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CHAPTER 5

Anti-Malaria Chemotherapy: State-of-the-Art in Prevention and Treatment and Novel Leads for Drug Development

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Abstract: Malaria is an infectious disease endemic to 106 countries of the tropical and subtropical regions of the world. According to the World Health Organization there were 216 million cases of malaria in 2010 that resulted in 655000 deaths. Children under the age of 5 are the most vulnerable, but approximately half of the world's population is at risk. Malaria is a febrile illness caused by parasitic protozoa of the genus *Plasmodium*, and transmitted exclusively by Anopheles mosquitoes. Control involves both prevention, through the use of indoor insecticide spraying with pyrethroids, insecticide treated bed nets, drug treatment of populations at high risk of infection and disease treatment. Malaria can be cured, but the development of resistance by *Plasmodium* is recurrent. Due to its high mortality and morbidity, the eradication of this disease has high priority in the UN 2000 Millennium Development Goals. As a result of renewed efforts, malaria related mortality decreased by 26% in the period 2000-2010, but control tools are limited. Presently there are no vaccines registered for this disease. The most deadly variant, caused by *Plasmodium falciparum*, is treated with artemisinin-based combination therapy with a 4-aminoquinoline or an amino alcohol. Recent reports of mosquito resistance to pyrethroid insecticides and of *Plasmodium* to artemisinin are serious causes for concern. The development of novel drugs remains a big challenge. This chapter highlights the state-of-the-art in malaria prevention and treatment. The literature published since 2000 on the development of new leads for chemotherapy is also reviewed.

Keywords: Malaria, antimalarial, *Plasmodium*, protozoa, *Anopheles*, infectious disease, pharmacology, artemisinin combination therapy, quinine, erythrocytes, antibiotics, aminoquinolines.

INTRODUCTION

Malaria is an infectious disease caused by parasitic protozoa of the genus *Plasmodium*, transmitted exclusively by the female *Anopheles* mosquito. It is

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endemic to 106 countries of the tropical and subtropical regions of the World. According to the World Health Organization, nearly 3.3 billion people were at risk of malaria in 2010, and in that year alone there were 216 million cases of which 655,000 resulted in death [1]. Due to its high mortality and morbidity, malaria is a disease of major importance. Although the figures for 2010 are staggering, they already represent a substantial improvement over the year 2000, when eradication of malaria was considered one of the priorities of the Millennium by the United Nations Organization, in its Millennium Development Goals. As a result of renewed interest, efforts and increased human and material resources for the fight against this disease, the number of reported cases of confirmed malaria decreased by more than 50% in 43 of the 99 countries with ongoing transmission during the period 2000 to 2010, and by 25 to 50% in another 8 countries [1]. It is estimated that during the same period the incidence of malaria decreased globally by 17%, and the mortality rate by 26% [1, 2].

Malaria is curable and controllable. When the parasite is inoculated into a human host by a feeding mosquito, it travels *via* the blood stream to the liver where it multiplies, returning afterwards to the blood stream where it infects the erythrocytes. The symptoms of malaria are initially similar to those of a minor systemic viral illness: headache, lassitude, fatigue, abdominal discomfort, muscle and joint aches, usually followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise [3]. Appropriate diagnostic means are also important initially, to identify the disease correctly. If the disease is not controlled initially by effective medicines or if there is a delay in treatment, it may develop into severe malaria, especially when *P. falciparum* is the causal agent. Severe malaria may have the following symptoms: coma (cerebral malaria), metabolic acidosis, severe anaemia, hypoglycaemia, acute renal failure or acute pulmonary edema and ultimately it may result in death if not treated [3]. This progress can be very rapid and can take place within hours or days. Normally the main objective is to prevent death, then to prevent disabilities and recrudescence [4]. Young children under 5 are usually the most susceptible to the disease, and indeed 86% of malaria deaths occur in children under 5 years of age. In areas where populations are exposed to a high rate of malarial inoculations, partial immunity to the clinical disease and its severe symptoms is acquired. Nevertheless, immunity changes during pregnancy. Travellers moving to endemic areas are also at high risk.

THE MALARIA PARASITES

There are five species of *Plasmodium* parasites (of the *Apicomplexa* phylum) that affect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* [2]. Nowadays *P. vivax* and *P. falciparum* are the most prevalent parasites. *Plasmodium falciparum* is the most dangerous. Malaria caused by this *Plasmodium* species often results in severe and life threatening conditions [5] and *P. falciparum* is responsible for approximately 90% of the deaths from malaria. In tropical countries *P. falciparum* is the most prevalent malaria parasite. *Plasmodium vivax* is more widely distributed and accounts for about 40% of the world malaria cases, but it is far less dangerous; it is also the dominant species outside Africa [3]. The other three species are less common. Although *P. vivax* and *P. ovale* very rarely cause fatal symptoms [3], these two species can form hypnozoites that stay dormant in the liver for long periods of time and can cause multiple relapses long after the first infection. Infection caused by *P. malariae* is often called "quartan" due to the periodic acute fevers it causes every four days, while with *P. vivax* and *P. ovale* this happens every third day; malaria caused by *P. vivax* is also known as "tertian". In *P. falciparum* malaria, the acute fevers occur at subtertian periods. *Plasmodium ovale* is the least distributed species, and is found only in sub-Saharan Africa, New Guinea and in the Philippines. *Plasmodium knowlesi* is a species that principally infects monkeys, but it has been causing an increasing number of human malaria cases in South-East Asia in recent years [3]. Parasitic *Plasmodium* species also infect birds, reptiles, chimpanzees and rodents.

THE LIFE CYCLE OF THE MALARIA PARASITE

Malaria is carried and transmitted to men by the female *Anopheles* mosquito only, when it bites to get a blood meal. The male mosquito only feeds on plant nectar and does not transmit the disease. The life cycle of the parasite [6], illustrated in Fig. (**1**), includes two stages: an exogeneous sexual phase (sporogony) with multiplication in the mosquito and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host. The asexual phase includes two stages: a phase in the parenchyma cells of the liver, where pre-erythrocytic schizogony occurs, and another in the red blood cells, the erythrocytic schizogony. While sucking blood, the mosquito transfers saliva containing the *Plasmodium* parasite into the blood stream of the host. At this

stage *Plasmodium* is in a highly infective sporozoite form. About 15 to 20 sporozoites are transferred. The parasite goes rapidly to the liver without being detected by the host's immune system and here starts the asexual liver stage in the life cycle of *Plasmodium*. The sporozoites invade the parenchyma cells of the liver where, by increasing their size and repeatedly replicating their nuclei and other organelles, they develop into tissue schizonts. In *P. vivax* and *P. ovale*, the sporozoites may remain in a dormant stage, the hypnozoites or develop into tissue schizonts. The hypnozoites can remain dormant in the liver for long periods of time, even years, and reactivate. In *P. falciparum* and *P. malariae* only schizonts form. The multinucleated schizonts subdivide into 10,000-30,000 identical daughter cells, consisting of a single nucleus and a narrow cytoplasmic ring, the merozoites. After one to two weeks the schizonts rupture releasing the merozoites into the blood stream. This starts the asexual erythrocytic phase of the parasite's life cycle.

Some merozoites invade the red blood cells, the erythrocytes, where they grow, absorbing the haemoglobin, and develop into trophozoites, or ring forms. Their nuclei divide 3 to 5 times into a variable number of small nuclei, then the cytoplasm divides, and as a result of this segmentation the blood schizonts are produced. The schizonts grow and subdivide into merozoites. After some time that varies from one *Plasmodium* species to another, the erythrocyte ruptures and releases 16-32 new merozoites into the blood stream. These new merozoites may also invade the erythrocytes and start a new erythrocyclic life cycle. This cycle, from erythrocyte invasion to schizont rupture lasts 48 h with *P. falciparum, P. vivax* and P. *ovale* and 72 h with *P. malariae*. After a number of asexual life cycles, some merozoites develop into sexual forms, the gametocytes, which are transferred to a mosquito during a blood meal. In the mosquito mid-gut the gametocytes reproduce sexually developing from diploid zygotes into motile ookinetes that attach themselves to the outer gut membrane, differentiating into oocysts. The oocysts divide many times producing thousands of infective sporozoites, which migrate to the salivary glands of the mosquito, ready to be transmitted to humans again, thus starting a new cycle.

