as ketones, and similar analogues could be studied.

13.1.5 Binding role of amines

Amines are extremely important functional groups in medicinal chemistry and are present in many drugs. They may be involved in hydrogen bonding, either as a hydrogen bond acceptor or a hydrogen bond donor (Fig. 13.10). The nitrogen atom has one lone pair of electrons and can act as a hydrogen bond acceptor for one hydrogen bond. Primary and secondary amines have N–H groups and can act as hydrogen bond donors. Aromatic and heteroaromatic amines act only as hydrogen bond donors because the lone pair interacts with the aromatic or heteroaromatic ring.

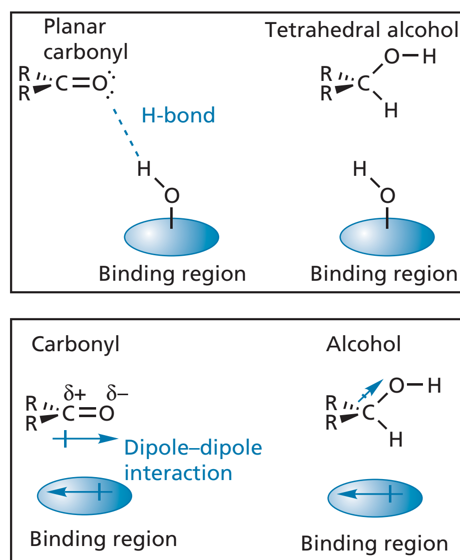


FIGURE 13.9 Effect on binding interactions following the reduction of a ketone or aldehyde.

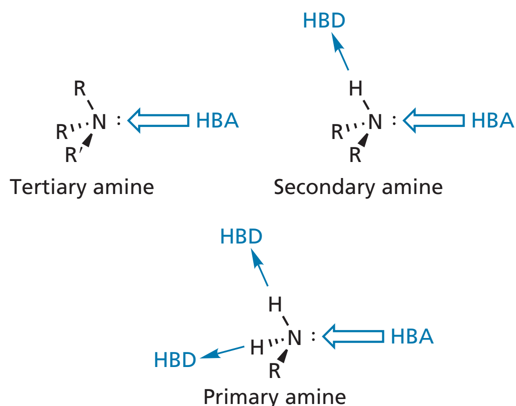


FIGURE 13.10 Possible binding interactions for amines.

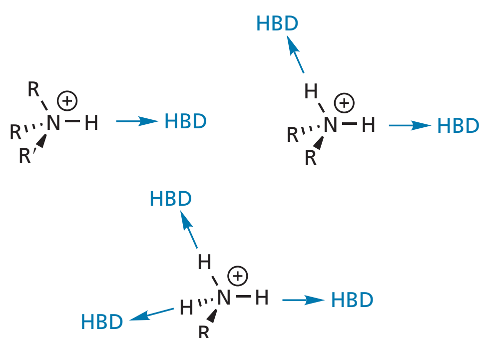


FIGURE 13.11 Possible hydrogen bonding interactions for ionized amines.

In many cases, the amine may be protonated when it interacts with its target binding site, which means that it is ionized and cannot act as a hydrogen bond acceptor. However, it can still act as a hydrogen bond donor and will form stronger hydrogen bonds than if it was not ionized (Fig. 13.11). Alternatively, a strong ionic interaction may take place with a carboxylate ion in the binding site (Fig. 13.12).

To test whether ionic or hydrogen bonding interactions are taking place, an amide analogue could be studied. This will prevent the nitrogen acting as a hydrogen bond acceptor, as the nitrogen's lone pair will interact with the neighbouring carbonyl group instead (Fig. 13.13). This interaction also prevents protonation of the nitrogen and rules out the possibility of ionic interactions. You might argue that the right-hand structure in Fig. 13.13a has a positive charge on the nitrogen and could still take part in an ionic interaction. However, this resonance structure represents one extreme and is never present as a distinct entity. The amide group as a whole is neutral, and so lacks the net positive charge required for ionic bonding.

It is relatively easy to form secondary and tertiary amides from primary and secondary amines, respectively,

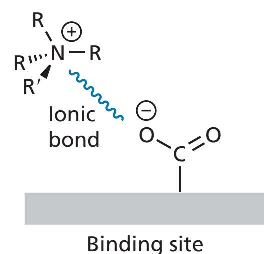


FIGURE 13.12 Ionic interaction between an ionized amine and a carboxylate ion (R^{1-4} = H, alkyl).

and it may be possible to carry out this reaction directly on the lead compound. A tertiary amide lacks the N–H group of the original secondary amine and would test whether this is involved as a hydrogen bond donor. The secondary amide formed from a primary amine still has a N–H group present, but the steric bulk of the acyl group should hinder it acting as a hydrogen bond donor.

Tertiary amines cannot be converted directly to amides, but if one of the alkyl groups is a methyl group, it is often possible to remove it with vinyloxycarbonyl chloride (VOC-Cl) to form a secondary amine, which could then be converted to the amide (Fig. 13.14). This demethylation reaction is extremely useful and has been used to good effect in the synthesis of morphine analogues (see Box 24.2 for the reaction mechanism).

13.1.6 Binding role of amides

Many of the lead compounds currently studied in medicinal chemistry are peptides or polypeptides consisting of amino acids linked together by peptide or amide bonds (section 2.1). Amides are likely to interact with binding sites through hydrogen bonding (Fig. 13.15). The carbonyl oxygen atom can act as a hydrogen bond acceptor and has the potential to form two hydrogen bonds. Both the lone pairs involved are in sp^2-hybridized orbitals which are located in the same plane as the amide group. The nitrogen cannot act as a hydrogen bond acceptor because the lone pair interacts with the neighbouring carbonyl group as described earlier. Primary and secondary amides have a N–H group, which allows the possibility of this group acting as a hydrogen bond donor.

The most common type of amide in peptide lead compounds is the secondary amide. Suitable analogues that could be prepared to test out possible binding interactions are shown in Fig. 13.16. All the analogues, apart from the primary and secondary amines, could be used to check whether the amide is acting as a hydrogen bond donor. The alkenes and amines could be tested to see whether the amide is acting as a hydrogen bond acceptor. However, there are traps for the unwary. The amide group is planar and does not rotate because of its partial double bond character. The ketone, the secondary amine,

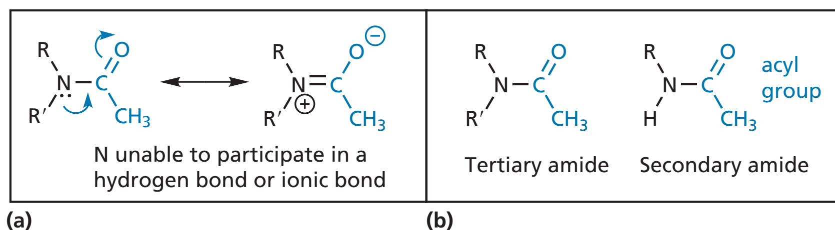


FIGURE 13.13 (**a**) Interaction of the nitrogen lone pair with the neighbouring carbonyl group in amides. (**b**) Secondary and tertiary amides.

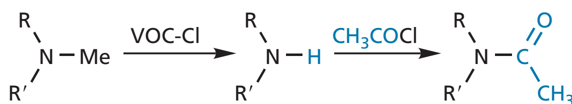


FIGURE 13.14 Dealkylation of a tertiary amine and formation of a secondary amide.

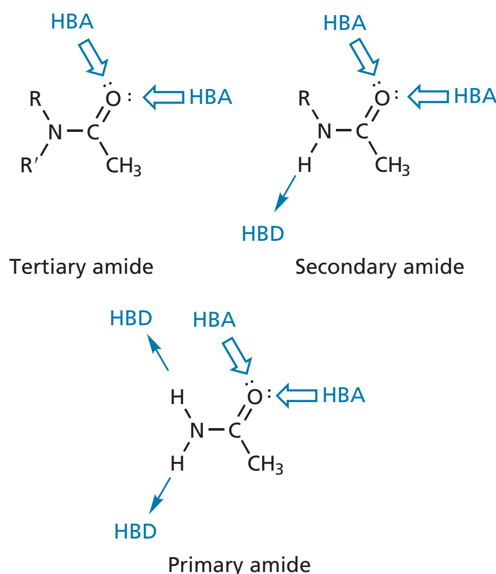


FIGURE 13.15 Possible hydrogen bonding interactions for amides.

and the tertiary amine analogues have a single bond at the equivalent position which *can* rotate. This would alter the relative positions of any binding groups on either side of the amide group and lead to a loss of binding, even if the amide itself was not involved in binding. Therefore, a loss of activity would not necessarily mean that the amide is important as a binding group. With these groups, it would only be safe to say that the amide group is not essential if activity is retained. Similarly, the primary amine and carboxylic acid may be found to have no activity, but this might be due to the loss of important binding groups in one half of the molecule. These particular analogues would only be worth considering if the amide group is peripheral to the molecule (e.g. R-NHCOMe or R-CONHMe) and not part of the main skeleton.

The alkene would be a particularly useful analogue to test because it is planar, cannot rotate, and cannot act as a hydrogen bond donor or hydrogen bond acceptor. However, the synthesis of this analogue may not be simple. In fact, it is likely that all the analogues described would have to be prepared using a full synthesis. Amides are relatively stable functional groups and, although several of the analogues described might be attainable directly from the lead compound, it is more likely that the lead compound would not survive the forcing conditions required.

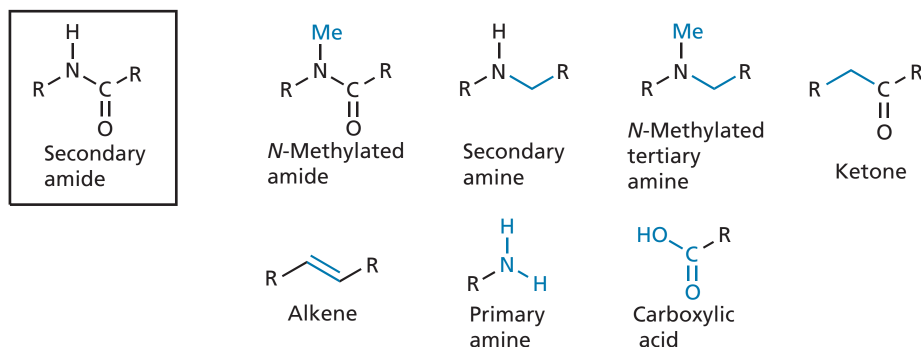


FIGURE 13.16 Possible analogues to test the binding interactions of a secondary amide.

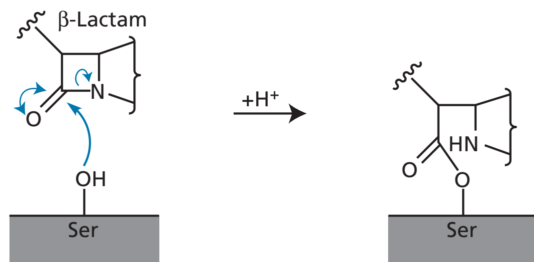


FIGURE 13.17 β-Lactam ring acting as an acylating agent.

Amides which are within a ring system are called lactams. They, too, can form intermolecular hydrogen bonds as described earlier in the chapter. However, if the ring is small and suffers ring strain, the lactam can undergo a chemical reaction with the target leading to the formation of a covalent bond. The best examples of this are the penicillins, which contain a four-membered β-lactam ring. This acts as an acylating agent and irreversibly inhibits a bacterial enzyme by acylating a serine residue in the active site (Fig. 13.17) (section 19.5.1.4).

13.1.7 Binding role of quaternary ammonium salts

Quaternary ammonium salts are ionized and can interact with carboxylate groups by ionic interactions (Fig. 13.18). Another possibility is an **induced dipole interaction** between the quaternary ammonium ion and any aromatic rings in the binding site. The positively charged nitrogen can distort the π electrons of the aromatic ring such that a dipole is induced, whereby the face of the ring is slightly negative and the edges are slightly positive. This allows an interaction between the slightly negative faces of the aromatic rings and the positive charge of the quaternary ammonium ion. This is also known as a **π–cation interaction**.

The importance of these interactions could be tested by synthesizing an analogue that has a tertiary amine group rather than the quaternary ammonium group. Of course, it is possible that such a group could ionize by becoming protonated and then interact in the same way. Converting the amine to an amide would prevent this possibility. The neurotransmitter **acetylcholine** has a quaternary ammonium group which is thought to bind to the binding site of its target receptor by ionic bonding and/or induced dipole interactions (section 22.5).

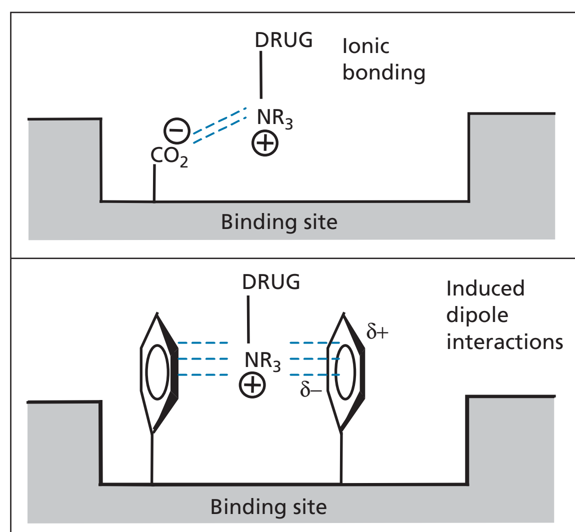


FIGURE 13.18 Possible binding interactions of a quaternary ammonium ion.

13.1.8 Binding role of carboxylic acids

The carboxylic acid group is reasonably common in drugs. It can act as a hydrogen bond acceptor or as a hydrogen bond donor (Fig. 13.19). Alternatively, it may exist as the carboxylate ion. This allows the possibility of an ionic interaction and/or a strong hydrogen bond where the carboxylate ion acts as the hydrogen bond acceptor. The carboxylate ion is also a good ligand for metal ion cofactors present in several enzymes, for example zinc metalloproteinases (section 21.7.1 and Case study 2).

In order to test the possibility of such interactions, analogues such as esters, primary amides, primary alcohols, and ketones could be synthesized and tested (Fig. 13.20). None of these functional groups can ionize, so a loss of activity could imply that an ionic bond is important. The primary alcohol could shed light on whether the carbonyl oxygen is involved in hydrogen bonding, whereas the ester and ketone could indicate whether the hydroxyl group of the carboxylic acid is involved in hydrogen bonding. It may be possible to synthesize the ester and amide analogues directly from the lead compound, but the reduction of a carboxylic acid to a primary alcohol requires harsher conditions and this sort

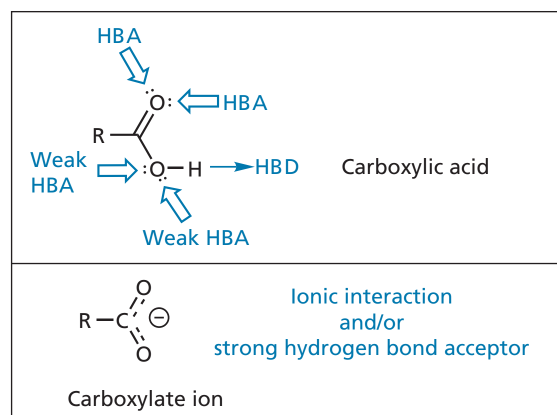


FIGURE 13.19 Possible binding interactions for a carboxylic acid and carboxylate ion.

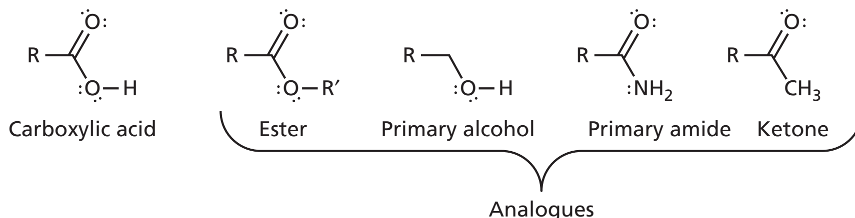


FIGURE 13.20 Analogues to test the binding interactions for a carboxylic acid.

of analogue would normally be prepared by a full synthesis. The ketone would also have to be prepared by a full synthesis.

13.1.9 Binding role of esters

An ester functional group has the potential to interact with a binding site as a hydrogen bond acceptor only (Fig. 13.21). The carbonyl oxygen is more likely to act as the hydrogen bond acceptor than the alkoxy oxygen (section 1.3.2), as it is sterically less hindered and has a greater electron density. The importance, or otherwise, of the carbonyl group could be judged by testing an equivalent ether, which would require a full synthesis.

Esters are susceptible to hydrolysis *in vivo* by metabolic enzymes called **esterases**. This may pose a problem if the lead compound contains an ester that is important to binding, as it means the drug might have a short lifetime *in vivo*. Having said that, there are several drugs that *do* contain esters and are relatively stable to metabolism thanks to electronic factors that stabilize the ester or steric factors that protect it.

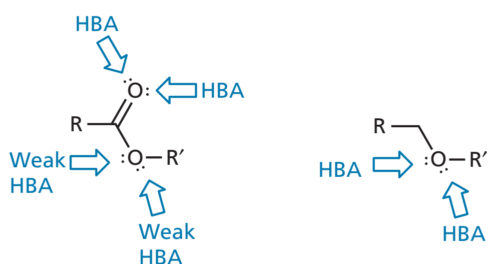


FIGURE 13.21 Possible binding interactions for an ester and an ether.

Esters that are susceptible to metabolic hydrolysis are sometimes used deliberately to mask a polar functional group, such as a carboxylic acid, alcohol, or phenol, in order to achieve better absorption from the gastrointestinal tract. Once in the blood supply, the ester is hydrolysed to release the active drug. This is known as a **prodrug** strategy (section 14.6).

Special mention should be made of the ester group in aspirin. Aspirin has an anti-inflammatory action resulting from its ability to inhibit an enzyme called **cyclooxygenase** (COX) which is required for **prostaglandin** synthesis. It is often stated that aspirin acts as an acylating agent and that its acetyl group is covalently attached to a serine residue in the active site of COX (Fig. 13.22). However, this theory has been disputed and it is stated that aspirin acts, instead, as a prodrug to generate salicylic acid, which then inhibits the enzyme through non-covalent interactions.

13.1.10 Binding role of alkyl and aryl halides

Alkyl halides involving chlorine, bromine, or iodine tend to be chemically reactive as the halide ion is a good leaving group. As a result, a drug containing an alkyl halide is likely to react with any nucleophilic group that it encounters and become permanently linked to that group by a covalent bond—an alkylation reaction (Fig. 13.23). This poses a problem, as the drug is likely to alkylate a large variety of macromolecules which have

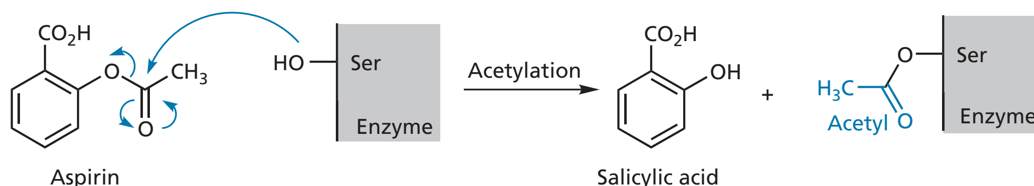


FIGURE 13.22 The disputed theory of aspirin acting as an acylating agent.

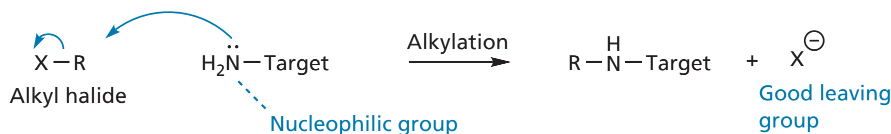


FIGURE 13.23 Alkylation of macromolecular targets by alkyl halides.

nucleophilic groups, especially amine groups in proteins and nucleic acids. It is possible to moderate the reactivity to some extent, but selectivity is still a problem and leads to severe side effects. These drugs are, therefore, reserved for life-threatening diseases, such as cancer (sections 9.3 and 21.2.3). Alkyl fluorides, however, are not alkylating agents because the C–F bond is strong and not easily broken. Fluorine is commonly used to replace a proton as it is approximately the same size, but has different electronic properties. It may also protect the molecule from metabolism (sections 13.3.7 and 14.2.4).

Aryl halides do not act as alkylating agents and pose less of a problem in that respect. As the halogen substituents are electron-withdrawing groups, they affect the electron density of the aromatic ring and this may have an influence on the binding of the aromatic ring. The halogen substituents chlorine and bromine are hydrophobic in nature and may interact favourably with hydrophobic pockets in a binding site. Hydrogen bonding is not important. Although halide ions are strong hydrogen bond acceptors, halogen substituents are poor hydrogen bond acceptors.

Aliphatic and aromatic analogues lacking the halogen substituent could be prepared by a full synthesis to test whether the halogen has any importance towards the activity of the lead compound.

13.1.11 Binding role of thiols and ethers

The thiol group (S–H) is known to be a good ligand for d-block metal ions and has been incorporated into several drugs designed to inhibit enzymes containing a zinc cofactor, for example the zinc metalloproteinases (section 21.7.1 and Case study 2). If the lead compound has a thiol group, the corresponding alcohol could be tested as a comparison. This would have a far weaker interaction with zinc.

An ether group (R′OR) might act as a hydrogen bond acceptor through the oxygen atom (Fig. 13.21). This could be tested by increasing the size of the neighbouring alkyl group to see whether it diminishes the ability of the group to take part in hydrogen bonding. Analogues where the oxygen is replaced with a methylene (CH_2) isostere should show significantly decreased binding affinity.

The oxygen atom of an aromatic ether is generally a poor hydrogen bond acceptor (section 1.3.2).

13.1.12 Binding role of other functional groups

A wide variety of other functional groups may be present in lead compounds that have no direct binding role, but could be important in other respects. Some may influence the electronic properties of the molecule (e.g. nitro groups or nitriles). Others may restrict the shape or conformation of a molecule (e.g. alkynes) (Box 13.3). Functional groups may also act as **metabolic blockers** (e.g. aryl halides) (section 14.2.4).

13.1.13 Binding role of alkyl groups and the carbon skeleton

The alkyl substituents and carbon skeleton of a lead compound are hydrophobic and may bind with hydrophobic regions of the binding site through van der Waals interactions. The relevance of an alkyl substituent to binding can be determined by synthesizing an analogue which lacks the substituent. Such analogues generally have to be synthesized using a full synthesis if they are attached to the carbon skeleton of the molecule. However, if the alkyl group is attached to nitrogen or oxygen, it may be possible to remove the group from the lead compound, as shown in Fig. 13.24. The analogues obtained may then be expected to have less activity if the alkyl group was involved in important hydrophobic interactions.

13.1.14 Binding role of heterocycles

A large diversity of heterocycles are found in lead compounds. Heterocycles are cyclic structures that contain

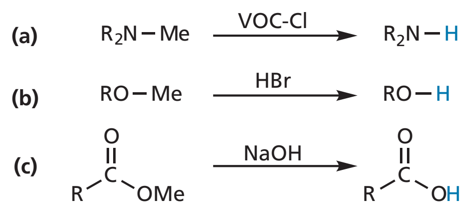


FIGURE 13.24 (**a**) *N*-Demethylation of a tertiary amine with vinyloxycarbonyl chloride (see Box 24.2 for mechanism). (**b**) Demethylation of a methyl ether using hydrogen bromide where nucleophilic substitution leads to an alcohol (or phenol) plus bromomethane. (**c**) Hydrolysis of an ester using sodium hydroxide where OH replaces OMe.

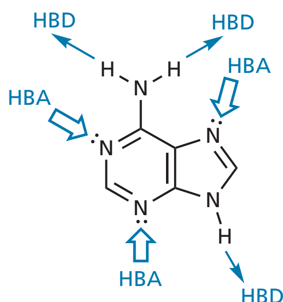


FIGURE 13.25 Possible hydrogen bonding interactions for adenine.

one or more heteroatoms, such as oxygen, nitrogen, or sulphur. Nitrogen-containing heterocycles are particularly prevalent. The heterocycles can be aliphatic or aromatic in character and have the potential to interact with binding sites through a variety of bonding forces. For example, the overall heterocycle can interact through van der Waals and hydrophobic interactions, while the individual heteroatoms present in the structure could interact by hydrogen bonding or ionic bonding.

As far as hydrogen bonding is concerned, there is an important directional aspect. The position of the heteroatom in the ring and the orientation of the ring in the binding site can be crucial in determining whether or not a good interaction takes place. For example, adenine can take part in six hydrogen bonding interactions: three as a hydrogen bond donor and three as a hydrogen bond acceptor. The ideal directions for these interactions are shown in Fig. 13.25. Van der Waals interactions are also possible to regions of the binding site above and below the plane of the ring system.

Heterocycles can be involved in quite intricate hydrogen bonding networks within a binding site. For example, the anticancer drug **methotrexate** contains a diaminopteridine ring system that interacts with its binding site as shown in Fig. 13.26.

If the lead compound contains a heterocyclic ring, it is worth synthesizing analogues containing a benzene ring or different heterocyclic rings to explore whether all the heteroatoms present are really necessary.

A complication with heterocycles is the possibility of **tautomers**. This played an important role in determining the structure of DNA (section 6.1.2). The structure of DNA consists of a double helix with base-pairing between two sets of heterocyclic nucleic acid bases. Base-pairing involves three hydrogen bonds between the base pair guanine and cytosine, and two hydrogen bonds between the base pair adenine and thymine (Fig. 13.27). The rings involved in the base-pairing are coplanar, allowing the optimum orientation for the hydrogen bond donors and hydrogen bond acceptors. This, in turn, means that the base pairs are stacked above each other allowing van der Waals interactions between the faces of each base pair. However, when Watson and Crick originally tried to devise a model for DNA, they incorrectly assumed that the preferred tautomers for the nucleic acid bases were as shown in the right-hand part of Fig. 13.27. With these tautomers, the required hydrogen bonding is not possible and would not explain the base-pairing observed in the structure of DNA.

In a similar vein, knowing the preferred tautomers of heterocycles can be important in understanding how drugs interact with their binding sites. This is amply illustrated in the design of the anti-ulcer agent cimetidine (section 25.2).

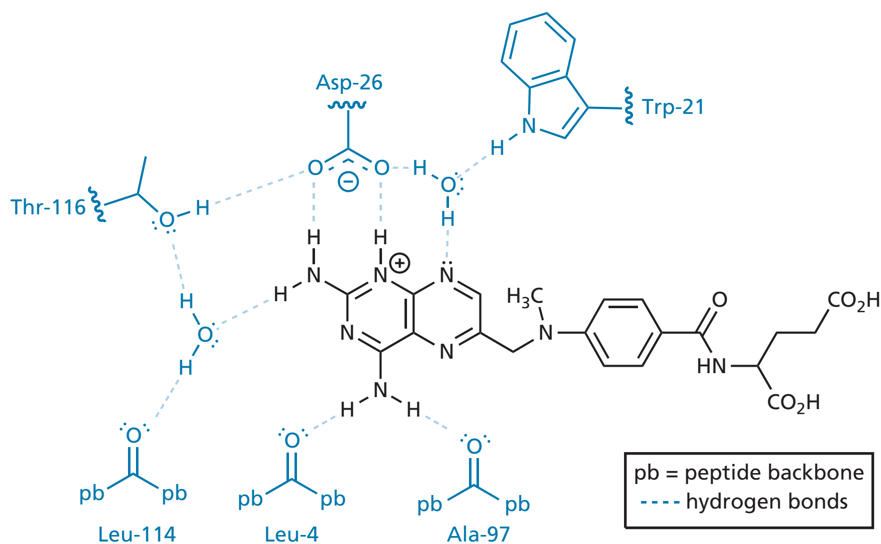


FIGURE 13.26 Binding interactions for the diaminopteridine ring of methotrexate in its binding site.

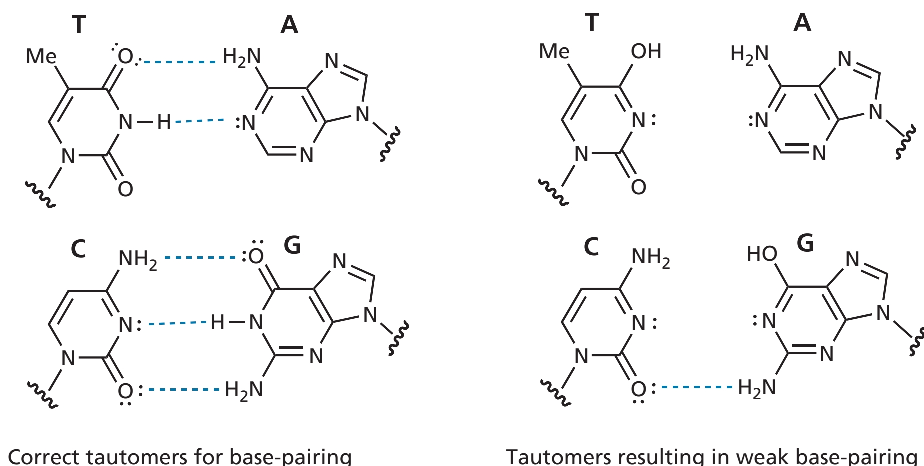


FIGURE 13.27 Base-pairing in DNA and the importance of tautomers.

With heterocyclic compounds, it is possible for a hydrogen bond donor and a hydrogen bond acceptor to be part of a conjugated system. Polarization of the electrons in the conjugated system permits π-**bond cooperativity**, where the strength of the hydrogen bond donor is enhanced by the hydrogen bond acceptor and *vice versa*. This has also been called **resonance-assisted hydrogen bonding**. This type of hydrogen bonding is possible for the hydrogen bond donors and acceptors for the nucleic acid base pairs (Fig. 13.28).

Note that not all heteroatoms in heterocyclic systems are able to act as good hydrogen bond acceptors. If a heteroatom's lone pair of electrons is part of an aromatic sextet of electrons, it is not available to form a hydrogen bond.

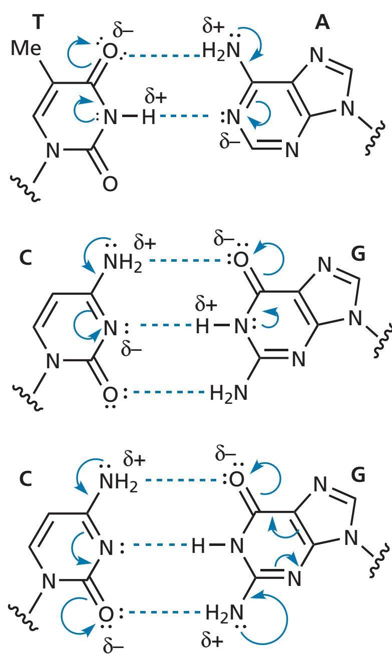


FIGURE 13.28 π-Bond cooperativity in hydrogen bonding.

13.1.15 Isosteres

Isosteres are atoms or groups of atoms which share the same valency and which have chemical or physical similarities (Fig. 13.29).

For example, SH, NH_2, and CH_3 are isosteres of OH, whereas S, NH, and CH_2 are isosteres of O. Isosteres can be used to determine whether a particular group is an important binding group or not by altering the character of the molecule in as controlled a way as possible. Replacing O with CH_2, for example, makes little difference to the size of the analogue, but will have a marked effect on its polarity, electronic distribution, and bonding. Replacing OH with the larger SH may not have such an influence on the electronic character, but steric factors become more significant.

Isosteric groups could be used to determine whether a particular group is involved in hydrogen bonding. For example, replacing OH with CH_3 would completely eliminate hydrogen bonding, whereas replacing OH with NH_2 would not.

The β-blocker **propranolol** has an ether linkage (Fig. 13.30). Replacement of the OCH_2 segment with the isosteres CH = CH, SCH_2, or CH_2CH_2 eliminates activity, whereas replacement with $NHCH_2$ retains activity (though reduced). These results show that the ether oxygen is important to the activity of the drug and suggests that it is involved in hydrogen bonding with the receptor.

The use of isosteres in drug design is described in section 13.3.7.

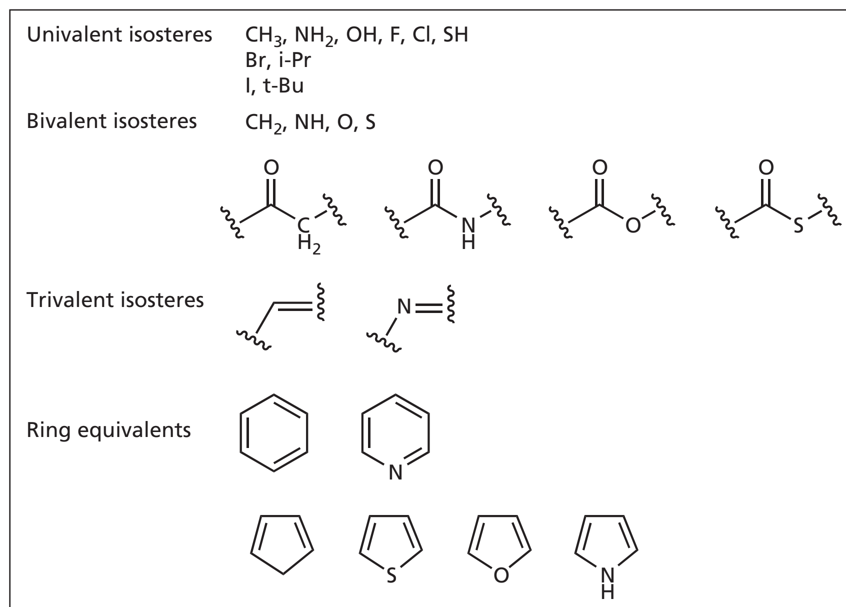


FIGURE 13.29 Examples of classic isosteres.

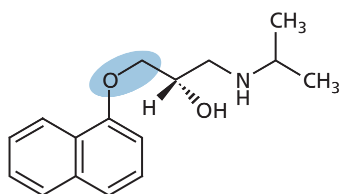


FIGURE 13.30 Propranolol.

13.1.16 Testing procedures

When investigating structure–activity relationships for drug–target binding interactions, biological testing should involve *in vitro* tests; for example inhibition studies on isolated enzymes or binding studies on membrane-bound receptors in whole cells. The results then show conclusively which binding groups are important in drug–target interactions. If *in vivo* testing is carried out, the results are less clear-cut because loss of activity may be due to the inability of the drug to reach its target rather than reduced drug–target interactions. However, *in vivo* testing may reveal functional groups that are important in protecting or assisting the drug in its passage through the body. This would not be revealed by *in vitro* testing.

NMR spectroscopy can also be used to test structure–activity relationships, as described in section 12.4.10.

As mentioned in the introduction, there is little point in designing a drug that has optimum interactions with its target if it has undesirable pharmacokinetic properties. Calculating a structure's hydrophobicity can provide an indication as to whether it is likely to suffer from pharmacokinetic problems. This is because hydrophobic drugs have been found to be more prone to adverse pharmacokinetic properties. For example, they are more likely to interact with other protein targets, resulting in unwanted side effects. They are generally less soluble, show poor permeability and are more likely to produce toxic metabolites. The hydrophobic nature of a drug can be calculated by its CLogD value (section 14.1). In recent years, several research groups have optimized drugs by optimizing **lipophilic efficiency** (LipE), where LipE = pK_i (or pIC_{50}) – CLogD. Drugs with a good level of lipophilic efficiency will have high activity (pK_i or pIC_{50}) and low hydrophobic character. Optimizing LipE involves a parallel optimization of potency and hydrophobicity. This quantitative method of optimizing both the pharmacodynamic and pharmacokinetic properties has been called **property-based drug design** and was used in the structure-based drug design of **crizotinib** (Box 13.4).

13.1.17 SAR in drug optimization

In this section, we have focused on SAR studies aimed at identifying important binding groups in a lead compound. SAR studies are also used in drug optimization, where the aim is to find analogues with better activity and selectivity. This involves further modifications of the lead compound to identify whether these are beneficial or detrimental to activity. This is covered in section 13.3 where the different strategies of optimizing drugs are discussed.

13.2 Identification of a pharmacophore

Once it is established which groups are important for a drug's activity, it is possible to move on to the next stage—the identification of the **pharmacophore**. The pharmacophore summarizes the important binding groups that are required for activity, and their relative positions in space with respect to each other. For example, if we discover that the important binding groups for our hypothetical drug glipine are the two phenol groups, the aromatic ring, and the nitrogen atom, then the pharmacophore is as shown in Fig. 13.31. Structure I shows the two-dimensional (2D) pharmacophore and structure II shows the three-dimensional (3D) pharmacophore. The latter specifies the relative positions of the important groups in space. In this case, the nitrogen atom is 5.063 Å from the centre of the phenolic ring and lies at an angle of 18° from the plane of the ring. Note that it is not necessary to show the specific skeleton connecting the important groups. Indeed, there are benefits in not doing so, as it is easier to compare the 3D pharmacophores from different structural classes of compound to see if they share a common pharmacophore. Three-dimensional pharmacophores can be defined using molecular modelling (section 17.11), which allows the definition of 'dummy bonds', such as the one in Fig. 13.31 between nitrogen and the centre of the aromatic ring. The centre of the ring can be defined by a **dummy atom** called a **centroid** (not shown).

An even more general type of 3D pharmacophore is the one shown as structure III (Fig. 13.31)—a bonding-type pharmacophore. Here, the bonding characteristics of each functional group are defined, rather than the group itself. Note also that the groups are defined as points in space. This includes the aromatic ring, which is defined by the centroid. All the points are connected by pharmacophoric triangles to define their positions. This allows the comparison of molecules which may have the same pharmacophore and binding interactions, but which use different functional groups to achieve these interactions. In this case, the phenol groups can act as hydrogen bond donors or acceptors, the aromatic ring can participate in van der Waals interactions, and the amine can act as a hydrogen bond acceptor or as an ionic centre if it is protonated. We shall return to the concept and use of 3D pharmacophores in sections 17.11 and 18.10.

Identifying 3D pharmacophores is relatively easy for rigid cyclic structures, such as the hypothetical glipine. With more flexible structures, it is not so straightforward because the molecule can adopt a large number of shapes or conformations which place the important binding groups in different positions relative to each other. Normally, only one of these conformations is recognized and bound by the binding site. This conformation is known as the **active conformation**. In order to identify the 3D pharmacophore, it is necessary to know the active conformation. There are various ways in which this might be done. Rigid analogues of the flexible compound could be synthesized and tested to see whether activity is retained (section 13.3.9). Alternatively, it may be possible to crystallize the target with the compound bound to the binding site. X-ray crystallography could then be used to identify the structure of the complex, as well as the active conformation of the bound ligand (section 17.10). Finally,

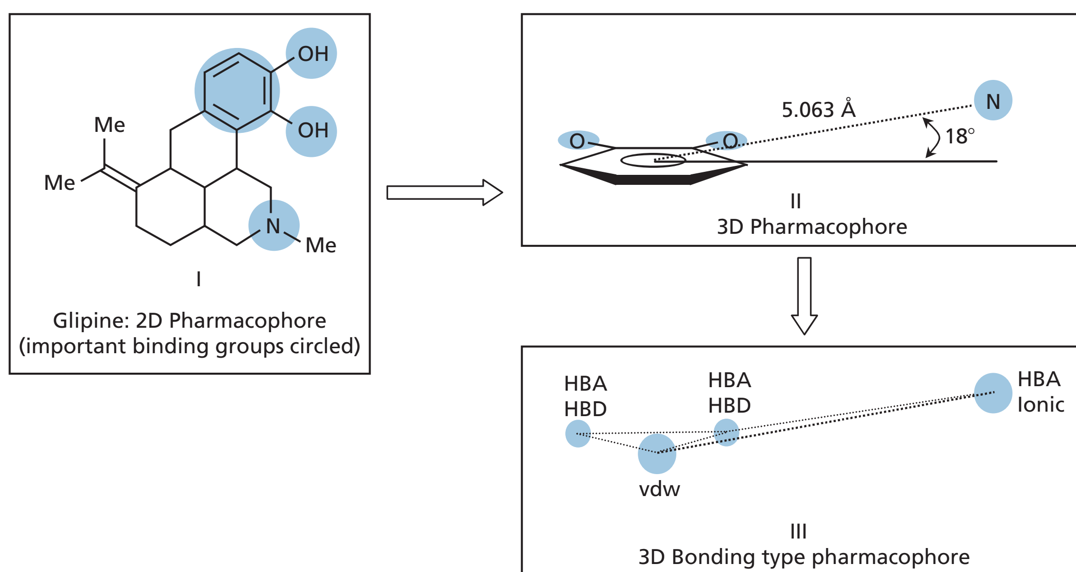


FIGURE 13.31 Pharmacophore for the fictitious structure glipine.

progress has been made in using NMR spectroscopy to solve the active conformation of isotopically labelled molecules bound to their binding sites.

