PART B

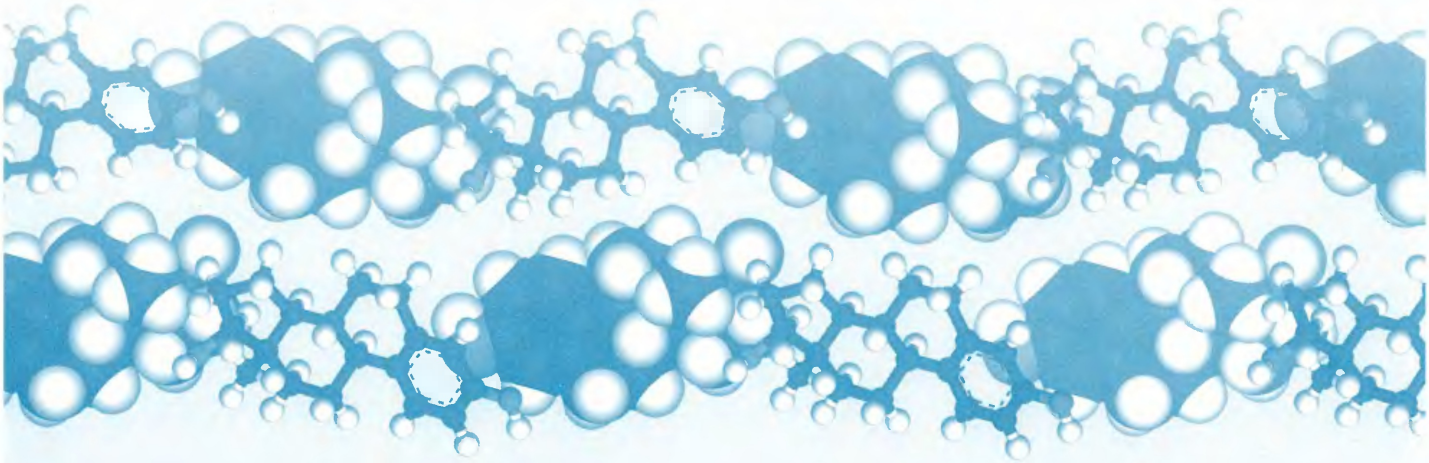
Pharmacodynamics and pharmacokinetics

The role of the medicinal chemist is to design and synthesize new drugs. In order to carry out that role, it is important to identify the particular target for a specific drug, and to establish how the drug interacts with that target to produce a biological effect. In chapters 2–6 of the previous section, we looked at the structure and function of various drug targets that are present in living systems. In this section we shall look at the general mechanisms by which drugs can produce a pharmacological or biological effect. This is an area of study known as pharmacodynamics.

Drugs are normally small molecules with a molecular weight less than 500 atomic mass units, and so they are much smaller than their macromolecular targets. As a result, they interact directly with only a small portion of the macromolecule. This is called a binding site. The binding site usually has a defined shape into which a drug must fit if it is to have an effect, and so it is important that the drug has the correct size and shape. However, there is more to drug action than just a good 'fit'. Once an active drug enters a binding site, a variety of intermolecular bonding interactions are set up which hold it there and lead to further effects, culminating eventually in a biological effect. For this to occur, the drug must have the correct functional groups and molecular skeleton capable of participating in these interactions.

Pharmacodynamics is the study of how a drug interacts directly with its target, and optimizing the pharmacodynamics is clearly important if we are to design an effective drug. Having said that, there are examples of compounds that interact extremely well with their target but which turn out to be useless in a clinical sense. That is because the compounds involved fail to reach their target in the body once they have been administered. There are various ways in which drugs can be administered, but generally the aim is to get the drug into the bloodstream such that it can then be carried to its particular target. Following the administration of a drug, there are a wide variety of hurdles and problems that have to be overcome. These include the efficiency with which a drug is absorbed into the bloodstream, how rapidly it is metabolized and excreted, and to what extent it is distributed round the body. This is an area of study known as pharmacokinetics, and we shall consider this in chapter 11.

Case Study 1 is a study on the clinically important statins used to lower cholesterol, and illustrates some of the principles of enzyme inhibitors mentioned in chapter 7.



7 Enzymes as drug targets

Many important drugs act as enzyme inhibitors. In other words, they hinder or prevent enzymes acting as catalysts for a particular reaction. We covered the structure and function of enzymes in chapter 3. In this chapter, we concentrate on how drugs target enzymes and inhibit their action.

7.1 Inhibitors acting at the active site of an enzyme

7.1.1 Reversible inhibitors

In chapter 3 we emphasized the importance of binding interactions between an enzyme and its substrate. If there are no interactions holding a substrate to the active site, then the substrate will drift in and drift back out again before there is a chance for it to react. Therefore, the more binding interactions there are, the stronger the substrate will bind, and the better the chance of reaction. But there is a catch! What happens if a substrate or product is bound *too* strongly to the active site (Fig. 7.1)?

The answer is that the enzyme becomes clogged up and is unable to accept any more substrate. Therefore, the binding interactions holding the substrate or the product to the enzyme must be properly balanced. They must be sufficiently strong that the substrate is held in the active site long enough for the reaction to occur, but weak enough to allow the product to leave. This bonding balancing act can be turned to great advantage if the medicinal chemist wishes to inhibit a particular enzyme, or to switch it off altogether. A molecule can be designed which is similar to the natural substrate or product, and can fit the active site, but which binds more strongly. It may not undergo any reaction when it is in the active site, but as long as it stays there, it blocks access to the natural substrate and the enzymatic reaction stops (Fig 7.2). This is known as competitive inhibition, as the drug is competing with the natural substrate for the active site. The longer the inhibitor is present in the active site, the greater the inhibition. Therefore, if the medicinal chemist knows which binding regions are present in an active site, and where they are, a range of molecules can be designed that can act as inhibitors.

Competitive inhibitors bind to the active site through intermolecular bonds and so the binding is reversible, allowing an equilibrium to occur between bound drug and unbound drug—a kind of 'yoyo' effect where the drug binds to the active site, is released, then binds again. This means that the inhibition caused by the drug is reversible. If the concentration of substrate increases, it competes more effectively with the drug for the active site, and so inhibition by the drug will be less effective (Box 7.1).

There are many examples of useful drugs that act as competitive inhibitors. For example, the **sulfonamides** act as antibacterial agents by inhibiting a bacterial enzyme in this fashion (section 19.4.1.5). Many diuretics used to

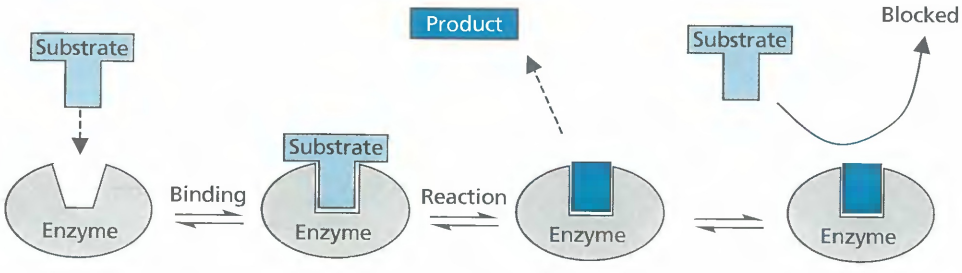


FIGURE 7.1 Example of an enzyme being 'clogged up' if the product remains bound.