Antimalarials usually act on different stages of the parasite's life cycle, and this distinction is commonly used to classify them. Table **1** shows the classification used. To treat someone suffering from malaria, a blood schizontocide and a gametocide are

needed if the protozoan is *P. falciparum*. If the protozoa causing the disease are *P. vivax* or *P. ovale*, a tissue schizontocide is also needed.

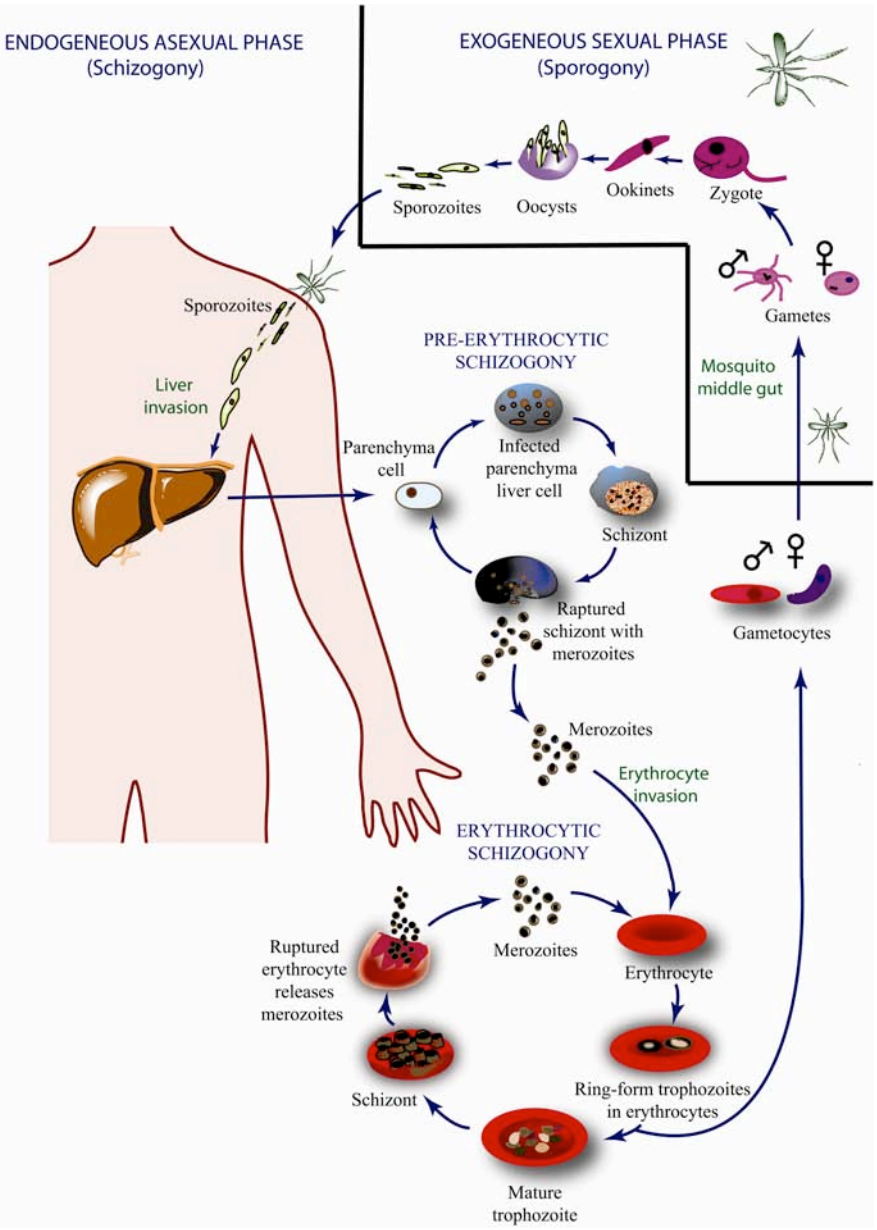


Figure 1: The life cycle of *P. falciparum*, with an exogeneous sexual phase (sporogony) with multiplication in certain *Anopheles* mosquitoes and an endogenous asexual phase (schizogony) with a liver stage and an erythrocytic stage of multiplication in man.

MALARIA CONTROL

Control involves both prevention, through the use of indoor insecticide spraying (mostly with pyrethroids), insecticide treated bed nets, drug treatment of populations at high risk of infection and disease treatment. Malaria can be cured. To maximize the effectiveness of the treatment and to prevent the development of resistance, combinations of drugs are recommended nowadays. Severe malaria, caused by *P. falciparum*, is often treated with artemisinin-based combination therapy (ACTs) with a 4-aminoquinoline or with an amino alcohol like quinine. Presently there are no vaccines registered for this disease, although attempts to develop one are underway. Recent reports of mosquito resistance to pyrethroid insecticides and of *Plasmodium* to artemisinin are serious causes for concern [1, 2]. Indeed, the development of resistance by *Plasmodium* is a continuous problem [8], and hence the development of novel drugs, to improve or substitute existing ones, is a continuous challenge. Affordability is yet another issue, since ACTs are nearly 10 times as expensive as the previous first-line drugs [9].

Table 1: Classification of Antimalarial Drugs According to the Stage of the Parasite's Life Cycle on Which they Act

Type of Drug	Drug Activity
Tissue Schizonticides	**They act on the liver forms of the parasite. When the primary exoerythrocytic stage is blocked, schizont development is impeded, and red blood cell invasion is prevented.** **These drugs are used for causal prophylaxis. *P. falciparum* is the most susceptible species.**
Blood Schizonticides	**They act on blood schizonts and related erythrocytic asexual forms of the parasite. These are the stages that are associated with acute disease, although sometimes there are only a few symptoms.** **They produce clinical cure.** **They also act on sexual erythrocytic forms of *P. vivax, P. ovale* and *P. malariae.* They don't act directly on the mature gametocytes of *P. falciparum.***
Gametocides	**They destroy the sexual forms of the parasite in the blood, the gametes. This prevents transmission of the infection to the mosquito.**
Sporontocides	**They prevent the development of oocysts and sporozoites in the mosquito. This prevents further transmission of malaria to humans.**
Hypnozoites	**Tissue schizonticides that act on the latent forms of the parasite that develop in the liver with *P. vivax* and *P. ovale*. They are used as anti-relapse drugs, preventing reactivation of the disease. These types of drugs are needed with these two species of *Plasmodium* to achieve radical cure of malaria.**

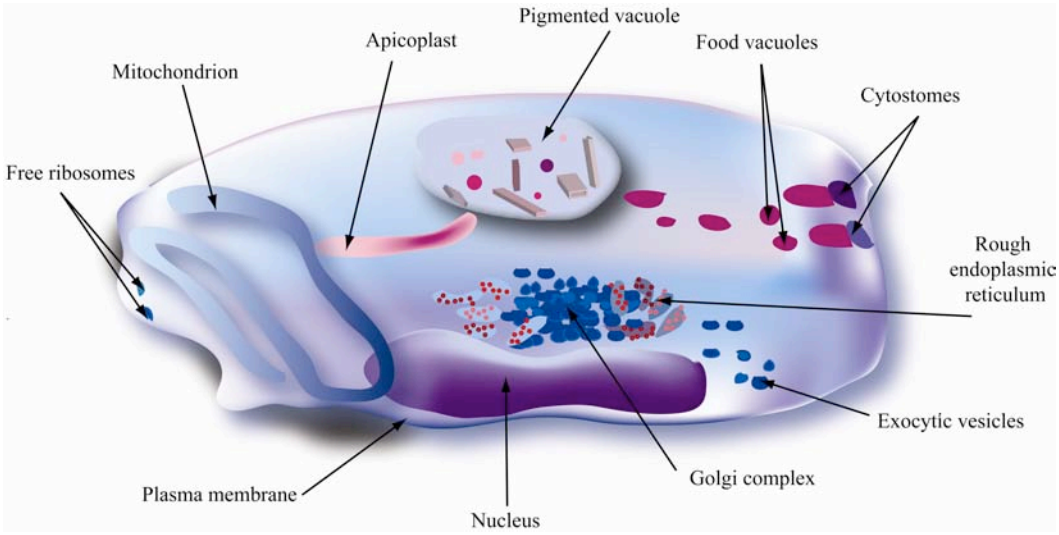


Figure 2: Schematic representation of the Mid-Trophozoite stage of *Plasmodium falciparum*, with its characteristic irregular outline [8].

