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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. First, the drug has to be tested to ensure that it is not only safe and effective, but can be administered in a suitable fashion. This involves preclinical and clinical trials covering toxicity, drug metabolism, stability, formulation, and pharmacological tests. Second, there are the various patenting and legal issues. Third, 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 such 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 tenfold 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.

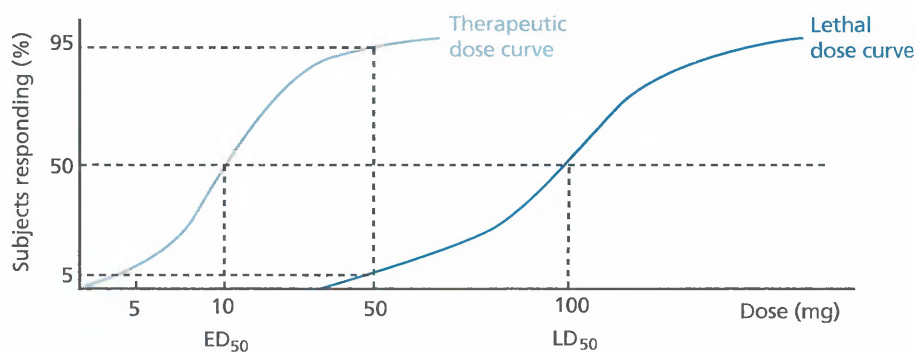


FIGURE 15.1 Comparison of therapeutic and lethal dose curves.

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 heart effects 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 five deaths and the two 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

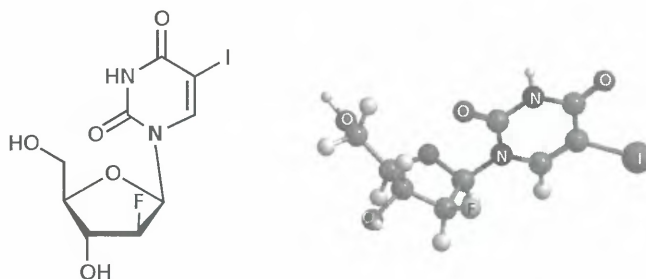


FIGURE 15.2 Fialuridine.

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.

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 (section 11.4). 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, since 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 (Box 15.1).

In order to carry out such studies, it is necessary to synthesize the drug, labelled with an isotope 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 , 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.

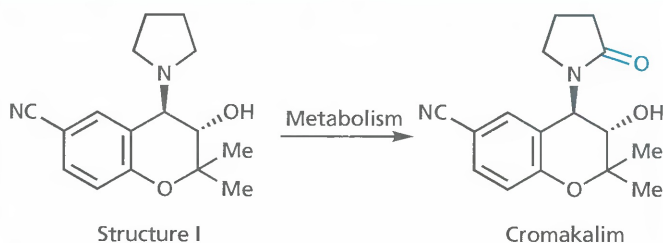
Deuterium or tritium can be very easily incorporated into any molecule containing an exchangeable proton such as those of an alcohol, carboxylic acid, or phenol. This is done simply by shaking a solution of the drug with D_2O or T_2O . Unfortunately, the label is just as easily lost as a result of proton exchange with water in the test animal. 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 that an isotope

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 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 anti-hypertensive agent.



Discovery of cromakalim.

could be lost in this way. 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 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, since 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. Preformulation 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, polymorphism, 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 dose unit. The dose 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 since 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 they do not leach through the plastic container into the preparation.

15.1.4 Clinical trials

Once the preclinical studies described above 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. 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 due to 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 of volunteers. 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 such as aspirin. 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, and 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 frequently used 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 on a life threatening disease such as AIDS or cancer, volunteer patients are used for phase I studies rather than healthy volunteers.

The decision 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 2 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. 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 3 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, and 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.

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) was used to treat rheumatoid arthritis for 5 years, before a clinical trial carried out after its release, showed that it was associated with increased risks of heart attack and stroke.

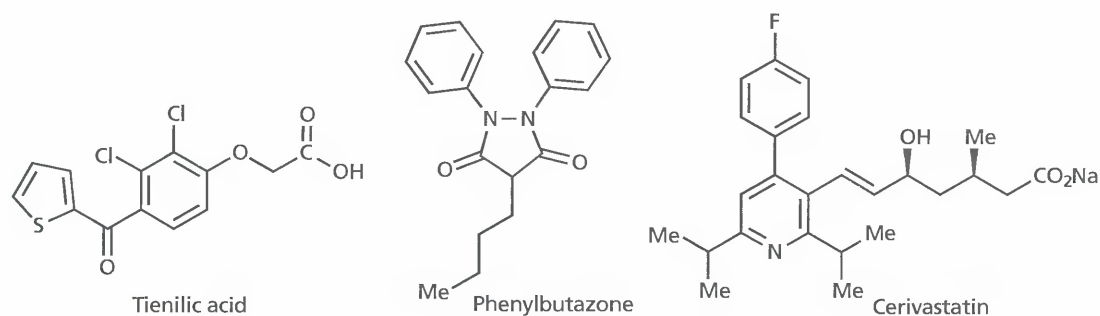


FIGURE 15.3 Drugs that have been removed from the market due to rare toxic side effects.

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 overall 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).

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. This means that most licensed drugs have been licensed for adults, and that around 40% of medicines given to children have never actually been tested on children. When it comes to prescribing for children, clinicians are left 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 the relative body weight of an adult and a child. 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 world wide. 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 the testing and prescribing of children's medicines. The European Medicines Agency now offers a license 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 that 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 safely be administered. 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 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 previously been revealed, 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 due to 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.

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. 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. Timing is important and the company ideally wants to have the pure enantiomer reaching the market just as the patent on the original racemate is expiring. It is not

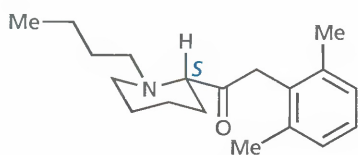


FIGURE 15.4 Levobupivacaine.

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 used for the same purposes as bupivacaine. Other examples of drugs that have undergone chiral switching include **salbutamol** (section 23.10.3) and **omeprazole** (section 25.3).

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 (EMA)** 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 adequately protected. An approval letter is finally 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 less 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 EMA.

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 demanding 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 the drug. 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 which 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 due to the pressures which are often placed on researchers to obtain their results as speedily as possible. This can lead to hasty decisions, resulting 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 drug.

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 that 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, and 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 closely studied 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 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 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

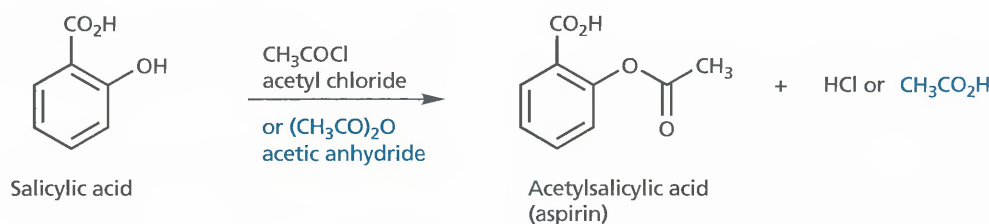
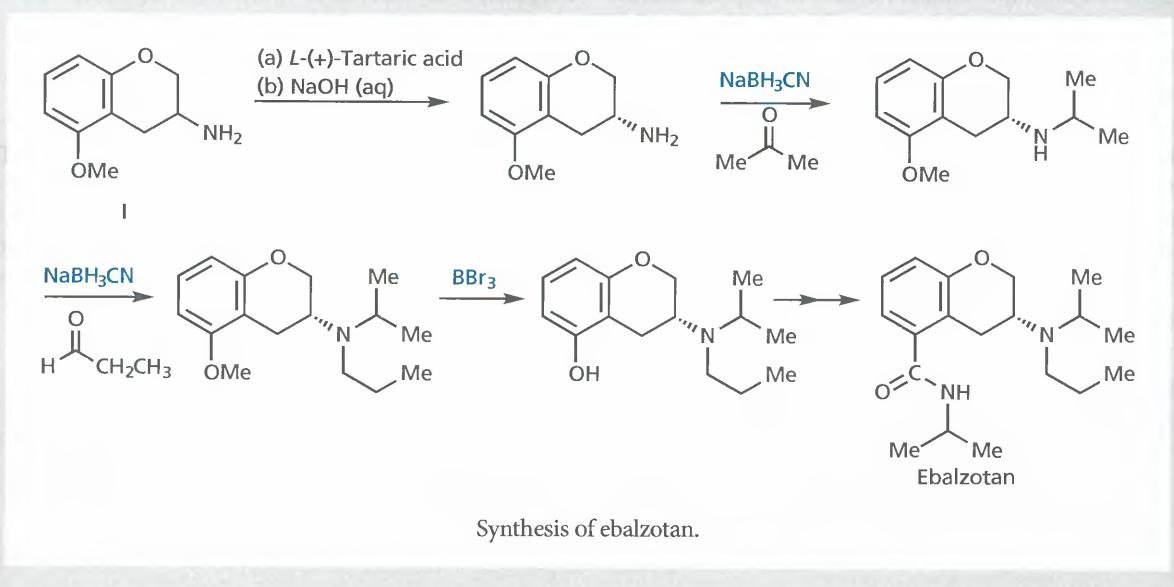


FIGURE 15.5 Synthesis of aspirin.

