CASE STUDY 3

Artemisinin and related antimalarial drugs

CS3.1 Introduction

Malaria is an ancient disease that has resulted in millions of deaths and much human misery. It is caused by a protozoal parasite which is carried by mosquitos and is transmitted between mosquitos and humans by mosquito bites. The malarial parasite is a microorganism belonging to the *Plasmodium* genus, of which there are four species: *vivax, falciparum, ovale*, and *malariae*. *Plasmodium falciparum* is the most dangerous of these and can result in death. The disease is currently associated with tropical countries, but, in the past, it was present in Europe and North America. Campaigns were carried out in the 1950s and 1960s to try and eradicate mosquitos by spraying their breeding grounds with dichlorodiphenyltrichloroethane (DDT). These efforts, along with the use of quinine-based drugs (Fig. CS3.1), successfully reduced the prevalence of malaria.

Quinine was the first of the antimalarial agents to be used and is still effective today. However, it can cause adverse reactions, such as ringing in the ears and partial deafness. Therefore, its use is currently limited to the treatment of malaria rather than as a **prophylactic**. A prophylactic is a protective agent that is administered to prevent a disease occurring. The agent that largely replaced quinine as the antimalarial drug of choice was **chloroquine**, which has far fewer side effects. This was introduced in the 1950s and, at one point, it was thought that the disease would be conquered. Unfortunately, from 1961 onwards, the parasite has developed resistance to chloroquine such that the drug is no longer effective in many malarial infected areas of the world, especially in sub-Saharan Africa. It is, therefore, crucial that new antimalarial therapies are discovered that can combat these drug-resistant strains. An added urgency comes from the belief that global warming might result in the return of the disease to North America and Europe. This is particular worrying with respect to the potentially fatal *P. falciparum*. Resistance appears to be a result of the parasite having a cell membrane protein which can pump the drug out of the cell. Fortunately, a new drug has been discovered in recent years that has been found to be active against these drug-resistant strains—artemisinin.

CS3.2 Artemisinin

For over 2000 years, Chinese herbalists have used concoctions or teas (called **qinghao**) obtained from an abundant Chinese plant called *Artemisia annua*. The herb was first described as a remedy for haemorrhoids in 168 BC, and the first mention of it as an antimalarial preparation was in 340 AD. Further references to the plant were made in 1596, when it was used for the treatment of chills and fever resulting from malaria.

In 1972, the active principle of the plant was isolated and identified as **artemisinin** (or **qinghaosu**). The compound caused great excitement because it was found to be effective against the particularly dangerous chloroquine-resistant *Plasmodium falciparum*, and also acted more

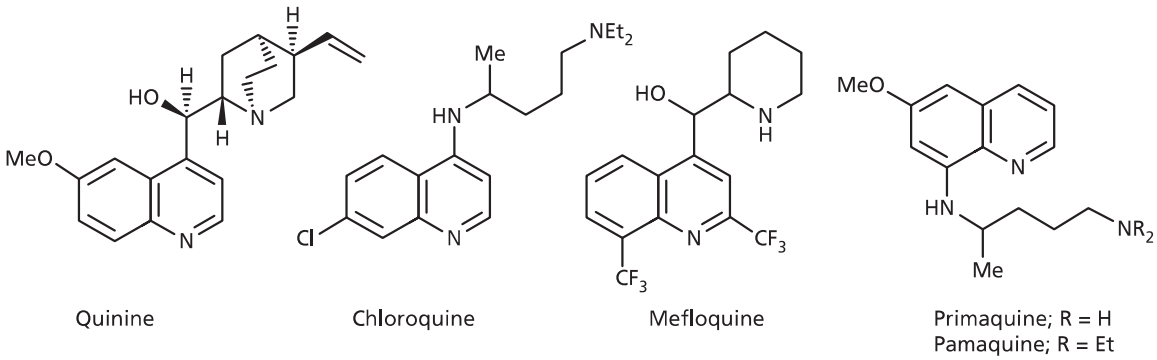


FIGURE CS3.1 Quinine and quinine-based antimalarial drugs.