Since an understanding of cell biology can lead to the development of better drugs, or to the exploitation of new drug targets, this is another area of intense research. The genome of *P. falciparum* has now been unveiled, and many present day studies focus on the development of molecular markers to identify genetic mutations related to antimalarial drug resistance [8]. A discussion of this subject is, however, beyond the scope of the present review. Nevertheless, to guide the reader through some of the aspects focused herein, Fig. (**2**) shows a schematic representation of the ultra-structure of *P. falciparum* in the mid trophozoite state. In this chapter, the state-of-the-art in malaria prevention and treatment is highlighted, as well as the pipeline of prospective drugs at several stages of clinical development. It ends with a brief overview of possible areas for future intervention.

CURRENTLY AVAILABLE ANTIMALARIAL DRUGS

The World Health Organization (WHO) recommends a number of drugs for the treatment and prophylaxis of malaria (Table **2**) [3, 7]. In this section, a brief look at their historical development, chemical and pharmaceutical properties, and the recommendations for their use, is presented. The pharmacokinetic parameters

indicated aim to be representative. In many cases more than one study was published.

Table 2: Main Drugs Currently Available for Malaria Treatment According to WHO [7]

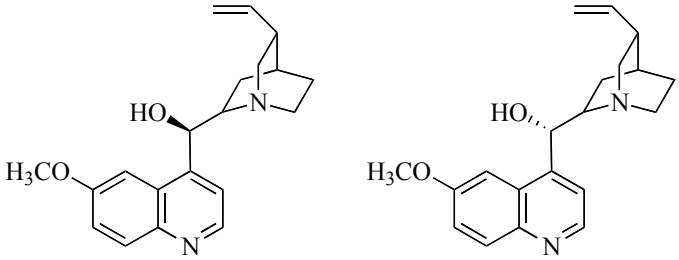
Chemical Class	Drug
Amino-alcohols	Quinine, quinidine, mefloquine, halofantrine, lumefantrine
4-Aminoquinolines	Chloroquine, amodiaquine, piperaquine
8-Aminoquinoline	Primaquine
Sulfonamides and sulfones	Sulfadoxine, sulfalene, dapsone
Diaminopyrimidine	Pyrimethamine
Biguanides	Proguanil, chlorproguanil
Naphthoquinone	Atovaquone
Sesquiterpene lactones	Artemisinin, arteether, artemether, artesunate, dihydroartemisinin
Antibiotics	Azythromycin, clindamycin, doxycycline, tetracycline

THE CINCHONA ALKALOIDS: QUININE AND QUINIDINE

Quinine

Quinine (QN) is the oldest antimalarial known [10]. A quinoline with a (8*S*,9*R*) quinuclidine methanol group, it is usually extracted from the bark of various species of the *Cinchona* tree, indigenous to Peru, where it is present in up to 13% of the dry matter. The medicinal properties of the bark were discovered by the Peruvian Quechua long ago. The plant was brought to Europe by the Jesuits in the 17th century, and it was already then used as an antimalarial drug. The powdered bark contains quinine and its diastereoisomer quinidine, both plasmodicidal alkaloids (Fig. **3**). A crude mixture of crystalline alkaloids was extracted from the cinchona bark by B. Gomes, in Portugal, in 1810 [11]. Quinine and cinchonine were later isolated by P. J. Pelletier and J. B. Caventou in Paris in 1820, and have been used as such since then. The first total synthesis was accomplished by R. B. Woodward and W. E. Dooring in 1944, and although a few more methods of total synthesis were developed since then, none is as economically viable as the extraction of the substance from the *Cinchona* tree bark. Quinine was the antimalarial drug of choice until the 1940s, but because of its side effects, other

drugs started to be used. Quinine is a highly effective blood schizonticide against all forms of human malaria, hence it is effective for the clinical cure of malaria [12]. Radical cure is possible with *P. falciparum*. It has no effect on the exo-erythrocytic phase or the gametocytic phase. It is however an effective gametocytocide for *vivax*, *ovale* and *malariae* malaria [12]. It is inactive against latent erythrocytic stages. Long experience with this drug proves its value. Quinine has its own characteristic side effects, like giddiness, ringing in the ears, tremors and blurred vision, which occur while the drug is being administered [3]. These symptoms subside when the administration of the drug is stopped. Given intravenously it lowers blood pressure. More serious effects may occur, but they are rare. Nowadays quinine is still a drug of choice for the immediate relief of the symptoms of severe *P. falciparum* malaria, and it is recommended by WHO as a first line alternative to artesunate or artemether. It is also the drug of choice for pregnant women [7]. After an initial emergency parenteral antimalarial treatment, and once the patient can take oral medicines, one of the possibilities for follow-up treatment, besides an ACT, is quinine plus an antibiotic like clindamycin or doxycycline [3]. In a study in Thailand, after standard treatment for severe falciparum malaria the mean parasite clearance time was 76 h [13]. QN with an antibiotic is also recommended as second-line option for the treatment of uncomplicated falciparum malaria [3], and this was the subject of a recent review [14]. It is usually active against most strains that are resistant to chloroquine. IC_{50} values measured against laboratory strains and clinical isolates of *P. falciparum* varied between 96 and 380 nM and between 136 and 286 nM respectively [6].



(-)-Quinine **1** (+)-Quinidine **2**

Figure 3: The first antimalarials, quinine and quinidine.

The mechanism of action of quinine is thought to involve inhibition of plasmodial haem polymerase [15-19]. In order to grow, the parasite breaks down the host's

haemoglobin to obtain amino acids for its own protein synthesis (Fig. **4**). This takes place inside a lysosome-like organelle, the food vacuole. Ferriprotoporphyrin IX, *i.e.,* free haem, is obtained as a by-product. Free haem is toxic to the parasite, and as part of the detoxification process free haem is converted into hemozoin, the malaria pigment crystals, by the parasite's haem polymerase. When the action of haem polymerase is blocked by the antimalarial, there is a build up of free haem that eventually leads to parasite death. In addition, QN can also block DNA transcription in the parasite, since it can become intercalated in the parasite's DNA.