We finish this section with a warning! A drawback with pharmacophores is their unavoidable emphasis on functional groups as the crucial binding groups. In many situations this is certainly true, but in other situations, it is not. It is not uncommon to find compounds that have the correct pharmacophore, but show disappointing activity and poor binding. It is important to realize that the overall skeleton of the molecule is involved in interactions with the binding site through van der Waals and hydrophobic interactions. The strength of these interactions can sometimes be crucial in whether a drug binds effectively or not, and the 3D pharmacophore does not take this into account. The pharmacophore also does not take into account the size of a molecule and whether it will fit the binding site. Finally, a functional group that is part of the pharmacophore may not be so crucial if an agent can form an alternative binding interaction with the binding site. For example, the phenol group is an important part of the analgesic pharmacophore for **morphine** and closely related analogues, but is less important for analgesics such as the **oripavines**. Other analgesics, such as **pethidine** and **methadone**, lack the phenol group entirely (Chapter 24).

KEY POINTS

- SARs define the functional groups or regions of a lead compound which are important to its biological activity.
- Functional groups, such as alcohols, amines, esters, amides, carboxylic acids, phenols, and ketones, can interact with binding sites by means of hydrogen bonding.
- Functional groups, such as aminium ions, quaternary ammonium salts, and carboxylate groups, can interact with binding sites by ionic bonding.
- Functional groups, such as alkenes and aromatic rings, can interact with binding sites by means of van der Waals interactions.
- Alkyl substituents and the carbon skeleton of the lead compound can interact with hydrophobic regions of binding sites by means of van der Waals interactions.
- Interactions involving dipole moments or induced dipole moments may play a role in binding a lead compound to a binding site.
- Reactive functional groups, such as alkyl halides, may result in irreversible covalent bonds being formed between a lead compound and its target.
- The relevance of a functional group to binding can be determined by preparing analogues where the functional group is modified or removed in order to see whether activity is affected by such a change.
- Some functional groups can be important to the activity of a lead compound for reasons other than target binding. They may play a role in the electronic or stereochemical properties of the compound, or they may have an important pharmacokinetic role.
- Replacing a group in the lead compound with an isostere (a group having the same valency) makes it easier to determine whether a particular property, such as hydrogen bonding, is important.
- *In vitro* testing procedures should be used to determine the SAR for target binding.
- The pharmacophore summarizes the groups which are important in binding a lead compound to its target, as well as their relative positions in three dimensions.

13.3 Drug optimization: strategies in drug design

Once the important binding groups and pharmacophore of the lead compound have been identified, it is possible to synthesize analogues that contain the same pharmacophore. But why is this necessary? If the lead compound has useful biological activity, why bother making analogues? The answer is that very few lead compounds are ideal. Most are likely to have low activity, poor selectivity, and significant side effects. They may also be difficult to synthesize, so there is an advantage in finding analogues with improved properties. We look now at strategies that can be used to optimize the interactions of a drug with its target in order to gain better activity and selectivity.

13.3.1 Variation of substituents

Varying easily accessible substituents is a common method of fine tuning the binding interactions of a drug.

13.3.1.1 Alkyl substituents

Certain alkyl substituents can be varied more easily than others. For example, the alkyl substituents of ethers, amines, esters, and amides are easily varied as shown in Fig. 13.32. In these cases, the alkyl substituent already present can be removed and replaced by another substituent. Alkyl substituents which are part of the carbon skeleton of the molecule are not easily removed, and it is usually necessary to carry out a full synthesis in order to vary them.

If alkyl groups are interacting with a hydrophobic pocket in the binding site, then varying the length and bulk of the alkyl group (e.g. methyl, ethyl, propyl, butyl,

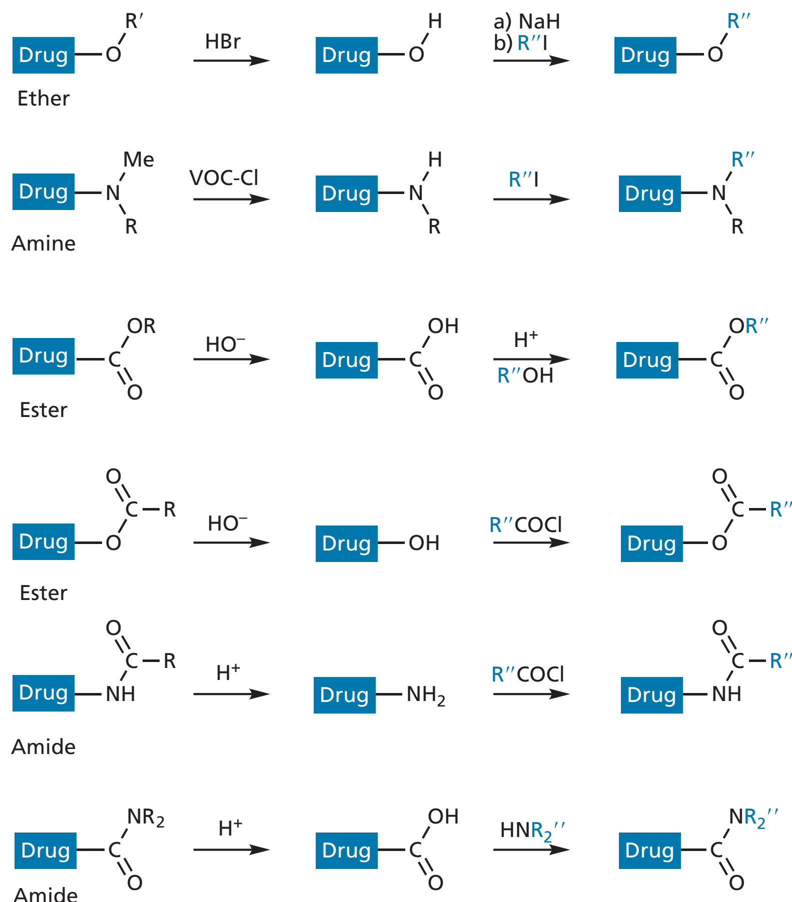


FIGURE 13.32 Methods of modifying an alkyl group.

isopropyl, isobutyl, or *t*-butyl) allows one to probe the depth and width of the pocket. Choosing a substituent that will fill the pocket will then increase the binding interaction (Fig. 13.33).

Larger alkyl groups may also confer selectivity on the drug. For example, in the case of a compound that interacts with two different receptors, a bulkier alkyl substituent may prevent the drug from binding to one of those receptors and so cut down side effects (Fig. 13.34). For example, **isoprenaline** is an analogue of **adrenaline** where a methyl group was replaced by an isopropyl group, resulting in selectivity for adrenergic β-receptors over adrenergic α-receptors (section 23.11.3).

13.3.1.2 Aromatic substituents

If a drug contains an aromatic ring, the position of substituents can be varied to find better binding interactions, resulting in increased activity (Fig. 13.35).

For example, the best anti-arrythmic activity for a series of benzopyrans was found when the sulphonamide substituent was at position 7 of the aromatic ring (Fig. 13.36).

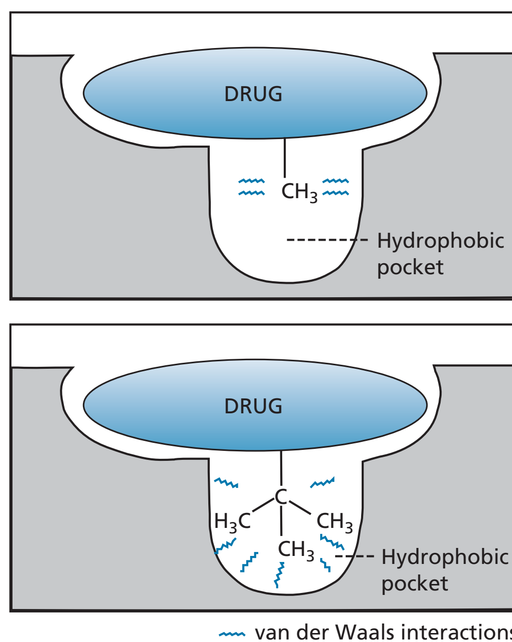


FIGURE 13.33 Variation of alkyl chain to fill a hydrophobic pocket.

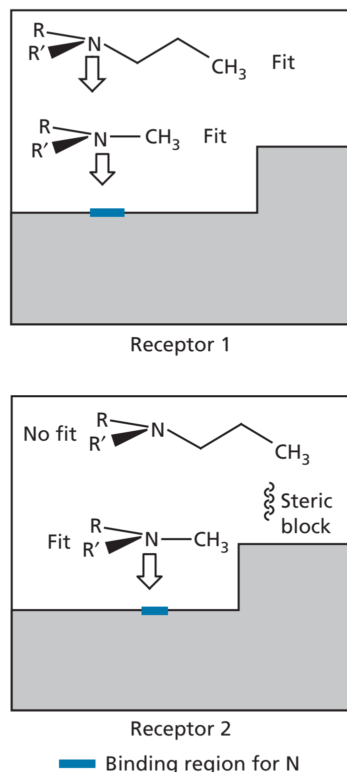


FIGURE 13.34 Use of a larger alkyl group to confer selectivity on a drug.

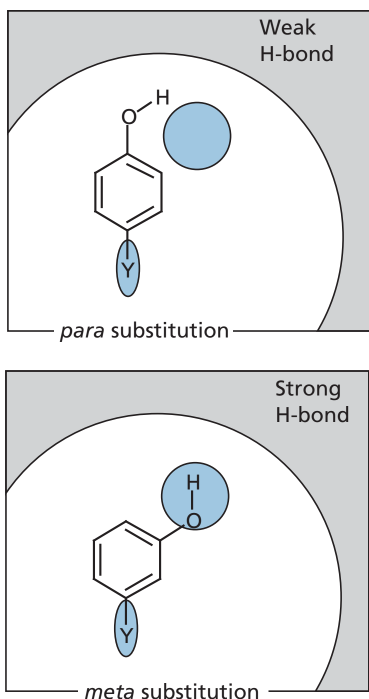


FIGURE 13.35 Varying the position of an aromatic substituent.

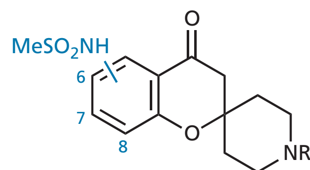


FIGURE 13.36 Benzopyrans.

Changing the position of one substituent may have an important effect on another. For example, an electron-withdrawing nitro group will affect the basicity of an aromatic amine more significantly if it is at the *para* position rather than the *meta* position (Fig. 13.37). At the *para* position, the nitro group will make the amine a weaker base and less liable to protonate. This would decrease the amine's ability to interact with ionic binding groups in the binding site, and decrease activity.

If the substitution pattern is ideal, then we can try varying the substituents themselves. Substituents have different steric, hydrophobic, and electronic properties, and so varying these properties may have an effect on binding and activity. For example, activity might be improved by having a more electron-withdrawing substituent, in which case a chloro substituent might be tried in place of a methyl substituent.

The chemistry involved in these procedures is usually straightforward, so these analogues are made as a matter of course whenever a novel drug structure is developed. Furthermore, the variation of aromatic or aliphatic substituents is open to **quantitative structure–activity studies** (QSARs), as described in chapter 18.

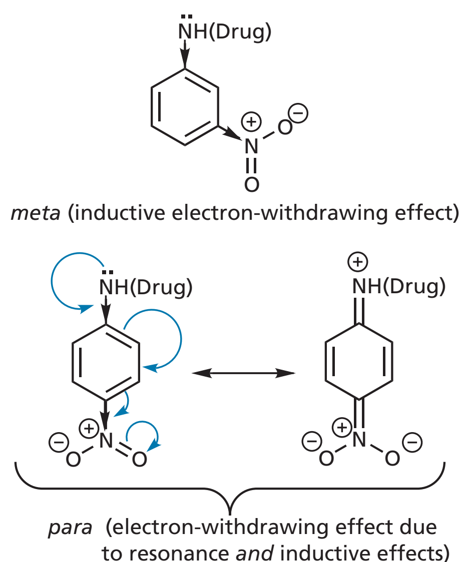


FIGURE 13.37 Electronic effects of different aromatic substitution patterns.

13.3.1.3 Synergistic effects

Finally, a warning! When varying substituents, it is normal to study analogues where only one substituent is added or altered at a time. In that way, one can identify those substituents that are good for activity and those that are not. However, it does not take into account the synergistic effect that two or more substituents may have on activity. For example, two substituents that are individually bad for activity may actually be beneficial for activity when they are both present. The design of the anticancer drug **sorafenib** provides an illustration of this effect (Box 21.10).

13.3.2 Extension of the structure

The strategy of extension involves the addition of another functional group or substituent to the lead compound in order to probe for extra binding interactions with the target. Lead compounds are capable of fitting the binding site and have the necessary functional groups to interact with some of the important binding regions present. However, it is possible that they do not interact with all the binding regions available. For example, a lead compound may bind to three binding regions in the binding site but fail to use a fourth (Fig. 13.38). Therefore, why not add extra functional groups to probe for that fourth region?

Extension tactics are often used to find extra hydrophobic regions in a binding site by adding various alkyl or arylalkyl groups. These groups can be added to functional groups, such as alcohols, phenols, amines, and carboxylic acids should they be present in the drug, as long as this does not disrupt important binding interactions that are already present. Alternatively, they could be built into the building blocks used in the synthesis of various analogues. By the same token, substituents containing polar functional groups could be added to probe for extra hydrogen bonding or ionic interactions. A good example of the use of extension tactics to increase binding interactions involves the design of the ACE inhibitor **enalaprilate** from the lead compound **succinyl proline** (see Case study 2, Figs. CS2.8 and 2.9).

Extension strategies are used to strengthen the binding interactions and activity of a receptor agonist or an enzyme inhibitor, but they can also be used to convert an agonist into an antagonist. This will happen if the extra binding interaction results in a different induced fit from that required to activate the receptor. As a result, the antagonist binds to an inactive conformation of the receptor and blocks access to the endogenous agonist. The strategy has also been used to alter an enzyme substrate into an inhibitor (Box 13.1).

The extension tactic has been used successfully to produce more active analogues of morphine (sections 24.6.2 and 24.6.4) and more active adrenergic agents (sections 23.9–23.11). It was also used to improve the activity and selectivity of the protein kinase inhibitor, **imatinib** (section 21.6.2.2). Other examples of the extension strategy can be found in Case studies 2, 5–7, and Box 17.6, as well as sections 19.7.7, 20.7.4, and 21.7.1.

An unusual example of an extension strategy is where a substituent was added to an enzyme substrate such that extra binding interactions took place with a neighbouring cofactor in the binding site. This resulted in the analogue acting as an inhibitor, rather than a substrate (Box 13.1).

13.3.3 Chain extension/contraction

Some drugs have two important binding groups linked together by a chain, in which case it is possible that the chain length is not ideal for the best interaction. Therefore, shortening or lengthening the chain length is a useful tactic to try (Fig. 13.39; see also Box 13.1, section 24.6.2, and Case study 2).

13.3.4 Ring expansion/contraction

If a drug has one or more rings that are important to binding, it is generally worth synthesizing analogues where one of these rings is expanded or contracted.

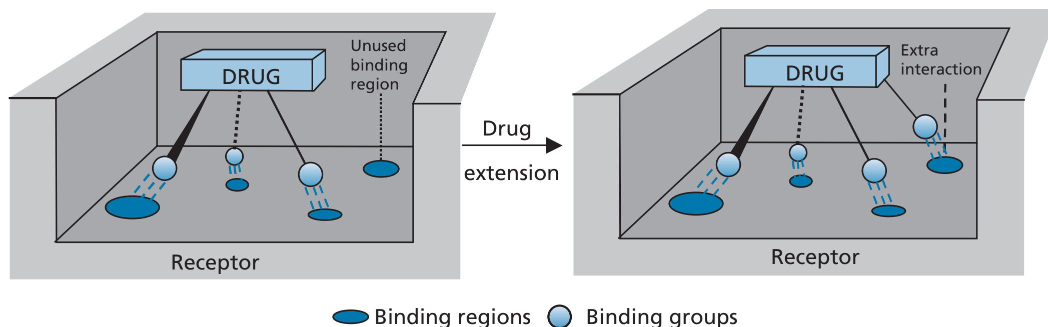
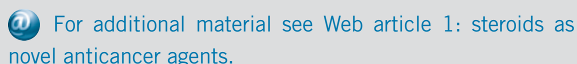


FIGURE 13.38 Extension of a drug to provide a fourth binding group.

BOX 13.1 Converting an enzyme substrate to an inhibitor by extension tactics

The enzyme **17β-hydroxysteroid dehydrogenase type 1** catalyses the conversion of **estrone** to the female steroid hormone **estradiol**, with the cofactor **NADH** acting as the reducing agent for the reaction (Fv. 1; see also Chapter 3, Figs. 3.10 and 3.11). Inhibition of this enzyme may prove useful in the treatment of estradiol-dependent tumours as the levels of estradiol present in the body would be lowered.

The cofactor NADH is bound next to estrone in the active site, and so it was reasoned that a direct bonding interaction between an estrone analogue and NADH would lock the analogue into the active site and block access to estrone itself. Therefore, the analogue would act as an enzyme inhibitor. Various substituents were added at position 16 to achieve this goal as crystallographic and molecular modelling studies had shown that such substituents would be ideally placed for an interaction with the cofactor. This led to a structure (Fig. 2) which showed promising activity as an inhibitor. The amide group interacts with the primary amide NADH by hydrogen bonding, while the pyridine ring interacts with the phosphate groups of the cofactor. A more conventional extension strategy was to add an ethyl group at C-2, which allowed additional van der Waals interactions with a small hydrophobic pocket in the active site. It was also observed that two protons acted as steric blockers and prevented NADH reducing the ketone group of the analogue.


For additional material see Web article 1: steroids as novel anticancer agents.

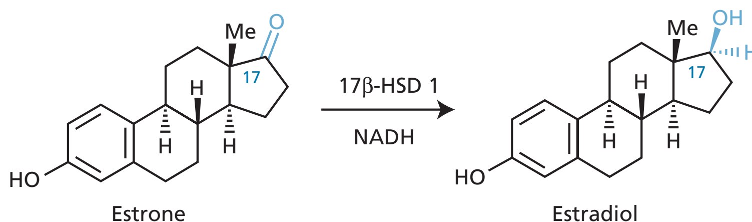


FIGURE 1 The enzyme-catalysed conversion of estrone to estradiol.

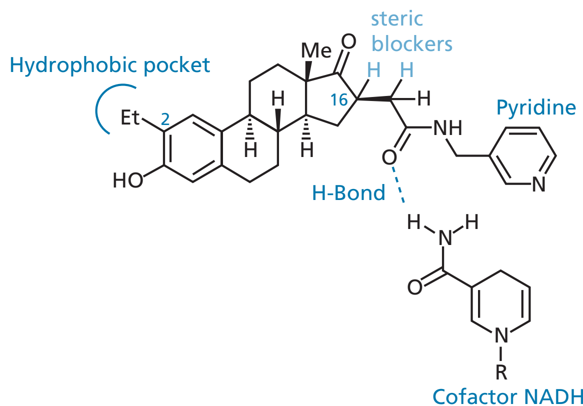


FIGURE 2 Extra binding interactions resulting from the extension strategy.

The principle behind this approach is much the same as varying the substitution pattern of an aromatic ring. Expanding or contracting a ring may put other rings in different positions relative to each other, and may lead to better interactions with specific regions in the binding site (Fig. 13.40).

Varying the size of a ring can also bring substituents into a good position for binding. For example, during the

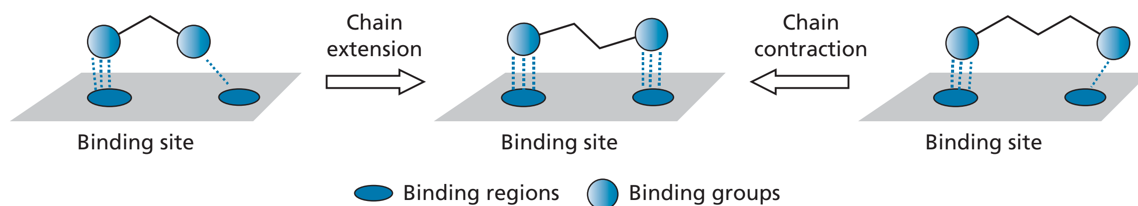


FIGURE 13.39 Chain contraction and chain extension.

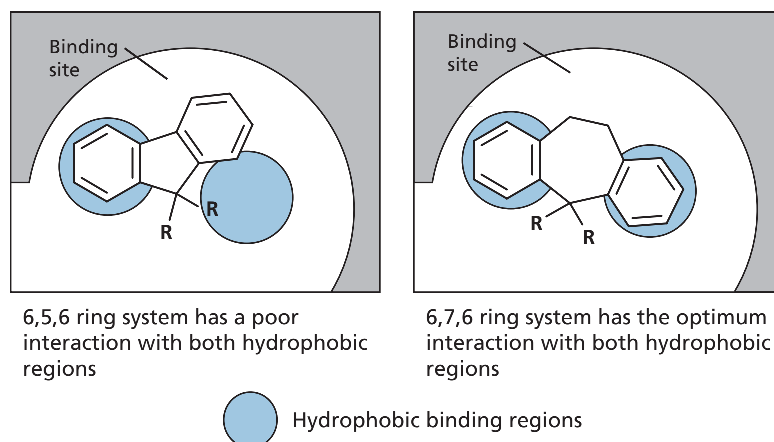


FIGURE 13.40 Ring expansion.

development of the anti-hypertensive agent **cilazaprilat** (another ACE inhibitor), the bicyclic structure I showed promising activity (Fig. 13.41). The important binding groups were the two carboxylate groups and the amide group. By carrying out various **ring contractions and expansions**, cilazaprilat was identified as the structure having the best interaction with the binding site.

13.3.5 Ring variations

A popular strategy used for compounds containing an aromatic or heteroaromatic ring is to replace the original ring with a range of other heteroaromatic rings of different ring size and heteroatom positions. For example, several non-steroidal anti-inflammatory agents (NSAIDs) have been reported, all consisting of a central ring with 1,2-biaryl substitution. Different pharmaceutical companies have varied the central ring to produce a range of active compounds (Fig. 13.42).

Admittedly, a lot of these changes are merely ways of avoiding patent restrictions (**'me too' drugs**), but there can often be significant improvements in activity, as well as increased selectivity and reduced side effects (**'me-better' drugs**). For example, the antifungal agent (I) (Fig. 13.43) acts against an enzyme present in both fungal and human cells. Replacing the imidazole ring of structure (I) with a 1,2,4-triazole ring to give UK 46245 resulted in better selectivity against the fungal form of the enzyme.

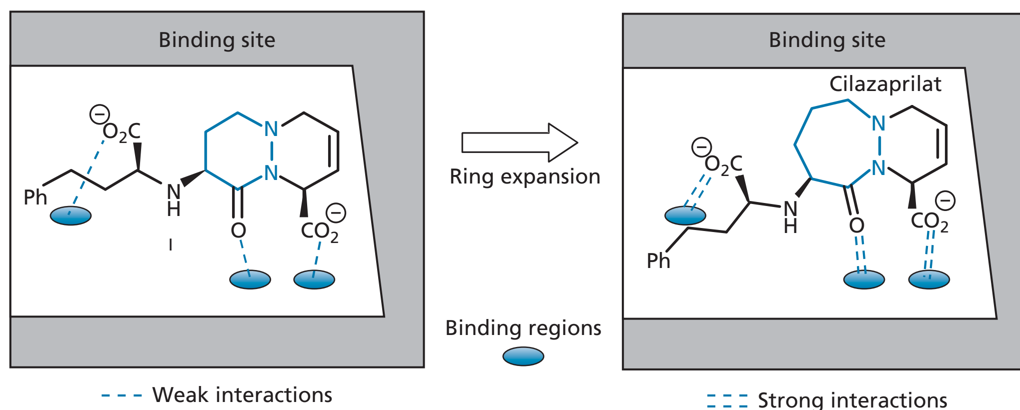


FIGURE 13.41 Development of cilazaprilat.

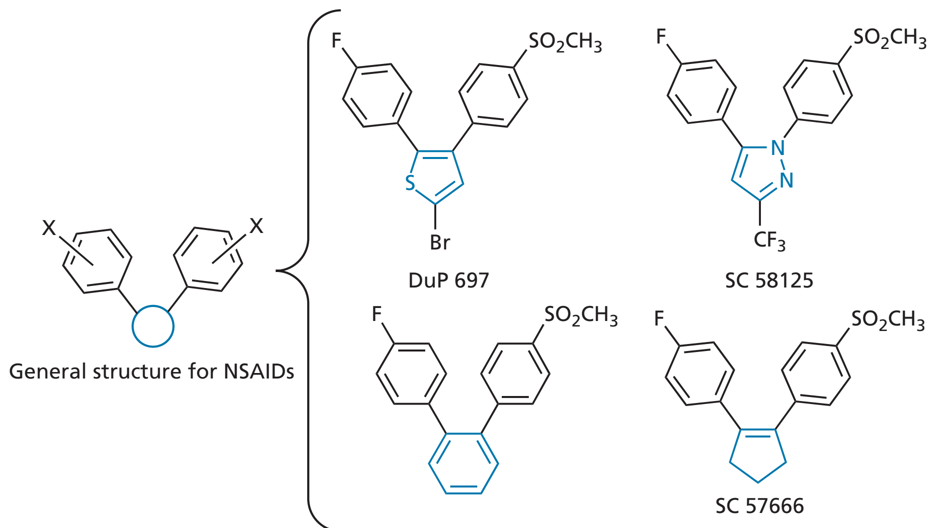


FIGURE 13.42 Non-steroidal anti-inflammatory drugs (NSAIDS).

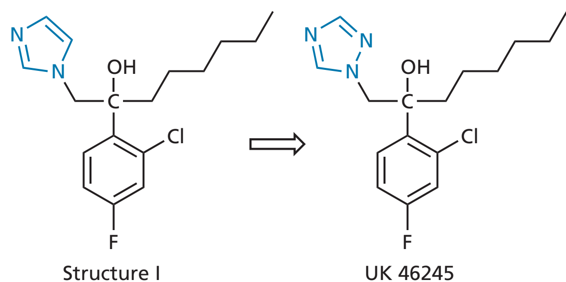


FIGURE 13.43 Development of UK 46245.

One advantage of altering an aromatic ring to a heteroaromatic ring is that it introduces the possibility of an extra hydrogen bonding interaction with the binding site, should a suitable binding region be available (*extension strategy*). For example, structure I (Fig. 13.44) was the lead compound for a project looking into novel antiviral agents. Replacing the aromatic ring with a pyridine ring resulted in an additional binding interaction with the target enzyme. Further development led eventually to the antiviral agent **nevirapine** (Fig. 13.44).

13.3.6 Ring fusions

Extending a ring by **ring fusion** can sometimes result in increased interactions or increased selectivity. One of the major advances in the development of the selective β-blockers was the replacement of the aromatic ring in **adrenaline** with a naphthalene ring system (**pronethalol**) (Fig. 13.45). This resulted in a compound that was able to distinguish between two very similar receptors—the α- and β-receptors for adrenaline. One possible explanation for this could be that the β-receptor has a larger van der Waals binding area for the aromatic system than the α-receptor, and can interact more strongly with pronethalol than with adrenaline. Another possible explanation is that the naphthalene ring system is sterically too big for the α-receptor, but is just right for the β-receptor.

13.3.7 Isosteres and bioisosteres

Isosteres (section 13.1.15) have often been used in drug design to vary the character of the molecule in a rational

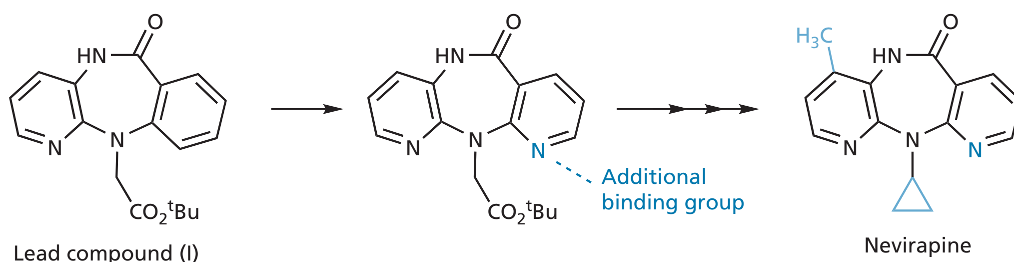


FIGURE 13.44 Development of nevirapine.

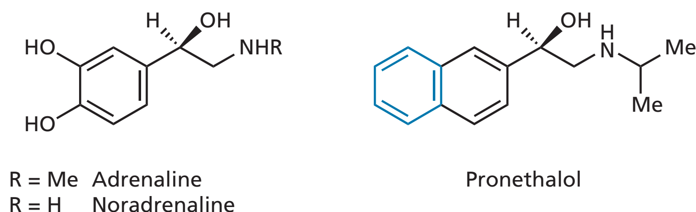


FIGURE 13.45 Structures of adrenaline, noradrenaline, and pronethalol.

way with respect to features such as size, polarity, electronic distribution, and bonding. Some isosteres can be used to determine the importance of size towards activity, whereas others can be used to determine the importance of electronic factors. For example, fluorine is often used as an isostere of hydrogen as it is virtually the same size. However, it is more electronegative and can be used to vary the electronic properties of the drug without having any steric effect.

The presence of fluorine in place of an enzymatically labile hydrogen can also disrupt an enzymatic reaction, as C–F bonds are not easily broken. For example, the antitumour drug **5-fluorouracil** described in section 21.3.2 is accepted by its target enzyme because it appears little different from the normal substrate—**uracil**. However, the mechanism of the enzyme-catalysed reaction is totally disrupted, as the fluorine has replaced a hydrogen which is normally lost during the enzyme mechanism.

Several non-classical isosteres have been used in drug design as replacements for particular functional groups. Non-classical isosteres are groups that do not obey the steric and electronic rules used to define classical isosteres, but which have similar physical and chemical properties. For example, the structures shown in Fig. 13.46 are non-classical isosteres for a thiourea group. They are all planar groups of similar size and basicity.

The term **bioisostere** is used in drug design and includes both classical and non-classical isosteres. A bioisostere is a group that can be used to replace another group while retaining the desired biological activity. For example, a cyclopropyl group has been used as a bioisostere for an alkene group in prodrugs (section 14.6.1.1) and opioid antagonists (section 24.6.2). Bioisosteres are often used to replace a functional group that is important for target binding, but is problematic in one way or another. For example, the thiourea group was present as an important binding group in early histamine antagonists, but was responsible for toxic side effects. Replacing it with bioisosteres allowed the important binding interactions to be retained for histamine antagonism but avoided the toxicity problems (section 25.2.6). Further examples of the use of bioisosteres are given in sections 14.1.6, 14.2.2, and 20.7.4. It is important to realize that bioisosteres are specific for a particular group of compounds and their target. Replacing a functional group with a bioisostere is not guaranteed to retain activity for every drug at every target.

As stated above, bioisosteres are commonly used in drug design to replace a problematic group while retaining activity. In some situations, the use of a bioisostere can actually increase target interactions and/or selectivity. For example, a pyrrole ring has frequently been used as a bioisostere for an amide. Carrying out this replacement on the dopamine antagonist **sultopride** led to increased activity and selectivity towards the dopamine D_3-receptor over the dopamine D_2-receptor (Fig. 13.47). Such agents show promise as antipsychotic agents that lack the side effects associated with the D_2-receptor.

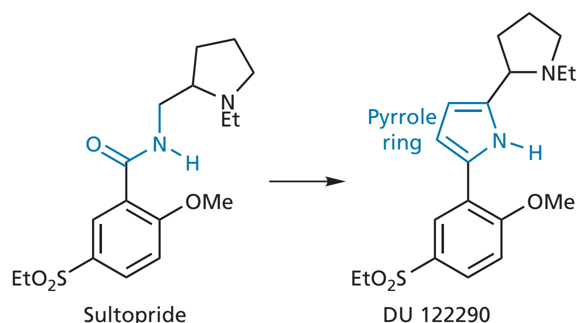


FIGURE 13.47 Introducing a pyrrole ring as a bioisostere for an amide group.

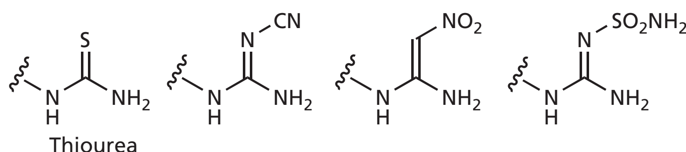


FIGURE 13.46 Non-classical isosteres for a thiourea group.

Introducing a bioisostere to replace a problematic group often involves introducing further functional groups that might form extra binding interactions with the target binding site (*Extension*, section 13.3.2). For example, a 10-fold increase in activity was observed for an antiviral agent when an *N*-acylsulphonamide was used as a bioisostere for a carboxylic acid (Fig. 13.48). The *N*-acylsulphonamide group introduces the possibility of further hydrogen bonding or van der Waals interactions with the binding site.

Transition-state isosteres are a special type of isostere used in the design of transition-state analogues. These are drugs that are used to inhibit enzymes (section 7.4). During an enzymatic reaction, a substrate goes through a transition state before it becomes product. It is proposed that the transition state is bound more strongly than either the substrate or the product, so it makes sense to design drugs based on the structure of the transition state rather than the structure of the substrate or the product. However, the transition state is inherently unstable and so transition-state isosteres are moieties that are used to mimic the crucial features of the transition state, but which are stable to the enzyme-catalysed reaction. For example, the transition state of an amide hydrolysis is thought to resemble the tetrahedral reaction intermediate shown in Fig. 13.49. This is a geminal diol, which is inherently unstable. The hydroxethylene moiety shown is a transition-state isostere because it shares the same tetrahedral geometry, retains one of the hydroxyl groups, and is stable to hydrolysis. Further examples of the use of transition-state isosteres are given in sections 20.7.4, 20.8.3, and 21.3.4, and Case studies 1 and 2.

13.3.8 Simplification of the structure

Simplification is a strategy which is commonly used on the often complex lead compounds arising from natural sources (see Box 13.2). Once the essential groups of such a drug have been identified by SAR, it is often possible to discard the non-essential parts of the structure without losing activity. Consideration is given to removing functional groups which are not part of the pharmacophore, simplifying the carbon skeleton (for example removing rings), and removing asymmetric centres.

This strategy is best carried out in small stages. For example, consider our hypothetical natural product glipine (Fig. 13.50). The essential groups have been highlighted and we might aim to synthesize simplified compounds in the order shown. These still retain the essential groups making up the pharmacophore.

Chiral drugs pose a particular problem. The easiest and cheapest method of synthesizing a chiral drug is to make the racemate. However, both enantiomers then have to be tested for their activity and side effects, doubling the number of tests that have to be carried out. This is because different enantiomers can have different activities. For example, compound **UH-301** (Fig. 13.51) is inactive as a racemate, whereas its enantiomers have opposing agonist and antagonist activity at the serotonin receptor (5-HT_{1A}). Another notorious example is **thalidomide**, where one of the enantiomers is teratogenic (section 21.8.1).

The use of racemates is discouraged and it is preferable to use a pure enantiomer. This could be obtained by separating the enantiomers of the racemic drug or carrying

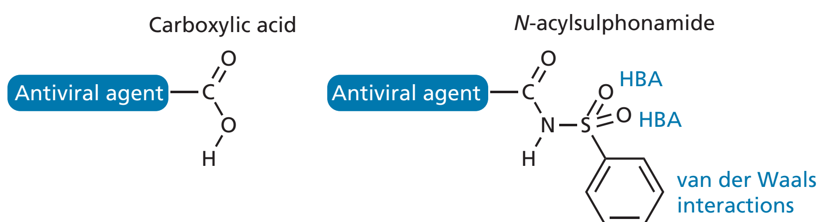


FIGURE 13.48 Extra binding interactions that might be possible when using an *N*-acylsulphonamide as a bioisostere for a carboxylic acid.

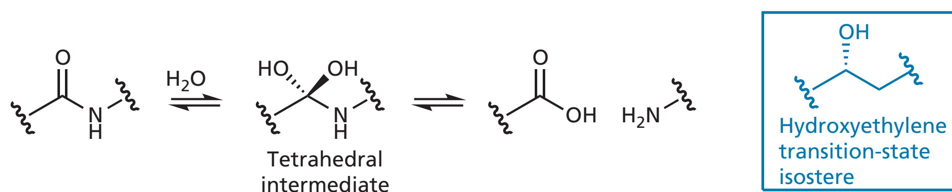


FIGURE 13.49 Example of a transition-state isostere designed to resemble a tetrahedral intermediate formed during an enzyme-catalysed reaction. The transition state is believed to resemble the tetrahedral intermediate.

BOX 13.2 Simplification

Simplification tactics have been used successfully with the alkaloid **cocaine**. Cocaine has local anaesthetic properties and its simplification led to the development of local anaesthetics which could be easily synthesized in the laboratory. One of the earliest was **procaine** (**Novocaine**), discovered in 1909 (Fig. 1). Simplification tactics have also proved effective in the design of simpler morphine analogues (section 24.6.3).

Simplification tactics were also used in the development of **devazepide** from the microbial metabolite **asperlicin**. The benzodiazepine and indole skeletons inherent in asperlicin are important to activity and have been retained. Both asperlicin and devazepide act as antagonists of a neuropeptide chemical messenger called **cholecystokinin** (CCK), which has been implicated in causing panic attacks. Therefore, antagonists may be of use in treating such attacks.

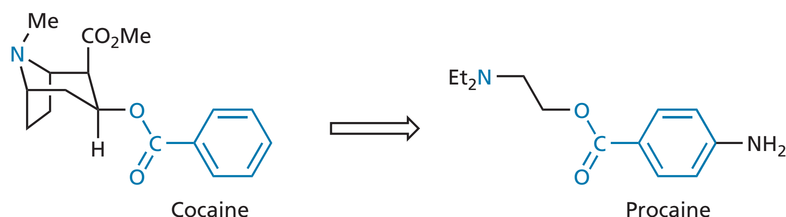


FIGURE 1 Simplification of cocaine (pharmacophore shown in colour).

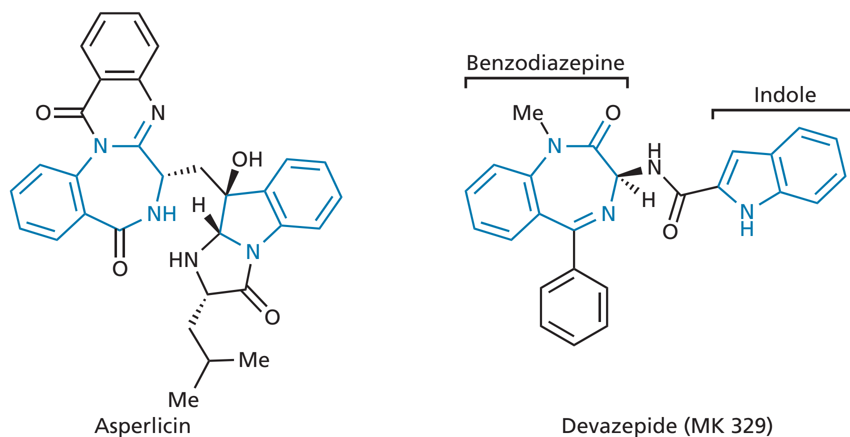


FIGURE 2 Simplification of asperlicin.

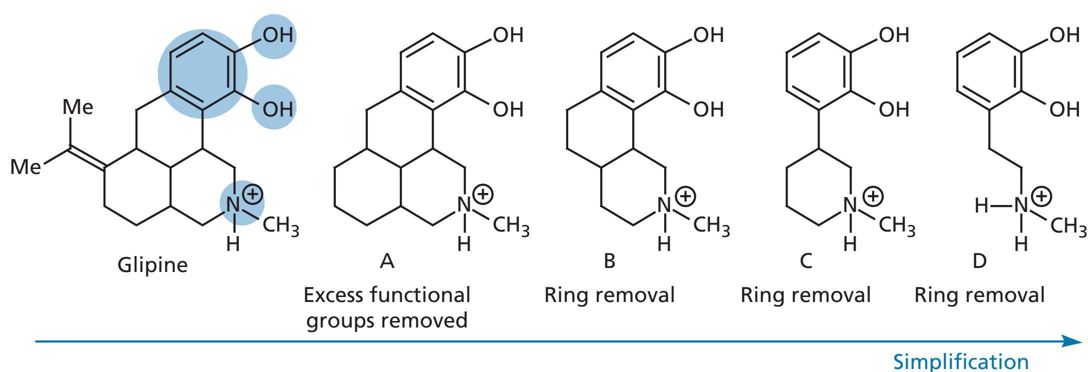


FIGURE 13.50 Glipine analogues.