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%. 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, BBr₃, which is corrosive, toxic and expensive, was replaced with HBr which is cheaper and less toxic.



scale up to the pilot plant, where about 100 kg is prepared for phase II and phase III clinical trials.

Because of the time scales 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 the minimum. For example, rather than isolating each intermediate in the synthetic sequence, it is better to move it 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.

Cost 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 overriding 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. 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.

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.

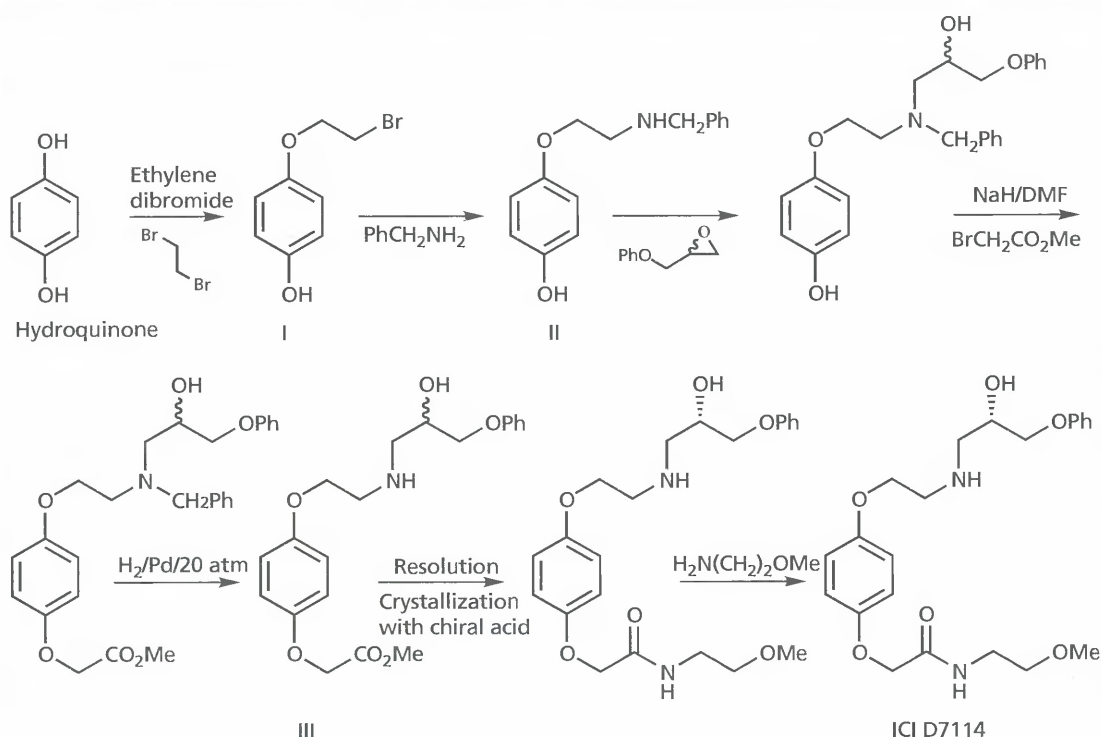


FIGURE 1 Research synthesis of ICI D7114.

BOX 15.3 Synthesis of ICI D7114 (Continued)

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 that 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 five 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.

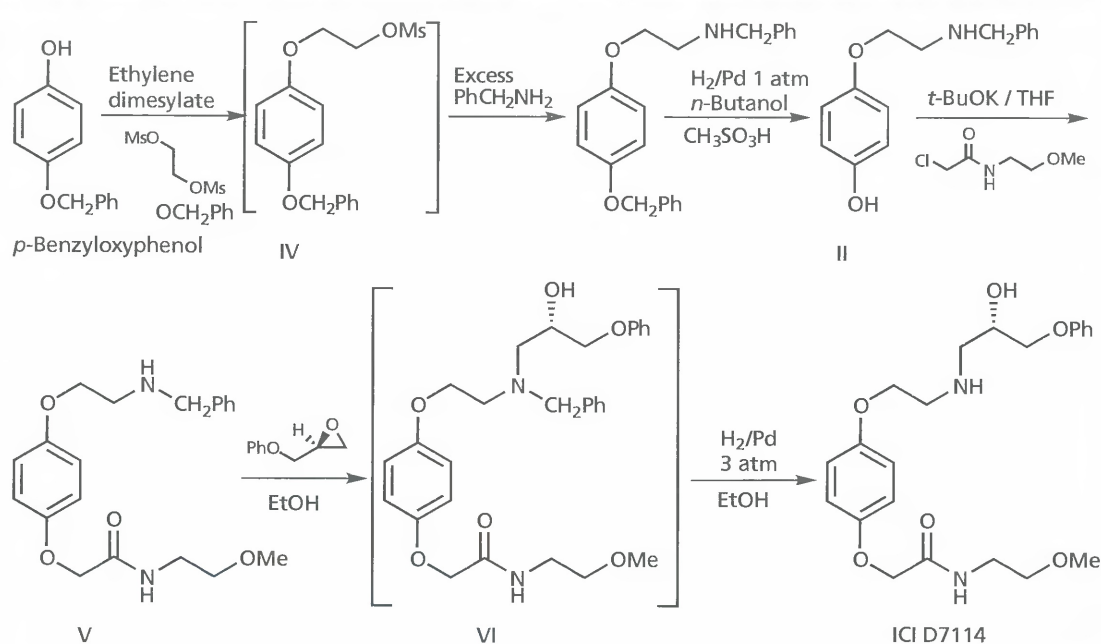


FIGURE 2 Revised synthetic route to ICI D7114.

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

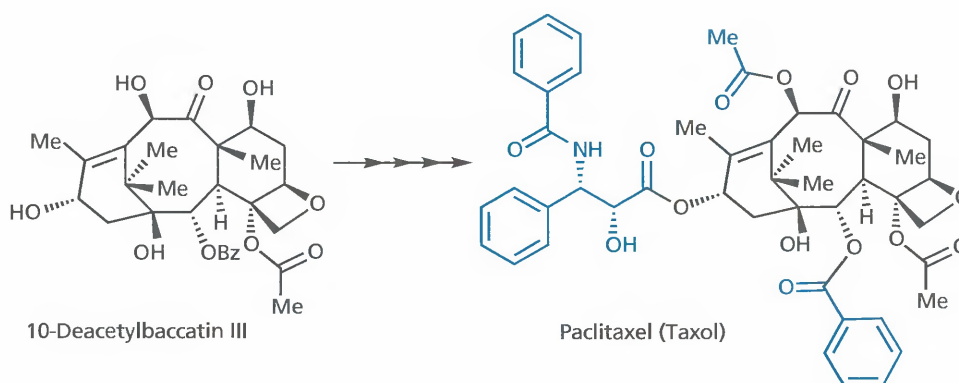


FIGURE 15.6 Semisynthetic synthesis of paclitaxel (Taxol).

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 could then be converted to the final product by conventional synthesis. This approach can have two advantages. First, the intermediate may be more easily extracted in higher yield than the final product itself. Second, 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).

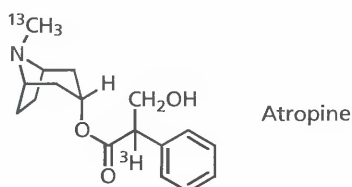
Another more recent example is that of paclitaxel. It is manufactured by extracting **10-deacetylbaccatin III** from the needles of the yew tree, then carrying out a four-stage synthesis (Fig. 15.6).

KEY POINTS

- Chemical development involves the development of a synthetic route that 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 that will satisfy these requirements.
- Process development aims to develop a production process that is safe, efficient, economic, environmentally friendly, and provides a product having 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.