Pharmacology of Quinine

Quinine is rapidly absorbed from the gastrointestinal tract. It is extensively metabolised *via* the cytochrome P450 enzyme CYP3A4 in the liver [3]. The initial metabolite is 3-hydroxyquinine (3-OH-QN) that has about 10% of the antimalarial activity. QN has a half-life of 10 hours [12] and its bioavailability is about 80% [20]. A mean plasma concentration of 2-5 mg L^{-1} is probably necessary to reduce parasitaemia in acute *P. vivax* malaria, and 5 mg L^{-1} to eliminate asexual parasites from the blood stream [12]. Slightly higher amounts are needed with *P. falciparum*, which vary with the strains. It is generally given as a 7-day course followed by an antibiotic like tetracycline, pyrimethamine-sulphadoxine (Fansidar), doxycycline or clindamycin. In a study of its pharmacokinetics on healthy individuals, a single oral dose of 600 mg quinine sulphate produced a maximum concentration in the blood of 11.2 mg L^{-1} after 2.8 h, and mean $AUC_{0\rightarrow\infty}$ 12 h mg L^{-1} [21]. The major metabolite 3-OH-QN reached a mean maximum concentration in the blood of 1.35 mg L^{-1} after 62.8 h [21]. These values are higher in patients with uncomplicated *P. falciparum* malaria. In areas of multi-drug resistant strains, 7-day regimens of quinine and tetracycline provide cure rates over 90% in patients with uncomplicated falciparum malaria [22]. The pharmacokinetics of quinine was determined recently on adult patients in Thailand. Patients were treated with the standard oral treatment of quinine (quinine sulphate, 10 mg kg^{-1} b.w. thrice daily for 7 days). It was found that a maximum concentration of 11.4 μg mL^{-1} of quinine was reached in the blood after 1.5 days. The active metabolite 3-OH-QN reached C_{max} after 2.5 days. The $AUC_{0\rightarrow7}$ was 10 fold higher for quinine than for its metabolite, and it was a mean of 51.7 d μg mL^{-1}. The mean parasite clearance time was 73 h. Some patients took

rifampicin at the same time, but in those cases the parasite clearance time (PCT) was the same, an indication that rifampicin did not contribute to kill the parasite. The maximum concentration of 3-OH-QN in the blood was 1.26 μg mL^{-1} [22]. The authors concluded that for cure a continuous concentration of 6 μg mL^{-1} is required for 7 days. The pharmacokinetics of quinine is not altered significantly during pregnancy [23, 24]. The pharmacokinetics of quinine is not affected when taken in combination with the antibiotics doxycycline [20] and clindamycin.

THE 4-AMINOQUINOLINES: CHLOROQUINE, PIPERAQUINE AND AMODIAQUINE

The fact that the availability of quinine depended on supplies of bark from Cinchona plantations led to a further search for synthetic analogues (Fig. **5**). Chloroquine (CQ) was discovered in this way [25-27]. Later the development of resistance and the desire to have antimalarials with improved properties and fewer side effects but which still retained the useful properties of CQ became additional driving forces for research in this area. The strategies adopted in which the 4-aminoquinoline ring is kept have been 1) to link two aminoquinoline moieties by linkers of various lengths and nature, 2) to modify the side chain by elongation or shortening, 3) to introduce lipophilic aromatic moieties into the side chain, and more recently 4) the synthesis of hybrid 4-aminoquinolines, that is drug hybrids containing both an aminoquinoline moiety, and a synthetic mimic of another current antimalarial molecule [28]. Following some of these approaches two important drugs widely used nowadays in the treatment of malaria were developed: piperaquine and amodiaquine. Although the precise mode of action of the 4-aminoquinolines is not yet completely understood, it is known that they accumulate in the food vacuole [11], and it is thought that like quinine, they target the processes that take place inside the digestive food vacuole related to the metabolism of haemoglobin and detoxification (Fig. **4**) [16].

Chloroquine

Chloroquine (CQ), initially named resochin, was developed by German scientist H. Andersag at Bayer AG as a substitute for quinine [25]. It is a totally synthetic

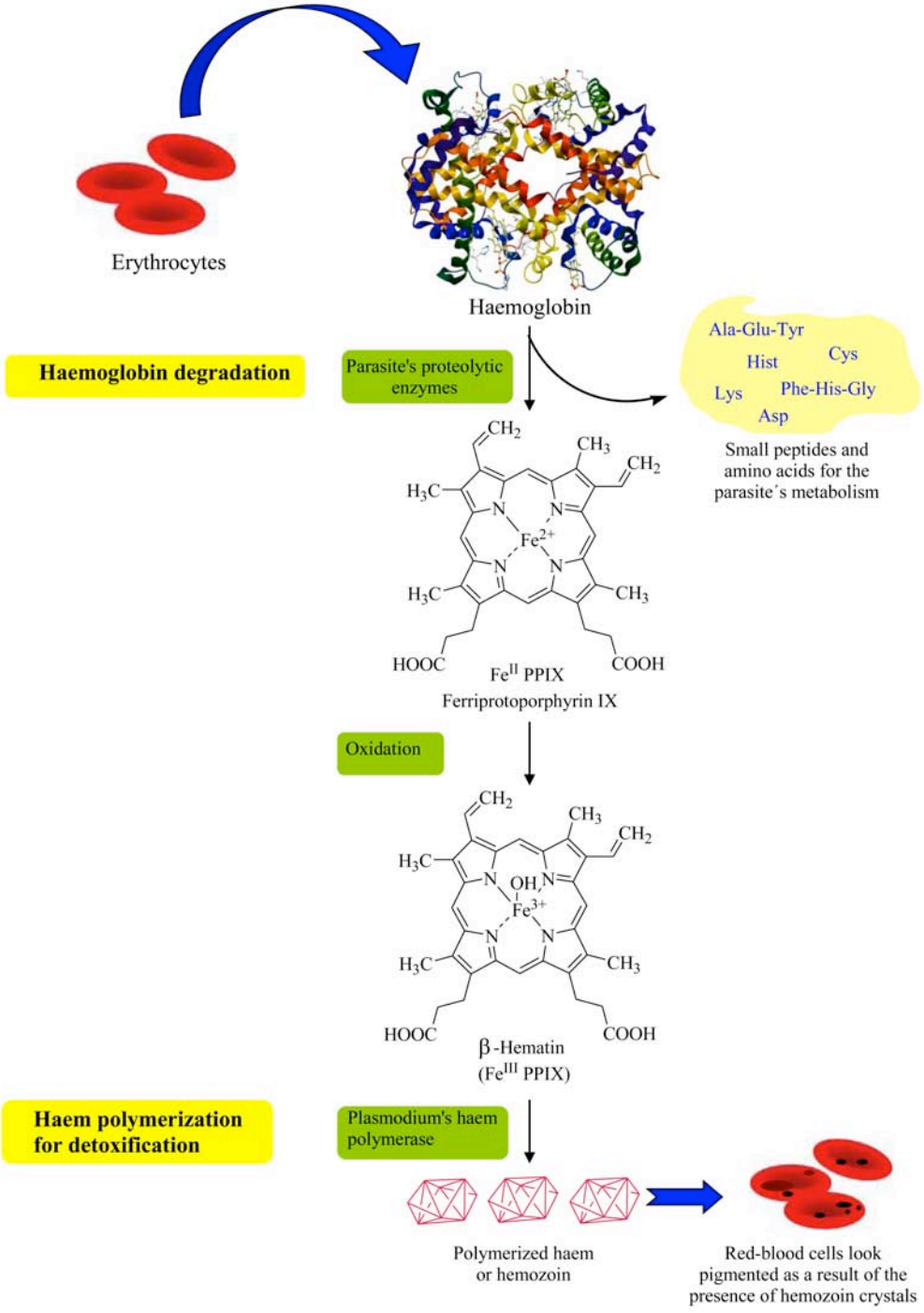
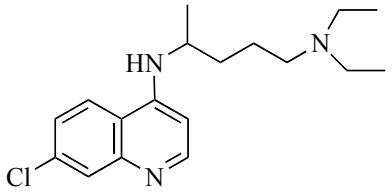


Figure 4: Haemoglobin digestion by *P. falciparum*.