quickly against chloroquine-sensitive strains. The **Walter Reed Army Institute of Research** in the USA was particularly interested in this new compound. Historically, more military casualties have resulted from malaria than from battle action. For example, during the Burmese campaign of World War II, a huge number of British and Indian soldiers were incapacitated by malaria, and had to be withdrawn from action. Many of the politically unstable countries in the world today are malarial infected areas, and so there is an obvious interest among the military to find novel antimalarial drugs for their troops.

Unfortunately, the only known source of *A. annua* was in China and the Chinese communist authorities were understandably reluctant to grant US army scientists free access into China. Negotiations were certainly not helped by US negotiators appearing in full dress uniform. As a result, the Americans were denied access to Chinese supplies and were forced to carry out a worldwide search to see if they could find an artemisinin-producing plant in a different country. Ironically enough, a suitable plant was eventually found growing in the US capital!

CS3.3 Structure and synthesis of artemisinin

The multicyclic structure of artemisinin (Fig. CS3.2) contains seven asymmetric centres and an unusual and unstable looking trioxane ring that includes an endoperoxide group. Despite, the unstable appearance of the molecule, it is stable to heat and light. Once the compound was identified, the next stage was to synthesize a range of analogues to investigate **structure–activity relationships** (**SAR**; section 13.1).

CS3.4 Structure–activity relationships

Artemisinin is a complex structure and, although it has been fully synthesized, this is not a practical method of obtaining it, or for producing a variety of different analogues. Consequently, analogues were prepared from artemisinin itself—a semi-synthetic approach. This was done by first reducing the lactone group of artemisinin to give **dihydroartemisinin** (Fig. CS3.3). This contains an alcoholic group which can then be alkylated to give various ethers such as **artemether** and **arteether**.

Esterifications can also be carried out on dihydroartemisinin; a particularly important ester is **sodium artesunate** from the reaction of artemisinin with succinic acid (Fig. CS3.4).

Dihydroartemisinin, artemether, arteether, and sodium artesunate are all more active than artemisinin itself, and so the lactone carbonyl group of artemisinin is not crucial to its antimalarial activity. A variety of other artemisinin

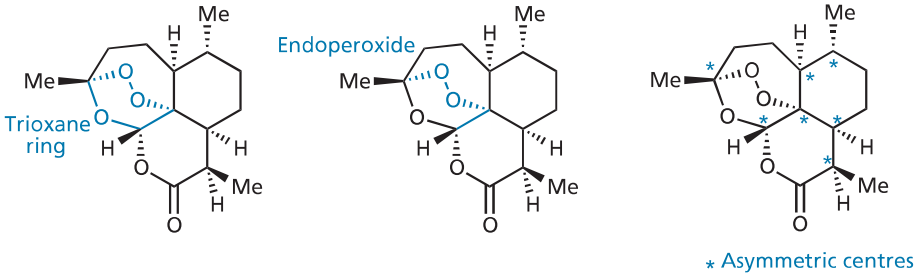


FIGURE CS3.2 Structure of artemisinin.

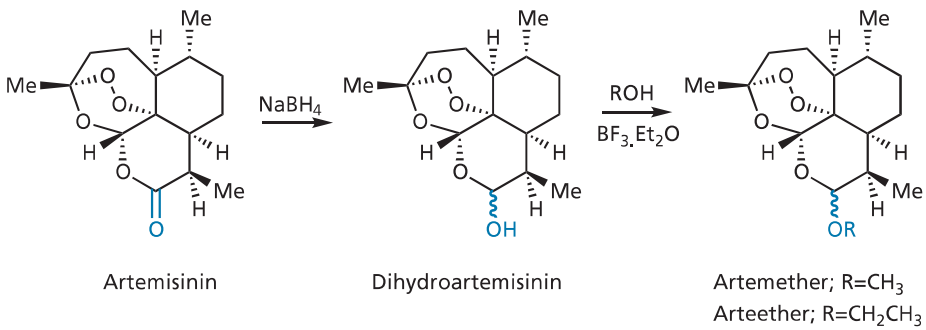


FIGURE CS3.3 Preparation of artemether and arteether.