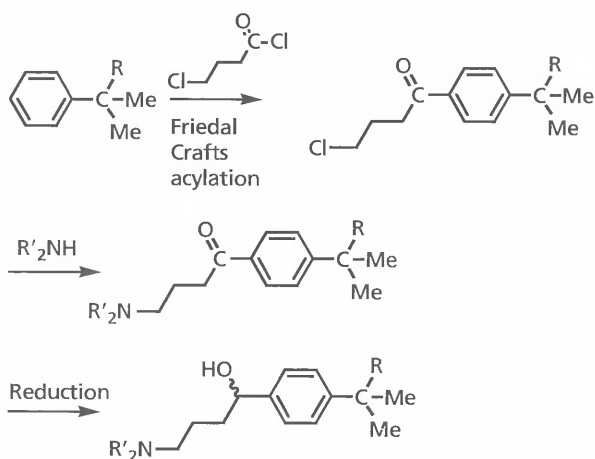
QUESTIONS

1. Discuss whether the doubly labelled atropine molecule shown below is suitable for drug metabolism studies.

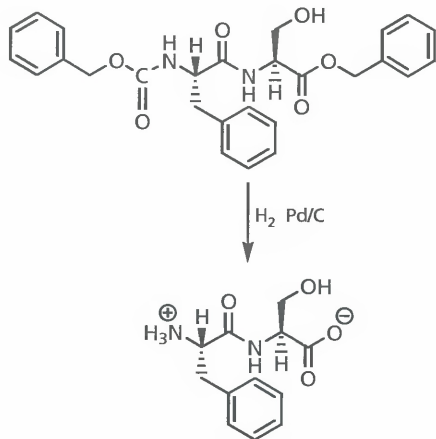


2. What is meant by a placebo and what sort of issues need to be considered in designing a suitable placebo?

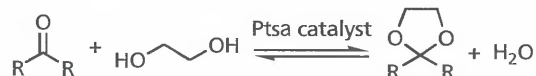
3. Usually, a 'balancing act' of priorities is required during chemical development. Explain what this means.
4. Discuss whether chemical development is simply a scale up exercise.
5. The following synthetic route was used for the initial synthesis of fexofenadine ($\text{R}=\text{CO}_2\text{H}$) – an analogue of terfenadine ($\text{R}=\text{CH}_3$). 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)



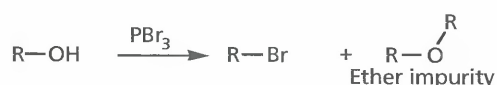
6. The following reaction was carried out using ethanol or water as solvents but gave poor yields in both cases. Suggest why this might be the case, and how these problems could be overcome.



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.



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

■ CASE STUDY 2

The design of ACE inhibitors

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. The **angiotensin-converting enzyme (ACE)** is a membrane-bound enzyme that 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. CS2.1).

Angiotensin II is an important hormone that causes blood vessels to constrict, resulting in a rise in blood pressure. Therefore, ACE inhibitors are potential anti-hypertensive 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 CS2.2 and is inhibited by **L-benzylsuccinic acid**.

The active site of carboxypeptidase (Fig. CS2.3) 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 ionically bound 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 most 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 hydrolysis product.

L-Benzylsuccinic acid binds as shown in Fig. CS2.4. 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



FIGURE CS2.1 Reaction catalysed by angiotensin-converting enzyme (ACE).

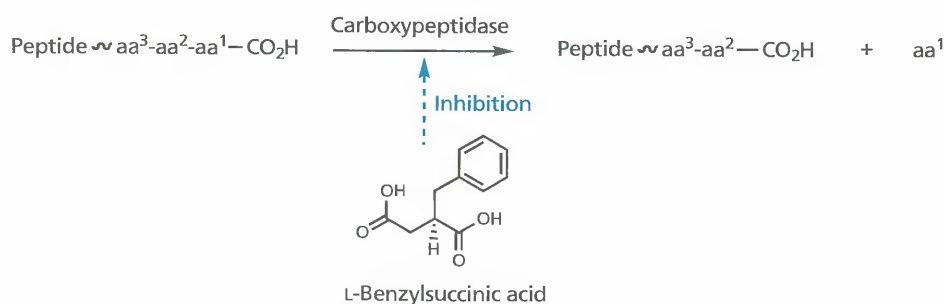


FIGURE CS2.2 Hydrolysis of a terminal amino acid from a peptide chain by the carboxypeptidase enzyme. The asymmetric centre of L-benzylsuccinic acid has the *R* configuration.

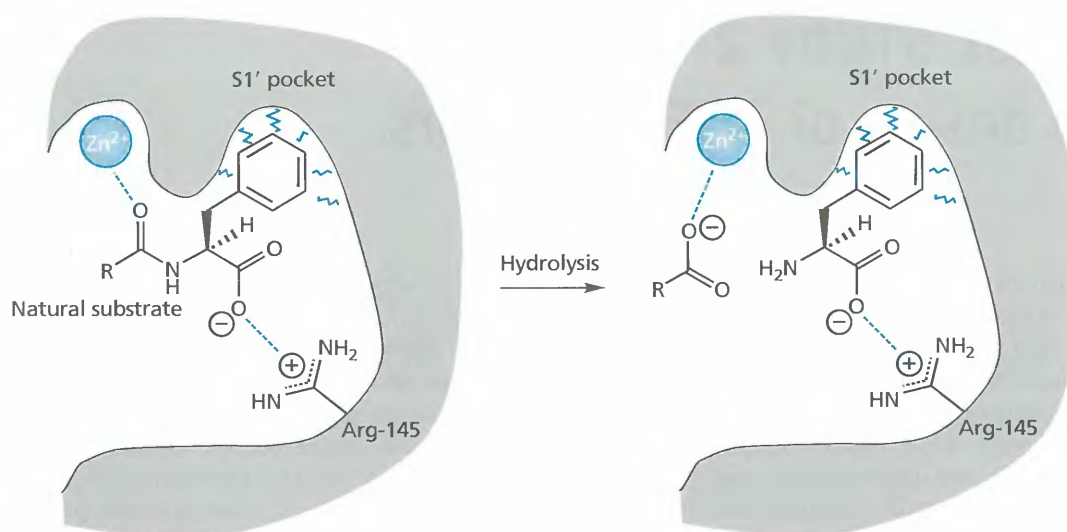


FIGURE CS2.3 Binding site interactions for a substrate bound to the active site of carboxypeptidase.

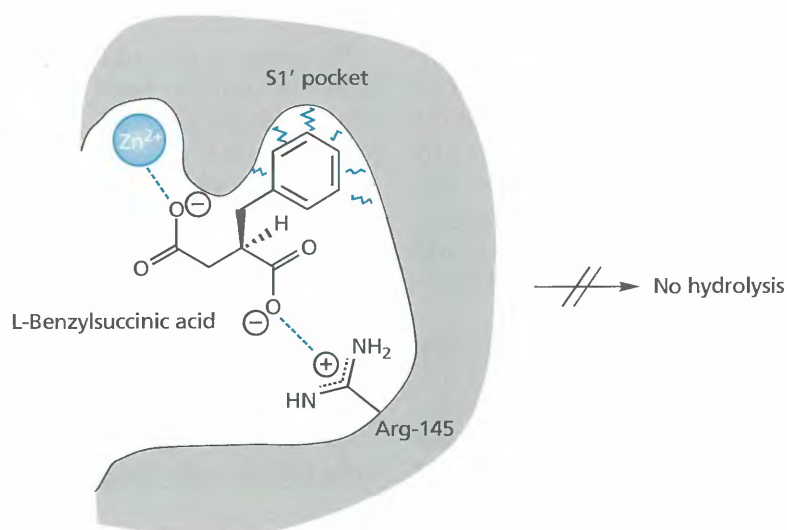


FIGURE CS2.4 Inhibition by L-benzylsuccinic acid (the *R*-enantiomer).

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 an 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 a 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. Since 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. CS2.5)—a nonapeptide which was isolated from the venom of the Brazilian pit viper. Although teprotide is a reasonably potent inhibitor, it is orally inactive, as it is susceptible to digestive enzymes. 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. CS2.6). It was now argued that there must be pockets available to

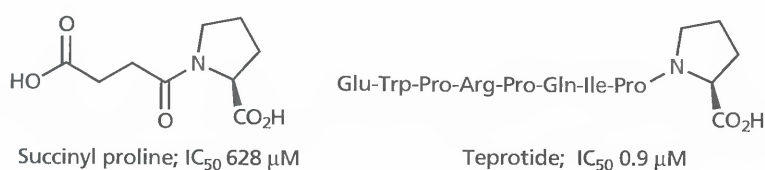


FIGURE CS2.5 Angiotensin-converting enzyme (ACE) inhibitors.