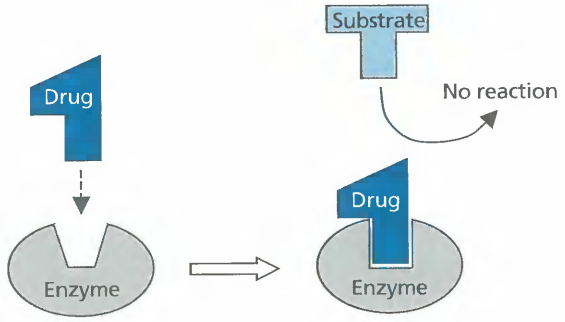


FIGURE 7.2 Competitive inhibition.

control blood pressure are competitive inhibitors, as are some antidepressants (section 23.12.5). Other examples include the statins (Case Study 1), ACE inhibitors (Case Study 2), and protease inhibitors (section 20.7.4). Indeed, most clinically useful enzyme inhibitors are of this nature.

As stated above, competitive inhibitors frequently bear some resemblance to the natural substrate, allowing them to be 'recognized' by the active site. Some of these inhibitors may have additional features which allow them to form extra binding interactions to regions of the active site that are not occupied by the substrate. This allows them to bind

BOX 7.1 A cure for antifreeze poisoning

Competitive inhibitors can generally be displaced by increasing the level of natural substrate. This feature has been useful in the treatment of accidental poisoning by antifreeze. The main constituent of antifreeze is **ethylene glycol**, which is oxidized in a series of enzymatic reactions to **oxalic acid**, which is toxic (Fig. 1). Blocking the synthesis of oxalic acid will lead to recovery.

The first step in this enzymatic process is the oxidation of ethylene glycol by **alcohol dehydrogenase (ADH)**. Ethylene glycol is acting here as a substrate, but we can view it as a competitive inhibitor because it is competing with the natural substrate for the enzyme (Fig. 2a). If the levels of natural substrate are increased, it will compete far better with ethylene glycol and prevent it from reacting (Fig. 2b). Toxic oxalic acid will no longer be formed and the unreacted ethylene glycol is eventually excreted from the body. The cure, then, is to administer high doses of the natural substrate—alcohol!

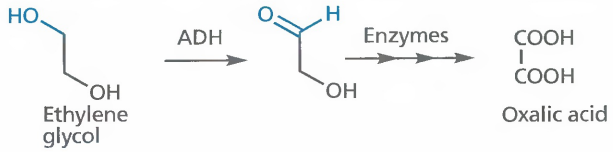


FIGURE 1 Formation of oxalic acid from ethylene glycol.

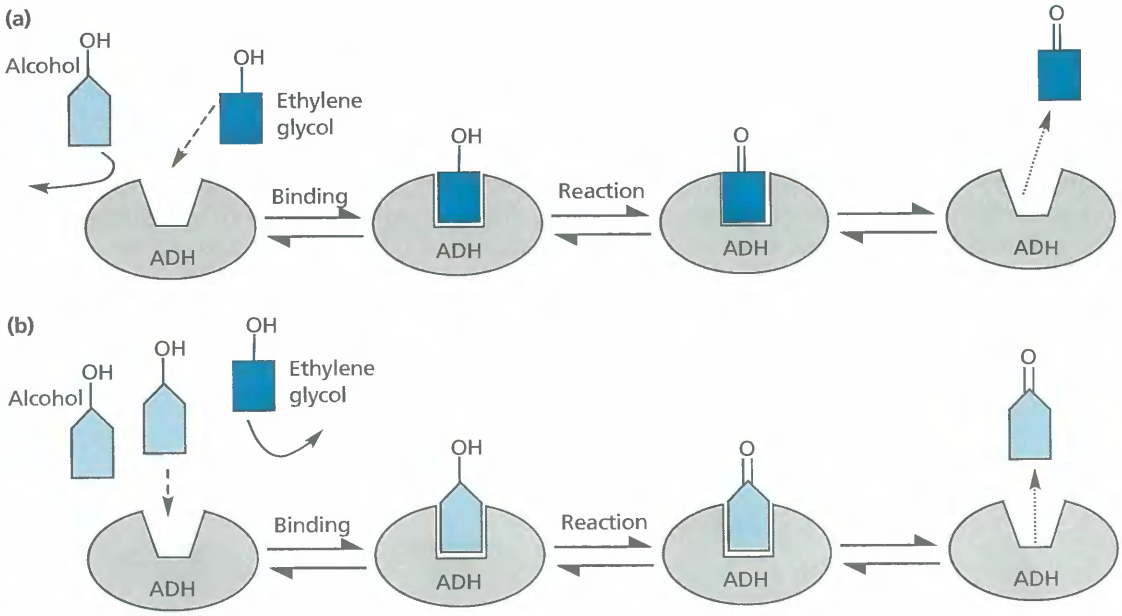


FIGURE 2 (a) Oxidation of ethylene glycol and (b) blocking with excess alcohol.

more strongly and to be more effective inhibitors. The statins described in Case Study 1 are a good example of this.

Although competitive inhibitors often bear some resemblance to the substrate, this is not always the case. As long as the drug has the right shape to fit the active site, and has functional groups that can interact with the binding regions available, it can still bind to the active site and inhibit the enzyme. Therefore, it is possible for drugs with a totally different skeleton to the substrate to act as competitive inhibitors. Such drugs may bind to a combination of binding regions within the active site, some of which are used by the substrate, and some of which are not.

It should also be remembered that the product of an enzyme catalysed reaction is bound to the active site before it is finally released, and so it is possible to have enzyme inhibitors which resemble the structure of the product more closely than the substrate. Other drugs are designed to mimic the transition state of the enzyme-catalysed reaction (section 7.4).

Finally, some competitive inhibitors bind to the active site, but do not compete with the substrate. How can this occur? The answer lies in the fact that the active site of some enzymes bind a substrate *and* an enzyme cofactor. Therefore, it is possible to have competitive inhibitors which bind to the region of the active site occupied by the cofactor, and compete with it rather than with the substrate. The kinase inhibitors described in section 21.6.2 are a good example of this. These agents compete with the cofactor ATP for the active site and not the protein substrate.

7.1.2 Irreversible inhibitors

There are some enzyme inhibitors that bind irreversibly to the active site and block the enzyme permanently. The most effective irreversible inhibitors are those that can react with an amino acid in the active site to form a covalent bond. Amino acids such as **serine** and **cysteine** which bear nucleophilic residues (OH and SH respectively) are present in several enzyme active sites, as they are often involved in the mechanism of the enzyme reaction (section 3.5.3). By designing an electrophilic drug that fits the active site, it is possible to alkylate these nucleophilic groups and hence permanently clog up the active site (Fig. 7.3). The nerve gases (section 22.15.2.1) are irreversible inhibitors of mammalian enzymes and are therefore highly toxic. In the same way, penicillins (section 19.5.1) are highly toxic to bacteria and act by irreversibly inhibiting an enzyme that is crucial to cell wall synthesis.

Not all irreversible inhibitors are highly toxic and several are used clinically. For example, disulfuram (Antabuse) (Box 12.6) is an irreversible inhibitor of the enzyme alcohol dehydrogenase and is used to treat alcoholism. The proton pump inhibitors described in section 25.3 are irreversible inhibitors and are used as antiulcer agents. The anti-obesity drug orlistat is also an irreversible inhibitor (Box 7.2). Having said that, it is generally better to inhibit an enzyme with a reversible inhibitor rather than an irreversible inhibitor. Since irreversible inhibitors have reactive functional groups, there is a risk that they might react with other proteins or nucleic acids and cause toxic side effects.

Irreversible enzyme inhibitors are not competitive inhibitors. Increasing the concentration of substrate will not reverse their inhibition since the inhibitors cannot be displaced from the active site. This can cause problems if the build up of a particular substrate leads to toxic side effects. For example, the monoamine oxidase inhibitors (MOAIs) block the metabolism of noradrenaline and have antidepressant activity (section 23.12.5). Unfortunately, the metabolism of substrates other than noradrenaline is also inhibited, leading to a build up of these compounds and serious side effects. More modern MAOIs have been designed to be reversible inhibitors in order to avoid this problem.