analogues have also been studied. For example, **deoxyartemisinin** (Fig. CS3.5) is a metabolite of artemisinin and is 300–1000-fold less active. **Deoxodeoxyartemisinin** is also poorly active, whereas **deoxoartemisinin** has a similar activity to arteether.

The results from these and other structures led to the conclusion that the endoperoxide group in the trioxane ring was the essential group required for antimalarial activity, and that this represented the **pharmacophore** (section 13.2) for antimalarial activity.

CS3.5 Mechanism of action

Artemisinin has a totally different mechanism of action from the quinine-based drugs and has, therefore, proved effective against chloroquine-resistant strains of malaria. The secret behind its action lies in the endoperoxide group. This acts as a molecular trigger for a kind of 'scattergun' action which causes severe damage within the parasite cell and ultimately leads to its death. As the group is acting as a 'trigger', something has to pull the trigger. This turns out to be iron ions and, in particular, ferrous ions. In the presence of these ions, a reduction of the endoperoxide group takes place which generates two possible radical species (Fig. CS3.6). Further reactions take place to generate a series of other cytotoxic free radicals and reactive electrophiles which alkylate, cross-link, and oxidize vital biomolecules within the parasite. Cell death is the result.

This explains the action of artemisinin on protozoal cells, but why does it not kill human cells as well? In particular, why does the drug not destroy red blood cells which are rich in iron-containing **haemoglobin**—the

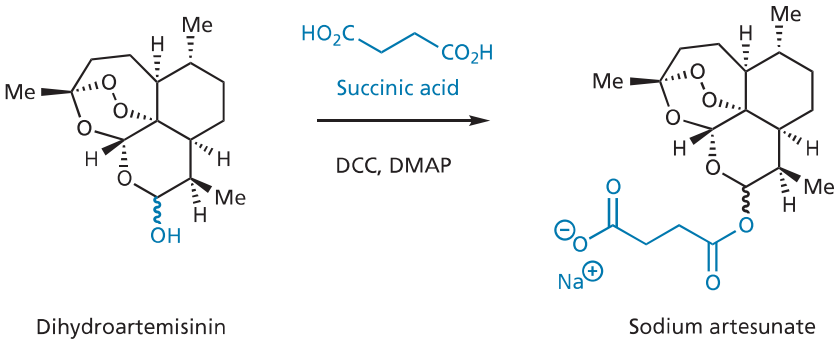


FIGURE CS3.4 Synthesis of sodium artesunate.

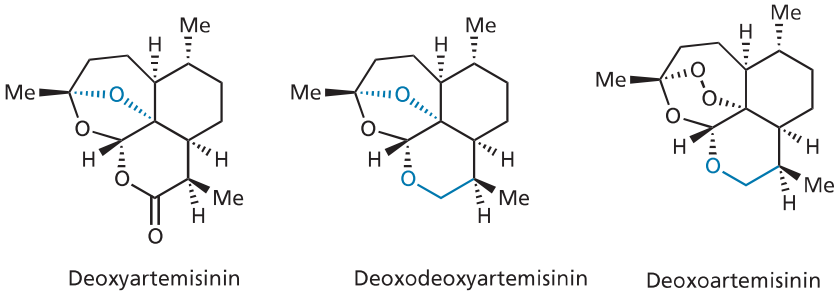


FIGURE CS3.5 Analogues of artemisinin.