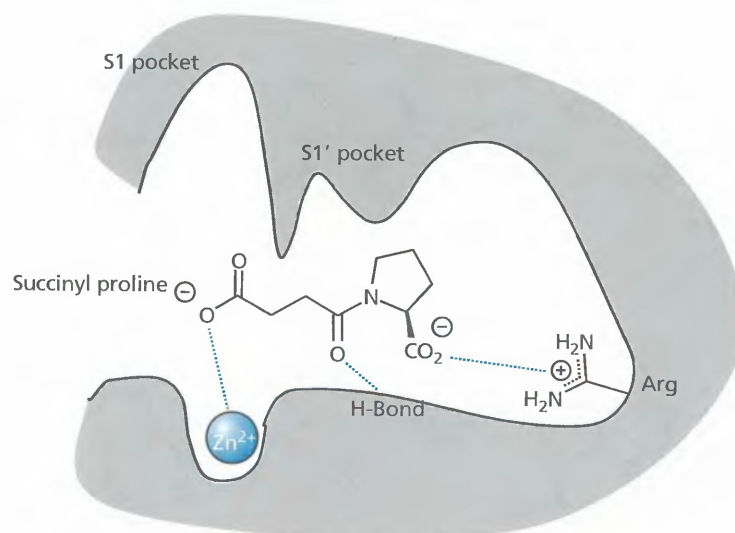


FIGURE CS2.6 Binding site interaction for succinyl proline in the active site of angiotensin-converting enzyme (ACE).

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 increase in activity (Fig. CS2.7). 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 re-introducing the carboxylate group as the zinc ligand, but as we have seen, this is a far weaker binding group for zinc. Therefore, the philosophy was to introduce groups that would be capable of forming extra binding interactions with the active site, and which would compensate for the poorer zinc ligand (extension strategy; section 13.3.2). First, 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. CS2.8). 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 was studied. Incorporating *L*-alanine introduced the methyl substituent that is

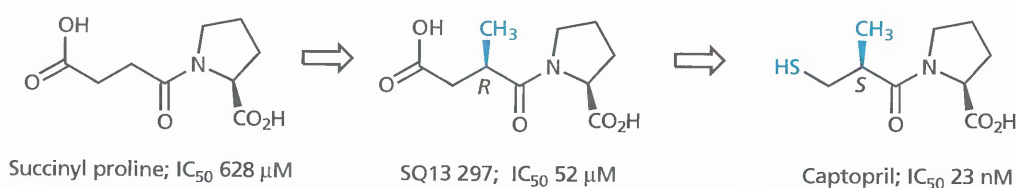


FIGURE CS2.7 Development of captopril.

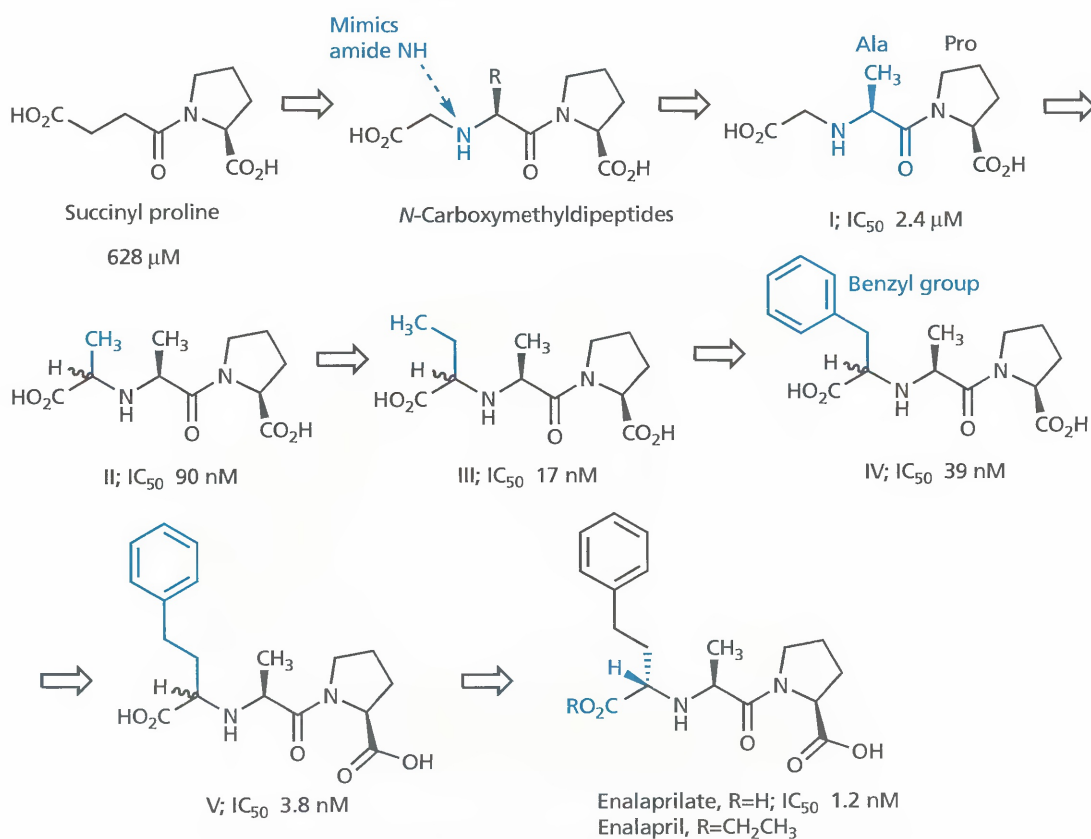


FIGURE CS2.8 Development of enalaprilate.

present in captopril (structure I, Fig. CS2.8). 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. CS2.6. 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-III; Fig. CS2.8). The analogues showed increased activity, with the ethyl analogue 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. These were now separated by chromatography for structure V, 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. CS2.9. **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. CS2.10) 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. ACE inhibitors do not bind in the way that was originally thought. 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. CS2.11). As a result, they have been described as transition-state analogues (section 7.4). This is because the transition state

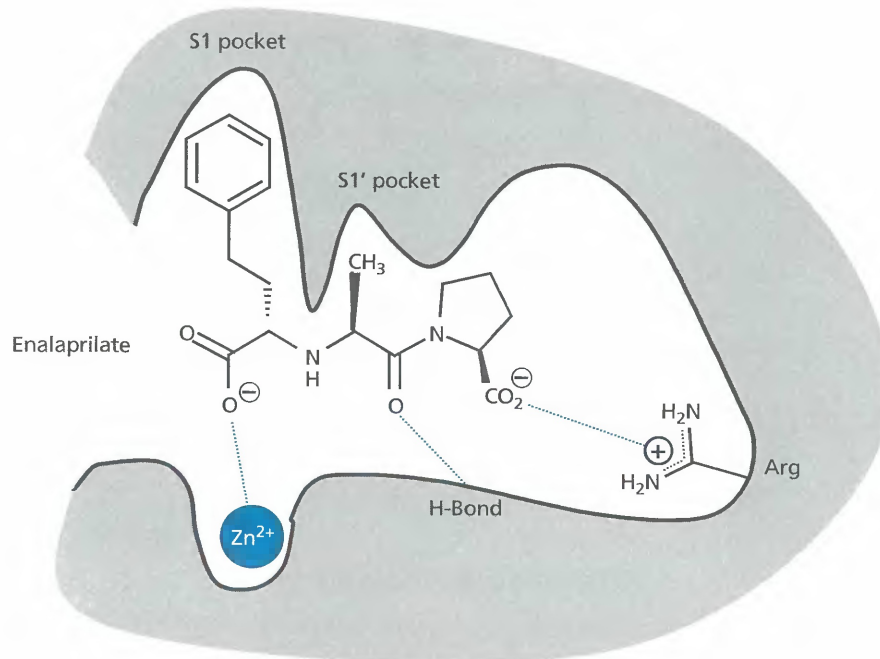


FIGURE CS2.9 Enalaprilate.

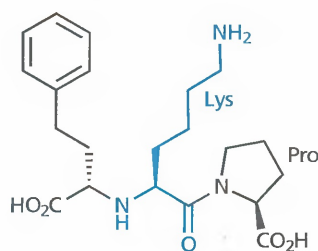


FIGURE CS2.10 Lisinopril.

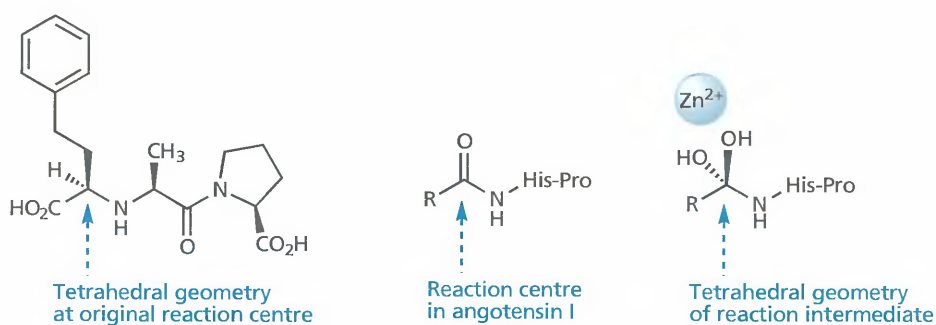


FIGURE CS2.11 Comparison of enalaprilate with angiotensin I and the reaction intermediate formed during enzyme-catalysed hydrolysis.