7.2 Inhibitors acting at allosteric binding sites

Allosteric binding sites were discussed in section 3.6 and are a means by which enzyme activity can be controlled by natural inhibitors. The allosteric binding site is at a different location on the enzyme to the active site, and when an inhibitor binds there an induced fit takes place which not only alters

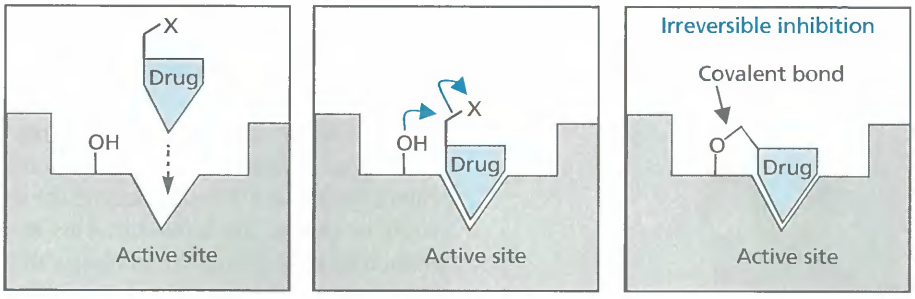
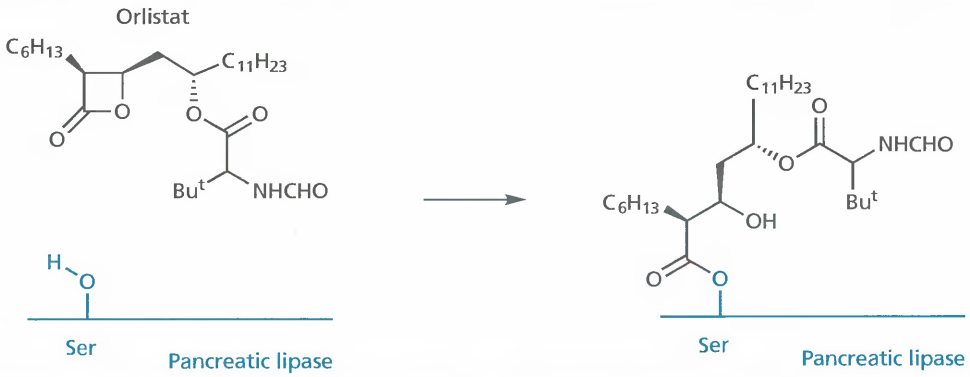


FIGURE 7.3 Irreversible inhibition of an enzyme with an alkylating agent. X, halogen leaving group.

BOX 7.2

Irreversible inhibition for the treatment of obesity

Fat in the diet is composed mainly of triglycerides which are digested in the small intestine to fatty acids and 2-monoglycerides. The digestion products are then absorbed and act as the building blocks for fat biosynthesis in the body. The enzyme pancreatic lipase is responsible for catalysing the digestion of fats, and so inhibition of this enzyme will result in a reduced absorption of glycerides and fatty acids from the gut. Consequently, less fat will be synthesized in the body. Orlistat is an anti-obesity drug that acts as an irreversible inhibitor of pancreatic lipase. It acylates a serine residue in the active site which is part of a catalytic triad of serine, histidine and aspartic acid (compare section 3.5.3).



the shape of the allosteric binding site, but also deforms the shape of the active site such that it becomes unrecognizable to the substrate. Drugs can be designed to mimic this natural control of the enzyme. If the drug binds through intermolecular interactions, the inhibition is reversible. If the drug contains a reactive group allowing it to form a covalent bond to the allosteric binding site, the inhibition is irreversible.

An enzyme with an allosteric binding site offers the medicinal chemist an extra option in designing an inhibitor. The chemist can not only design drugs which are based on the structure of a substrate or a cofactor which bind directly to the active site, but can also design drugs based on the structure of the endogenous compound that naturally binds to the allosteric binding site and controls the activity of the enzyme.

The drug **6-mercaptopurine** (Fig. 7.4), used in the treatment of leukaemia, is an example of an allosteric inhibitor. It inhibits the first enzyme involved in the synthesis of purines (section 6.1) and therefore blocks purine synthesis. This in turn blocks DNA synthesis.

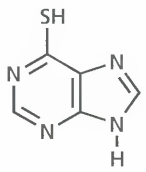


FIGURE 7.4 6-Mercaptopurine.

7.3 Uncompetitive and non-competitive inhibitors

Uncompetitive inhibitors are inhibitors that can only bind reversibly to an enzyme when the substrate is already bound to the active site. In other words, the inhibitor binds to the enzyme–substrate complex. In this situation, increasing the substrate concentration will not overcome inhibition as it would with competitive inhibitors. Indeed, the level of inhibition is dependent on sufficient substrate being present to form the enzyme–substrate complexes. Therefore, uncompetitive inhibitors are less effective at low substrate concentrations. Uncompetitive inhibitors are not very common.

In theory, a non-competitive inhibitor is one which binds to an allosteric binding site and inhibits an enzyme-catalysed reaction without affecting the ability of the substrate to bind to the active site. This would occur if the induced fit arising from the binding of the allosteric inhibitor distorts the active site sufficiently to prevent the catalytic mechanism taking place, but not the binding process.

In practice, this ideal situation is extremely rare if it even occurs at all. It is almost inevitable that any active site distortion affecting the catalytic process will also affect substrate binding. Therefore, those inhibitors which inhibit the catalytic process without preventing substrate binding, normally cause some inhibition of substrate binding. This is known as mixed inhibition since it is neither pure competitive inhibition nor pure non-competitive inhibition.

7.4 Transition-state analogues: renin inhibitors

An understanding of an enzyme mechanism can help medicinal chemists design more powerful inhibitors. For example, it is possible to design inhibitors which bind so strongly to the active site (using non-covalent forces) that they are effectively irreversible inhibitors—a bit like inviting someone for dinner and finding that they have moved in on a permanent basis. One way of doing this is to design a drug that resembles the transition state for the catalysed reaction. Such a drug should bind more strongly than either the substrate or the product, and be a strong inhibitor as a result. Such compounds are known as **transition-state analogues or inhibitors**.

The use of transition-state analogues has been particularly effective in the development of renin inhibitors (Fig. 7.5). Renin is a protease enzyme which is responsible for hydrolysing a specific peptide bond in the protein angiotensinogen to form angiotensin I. Angiotensin I is further converted to angiotensin II, which acts to constrict blood vessels and retain fluid in the kidneys, both of which lead to a rise in blood pressure. Therefore, an inhibitor of renin should act as an antihypertensive agent (i.e. lower blood pressure) by preventing the first stage in this process.

Renin contains two aspartyl residues and a bridging water molecule in the active site which are crucial to the mechanism of action by which an amide bond in the substrate is hydrolysed (Fig. 7.6). In the first stage of this mechanism, a tetrahedral intermediate is formed. In order to form this intermediate, the reaction mechanism has to proceed through a high energy transition state, and it is this transition state that we wish to mimic with a transition-state analogue. However, it is not possible to isolate such a high energy species in order to study its structure, so how can one design a drug to mimic it? The answer is to base the design of the drug on the reaction intermediate. The rationale for this is as follows. Since the intermediate is less stable than the substrate, it is presumed that it is closer in character to the transition state. This in turn implies that the transition state is more tetrahedral in character than planar. Therefore, drugs based on the structure of the tetrahedral intermediate are more likely to mimic the transition state.

The intermediate itself is reactive and easily cleaved. Therefore, an analogue has to be designed which binds as strongly as it, but is stable to hydrolysis. This can be

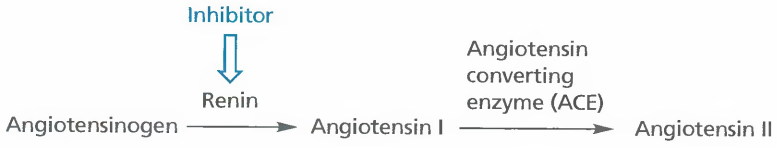


FIGURE 7.5 Inhibition of renin to block the synthesis of angiotensin I and angiotensin II.