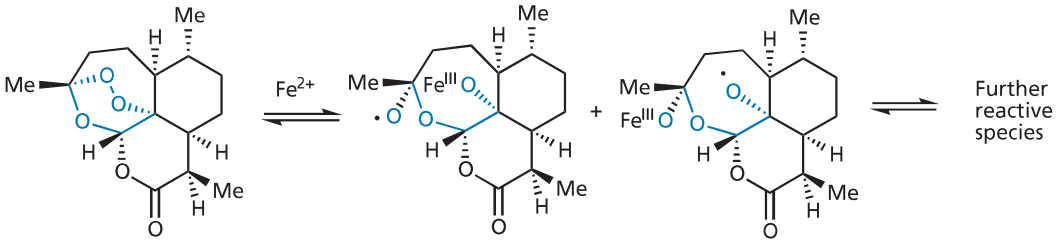


FIGURE CS3.6 Activation of artemisinin by ferrous ions.

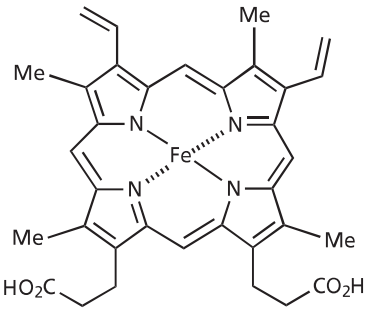


FIGURE CS3.7 Structure of heme.

protein responsible for carrying oxygen from the lungs to the rest of the body?

To answer these questions, we need to consider the life cycle of the parasite. This is quite a complex process involving both humans and mosquitos, but part of the parasite's life cycle in humans involves the invasion of red blood cells. As mentioned earlier, red blood cells contain haemoglobin. This is a protein that contains an iron (II)-centred porphyrin called **heme** (Fig. CS3.7). The porphyrin and the ferrous ion are buried deep within the protein and are effectively shielded. This explains why artemisinin is not toxic to normal, uninfected red blood cells. The ferrous iron, which would trigger its destructive capability, is 'hidden from view'.

When the malarial parasite infects red blood cells, it breaks down haemoglobin as a food source to provide itself with amino acids. This, of course, releases heme into the parasite cell. The ferrous ion present in heme can now react with artemisinin leading to the parasite's demise. Therefore, artemisinin and its analogues can be viewed as **prodrugs** (section 14.6) which are activated as a result of the parasite's own destructive tendencies to haemoglobin—poetic justice really!

A lot of research has been carried out to investigate the detailed radical mechanisms that follow on from the two radical products shown in Fig. CS3.6. The story is quite complex, but there is evidence that a particularly important mechanistic route for high antimalarial activity is the formation of a C-4 radical via 1,5-hydrogen atom abstraction (Fig. CS3.8). This produces the major metabolite that is observed for artemisinin, and also generates a highly reactive ferric hydroxide species which can go on to cause havoc within the cell.

Support for this theory comes from the activities of the simplified artemisinin analogues shown in Fig. CS3.9. Structure II is twice as active as artemisinin *in vitro*, whereas structures I and III are 100-fold less active. The 1,5-hydrogen shift shown in Fig. CS3.8 is not possible for structures I and III where the crucial hydrogen atom has been replaced with an α-methyl group. These compounds still react with the ferrous ion, but the 1,5-hydrogen shift is blocked. There is some evidence that the β-alkyl group at position 4 of structure II enhances activity, possibly by stabilizing the radical at position 4.

CS3.6 Drug design and development

As artemisinin is poorly soluble in both water and oil, early research was aimed at producing analogues which

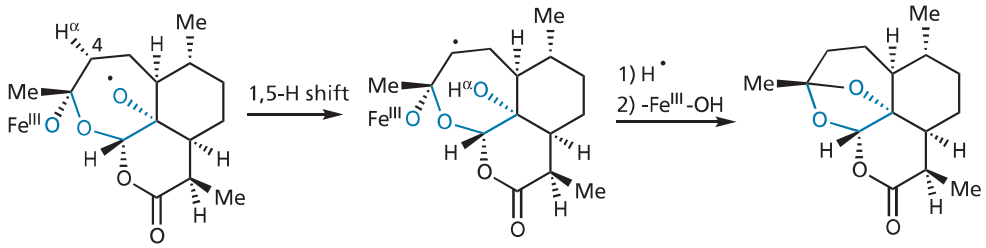


FIGURE CS3.8 Generation of a C-4 radical by 1,5-hydrogen atom abstraction.