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 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. CS2.12).

Keto-ACE analogues 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.

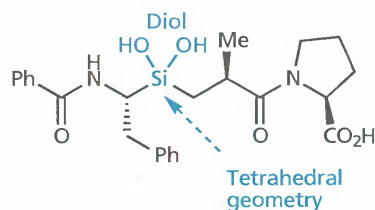


FIGURE CS2.12 Example of a silanediol transition-state analogue.

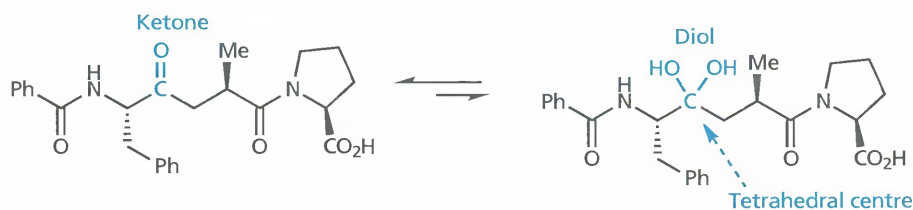


FIGURE CS2.13 Keto-ACE inhibitors.

BOX CS 2.1 Synthesis of captopril and enalaprilate

There are several methods of synthesising captopril. One method is to take the *t*-butyl ester of proline and react it with the ester (I) (Fig. 1). A nucleophilic substitution takes place to form the amide (II). The *t*-butyl protecting group can then be removed in the presence of acid to give captopril.

Enalaprilate can be synthesized by reacting the ketoacid (III) with alanylproline. Two diastereomers are formed that are separated by chromatography.

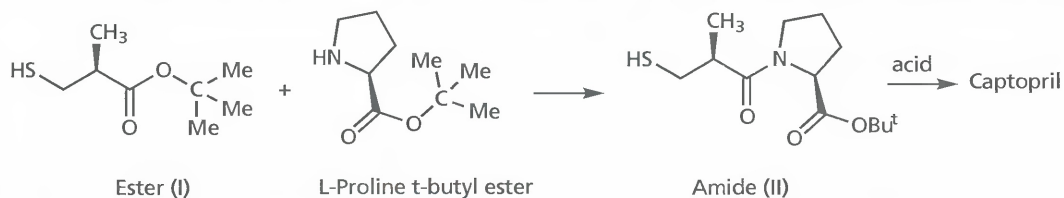


FIGURE 1 Synthesis of captopril.

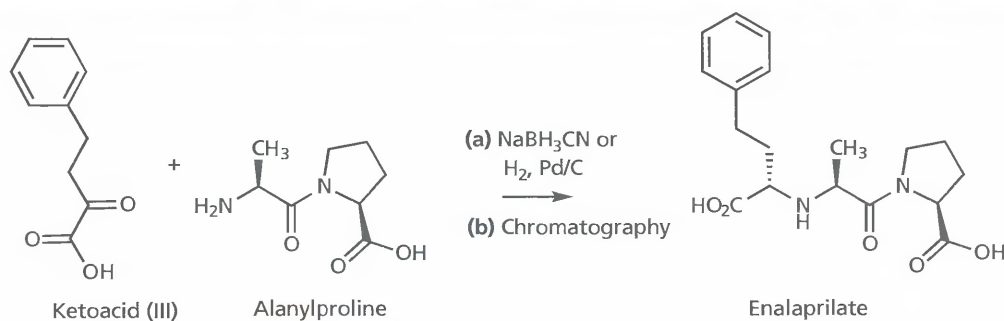


FIGURE 2 Synthesis of enalaprilate.

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■ CASE STUDY 3

Artemisinin and related antimalarial drugs

Introduction

Malaria is an ancient disease that has resulted in millions of deaths and much human misery. It is caused by a protozoal parasite that 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 60s to try and eradicate mosquitos by spraying their breeding grounds with 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 (as the author can testify!). 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 particularly worrying with respect to the potentially fatal *Plasmodium falciparum*. Resistance appears to be a result of the parasite having a cell membrane protein that 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.

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 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

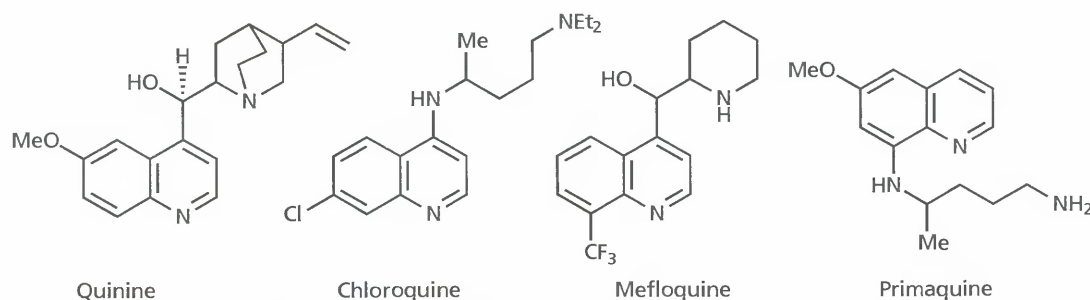


FIGURE CS3.1 Quinine and quinine-based antimalarial drugs.

than from battle action. For example, during the Burmese campaign of the Second World War, a huge number of British and Indian soldiers were incapacitated by malaria, and had to be withdrawn from action. Many of the 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 *Artemisia 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 American 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 American capital!

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).

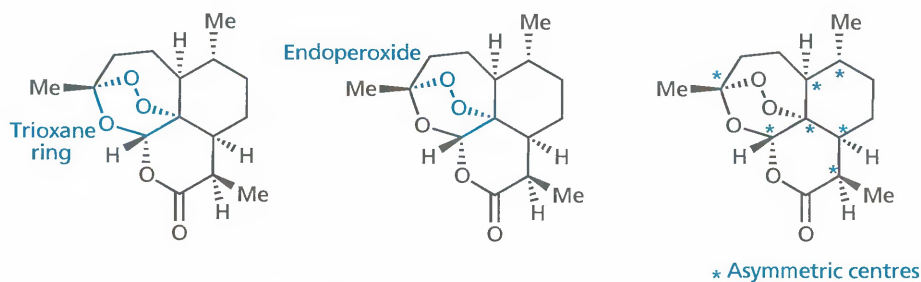


FIGURE CS3.2 Structure of artemisinin.

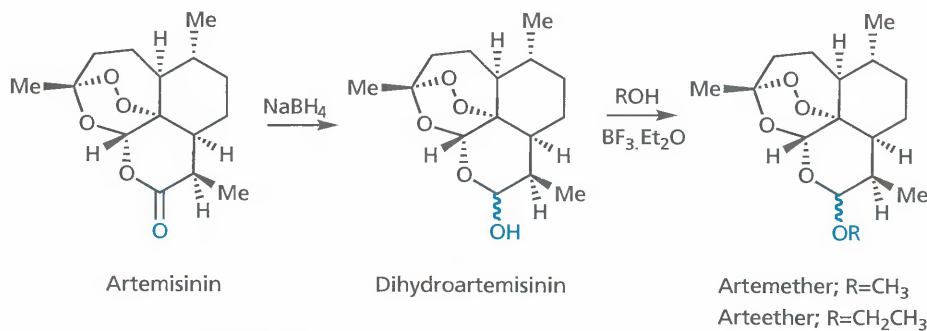


FIGURE CS3.3 Preparation of artemether and arteether.

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 that can then be alkylated to give various ethers such as **artemether** and **arteether**.

Esterifications can also be carried out on dihydroartemisinin, and 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 analogues have also been studied. For example, deoxyartemisinin (Fig. CS3.5) is a metabolite of artemisinin, and is 300–1000-fold less active. Deoxydeoxyartemisinin is also poorly active, whereas deoxyartemisinin 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.

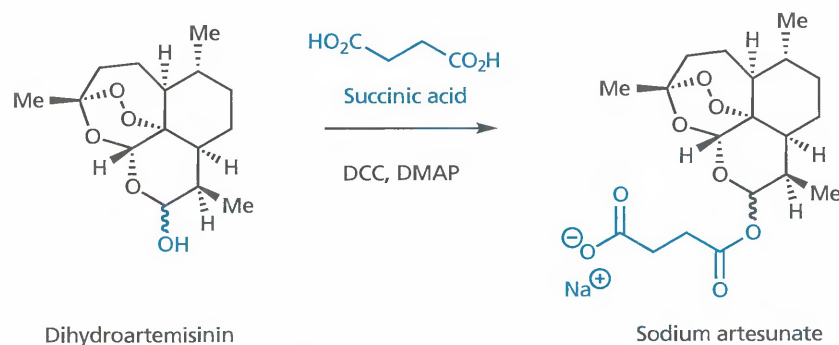


FIGURE CS3.4 Synthesis of sodium artesunate.