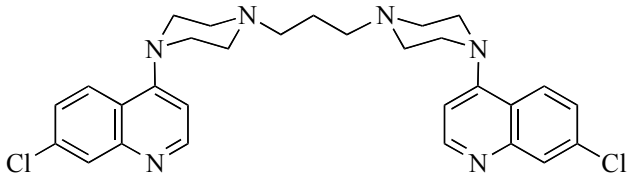
drug, a 7-chloroquinoline with a flexible pentadiaminoalkane side chain at position 4 (**3**, Fig. **5**). Due to its high effectiveness, fast action, low toxicity and low cost, it became the most successful antimalarial drug ever used. It was the drug recommended by WHO for its Global Malaria Eradication Programme launched in 1955 [6]. Together with DDT spraying, landscaping measures and surveillance in national programmes, thousands of lives were saved and the final result was the eradication of malaria from areas with temperate climates and seasonal malaria transmission. In 1975 WHO declared that Europe was free of malaria. However, due to the extensive use of this drug, *Plasmodium falciparum* started to develop resistance in the 1960s independently in four different regions of the world, and nowadays resistance has spread to almost the entire malaria endangered area. More than 80% of wild isolates are resistant to CQ [6]. Nevertheless, CQ is still being used as a first line option in countries where strains are sensitive. It remains effective against most strains of *P. vivax*, *P. ovale* and *P. malariae*, but resistance to *P. vivax* is increasing. It is the drug recommended by WHO for the treatment of vivax malaria in areas where this drug is effective [7]. The treatment should be followed with a 14-day course of primaquine, to eradicate the persistent liver stages and hence prevent relapse [1]. It is still also the drug of choice to prevent malaria, used for prophylaxis by travellers going to sensitive areas. In addition, it is also recommended for the treatment of malariae and ovale malaria [1].

CQ is a very potent schizonticidal drug effective against erythrocytic stages of all *Plasmodium* species [12]. It has no effect on sporozoites or hypnozoites. It is gametocytocidal against *P. vivax, P. ovale*, and *P. malariae* and it is also effective against immature gametocytes of *P. falciparum*. However, it is ineffective against the mature ones [12]. The way it acts is not entirely clear, but like other aminoquinolines and also quinine, it targets metabolic processes that take place inside the food vacuole. CQ concentrates in the food vacuole, and an accumulation of undigested haemoglobin may be observed [11]. Hence it seems that it inhibits the process of haemoglobin degradation. Like quinine, it also intercalates with DNA and it fragments parasitic RNA. It can kill plasmodia at a concentration three orders of magnitude lower than the concentration at which it is toxic to mammalian cells [11]. When *P. falciparum* infection is safely excluded,

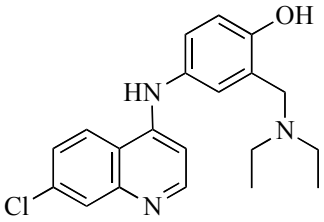
chloroquine remains the standard of care for *P. malariae*, *P. ovale* and for *P. vivax* malaria [29].



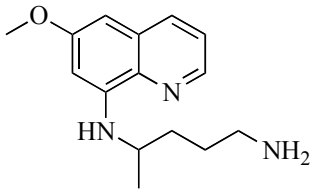
Chloroquine **3**



Piperaquine **4**



Amodiaquine **5**



Primaquine **6**

Figure 5: The aminoquinolines.

Pharmacology of Chloroquine

CQ is rapidly absorbed from the gastrointestinal tract after oral administration, and it has a high bioavailability of between 80 and 90% [30]. Only about 50% is metabolized, predominantly to desethylchloroquine, which has similar antimalarial activity against *P. falciparum*. Bisdesethylchloroquine and 7-chlor-4-aminoquinoline are minor metabolites. The half-life is estimated at 1-2 months [3]. Recently there was a study on the pharmacokinetics of chloroquine in healthy volunteers [31], who received the standard WHO three-day CQ regimen: 1,000 mg CQ (600 mg base) on days 1 and 2, and 500 mg (300 mg base) on day 3. It was found that a maximum concentration of CQ of 16.5 kg μg mL^{-1} was present in the plasma after 6.6 h, and $AUC_{0\rightarrow\infty}$ was 1,690 kg h μg mL^{-1}. The pharmacokinetics of the metabolite desethylchloroquine was as follows: a maximum concentration of 4.99 kg μg mL^{-1} was present in the plasma after 13.2 h, and $AUC_{0\rightarrow\infty}$ was 761 kg h μg mL^{-1}. In this study the authors also determined the pharmacokinetics of chloroquine in the presence of azithromycin. However, although it has been observed by others that the effectiveness of these compounds

increases when they are administered together [32], this study did not reveal a pharmacokinetic interaction between the two drugs. The therapeutic dose is 10 mg kg^{-1} b.w. At 20 mg kg^{-1} CQ causes serious side effects, 30 mg kg^{-1} is potentially lethal. In long-term prophylaxis, it may cause serious and irreversible side effects, such as neuromyopathy, retinopathy, erythema multiform and bone-marrow toxicity, but this is rare [6]. The effective concentration against *P. vivax* is 15 ng mL^{-1} in plasma and 30 ng mL^{-1} in serum [7]. Since the concentrations in whole blood are usually several times higher than those in plasma or serum, and the metabolite monodesethylchloroquine can also act against *P. vivax*, as it does against *P. falciparum*, whole-blood concentrations of 70-90 ng mL^{-1} of chloroquine and its metabolite should be adequate to suppress *P. vivax* [7].

Parasite clearance in the blood stream occurs within 48-72 h. CQ is well tolerated by pregnant women and children. In a study with pregnant and non-pregnant women with acute *P. vivax* malaria, the pharmacokinetics was found to be similar in both groups [33]. When 25 mg CQ base/kg were administered over 3 days to pregnant women, a peak plasma concentration of 960.5 ng mL^{-1} was observed after 3 h, with $AUC_{0-\infty}$ 122,216 h ng mL^{-1}. The half-life was 180.4 h. These values were similar to those obtained with non-pregnant women. At day 28 there was 100% cure, but by day 42 this value had dropped to 83% for the first group, 69% for the second.

Piperaquine

Piperaquine (PPQ) is a totally synthetic bis(quinolyl)piperazine (**4**, Fig. **5**) developed independently in the 1960s by researchers in China, at the Shanghai Research Institute of Pharmaceutical Industry, and at Rhone Poulenc in France. It was used extensively for twenty years, particularly in China, for prophylaxis and treatment of falciparum and vivax malaria [34]. It is active against exoerythrocytic forms of the parasite and it is also is a fast-acting blood schizonticide. It is thought to act by a mechanism similar to that of chloroquine and other aminoquinolines, accumulating in the lysosomal food vacuole, where it interferes with the haem metabolism. Its activity against resistant parasites is thought to be due to its large steric bulk, which prevents it from fitting into the binding site of the *trans*-membrane protein *Pf*CRT (*P. falciparum* chloroquine

resistance transporter), a membrane transporter protein that, as a result of a genetic modification by a gene which underwent a mutation, *pfcrt*, is thought to be responsible for resistance [6]. The transporter protein reduces the concentration of these drugs in the food vacuole, preventing them from exerting their antiparasitic action. The effectiveness of piperaquine may also be due to the fact that it is more effectively trapped inside the acidic food vacuole as a result of its four negative charges [6]. However, nowadays widespread resistance to piperaquine has also developed. There are also reports of cross-resistance with chloroquine [6]. Cross-resistance occurs when two drugs have similar mechanisms of resistance, and the parasite being resistant to one, is also resistant to the other. There are also reports that cross-resistance between PPQ and dihydroartemisinin can be induced *in vitro*. In Africa, where piperaquine has not been introduced for treatment, wild isolates of *P. falciparum* are still sensitive to this drug, with IC_{50} values of 40 nM [35]. Piperaquine is well tolerated and its most important side-effect is an increase in blood pressure [6].