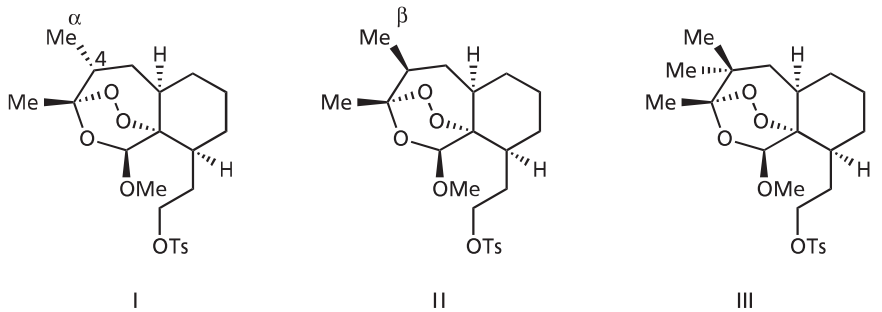


FIGURE CS3.9 Simplified analogues of artemisinin.

BOX CS3.1 Clinical properties of artemisinin and analogues

Artemisinin has proved highly effective in treating malaria, but there are problems related with its use. First of all, it is not water soluble and it has to be administered by intramuscular injection. It is also found that malaria re-occurs in up to 25% of patients treated after 1 month. Artemether and arteether are more hydrophobic than artemisinin and can be administered more easily in the field by injection in oil. They are also more potent. Sodium artesunate is also used clinically. Owing to the ionized carboxylate group, sodium artesunate is water soluble and can be administered by intravenous injection.

Currently, artemisinin, artemether, and sodium artesunate are used clinically. These compounds are now considered to be an essential component of **artemisinin combination therapy** (ACT) against drug-resistant malaria. They show brisk and potent activity, while cross-resistance with the more traditional antimalarial drugs is unlikely owing to the different mechanism of action.

Drawbacks for these drugs include a short plasma half-life, which is typically less than an hour, and rapid elimination This means that the drug is cleared from the system within a day of administration, leaving the longer-lived drugs of the combination therapy to continue the battle alone. This increases the risk of drug-resistant parasites emerging.

would be more soluble in one or other of these media. Dihydroartemisinin was found to be twice as active as artemisinin itself and was the gateway to the synthesis of a range of ethers and esters (Figs. CS3.4 and CS3.5). Many of these were found to have enhanced activity, as well as better solubility. The most interesting of these are artemether and arteether which, being more hydrophobic in nature, are more soluble in oil. Among the esters, the most interesting compound is sodium artesunate, which is ionized and water soluble.

Research has also been carried out with the aim of designing an antimalarial agent that can be synthesized easily and which has the same mechanism of action as the lead compound, artemisinin. As with many lead compounds of complex structure, the strategy of simplification (section 13.3.8) has been used. Artemisinin has a complex tetracyclic structure with seven asymmetric centres, which makes it far too complex to synthesize economically in the laboratory. A variety of simpler structures retaining the trioxane ring have been synthesized—one of the most interesting of these is **fenozan**, which has a tricyclic ring system as its core and two asymmetric centres (Fig. CS3.10). This structure shows comparable activity to arteether and sodium artesunate against some malarial strains.

Other simplified structures having comparable activity to artemisinin or its semi-synthetic analogues include bicyclic spiroalkyl trioxanes (Fig. CS3.10), which are as active as artemisinin in mice experiments, and the trioxanes shown in Fig. CS3.11, which have comparable activity to artemisinin *in vitro*.