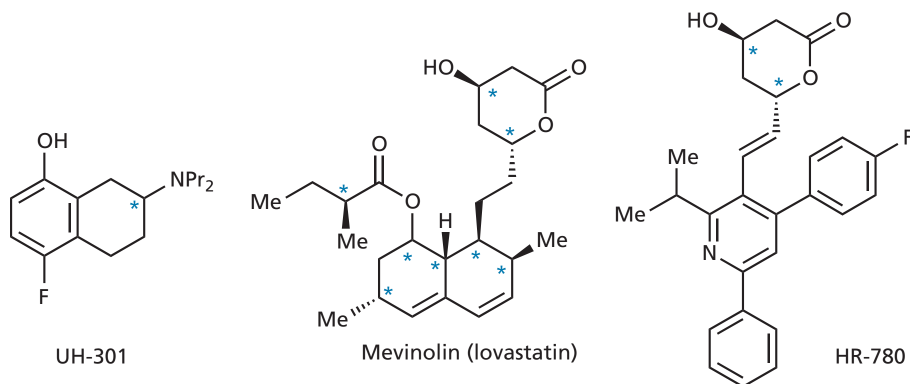


FIGURE 13.51 UH-301, mevinolin, and HR 780.

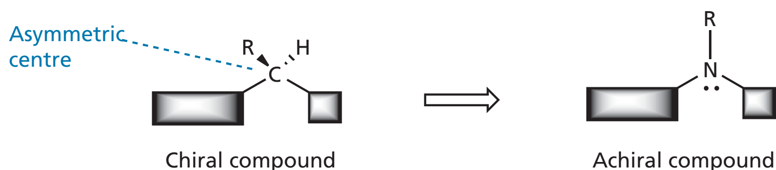


FIGURE 13.52 Replacing an asymmetric carbon with nitrogen.

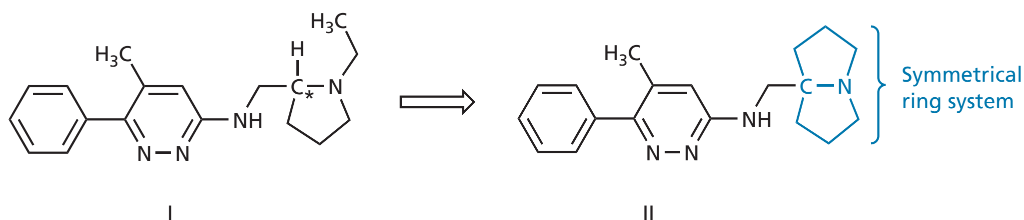


FIGURE 13.53 Introducing symmetry.

out an asymmetric synthesis. Both options inevitably add to the cost of the synthesis and so designing a structure that lacks some, or all, of the asymmetric centres can be advantageous and represents a simplification of the structure. For example, the cholesterol-lowering agent **mevinolin** has eight asymmetric centres, but a second generation of cholesterol-lowering agents has been developed which contain far fewer (e.g. **HR 780**; Fig. 13.51; see also Case study 1).

Various tactics can be used to remove asymmetric carbon centres. For example, replacing the carbon centre with nitrogen has been effective in many cases (Fig. 13.52). An illustration of this can be seen in the design of thymidylate synthase inhibitors described in Case study 5. However, it should be noted that the introduction of an amine in this way may well have significant effects on the pharmacokinetics of the drug in terms of log*P*, basicity, polarity, etc. (see Chapters 11 and 14).

Another tactic is to introduce symmetry where originally there was none. For example, the muscarinic agonist (II) was developed from (I) in order to remove asymmetry. Both structures have the same activity.

Simplification strategies have been applied extensively in many areas of medicinal chemistry, some of which are described in this text, for example antiprotozoal agents (Case studies 3 and 4), local anaesthetics (section 17.9), antibacterial agents (section 19.5.5.2), antiviral agents (section 20.7.4.8), anticancer agents (sections 21.2.1, 21.2.3.3, and 21.5.2), muscarinic antagonists (section 22.9.2.2), and opioids (section 24.6.3). The advantage of simpler structures is that they are easier, quicker, and cheaper to synthesize in the laboratory. Usually, the complex lead compounds obtained from natural sources are impractical to synthesize and have to be extracted from the source material—a slow, tedious, and expensive business. Removing unnecessary functional groups can also be advantageous in removing side effects if these groups interact with other targets or are chemically reactive. There are, however, potential disadvantages in oversimplifying molecules. Simpler molecules are often more flexible and can sometimes bind differently to their targets compared with the original lead compound, resulting in different effects. It is best to simplify in small stages, checking that the desired activity is retained at

each stage. Oversimplification may also result in reduced activity, reduced selectivity, and increased side effects. We shall see why in the next section (section 13.3.9).

13.3.9 Rigidification of the structure

Rigidification has often been used to increase the activity of a drug or to reduce its side effects. In order to understand why this tactic can work, let us consider again our hypothetical neurotransmitter from Chapter 5 (Fig. 13.54). This is quite a simple, flexible molecule with several rotatable bonds that can lead to a large number of conformations or shapes. One of these conformations is recognized by the receptor and is known as the **active conformation**. The other conformations are unable to interact efficiently with the receptor and are inactive conformations. However, it is possible that a different receptor exists which *is* capable of binding one of these alternative conformations. If this is the case, then our model neurotransmitter could switch on two different receptors and give two different biological responses, one which is desired and one which is not.

The body's own neurotransmitters are highly flexible molecules (section 4.2), but, fortunately, the body is efficient at releasing them close to their target receptors, then quickly inactivating them so that they do not make the journey to other receptors. This is not the case for drugs. They have to be sturdy enough to travel throughout the body and will interact with all the receptors that are prepared to accept them. The more flexible a drug molecule is, the more likely it will interact with more than one receptor and produce other biological responses (side effects). Too much flexibility is also bad for oral bioavailability (section 11.3).

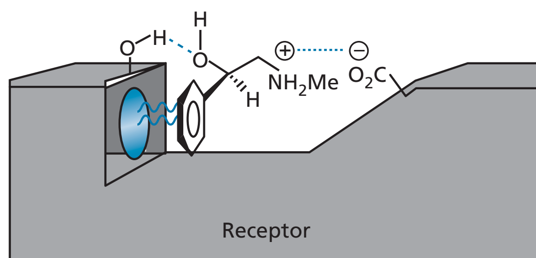


FIGURE 13.54 Active conformation of a hypothetical neurotransmitter.

The strategy of rigidification is to make the molecule more rigid, such that the active conformation is retained and the number of other possible conformations is decreased. This should reduce the possibility of other receptor interactions and side effects. This same strategy should also increase activity. By making the drug more rigid, it is more likely to be in the active conformation when it approaches the target binding site and should bind more readily. This is also important when it comes to the thermodynamics of binding. A flexible molecule has to adopt a single active conformation in order to bind to its target, which means that it has to become more ordered. This results in a decrease in entropy and, as the free energy of binding is related to entropy by the equation $\Delta G = \Delta H - T\Delta S$, any decrease in entropy will adversely affect ΔG. In turn, this lowers the binding affinity (K_i), which is related to ΔG by the equation $\Delta G = -RT\ln K_i$. A totally rigid molecule, however, is already in its active conformation and there is no loss of entropy involved in binding to the target. If the binding interactions (ΔH) are exactly the same as for the more flexible molecule, the rigid molecule will have the better overall binding affinity.

Incorporating the skeleton of a flexible drug into a ring is the usual way of locking a conformation—for our model compound the analogue shown in Fig. 13.55 would be suitably rigid.

A ring was used to rigidify the acyclic pentapeptide shown in Fig. 13.56. This is a highly flexible molecule that acts as an inhibitor of a proteolytic enzyme. It was decided to rigidify the structure by linking the asparagine residue with the aromatic ring of the phenylalanine residue to form a macrocyclic ring. The resulting structure showed a 400-fold increase in activity.

Similar rigidification tactics have been useful in the development of the anti-hypertensive agent **cilazapril** (Fig. 12.12) from captopril, and the development of the

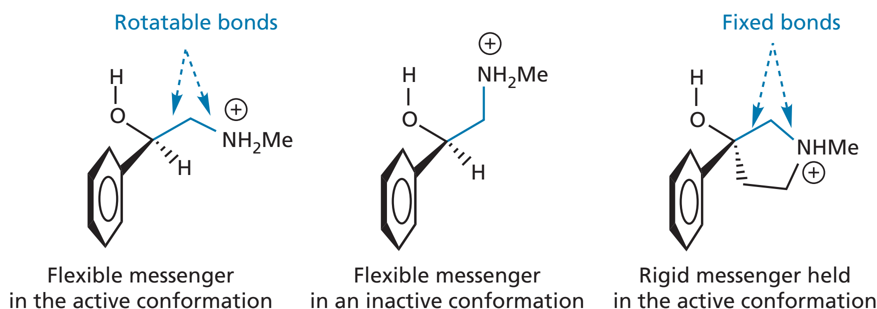


FIGURE 13.55 Rigidification of a molecule by locking rotatable bonds within a ring.

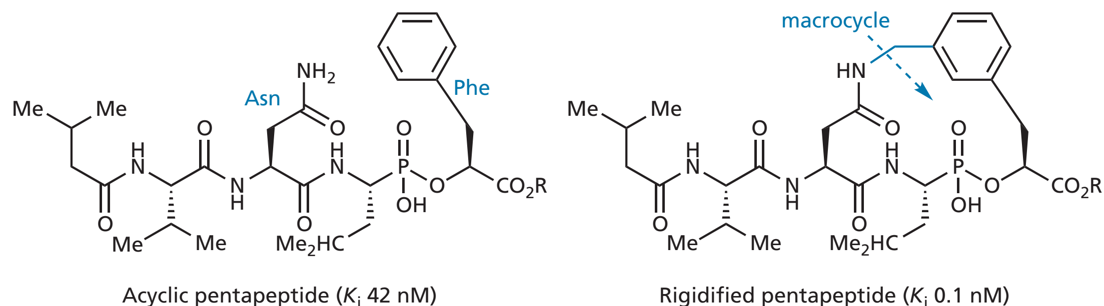


FIGURE 13.56 Rigidification of an acyclic pentapeptide.

sedative **etorphine** (section 24.6.4). Other examples of rigidification can be seen in sections 21.7.1 and 25.2.8.1.

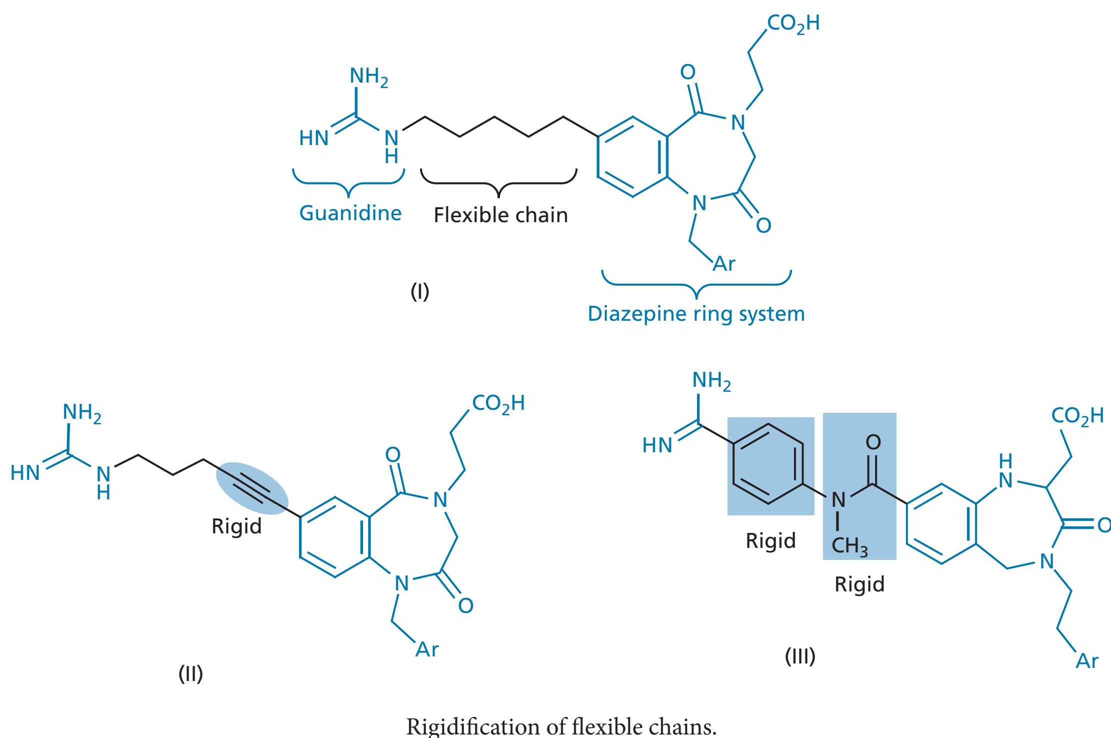
Locking a rotatable bond into a ring is not the only way a structure can be rigidified. A flexible side chain can be partially rigidified by incorporating a rigid functional group such as a double bond, alkyne, amide, or aromatic ring (see Box 13.3).

For additional material see Web article 5: the design of a serotonin antagonist as a possible anxiolytic agent.

Rigidification also has potential disadvantages. Rigidified structures may be more complicated to synthesize. There is also no guarantee that rigidification will retain the active conformation; it is perfectly possible that

BOX 13.3 Rigidification tactics in drug design

The diazepine (I) is an inhibitor of platelet aggregation, and binds to its target receptor by means of a guanidine functional group and a diazepine ring system. These binding groups are linked together by a highly flexible chain. Structures (II) and (III) are examples of active compounds where the connecting chain between the guanidine group and the bicyclic system has been partially rigidified by the introduction of rigid functional groups.



Rigidification of flexible chains.

rigidification will lock the compound into an inactive conformation. Another disadvantage involves drugs acting on targets which are prone to mutation. If a mutation alters the shape of the binding site, then the drug may no longer be able to bind, whereas a more flexible drug may adopt a different conformation that *could* bind.

13.3.10 Conformational blockers

We have seen how rigidification tactics can restrict the number of possible conformations for a compound. Another tactic that has the same effect is the use of **conformational blockers**. In certain situations, a quite simple substituent can hinder the free rotation of a single bond. For example, introducing a methyl substituent to the dopamine (D_3) antagonist (I in Fig. 13.57) gives structure II and results in a dramatic reduction in affinity. The explanation lies in a bad steric clash between the new methyl group and an *ortho* proton on the neighbouring ring which prevents both rings being in the same plane. Free rotation around the bond between the two rings is no longer possible and so the structure adopts a conformation where the two rings are at an angle to each other. In structure I, free rotation around the connecting bond allows the molecule to adopt a conformation where the aromatic rings are co-planar—the active conformation

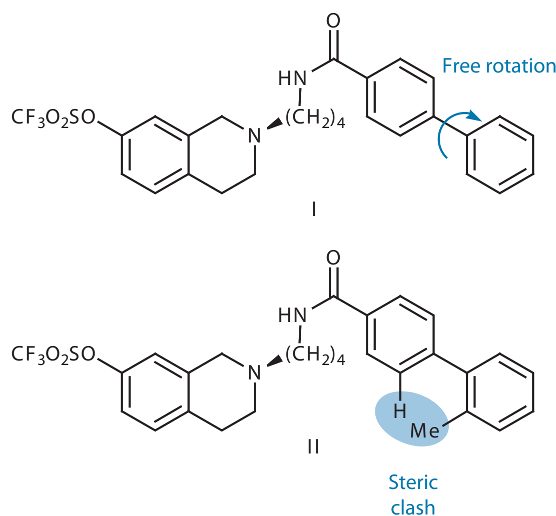


FIGURE 13.57 Introducing rigidity by conformational blocking.

for the receptor. In this case, a conformational blocker 'rejects' the active conformation. Examples of a conformational blocker favouring the active conformation can be seen with 4-methylhistamine (section 25.2.2.2), the design of a serotonin antagonist (see Web article 5), and the development of the anticancer agent imatinib (section 21.6.2.2). In the last case, conformational restraint not only increased activity, but also introduced selectivity between two similar target binding sites.

Rigidification is also possible through intramolecular hydrogen bonding, which may help to stabilize particular conformations (Fig. 13.58).

13.3.11 Structure-based drug design and molecular modelling

So far we have discussed the traditional strategies of drug design. These were frequently carried out with no knowledge of the target structure, and the results obtained were useful in providing information about the target binding site. Clearly, if a drug has an important binding group, there must be a complementary binding region present in the binding site of the receptor or enzyme.

If the macromolecular target can be isolated and crystallized, then it may be possible to determine the structure using X-ray crystallography. Unfortunately, this does not reveal where the binding site is, and so it is better to crystallize the protein with a known inhibitor or antagonist (ligand) bound to the binding site. X-ray crystallography can then be used to determine the structure of the complex and this can be downloaded to a computer. Molecular modelling software is then used to identify where the ligand is and thus identify the binding site. Moreover, by measuring the distances between the atoms of the ligand and neighbouring atoms in the binding site, it is possible to identify important binding interactions between the ligand and the binding site. Once this has been done, the ligand can be removed from the binding site *in silico* and novel lead compounds can be inserted *in silico* to see how well they fit. (The term *in silico* indicates that the virtual process concerned is being carried out on a computer using molecular modelling software.) Regions in the binding site which are not occupied by the lead compound can be identified and used to guide the medicinal chemist as to what modifications and additions can be made to design

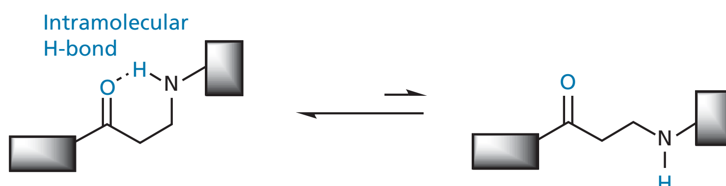
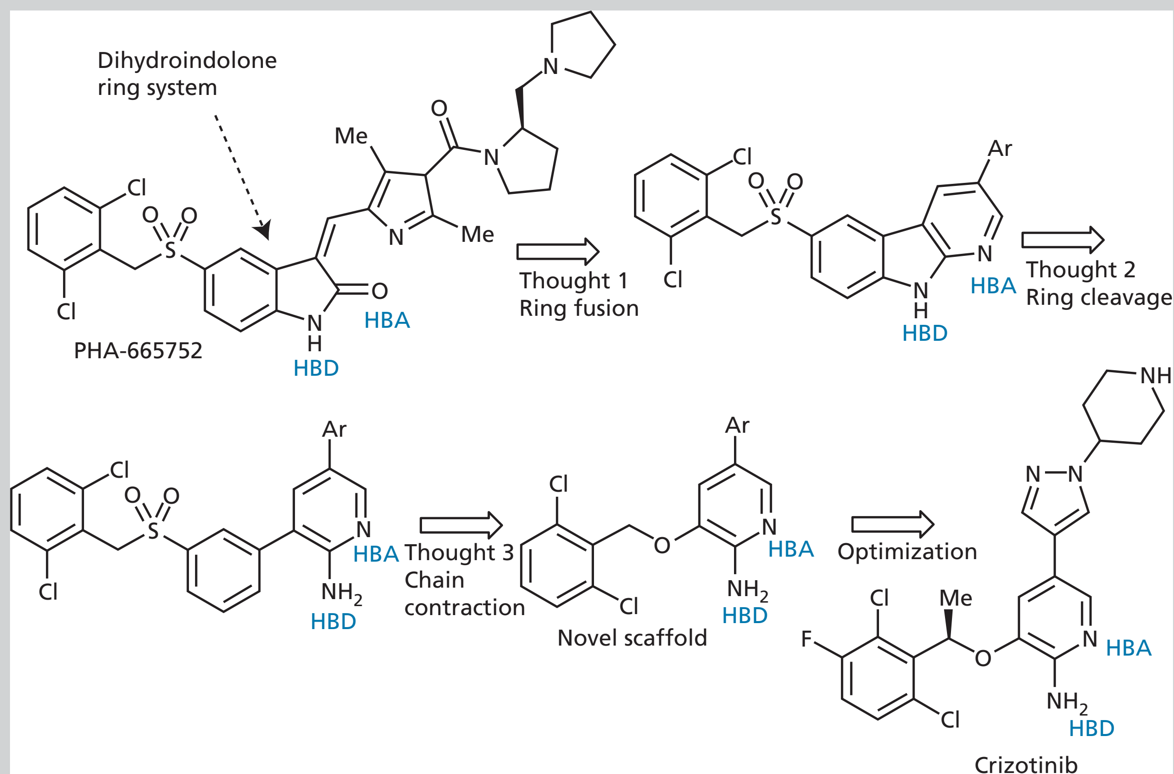


FIGURE 13.58 Rigidification involving an intramolecular hydrogen bond.

BOX 13.4 The structure-based drug design of crizotinib

Structure-based drug design is normally used to observe the binding interactions of a ligand and then to identify modifications that will result in better interactions and greater activity. This approach was used in the design of a recently approved anticancer agent called crizotinib, and included a substantial modification which totally altered the scaffold of the molecule. **PHA-665752** was the starting point for this research and had been obtained from structure-based drug design of a previous lead compound. However, it had a large molecular weight and was too hydrophobic to be orally active. The structure was co-crystallized with the target enzyme and the crucial binding interactions were identified. These included the dihydroindolone ring system which formed two important hydrogen bonds (hydrogen bond donor and hydrogen bond acceptor), as well as the dichloroaromatic ring. As a result of this study, it was noted that much of the scaffold connecting these binding groups was redundant, and so a much simpler, less hydrophobic skeleton was designed which would position the important binding groups in a similar but more efficient manner. The thought process behind this design involved a ring fusion, ring cleavage, and chain contraction. When the novel structures were synthesized, they were found to bind as predicted, and further structure-based drug design was used in the optimization process leading to crizotinib.



a new drug that occupies more of the available space and binds more strongly. The drug can then be synthesized and tested for activity. If it proves active, the target protein can be crystallized with the new drug bound to the binding site, and then X-ray crystallography and molecular modelling can be used again to identify the structure of the complex to see if binding took place as expected. This approach is known as structure-based drug design. Examples of the use of structure-based drug design can be found in Case studies 2, and 5, Box 13.4, sections 14.9.1, 20.7.3.2, 20.9, 21.6.2, and 20.7.4, and Web article 5.

A related process is known as *de novo* drug design (section 17.15). This involves the design of a novel drug structure, based on a knowledge of the binding site alone. This is quite a demanding exercise, but there are examples where *de novo* design has successfully led to a novel lead compound which can then be the starting point for structure-based drug design (see Case study 5 and section 20.7.4.4).

Structure-based drug design cannot be used in all cases. Sometimes the target for a lead compound may not have been identified and, even if it has, it may not be possible to crystallize it. This is particularly true for membrane-bound proteins. One way round this is to identify a protein which is thought to be similar to the target protein, and which *has* been crystallized and

studied by X-ray crystallography. The structural and mechanistic information obtained from that analogous protein can then be used to design drugs for the target protein (see Case studies 2 and 5).

Molecular modelling can also be used to study different compounds which are thought to interact with the same target. The structures can be compared and the important pharmacophore identified (section 17.11), allowing the design of novel structures containing the same pharmacophore. Compound databanks can be searched for those pharmacophores to identify novel lead compounds (section 17.13).

There are many other applications of molecular modelling in medicinal chemistry, some of which are described in Chapter 17. However, a word of caution is worth making at this stage. Molecular modelling studies tackle only one part of a much bigger problem—the design of an effective drug. True, one might design a compound that binds perfectly to a particular enzyme or receptor *in silico*, but if the drug cannot be synthesized or never reaches the target protein in the body, it is useless.

There have also been various examples where a binding site has altered shape in an unpredictable way to accommodate ligands that would not normally be expected to bind. Examples include binding sites for the **statins** (Case study 1) and an anti-inflammatory steroid (Box 8.1). Another example involves the dimeric structure of **galantamine** which has been studied as an inhibitor of the enzyme **acetylcholinesterase** (section 22.15.2).

13.3.12 Drug design by NMR spectroscopy

The use of NMR spectroscopy in designing lead compounds has already been discussed in section 12.4.10. This can also be seen as a method of drug design as the focus is not only on designing a lead compound, but in designing a *potent* lead compound. Usually, drug design aims to optimize a lead compound once it has been discovered. In the NMR method, the component parts **(epitopes)** are optimized first to maximize binding interactions, then linked together to produce the final compound.

NMR is also being increasingly used to identify the structure of target proteins that cannot be crystallized and studied by X-ray crystallography. Once the structure has been identified, molecular modelling techniques can be used for drug design, as described in section 13.3.11.

13.3.13 The elements of luck and inspiration

It is true to say that drug design has become more rational, but the role of chance or the need for hard-working, mentally alert medicinal chemists has not yet been eliminated. Most of the drugs currently on the market were developed by a mixture of rational design, trial and error, hard graft, and pure luck. There are a growing number of drugs that were developed by rational design, such as the ACE inhibitors (Case study 2), thymidylate synthase inhibitors (Case study 5), HIV protease inhibitors (section 20.7.4), neuraminidase inhibitors (section 20.8.3), **pralidoxime** (section 22.14), and **cimetidine** (section 25.2), but they are still in the minority.

Frequently, the development of drugs is helped by reading the literature to see what works on related compounds and what doesn't, then trying out similar alterations to one's own work. It is often a case of groping in the dark, with the chemist asking whether the addition of a group at a certain position will have a steric, electronic, or interactive effect. Even when drug design is carried out on rational lines, good fortune often has a role to play, for example the discovery of the β-blocker **propranolol** (section 23.11.3).

Finally, there are some cases where the use of logical step-by-step modifications to a structure fails to result in significantly improved activity. In such cases, there may be some advantage in synthesizing a large range of structures with different substituents or modifications in the hope of striking lucky. This is illustrated in the development of the anticancer agent **sorafenib** (Box 21.10). The breakthrough here was the discovery of an active structure which contained two substituents that were known to be bad for activity when only one or other was present. When both were present, however, there was a beneficial synergistic effect.

13.3.14 Designing drugs to interact with more than one target

Many diseases require a cocktail of drugs interacting with different targets to provide suitable treatments. A better approach would be to design agents that interact with two or more targets in a controlled fashion in order to reduce the number of drugs that have to be taken. This is known as **multi-target drug discovery** (MTDD) (section 12.2.7). There have been two approaches to designing such multi-target-directed ligands. One is to design agents from known drugs and pharmacophores such that the new agent has the combined properties of the drugs involved. The other approach is to start from a lead compound which has activity against a wide range of targets, and then modify the structure to try and narrow the activity down to the desired targets.

13.3.14.1 Agents designed from known drugs

In the former approach, individual drugs have been linked together to form dimeric structures. The advantage of this approach is that there is a good chance that the

resulting dimer will have a similar selectivity and potency to the original individual drugs for both intended targets. The disadvantage is the increased number of functional groups and rotatable bonds, which may have detrimental effects on whether the resulting dimer is orally active or not. There is also the problem that attaching one drug to another may block each individual component binding to its target binding site. Nevertheless, the design of dimers has been successful in a number of fields. Dimers can be defined as homodimeric or heterodimeric depending on whether the component drugs are the same or not. Homodimeric and heterodimeric opioid ligands have been synthesized to take advantage of the fact that opioid receptors form homodimeric and heterodimeric arrays in certain tissues of the body (section 24.9.2).

There is also great potential for dimers in the treatment of Alzheimer's disease. The **acetylcholinesterase** enzyme has an active site and a peripheral binding site, both of which play a role in the symptoms of the disease. Dimers have been designed that can interact with both of these sites and act as **dual-action agents** (section 22.15.2). Research is also being carried out to design triple-action agents that will interact with the two binding sites in the acetylcholinesterase enzyme plus a totally different target that is also involved in the symptoms or development of the disease.

Enzyme inhibitors have also been designed that contain structural components of the substrate and cofactor of **17β-hydroxysteroid dehydrogenase type 1** (see Web article 1).

A nice method of designing dual-action drugs is to consider the pharmacophores of two different drugs, and to then design a hybrid structure where the two pharmacophores are merged. Such drugs are called **hybrid drugs**. One example of this is **ladostigil** (Fig. 13.59), which is a hybrid structure of the acetylcholinesterase inhibitor **rivastigmine** and the monoamine oxidase inhibitor **rasagiline**. The feature in blue indicates the structural components of ladostigil that are present in both component drugs.

Another method is to design a **chimeric drug** that contains key pharmacophore features from two different drugs. For example, a structure containing features of **2-methoxyestradiol** and **colchicine** has been synthesized as a potential anticancer agent (Fig 13.60). Although both of the parent structures have anticancer activity, they have serious drawbacks. 2-Methoxyestradiol is metabolized rapidly, while colchicine has toxic side effects. The chimeric structure also has anticancer activity, but improved pharmacokinetic properties.

13.3.14.2 Agents designed from non-selective lead compounds

The second approach to designing multi-target drugs is to identify a lead compound that already shows the ability to interact with a wide variety of targets. Such an agent is termed a **promiscuous ligand** or a **dirty drug**. Linear polyamines have been suggested as ideal lead compounds in this approach as they have several amine groups that can act as good binding groups to protein targets. Moreover, the flexibility of the structure means that an active conformation is likely to exist for a large number of protein targets. The challenge is then to modify the structure such that it shows selectivity towards the desired targets. This approach has been used in the design of an agent which shows activity, both as an acetylcholinesterase inhibitor and a muscarinic antagonist (section 22.15.3). Such agents may be useful in the treatment of Alzheimer's disease.

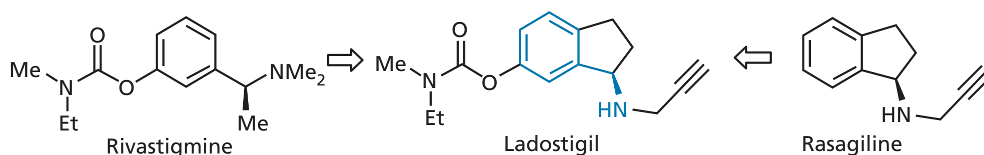


FIGURE 13.59 Design of the hybrid drug ladostigil.

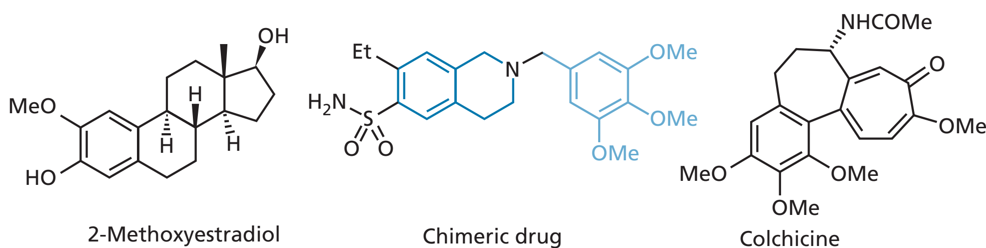


FIGURE 13.60 Design of a chimeric drug.

Multi-tyrosine receptor kinase inhibitors have also been developed as anticancer agents (section 21.6.2.5).

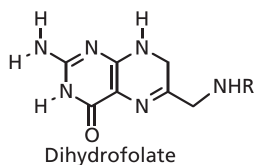
KEY POINTS

- Drug optimization aims to maximize the interactions of a drug with its target binding site in order to improve activity and selectivity, and to minimize side effects. Designing a drug that can be synthesized efficiently and cheaply is another priority.
- The length and size of alkyl substituents can be modified to fill up hydrophobic pockets in the binding site or to introduce selectivity for one target over another. Alkyl groups attached to heteroatoms are most easily modified.
- Aromatic substituents can be varied in character and/or ring position.
- Extension is a strategy where extra functional groups are added to the lead compound in order to interact with extra binding regions in the binding site.
- Chains connecting two important binding groups can be modified in length in order to maximize the interactions of each group with the corresponding binding regions.
- Ring systems can be modified to maximize binding interactions through strategies such as expansion, contraction, variation, or fusion with other rings.
- Classical and non-classical isosteres are frequently used in drug optimization.
- Simplification involves removing functional groups from the lead compound that are not part of the pharmacophore. Unnecessary parts of the carbon skeleton or asymmetric centres can also be removed in order to design drugs that are easier and cheaper to synthesize. Oversimplification can result in molecules that are too flexible, resulting in decreased activity and selectivity.
- Rigidification is applicable to flexible lead compounds. The aim is to reduce the number of conformations available while retaining the active conformation. Locking rotatable rings into ring structures or introducing rigid functional groups are common methods of rigidification.
- Conformational blockers are groups which are introduced into a lead compound to reduce the number of conformations that the molecule can adopt.
- Structure-based drug design makes use of X-ray crystallography and computer-based molecular modelling to study how a lead compound and its analogues bind to a target binding site.
- NMR studies can be used to determine protein structure and to design novel drugs.
- Serendipity plays a role in drug design and optimization.
- Multi-target-directed ligands can be designed by linking or merging established drugs, or by modifying a lead compound that interacts with a large number of targets.

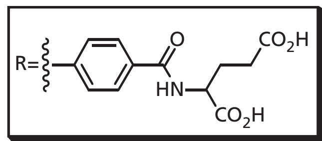
QUESTIONS

1. **DU 122290** was developed from **sultopride** (Fig. 13.47) and shows improved activity and selectivity. Suggest possible reasons for this.

2. Methotrexate inhibits the enzyme dihydrofolate reductase. The pteridine ring system of methotrexate binds to the binding site as shown in Fig. 13.26. Suggest how dihydrofolate (the natural substrate for the enzyme) might bind.

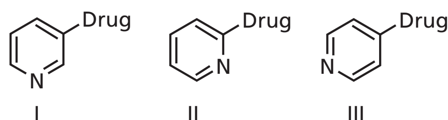


Dihydrofolate



3. A lead compound containing a methyl ester was hydrolysed to give a carboxylic acid. An *in vivo* bioassay suggested that the ester was active and the acid was inactive. However, an *in vitro* bioassay suggested that the ester was inactive and the acid was active. Explain these contradictory results.

4. A lead compound contains an aromatic ring. The following structures were made as analogues. Structures I and II were similar in activity to the lead compound, whereas structure III showed a marked increase in activity. Explain these results and describe the strategies involved.



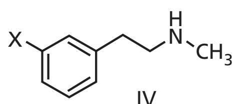
5. The pharmacophore of cocaine is shown in Box 13.2. Identify possible cyclic analogues that are simpler than cocaine and which would be expected to retain activity.

6. Procaine (Box 13.2) has been a highly successful local anaesthetic and yet there are three bonds between the important ester and amine binding groups, compared with

four in cocaine. This might suggest that these groups are too close together in procaine. In fact, this is not the case. Suggest why not.

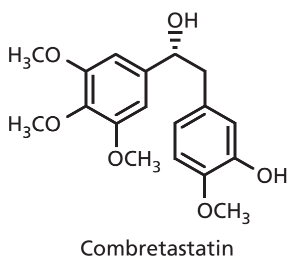
7. The aromatic amine on procaine is not present in cocaine. Comment on its possible role.

8. Explain how you would apply the principles of rigidification to structure IV below in order to improve its pharmacological properties. Give two specific examples of rigidified structures.

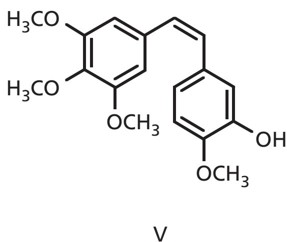


IV

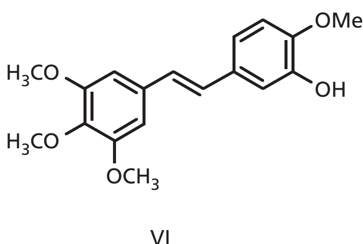
9. Combretastatin is an anticancer agent discovered from an African plant. Analogue V is more active than combretastatin, whereas analogue VI is less active. What strategy was used in designing analogues V and VI? Why is analogue V more active and analogue VI less active than combretastatin?



Combretastatin

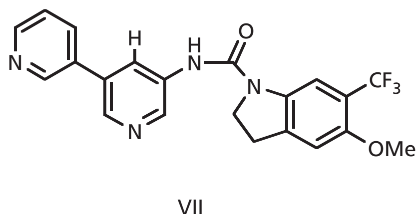


V

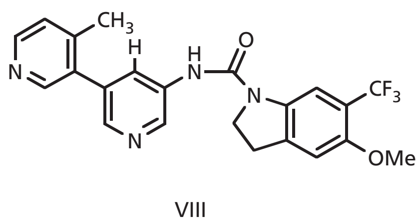


VI

10. Structure VII is a serotonin antagonist. A methyl group has been introduced into analogue VIII, resulting in increased activity. What role does the methyl group play and what is the term used for such a group? Explain why increased activity arises.



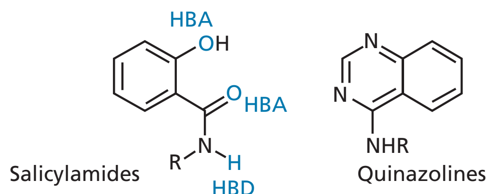
VII



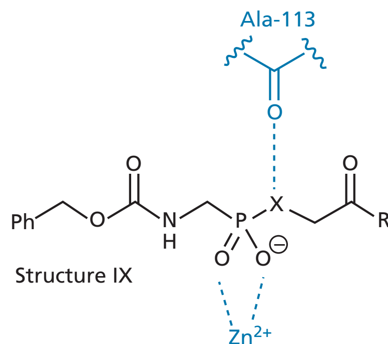
VIII

11. Explain what kind of drug design strategies were carried out in the design of enalaprilate (Case study 2).

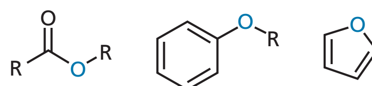
12. Salicylamides are inhibitors for an enzyme called **scytalone dehydratose**. SAR shows that there are three important hydrogen bonding interactions. Explain whether you think quinazolines could act as a bioisostere for salicylamides.



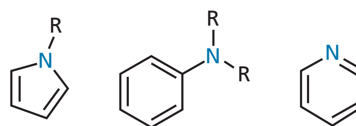
13. Structure IX (X = NH) is an inhibitor of a metalloenzyme called **thermolysin** and forms interactions as shown. Explain why the analogue (X = O) has reduced binding affinity by a factor of 1000 and why the analogue (X = CH_2) has roughly the same binding affinity.



14. Suggest why the oxygen atoms in the following structures are poor hydrogen bond acceptors.



15. Compare the ability of the nitrogen atoms in the following structures to act as hydrogen bond acceptors.



FURTHER READING

Acharya, K. R., Sturrock, E. D., Riordan, J. F., and Ehlers, M. R. (2003) ACE revisited: a new target for structure-based drug design. *Nature Reviews Drug Discovery* **2**, 891–902.

Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., and Kraut, J. (1982) Crystal structures of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7 Å resolution. I. General features and binding of methotrexate. *Journal of Biological Chemistry* **257**, 13650–13662.

Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., and Melchiorre, C. (2008) Multi-target-directed ligands to combat neurodegenerative diseases, *Journal of Medicinal Chemistry* **51**, 347–372.

Cui, J. J., Tran-Dubé, M., Shen, H., Nambu, M., Kung, P. P., Pairish, M., et al. (2011) Structure based drug design of crizotinib, *Journal of Medicinal Chemistry* **54**, 6342–6363.

Hruby, V. J. (2002) Designing peptide receptor agonists and antagonists. *Nature Reviews Drug Discovery* **1**, 847–858.

Jeffrey, G. A. (1991) *Hydrogen Bonding in Biological Structures*. Springer-Verlag, New York.

Khan, A. R., Parrish, J. C., Fraser, M. E., Smith, W. W., Bartlett, P. A., and James, M. N. (1998) Lowering the entropic barrier for binding conformationally flexible inhibitors to enzymes. *Biochemistry* **37**, 16839–16845.

Kubinyi, H. (2001) Hydrogen bonding: The last mystery in drug design? In: Testa, B. (ed.) *Pharmacokinetic Optimisation in Drug Research*. Wiley-VCH, Zurich.

Luca, S., White, J. F., Sohal, A. K., Filippov, D. V., van Boom, J. H., Grisshammer, R., and Baldus, M. (2003) The conformation of neurotensin bound to its G protein-coupled receptor. *Proceedings of the National Academy of Sciences of the USA* **100**, 10706–10711.

Meyer, E. G., Botos, I., Scapozza, L., and Zhang, D. (1995) Backward binding and other structural surprises. *Perspectives in Drug Discovery and Design* **3**, 168–195.

Morphy R. and Rankovic, Z. (2005) Designed multiple ligands. An emerging drug discovery paradigm. *Journal of Medicinal Chemistry* **48**, 6523–6543.

Morphy, R., Kay, C., and Rankovic, Z. (2004), From magic bullets to designed multiple ligands, *Drug Discovery Today* **9**, 641–651.

Pellecchia, M., Sem, D. S., and Wuthrich, K. (2002) NMR in drug discovery. *Nature Reviews Drug Discovery* **1**, 211–219.

Rees, D. C., Congreve, M., Murray, C. W., and Carr, R. (2004) Fragment-based lead discovery. *Nature Reviews: Drug Discovery* **3**, 660–672.

Titles for general further reading are listed on p. 763

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 11.10 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 too hydrophilic do not cross the cell membranes of the gut wall easily. One way round this is to inject them, but they cannot be used against intracellular targets as 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. It has also been observed that toxic metabolites are more likely to be formed from hydrophobic drugs.