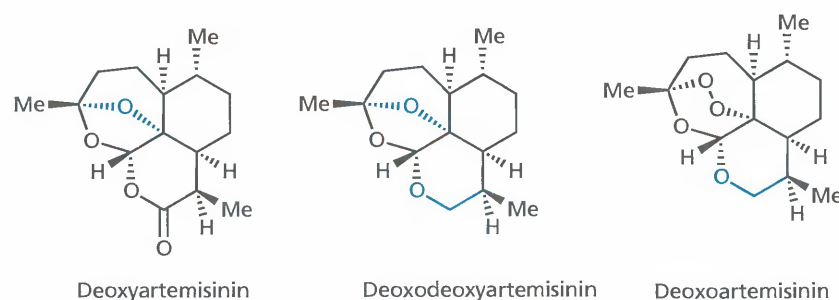


FIGURE CS3.5 Analogues of artemisinin.

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. Since 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 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 above, red blood cells contain haemoglobin. This is a protein that contains an iron(II)-centred porphyrin called heme (Fig. CS 3.7). The porphyrin and the ferrous ion are buried deep within the protein

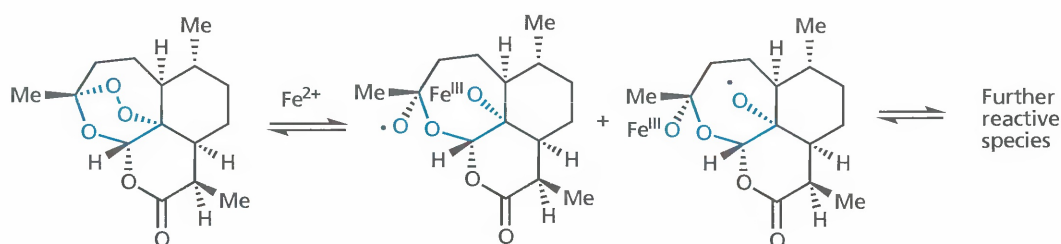


FIGURE CS3.6 Activation of artemisinin by ferrous ions.

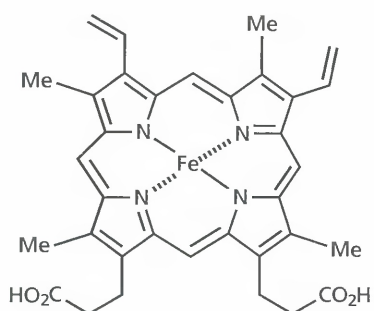


FIGURE CS3.7 Structure of heme.

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 only 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 that 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 there is 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 stabilising the radical at position 4.

Drug design and development

Since artemisinin is poorly soluble in both water and oil, early research was aimed at producing analogues which would be more soluble in one or other of these mediums. 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 (Fig. 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.

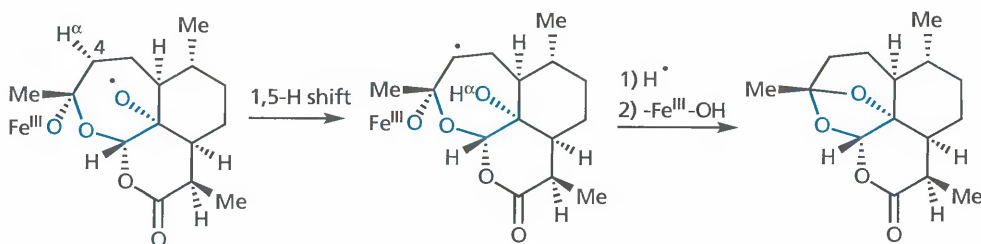


FIGURE CS3.8 Generation of a C-4 radical by 1,5-hydrogen atom abstraction.

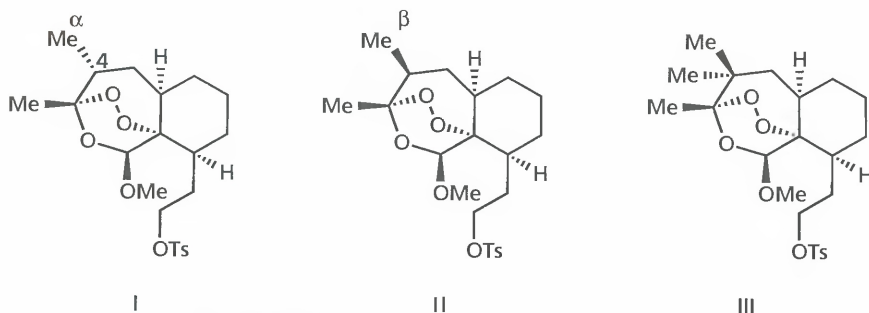


FIGURE CS3.9 Simplified analogues of artemisinin.

BOX CS 3.1 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 reoccurs 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. Due 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, due to the different mechanism of action.

Drawbacks for these drugs include a short plasma half-life, which is typically less than 1 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.

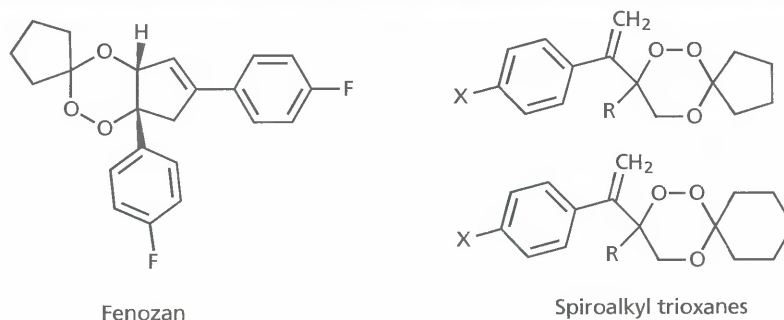


FIGURE CS3.10 Fenozan and spiroalkyl trioxanes.

Research has also been carried out with the aim of designing an antimalarial agent that can be easily synthesized, 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 has been synthesized, and 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 semisynthetic 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 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

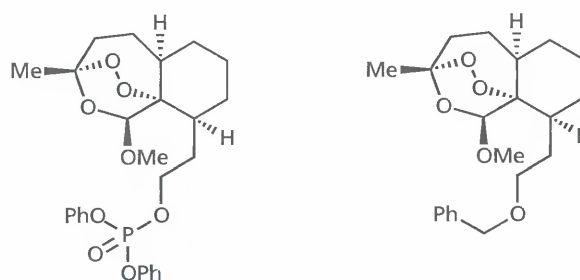


FIGURE CS3.11 Trioxanes having comparable activity to artemisinin.

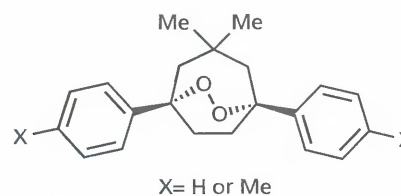


FIGURE CS3.12 Symmetrical analogues of artemisinin.

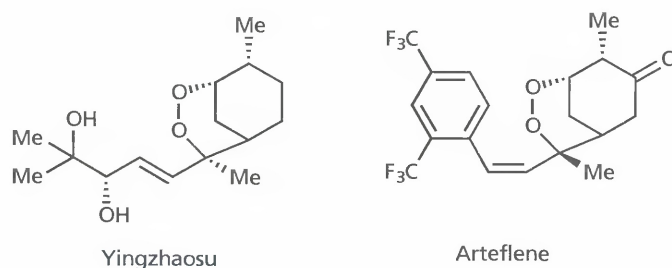


FIGURE CS3.13 Yingzhaosu A and arteflene.

traditional Chinese herbal remedy for fever (*Artemisia 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 that 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.

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■ 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, both 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

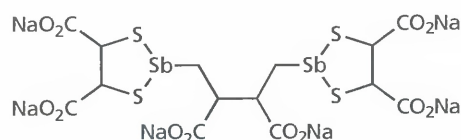


FIGURE CS4.2 Stibocaptate.

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 ultimately led to the discovery of oxamniquine (Fig. CS4.1), which met all but one of those goals.