Pharmacology of Piperaquine

Piperaquine is highly lipophilic and basic, poorly soluble in water [37]. It has a long half-life, of 20 to 30 days [38]. Five metabolites of piperaquine have been identified recently in the urine of 2 healthy individuals, after administration of tablets containing PPQ and DHA together with a fatty meal (PPQ 120 mg, DHA 960 mg), but only two were present in blood and plasma and appear to be the main metabolites: piperaquine N-oxide and 3-[4-(7-choro-quinolin-4-yl)-piperazin-1-yl]-propionic acid [38].

The pharmacokinetics has been determined in a study with healthy Vietnamese adults [39]. Single doses of 500 mg of piperaquine were administered, and the maximum concentration of the drug was found to be 69.6 ng mL^{-1} after 4 hours, with $AUC_{0-\infty}$ 4,484 ng mL kg^{-1}, and a half-life of 483 h. A single dose of 1000 mg, gave similar parameters, with a peak blood plasma concentration of 195.5 ng mL^{-1} being observed after 4 hours, $AUC_{0-\infty}$ 13,370 ng mL kg^{-1} and a half-life of 494 h. When used to treat falciparum malaria, PPQ is conventionally given as four equal doses of between 2.8 and 10.8 mg base kg^{-1} [40].

The pharmacokinetic profile of this highly lipid-soluble drug with slow absorption, good bioavailability, long elimination half-life, large volume of distribution and its low cost, make it a promising partner for combination therapies with artemisinins for example. A fixed combination with dihydroartemisinin is now one of the ACTs recommended by WHO for malaria treatment [3]. Although the drug was used extensively in China, only a few publications are available in the English literature on its pharmacokinetics in individuals with malaria, and the studies were carried out using the PPQ-DHA fixed combination antimalarials. For further details see under Artemisinin-Based Combination Therapies).

Amodiaquine

Structure-activity relationship studies have shown that antimalarial activity is retained when the side chain of chloroquine is shortened or lengthened. Some of these modifications led to the development of amodiaquine (AQ) (**5**, Fig. **5**), a drug with higher lipophilicity as a result of the incorporation of an aromatic ring in the side chain, a 4-aminophenol group [28]. Its potential for malaria treatment was discovered in 1946, and it is thought to function as a blood schizonticide by a mechanism similar to that of chloroquine. It produces clinical cure of all types of human malaria and radical cure of falciparum and malariae infections [12]. It is an excellent suppressive agent. It is also a gametocytocidal agent against *P. vivax*, *P. ovale* and *P. malariae*, and it is effective against immature gametocytes of *P. falciparum*. Over the years resistance has developed against this antimalarial [41], and there is even cross-resistance with chloroquine [42]. However, in areas where CQ resistance has been identified, amodiaquine is still more effective, and hence it remains an important drug. Nowadays it is used in several countries as a first-line drug, particularly in combination therapies [7]. The minimum concentration of amodiaquine required to inhibit, *in vitro*, the maturation of blood schizonts of a sensitive strain of *P. falciparum* (*e.g.,* strain Uganda 1) is 50 μg salt L^{-1} of blood [12]. For a CQ-resistant Vietnam (Marks) strain, it is 100 μg salt L^{-1} of blood [12].

Amodiaquine causes some side effects. Hepatoxicity may occur after prophylatic use for a period of 3 weeks to 10 months, but the chances of this occurring are 1

in 15,500. Another pathology which has been associated with long term use is agranulocytosis, a severe condition characterized by low white blood cell counts and hence a higher susceptibility to infections as a result of a suppressed immune system. The incidence is also low, 1 in 2,100, but for these reasons amodiaquine is not recommended nowadays for long-term treatments or prophylaxis [27]. It is assumed to be safe when used in short therapeutic regimes [27]. Although some studies suggest that amodiaquine at standard doses is not teratogenic, there is yet not enough data on its safety and pharmacokinetics in pregnancy [43]. Artesunate plus amodiaquine is nowadays an ACT recommended for malaria therapy by WHO (see under Artemisinin-Based Combination Therapies).

Pharmacology of Amodiaquine

Amodiaquine has low bioavailability. However, it is considered to be a prodrug of desethylamodiaquine (DAQ), the main metabolite that forms rapidly in the liver, and a potent antimalarial with a plasma concentration 6- to 7-fold higher than the parent drug. DEA has a long elimination half-life of more than 100 h. The use of AQ to treat uncomplicated falciparum malaria has been recently reviewed [41]. In a study in which amodiaquine hydrochloride (3 mg base kg^{-1}) was given by constant rate intravenous injection over 10 minutes to healthy male volunteers, and over 4 h by constant rate infusion to adults with falciparum malaria (10 mg base kg^{-1}), a peak plasma concentration of 415 ng mL^{-1} and of 322 ng mL^{-1} was found in healthy volunteers and patients respectively [44]. The data obtained in this study suggested that parenteral amodiaquine should be given by constant rate intravenous infusion and that an effective dose would be the administration of 5 mg base kg^{-1} every 8 hours to a total of 25 mg kg^{-1}. Amodiaquine (25-30 mg base kg^{-1} body weight given over 3 days) has been used effectively for the treatment of chloroquine-resistant vivax malaria [3]. Primaquine must be added for radical cure.

The pharmacokinetics was studied in patients with uncomplicated falciparum malaria in Zambia [45]. Two different oral dose regimens were followed: either 10 mg kg^{-1} AQ on day 1, and 5 mg at 6 h, 24 h, and 48 h later or 10 mg kg^{-1} on day 1 and 24 h later, and 5 mg 48 h later. For AQ, the maximum concentration in the plasma was 21 ng mL^{-1} after 2.0 h, $AUC_{0\rightarrow36}$ was 77 h ng mL^{-1}, and the half-life 3.7 h. DAQ had a maximum concentration in the plasma after 3.9 h of

161 ng mL^{-1}, $AUC_{0\rightarrow6}$ was 621 h ng mL^{-1}. The profiles in malaria patients are similar to those in healthy individuals, although the time to reach the maximum concentration in the plasma was three times higher in malaria patients.

Primaquine

Primaquine (PQ) is an 8-aminoquinoline (**6**, Fig. **5**). It has a chiral centre, but the commercially available preparations are all racemic [46]. It was introduced as an antimalarial in the United States of America by Elderfield and co-workers in 1946, after an intensive search for a drug to prevent relapse of malaria in American troops during the war in the Pacific [47]. Pamaquine was used then, but it was too toxic. PQ is active against all exoerythrocytic forms of the parasite [48]. Asexual stages of *P. falciparum* are not susceptible to primaquine, but those of *P. vivax* are [3]. PQ is gametocytocidal against *P. falciparum*, including multidrug-resistant *P. falciparum* and has significant blood stage activity against *P. vivax.* It is also active against gametocytes of other species that cause human malaria. In fact PQ is the only drug known to act on mature gametocytes, *i.e.,* those present in the blood stream when malaria symptoms are observable [3]. It is also highly effective on hypnozoites that can persist in the liver for long periods of time and cause relapses [3]. Although PQ is active against asexual blood stages, its activity is low, and it is normally used with another antimalarial, a schizonticide, in the case of acute attacks [48].

The mechanism of action of primaquine is still unclear [6]. It has been suggested that it may have an antagonistic effect on ubiquinone, with subsequent inhibition of electron transport in mitochondrial respiration [6]. Another possibility is that reactive metabolites create oxidative stress in the parasite's cells [49]. Primaquine is generally well tolerated, but in patients with glucose phosphate dehydrogenase (G6PD) deficiency it can cause haemolytic anaemia, which may be severe and life threatening [3]. Qualitative screening tests can be used to identify individuals who are G6PD deficient, prior to prescription of the drug [50]. There are however a few exceptions: those who have one of the milder G6PD deficient variants and recent haemolysis and some women who are heterozygous for the gene and also have mild deficiency. This side effect is known to be due to intra-erythrocytic oxidative stress mediated by redox-active metabolites [48]. Methemoglobinemia

may also occur. In addition, primaquine should not be given to pregnant women and to nursing women. It is recommended for children, but not to infants. Anti-relapse treatment with PQ should only be used when there is confirmation that vivax malaria occurs [3].