Simple, symmetrical endoperoxides have also been synthesized (Fig. CS3.12). These have been designed to

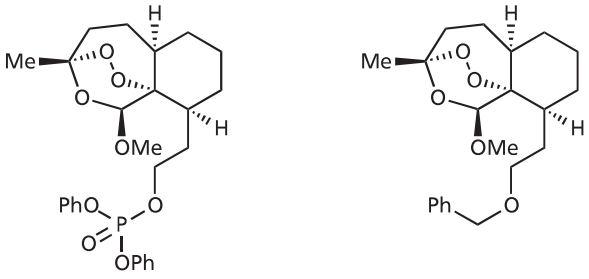
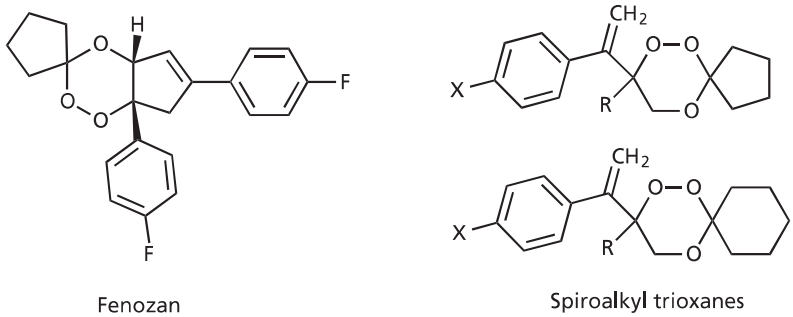


FIGURE CS3.11 Trioxanes having comparable activity to artemisinin.



Fenozan

Spiroalkyl trioxanes

FIGURE CS3.10 Fenozan and spiroalkyl trioxanes.

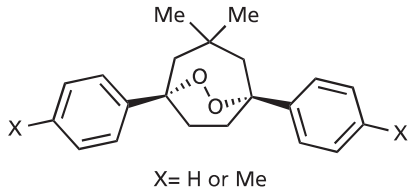


FIGURE CS3.12 Symmetrical analogues of artemisinin.

take advantage of the proposed 1,5-H abstraction mechanism described in Fig. CS3.8. The advantage of a symmetrical artemisinin is that degradation can occur in the same manner regardless of which oxygen reacts with iron. The potency of this compound is about a seventh of artemisinin *in vitro*, but this is still considered to be high.

Yingzhaosu A (Fig. CS3.13) is a naturally occurring endoperoxide which was isolated in 1979 from a traditional Chinese herbal remedy for fever (*Artabotrys uncinatus*) and shows antimalarial activity. However, the plant is a rare ornamental vine, and extraction of the natural compound is difficult and erratic. A synthesis was devised to produce a synthetic analogue of the structure, resulting in the discovery of **arteflene**, which is half as active as artemisinin.

To date, none of the simplified structures described have found widespread use as clinical agents, but there would be clear benefits in having a simple synthetic structure which would have the same mechanism of action as artemisinin, and which could be produced efficiently and cheaply for a market that cannot afford expensive drugs.

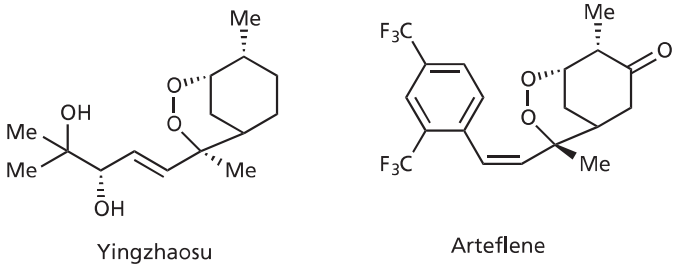


FIGURE CS3.13 Yingzhaosu A and arteflene.

FURTHER READING

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