Lucanthone was chosen as the lead compound since it was orally active and 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, and 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

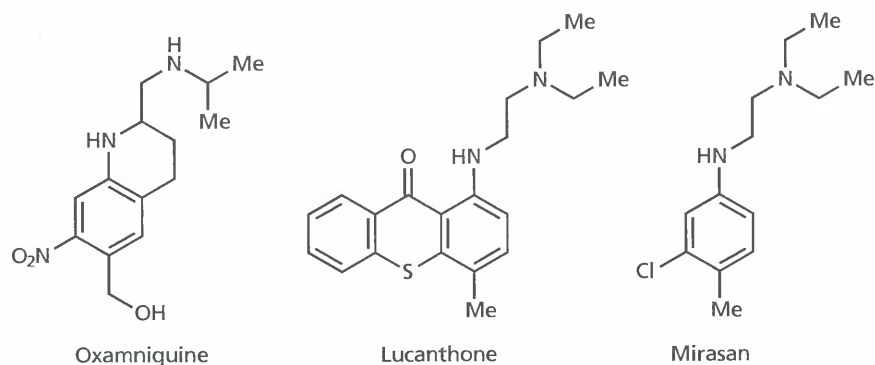


FIGURE CS4.1 Oxamniquine, lucanthone and mirasan.

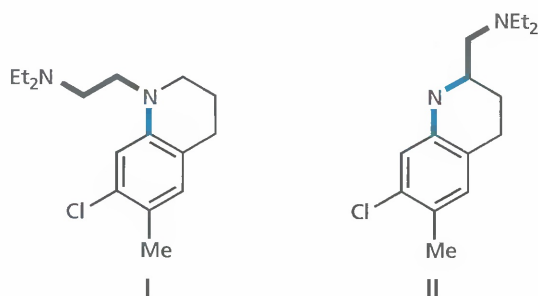


FIGURE CS4.3 Bicyclic structures I and II (restricted bonds in colour).

substituent, positioned where the sulfur 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 to binding the drug to a target binding site, and would adopt a particular conformation in order to bind efficiently. 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.

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 (sections 14.1.3 and 14.1.4). The electronic effect of a substituent on a distant functional group is a useful strategy in drug design (sections 13.3.1.2, 14.1.4 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).

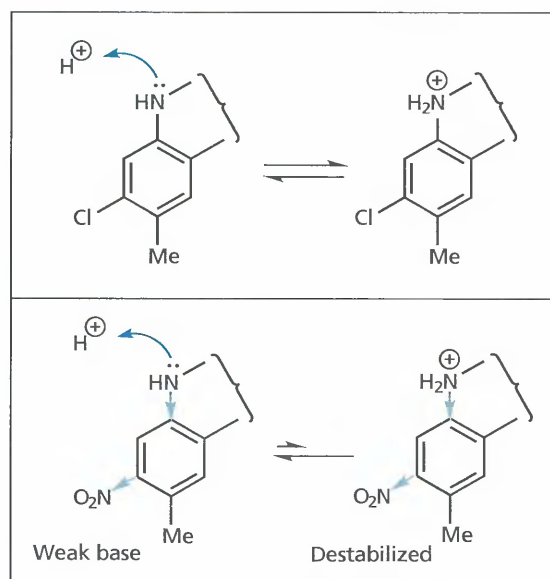


FIGURE CS4.4 Effect of aromatic substituents on pK_a .

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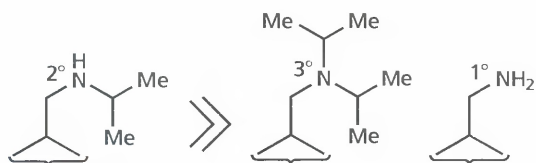


FIGURE CS4.5 Relative activity of amino side chains.

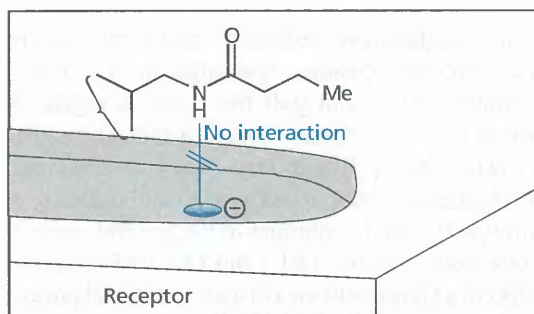
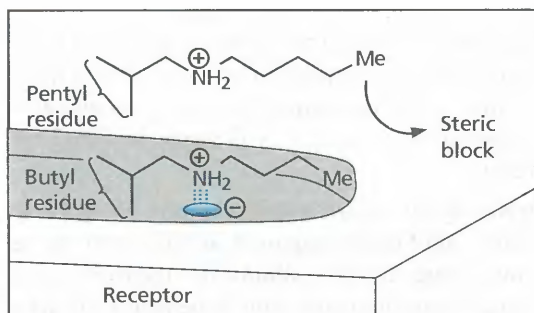


FIGURE CS4.6 Proposed ionic binding interaction.

- 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 binding to the binding site. Acyl groups eliminated activity altogether, 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).

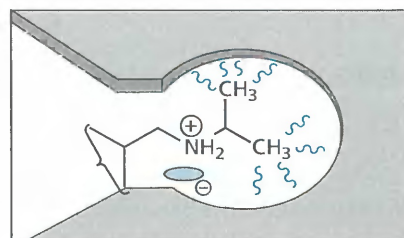
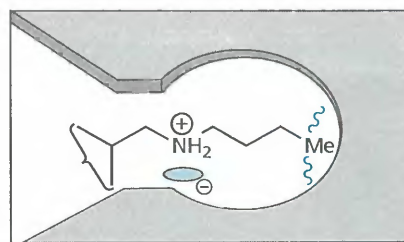


FIGURE CS4.7 Branching of the alkyl chain.

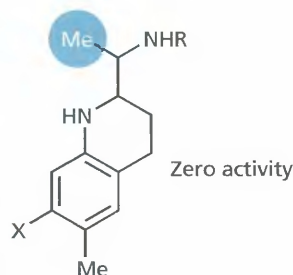


FIGURE CS4.8 Addition of a methyl group.

- 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. This result suggests the opposite.

The optimum structure based on these results was structure III (Fig. CS4.10). It has one asymmetric centre and, as one might expect, the activity was much greater in one enantiomer than it was in the other.

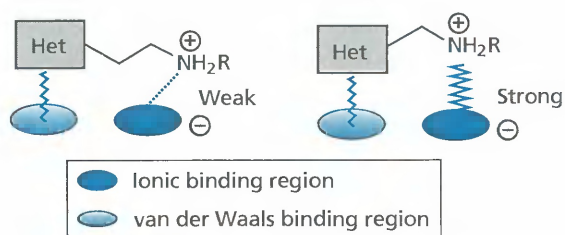


FIGURE CS4.9 Effect of extension of the side chain.

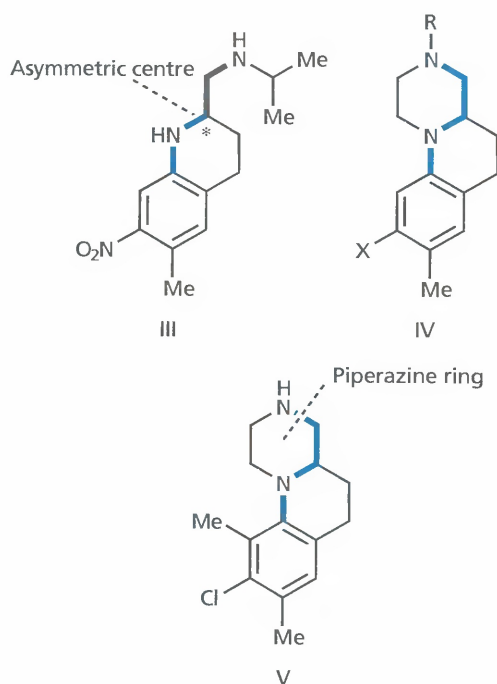


FIGURE CS4.10 The optimum structure (III) and the tricyclic structures (IV) and (V) (restricted bonds in colour).

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 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 14.1.4).

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.

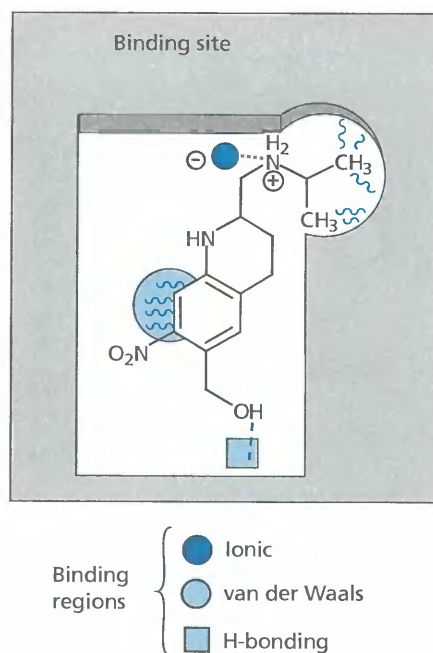


FIGURE CS4.11 Proposed binding interactions for oxamniquine to a binding site.