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. Other experimental procedures to determine log P include high-performance liquid chromatography (HPLC) and automated potentiometric titration procedures. It is also possible to calculate log P values for a given structure using suitable software programs. 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 quantitative structure–activity relationships (QSARs), 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

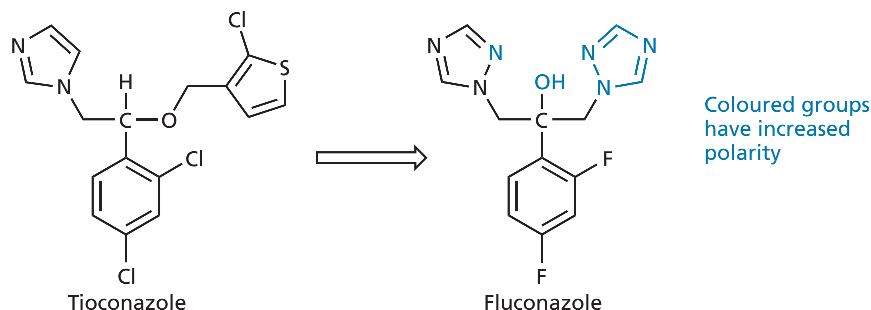


FIGURE 14.1 Increasing polarity in antifungal agents.

(section 11.3), and so the tactics of rigidification described in section 13.3.9 can be useful in improving drug absorption.

14.1.1 Masking polar functional groups to decrease 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. Masking such groups would decrease binding interactions and lower activity. 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).

14.1.2 Adding or removing 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 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. 14.1). Another example can be found in Case study 1 where a polar sulphonamide 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 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. 14.2 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 Varying hydrophobic substituents to vary polarity

Polarity can be varied by the addition, removal, or variation of suitable hydrophobic substituents. For example,

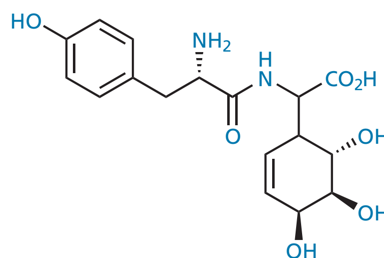


FIGURE 14.2 Excess polarity (coloured) in a drug.

extra alkyl groups could be included within the carbon skeleton of the molecule to increase hydrophobicity if the synthetic route permits. Alternatively, alkyl groups already present might be replaced with larger groups. 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 found to modify the hydrophobicity of a compound. The addition of halogen substituents also increases hydrophobicity. Chloro or fluoro substituents are commonly used, and, less commonly, a bromo substituent.

14.1.4 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 (section 11.3). 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 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. 14.3) has anti-thrombotic 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.

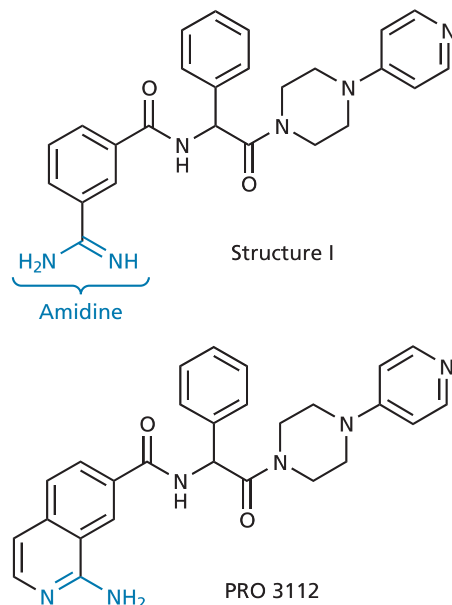


FIGURE 14.3 Varying basicity in anti-thrombotic agents.

14.1.5 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).

14.1.6 Bioisosteres for polar groups

The use of bioisosteres has already been described in section 13.3.7 in the design of compounds with improved target interactions. Bioisosteres have also been used as substitutes for important functional groups that 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 bioisosteres have been used for carboxylic acids, but among the most popular is a 5-substituted tetrazole ring (Fig. 14.4). 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

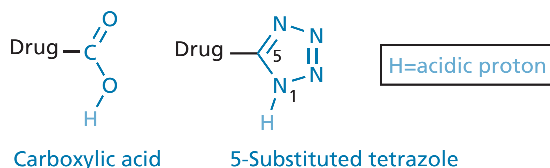


FIGURE 14.4 5-Substituted tetrazole ring as a bioisostere for a carboxylic acid.

BOX 14.1 The 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.

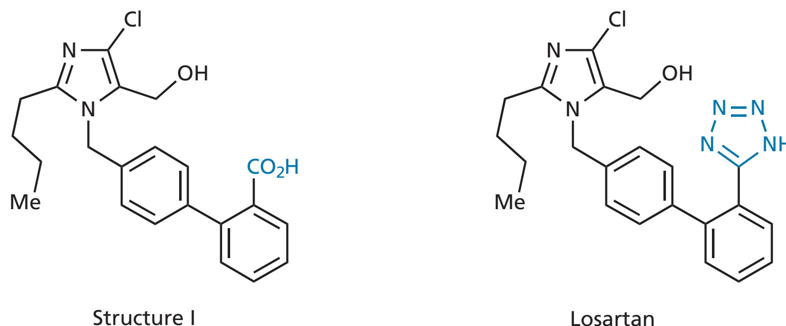


FIGURE 1 Development of losartan.

result (see Box 14.1). They are also resistant to many of the metabolic reactions that occur on carboxylic acids. *N*-Acylsulphonamides have also been used as bioisosteres for carboxylic acids (section 13.3.7).

Phenol groups are also commonly present in drugs but are susceptible to metabolic conjugation reactions. Various bioisosteres involving amides, sulphonamides, or heterocyclic rings have been used where an N–H group mimics the phenol O–H group.

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 anti-rheumatic agent **D 1927** serves as a steric shield and blocks hydrolysis of the terminal peptide bond (Fig. 14.5). 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 [section 22.7.1, SCh 226374 (Fig. 21.46), and CGS27023 (Fig. 21.64)].

For additional material see Web article 5: the design of a serotonin antagonist as a possible anxiolytic agent.

14.2.2 Electronic effects of bioisosteres

Another popular tactic used to protect a labile functional group is to stabilize the group electronically using a bioisostere. Isosteres and non-classical isosteres are frequently used as bioisosteres (see also sections 13.1.15, 13.3.7, and 14.1.6). For example, replacing the methyl

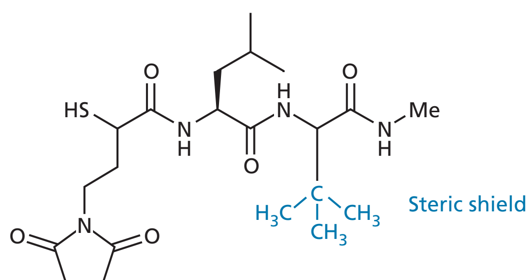


FIGURE 14.5 The use of a steric shield to protect the anti-rheumatic agent D 1927.

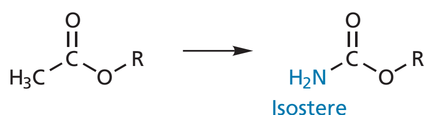


FIGURE 14.6 Isosteric replacement of a methyl group with an amino group.

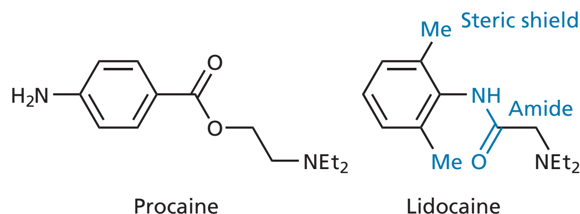


FIGURE 14.7 Steric and electronic modifications which make lidocaine a longer-lasting local anaesthetic compared with procaine.

group of an ethanoate ester with NH_2 results in a urethane functional group which is more stable than the original ester (Fig. 14.6). 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 as it can feed electrons into the carboxyl group and stabilize it from hydrolysis. The cholinergic agonist **carbachol** is stabilized in this way (section 22.7.2), 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 often specific to a particular area of medicinal chemistry. 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 Steric and electronic modifications

Steric hindrance and electronic stabilization have often been used together to stabilize labile groups. For example, **procaine** (Fig. 14.7) is a good, but short-lasting, local anaesthetic because its ester group is quickly hydrolysed. Changing the ester group to the less reactive amide group reduces susceptibility to 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**. Further successful examples of steric and electronic modifications are demonstrated by **oxacillin** (Box 19.5) and **bethanechol** (section 22.7.3).

14.2.4 Metabolic blockers

Some drugs are metabolized by the introduction of polar groups at particular positions in their skeleton. For example, steroids can be oxidized at position 6 of the tetracyclic ring to introduce a polar hydroxyl group. The introduction of this group allows the formation of polar conjugates which can be eliminated quickly from the system. By introducing a methyl group at position 6, metabolism is blocked and the activity of the steroid is prolonged. The oral contraceptive megestrol acetate is an agent which contains a 6-methyl blocking group.

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. 14.9) 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

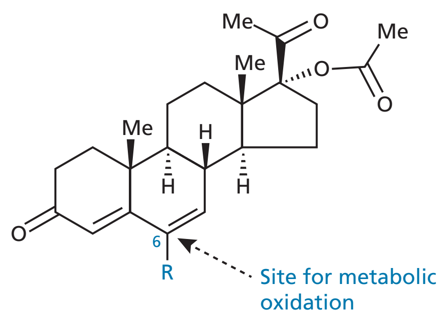


FIGURE 14.8 Metabolically susceptible steroid (R = H), metabolite (R = OH), and megestrol acetate (R = Me).

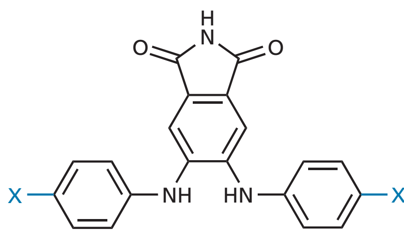


FIGURE 14.9 The use of fluorine substituents as metabolic blockers (X = H, CGP 52411; X = OH, metabolite; X = F, CGP 53353).

as an anticancer agent and was found to undergo oxidative metabolism at the *para*-position of the aromatic rings. Fluoro-substituents were successfully added in the analogue **CGP 53353** to block this metabolism. This tactic was also applied successfully in the design of **gefitinib** (section 21.6.2.1). Fluorine has now been used extensively to block metabolism in a variety of structural situations.

Another approach which is actively being explored is to replace a hydrogen atom with a deuterium isotope. The covalent bond between carbon and deuterium is twice as strong as that between carbon and hydrogen, and this might help to block metabolic mechanisms.

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 11.5.2). These acids can then be quickly eliminated from the body. Other common metabolic reactions include aliphatic and aromatic *C*-hydroxylations, *N*- and *S*-oxidations, *O*- and *S*-dealkylations, and deaminations (section 11.5).

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. 14.10). 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.

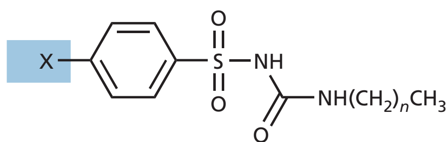


FIGURE 14.10 Tolbutamide (X = Me; *n* = 3) and chlorpropamide (X = Cl; *n* = 2).

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. 14.11). 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. As 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 (I in Fig. 14.12) 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. 14.12).

Removing the OH or replacing it with a methyl group prevents metabolism, but also prevents the important hydrogen bonding interactions with the binding site.

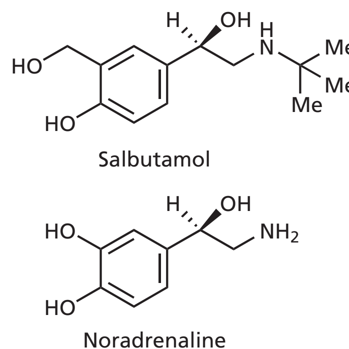


FIGURE 14.11 Salbutamol and noradrenaline.

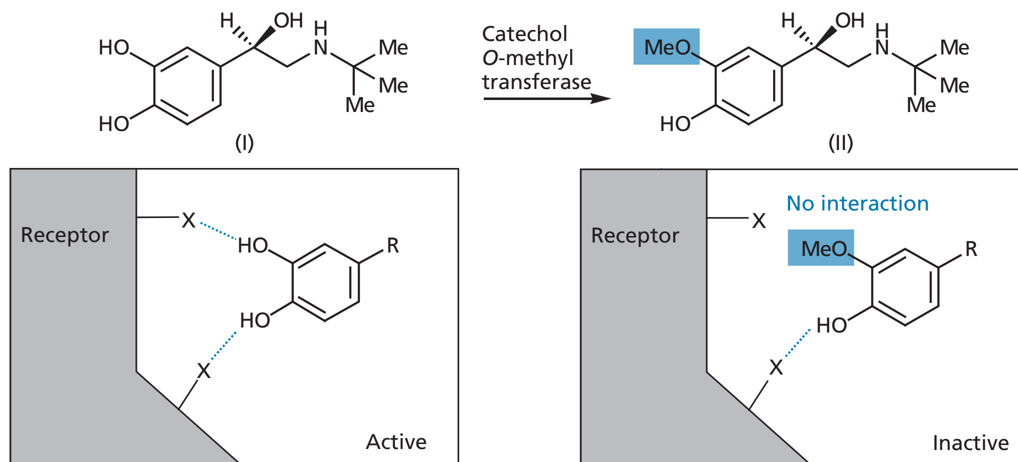


FIGURE 14.12 Metabolic methylation of a noradrenaline analogue. X denotes an electronegative atom.

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. 14.13). Beyond that, activity is lost because the OH group is out of range or the substituent is 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 in any part of that available volume. Another example of a successful **group shift** strategy can be seen in Case study 7.

Shifting an important binding group that is metabolically susceptible cannot be guaranteed to work in every situation. It may well 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 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. 14.1).

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 (see Web article 5).

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

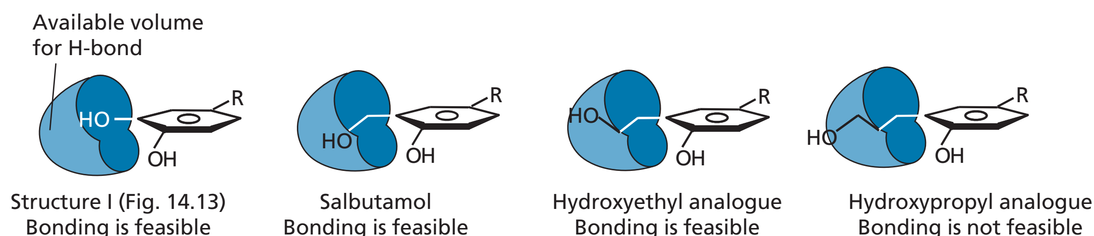


FIGURE 14.13 Viewing a binding region as an available volume.

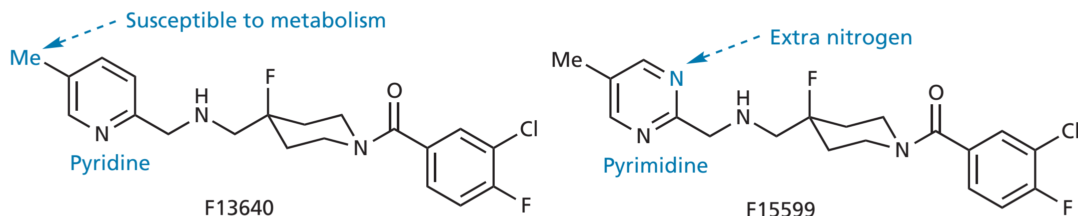


FIGURE 14.14 Stabilizing an aromatic or heteroaromatic methyl substituent by adding a nitrogen to the ring.

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, as lowering the electron density in the ring also helps to make the methyl substituent more resistant to metabolism. For example, F13640 underwent phase II clinical trials as an analgesic (Fig. 14.14). 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.

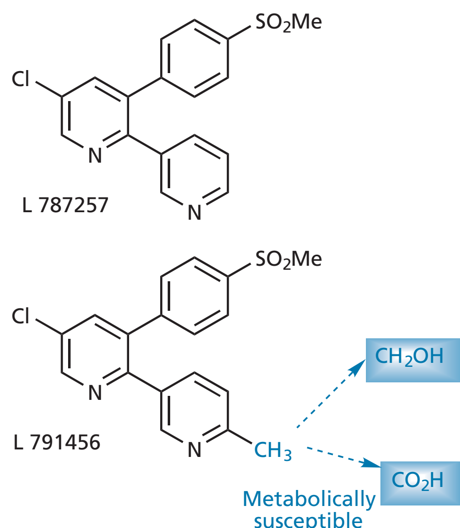


FIGURE 14.15 Adding a metabolically labile methyl group to shorten a drug's lifetime.

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 (see 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 the resulting compound (**L 791456**) was metabolically oxidized to a polar alcohol, as well as to a carboxylic acid (Fig 14.15).

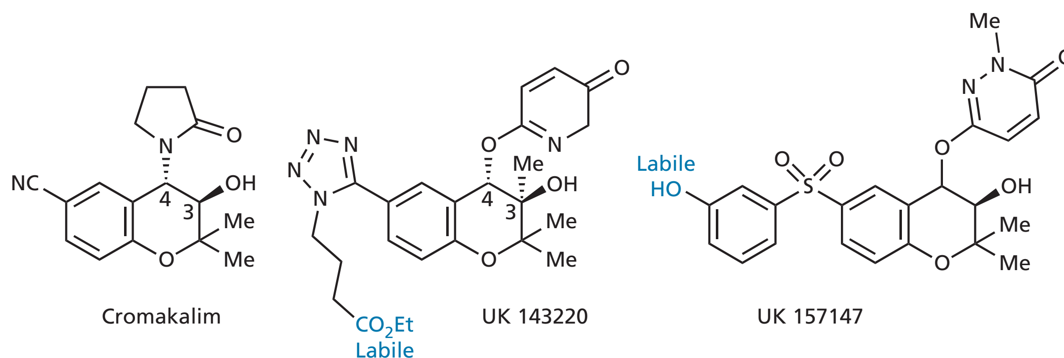
Another example is the analgesic **remifentanil** (section 24.6.3.4), where ester groups were incorporated to make it a short-lasting agent. The beta-blocker **esmolol** was also designed to be a short-acting agent by introducing an ester group (section 23.11.3.4).

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 degrades spontaneously 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 22.10.2.4). 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.

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.

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 is a result of electronic factors.
- Metabolically stable groups can be added to block metabolism at certain positions.
- Groups which 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 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 which are slowly metabolized may linger too long in the body and cause side effects.
- Groups which 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 the enzyme serves to activate an anticancer prodrug, and the antibody directs 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**, which are covered in sections 21.9.4 and 21.9.5 respectively. Antibodies are also being studied as a means of targeting viruses (section 20.12.5).

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 be done by using a fully ionized drug that is incapable of crossing cell membranes. For example, highly ionized sulphonamides are used against gastrointestinal infections (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 (CNS). By increasing the polarity of drugs, they are less likely to cross the blood–brain barrier (section 11.4.5), and this means they are less likely to have CNS side effects. Achieving selectivity for the CNS over the peripheral regions of the body is not so straightforward. In order to achieve that, the drug would have to be designed to cross the blood–brain barrier efficiently, while being metabolized rapidly to inactive metabolites in the peripheral system.

14.4.4 Targeting with membrane tethers

Several drug targets are associated with cell membranes and one way of targeting drugs to these targets is to attach membrane tethers to the drug such that the molecule is anchored in the membrane close to the target. The antibacterial agent **teicoplanin** is one such example and is discussed in section 19.5.5.2. Another membrane-tethered drug has been designed to inhibit the enzyme **β-secretase**, with the ultimate aim of treating Alzheimer's disease (AD). This enzyme generates the proteins that are responsible for the toxic protein aggregates found in the brains of AD sufferers, and does so mainly in cellular organelles called **endosomes**. A peptide transition-state inhibitor has been linked to a sterol such that it is taken into endosomes by endocytosis. The sterol then acts as the membrane tether to lock the drug in position, such that it targets β-secretase in endosomes rather than β-secretase in other locations. Potential agents for AD treatment are also being targeted to mitochondria where AD leads to the generation of radicals and oxidation reactions that are damaging to the cell. **MitoQ** (Fig. 14.16) is an agent undergoing clinical trials which contains an antioxidant prodrug linked to a hydrophobic triphenylphosphine moiety. The latter group aids the drug's entry into mitochondria, then tethers it to the phospholipid bilayers of the mitochondria membrane. The quinone ring system is reduced rapidly to the active quinol form which can then act as an antioxidant to neutralize free radicals. A different approach for targeting antioxidant drugs to mitochondria has been to modify known antibacterial agents (e.g. **gramicidin S**) such that they act as antioxidants rather than antibacterial agents. The rationale here

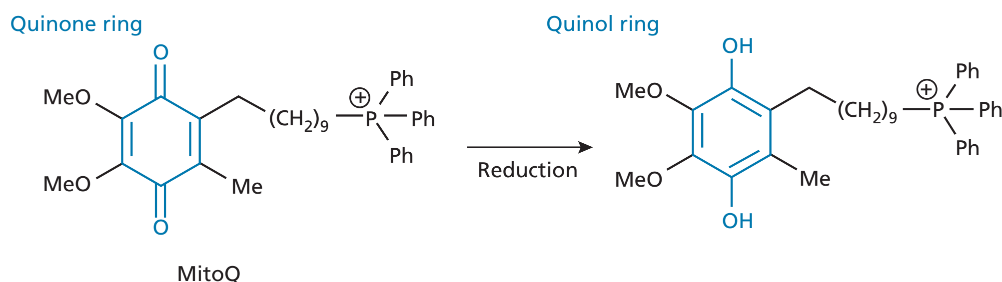


FIGURE 14.16 MitoQ acting as a prodrug.

is that the mitochondria membrane is similar in nature to bacterial cell membranes, and so antibacterial agents may show selectivity for mitochondrial membranes over cell membranes.

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 that 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 11.5 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).

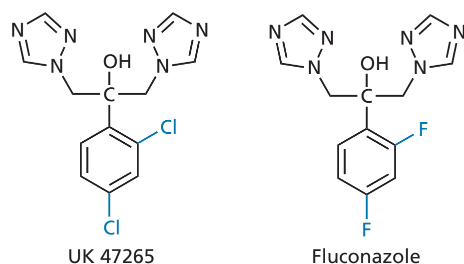


FIGURE 14.17 Varying aromatic substituents to reduce toxicity.

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 CNS 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

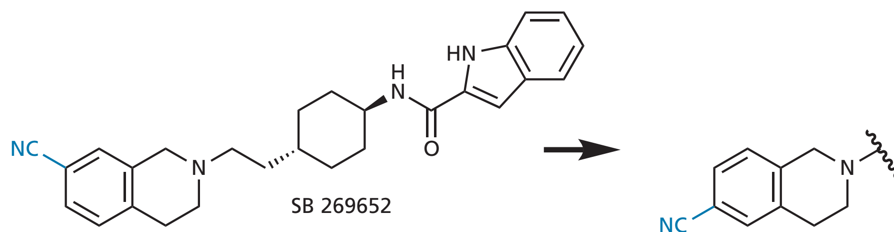


FIGURE 14.18 Varying substituent positions to reduce side effects.

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, $OCONR_2$, OAr). The inductive effect of these groups aids the hydrolytic mechanism by stabilizing the alkoxide 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 by the acid conditions of the stomach or the more alkaline conditions of the intestine before it reaches the blood supply. To that end, it may be necessary to make the ester more stable. For example, cyclopropanecarboxylic acid esters have been studied as potential prodrugs because the cyclopropane ring has the ability to stabilize the carbonyl group of a neighbouring ester (Fig. 14.21). In this respect, it is acting as a bioisostere for a double bond (see also section 13.3.7). A conjugated double bond stabilizes a neighbouring carbonyl group due to interaction of the π-systems involved. It is proposed that the σ-bonds of a cyclopropane ring are orientated correctly to allow a hyperconjugative interaction that has a similar stabilizing effect on a neighbouring carbonyl group. The interaction proposed involves hyperconjugative donation to the anti-bonding π orbital of the carbonyl group.

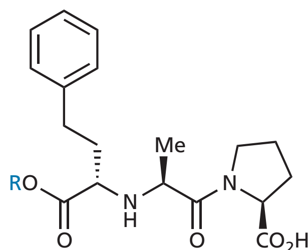


FIGURE 14.19 Enalapril (R = Et); Enalaprilate (R = H).

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 anti-epileptics take advantage of this reaction, for example **hexobarbitone** (Fig. 14.22).

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

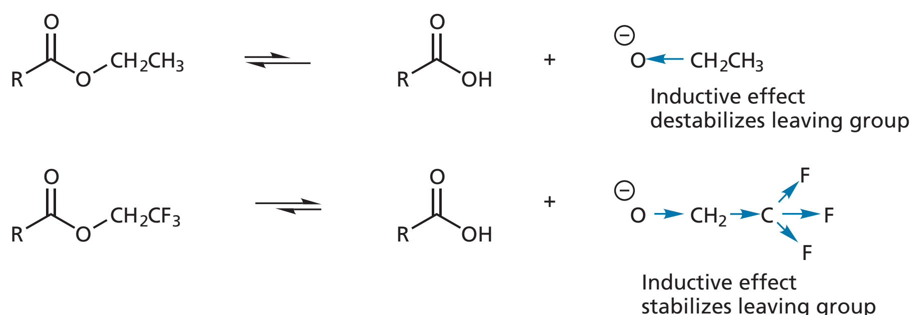


FIGURE 14.20 Inductive effects on the stability of leaving groups.

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 (Fig. 1).

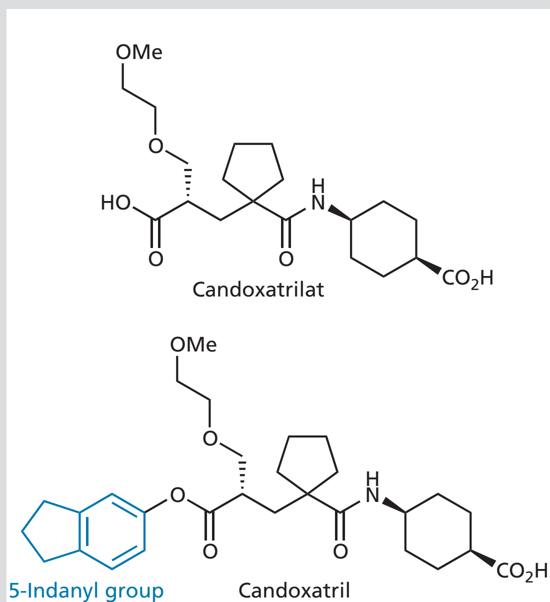


FIGURE 1 Protease inhibitors.

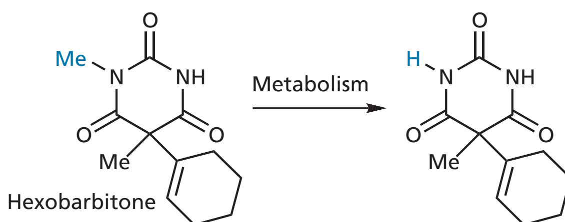


FIGURE 14.22 *N*-Demethylation of hexobarbitone.

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** (section 11.5.5), 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, as 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 from where it 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 containing a large lipophilic group and is only released into the blood supply following slow dissociation of the ion complex.

Similarly, lipophilic esters of the antipsychotic drug **fluphenazine** are used to prolong its action (Fig. 14.27).

levodopa (Fig. 14.23). 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 as 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, 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.

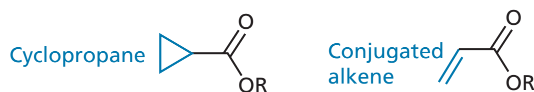


FIGURE 14.21 Cyclopropane carboxylic acid esters as prodrugs and bioisosteres for α,β-unsaturated esters.

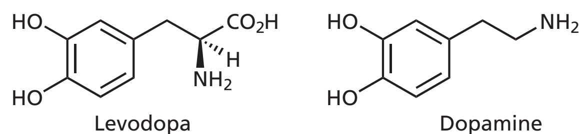


FIGURE 14.23 Levodopa and dopamine.

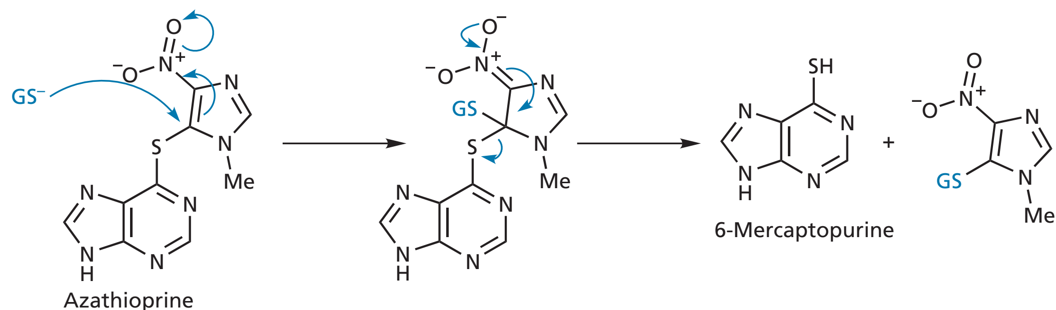


FIGURE 14.24 Azathioprine acts as a prodrug for 6-mercaptopurine (GS = glutathione).

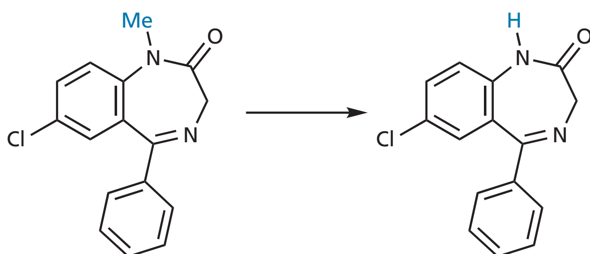


FIGURE 14.25 Valium (diazepam) as a possible prodrug for nordazepam.

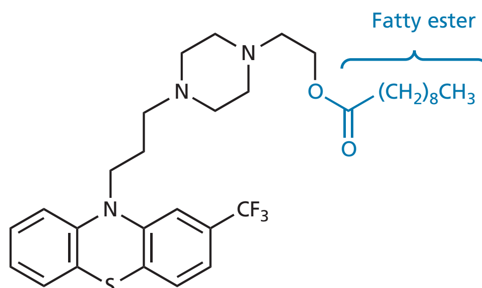


FIGURE 14.27 Fluphenazine decanoate.

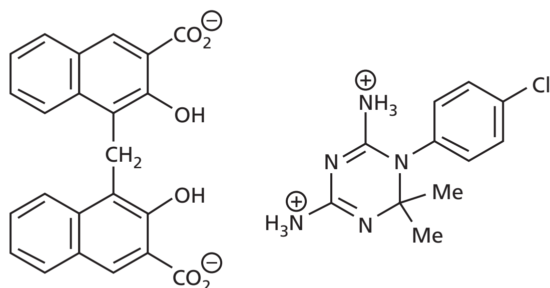


FIGURE 14.26 Cycloguanil pamoate.

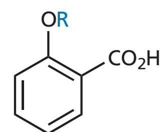


FIGURE 14.28 Aspirin (R = $COCH_3$) and salicylic acid (R = H).

The prodrug is given by intramuscular injection and slowly diffuses from fat tissue into the blood supply, where it is rapidly hydrolysed.

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 because of the 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 **pargyline** 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 activated by a viral enzyme which is only present in infected cells (sections 9.5 and 20.6.1). In a similar vein, the

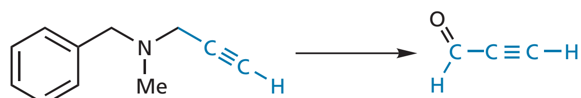
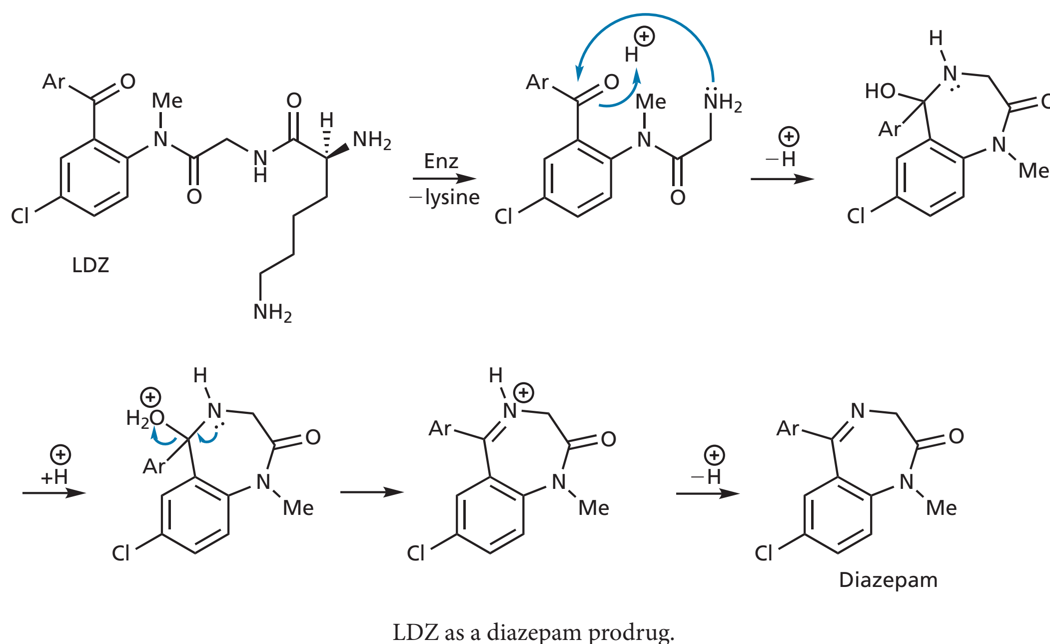


FIGURE 14.29 Pargyline as a prodrug for propiolaldehyde.

BOX 14.4 Prodrugs masking toxicity and side effects

LDZ is an example of a diazepam prodrug which 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).



LDZ as a diazepam prodrug.

anti-schistosomal 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 to prevent them dissolving 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 is present. It does not dissolve easily on the tongue and is quickly hydrolysed once swallowed.

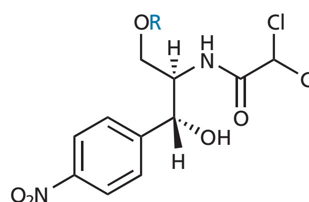


FIGURE 14.30 Chloramphenicol (R = H) and chloramphenicol prodrugs; chloramphenicol palmitate (R = $CO(CH_2)_{14}CH_3$); chloramphenicol succinate (R = $CO(CH_2)_2CO_2H$).

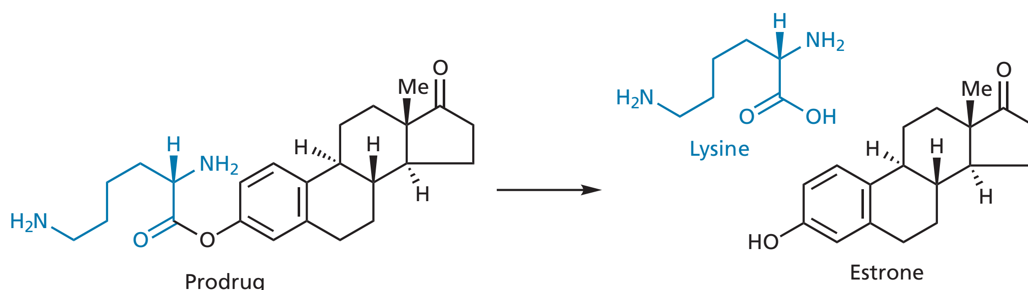
14.6.5 Prodrugs to improve water solubility

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

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 **estrone** 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 estrone to improve water solubility and absorption.

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 this is avoided by using a phosphate ester prodrug which has much better solubility because of the ionic phosphate group (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 degrades spontaneously 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.

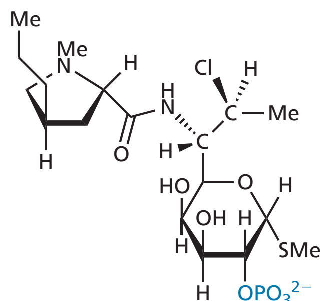


FIGURE 14.31 Clindamycin phosphate.

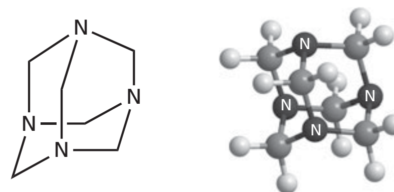


FIGURE 14.32 Methenamine.

Prodrugs of sulphonamides have also been used to target 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.

Finally, the **proton pump inhibitors** are prodrugs that are activated by the acid conditions of the stomach (section 25.3).

14.6.7 Prodrugs to increase chemical stability

The antibacterial agent **ampicillin** decomposes in concentrated aqueous solution as a result of intramolecular

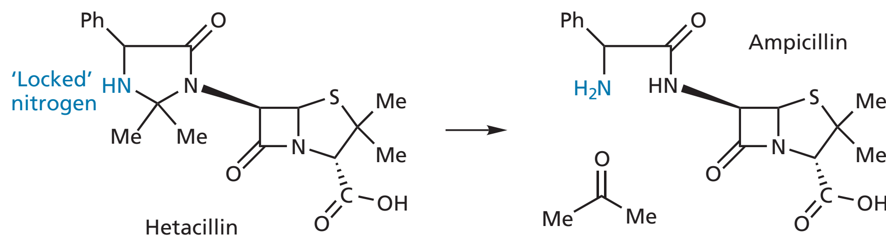


FIGURE 14.33 Hetacillin and ampicillin.

attack of the side chain amino group on the lactam ring (section 19.5.1.8). **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. In the field of antiviral agents, cyclopropane carboxylic acid esters (section 14.6.1.1) are being studied as potential prodrugs of aciclovir in order to prolong chemical stability in solution.

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 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 which 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 which contain metabolically susceptible polar groups are useful in improving water solubility. They are particularly useful for drugs which have to be injected or for drugs which are too hydrophobic for effective absorption from the gut.
- Prodrugs which are susceptible to pH or chemical degradation can be effective in targeting drugs or increasing stability in solution prior to injection.
- Prodrugs which 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 (sections 7.5 and 19.5.4.1).

The antiviral preparation **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, which 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).

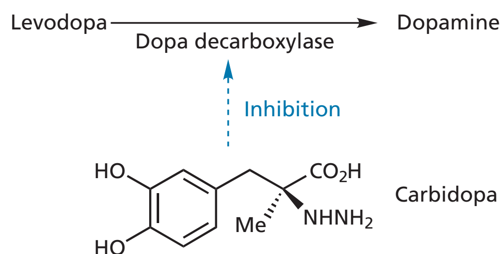


FIGURE 14.34 Inhibition of levodopa decarboxylation.

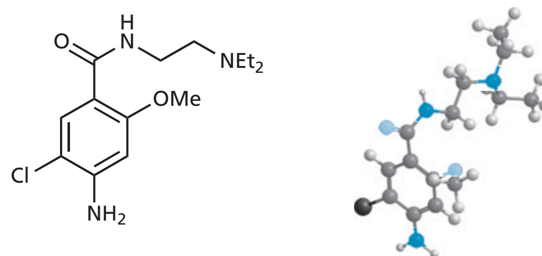


FIGURE 14.35 Metoclopramide.

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 CNS (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, as 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).

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 removed rapidly 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 which is administered alongside another drug to enhance the latter's activity.
- Many sentry drugs protect their partner drug by inhibiting an enzyme which acts on the latter.
- Other drugs have 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, to see how feasible it is to use them as drugs.

14.8.1 Neurotransmitters

Many non-peptide neurotransmitters are simple molecules which can be prepared easily 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 synthesized easily, but they cannot be used effectively 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 around 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 quantities 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 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. Another example 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**.

Recombinant enzymes have also been produced. For example, **glucarpidase** is a carboxypeptidase enzyme which was recently approved in 2012. It is administered to cancer patients with failed kidneys when they are taking the anticancer drug **methotrexate**. The enzyme serves to metabolize methotrexate and prevent it from reaching toxic levels. Other examples of recombinant enzymes used in the clinic include **agalsidase beta** for the treatment of **Fabry disease** and **imiglucerase** for the treatment of **Gaucher's disease**.

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 that 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 **severe combined immunodeficiency** (SCID) syndrome respectively. SCID 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 enlargement of the skull, jaw, hands, and feet as a result of the excessive production of growth hormone. **Pegfilgrastim** is the PEGylated form of **filgrastim** (**recombinant human granulocyte-colony stimulating factor**) and is used as an anticancer agent.

PEGylation has also been used to protect liposomes for drug delivery (section 11.10).