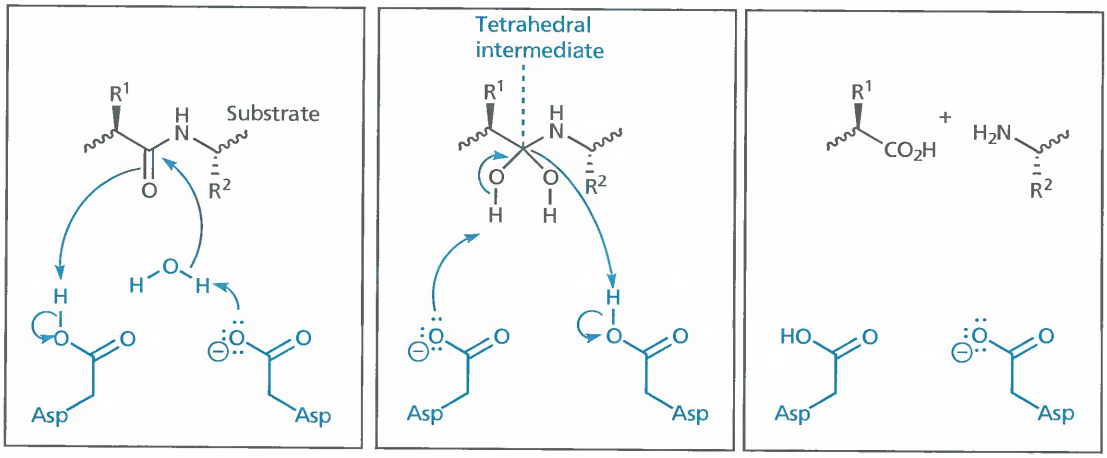


FIGURE 7.6 Mechanism of renin-catalysed hydrolysis.

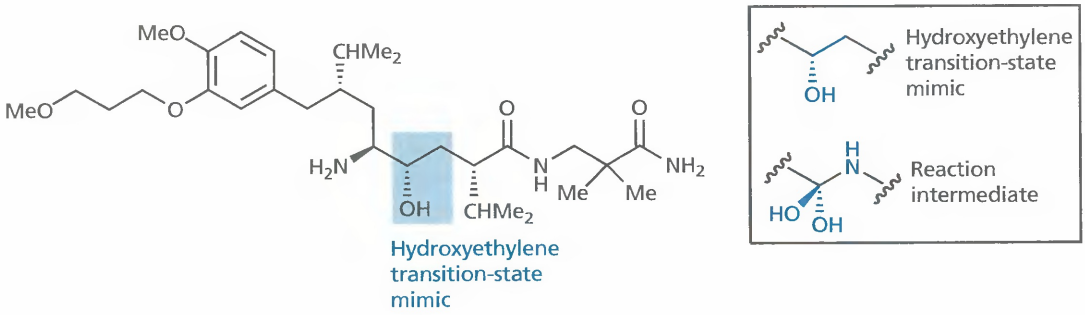


FIGURE 7.7 Aliskiren.

done by introducing a feature that mimics the tetrahedral structure of the intermediate, but has no leaving group for the second part of the reaction mechanism. A variety of mimics has been tried and a hydroxyethylene moiety has proved effective (e.g. aliskiren; Fig. 7.7). The hydroxyethylene group has the required tetrahedral geometry and one of the two hydroxyl groups required for good binding. It is stable to hydrolysis because there is no leaving group present. Aliskiren was approved by the US FDA in 2007 for the treatment of hypertension.

Similar strategies have been used successfully to design antiviral agents which act as transition-state analogue inhibitors for the HIV protease enzyme (section 20.7.4). The statins can also be viewed as transition-state analogues (Case Study 1), as can some ACE inhibitors (Case Study 2).

7.5 Suicide substrates

Transition-state analogues can be viewed as *bona fide* visitors to an enzyme's active site that become stubborn squatters once they have arrived. Other apparently harmless visitors can turn into lethal assassins once they have bound to their target enzyme. Such agents are designed to undergo an enzyme-catalysed transformation which converts them into a highly reactive species that forms a covalent bond to the active site. One example of this is the unnatural amino acid **trifluoroalanine** which irreversibly inhibits the enzyme **alanine transaminase** (Fig. 7.8).

The normal mechanism for the transamination reaction is shown in Fig. 7.9 (R=H) and involves the condensation of alanine and **pyridoxal phosphate** to give an imine. A proton is lost from the imine to give a dihydropyridine intermediate. This reaction is catalysed by a basic amino acid provided by the enzyme, and by the electron-withdrawing effects of the protonated pyridine ring. The dihydropyridine structure now formed is hydrolysed to give the products.

Trifluoroalanine contains three fluorine atoms, which are very similar in size to the hydrogen atoms in alanine. The molecule is therefore able to fit into the active site of the enzyme and take alanine's place. The reaction mechanism proceeds as before to give the dihydropyridine intermediate. However, at this stage, an alternative mechanism now becomes possible (Fig. 7.9; R=F). A fluoride atom is electronegative and can therefore act as a leaving group. When this happens, a powerful alkylating agent is formed which can irreversibly alkylate any nucleophilic group present in the enzyme's active site. A covalent bond is now formed and the active site is unable to accept further substrate. As a result, the enzyme is irreversibly inhibited.

Drugs that operate in this way are often called **mechanism-based inhibitors** or **suicide substrates** because the enzyme is committing suicide by reacting with them (Box 7.3). The great advantage of this approach is that the alkylating agent is generated at the site where it is meant to act and is therefore highly selective for the target enzyme. If the alkylating group had not been disguised in this way, the drug would have alkylated the first nucleophilic group it met in the body and would have shown little or no selectivity. The uses of alkylating agents and the problems associated with them are discussed in sections 9.3 and 21.2.3.

Inhibiting the transaminase enzyme has no medicinal application; the enzyme is crucial to mammalian biochemistry, and inhibiting it would be toxic to the host. The main use for suicide substrates has been in labelling specific enzymes. The substrates can be labelled with radioactive isotopes and reacted with their target enzyme in order to locate the enzyme in tissue preparations. However, the suicide substrate approach has potential therapeutic applications against enzymes that are unique to foreign invaders such as bacteria, protozoa, and fungi. **Clavulanic acid** (section 19.5.4.1) can be classed as a suicide substrate and is present in the antibacterial preparation called Augmentin. Another interesting example of a suicide substrate is **5-fluorodeoxyuracil monophosphate** (5-FdUMP). The anticancer agent **5-fluorouracil** is used to treat cancers of the breast, liver, and skin, and is converted to 5-FdUMP in the body. This then acts as a suicide substrate for the enzyme thymidylate synthase (section 21.3.2).

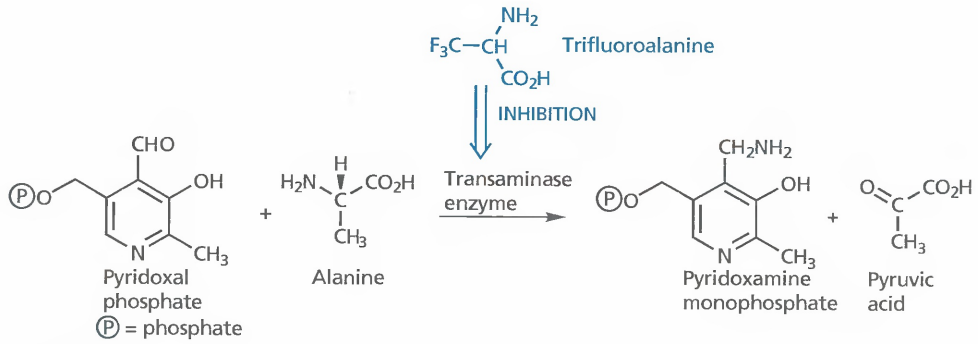


FIGURE 7.8 The reaction catalysed by alanine transaminase, which is inhibited by trifluoroalanine.