So far there is no unambiguous evidence of the development of resistance against primaquine [7], although variations in the sensitivity of hypnozoites to the drug may be observed from region to region. This lack of resistance development is difficult to understand, and besides the drug's physical and chemical properties, it is thought that this may be related to its low half-life or its ability to sterilise the parasite's gametocytes [51]. The anti-relapse effect of primaquine is a function of the total dose rather than the duration of the treatment [7]. It is recommended that a lack of optimum response should be compensated for through the use of different regimens [7].

Nowadays, six decades after its introduction as an antimalarial, PQ is still the only transmission-blocking antimalarial available. It is recommended by WHO as an antigametocyte medicine to be used as a single dose in combination with an ACT for the treatment of uncomplicated falciparum malaria [3]. It is also used for primary prophylaxis against all species of malaria and for anti-relapse therapy in persons exposed to *P. vivax* and *P. ovale* [48]. Primaquine is recommended by WHO as first line drug for the treatment of *P. vivax* and *P. ovale* malaria in combination with chloroquine with the indication that the usual dose should be lowered when there is mild mild-to-moderate G6PD deficiency, but it is contra-indicated in severe G6PD deficiency [3]. It produces radical cure.

Although primaquine has always been used as a racemate, a few studies have shown that there is a difference in the metabolic rate as well as the hepatotoxicity of the two enantiomers, which could have implications for treatment and if explored, it could lead to future improvements of this valuable antimalarial [52].

Pharmacology of Primaquine

Primaquine is absorbed rapidly and almost completely from the gastrointestinal tract. The maximum concentration in the body is reached within 1 to 4 h. It is

metabolised in the liver primarily to carboxyprimaquine, which has much lower activity [53], and other unidentified minor metabolites. The half-life is variable, ranging from 1 to 16 h, in patients and healthy volunteers [20]. It has a bioavailability of 96% [54]. The pharmacokinetics has been studied in healthy volunteers in Thailand. After an oral dose of 15 mg, a mean peak plasma concentration of primaquine of 65 ng mL^{-1} was achieved after 2 h, and the mean AUC was 468 h ng mL^{-1}. The mean elimination half-life was 4.4 h. Almost identical results were obtained in the mean plasma concentration-time profile of the drug after the volunteers had a chronic dose (15 mg daily for 14 days) [55]. Nevertheless, there were marked interindividual differences in the pharmacokinetics observed after the chronic therapy.

The recommended dose of primaquine is 15 mg for 14 days to prevent relapses. This dose may be increased to eliminate hypnozoites fully with some *P. vivax* strains. For radical cure a blood schizonticide is usually given at the same time [12].

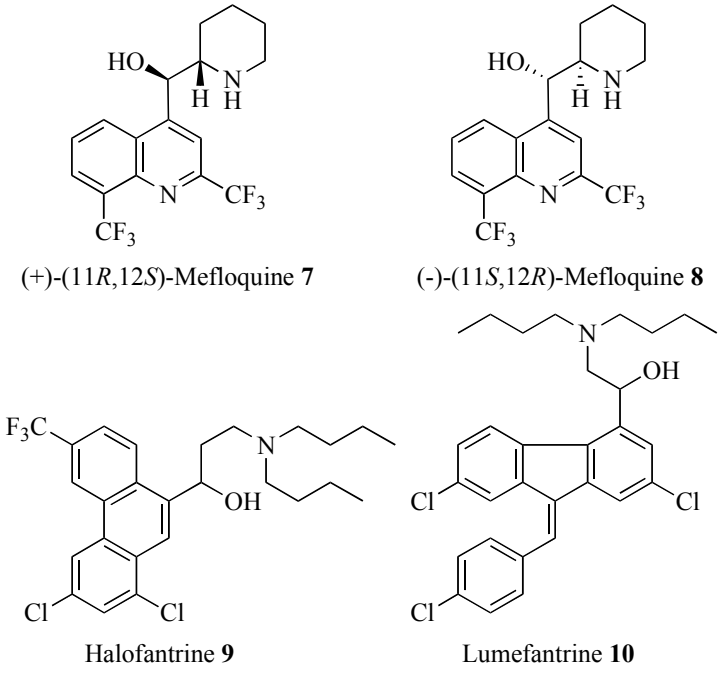
THE AMINO ALCOHOLS: MEFLOQUINE, HALOFANTRINE AND LUMEFANTRINE

These antimalarial compounds have in common an aryl amino alcohol unit, which is also found in quinine and quinidine, but for historical reasons they are described separately in this review (Fig. **6**). They exert their toxicity on the erythrocytic stages of the *Plasmodium*'s life cycle [10]. It is thought that their mechanisms of action involve the metabolic processes that take place inside the food vacuole, like the cinchona alkaloids and the 4-aminoquinolines [11].

Mefloquine

Mefloquine (MQ), the most widely used drug of this class of antimalarials, is a quinoline methanol derivative developed at the Water Reed Army Research Institute, United States of America (**7**, Fig. **6**). This totally synthetic drug was selected from about 300 analogues of quinine synthesized in the 1970s, and it started to be used for therapy in 1985 as Lariam [6]. Mefloquine is effective against all forms of malaria, and it is used for treatment and prevention [56]. In

2000 it was the most widely used drug for the treatment of multidrug resistant falciparum malaria [57]. It has high activity against most chloroquine-resistant *Plasmodium* strains, *e.g.,* IC_{50} = 8.4 nM (CQ-sensitive laboratory strain D6), IC_{50} = 3.4 nM (CQ-resistant strain W2) and IC_{50} = 6.2-10.7 nM (CQ-resistant isolates from Cameroon) [6]. Mefloquine has been widely used in Asia. Over the years considerable resistance has developed. Since it is highly effective against CQ-resistant vivax malaria, it was recommended as a monotherapy for treatment, for example in Cambodia - Greater Mekong sub-region, from 1993 to 2000 [7]. As resistance gradually build-up throughout the country, the first line treatment policy was changed to a three-day course of artesunate plus mefloquine. When it adopted this policy, Cambodia became the first country to have an ACT for first line treatment as national policy [7].



(+)-(11*R*,12*S*)-Mefloquine **7**

(-)-(11*S*,12*R*)-Mefloquine **8**

Halofantrine **9**

Lumefantrine **10**

Figure 6: The erythro optical isomers of mefloquine and other aminoalcohols currently in use to treat malaria.

Mefloquine is a blood schizonticide, effective against erythrocytic stages of the parasites. It has no effect on the exo-erythrocytic phase or on gametocytes in *P. falciparum*. Its mode of action is different from that of the 4-aminoquinolines,

although it also targets the food vacuole. It is known that it makes it swell. It is thought that mefloquine inhibits vesicular docking indirectly or that, due to its high affinity for phospholipids on the membrane, it causes an antagonistic effect on vesicular docking [58].

Mefloquine may cause minor side effects, such as nausea, vomiting, abdominal pain, anorexia, diarrhoea, headache, dizziness, loss of balance, dysphoria, somnolence and sleep disorders, notably insomnia and abnormal dreams [3]. Since prophylatic use of mefloquine has been associated with neuropsychiatric side effects such as insomnia, depression and panic attacks in 5-29% of all those using it, prophylatic use is forbidden for people who require unimpeded abilities such as air-crew members [6]. Mefloquine is safe to use on children and has been used in pregnancy, but it was associated with an increase in stillbirths in Thailand, and it became less favoured for this purpose since then [59, 60].