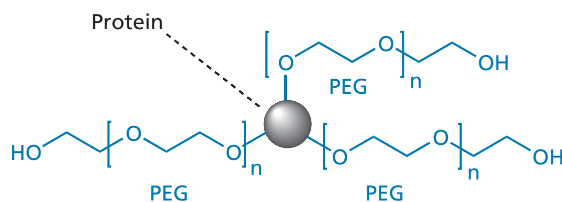


FIGURE 14.36 PEGylated protein.

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 or macromolecule, they have great potential in targeting cancer cells or viruses. Alternatively, they could be used to carry drugs or poisons to specific targets (see sections 14.4.1, 20.11.5, 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. As 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 which spawned 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 mouse-like 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 immunosuppressants, antiviral agents (section 20.11.5), 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, thus preventing it from acting in this way.

Another example is **adalimumab**, which was launched in 2003 and was 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 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. **Ranibizumab** is a fragment of the monoclonal antibody **bevacizumab** used in cancer therapy (section 21.9.1), and is used for the treatment of a condition that results in age-related vision loss. Other monoclonal antibodies undergoing clinical trials include **reslizumab** for the treatment of asthma. **Denosumab** is a fully humanized monoclonal antibody that was approved in 2009 for the treatment of osteoporosis. **Belimumab** was approved in 2011 for the treatment of lupus—an autoimmune disease, while **natalizumab** was approved in 2004 for the treatment of multiple sclerosis.

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 in order to collect antibodies 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.

An interesting idea under investigation involves coating the inside of nanotubes with antibodies that can recognize infectious agents, such as viruses. It is hoped that such nanotubes could be administered to trap and remove viruses from the blood supply.

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), luteinizing hormone-releasing hormone 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 because many of the new targets in medicinal chemistry involve peptides as receptor ligands or as enzyme substrates, for example the protein kinases. 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 try and improve bioavailability and attain more acceptable levels in the blood supply. This usually involves strategies aimed at disguising or reducing the peptide nature of the lead compound to generate a structure which is more easily absorbed from the gastrointestinal tract, and is more resistant to digestive and metabolic enzymes. Such analogues are known as **peptidomimetics**.

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 either more stable to hydrolytic attack by peptidase enzymes or binds less readily to the relevant active sites. For example, a peptide bond might be replaced by an alkene (Fig. 14.37). If the compound retains activity, then the alkene represents a

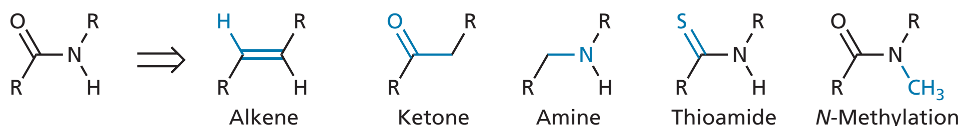


FIGURE 14.37 Examples of functional groups that might be used to replace a peptide bond.

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 (see 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 sulphur 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 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 that 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 also become unrecognizable to the desired target.

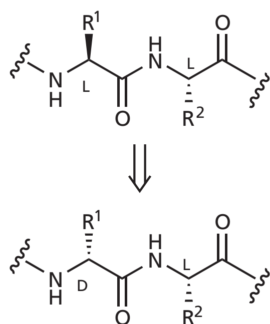


FIGURE 14.38 Replacing an L-amino acid with a D-amino acid. The common L-amino acids have the *R*-configuration except for L-cysteine, which has the *S*-configuration.

A third strategy is to replace natural amino acid residues with unnatural ones. This is a tactic that has 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 side chains 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 less recognizable 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 (see also sections 20.7.4.6 and 20.7.4.7).

Peptidomimetics are often hydrophobic 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 that would result in a decreased binding affinity.

Another potential problem with peptide lead compounds is that they are invariably flexible molecules with a large number of freely rotatable bonds. Flexibility has been shown to be detrimental to oral bioavailability

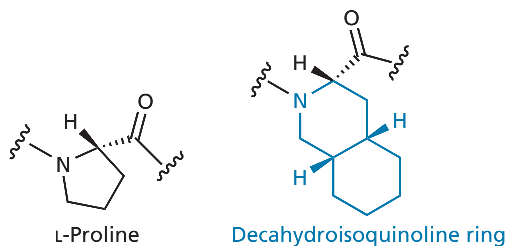


FIGURE 14.39 Replacing a natural residue with an unnatural one.

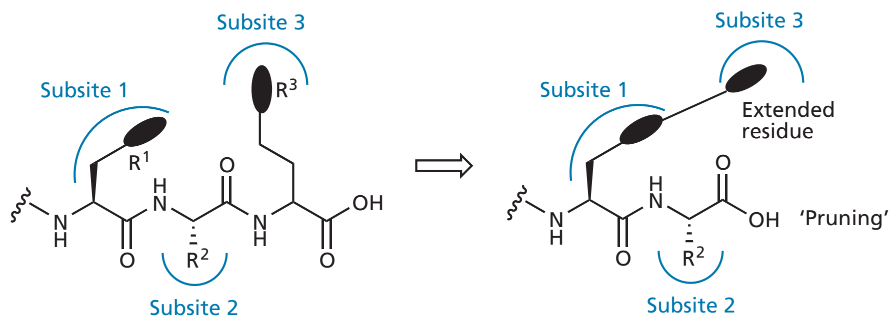


FIGURE 14.40 Extended residues.

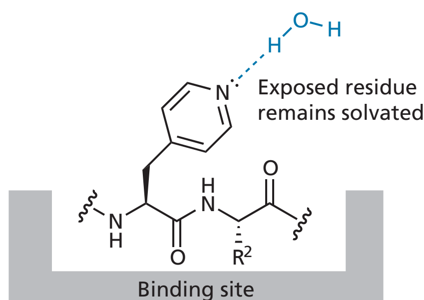


FIGURE 14.41 Altering exposed residues to increase water solubility.

(section 11.3) 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 a stable molecular scaffold that contains substituents capable of mimicking the side chains of amino acids. The substituents should be positioned correctly to match the positions of amino acid side chains 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 helical conformation when they bind to their receptor. 1,1,6-Trisubstituted indanes have been designed to mimic three consecutive amino acid side chains 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 11.3). 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 (section 21.4.2). 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.

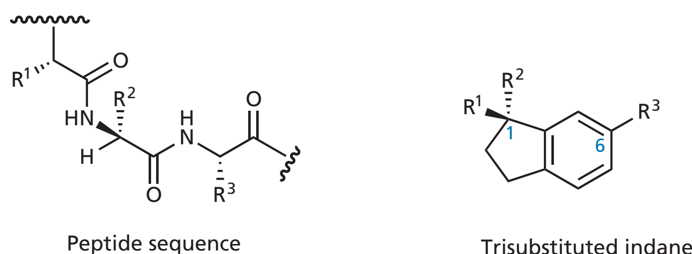


FIGURE 14.42 Trisubstituted indanes as a peptidomimetic for a tripeptide sequence in an α-helix.

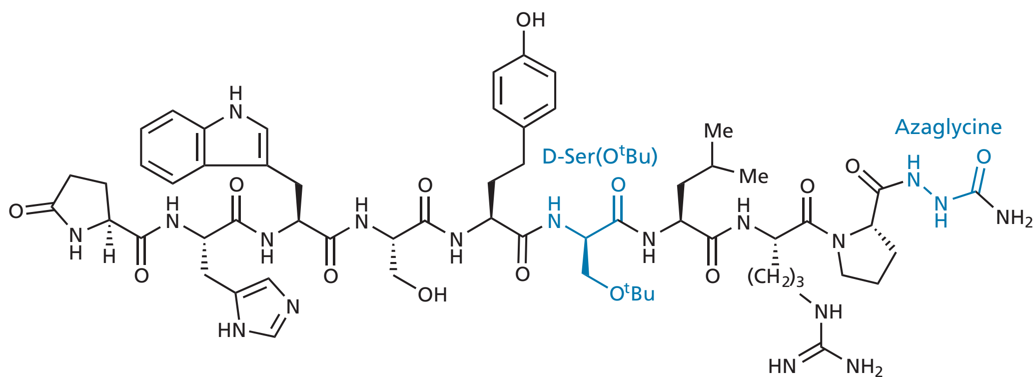


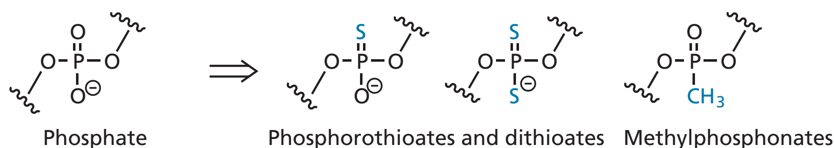
FIGURE 14.43 Goserelin (Zoladex). Moities in blue increase metabolic resistance and receptor affinity.

14.10 Oligonucleotides as drugs

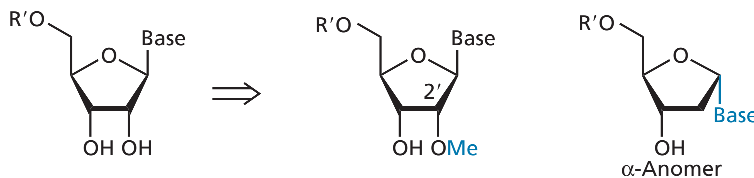
Oligonucleotides are being studied as **antisense drugs** and **aptamers**. The rationale and therapeutic potential of these agents are described in sections 9.7.2 and 10.5. However, there are disadvantages to the use of oligonucleotides as drugs, as they are rapidly degraded by enzymes called **nucleases.** 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 methoxy 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 of 18 deoxynucleotides linked by a phosphorothioate

Phosphate modifications



Sugar modifications



Base modifications

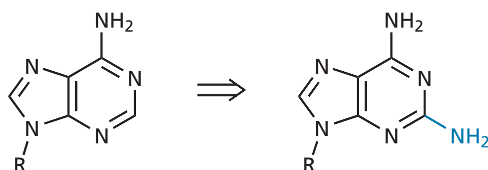


FIGURE 14.44 Modifications on oligonucleotides.

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 treatment of cancer. The drug is currently undergoing Phase III clinical trials in combination with the anticancer drugs **docetaxel** and **irenotecan.**

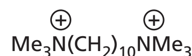
Phosphorothioate oligonucleotides are also being investigated which will target the genetic instructions for **Raf** and **PKCγ**, two proteins which are involved in signal transduction pathways. These too have potential as anticancer drugs.

KEY POINTS

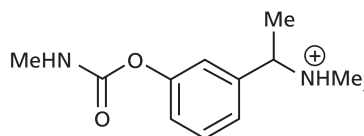
- Neurotransmitters are not effective as drugs as they have a short lifetime in the body, and have poor selectivity for the various types and subtypes of a particular target.
- Hormones are more suitable as drugs and several are used clinically. Others are susceptible to digestive or metabolic enzymes, 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 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 they are no longer recognized by relevant enzymes.
- Antisense molecules have been designed to inhibit the m-RNA molecules that code for the proteins which suppress cell death.

QUESTIONS

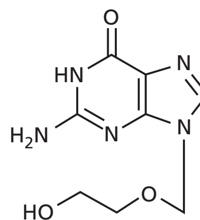
1. Suggest a mechanism by which methenamine (Fig. 14.32) is converted to formaldehyde under acid conditions.
2. Suggest a mechanism by which ampicillin (Fig. 14.33) decomposes in concentrated solution.
3. Carbidopa (Fig. 14.34) protects levodopa from decarboxylation in the peripheral blood supply, but 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 which might have better stability.
5. Decamethonium is a neuromuscular blocking agent which requires both positively charged nitrogen groups to be present. Unfortunately, it is slowly metabolized and lasts too long in the body. Suggest analogues which 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.



Decamethonium

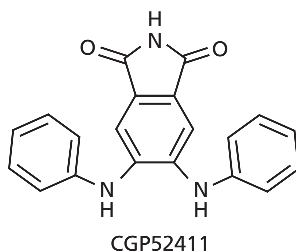


Miotine

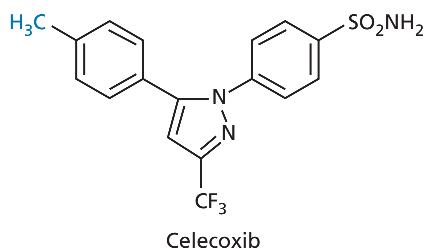


Aciclovir

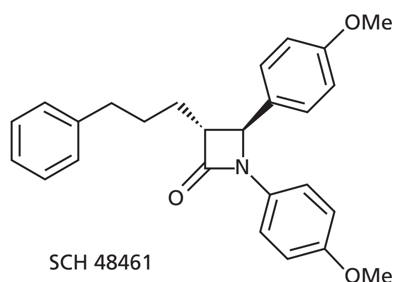
8. CGP 52411 is a useful inhibitor of a protein kinase enzyme. Studies on structure–activity relationships demonstrate that substituents on the aromatic rings such as Cl, Me, or OH are bad for activity. Drug metabolism studies show that *para*-hydroxylation occurs to produce inactive metabolites. How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



CGP52411



Celecoxib



SCH 48461

9. 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?

10. 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

Berg, C., Neumeyer, K., and Kirkpatrick, P. (2003) Teriparatide. *Nature Reviews Drug Discovery,* **2**, 257–258.

Bolgnesi, M. L., Matera, R., Minarini, A. Rosini, M., and Melchiorre, C. (2009) Alzheimer's disease: new approaches to drug discovery, *Current Opinions in Chemical Biology* **13**, 303–308.

Burke, M. (2002) Pharmas market. *Chemistry in Britain* June, 30–32.

Duncan, R. (2003) The dawning era of polymer therapeutics. *Nature Reviews Drug Discovery* **2**, 347–360.

Ezzell, C. (2001) Magic bullets fly again. *Scientific American* October, 28–35.

Ganellin, C. R. and Roberts, S. M. (eds) (1994) Fluconazole, an orally active antifungal agent. In: *Medicinal Chemistry—The Role of Organic Research in Drug Research*, 2nd edn. Academic Press, London.

Harris, J. M. and Chess, R. B. (2003) Effect of pegylation on pharmaceuticals. *Nature Reviews Drug Discovery* **2**, 214–221.

Herr, R. J. (2002) 5-Substituted-1H-tetrazoles as carboxylic acid isosteres: medicinal chemistry and synthetic methods. *Bioorganic and Medicinal Chemistry* **10**, 3379–3393.

Matthews, T., Salgo, M., Greenber, M., Chung, J., DeMasi, R., and Bolognesi, D. (2004) Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes. *Nature Reviews Drug Discovery* **3**, 215–225.

Moreland, L., Bate, G., and Kirkpatrick, P. (2006) *Abatacept Nature Reviews Drug Discovery* **5**, 185–186.

Opalinska, J. B. and Gewirtz, A. M. (2002) Nucleic-acid therapeutics: basic principles and recent applications. *Nature Reviews Drug Discovery* **1**, 503–514.

Pardridge, W. M. (2002) Drug and gene targeting to the brain with molecular Trojan horses. *Nature Reviews Drug Discovery* **1**, 131–139.

Reichert, J. M. and Dewitz, M. C. (2006) Anti-infective monoclonal antibodies: perils and promise of development. *Nature Reviews Drug Discovery* **5**, 191–195.

Rotella, D. P. (2002) Phosphodiesterase 5 inhibitors: current status and potential applications, *Nature Reviews Drug Discovery* **1**, 674–682.

Titles for general further reading are listed on p. 763.

15 Getting the drug to market

The methods by which lead compounds are discovered were discussed in Chapter 12. In Chapters 13 and 14, we looked at how lead compounds can be optimized to improve their target interactions and pharmacokinetic properties. In this chapter, we look at the various issues that need to be tackled before a promising-looking drug candidate reaches the clinic and goes into full-scale production. This final phase is significantly more expensive in terms of time and money than either lead discovery or drug design, and many drugs will fall by the wayside. On average, for every 10,000 structures synthesized during drug design, 500 will reach animal testing, 10 will reach phase I clinical trials, and only 1 will reach the market place. The average overall development cost of a new drug was recently estimated as $800 million or £444 million.

Three main issues are involved in getting the drug to the market. Firstly, the drug has to be tested to ensure that it is safe and effective, and can be administered in a suitable fashion. This involves preclinical and clinical trials covering toxicity, drug metabolism, stability, formulation, and pharmacological tests. Secondly, there are the various patenting and legal issues. Thirdly, the drug has to be synthesized in ever-increasing quantities for testing and eventual manufacture. This is a field known as chemical and process development. Many of these issues have to be tackled in parallel.

15.1 Preclinical and clinical trials

15.1.1 Toxicity testing

One of the first priorities for a new drug is to test if it has any toxicity. This often starts with *in vitro* tests on genetically engineered cell cultures and/or *in vivo* testing on transgenic mice to examine any effects on cell reproduction and to identify potential carcinogens. Any signs of carcinogenicity would prevent the drug being taken any further.

The drug is also tested for acute toxicity by administering sufficiently large doses *in vivo* to produce a toxic effect or death over a short period of time. Different animal species are used in the study and the animals are dissected to test whether particular organs are affected. Further studies on acute toxicity then take place over a period of months, where the drug is administered to laboratory animals at a dose level expected to cause toxicity but not death. Blood and urine samples are analysed over that period and then the animals are killed so that tissues can be analysed by pathologists for any sign of cell damage or cancer.

Finally, long-term toxicology tests are carried out over a period of years at lower dose levels to test the drug for chronic toxic effects, carcinogenicity, special toxicology, mutagenicity, and reproduction abnormalities.

The toxicity of a drug used to be measured by its **LD_{50}** value (the lethal dose required to kill 50% of a group of animals). The ratio of LD_{50} to **ED_{50}** (the dose required to produce the desired effect in 50% of test animals) is known as the **therapeutic ratio** or **therapeutic index**. A therapeutic ratio of 10 indicates an LD_{50}:ED_{50} ratio of 10:1. This means that a 10-fold increase in the ED_{50} dose would result in a 50% death rate. The dose–response curves for a drug's therapeutic and lethal effects can be compared to determine whether the therapeutic ratio is safe or not (Fig. 15.1). Ideally, the curves should not overlap on the x-axis, which means that the more gradual the two slopes, the riskier the drug will be. The graph provided (Fig. 15.1) shows the therapeutic and lethal dose–response curves for a sedative. Here, a 50-mg dose of the drug will act as a sedative for 95% of the test animals, but will be lethal for 5%. Such a drug would be unacceptable, even though it is effective in 95% of cases treated.

A better measure of a drug's safety is to measure the ratio of the lethal dose for 1% of the population to the effective dose for 99% of the population. A sedative drug with the ratio LD_1:ED_{99} of 1 would be safer than the one shown in Fig. 15.1.

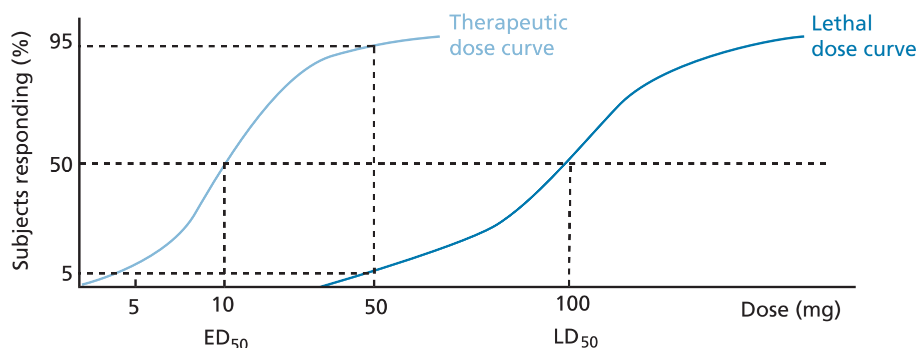


FIGURE 15.1 Comparison of therapeutic and lethal dose curves.

However, LD values and therapeutic ratios are not the best indicators of a drug's toxicity as they fail to register any non-lethal or long-term toxic effects. Therefore, toxicity testing should include a large variety of different *in vitro* and *in vivo* tests designed to reveal different types of toxicity. This is not foolproof, however, and a new and unexpected toxic effect may appear during later clinical trials, which will require the development of a new test. For example, when **thalidomide** was developed, nobody appreciated that drugs could cause fetal deformities, and so there was no test for this. Moreover, even if there had been such tests available, only *in vivo* tests on rabbits would have detected the potential risk.

Many promising drugs fail toxicity testing—a frustrating experience indeed for the drug design teams. For example, the antifungal agent **UK 47265** (Fig. 14.17) was an extremely promising antifungal agent, but *in vivo* tests on mice, dogs, and rats showed that it had liver toxicity and was potentially **teratogenic**. The design team had to synthesize more analogues and finally discovered the clinically useful drug **fluconazole** (section 14.5). A variety of other drugs have had to be withdrawn at a late stage in their development because they were found to have potentially serious effects on the heart, which could result in heart failure caused by inhibition of calcium ion channels known as **HERG K^+ ion channels**. As a result of this, *in vivo* and *in vitro* tests are now carried out at an early stage in drug development to detect this kind of activity (Box 12.3).

It should also be borne in mind that it is rare for a drug to be 100% pure. There are bound to be minor impurities present arising from the synthetic route used, and these may well have an influence on the toxicity of the drug. The toxicity results of a drug prepared by one synthetic route may not be the same for the same drug synthesized by a different route, and so it is important to establish the manufacturing synthesis as quickly as possible (section 15.3).

Another aim of toxicity testing is to discover what dose levels are likely to be safe for future clinical trials. Animal toxicity tests do not, however, always highlight potential problems, and the toxic properties in test animals may differ from those ultimately observed in humans. For example, clinical trials were started for the antiviral agent **fialuridine** (Fig. 15.2) after it had passed toxicity tests on animals. However, the clinical trials had to be stopped when it was found that the drug had severe liver and kidney toxicity. Half the patients (15 in total) suffered liver failure, resulting in 5 deaths, and the 2 survivors requiring liver transplants. It was later found that the drug was incorporated into mitochondrial DNA—something that was not observed in the animal toxicity tests.

Having said that, it is unlikely that the thorny problem of animal testing will disappear for a long time. There are so many variables involved in a drug's interaction with the body that it is impossible to anticipate them all. One has also to take into account that the drug will be metabolized to other compounds, all with their own range of biological properties. It appears impossible, therefore, to predict whether a potential drug will be safe by *in vitro* tests alone. Therein, lies the importance of animal experiments. Only animal tests can test for the unexpected. Unless we are prepared to volunteer ourselves as guinea pigs, animal experiments will remain an essential feature of preclinical trials for many years to come.

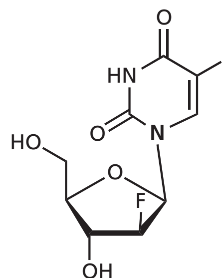


FIGURE 15.2 Fialuridine.

15.1.2 Drug metabolism studies

The body has an arsenal of metabolic enzymes that can modify foreign chemicals in such a way that they are rapidly excreted (sections 11.5 and 11.6). The structures formed from these reactions are called drug metabolites, and it is important to find out what metabolites are formed from any new drug. The structure and stereochemistry of each metabolite has to be determined and the metabolite tested to see what sort of biological activity it might have. This is a safety issue, as some metabolites might prove toxic and others may have side effects that will affect the dose levels that can be used in clinical trials. Ideally, any metabolites that are formed should be inactive and quickly excreted. However, it is quite likely that they will have some form of biological activity (see Box 15.1).

In order to carry out such studies, it is necessary to synthesize the drug with an isotopic label, such as deuterium (^2H or D), carbon-13 (^{13}C), tritium (^3H or T), or carbon-14 (^{14}C). This makes it easier to detect any metabolites that might be formed. Metabolites containing radioisotopes, such as ^3H and ^{14}C, can be detected at small levels by measuring their β radiation. Metabolites containing stable heavy isotopes, such as deuterium, can be detected by mass spectrometry or, in the case of ^{13}C, nuclear magnetic resonance (NMR) spectroscopy.

Normally, a synthesis is carried out to include the isotopic label at a specific position in the molecule. It may be possible to use the established synthetic route for the drug, but, in many cases, a different route may have to be developed in order to incorporate the label in an efficient manner. Usually, it is preferable to include the label at the latest possible stage of the synthesis. It is not necessary to label every single molecule of the drug, as detection methods are sensitive enough to detect the label, even if only a small proportion of the molecules are labelled.

Deuterium or tritium can be very easily incorporated into any molecule containing an exchangeable proton, such as those of an alcohol, carboxylic acid, or phenol. This is done by simply shaking a solution of the drug with D_2O or T_2O. Unfortunately, the label is just as easily lost as a result of proton exchange with water during *in vivo* testing. Therefore, it is best to carry out a synthesis that places the label on the carbon skeleton of the drug. Nevertheless, there is always the possibility that deuterium or tritium could be lost through a metabolic reaction, such that the metabolite is not detected.

Introducing a carbon isotope often means devising a different synthetic route from the normal one. The effort is often worthwhile, though, as there is less chance of the isotope being lost as a result of a metabolic reaction. Having said that, it is not impossible for an isotope to be lost in this way. For example, labelling an *N*-methyl group is asking for problems, as *N*-demethylation is a well-known metabolic reaction.

Once a labelled drug has been synthesized, a variety of *in vitro* and *in vivo* tests can be carried out. *In vivo* tests are carried out by administering the labelled drug to a test animal in the normal way, then taking blood and urine samples for analysis to see if any metabolites

BOX 15.1 Drug metabolism studies and drug design

Drug metabolism studies can sometimes be useful in drug design. On several occasions it has been found that an active drug *in vivo* is inactive *in vitro*. This is often a sign that the structure is not really active at all, but is being converted to the active drug by metabolism. The story of **oxamniquine** (Case study 3) illustrates this. Another example was the discovery that the antihypertensive structure I (Fig. 1) was less active *in vitro* than it was *in vivo*, implying that it was being converted into an active metabolite. Further studies led to the discovery that the active metabolite was **cromakalim**, which proved superior to structure I as an antihypertensive agent.

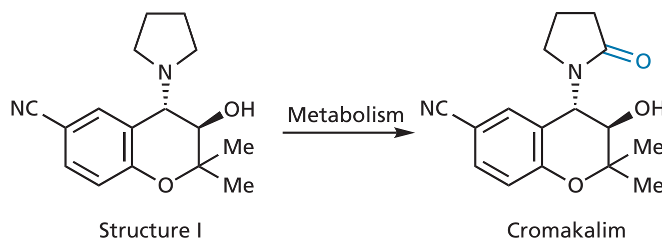


FIGURE 1 Discovery of cromakalim.

have been formed. For radiolabelled drugs, this can be done by using high-performance liquid chromatography (HPLC) with a radioactivity detector. It is important to choose the correct animal for these studies, as there are significant metabolic differences across different species. *In vivo* drug metabolism tests are also carried out as part of phase I clinical trials to see whether the drug is metabolized differently in humans from any of the test animals.

In vitro drug metabolism studies can also be carried out using perfused liver systems, liver microsomal fractions, or pure enzymes. Many of the individual cytochrome P450 enzymes that are so important in drug metabolism are now commercially available.

15.1.3 Pharmacology, formulation, and stability tests

Although the pharmacology of the drug may have been studied during the drug discovery and drug design stages, it is usually necessary to carry out more tests to see whether the drug has activity at targets other than the intended one, and to gain a better insight into the drug's mechanism of action. These studies also determine a dose–response relationship and define the drug's duration of action.

Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. For orally taken drugs, this usually involves incorporating the drug into a tablet or a capsule. It is important to appreciate that a tablet contains a variety of other substances apart from the drug itself, and studies have to be carried out to ensure that the drug is compatible with these other substances. Pre-formulation involves the characterization of a drug's physical, chemical, and mechanical properties in order to choose what other ingredients should be used in the preparation. Formulation studies then consider such factors as particle size, salt forms, crystal polymorphism, solvates, pH, and solubility, as all of these can influence **bioavailability** and, hence, the activity of a drug. The drug must be combined with inactive additives by a method which ensures that the quantity of drug present is consistent in each dosage unit. The dosage should have a uniform appearance, with an acceptable taste, tablet hardness, or capsule disintegration.

It is unlikely that these studies will be complete by the time clinical trials commence. This means that simple preparations are developed initially for use in phase I clinical trials (section 15.1.4.1). These typically consist of hand-filled capsules containing a small amount of the drug and a diluent. Proof of the long-term stability of these formulations is not required, as they will be used in a matter of days. Consideration has to be given to what is called the **drug load**—the ratio of the active drug to the total contents of the dose. A low drug load may cause homogeneity problems. A high drug load may pose flow problems or require large capsules if the compound has a low bulk density.

By the time phase III clinical trials are reached (section 15.1.4.3), the formulation of the drug should have been developed to be close to the preparation that will ultimately be used in the market. A knowledge of stability is essential by this stage and conditions must have been developed to ensure that the drug is stable in the preparation. If the drug proves unstable, it will invalidate the results from clinical trials as it would be impossible to know what the administered dose actually was. Stability studies are carried out to test whether temperature, humidity, ultraviolet light, or visible light have any effect, and the preparation is analysed to see if any degradation products have been formed. It is also important to check whether there are any unwanted interactions between the preparation and the container. If a plastic container is used, tests are carried out to see whether any of the ingredients become adsorbed on to the plastic, and whether any plasticizers, lubricants, pigments, or stabilizers leach out of the plastic into the preparation. Even the adhesives for the container label need to be tested to ensure that they do not leach through the plastic container into the preparation. Despite extensive testing, there is always the possibility that an unexpected problem might occur that can result in contamination of drugs. For example, several batches of paracetamol had to be withdrawn from the market in 2009 because they had a musty smell and caused nausea and stomach pains. It was discovered that the batches were contaminated with the breakdown product of a fungicide that had been applied to the wooden pallets used to transport packaging materials.

15.1.4 Clinical trials

Once the preclinical studies described in the previous sections have been completed, the company decides whether to proceed to clinical trials. Usually, this will happen if the drug has the desired effect in animal tests, demonstrates a distinct advantage over established therapies, and has acceptable pharmacokinetics, few metabolites, a reasonable half-life, and no serious side effects. Clinical trials are the province of the clinician rather than the scientist, but this does not mean that the research team can wash its hands of the candidate drug and concentrate on other things. Many promising drug candidates fail this final hurdle and further analogues may need to be prepared before a clinically acceptable drug is achieved. For example, a study carried out for the period 1990–2002 showed that there was an average failure rate of 90% for the drugs

that reached clinical trials. Clinical trials involve testing the drug on volunteers and patients, so the procedures involved must be ethical and beyond reproach. These trials can take 5–7 years to carry out, involve hundreds to thousands of patients, and be extremely expensive. There are four phases of clinical trials.

15.1.4.1 Phase I studies

Phase I studies take about a year and involve 100–200 volunteers. They are carried out on healthy human volunteers to provide a preliminary evaluation of the drug's safety, its pharmacokinetics, and the dose levels that can be administered, but they are not intended to demonstrate whether the drug is effective or not.

The drug is tested at different dose levels to see what levels can be tolerated. For each dose level, 6–12 subjects are given the active drug and 2–4 subjects are given a **placebo**. Normally, the initial dose is a tenth of the highest safest dose used in animal testing. Pharmacokinetic studies are then carried out in order to follow the drug and its metabolites. After a full safety assessment has been made, a higher dose is given, and this is continued until mild adverse effects are observed. This indicates the maximum tolerated dose and further studies will then concentrate on smaller doses.

During the study, volunteers do not take medication, caffeine, alcohol, or cigarettes. This is to avoid any complications that might arise because of drug–drug interactions (section 11.4.6). As a result, these effects may appear in later phase studies. Studies are carried out early on, however, to determine whether there are any interactions between the drug and food. This is essential in order to establish when the dose should be taken relative to meals.

Another study involves 4–8 healthy volunteers being given a radiolabelled drug in order to follow the absorption, distribution, and excretion of the drug. These studies also determine how the drug is metabolized in humans.

Studies may be carried out on special age groups. For example, drugs intended for Alzheimer's disease are tested on healthy, elderly volunteers to test the drug's pharmacokinetics in that particular population. Studies may also be carried out to test whether there are interactions with any other drugs likely to be taken by such a cohort. For example, a drug for Alzheimer's disease will be used mostly on elderly patients who are likely to be taking drugs such as diuretics or anticoagulants. Special studies may be carried out on volunteers with medical conditions that will affect the pharmacokinetics of the drug. These include patients with abnormal rates of metabolism, liver or kidney problems, inflammatory bowel disease, or other gastrointestinal diseases.

Bioavailability refers to the fraction of administered drug that reaches the blood supply in a set period of time. This can vary depending on a variety of factors, such as the crystal form of the drug, whether the drug is administered as a tablet or a capsule, or a variation in the constituents of a tablet or capsule. As a result, it is important to check that bioavailability remains the same should there be any alteration to the manufacturing, formulation, or storage processes. Such checks are called **bioequivalence** studies. For example, bioequivalence studies are required when different dosage forms are used in the early and late phases of clinical trials. Powder-filled capsules are used frequently in phase I, whereas tablets are used in phases II and III. Therefore, it is necessary to establish that these formulations show bioequivalence in healthy volunteers. In addition, it has to be demonstrated that dissolution of both formulations is similar.

In situations where the drug is potentially toxic and is to be used for a life threatening disease, such as AIDS or cancer, volunteer patients are used for phase I studies rather than healthy volunteers.

The decision on whether to proceed to phase II can be difficult, as only a limited amount of safety data is available. Any adverse effects that are observed may or may not be due to the drug. For example, abnormal liver function in a healthy patient may be due to the drug or to alcohol. Nevertheless, evidence of a serious adverse effect will usually result in clinical trials being terminated.

15.1.4.2 Phase II studies

Phase II studies generally last about two years and may start before phase I studies are complete. They are carried out on patients to establish whether the drug has the therapeutic property claimed, to study the pharmacokinetics and short-term safety of the drug, and to define the best dose regimen. Phase II trials can be divided into early and late studies (IIa and IIb respectively).

Initial trials (phase IIa) involve a limited number of patients to see if the drug has any therapeutic value at all, and to see if there are any obvious side effects. If the results are disappointing, clinical trials may be terminated at this stage.

Later studies (IIb) involve a larger numbers of patients. They are usually carried out as double-blind, placebo-controlled studies. This means that the patients are split into two groups where one group receives the drug and the other group receives a placebo. In a double-blind study, neither the doctor nor the patient knows whether a placebo or drug is administered. In the past, it has been found that investigators can unwittingly 'give the game away' if they know which patient is getting the actual drug. The studies demonstrate whether the patients receiving the drug show an improvement relative to the

patients receiving the placebo. The placebo effect can be particularly marked for patients involved in trials for novel antidepressives or anxiolytics. Different dosing levels and regimes are also determined to find the most effective. Most phase II trials require 20–80 patients per dose group to demonstrate efficacy.

Some form of rescue medication may be necessary for those patients taking a placebo. For example, it would be unethical to continue asthmatic patients on a placebo if they suffer a severe asthmatic attack. A conventional drug would be given and its use documented. The study would then compare how frequently the placebo group needed to use the rescue medicine compared with those taking the new drug. With life-threatening diseases, such as AIDS or cancer, the use of a placebo is not ethical and an established drug is used as a standard comparison.

The **endpoint** is the measure that is used to determine whether a drug is successful or not. It can be any parameter that is relevant, measurable, sensitive, and ethically acceptable. Examples of endpoints include blood assays, blood pressure, tumour regression, and the disappearance of an invading pathogen from tissues or blood. Less defined endpoints include perception of pain, use of rescue medications, and level of joint stiffness.

15.1.4.3 Phase III studies

Phase III studies normally take about three years and can be divided into phases IIIa and IIIb. These studies may begin before phase II studies are completed. The drug is tested in the same way as in phase II, using double-blind procedures, but on a much larger sample of patients. Patients taking the drug are compared with patients taking a placebo or another available treatment. Comparative studies of this sort must be carried out without bias and this is achieved by randomly selecting the patients—those who will receive the new drug and those who will receive the alternative treatment or placebo. Nevertheless, there is always the possibility of a mismatch between the two groups with respect to factors such as age, race, sex, or disease severity, and so the greater the number of patients in the trial the better.

Phase IIIa studies establish whether the drug is really effective or whether any beneficial effects are psychological. They also allow further 'tweaking" of dose levels to achieve the optimum dose. Any side effects not previously detected may be picked up with this larger sample of patients. If the drug succeeds in passing phase IIIa, it can be registered. Phase IIIb studies are carried out after registration, but before approval. They involve a comparison of the drug with those drugs that are already established in the field.

In certain circumstances where the drug shows a clear beneficial effect early on, the phase III trials may be terminated earlier than planned. Some patients in the phase III studies will be permitted to continue taking the drug if it has proved effective, and will be monitored to assess the long-term safety of the drug. However, serious side effects observed during phase III may result in early termination of the clinical trials and the abandonment of further development. For example, the development of Pfizer's **torcetrapib** (a cholesterol-lowering agent) was terminated in 2006 when it was discovered that there was a statistically increased risk of death associated with its use. The drug had been developed over a period of 16 years at a cost of $800 million and represented one of the costliest failures in pharmaceutical history.

15.1.4.4 Phase IV studies

The drug is now placed on the market and can be prescribed, but it is still monitored for effectiveness and for any rare or unexpected side effects. In a sense, this phase is a never-ending process as unexpected side effects may crop up many years after the introduction of the drug. In the UK, the medicines committee runs a voluntary yellow card scheme where doctors and pharmacists report suspected adverse reactions to drugs. This system has revealed serious side effects for a number of drugs after they had been put on the market. For example, the β-blocker **practolol** had to be withdrawn after several years of use because some patients suffered blindness and even death. The toxic effects were unpredictable and are still not understood, and so it has not been possible to develop a test for this effect.

The diuretic agent **tienilic acid** (Fig. 15.3) had to be withdrawn from the market because it damaged liver cells in 1 out of every 10,000 patients. The anti-inflammatory agent **phenylbutazone** (Fig. 15.3) can cause a rare, but fatal, side effect in 22 patients out of every million treated with the drug! Such a rare toxic effect would clearly not be detected during phase III trials. A more recent example is **cerivastatin** (Fig. 15.3), which was marketed as a potent anticholesterol drug (Case study 1). Unfortunately, it had to be withdrawn in 2001 as a result of adverse drug–drug interactions which resulted in muscle damage and the deaths of 40 people worldwide. **Rofecoxib** (VIOXX) (Fig. 7.11) was used to treat rheumatoid arthritis for five years before a clinical trial carried out after its release showed that it was associated with increased risks of heart attack and stroke. The drug was withdrawn voluntarily by Merck in 2004, but in the 5 years it had been on the market, rofecoxib had been prescribed to 1.3 million patients in the USA and to 700,000 patients in 80 other countries. Annual profits from the drug had reached $1.2 billion, which represented 18% of Merck's net income. The loss of this income was so serious that the company's share price dropped 27% in value in a single day. Not only that,

the company was faced with a lengthy litigation battle as thousands of patients sought compensation for alleged personal injuries resulting from the use of the drug.

The withdrawal of drugs could, potentially, be avoided if the genomes of individual patients were 'fingerprinted' to establish who might be at risk from rare toxic effects—a process known as **personalized medicine** (see also section 21.1.11). For example, genetic fingerprinting has been used to determine doses for the anticoagulant drug **warfarin** (Fig. 11.7) for different individuals. Similarly, genetic fingerprinting has been used to identify those patients most likely to respond to the anticancer drug **panitumumab** (Box 21.12). It may also be possible to re-establish drugs that have previously been withdrawn if a biomarker can be developed that identifies those patients at risk. For example, the anti-inflammatory agent **lumiracoxib** (Fig. 15.3) is a selective inhibitor for the **cyclooxygenase-2** enzyme and was introduced to the European market in 2006, only to be withdrawn a year later as a result of severe liver toxicity in a small number of patients. A genetic biomarker has now been identified that can determine which patients are at risk to these side effects, and there is a possibility that the drug may be re-introduced.

15.1.4.5 Ethical issues

In phases I–III of clinical trials, the permission of the patient is mandatory. However, ethical problems can still arise. For example, unconscious patients and mentally ill patients cannot give consent, but might benefit from the improved therapy. Should one include them or not? The ethical problem of including children in clinical trials is also a thorny issue, and so most clinical trials exclude them. As most licensed drugs have been licensed for adults, it means that around 40% of medicines given to children have never actually been tested on that age group. When it comes to prescribing for children, clinicians are faced with the problem of deciding what dose levels to use, and simple arithmetic mistakes made by tired health staff can have tragic consequences. Furthermore, children are not small adults. It is not a simple matter of modifying dose levels based purely on relative body weight. The pharmacodynamic and pharmacokinetic properties of a drug are significantly different in a child compared with an adult. For example, drug metabolism varies considerably with the age and development of a child. Adverse side effects also differ. The **grey baby syndrome** associated with **chloramphenicol** is one such example (Box 19.15).