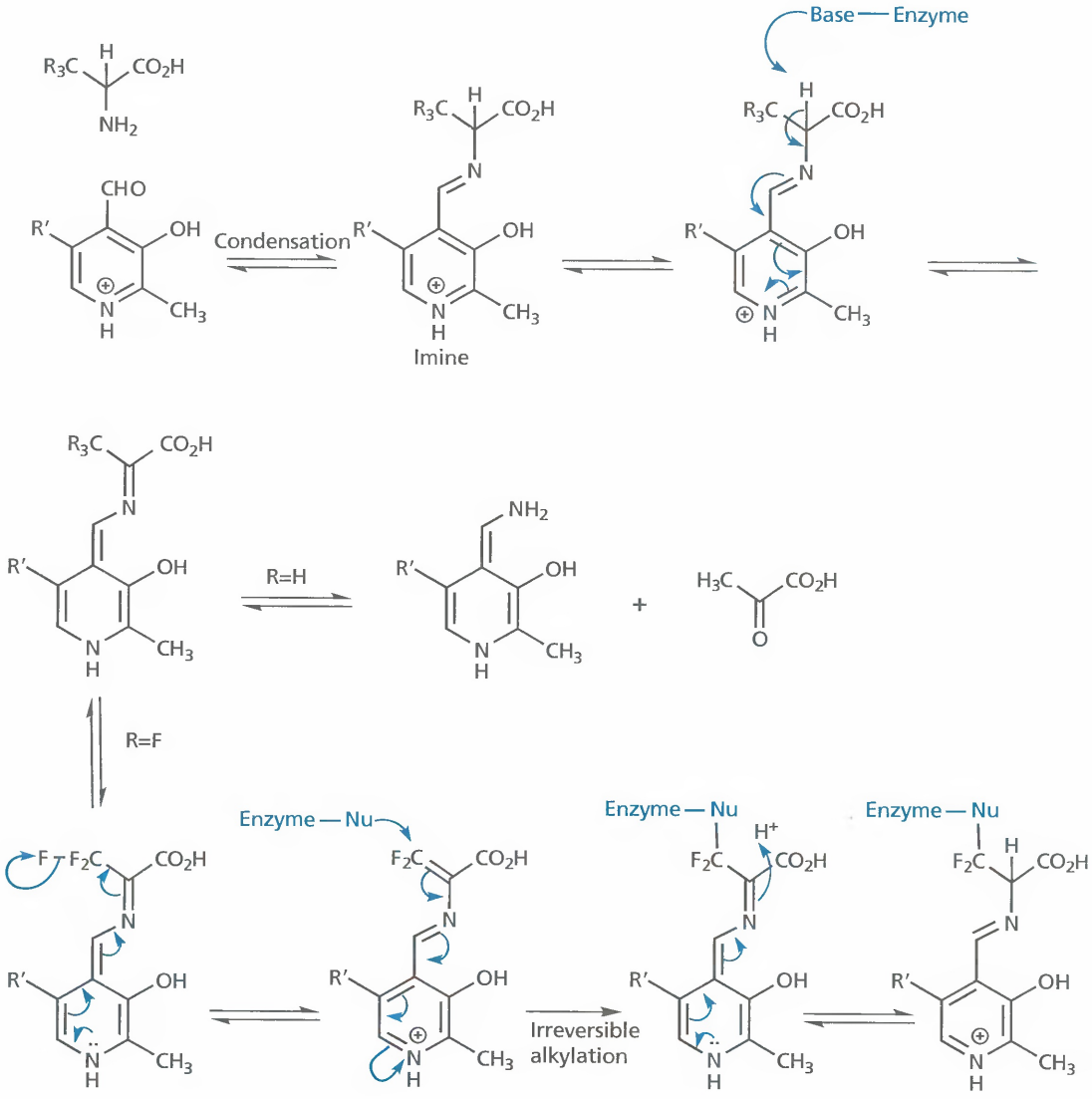
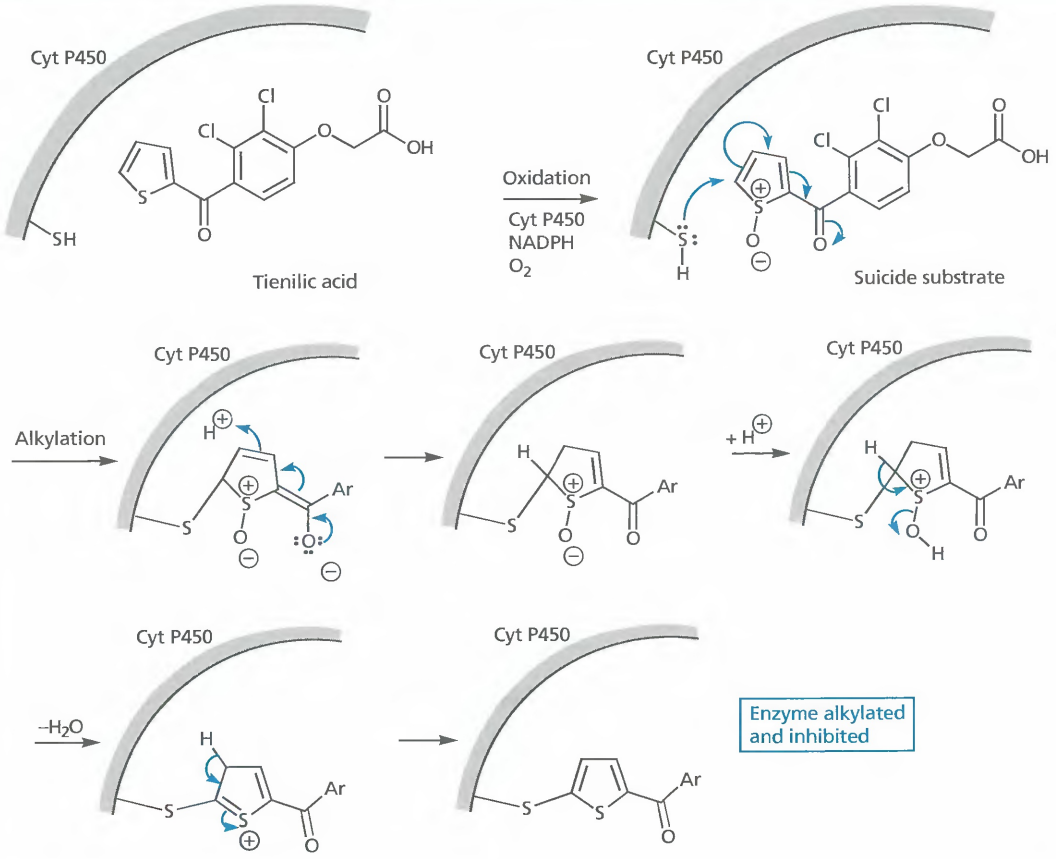


FIGURE 7.9 Mechanism for the transamination reaction and its irreversible inhibition.

BOX 7.3 Suicide substrates

Suicide substrates are agents which are converted to highly reactive species when they undergo an enzyme-catalysed reaction. They form covalent bonds to the enzyme and inhibit it irreversibly. In some cases, this can cause toxicity. For example, the diuretic agent **tienilic acid** had to be withdrawn from the market because it was found to act as a suicide substrate for the cytochrome P450 enzymes involved in drug metabolism (section 11.4.2). Unfortunately, the metabolic reaction carried out by these enzymes converted tienilic acid to a thiophene sulfoxide which proved highly electrophilic. This encouraged a Michael reaction leading to alkylation of a thiol group in the enzyme's active site. Loss of water from the thiophene sulfoxide restored the thiophene ring and resulted in the formation of a covalent link to the enzyme, thus inhibiting the enzyme irreversibly.