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 11.4.2) 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). 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 *Schistosoma mansoni*. Side effects are relatively mild compared to 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, 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.

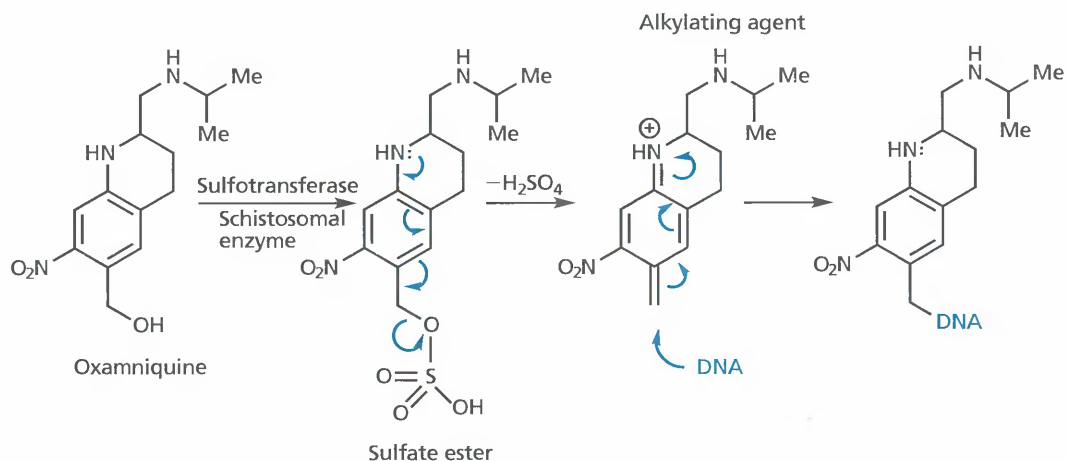


FIGURE CS4.12 Mechanism by which oxamniquine might dissociate to form an alkylating agent.

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 sulfotransferase 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 sulfate ester (Fig. CS4.12). The ester is a

BOX CS 4.1 Synthesis of oxamniquine

One method of synthesizing oxamniquine is to start from the quinoline structure (I). 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.

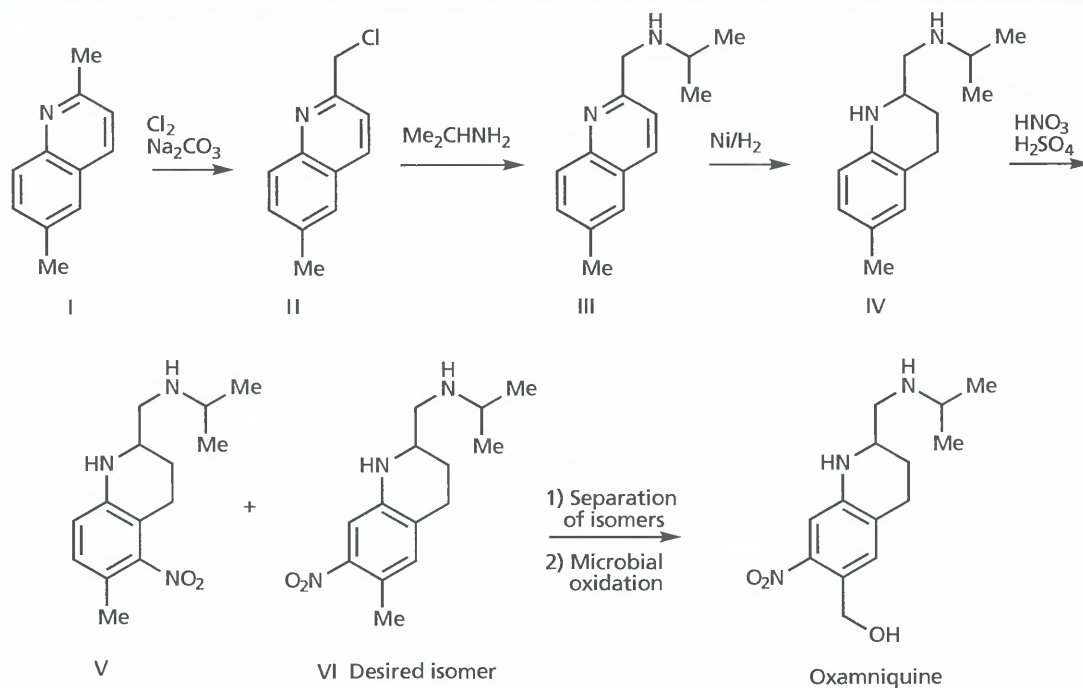


FIGURE 1 Synthesis of oxamniquine.

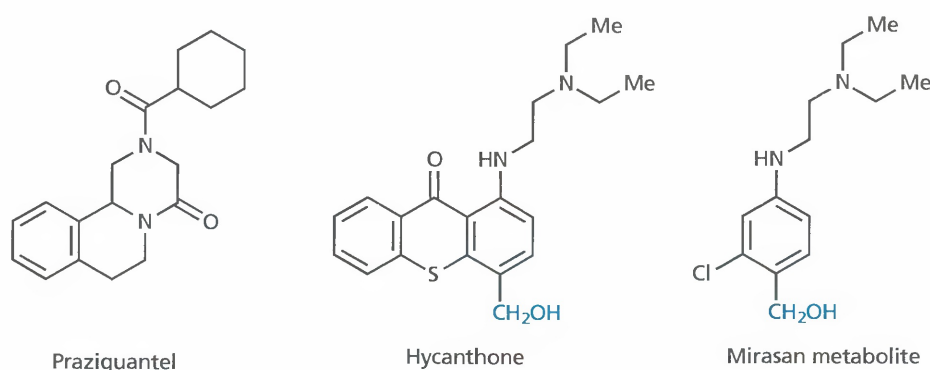


FIGURE CS4.13 Hycanthone and praziquantel.

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 SAR results previously described, 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), and the target binding site is the active site of the parasitic sulfotransferase enzyme.

Other agents

The knowledge that the CH₂OH 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 oxidised *in vivo* to

a metabolite called **hycanthone** (Fig. CS4.13), which then acted 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 oxidised *in vivo* to a metabolite (Fig. CS4.13) that proved to be active in a range of species. However, the metabolite was never used clinically. It is likely that the observed inactivity of mirasan in monkeys is due to it being resistant to metabolic oxidation in that species.

Praziquantel (Fig. CS4.13) is now the recommended treatment for schistosomiasis in the UK, since 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

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PART D

Tools of the trade

In section D, we shall study three topics that are invaluable tools in the discovery and design of drugs. The topics covered are quantitative structure–activity relationships, combinatorial chemistry, and the use of computers in medicinal chemistry. It should be emphasized that these are not the only topics that could be considered as ‘tools of the trade’. For example, it could easily be argued that organic synthesis should be included, since a knowledge of synthesis is crucial for drug design. There is no point designing drugs that are impossible to synthesize. However, organic synthesis is a major area in itself and a single chapter would do no justice to it, especially when there are many excellent undergraduate texts covering the subject in depth.

Combinatorial chemistry is the subject of chapter 16. This is a method of rapidly preparing large numbers of compounds in an automated or semi-automated fashion. The technique was developed to meet the urgent need for new lead compounds for the ever increasing number of novel targets discovered by genomic and proteomic projects. It is now an effective method of producing large numbers of analogues for drug development and for studies into structure activity relationships.

In chapter 17, we look at some of the operations that can be carried out using computers, and which aid the drug design process. Computers and molecular modelling software packages have now become an integral part of the drug design process, and have been instrumental in a more scientific approach to medicinal chemistry.

In chapter 18, we look at Quantitative Structure–Activity Relationships (QSAR). This topic has been around for many years and it is a well established tool in medicinal chemistry. QSAR attempts to relate the physicochemical properties of compounds to their biological activity in a quantitative fashion by the use of equations. In traditional QSAR, this typically involves studying a series of analogues with different substituents, and studying how the physicochemical properties of the substituents affect the biological activities of the analogues. Typically, the hydrophobic, steric and electronic properties of each substituent are considered when setting up a QSAR equation. With the advent of computers and suitable software programs, traditional QSAR studies have been largely superseded by three-dimensional quantitative structure–activity relationships (3D QSAR), where the physicochemical properties of the complete molecule are calculated and then related to biological activity.

All of these tools are extremely important in medicinal chemistry research, but they are meant to be used in combination with each other and should also be used when they are appropriate. Critics have sometimes argued that none of these tools has ever resulted in a clinically useful drug *per se*. That is not the point. One cannot build a house with just a hammer, so it is unrealistic to suggest that a drug can be discovered by using a single scientific tool. The case studies in this section illustrate how the tools mentioned in chapters 16–18 are applied alongside more traditional drug design strategies.