Regulatory and professional bodies are actively addressing these issues in different countries worldwide. For example, the **Medicines for Children Research Network** was set up in England with the aim of carrying out high quality clinical studies on children for both established drugs and new chemical entities. In 2005, the **British National Formulary for Children** was published and there is pressure for European-wide regulation for

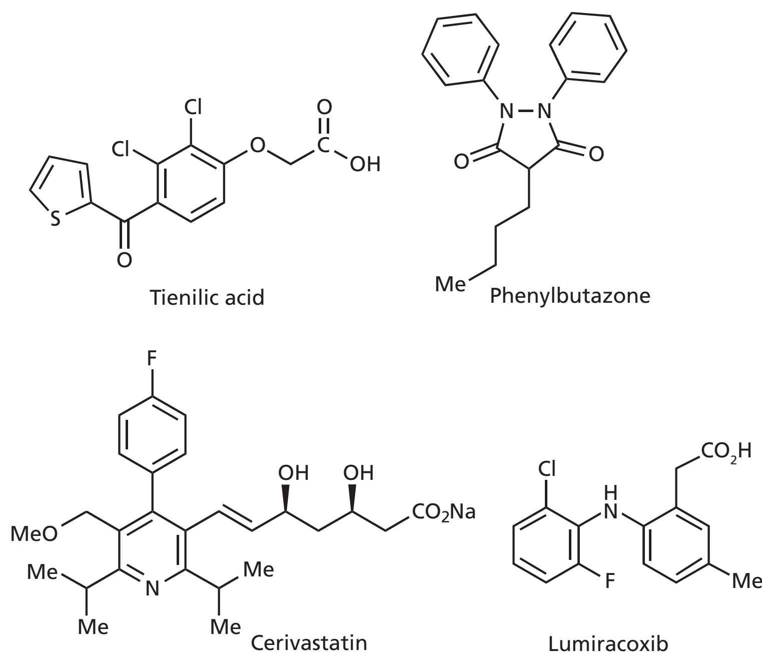


FIGURE 15.3 Drugs which have been removed from the market as a result of rare toxic side effects.

the testing and prescribing of children's medicines. The European Medicines Agency now offers a licence extension for new drugs to companies who have included testing on children. A newly created licence called the **Paediatric Use Marketing Authorisation** (PUMA) has also been introduced for established drugs.

KEY POINTS

- Toxicity tests are carried out *in vitro* and *in vivo* on drug candidates to assess acute and chronic toxicity. During animal studies, blood and urine samples are taken for analysis. Individual organs are analysed for tissue damage or abnormalities. Toxicity testing is important in defining what the initial dose level should be for phase I clinical trials.
- Drug metabolism studies are carried out on animals and humans to identify drug metabolites. The drug candidate is labelled with an isotope in order to aid the detection of metabolites.
- Pharmacology tests are carried out to determine a drug's mechanism of action and to determine whether it acts at targets other than the intended one.
- Formulation studies aim to develop a preparation of the drug which can be administered during clinical trials and beyond. The drug must remain stable in the preparation under a variety of environmental conditions.
- Clinical trials involve four phases. In phase I, healthy volunteers are normally used to evaluate the drug's safety, its pharmacokinetics, and the dose levels that can be administered safely. Phase II studies are carried out on patients to assess whether the drug is effective, to give further information on the most effective dose regimen, and to identify side effects. Phase III studies are carried out on larger numbers of patients to ensure that results are statistically sound and to detect less common side effects. Phase IV studies are ongoing and monitor the long-term use of the drug in specific patients, as well as the occurrence of rare side effects.

15.2 Patenting and regulatory affairs

15.2.1 Patents

Having spent enormous amounts of time and money on research and development, a pharmaceutical company, quite rightly, wants to reap the benefit of all its hard work. To do so, it needs to have the exclusive rights to sell and manufacture its products for a reasonable period of time, and at a price which will not only recoup its costs, but that will generate sufficient profits for further research and development. Without such rights, a competitor could synthesize the same product without suffering the expense involved in designing and developing it.

Patents allow companies the exclusive right to the use and profits of a novel pharmaceutical for a limited term. In order to gain a patent, the company has to first submit or file the patent. This should reveal what the new pharmaceutical is, what use it is intended for, and how it can be synthesized. This is no straightforward task. Each country has its own patents, so the company has to first decide in which countries it is going to market its new drug and then file the relevant patents. Patent law is also very precise and varies from country to country. Therefore, submitting a patent is best left to the patent attorneys and lawyers who are specialists in the field. The cost and effort involved in obtaining patents from different countries can be reduced in two ways. First, a patent application can be made to the **European Patent Office** (**EPO**). If it is approved, a European patent is granted, which can then be converted to country-specific patents relevant to the individual countries belonging to the **European Patent Convention** (**EPC**). There are 27 such countries and the applicant can decide how many of the 27 individual patents should be taken out. A second approach is to file an international application which designates one or more of the 122 countries who have signed up to the **Patent Cooperation Treaty** (**PCT**). An **International Search Report** (**ISR**) and **International Preliminary Examination Report** (**IPER**) can be obtained, which can then be used when applying for patents from individual countries. No PCT or international patents are awarded, but the reports received help the applicant to decide which patent applications to individual countries are likely to succeed.

Once a patent has been filed, the patent authorities decide whether the claims are novel and whether they satisfy the necessary requirements for that patent body. One universal golden rule is that the information supplied has not been revealed previously, either in print or by word of mouth. As a result, pharmaceutical companies only reveal their work after the structures involved have been safely patented.

It is important that a patent is filed as soon as possible. Such is the competition between the pharmaceutical companies that it is highly likely that a novel agent discovered by one company may be discovered by a rival company only weeks or months later. This means that patents are filed as soon as a novel agent or series of agents is found to have significant activity. Usually, the patent is filed before the research team has had the chance to start all the extensive preclinical tests that need to be carried out on novel drugs. It may not even have synthesized all the possible structures it is intending to make. Therefore, the team is in no position to identify which specific compound in a series of structures is likely

to be the best drug candidate. As a result, most patents are designed to cover a series of compounds belonging to a particular structural class, rather than one specific structure. Even if a specific structure has been identified as the best drug candidate, it is best to write the patent to cover a series of analogues. This prevents a rival company making a close analogue of the specified structure and selling it in competition. All the structures that are to be protected by the patent should be specified in the patent, but only a representative few need to be described in detail.

Patents in most countries run for 20 years after the date of filing. This sounds a reasonable time span, but it has to be remembered that the protection period starts from the time of filing, not from when the drug comes onto the market. A significant period of patent protection is lost because of the time required for preclinical tests, clinical trials, and regulatory approval. This often involves a period of 6–10 years. In some cases, this period may threaten to be even longer, in which case the company may decide to abandon the project as the duration of patent protection would be deemed too short to make sufficient profits. This illustrates the point that not all patents lead to a commercially successful product.

The income obtained from a successful drug is so important to a company's financial viability that **pay-for-delay deals** have become a growing trend in the pharmaceutical sector for drugs that are nearing the end of their patent lifetime. These involve a pharmaceutical company making a deal with a manufacturer that specializes in producing off-patent drugs or **generics**. The generic manufacturer receives a lump sum if it agrees to delay manufacturing the generic version for an agreed time period (typically a year), allowing the inventor to gain several months of additional income. One reason for this trend is the fact that the patents of many profitable small drug molecules are due to run out in the period 2010–14. As there are fewer drugs now reaching the market to take their place, this has become known as the **patent cliff** and it has caused many large pharmaceutical companies to rethink their business strategy. Many firms are now outsourcing projects to other companies and concentrating more on research into novel drug delivery processes or combination therapies using existing drugs.

Patents can be taken out to cover specific products, the medicinal use of the products, the synthesis of the products, or, preferably, all three of these aspects. Taking out a patent which only covers the synthesis of a novel product offers poor patent protection. A rival company could quite feasibly develop a different synthesis to the same structure and then sell it legally.

One of the current issues in the patent area is **chiral switching**. In the period 1983–87, 30% of approved drugs were pure enantiomers, 29% were racemates, and 41% were achiral. Nowadays, most of the drugs reaching the market are either achiral or pure enantiomers. The problem with racemates is that each enantiomer usually has a different level of activity. Moreover, the enantiomers often differ in the way they are metabolized and in their side effects. Consequently, it is better to market the pure enantiomer rather than the racemate. The issue of chiral switching relates mostly to racemic drugs that have been on the market for several years and are approaching the end of their patent life. By switching to the pure enantiomer, companies can argue that it is a new invention and take out a new patent. Timing is important and, ideally, the company wants to have the pure enantiomer reaching the market just as the patent on the original racemate is expiring. However, they have to prove that the pure enantiomer is an improvement on the original racemate and that they could not reasonably have been expected to know that when the racemate was originally patented. A full appreciation of how different enantiomers can have different biological properties was realized in the 1980s, and so chiral switches have normally been carried out on racemic drugs that reached the market before that date. It is not possible to patent a new drug in its racemic form today then expect to market it later as the pure enantiomer, as the issue of stereoisomerism is now an established fact.

Bupivacaine is an example of an established chiral drug that has undergone chiral switching. It is a long-lasting local anaesthetic used as a spinal and epidural anaesthetic for childbirth and hip replacements, and acts by blocking sodium ion channels in nerve axons. Unfortunately, it also affects the heart, which prevents it being used for intravenous injections. The *S*-enantiomer of bupivacaine is called **levobupivacaine** (Fig. 15.4). It has less severe side effects and is a safer local anaesthetic. Other examples of drugs that have undergone chiral switching include **salbutamol** (section 23.10.3) and **omeprazole** (section 25.3). **Armodafinil** (Fig. 15.4) is another example of chiral switching and was approved in 2007 for the treatment of excess sleepiness. It is an enantiomer of the racemic drug **modafinil**.

New patents can also be taken out on an existing drug if it is marketed as a new salt, or if the formulation is altered in a significant way. Finally, there may be scope for pharmaceutical companies to develop patented drugs in veterinary medicine rather than human medicine.

A looming threat to the patents and profits of the pharmaceutical industry relates to rules called the **Trade Related Aspects of Intellectual Property Rights** (TRIPS), which are set out by the **World Trade Organization** (WTO). Under these rules a country can grant a company a compulsory licence to produce a patented drug if that country faces a national health crisis. In 2012, India used these rules to allow one of its generics companies to produce the currently patented anticancer

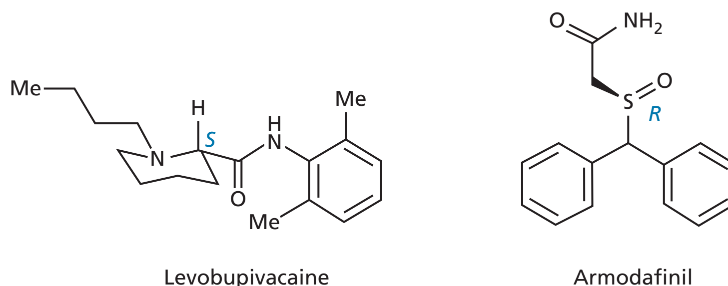


FIGURE 15.4 Levobupivacaine and armodafinil.

drug **sorafenib** at a fraction of its current cost, because it was seen as a life-saving drug. This might set a precedent which would result in any novel, life-saving drug being prone to similar compulsory licences. If so, there is a risk that the pharmaceutical industry would cut back its commitment to the design of novel anticancer drugs.

15.2.2 Regulatory affairs

15.2.2.1 The regulatory process

Regulatory bodies such as the **Food and Drug Administration** (**FDA**) in the USA and the **European Agency for the Evaluation of Medicinal Products** (**EMEA**) in Europe come into play as soon as a pharmaceutical company believes it has a useful drug. Before clinical trials can begin, the company has to submit the results of its scientific and preclinical studies to the relevant regulatory authority. In the USA, this takes the form of an **Investigational Exemption to a New Drug Application** (**IND**), which is a confidential document submitted to the FDA. The IND should contain information regarding the chemistry, manufacture, and quality control of the drug, as well as information on its pharmacology, pharmacokinetics, and toxicology. The FDA assesses this information and then decides whether clinical trials can begin. Dialogue then continues between the FDA and the company throughout the clinical trials. Any adverse results must be reported to the FDA, who will discuss with the company whether the trials should be stopped.

If the clinical trials proceed smoothly, the company applies to the regulatory authority for marketing approval. In the USA, this involves the submission of a **New Drug Application** (**NDA**) to the FDA; in Europe, the equivalent submission is called a **Marketing Authorization Application (MAA)**. An NDA or MAA is typically 400–700 volumes in size, with each volume containing 400 pages! The application has to state what the drug is intended to do, along with scientific and clinical evidence for its efficacy and safety. It should also give details of the chemistry and manufacture of the drug, as well as the controls and analysis which will be in place to ensure that the drug has a consistent quality. Any advertising and marketing material must be submitted to ensure that it makes accurate claims and that the drug is being promoted for its intended use. The labelling of a drug preparation must also be approved to ensure that it instructs physicians about the mechanism of action of the drug, the medical situations for which it should be used, and the correct dosing levels and frequency. Possible side effects, toxicity, or addictive effects should be detailed, as well as special precautions which might need to be taken (e.g. avoiding drugs that interact with the preparation).

The FDA has inspectors who will visit clinical investigators to ensure that their records are consistent with those provided in the NDA, and that patients have been protected adequately. Finally, an approval letter is given to the company and the product can be launched, but the FDA will continue to monitor the promotion of the product, as well as further information regarding any unusual side effects.

Once an NDA is approved, any modifications to a drug's manufacturing synthesis or analysis must be approved. In practice, this means that the manufacturer will stick with the route described in the NDA and perfect that, rather than consider alternative routes.

An abbreviated NDA can be filed by manufacturers who wish to market a generic variation of an approved drug whose patent life has expired. The manufacturer is only required to submit chemistry and manufacturing information, and demonstrate that the product is comparable with the product already approved.

The term **new chemical entity** (**NCE**) or **new molecular entity** (**NME**) refers to a novel drug structure. In the 1960s about 70 NCEs reached the market each year, but this had dropped to fewer than 30 per year by 1971. In part, this was due to more stringent testing regulations that were brought in after the thalidomide disaster. Another factor was the decreasing number of lead compounds available at the time. Since the 1970s, there has

been an emphasis on understanding the mechanism of a disease and designing new drugs in a scientific fashion. Although the approach has certainly been more scientific than the previous trial and error approach, the number of NCEs reaching the market is still low. In 2002, only 18 NCEs were approved by the FDA and 13 by the EMEA.

15.2.2.2 Fast-tracking and orphan drugs

The regulations of many regulatory bodies include the possibility of **fast-tracking** certain types of drug, so that they reach the market as quickly as possible. Fast-tracking is made possible by requiring a smaller number of phase II and phase III clinical trials before the drug is put forward for approval. Fast-tracking is carried out for drugs that show promise for diseases where no current therapy exists and for drugs that show distinct advantages over existing ones in the treatment of life-threatening diseases, such as cancer. An example of a fast tracked drug is **oseltamivir** (Tamiflu) for the treatment of flu (section 20.8.3.4).

Orphan drugs are drugs that are effective against relatively rare medical problems. In the USA, an orphan drug is defined as one that is used for less than 200,000 people. Because there is a smaller market for such drugs, pharmaceutical companies may be less likely to reap huge financial benefits and may decide not to develop and market an orphan drug. Therefore, financial and commercial incentives are given to firms in order to encourage the development and marketing of such drugs. The attitude of pharmaceutical companies towards orphan drugs may well change following the therapeutic and financial success of **imatinib**—an orphan drug which was designed against a specific form of cancer (section 21.6.2.2). Although there are not many patients for a specific orphan disease, it is estimated that tens of millions of patients in Europe suffer from some form of orphan disease, and so there is a significant market to be tapped.

15.2.2.3 Good laboratory, manufacturing, and clinical practice

Good Laboratory Practice (**GLP**) and **Good Manufacturing Practice** (**GMP**) are scientific codes of practice for a pharmaceutical company's laboratories and production plants. They detail the scientific standards that are necessary and the company must prove to regulatory bodies that it is adhering to these standards.

GLP regulations apply to the various research laboratories involved in pharmacology, drug metabolism, and toxicology studies. GMP regulations apply to the production plant and chemical development laboratories. They encompass the various manufacturing procedures used in the production of the drug, as well as the procedures used to ensure that the product is of a consistently high quality.

As part of GMP regulations, the pharmaceutical company is required to set up an independent quality control unit which monitors a wide range of factors including employee training, the working environment, operational procedures, instrument calibration, batch storage, labelling, and the quality control of all solvents, intermediates, and reagents used in the process. The analytical procedures which are used to test the final product must be defined, as well as the specifications that have to be met. Each batch of drug that is produced must be sampled to ensure that it passes those specifications. Written operational instructions must be in place for all special equipment (e.g. freeze dryers), and standard operating procedures (SOPs) must be written for the use, calibration, and maintenance of equipment.

Detailed and accurate paperwork on the above procedures must be available for inspection by the regulatory bodies. This includes calibration and maintenance records, production reviews, batch records, master production records, inventories, analytical reports, equipment cleaning logs, batch recalls, and customer complaints. Although record-keeping is crucial, it is possible that the extra paperwork involved can stifle innovations in the production process.

Investigators involved in clinical research must demonstrate that they can carry out the work according to **Good Clinical Practice** (**GCP**) regulations. The regulations require proper staffing, facilities, and equipment for the required work, and each test site involved must be approved. There must also be evidence that a patient's rights and well-being are properly protected. In the USA, approval is given by the **Institutional Review Board** (**IRB**). While the work is in progress, regulatory authorities may carry out data audits to ensure that no research misconduct is taking place (e.g. plagiarism, falsification of data, poor research procedures, etc.). In the UK, the **General Medical Council** or the **Association of British Pharmaceutical Industry** can discipline unethical researchers. Problems can arise as a result of the pressures that are often placed on researchers to obtain their results as speedily as possible. This can lead to hasty decisions, which result in mistakes and poorly thought-out procedures. There have also been individuals who have deliberately falsified results or have cut corners. Sometimes personal relationships can prove a problem. The investigator can be faced with a difficult dilemma between doing the best for the patient and maintaining good research procedures. Patients may also mislead clinicians if they are desperate for a new cure and falsify their actual condition in order to take part in the trial.

Other patients have been known to get their drugs analysed to see if they are getting a placebo or the drug.

15.2.2.4 Analysis of cost versus benefits

A medicine that is successfully licensed and reaches the market has one other hurdle to negotiate—a cost versus benefit analysis carried out by individual government authorities. For example, the UK's **National Institute for Health and Clinical Excellence** (**NICE**) determines whether novel drugs should be used by the National Health Service and have turned their thumbs down at several approved anticancer drugs, such as **lapatinib**, **dasatinib**, **sorafenib**, **nilotinib**, **bevacizumab**, and **temsirolimus**. The decisions of NICE have a significant economic impact on world-wide pharmaceutical sales, as more than 60 other countries adopt the NICE guidelines rather than carrying out their own cost/benefit analysis.

KEY POINTS

- Patents are taken out as soon as a useful drug has been identified. They cover a structural class of compounds rather than a single structure.
- A significant period of the patent is lost as a result of the time taken to get a drug to the market place.
- Patents can cover structures, their medicinal use, and their method of synthesis.
- Regulatory bodies are responsible for approving the start of clinical trials and the licensing of new drugs for the market place.
- Drugs that show promise in a field which is devoid of a current therapy may be fast-tracked.
- Special incentives are given to companies to develop orphan drugs. These are drugs that are effective in rare diseases.
- Pharmaceutical companies are required to abide by professional codes of practice known as Good Laboratory Practice, Good Manufacturing Practice, and Good Clinical Practice.

15.3 Chemical and process development

15.3.1 Chemical development

Once a compound goes forward for preclinical tests, it is necessary to start the development of a large-scale synthesis as soon as possible. This is known as chemical development and is carried out in specialist laboratories. To begin with, a quantity of the drug may be obtained by scaling up the synthetic route used by the research laboratories. In the longer term, however, such routes often prove unsuitable for large-scale manufacture. There are several reasons for this. During the drug discovery/design phase, the emphasis is on producing as many different compounds as possible in as short a period of time as possible. The yield is unimportant as long as sufficient material is obtained for testing. The reactions are also done on a small scale, which means that the cost is trivial, even if expensive reagents or starting materials are used. Hazardous reagents, solvents, or starting materials can also be used because of the small quantities involved.

The priorities in chemical development are quite different. A synthetic route has to be devised which is straightforward, safe, cheap, efficient, high yielding, has the minimum number of synthetic steps, and will provide a consistently high-quality product which meets predetermined specifications of purity.

During chemical development, the conditions for each reaction in the synthetic route are studied closely and modified in order to get the best yields and purity. Different solvents, reagents, and catalysts may be tried. The effects of temperature, pressure, reaction time, excess reagent or reactant, concentration, and method of addition are studied. Consideration is also given to the priorities required for scale-up. For example, the original synthesis of aspirin from salicylic acid involved acetylation with acetyl chloride (Fig. 15.5). Unfortunately, the by-product from this is hydrochloric acid, which is corrosive and environmentally hazardous. A better synthesis involves acetic anhydride as the acylating agent. The by-product formed here is acetic acid, which does not have the unwanted properties of hydrochloric acid and can also be recycled.

Therefore, the final reaction conditions for each stage of the synthesis may be radically different from the original conditions, and it may even be necessary to abandon the original synthesis and devise a completely different route (Box 15.3).

Once the reaction conditions for each stage have been optimized, the process needs to be scaled up. The priorities here are cost, safety, purity, and yield. Expensive or hazardous solvents or chemicals should be avoided and replaced by cheaper, safer alternatives. Experimental procedures may have to be modified. Several operations carried out on a research scale are impractical on a large scale. These include the use of drying agents, rotary evaporators, and separating funnels. Alternative large-scale procedures for these operations are, respectively, removing water as an azeotrope, distillation, and stirring the different phases.

There are several stages in chemical development. In the first stage, about a kilogram of drug is required for short-term toxicology and stability tests, analytical research, and pharmaceutical development. Often, the

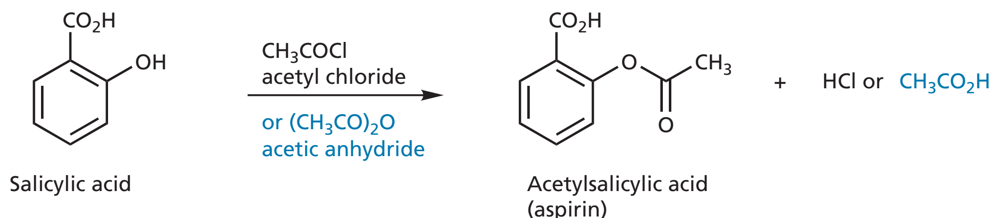


FIGURE 15.5 Synthesis of aspirin.

original synthetic route will be developed quickly and scaled up in order to produce this quantity of material, as time is of the essence. The next stage is to produce about 10 kg for long-term toxicology tests, as well as for formulation studies. Some of the material may also be used for phase I clinical trials. The third stage involves a further scale up to the pilot plant, where about 100 kg is prepared for phase II and phase III clinical trials.

Because of the timescales involved, the chemical process used to synthesize the drug during stage 1 may differ markedly from that used in stage 3. However, it is important that the quality and purity of the drug remains as constant as possible for all the studies carried out. Therefore, an early priority in chemical development is to optimize the final step of the synthesis and to develop a purification procedure which will consistently give a high-quality product. The **specifications** of the final product are defined and determine the various analytical tests and purity standards required. These define predetermined limits for a range of properties, such as melting point, colour of solution, particle size, polymorphism, and pH. The product's chemical and stereochemical purities must also be defined, and the presence of any impurities or solvent should be identified and quantified if they are present at a level greater than 1%. Acceptable limits for different compounds are proportional to their toxicity. For example, the specifications for ethanol, methanol, mercury, sodium, and lead are 2%, 0.05%, 1 ppm, 300 ppm, and 2 ppm respectively. Carcinogenic compounds, such as benzene or chloroform, should be completely absent, which means, in practice, that they must not be used as solvents or reagents in the final stages of the synthesis.

All future batches of the drug must meet these specifications. Once the final stages have been optimized, future development work can then look to optimize or alter the earlier stages of the synthesis (Box 15.2).

In some development programmes, the structure originally identified as the most promising clinical prospect may be supplanted by another structure that demonstrates better properties. The new structure may be a close analogue of the original compound, but such a change can have radical effects on chemical development and require totally different conditions to maximize the yields for each synthetic step.

15.3.2 Process development

Process development aims to ensure that the number of reactions in the synthetic route is as small as possible and that all the individual stages in the process are integrated with each other, such that the full synthesis runs smoothly and efficiently on a production scale. The aim is to reduce the number of operations to a minimum. For example, rather than isolating each intermediate in the synthetic sequence, it is better to move it in solution directly from one reaction vessel to the next for the subsequent step. Ideally, the only purification step carried out is on the final product.

Environmental and safety issues are extremely important. Care is taken to minimize the risk of chemicals escaping into the surrounding environment, and chemical recycling is carried out as much as possible. The use of 'green technology' such as electrochemistry, photochemistry, ultrasound, or microwaves may solve potential environmental problems.

Keeping costs low is a high priority and it is more economic to run the process such that a small number of large batches are produced rather than a large number of small batches. Extreme care must be taken over safety. Any accident in a production plant has the potential to be a major disaster, so there must be strict adherence to safety procedures, and close monitoring of the process when it is running. However, the over-riding priority is that the final product should still be produced with a consistently high purity in order to meet the required specifications.

Process development is very much aimed at optimizing the process for a specific compound (Box 15.3). If the original structure is abandoned in favour of a different analogue, the process may have to be rethought completely.

The regulatory authorities require that every batch of a drug product is analysed to ensure that it meets the required specifications, and that all impurities present at more than 1% are characterized, identified, and quantified.

BOX 15.2 Synthesis of ebalzotan

Ebalzotan is an antidepressant drug produced by Astra which works as a selective serotonin (5-HT_{1A}) antagonist. The original synthesis from structure I involved six steps and included several expensive and hazardous reagents, resulting in a paltry overall yield of 3.7% (Fig. 1). Development of the route involved the replacement of ‘problem’ reagents and optimization of the reaction conditions, leading to an increase in the overall yield to 15%. Thus, the expensive and potentially toxic reducing agent sodium cyanoborohydride was replaced with hydrogen gas over a palladium catalyst. In the demethylation step, BBr_3, which is corrosive, toxic, and expensive, was replaced with the cheaper and less toxic HBr.

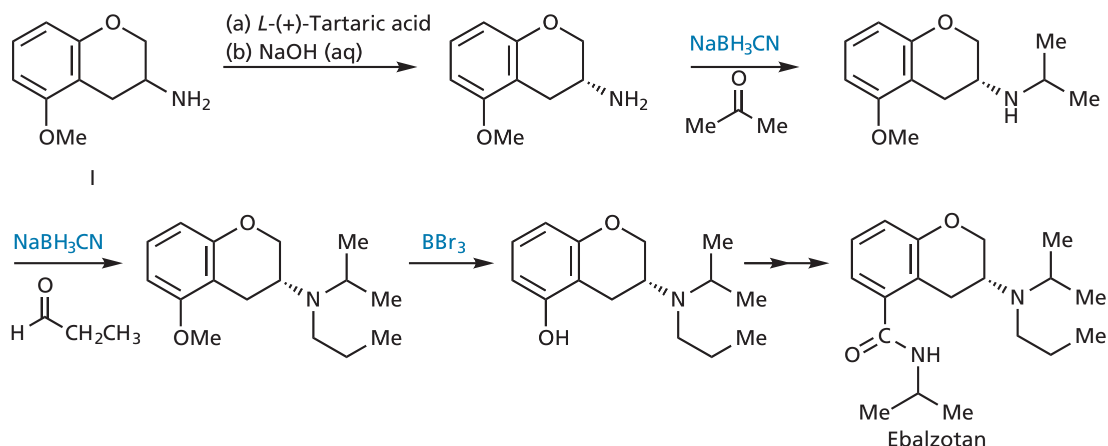


FIGURE 1 Synthesis of ebalzotan.

BOX 15.3 Synthesis of ICI D7114

ICI D7114 is an agonist at adrenergic β_3-receptors and was developed for the treatment of obesity and non-insulin-dependent diabetes, The original synthetic route used in the research laboratory is shown in Fig. 1.

The overall yield was only 1.1% and there were various problems in applying the route to the production scale. The first reaction involves hydroquinone and ethylene dibromide, both of which could react twice to produce side products. Moreover, ethylene dibromide is a carcinogen, and toxic vinyl bromide is generated as a volatile side product during the reaction. A chromatographic separation was required after the second stage to remove a side product—a process which is best avoided on a large scale. The use of high-pressure hydrogenation at 20 times atmospheric pressure to give structure III was not possible on the plant scale, as the equipment available could only achieve 500 kP (about 5 times atmospheric pressure). Finally, the product has an asymmetric centre and so it was necessary to carry out a resolution. This involved forming a salt with a chiral acid and carrying out eight crystallizations—a process that would be totally unsuitable at production scale.

The revised synthetic route shown in Fig. 2 avoided these problems and improved the overall yield to 33%. To avoid the possibility of any dialkylated side product being formed, *para*-benzyloxyphenol was used as starting material. Ethylene dimesylate was used in place of the carcinogenic ethylene dibromide. As a result, vinyl bromide was no longer generated as a side product. The alkylated product (IV) was not isolated, but was treated *in situ* with benzylamine, thus cutting down the number of operations involved. Hydrogenolysis of the benzyl ether group was carried out in the presence of methanesulfonic acid, the latter helping to prevent hydrogenolysis of the *N*-benzyl group. This gave structure II which was one of the intermediates in the original synthesis. Alkylation gave structure V and an asymmetric reaction was carried out with the epoxide to avoid the problem of resolution. The product from this reaction (VI) could be hydrogenated to the final product *in situ* without the need to isolate VI, again cutting down the number of operations.

(*Continued*)

BOX 15.3 Synthesis of ICI D7114 (*Continued*)

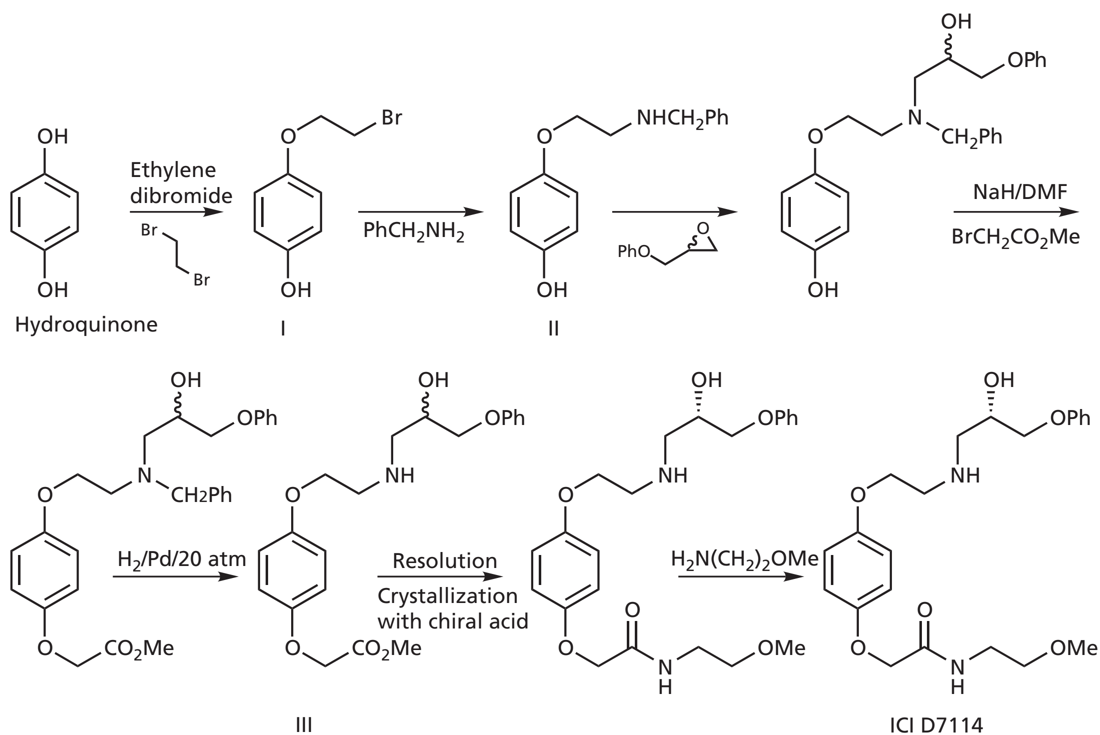


FIGURE 1 Research synthesis of ICI D7114.

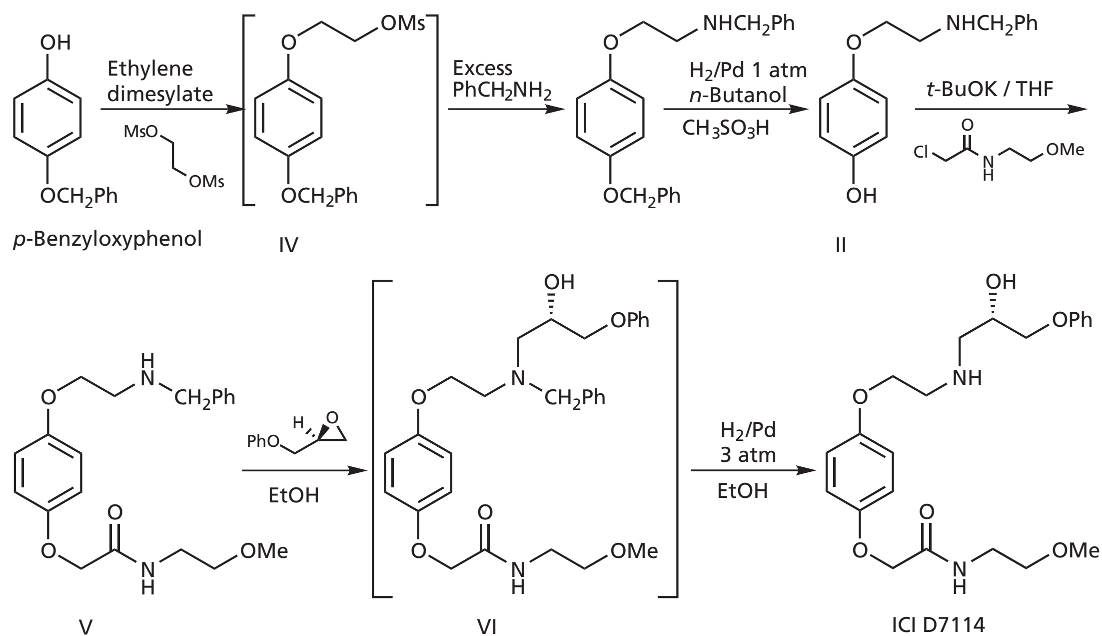


FIGURE 2 Revised synthetic route to ICI D7114.

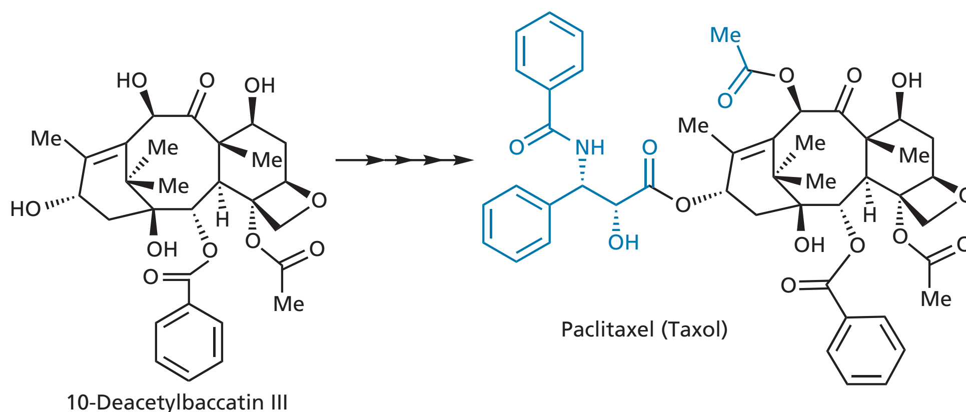


FIGURE 15.6 Semi-synthetic synthesis of paclitaxel (Taxol).

The identification of impurities is known as **impurity profiling** and typically involves their isolation by preparative HPLC, followed by NMR spectroscopy or mass spectrometry to identify their structure.

15.3.3 Choice of drug candidate

The issues surrounding chemical and process development can affect the choice of which drug candidate is taken forward into drug development. If it is obvious that a particular structure is going to pose problems for large-scale production, an alternative structure may be chosen which poses fewer problems, even if it is less active.

15.3.4 Natural products

Not all drugs can be fully synthesized. Many natural products have quite complex structures that are too difficult and expensive to synthesize on an industrial scale. These include drugs such as **penicillin**, **morphine**, and **paclitaxel (Taxol)**. Such compounds can only be harvested from their natural source—a process which can be tedious, time-consuming, and expensive, as well as being wasteful on the natural resource. For example, four mature yew trees have to be cut down to obtain enough paclitaxel to treat one patient! Furthermore, the number of structural analogues that can be obtained from harvesting is severely limited.

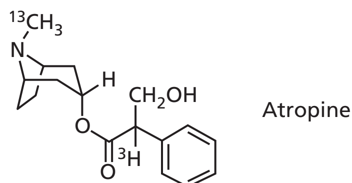
Semi-synthetic procedures can sometimes get round these problems. This often involves harvesting a biosynthetic intermediate from the natural source, rather than the final compound itself. The intermediate can then be converted to the final product by conventional synthesis. This approach can have two advantages. Firstly, the intermediate may be more easily extracted in higher yield than the final product itself. Secondly, it may allow the possibility of synthesizing analogues of the final product. The semi-synthetic penicillins are an illustration of this approach (section 19.5.1.6). Another, more recent, example is that of paclitaxel. It is manufactured by extracting **10-deacetylbaccatin III** from the needles of the yew tree, then carrying out a four-stage synthesis (Fig. 15.6).

KEY POINTS

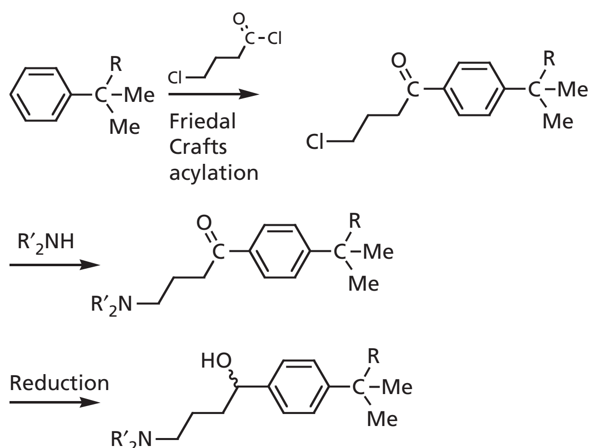
- Chemical development involves the development of a synthetic route which is suitable for the large-scale synthesis of a drug.
- The priorities in chemical development are to develop a synthetic route which is straightforward, safe, cheap, efficient, has the minimum number of synthetic steps, and will provide a consistently good yield of high-quality product that meets predetermined purity specifications.
- An early priority in chemical development is to define the purity specifications of the drug and to devise a purification procedure which will satisfy these requirements.
- Process development aims to develop a production process which is safe, efficient, economic, environmentally friendly, and provides a product that has consistent yield and quality to satisfy purity specifications.
- Drugs derived from natural sources are usually produced by harvesting the natural source or through semi-synthetic methods.

QUESTIONS

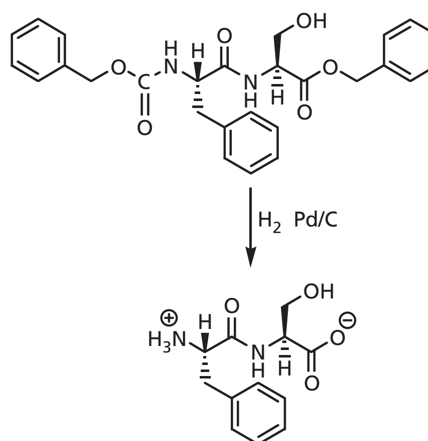
1. Discuss whether the doubly labelled atropine molecule shown below is suitable for drug metabolism studies.



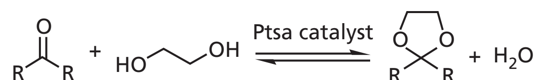
2. What is meant by a placebo and what sort of issues need to be considered in designing a suitable placebo?
3. Usually, a 'balancing act' of priorities is required during chemical development. Explain what this means.
4. Discuss whether chemical development is simply a scale-up exercise.
5. The following synthetic route was used for the initial synthesis of fexofenadine (R=CO_2H)—an analogue of terfenadine (R=CH_3). The synthesis was suitable for the large-scale synthesis of terfenadine, but not for fexofenadine. Suggest why not. (Hint: consider the electronic effects of R.)



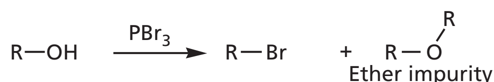
6. The following reaction was carried out using ethanol or water as solvents, but gave poor yields in both cases. Suggest why this might be the case and how these problems could be overcome.