Irreversible inhibition of cytochrome P450 by tienilic acid.

7.6 Isozyme selectivity of inhibitors

Identification of isozymes that are selective for certain tissues allows the possibility of designing tissue-selective enzyme inhibitors (Box 7.4).

For example, the non-steroidal anti-inflammatory drug (NSAID) **indometacin** (Fig. 7.10) is used to treat inflammatory diseases such as rheumatoid arthritis, and works by inhibiting the enzyme **cyclooxygenase**. This enzyme is involved in the biosynthesis of **prostaglandins**—agents which are responsible for the pain and inflammation of rheumatoid arthritis. Inhibiting the enzyme lowers prostaglandin levels and alleviates the symptoms of the disease. However, the drug also inhibits the synthesis of beneficial prostaglandins in the gastrointestinal tract and the kidney. It has been discovered that cyclooxygenase has two isozymes, COX-1 and COX-2. Both isozymes carry out the same reactions, but COX-1 is the isozyme that is active under normal healthy conditions. In rheumatoid

BOX 7.4 Designing drugs to be isozyme selective

Designing drugs to be isozyme selective means that they can be designed to act on different diseases despite acting on the same enzyme. This is because isozymes differ in substrate specificity and are distributed differently in the body. **Monoamine oxidase (MAO)** is one of the enzymes responsible for the metabolism of important neurotransmitters such as **dopamine, noradrenaline**, and **serotonin** (section 4.2) and exists in two isozymic forms (MAO-A and MAO-B). These isozymes differ in substrate specificity, tissue distribution, and primary structure, but carry out the same reaction by the same mechanism (Fig. 1). MAO-A is selective for noradrenaline and serotonin whereas MAO-B is selective for dopamine. MAO-A inhibitors such as **clorgiline** are used clinically as antidepressants while MAO-B inhibitors such as **selegiline** are administered with **levodopa** for the treatment of Parkinson's disease (Fig. 2). MAO-B inhibition protects levodopa from metabolism. Clorgiline and selegiline are thought to act as suicide substrates where they are converted by the enzyme to reactive species which react with the enzyme and form covalent bonds. The amine and alkyne functional groups present in both drugs are crucial to this process.

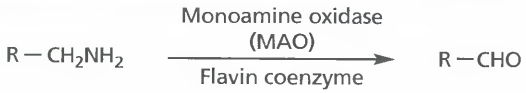


FIGURE 1 Reaction catalysed by MAO.

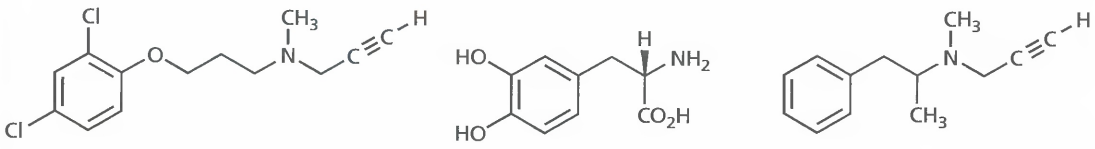


FIGURE 2 Clorgiline, levodopa and selegiline.

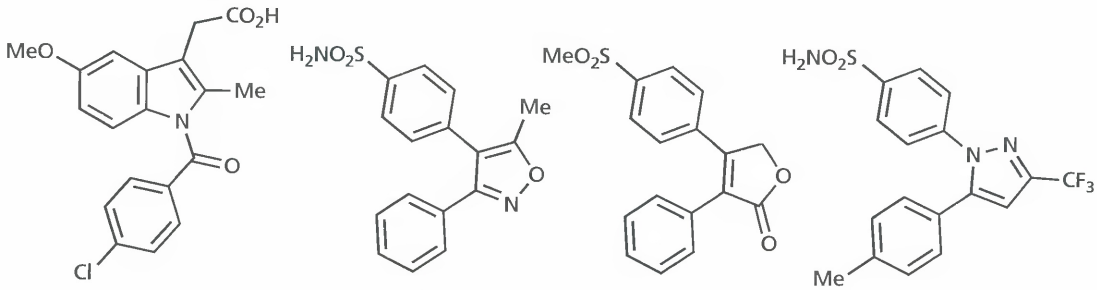


FIGURE 7.10 Cyclooxygenase inhibitors.

arthritis, the normally dormant COX-2 becomes activated and produces excess inflammatory prostaglandins. Therefore, drugs such as **valdecoxib**, **rofecoxib**, and **celecoxib** have been developed to be selective for the COX-2 isozyme, so that only the production of inflammatory prostaglandins is reduced. Rofecoxib was authorized in 1999, but had to be withdrawn in September 2004 as it was linked to an increased risk of heart attack and stroke when taken over a period of 18 months or so.

7.7 Medicinal uses of enzyme inhibitors

7.7.1 Enzyme inhibitors used against microorganisms

Inhibitors of enzymes have been extremely successful in the war against infection. If an enzyme is crucial to a microorganism, then switching it off will clearly kill the

cell or prevent it from growing. Ideally, the enzyme chosen should be one that is not present in our own bodies, and fortunately such enzymes exist because of the significant biochemical differences between bacterial cells and our own. Nature, of course, is well ahead in this game. For example, many fungal strains produce metabolites such as **penicillin** which are toxic to bacteria but not to themselves. This gives fungi an advantage over their microbiological competitors when competing for nutrients.

Although it is preferable to target enzymes that are unique to the foreign invader, it is still possible to target enzymes that are present in both bacterial and mammalian cells, as long as there are significant differences between them. Such differences are perfectly feasible. Although the enzymes in both species may have derived from a common ancestral protein, they have evolved and mutated separately over several million years. Identifying these differences allows the medicinal chemist to design drugs that will bind and act selectively against the bacterial enzyme. Chapter 19 covers antibacterial agents such as the sulfonamides, penicillins, and cephalosporins, all of which act by inhibiting enzymes.

7.7.2 Enzyme inhibitors used against viruses

Enzyme inhibitors are also extremely important in the battle against viral infections (e.g. herpesvirus and HIV). Successful antiviral drugs include **aciclovir** for herpes, and drugs such as **zidovudine** and **saquinavir** for HIV (see chapter 20).

7.7.3 Enzyme inhibitors used against the body's own enzymes

Drugs that act against the body's own enzymes are important in medicine. Some examples are given in Table 7.1. Agents known as **anticholinesterases**, which are used in a variety of diseases including Alzheimer's disease, are discussed in sections 22.14–22.17. Antidepressant agents known as monoamine oxidase inhibitors are discussed

TABLE 7.1 Medicinally useful drugs that act against enzymes in the body.

Drug	Target enzyme	Field of therapy
Aspirin	Cyclooxygenase	Anti-inflammatory
Captopril and enalapril	Angiotensin converting enzyme (ACE)	Antihypertension
Simvastatin	HMG-CoA reductase	Lowering of cholesterol levels
Desipramine	Monoamine oxidase	Antidepressant
Clorgiline	Monoamine oxidase-A	Antidepressant
Selegiline	Monoamine oxidase-B	Treatment of Parkinson's disease
Methotrexate	Dihydrofolate reductase	Anticancer
5-Fluorouracil	Thymidylate synthase	Anticancer
Gefitinib and imatinib	Tyrosine kinases	Anticancer
Sildenafil (Viagra)	Phosphodiesterase enzyme (PDE5)	Treatment of male erectile dysfunction
Allopurinol	Xanthine oxidase	Treatment of gout
Zidovudine	HIV reverse transcriptase	AIDS therapy
Saquinavir	HIV protease	AIDS therapy
Aciclovir	Viral DNA polymerase	Treatment of herpes
Penicillins and cephalosporins	Bacterial transpeptidase	Antibacterial
Clavulanic acid	Bacterial β-lactamases	Antibacterial
Sulfonamides	Dihydropteroate synthetase	Antibacterial
Fluoroquinolones	Bacterial topoisomerases	Antibacterial
Ro41–0960	Catechol-*O*-methyltransferase	Treatment of Parkinson's disease
Omeprazole	H^+/K^+ ATPase proton pump	Ulcer therapy
Organophosphates	Acetylcholinesterase	Treatment of myasthenia gravis, glaucoma, and Alzheimer's disease
Acetazolamide	Carbonic anhydrase	Diuretic
Zileutin	5-Lipoxygenase	Anti-asthmatic

BOX 7.5 Action of toxins on enzymes

The toxicity of several poisons, toxins and heavy metals result from their action on enzymes. Heavy metals such as lead, cadmium and mercury have teratogenic effects leading to babies being born with malformed limbs. The worst case of mercury poisoning was in Japan, where a local population ate fish contaminated with methyl mercury (MeHg) that had been used as an agricultural fungicide. The compound inactivates enzymes by reacting with the thiol groups (R–SH) of cysteine residues to form covalent bonds (R–S–HgMe).

Mercury poisoning can also affect enzymes in the central nervous system leading to strange behaviour. For example, mercury nitrate was used by hat makers to soften and shape animal furs, and inevitably some of the chemical was absorbed through the skin. So many in the trade were poisoned in this way that their peculiar manner of behaviour led to the phrase 'mad as a hatter'.

The poison arsenite (AsO_3^{3-}) reacts with the thiol groups of an enzyme cofactor called dihydrolipoate which is a prosthetic group (section 3.5.4) in some enzymes (Fig. 1). It is possible to reverse the poisoning by administering reagents with adjacent thiol groups that displace the arsenic from the cofactor. **2,3-Dimercaptopropanol** was developed after World War 1 as an antidote to an arsenic-based chemical weapon called **lewisite**.

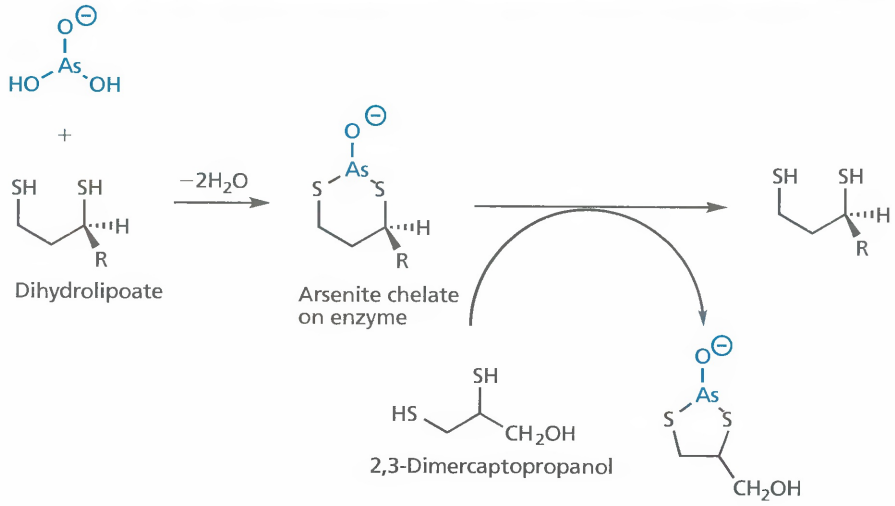


FIGURE 1 Mechanism of arsenite poisoning and its treatment.

in section 23.12.5. Proton pump inhibitors, used as antiulcer agents, are discussed in section 25.3. Anticancer drugs that inhibit enzymes are discussed in sections 21.3, 21.6, and 21.7.

The search continues for new enzyme inhibitors, especially those that are selective for a specific isozyme, or act against recently discovered enzymes. Some current research projects include investigations into inhibitors of the **COX-2 isozyme** (sections 7.6 and 21.7.2), **matrix metalloproteinases** (antiarthritic and anticancer drugs; section 21.7.1), **aromatases** (anticancer agents; section 21.4.5), and **caspases.** The caspases are implicated in the processes leading to cell death. Inhibitors of caspases may have potential in the treatment of stroke victims (Box 12.1). A vast amount of research is also taking place on inhibitors of a family of enzymes known as **kinases.** These enzymes catalyse the phosphorylation of proteins and play an important role in signalling pathways within cells (chapter 5 and section 21.6.2).

KEY POINTS

- Enzyme inhibition is reversible if the drug binds through intermolecular interactions. Irreversible inhibition results if the drug reacts with the enzyme and forms a covalent bond.
- Competitive inhibitors bind to the active site and compete with either the substrate or the cofactor.
- Allosteric inhibitors bind to an allosteric binding site that is different from the active site. They alter the shape of the enzyme such that the active site is no longer recognizable.

- Transition-state analogues are enzyme inhibitors that are designed to mimic the transition state of an enzyme-catalysed reaction mechanism. They bind more strongly than either the substrate or the product.
- Suicide substrates are molecules that act as substrates for a target enzyme, but which are converted into highly reactive species as a result of the enzyme catalysed reaction mechanism. These species react with amino acid residues present in the active site to form covalent bonds, and act as irreversible inhibitors.
- Drugs that selectively inhibit isozymes are less likely to have side effects and be more selective in their effect.
- Enzyme inhibitors are used in a wide variety of medicinal applications.

7.8 Enzyme kinetics

Studies of enzyme kinetics are extremely useful in determining the properties of an enzyme inhibitor.

7.8.1 Lineweaver–Burk plots

The Michaelis–Menten equation described in section 3.8 can be modified by taking its reciprocal such that a straight line plot is obtained (the Lineweaver–Burk plot) (Figure 7.11a):

$$\frac{1}{\text{rate}} = \frac{1}{\text{rate}_{max}} + \frac{K_M}{\text{rate}_{max}[S]}$$

Plots such as these can be used to determine whether an inhibitor is competitive, uncompetitive or non-competitive (Figs. 7.11b and 7.12). The reaction rate of the enzyme reaction is measured with respect to varying substrate concentration, with and without an inhibitor being present.

In the case of competitive inhibition, the lines cross the y-axis at the same point (i.e. the maximum rate of the enzyme is unaffected), but the slopes are different. The fact that the maximum rate is unaffected reflects the fact that the inhibitor and substrate are competing for the same active site and that increasing the substrate concentration sufficiently will overcome the inhibition. The increase in the slope that results from adding an inhibitor

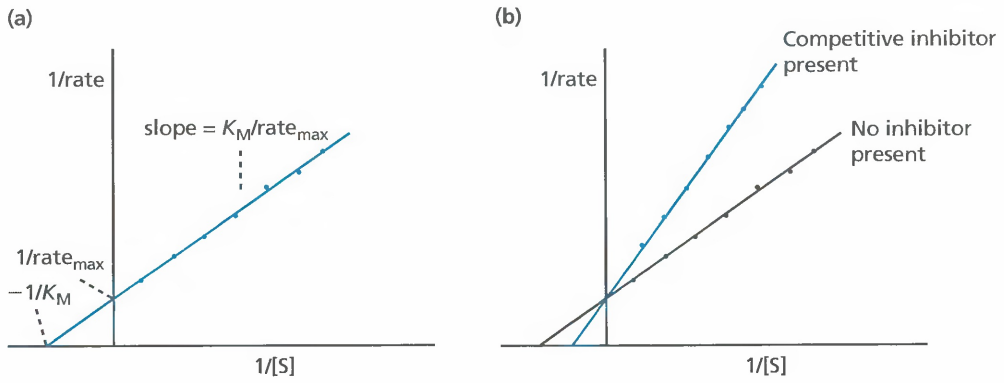


FIGURE 7.11 (a) Lineweaver–Burk plot. (b) Lineweaver–Burk plots with and without a competitive inhibitor present.