7. The following reaction was carried out with heating under reflux at 110°C. However, the yield was higher when the condenser was set for distillation. Explain.



8. What considerations do you think have to be taken into account when choosing a solvent for scale up? Would you consider diethyl ether or benzene as a suitable solvent?
9. Phosphorus tribromide was added to an alcohol to give an alkyl bromide, but the product was contaminated with an ether impurity. Explain how this impurity might arise and how the reaction conditions could be altered to avoid the problem.



FURTHER READING

Preclinical trials

Cavagnaro, J. A. (2002) Preclinical safety evaluation of biotechnology-derived pharmaceuticals. *Nature Reviews Drug Discovery* **1**, 469–475.

Lindpaintner, K. (2002) The impact of pharmacogenetics and pharmacogenomics on drug discovery. *Nature Reviews Drug Discovery* **1**, 463–469.

Matfield, M. (2002) Animal experimentation: the continuing debate. *Nature Reviews Drug Discovery* **1**, 149–152.

Nicholson, J. K., Connelly, J., Lindon, J. C., and Holmes, E. (2002) Metabonomics: a platform for studying drug toxicity and gene function. *Nature Reviews Drug Discovery* **1**, 153–161.

Pritchard, J. F. (2003) Making better drugs: decision gates in non–clinical drug development. *Nature Reviews Drug Discovery* **2**, 542–553.

Ulrich, R. and Friend, S. H. (2002) Toxicogenomics and drug discovery: will new technologies help us produce better drugs? *Nature Reviews Drug Discovery* **1**, 84–88.

Chemical and process development

Collins, A. N., Sheldrake, G. N., and Crosby, J. (eds) (1997) *Chirality in Industry I and II*. John Wiley and Sons, Chichester.

Delaney, J. (2009) Spin-outs: protecting your assets. *Chemistry World* July, 54–55.

Lee, S. and Robinson, G. (1995) *Process Development: Fine Chemicals from Grams to Kilograms.* Oxford University Press, Oxford.

Repic, O. (1998) *Principles of Process Research and Chemical Development in the Pharmaceutical Industry.* John Wiley and Sons, Chichester.

Saunders, J. (2000) *Top Drugs: Top Synthetic Routes.* Oxford University Press, Oxford.

Patenting

Agranat, I., Caner, H., and Caldwell, J. (2002) Putting chirality to work: the strategy of chiral switches. *Nature Reviews Drug Discovery* **1**, 753–768.

Southall, N. T. and Ajay (2006) Kinase patent space visualization using chemical replacements. *Journal of Medicinal Chemistry* **49**, 2103–2109.

Webber, P. M. (2003) Protecting your inventions: the patent system. *Nature Reviews Drug Discovery* **2**, 823–830.

Regulatory affairs

Engel, L. W. and Straus, S. E. (2002) Development of therapeutics: opportunities within complementary and alternative medicine. *Nature Reviews Drug Discovery* **1**, 229–237.

Haffner, M. E., Whitley, J., and Moses, M. (2002) Two decades of orphan product development. *Nature Reviews Drug Discovery* **1**, 821–825.

Houlton, S. (2010) Recalling pharma. *Chemistry World* July, 18–19.

Maeder, T. (2003) The orphan drug backlash. *Scientific American* May, 71–77.

Perks, B. (2011) Faking it. *Chemistry World* January, 56–59.

Perks, B. (2011) Orphans come in from the cold. *Chemistry World* September, 60–63.

Reichert, J. M. (2003) Trends in development and approval times for new therapeutics in the United States. *Nature Reviews Drug Discovery* **2**, 695–702.

Clinical trials

Issa, A. M. (2002) Ethical perspectives on pharmacogenomic profiling in the drug development process. *Nature Reviews Drug Discovery* **1**, 300–308.

Lewcock, A. (2010) Medicine made to measure. *Chemistry World* July, 56–61.

Schreiner, M. (2003) Paediatric clinical trials: redressing the balance. *Nature Reviews Drug Discovery* **2**, 949–961.

Sutcliffe, A. G. and Wong, I. C. K. (2006) Rational prescribing for children. *British Medical Journal* **332**, 1464–1465.

Titles for general further reading are listed on p. 763.

CASE STUDY 2

The design of angiotensin-converting enzyme (ACE) inhibitors

Angiotensin-converting enzyme (ACE) is a key component of the biosynthetic pathway that generates the hormone **angiotensin II** (Fig. CS2.1). The pathway involves the conversion of **angiotensinogen** to **angiotensin I** catalysed by the enzyme **renin**, followed by the conversion of angiotensin I to angiotensin II, catalysed by ACE. Angiotensin II is a potent vasoconstrictor which results in increased blood pressure, and so drugs that block the synthesis or actions of this hormone can act as antihypertensives. At present, there are three main categories of drugs affecting the biosynthesis or actions of angiotensin II: **renin inhibitors**, **ACE inhibitors**, and **angiotensin II receptor antagonists**. In this case study, we will concentrate on the ACE inhibitors. Renin inhibitors and angiotensin II antagonists are mentioned in section 7.4 and Box 14.1 respectively.

The design of ACE inhibitors demonstrates how it is possible to design drugs for a protein target in a rational manner, even if the structure of the target has not been determined. ACE is a membrane-bound enzyme which has been difficult to isolate and study. It is a member of a group of enzymes called the **zinc metalloproteinases** and catalyses the hydrolysis of a dipeptide fragment from the end of a decapeptide called **angiotensin I** to give the octapeptide **angiotensin II** (Fig. CS2.2).

Angiotensin II is an important hormone that causes blood vessels to constrict, resulting in a rise in blood pressure. Therefore, ACE inhibitors are potential antihypertensive agents because they inhibit the production of angiotensin II. Although the enzyme ACE could not be isolated, the design of ACE inhibitors was helped by studying the structure and mechanism of another zinc metalloproteinase that could—an enzyme called **carboxypeptidase.** This enzyme splits the terminal amino acid from a peptide chain as shown in Fig. CS2.3 and is inhibited by **L-benzylsuccinic acid**.

FIGURE CS2.1 Biosynthesis of angiotensin II.

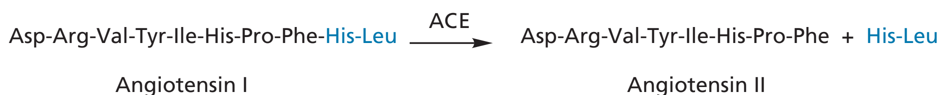


FIGURE CS2.2 Reaction catalysed by angiotensin converting enzyme (ACE).

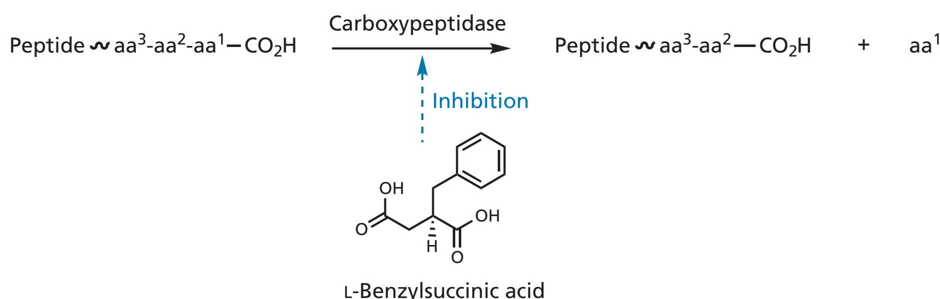


FIGURE CS2.3 Hydrolysis of a terminal amino acid from a peptide chain by the carboxypeptidase enzyme. The asymmetric centre of L-benzylsuccinic acid has the *R* configuration.

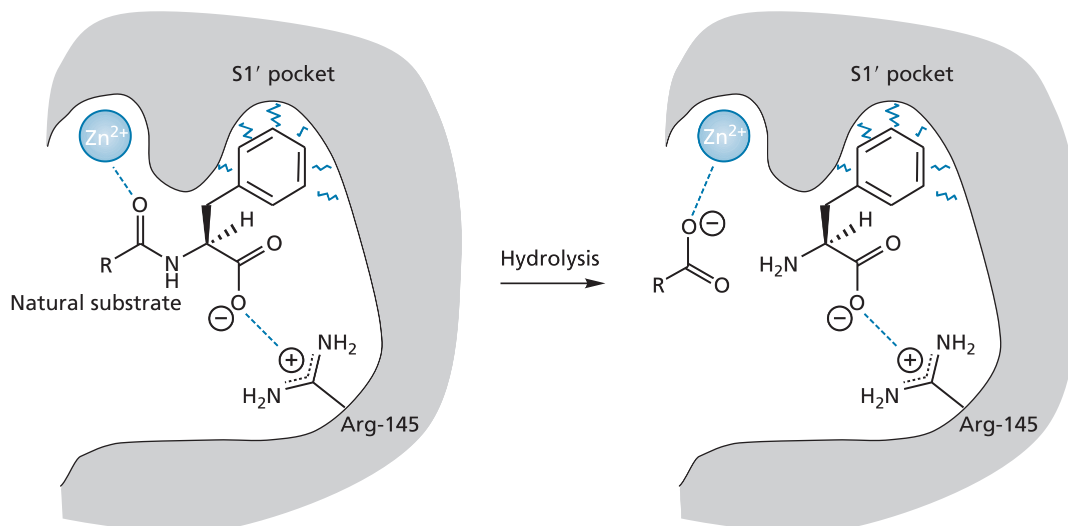


FIGURE CS2.4 Binding site interactions for a substrate bound to the active site of carboxypeptidase.

The active site of carboxypeptidase (Fig. CS2.4) contains a charged arginine unit (Arg-145) and a zinc ion, which are both crucial in binding the substrate peptide. The peptide binds such that the terminal carboxylic acid is bound ionically to the arginine unit, while the carbonyl group of the terminal peptide bond is bound to the zinc ion. There is also a hydrophobic pocket called the S1′ pocket which can accept the side chain of the terminal amino acid. Aromatic rings are found to bind strongly to this pocket and this explains the specificity of the enzyme towards peptide substrates containing an aromatic amino acid at the *C*-terminus (Phe in the example shown). Hydrolysis of the terminal peptide bond then takes place aided by the zinc ion, which plays a crucial role in the mechanism by polarizing the carbonyl group and making the amide group more susceptible to hydrolysis.

The design of the carboxypeptidase inhibitor L-benzylsuccinic acid was based on the hydrolysis products arising from this enzymatic reaction. The benzyl group was included to occupy the S1′ pocket, while the adjacent carboxylate anion was present to form an ionic interaction with Arg-145. The second carboxylate was present to act as a ligand to the zinc ion, mimicking the carboxylate ion of the other hydrolysis product.

L-Benzylsuccinic acid binds as shown in Fig. CS2.5. However, it cannot be hydrolysed as there is no peptide bond present and so the enzyme is inhibited for as long as the compound stays attached.

An understanding of the above mechanism and inhibition helped in the design of ACE inhibitors. First of all, it was assumed that the active site contained the same zinc ion and arginine group. However, as ACE splits a dipeptide unit from the peptide chain rather than a single amino acid, these groups are likely to be further apart, and so an analogous inhibitor to benzylsuccinic acid would be a succinyl-substituted amino acid. The next step was to choose which amino acid to use. Unlike carboxypeptidase, ACE shows no specificity for peptide substrates containing any particular *C*-terminal amino acid and so the binding pocket for the *C*-terminal side chain must be different for the two enzymes. The relevant pockets would be S2′ for ACE (not shown in the diagrams) and S1′ for the carboxypeptidase enzyme. As there was no selectivity shown for peptide substrates, it was decided to study peptides that acted as ACE inhibitors, and to identify whether any *C*-terminal amino acids were commonly present in these structures. The amino acid proline is the *C*-terminal amino acid in a known ACE inhibitor called **teprotide** (Fig. CS2.6)—a nonapeptide which was isolated from the venom of the Brazilian pit viper. Although teprotide is a reasonably potent inhibitor, it is susceptible to digestive enzymes and is orally inactive. Other ACE inhibitors found in snake venoms also contain the terminal proline group, implying that the ring might be involved in some binding interaction with the binding site. **Succinyl proline** was the end result of this design philosophy.

Succinyl proline was found to be a weak, but specific, inhibitor of ACE, and it was proposed that both carboxylate groups were ionized—one interacting with the arginine group and one with the zinc ion (Fig. CS2.7). It was now argued that there must be pockets available to accommodate amino acid side chains on either side of the reaction centre (pockets S1 and S1′). The strategy of extension (section 13.3.2) was now used to find a group that would fit the S1′ pocket and increase the binding affinity. A methyl group fitted the bill and resulted in an

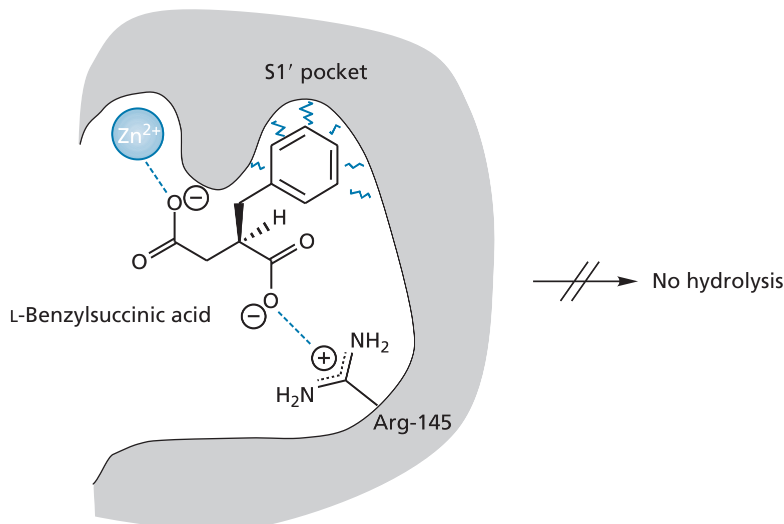


FIGURE CS2.5 Inhibition by L-benzylsuccinic acid (the *R*-enantiomer).

FIGURE CS2.6 Angiotensin converting enzyme (ACE) inhibitors.

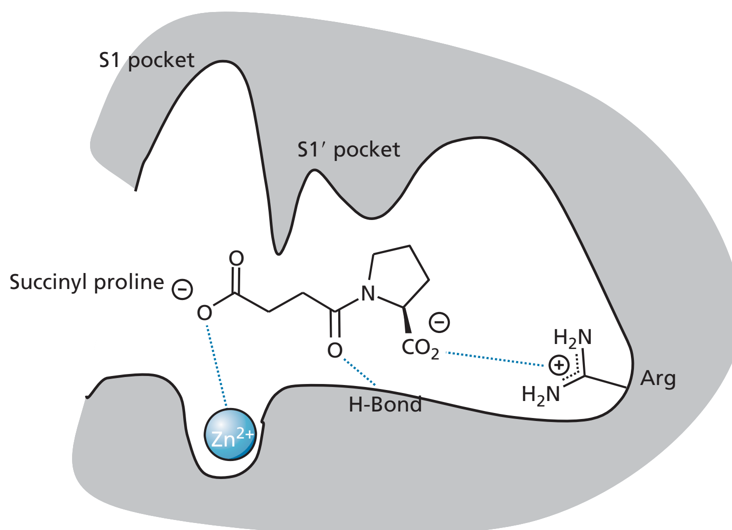


FIGURE CS2.7 Binding site interaction for succinyl proline in the active site of angiotensin converting enzyme (ACE).

increase in activity (Fig. CS2.8). The next step was to see whether there was a better group than the carboxylate ion to interact with zinc, and it was discovered that a thiol group led to dramatically increased activity. This resulted in **captopril**, which was the first non-peptide ACE inhibitor to become commercially available. The stereochemistry of the methyl substituent in both SQ13 297 and captopril is important for activity since the opposite enantiomers show 100-fold less activity.

The most common side effects associated with captopril are rashes and loss of taste, which are thought to be associated with the thiol group. Therefore, other workers sought to find an ACE inhibitor that was as potent as captopril, but which lacked the thiol group. This meant

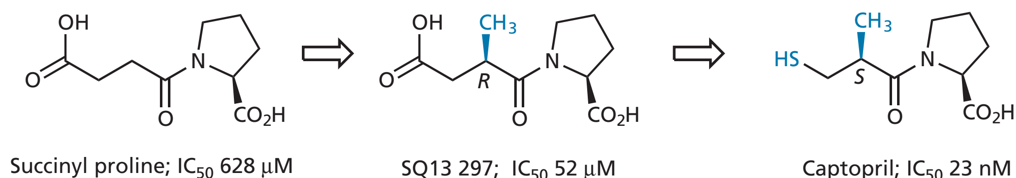


FIGURE CS2.8 Development of captopril.

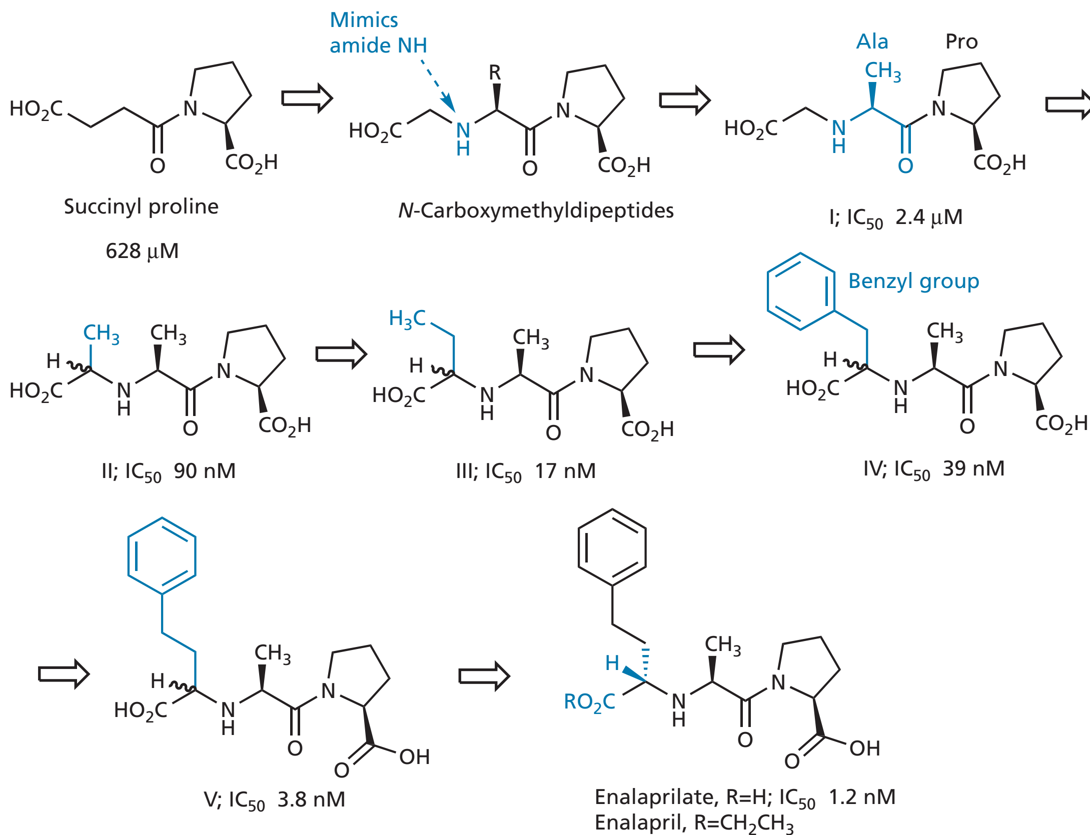


FIGURE CS2.9 Development of enalaprilate.

re-introducing the carboxylate group as the zinc ligand, despite the fact that it is a far weaker binding group for zinc. To compensate for this, groups were introduced that could form extra binding interactions with the active site (**extension strategy**; section 13.3.2). Firstly, it was decided to extend the chain length of succinyl proline in order to introduce an NH group. The rationale was that the NH group would mimic the amide NH of the peptide link that would normally be hydrolysed by the enzyme (Fig. CS2.9). It seemed reasonable to assume that this group could be involved in some kind of hydrogen bonding with the active site. Introducing the NH group meant that a second amino acid had now been introduced into the structure, and so a series of *N*-carboxymethyl dipeptides were studied. Incorporating L-alanine introduced the methyl substituent that is present in captopril (structure I, Fig. CS2.9). The activity of this compound was better than succinyl proline, but greatly inferior to captopril. Therefore, it was decided to 'grow' a substituent from the penultimate carbon atom in the structure to search for the binding pocket S1 shown in Fig. CS2.7. This pocket would normally accept the phenylalanine side chain of angiotensin I and should be hydrophobic in nature. Therefore, methyl and ethyl substituents were introduced (structures II and III; Fig. CS2.9). The analogues showed increased activity, with the ethyl analogue

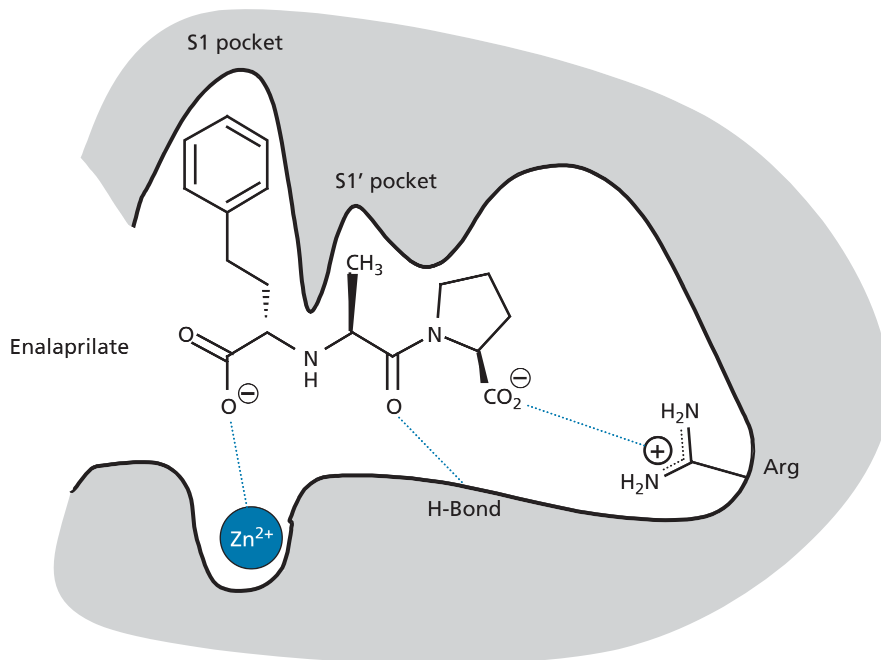


FIGURE CS2.10 Enalaprilate.

proving as effective as captopril. Activity dropped slightly with the introduction of a benzyl group, but a chain extension (section 13.3.3) led to a dramatic increase in activity, such that structure V proved to be more active than captopril.

The addition of a new substituent to structures II–V meant that a new asymmetric centre had been introduced and all these structures had been tested as mixtures of diastereomers—the *R,S,S* and *S,S,S* diastereomers. The diastereoisomers of structure V were now separated by chromatography and the *S,S,S* diastereomer was found to be 700 times more active than the *R,S,S* diastereomer. This structure was named **enalaprilate** and is proposed to bind to the active site as shown in Fig. CS2.10. **Enalapril** is the ethyl ester prodrug of enalaprilate and is used clinically (**prodrugs**; section 14.6.1.1). The prodrug is absorbed more easily from the gut than enalaprilate itself and is converted to enalaprilate by esterase enzymes.

Lisinopril (Fig. CS2.11) is another successful ACE inhibitor which is similar to enalaprilate, but where the methyl substituent has been extended to an aminobutyl substituent—the side chain for the amino acid lysine. In 2003, a crystal structure of ACE complexed with lisinopril was finally determined by X-ray crystallography. This provided a detailed picture of the three-dimensional structure of ACE, and how lisinopril binds to the active site. In fact, there is a marked difference in structure between ACE and carboxypeptidase A, which means that ACE inhibitors do not bind as thought originally. For example, the ionic interaction originally thought to involve an arginine residue involves a lysine residue instead. Now that an accurate picture has been obtained of the active site and the manner in which lisinopril binds, it is possible that a new generation of ACE inhibitors will be designed with improved binding characteristics using structure-based drug design (section 13.3.11).

Both enalaprilate and lisinopril have a tetrahedral geometry at what would normally be the reaction centre for the enzyme-catalysed reaction (Fig. CS2.12). As a result, they have been described as transition-state analogues (section 7.4). This is because the transition state for the enzyme-catalysed reaction would be expected to be similar in nature to the tetrahedral intermediate formed during the hydrolysis mechanism.

There has been recent research into finding newer ACE inhibitors which would be more similar in nature

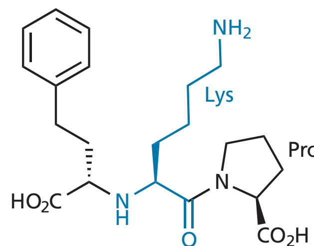


FIGURE CS2.11 Lisinopril.

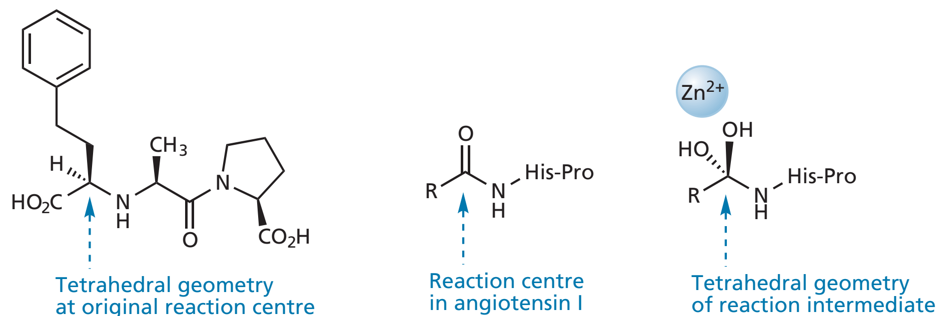


FIGURE CS2.12 Comparison of enalaprilate with angiotensin I and the reaction intermediate formed during enzyme-catalysed hydrolysis.

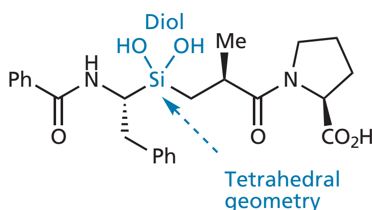


FIGURE CS2.13 Example of a silanediol transition-state analogue.

to the transition state. For example, silanediol structures have been studied which contain a tetrahedral centre. The diol mimics the hydrated carbonyl of the reaction intermediate and interacts with the zinc ion in the active site (Fig. CS2.13).

Keto-ACE analogues (Fig. CS2.14) are also being investigated as more selective ACE inhibitors. The hydrated form of the ketone group acts as the transition-state analogue in these cases.

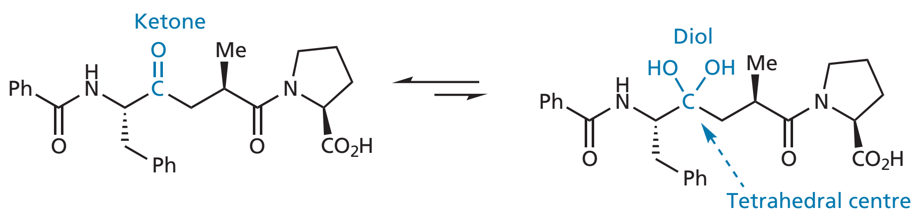


FIGURE CS2.14 Keto-ACE inhibitors.

BOX CS2.1 Synthesis of captopril and enalaprilate

There are several methods of synthesizing captopril. One method is to take the t-butyl ester of proline and react it with the carboxylic acid in the presence of a coupling agent (I) to form the amide (II) (Fig. 1). The t-butyl and acetyl protecting groups can then be removed in the presence of acid and base respectively to give captopril.

Enalaprilate can be synthesized by reacting the ketoacid (III) with L-analyl-L-proline. Two diastereomers are formed which are separated by chromatography.

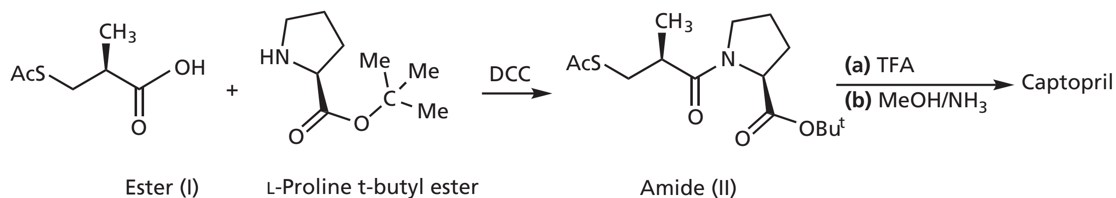


FIGURE 1 Synthesis of captopril.

(*Continued*)

BOX CS2.1 Synthesis of captopril and enalaprilate (*Continued*)

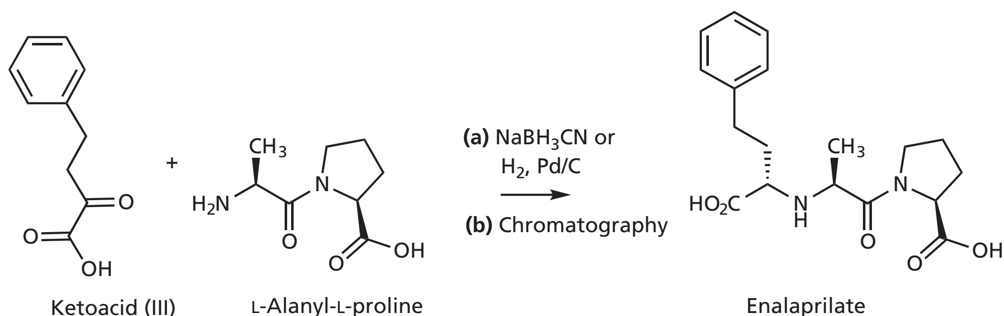


FIGURE 2 Synthesis of enalaprilate.

FURTHER READING

Acharya, K. R., Sturrock, E. D., Riordan, J. F., and Ehlers, M. R. (2003) ACE revisited: a new target for structure-based drug design. *Nature Reviews Drug Discovery* **2**, 891–902.

Ganellin, C. R. and Roberts, S. M. (eds) (1994) Angiotensin-converting enzyme (ACE) inhibitors and the design of cilazapril. In: *Medicinal Chemistry—The Role of Organic Research in Drug Research*, 2nd edn. Academic Press, London.

Kim, J. and Sieburth, S. M. (2004) Silanediol peptidomimetics. Evaluation of four diastereomeric ACE inhibitors. *Bioorganic and Medicinal Chemistry Letters* **14**, 2853–2856.

Natesh, R., Schwager, S. L., Sturrock, E. D., and Acharya, K. R. (2003) Crystal structure of the human angiotensin-converting enzyme–lisinopril complex. *Nature* **420**, 551–554.

Nchinda, A. T., Chibale, K., Redelinghuys, P., and Sturrock, E. D. (2006) Synthesis of novel keto-ACE analogues as domain-selective angiotensin I-converting enzyme inhibitors. *Bioorganic and Medicinal Chemistry Letters* **16**, 4612–4615.

Ondetti, M. A., Rubin, B., and Cushman, D. W. (1977) Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* **196**, 441–444 [captopril].

Patchett, A. A., Harris, E., Tristram, E. W., Wyvratt, M. J., Wu, M. T., Taub, D., et al. (1980) A new class of angiotensin-converting enzyme inhibitors. *Nature* **288**, 280–283.

Saunders, J. (ed.) (2000) Inhibitors of angiotensin converting enzyme as effective antihypertensive agents. In: *Top Drugs: Top Synthetic Routes*. Oxford University Press, Oxford.

Zaman, M. A., Oparil, S., and Calhoun, D. A. (2002) Drugs targeting the renin-angiotensin-aldosterone system. *Nature Reviews Drug Discovery* **1**, 621–636.

CASE STUDY 3

Artemisinin and related antimalarial drugs

CS3.1 Introduction

Malaria is an ancient disease that has resulted in millions of deaths and much human misery. It is caused by a protozoal parasite which is carried by mosquitos and is transmitted between mosquitos and humans by mosquito bites. The malarial parasite is a microorganism belonging to the *Plasmodium* genus, of which there are four species: *vivax, falciparum, ovale*, and *malariae*. *Plasmodium falciparum* is the most dangerous of these and can result in death. The disease is currently associated with tropical countries, but, in the past, it was present in Europe and North America. Campaigns were carried out in the 1950s and 1960s to try and eradicate mosquitos by spraying their breeding grounds with dichlorodiphenyltrichloroethane (DDT). These efforts, along with the use of quinine-based drugs (Fig. CS3.1), successfully reduced the prevalence of malaria.

Quinine was the first of the antimalarial agents to be used and is still effective today. However, it can cause adverse reactions, such as ringing in the ears and partial deafness. Therefore, its use is currently limited to the treatment of malaria rather than as a **prophylactic**. A prophylactic is a protective agent that is administered to prevent a disease occurring. The agent that largely replaced quinine as the antimalarial drug of choice was **chloroquine**, which has far fewer side effects. This was introduced in the 1950s and, at one point, it was thought that the disease would be conquered. Unfortunately, from 1961 onwards, the parasite has developed resistance to chloroquine such that the drug is no longer effective in many malarial infected areas of the world, especially in sub-Saharan Africa. It is, therefore, crucial that new antimalarial therapies are discovered that can combat these drug-resistant strains. An added urgency comes from the belief that global warming might result in the return of the disease to North America and Europe. This is particular worrying with respect to the potentially fatal *P. falciparum*. Resistance appears to be a result of the parasite having a cell membrane protein which can pump the drug out of the cell. Fortunately, a new drug has been discovered in recent years that has been found to be active against these drug-resistant strains—artemisinin.

CS3.2 Artemisinin

For over 2000 years, Chinese herbalists have used concoctions or teas (called **qinghao**) obtained from an abundant Chinese plant called *Artemisia annua*. The herb was first described as a remedy for haemorrhoids in 168 BC, and the first mention of it as an antimalarial preparation was in 340 AD. Further references to the plant were made in 1596, when it was used for the treatment of chills and fever resulting from malaria.

In 1972, the active principle of the plant was isolated and identified as **artemisinin** (or **qinghaosu**). The compound caused great excitement because it was found to be effective against the particularly dangerous chloroquine-resistant *Plasmodium falciparum*, and also acted more

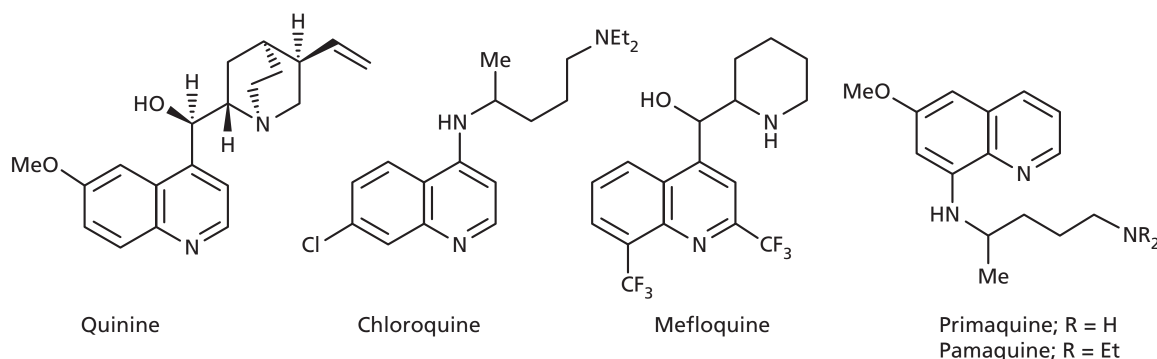


FIGURE CS3.1 Quinine and quinine-based antimalarial drugs.

quickly against chloroquine-sensitive strains. The **Walter Reed Army Institute of Research** in the USA was particularly interested in this new compound. Historically, more military casualties have resulted from malaria than from battle action. For example, during the Burmese campaign of World War II, a huge number of British and Indian soldiers were incapacitated by malaria, and had to be withdrawn from action. Many of the politically unstable countries in the world today are malarial infected areas, and so there is an obvious interest among the military to find novel antimalarial drugs for their troops.

Unfortunately, the only known source of *A. annua* was in China and the Chinese communist authorities were understandably reluctant to grant US army scientists free access into China. Negotiations were certainly not helped by US negotiators appearing in full dress uniform. As a result, the Americans were denied access to Chinese supplies and were forced to carry out a worldwide search to see if they could find an artemisinin-producing plant in a different country. Ironically enough, a suitable plant was eventually found growing in the US capital!

CS3.3 Structure and synthesis of artemisinin

The multicyclic structure of artemisinin (Fig. CS3.2) contains seven asymmetric centres and an unusual and unstable looking trioxane ring that includes an endoperoxide group. Despite, the unstable appearance of the molecule, it is stable to heat and light. Once the compound was identified, the next stage was to synthesize a range of analogues to investigate **structure–activity relationships** (**SAR**; section 13.1).

CS3.4 Structure–activity relationships

Artemisinin is a complex structure and, although it has been fully synthesized, this is not a practical method of obtaining it, or for producing a variety of different analogues. Consequently, analogues were prepared from artemisinin itself—a semi-synthetic approach. This was done by first reducing the lactone group of artemisinin to give **dihydroartemisinin** (Fig. CS3.3). This contains an alcoholic group which can then be alkylated to give various ethers such as **artemether** and **arteether**.

Esterifications can also be carried out on dihydroartemisinin; a particularly important ester is **sodium artesunate** from the reaction of artemisinin with succinic acid (Fig. CS3.4).

Dihydroartemisinin, artemether, arteether, and sodium artesunate are all more active than artemisinin itself, and so the lactone carbonyl group of artemisinin is not crucial to its antimalarial activity. A variety of other artemisinin

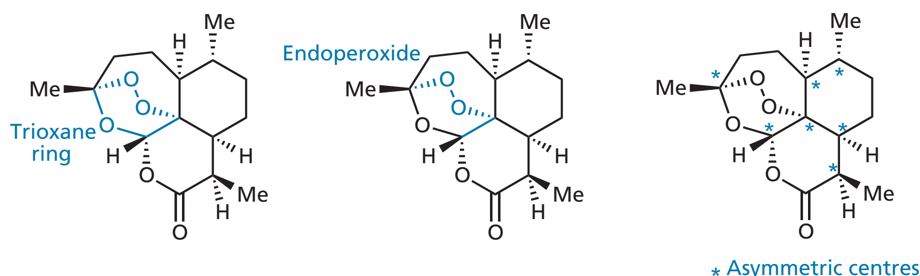


FIGURE CS3.2 Structure of artemisinin.

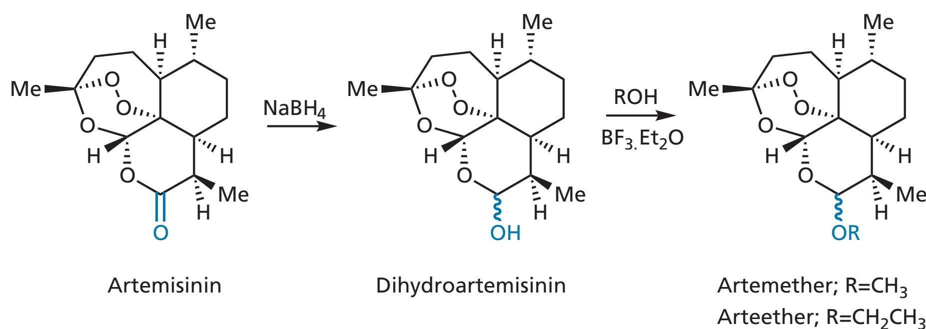


FIGURE CS3.3 Preparation of artemether and arteether.

analogues have also been studied. For example, **deoxyartemisinin** (Fig. CS3.5) is a metabolite of artemisinin and is 300–1000-fold less active. **Deoxodeoxyartemisinin** is also poorly active, whereas **deoxoartemisinin** has a similar activity to arteether.

The results from these and other structures led to the conclusion that the endoperoxide group in the trioxane ring was the essential group required for antimalarial activity, and that this represented the **pharmacophore** (section 13.2) for antimalarial activity.

CS3.5 Mechanism of action

Artemisinin has a totally different mechanism of action from the quinine-based drugs and has, therefore, proved effective against chloroquine-resistant strains of malaria. The secret behind its action lies in the endoperoxide group. This acts as a molecular trigger for a kind of 'scattergun' action which causes severe damage within the parasite cell and ultimately leads to its death. As the group is acting as a 'trigger', something has to pull the trigger. This turns out to be iron ions and, in particular, ferrous ions. In the presence of these ions, a reduction of the endoperoxide group takes place which generates two possible radical species (Fig. CS3.6). Further reactions take place to generate a series of other cytotoxic free radicals and reactive electrophiles which alkylate, cross-link, and oxidize vital biomolecules within the parasite. Cell death is the result.