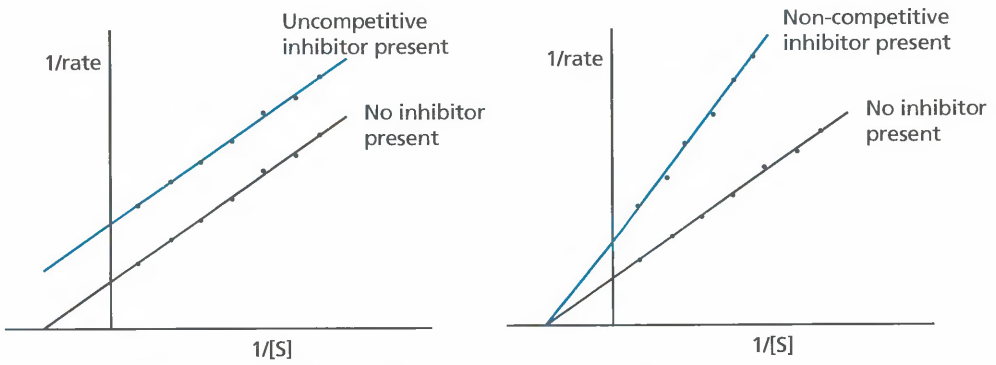


FIGURE 7.12 Lineweaver–Burk plots with and without an uncompetitive inhibitor or non-competitive inhibitor.

is a measure of how strongly the inhibitor binds to the enzyme.

In the case of an uncompetitive inhibitor, the lines are parallel and cross the y-axis at different points, indicating that the maximum rate for the enzyme has been reduced. For a reversible, non-competitive inhibitor, the lines have the same intercept point on the x-axis (i.e. K_M is unaffected), but have different slopes and different intercepts on the y-axis. Therefore, the maximum rate for the enzyme has been reduced.

Lineweaver–Burk plots are extremely useful in determining the nature of inhibition, but they have their limitations and are not applicable to enzymes that are under allosteric control.

7.8.2 Comparison of inhibitors

When comparing the activity of enzyme inhibitors, the IC_{50} value is often quoted. This is the concentration of inhibitor required to reduce the activity of the enzyme by 50%. Compounds with a high IC_{50} are less powerful inhibitors than those with a low IC_{50}, as a higher concentration of the former is required to attain the same level of inhibition.

K_i values are also reported in enzyme inhibition studies. K_i is the apparent inhibition constant for the following equilibrium between the enzyme and the inhibitor:

$$EI \rightleftharpoons E + I \qquad K_I = \frac{[E]\,[I]}{[EI]}$$

It can be shown that $IC_{50} = K_i + [E]_{total}/2$. If the concentration of inhibitor required to inhibit the enzyme by 50% is much greater than the concentration of enzyme, then K_i is much larger than $[E]_{total}$ and this equation approximates to $IC_{50} = K_i$.

IC_{50} and K_i values are measured in assays involving isolated enzymes. However, it is often useful to carry out enzyme inhibition studies where the enzyme is present in whole cells or tissues. In these studies, a cellular effect resulting from enzyme activity is monitored. EC_{50} values represent the concentration of inhibitor required to reduce that particular cellular effect by 50%. It should be noted that the effect being measured may be several stages downstream from the enzyme reaction concerned.

KEY POINTS

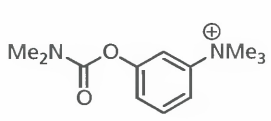
- The Michaelis–Menten equation relates the rate of an enzyme-catalysed reaction to substrate concentration.
- Lineweaver–Burk plots are derived from the Michaelis–Menten equation and are used to determine whether inhibition is competitive, uncompetitive or non-competitive.
- The activity of different enzymes can be compared by measuring values of EC_{50}, K_i or IC_{50}.

QUESTIONS

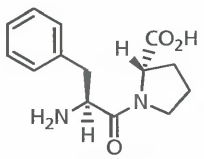
1. It is known that the amino acid at position 523 of the cyclooxygenase enzyme is part of the active site. In the isoenzyme COX-1, this amino acid is isoleucine, whereas in COX-2, it is valine. Suggest how such information could be used in the design of drugs that selectively inhibit COX-2.

2. Neostigmine is an inhibitor of acetylcholinesterase. The enzyme attempts to catalyse the same reaction on neostigmine as it does with acetylcholine. However, a stable intermediate is formed which prevents completion of the process and which results in a molecule being covalently linked to the active site. Identify the stable intermediate and explain why it is stable.

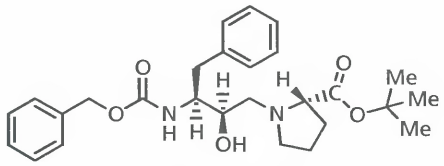
3. The human immunodeficiency virus contains a protease enzyme that is capable of hydrolysing the peptide bond of L-Phe-L-Pro. Structure I was designed as a transition-state inhibitor of the protease enzyme. What is a transition-state inhibitor and how does structure I fit the description of a transition-state inhibitor? What is meant by IC_{50} 6500 nM?



Neostigmine



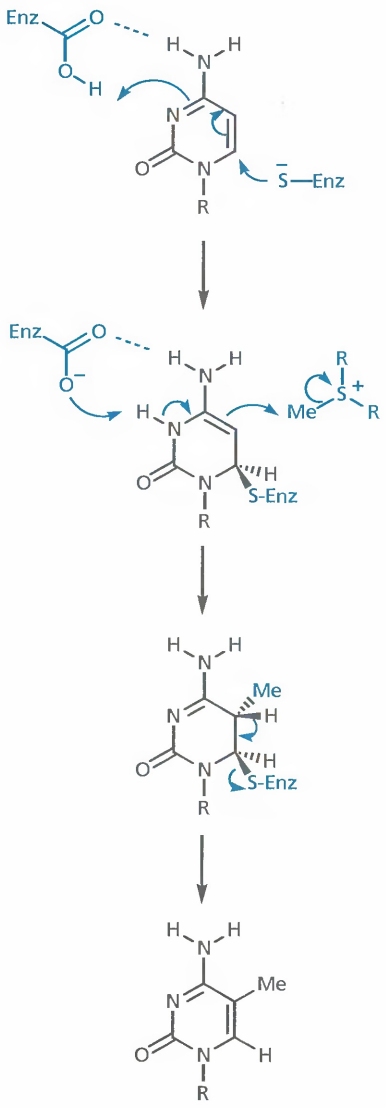
L-Phe-L-Pro



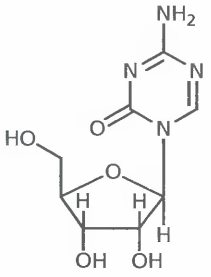
(I) IC_{50} 6500 nM

4. Why should a transition state be bound more strongly to an enzyme than a substrate or a product?

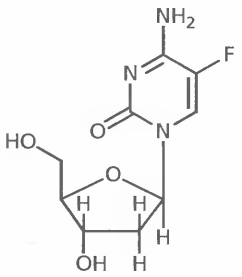
5. The methylation of cytosine residues in DNA plays a role in the regulation of transcription and is catalysed by the enzyme DNA methyltransferase. The mechanism is as follows.



5-Azacytidine and 5-fluoro-2′-deoxycytidine are mechanism-based inhibitors of the DNA methyltransferase. Explain why.



5-Azacytidine



5-Fluoro-2′-deoxycytidine

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