This explains the action of artemisinin on protozoal cells, but why does it not kill human cells as well? In particular, why does the drug not destroy red blood cells which are rich in iron-containing **haemoglobin**—the

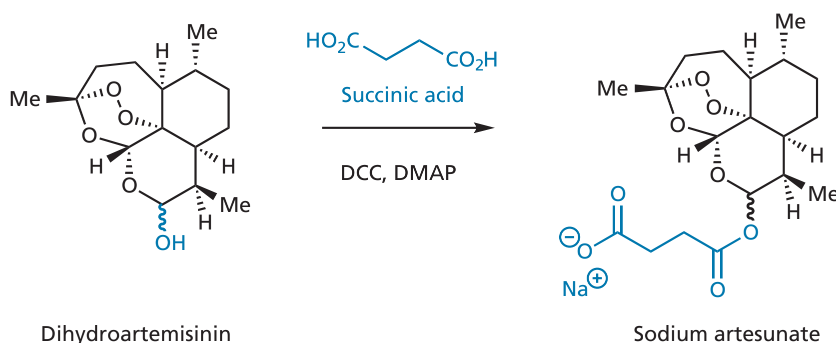


FIGURE CS3.4 Synthesis of sodium artesunate.

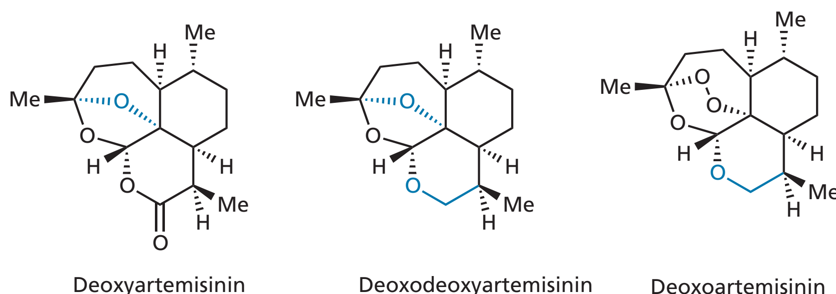


FIGURE CS3.5 Analogues of artemisinin.

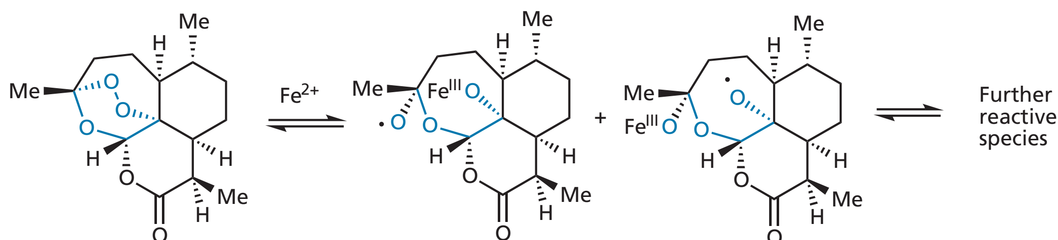


FIGURE CS3.6 Activation of artemisinin by ferrous ions.

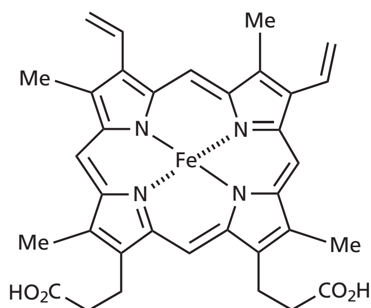


FIGURE CS3.7 Structure of heme.

protein responsible for carrying oxygen from the lungs to the rest of the body?

To answer these questions, we need to consider the life cycle of the parasite. This is quite a complex process involving both humans and mosquitos, but part of the parasite's life cycle in humans involves the invasion of red blood cells. As mentioned earlier, red blood cells contain haemoglobin. This is a protein that contains an iron (II)-centred porphyrin called **heme** (Fig. CS3.7). The porphyrin and the ferrous ion are buried deep within the protein and are effectively shielded. This explains why artemisinin is not toxic to normal, uninfected red blood cells. The ferrous iron, which would trigger its destructive capability, is 'hidden from view'.

When the malarial parasite infects red blood cells, it breaks down haemoglobin as a food source to provide itself with amino acids. This, of course, releases heme into the parasite cell. The ferrous ion present in heme can now react with artemisinin leading to the parasite's demise. Therefore, artemisinin and its analogues can be viewed as **prodrugs** (section 14.6) which are activated as a result of the parasite's own destructive tendencies to haemoglobin—poetic justice really!

A lot of research has been carried out to investigate the detailed radical mechanisms that follow on from the two radical products shown in Fig. CS3.6. The story is quite complex, but there is evidence that a particularly important mechanistic route for high antimalarial activity is the formation of a C-4 radical via 1,5-hydrogen atom abstraction (Fig. CS3.8). This produces the major metabolite that is observed for artemisinin, and also generates a highly reactive ferric hydroxide species which can go on to cause havoc within the cell.

Support for this theory comes from the activities of the simplified artemisinin analogues shown in Fig. CS3.9. Structure II is twice as active as artemisinin *in vitro*, whereas structures I and III are 100-fold less active. The 1,5-hydrogen shift shown in Fig. CS3.8 is not possible for structures I and III where the crucial hydrogen atom has been replaced with an α-methyl group. These compounds still react with the ferrous ion, but the 1,5-hydrogen shift is blocked. There is some evidence that the β-alkyl group at position 4 of structure II enhances activity, possibly by stabilizing the radical at position 4.

CS3.6 Drug design and development

As artemisinin is poorly soluble in both water and oil, early research was aimed at producing analogues which

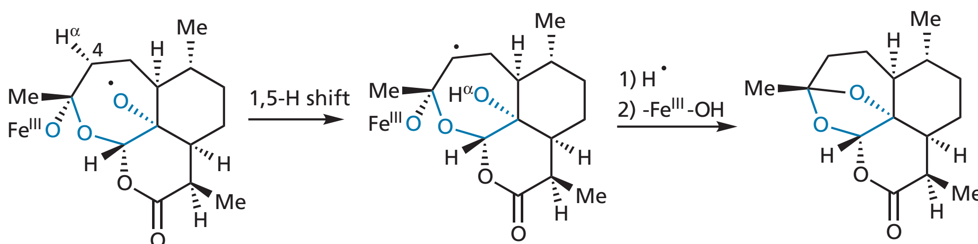


FIGURE CS3.8 Generation of a C-4 radical by 1,5-hydrogen atom abstraction.

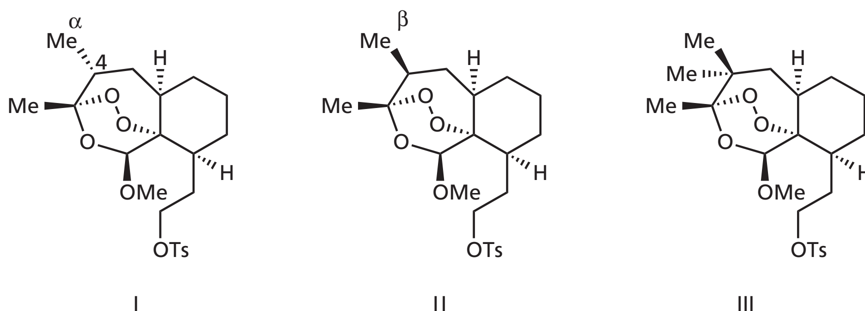


FIGURE CS3.9 Simplified analogues of artemisinin.

BOX CS3.1 Clinical properties of artemisinin and analogues

Artemisinin has proved highly effective in treating malaria, but there are problems related with its use. First of all, it is not water soluble and it has to be administered by intramuscular injection. It is also found that malaria re-occurs in up to 25% of patients treated after 1 month. Artemether and arteether are more hydrophobic than artemisinin and can be administered more easily in the field by injection in oil. They are also more potent. Sodium artesunate is also used clinically. Owing to the ionized carboxylate group, sodium artesunate is water soluble and can be administered by intravenous injection.

Currently, artemisinin, artemether, and sodium artesunate are used clinically. These compounds are now considered to be an essential component of **artemisinin combination therapy** (ACT) against drug-resistant malaria. They show brisk and potent activity, while cross-resistance with the more traditional antimalarial drugs is unlikely owing to the different mechanism of action.

Drawbacks for these drugs include a short plasma half-life, which is typically less than an hour, and rapid elimination This means that the drug is cleared from the system within a day of administration, leaving the longer-lived drugs of the combination therapy to continue the battle alone. This increases the risk of drug-resistant parasites emerging.

would be more soluble in one or other of these media. Dihydroartemisinin was found to be twice as active as artemisinin itself and was the gateway to the synthesis of a range of ethers and esters (Figs. CS3.4 and CS3.5). Many of these were found to have enhanced activity, as well as better solubility. The most interesting of these are artemether and arteether which, being more hydrophobic in nature, are more soluble in oil. Among the esters, the most interesting compound is sodium artesunate, which is ionized and water soluble.

Research has also been carried out with the aim of designing an antimalarial agent that can be synthesized easily and which has the same mechanism of action as the lead compound, artemisinin. As with many lead compounds of complex structure, the strategy of simplification (section 13.3.8) has been used. Artemisinin has a complex tetracyclic structure with seven asymmetric centres, which makes it far too complex to synthesize economically in the laboratory. A variety of simpler structures retaining the trioxane ring have been synthesized—one of the most interesting of these is **fenozan**, which has a tricyclic ring system as its core and two asymmetric centres (Fig. CS3.10). This structure shows comparable activity to arteether and sodium artesunate against some malarial strains.

Other simplified structures having comparable activity to artemisinin or its semi-synthetic analogues include bicyclic spiroalkyl trioxanes (Fig. CS3.10), which are as active as artemisinin in mice experiments, and the trioxanes shown in Fig. CS3.11, which have comparable activity to artemisinin *in vitro*.

Simple, symmetrical endoperoxides have also been synthesized (Fig. CS3.12). These have been designed to

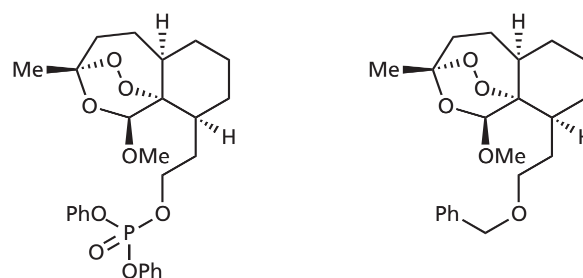


FIGURE CS3.11 Trioxanes having comparable activity to artemisinin.

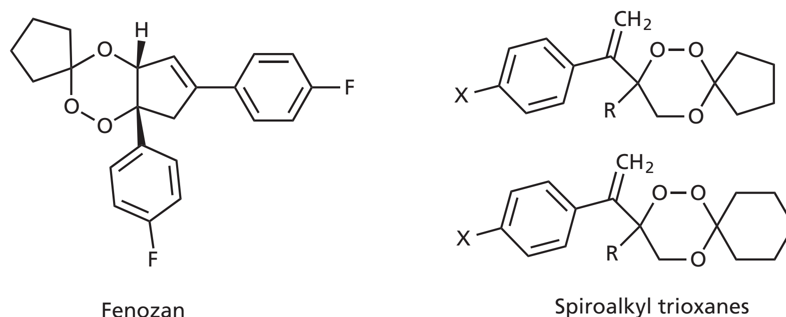


FIGURE CS3.10 Fenozan and spiroalkyl trioxanes.

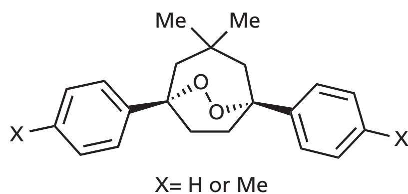
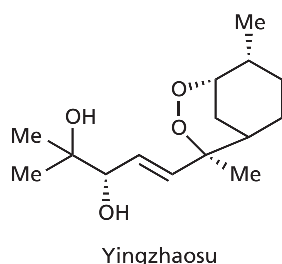


FIGURE CS3.12 Symmetrical analogues of artemisinin.

take advantage of the proposed 1,5-H abstraction mechanism described in Fig. CS3.8. The advantage of a symmetrical artemisinin is that degradation can occur in the same manner regardless of which oxygen reacts with iron. The potency of this compound is about a seventh of artemisinin *in vitro*, but this is still considered to be high.

Yingzhaosu A (Fig. CS3.13) is a naturally occurring endoperoxide which was isolated in 1979 from a traditional Chinese herbal remedy for fever (*Artabotrys uncinatus*) and shows antimalarial activity. However, the plant is a rare ornamental vine, and extraction of the natural compound is difficult and erratic. A synthesis was devised to produce a synthetic analogue of the structure, resulting in the discovery of **arteflene**, which is half as active as artemisinin.

To date, none of the simplified structures described have found widespread use as clinical agents, but there would be clear benefits in having a simple synthetic structure which would have the same mechanism of action as artemisinin, and which could be produced efficiently and cheaply for a market that cannot afford expensive drugs.



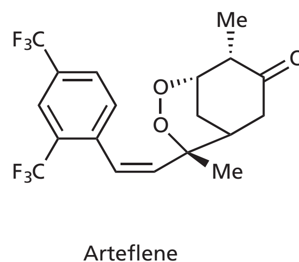


FIGURE CS3.13 Yingzhaosu A and arteflene.

FURTHER READING

Cumming, J. N., Ploypradith, P., and Posner, G. H. (1997) Antimalarial activity of artemisinin (Qinghaosu) and related trioxanes: mechanism(s) of action. *Advances in Pharmacology* **37**, 253–297.

Davies, J. (2010) Cultivating the seeds of hope. *Chemistry World* June, 50–53.

Drew, M. G. Metcalfe, J., Dascombe, M. J., and Ismail, F. M. (2006) Reactions of artemisinin and arteether with acid: implications for stability and mode of antimalarial action. *Journal of Medicinal Chemistry* **49**, 6065–6073.

Olliaro, P.L., Haynes, R. K., Meunier, B., and Yuthavong, Y. (2001) Possible modes of action of the artemisinin-type compounds. *Trends in Parasitology* **17**, 122–126.

Posner, G.H. (2007) Malaria-infected mice are cured by a single dose of novel artemisinin derivatives. *Journal of Medicinal Chemistry* **50**, 2516–2519.

Posner, G. H. and O'Neill, P. M. (2004) Knowledge of the proposed chemical mechanism of action and cytochrome P450 metabolism of antimalarial trioxanes like artemisinin allows rational design of new antimalarial peroxides. *Accounts of Chemical Research* **37**, 397–404.

Wu, Y. (2002) How might qinghaosu (artemisinin) and related compounds kill the intraerythrocytic malaria parasite? A chemist's view. *Accounts of Chemical Research* **35**, 255–259.

CASE STUDY 4

The design of oxamniquine

The development of **oxamniquine** (Fig. CS4.1) by Pfizer pharmaceuticals is a nice example of how traditional strategies can be used in the development of a drug where the molecular target is unknown. It also demonstrates that strategies can be used in any order and may be used more than once.

Oxamniquine is an important drug in developing countries, used in the treatment of **schistosomiasis** (**bilharzia**). After malaria, this disease is the most endemic parasitic disease in the world, affects an estimated 200 million people, and is responsible for almost 500,000 deaths each year. Urinary infection can cause bladder cancer, while intestinal infection can result in liver damage. The disease is caused by small flatworms called **schistosomes** which are contracted by swimming or wading in infected water. The parasites can rapidly penetrate human skin in the larval form and, once they are in the blood supply, the larvae develop into adult flatworms. The females then produce eggs that become trapped in organs and tissues, leading to inflammation and a long, debilitating disease that can last up to 20 years. In severe cases, the disease can be fatal. There are three pathogenic species of the parasite—*Schistosoma mansoni, S. haematobium*, and *S. japonicum*.

In the early 1960s, the only drugs available were the tricyclic structure **lucanthone** (Fig. CS4.1) and antimonials, such as **stibocaptate** (Fig. CS4.2). However, stibocaptate and lucanthone had serious drawbacks as therapeutics. Stibocaptate was orally inactive, while both drugs required frequent dosing regimens and produced toxic side effects. For example, lucanthone had to be administered 3–5 times per day, and could cause nausea and vomiting. More seriously, it could result in severe toxic effects on the heart and the central nervous system. Finally, it was not effective against all three of the pathogenic strains.

In 1964, Pfizer initiated a project aimed at developing an orally active, non-toxic agent that would be effective as a single dose against all three pathogenic strains, and which would be affordable for patients in developing countries. This research led, ultimately, to the discovery of oxamniquine (Fig. CS4.1), which met all but one of those goals.

Lucanthone was chosen as the lead compound because it was orally active; it was decided to try *simplifying* (*section 13.3.8*) the structure to see whether the tricyclic system was really necessary. Several compounds were made—the most interesting structure was one where the two rings seen on the left in Fig. CS4.1 had been removed. This gave a compound called **mirasan** (Fig. CS4.1), which retained the right-hand aromatic ring containing the methyl and β-aminoethylamino side chains *para* to each other. *Varying substituents* (*section 13.3.1.2*) showed that an electronegative chloro substituent, positioned where the sulphur atom had been was beneficial to activity. Mirasan was active against the bilharzia parasite in mice, but not in humans.

It was now reasoned that the β-aminoethylamino side chain was important in binding the drug to a target binding site and would adopt a particular conformation

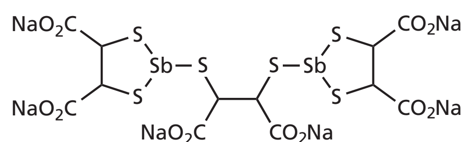


FIGURE CS4.2 Stibocaptate.

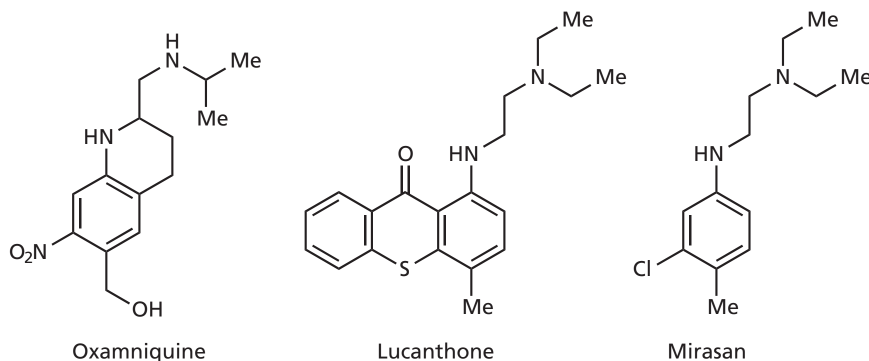


FIGURE CS4.1 Oxamniquine, lucanthone, and mirasan.

in order to bind efficiently (*active conformation; section 13.2*). This conformation would be only one of many conformations available to a flexible molecule such as mirasan, and so there would only be a limited chance of it being adopted at any one time. Therefore, it was decided to restrict the number of possible conformations by incorporating the side chain into a ring (*rigidification; section 13.3.9*). This would increase the chances of the molecule having the correct conformation when it approached its target binding site. There was the risk that the active conformation itself would be disallowed by this tactic and, so, rather than incorporate the whole side chain into a ring, compounds were first designed such that only portions of the chain were included.

The bicyclic structure (I in Fig. CS4.3) contains one of the side chain bonds fixed in a ring to prevent rotation round that bond. It was found that this gave a dramatic improvement in activity. The compound was still not active in humans, but, unlike mirasan, it was active in monkeys. This gave hope that the chemists were on the right track. Further *rigidification* led to structure II in Fig. CS4.3, where two of the side chain bonds were constrained. This compound showed even more activity in mouse studies and it was decided to concentrate on this compound.

By now the structure of the compound had been altered significantly from mirasan. When this is the case, it is advisable to check whether past results still hold true. For example, does the chloro group still have to be *ortho* to the methyl group? Can the chloro group be changed for something else? Novel structures may fit the binding site slightly differently from the lead compound, such that the binding groups are no longer in the optimum positions for binding.

Therefore, structure II was modified by *varying substituents* and *substitution patterns* on the aromatic ring (*section 13.3.1.2*), and by *varying alkyl substituents* on the amino groups (*section 13.3.1.1*). Chains were also *extended* (*section 13.3.3*) to search for other possible binding regions.

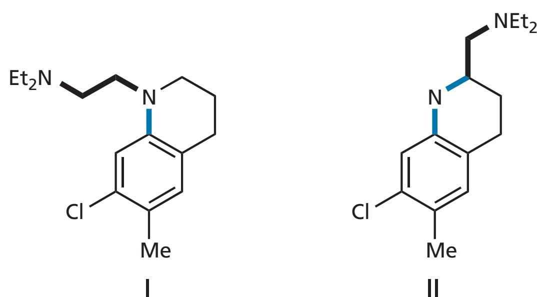


FIGURE CS4.3 Bicyclic structures I and II (restricted bonds in colour).

The results and possible conclusions were as follows.

- It was possible to vary substituents on the aromatic ring, but the substitution pattern itself could not be altered and was essential for activity. Altering the substitution pattern presumably places the essential binding groups out of position with respect to their binding regions.
- Replacing the chloro substituent with more electronegative substituents improved activity, with the nitro group being the best substituent. Therefore, an electron-deficient aromatic ring is beneficial to activity. One possible explanation for this could be the effect of the neighbouring aromatic ring on the basicity of the cyclic nitrogen atom. A strongly electron-deficient aromatic ring would pull the cyclic nitrogen's lone pair of electrons into the ring, thus reducing its basicity (Fig. CS4.4). This, in turn, might alter the pK_a of the drug such that it is less ionized and is able to pass through the cell membranes of the gut and target cells more easily (see sections 14.1.4 and 14.1.5). The electronic effect of a substituent on a distant functional group is a useful strategy in drug design (see sections 13.3.1.2, 14.1.5, and 19.5.1.8).
- The best activities were found if the amino group on the side chain was secondary rather than primary or tertiary (Fig. CS4.5).
- The alkyl group on this nitrogen could be increased up to four carbon units with a corresponding increase in activity. Longer chains led to a reduction in activity. The latter result might imply that large substituents are too bulky and prevent the drug from fitting the binding site. Acyl groups eliminated activity altogether,

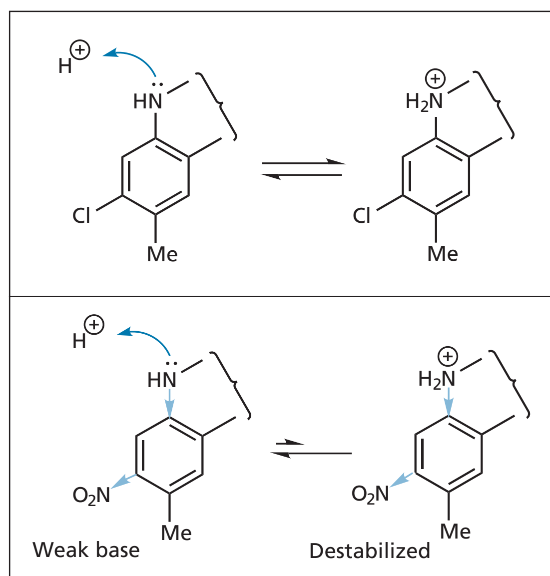


FIGURE CS4.4 Effect of aromatic substituents on pK_a.

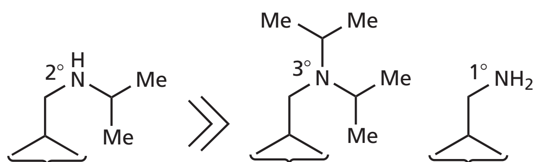


FIGURE CS4.5 Relative activity of amino side chains.

emphasizing the importance of this nitrogen atom. Most likely, it is ionized and interacts with the binding site through an ionic bond (Fig. CS4.6).

- Branching of the alkyl chain increased activity. A possible explanation is that branching increases van der Waals interactions to a hydrophobic region of the binding site (Fig. CS4.7). Alternatively, the lipophilicity of the drug might be increased, allowing easier passage through cell membranes.
- Putting a methyl group on the side chain eliminated activity (Fig. CS4.8). A methyl group is a bulky group compared with a proton and it is possible that it prevents the side chain taking up the correct binding conformation—*conformational blocking* (*section 13.3.10*).
- Extending the length of the side chain by an extra methylene group eliminated activity (Fig. CS4.9). This tactic was tried in case the binding groups were not far enough apart for optimum binding (*chain extension; section 13.3.3*). This result suggests the opposite.

The optimum structure based on these results was structure III (Fig. CS4.10). It has one asymmetric centre and,

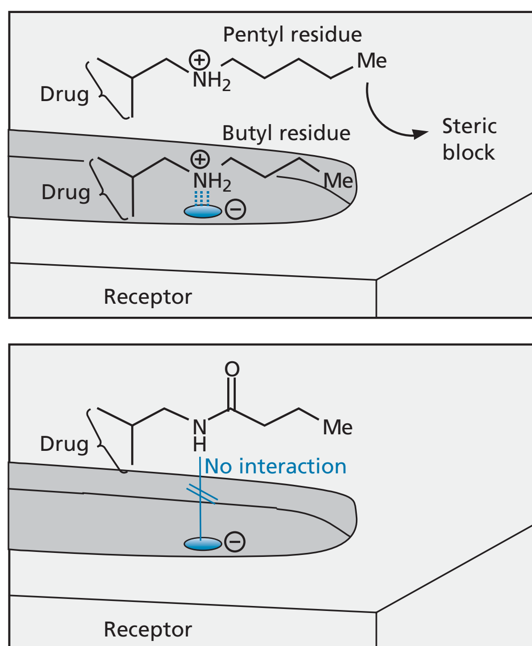


FIGURE CS4.6 Proposed ionic binding interaction.

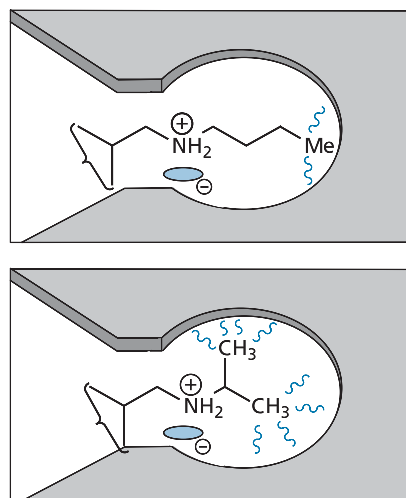


FIGURE CS4.7 Branching of the alkyl chain.

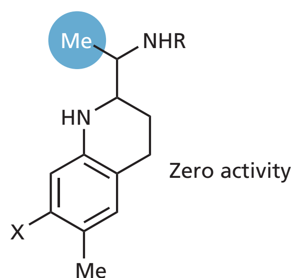


FIGURE CS4.8 Addition of a methyl group.

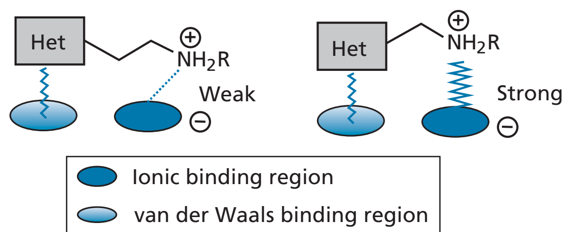


FIGURE CS4.9 Effect of extension of the side chain.

as one might expect, the activity was much greater for one enantiomer than it was for the other.

The tricyclic structure IV (Fig. CS4.10) was also constructed. In this compound, the side chain is fully incorporated into a ring structure, drastically restricting the number of possible conformations (*rigidification*). As mentioned earlier, there was a risk that the active conformation would no longer be allowed, but, in this case, good activity was still obtained. The same *variations* as above were carried out to show that a secondary amine was essential and that an electronegative group on the aromatic ring was required. However, some conflicting

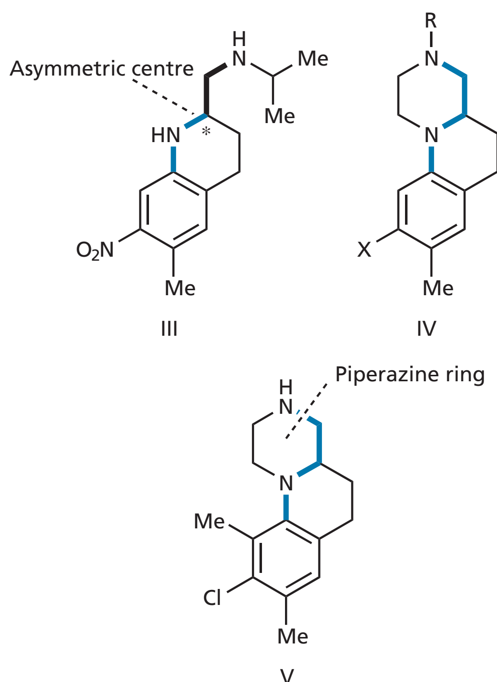


FIGURE CS4.10 The optimum structure (III) and the tricyclic structures (IV) and (V) (restricted bonds in colour).

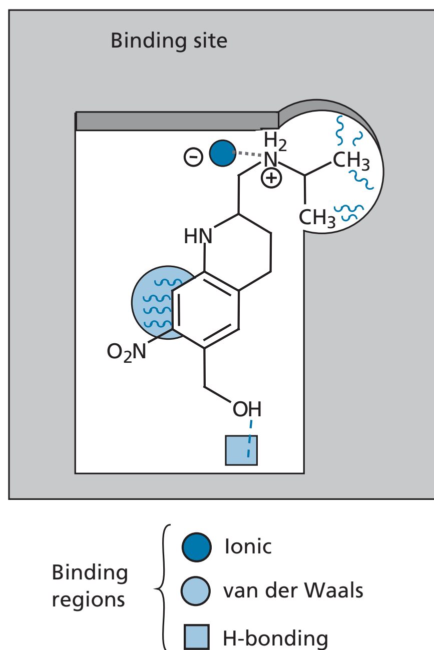


FIGURE CS4.11 Proposed binding interactions for oxamniquine to a binding site.

results were obtained compared with the previous results for structure III. A chloro substituent on the aromatic ring was better than a nitro group and it could be in either of the two possible *ortho* positions relative to the methyl group. These results demonstrate that optimizing substituents in one structure does not necessarily mean that they will be optimum in another. One possible explanation for the chloro substituent being better than the nitro is that a less electronegative substituent is required to produce the optimum pK_a or basicity for membrane permeability (section 14.1.5).

Adding a further methyl group to the aromatic ring to give structure (V) (Fig. CS4.10) increased activity. It was proposed that the bulky methyl group could interact with the piperazine ring, causing it to twist out of the plane of the other two rings (*conformational blocker*). The resulting increase in activity suggests that a better fitting conformation is obtained for the binding site.

Compound V was three times more active than structure III. However, structure III was chosen for further development. The decision to choose III rather than V was based on preliminary toxicity results, as well as the fact that it was cheaper to synthesize. Structure III is a simpler molecule and, in general, simpler molecules are easier and cheaper to synthesize.

Further studies on the metabolism of related compounds then revealed that the aromatic methyl group on these compounds is oxidized to a hydroxymethylene group (section 11.5.2) and that the resulting metabolites were more active compounds. This suggested that structure III was acting as a prodrug (section 14.6). Therefore, the methyl group on III was replaced by a hydroxymethylene group to give oxamniquine (Fig. CS4.11). It was proposed that the new hydroxyl group may be involved in an extra hydrogen bonding interaction with the binding site. The drug was put on the market in 1975, 11 years after the start of the project. Oxamniquine is effective as a single oral dose for treating infections of *S. mansoni.* Side effects are relatively mild compared with those of lucanthone, the most frequent being dizziness, drowsiness, and headache, which can last for a few hours after administration. Although the drug is not effective against all three strains of the parasite, it met all the other goals of the project and proved to be a highly successful drug. It is still used today in countries such as Brazil. The contribution that the drug made to tropical medicine earned Pfizer the Queen's Award for Technological Achievement in 1979.

CS4.1 Mechanism of action

When oxamniquine was being developed, its mechanism of action and target binding site were unknown. Oxamniquine is now known to inhibit nucleic acid synthesis in schistosomal cells. The mechanism of action is thought to involve prior activation of the drug by a

sulphotransferase enzyme that is present in parasitic cells, but not in mammalian cells. Once oxamniquine is bound to the active site of the schistosomal enzyme, the hydroxyl group is converted to a sulphate ester (Fig. CS4.12). The ester is a much better leaving group than the original hydroxyl group and so the molecule is now set up to dissociate. This is aided by the *para*-substituted nitrogen which can feed its lone pair of electrons into the aromatic ring. The structure that is formed is an alkylating agent which alkylates the DNA of the parasite and prevents DNA replication. This theory fits in nicely with the structure–activity relationship (SAR) results described previously, which emphasize the importance of the hydroxyl group, the aromatic amine, and the electron-deficient aromatic ring. It also explains why the agent is selectively toxic for the parasite rather than mammalian cells. Therefore, oxamniquine is acting as a prodrug (section 14.6.6), which is activated by the parasitic sulphotransferase enzyme.

CS4.2 Other agents

The knowledge that the CH_2OH group is crucial to the activity and mechanism of action of oxamniquine led to further investigations on lucanthone and mirasan. Studies showed that lucanthone was being oxidized *in vivo* to a metabolite called **hycanthone** (Fig. CS4.13), which then acts as a prodrug in a similar manner to oxamniquine. Hycanthone was shown to be more active than lucanthone and replaced it for the treatment of schistosomal infection. It was widely used in the 1970s, at which time it was considered the best treatment available. However, it suffered the same toxic side effects as lucanthone and was suspected of being mildly carcinogenic. It was gradually withdrawn from the market to be replaced by safer and more potent agents, such as oxamniquine.

Mirasan was also found to be oxidized *in vivo* to a metabolite (Fig. CS4.13) that proved to be active in a

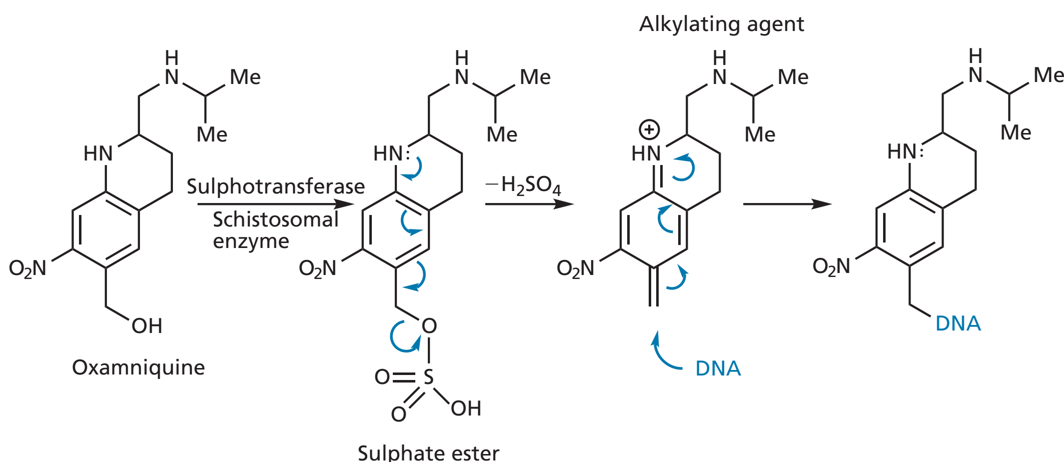


FIGURE CS4.12 Mechanism by which oxamniquine might dissociate to form an alkylating agent.

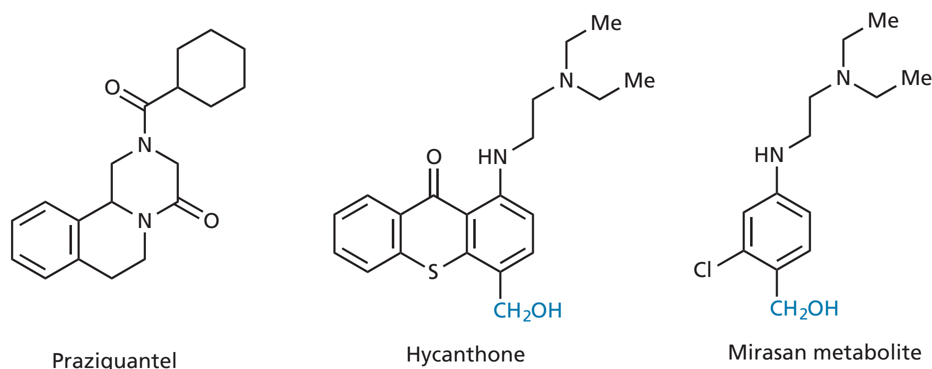


FIGURE CS4.13 Other agents.

BOX CS4.1 Synthesis of oxamniquine

One method of synthesizing oxamniquine is to start from the quinoline structure (I) (Fig. 1). The methyl substituent on the heterocyclic ring is selectively chlorinated and the alkyl chloride (II) undergoes a nucleophilic substitution with 2-aminopropane to form structure III. Reduction with hydrogen gas over a nickel catalyst gives the tetrahydroquinoline IV, which is nitrated to give a mixture of isomers. These are separated and the desired isomer is then hydroxylated in the presence of the fungus *Aspergillus sclerotiorum*. Microbial enzymes catalyse the oxidation reaction.

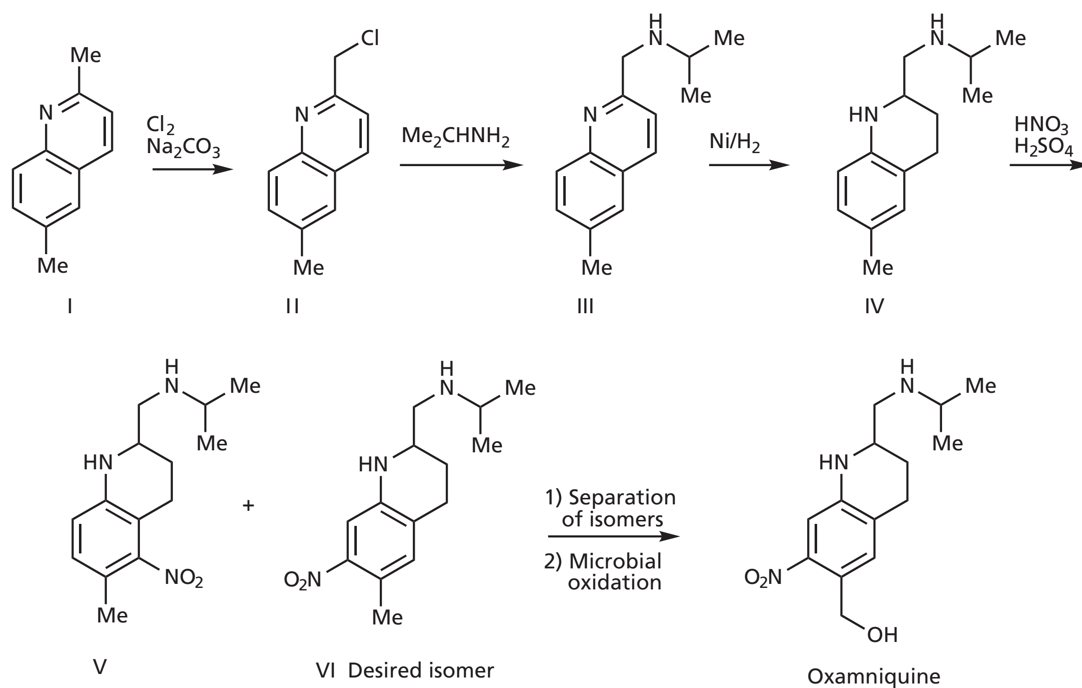


FIGURE 1 Synthesis of oxamniquine.

range of species. However, the metabolite was never used clinically. It is likely that the observed inactivity of mirasan in monkeys is a result of it being resistant to metabolic oxidation in that species.

Praziquantel (Fig. CS4.13) is now the recommended treatment for schistosomiasis in the UK, as it is active against all three parasitic strains. Unfortunately, it is more expensive than oxamniquine which limits its use in less affluent nations. New agents would be desirable, but schistosomicides are not economically attractive to the pharmaceutical industry.

FURTHER READING

Cioli, D., Pica-Mattoccia, L., and Archer, S. (1995) Antischistosomal drugs: past, present … and future? *Pharmacology and Therapeutics* **68**, 35–85.

Filho, S. B., Gargioni, C., Silva Pinto, P. L., Chiodelli, S. G., Gurgel Vellosa, S. A., da Silva, R. M., and da Silviera, M. A. (2002) Synthesis and evaluation of new oxamniquine derivatives. *International Journal of Pharmaceutics* **233**, 35–41.

Filho, R. P., de Souza Menezes, C. M., Pinto, P. L., Paula, G. A., Brandt, C. A., and da Silviera, M. A. (2007) Design, synthesis, and in vivo evaluation of oxamniquine methacrylate and acrylamide prodrugs. *Bioorganic and Medicinal Chemistry* **15**, 1229–1236.

Roberts, S. M., Price, B. J. (eds) (1985) Oxamniquine: a drug for the tropics. In: *Medicinal Chemistry—The Role of Organic Research in Drug Research*. Academic Press, London.