Mefloquine is used in malaria therapy as the erythro racemate, but there has been interest in studying if one of the enantiomers could be more efficient to treat malaria or to have fewer undesirable side effects. *In vitro* the (+) enantiomers have higher IC_{50} values than the (-)-enantiomers by a factor of 1.6-1.8 on some strains of *P. falciparum* (D6 and W2) [61]. Recent research shows that the pharmacokinetics of the two enantiomers is different [62, 63], and in young children the differences are higher [64]. The (-)-enantiomer is present in plasma in higher concentrations and it has a longer half-life. There is also evidence that the (+)-enantiomer is more effective to treat malaria [65]. In addition it has been shown that the (-)-enantiomer binds to adenosine receptors in the central nervous system, which may explain some of the psychotropic effects associated with this antimalarial [66]. It has been suggested that the better *in vitro* antimalarial activity of the (+)-enantiomer could be accounted for by a stereoselective specific transport in the food vacuole of the parasite through the Pgh-1 transporter [67]. Recent data however suggests that although there is a difference in the safety and tolerability profiles between the (+)-enantiomer and racemic mefloquine, the overall difference does not appear to suggest that a replacement is necessary [68]. Nowadays mefloquine is recommended as an ACT in combination with artesunate for malaria therapy (See under Artemisinin-Based Combination Therapies).

Pharmacology of Mefloquine

Mefloquine is well absorbed from the gastrointestinal tract. It is converted by hepatic microsomal enzymes into its carboxy metabolite, a process probably mediated by CYP3A4 [20]. This metabolite is inactive against *P. falciparum*. MQ has a long elimination half-life of 21 days, which decreases to 14 days in malaria patients [3]. In the treatment of multidrug-resistant falciparum malaria, it has been used in doses ranging from 15 to 25 mg of base/kg of body weight [12]. The time taken for a maximum plasma concentration to be reached varies from individual to individual. The pharmacokinetics of MQ was studied in Thai male patients with acute, uncomplicated falciparum malaria [69]. An initial dose of 750 mg MQ was used, followed by 500 mg 6 h later. A mean maximum concentration of 2,212 ng mL^{-1} was reached after 20.3 h. The mean AUC was 17.2 d μg mL^{-1}, and the half-life 12 days. The mean parasite clearance time was 82.3 h. Some patients were treated concurrently with artesunate and mefloquine, and in those, the parasite clearance time was shorter, 47.5 h.

The concentration of mefloquine that gives a 95% protective level in areas with chloroquine-resistant *Plasmodium falciparum* is 620 ng mL^{-1} [60]. A single oral dose of 1.0 g of mefloquine was found to clear chloroquine-resistant *P. falciparum* parasites in 103.1 ± 18.0 h in semi-immune patients [70]. As for chloroquine-resistant vivax malaria, a single dose of 15 mg base kg^{-1} body weight given over three days has been found to be highly effective for treatment, giving 100% cure [71].

Halofantrine

Halofantrine (HF) is another synthetic quinine analogue, an amino alcohol (**9**, Fig. **6**) belonging to the class of 9-phenantrenmethanols, developed at the Water Reed Army Research Institute, United States of America in 1972. It was introduced for therapy in 1988, and marketed as Halfan. It was thought that since this class of compounds had not yet been used for malaria treatment, the risk of cross-resistance was minimal. HF is a highly lipophilic drug, insoluble in water. It is administered as a racemate, and its (+) and (-) enantiomers do not show a difference in activity *in vitro* [72]. It is a blood schizonticidal effective against asexual forms of *P. falciparum* and *P. vivax*. It has no action on sporozoites,

gametocytes or hypnozoites in the liver [73]. Halofantrine is active against chloroquine-resistant *Plasmodium* strains, with a mean IC_{50} of 1.2 nM (Cameroon wild isolates); against chloroquine-sensitive isolates, mean IC_{50} values of 1.5 nM were observed [6]. Its mechanism of action is thought to be similar to that of mefloquine [6]. Halofantrine is effective against vivax malaria [12] and multidrug-resistant (including mefloquine) *P. falciparum* malaria [58], but nowadays it has been withdrawn from the market in a few countries [74], for safety, due to its cardiotoxicity. It can cause significant prolongation of the QT_c interval and hence cardiac arrhythmias [12], which could result in death. It is contraindicated in patients with a history of irregular heartbeat.

Pharmacology of Halofantrine

Halofantrine is poorly absorbed after oral administration, and its bioavailability is variable, <36 %, making it difficult to determine precisely its pharmacokinetics. Bioavailability increases with high-fat meals, but it is contra-indicated with food, because of the increased risk of cardiotoxicity. The maximum plasma concentration is usually reached within 6 h [73]. The elimination half-life is 1-2 days, and that of its main metabolite, N-desbutylhalofantrine, which is also an effective antimalarial, is 3-5 days. A typical dose that has been used for the treatment of malaria is 3 × 500 mg at 6 h intervals [73]. Healthy volunteers who were given this regime of halofantrine hydrochloride, and fed 2 hours before the second and third doses, showed three to five-fold increases in absorption. A mean maximum plasma concentration of 3200 ng mL^{-1} was attained 9 to 17 hours after this multiple dose [6].

Data pooled for 1474 patients who were given the same oral multiple dose of halofantrine, showed that in the case of acute uncomplicated *P. falciparum* infections, the mean parasite clearance time was 57.9 h, and 57.3 h in the case of *P. vivax* infections [73]. A 24 mg base kg^{-1} b.w. dose divided into three portions taken over a period of 12 hours has been shown to be efficient to treat vivax malaria [12].

Lumefantrine

Lumefantrine (LU) also known as benflumetol (**10**, Fig. **6**), is another aryl amino alcohol, a fluorine derivative, used in medicine as a racemate. It was developed in

the 1970s in the Academy of Military Medical Sciences in Beijing, China. Lumefantrine is a blood schizonticide, effective against erythrocytic stages of *P. falciparum* [58]. It has a lower antimalarial activity than halofantrine. IC_{50} = 34-44 nM (laboratory strains) and 11.9 nM (Cameroon parasites) [6]. It is very effective against chloroquine resistant *P. falciparum* strains. The possibility of the enantiomers of lumefantrine differing in their activity against *Plasmodium* parasites has been investigated. However, (+)-lumefantrine, (-)-lumefantrine and racemic lumefantrine were found to be highly active, potent inhibitors of schizont maturation in fresh, natural isolates of *P. falciparum* but with similar potencies *i.e.,* the mean EC-50 values are 8.87, 9.71 and 12.44 nmol L^{-1} blood-medium-mixture, respectively [75]. Although lumefantrine is structurally similar to halofantrine, it does not have the dangerous cardiac side effects, and also no other significant toxicity [6]. It is very well tolerated, and only minor side effects such as nausea, abdominal discomfort, headache or dizziness have been reported, which are also characteristic symptoms of malaria [6]. Lumefantrine is not available as a monotherapy, and it has never been used alone for the treatment of malaria [6]. Presently it is available as combination therapy with artemether, marketed as Riamet or Coartem. It was registered first in China as an ACT in 1992, and it is nowadays one of the ACTs recommended by WHO for malaria therapy. At present it is used in 56 countries as first- or second-line treatment [3]. Other characteristics of this ACT are discussed under Artemisinin-Based Combination Therapies. Lumefantrine is thought to function by a mechanism similar to that of quinine, mefloquine and halofantrine, targeting the parasite's food vacuole.

Pharmacology of Lumefantrine

Data on the pharmacokinetics of lumefantrine is not readily accessible, since most of the preclinical research was done in China, without being published [20]. Recent reports concentrate on the properties of combination therapies. It is also known that lumefantrine is extensively metabolized in the liver primarily by cytochrome P450 3A4 [58]. The major metabolite is desbutyl-lumefantrine (DBL). So far there has not been much supportive pharmacokinetic data to show the effect of this metabolite in treatment [76]. Recent data shows that DBL is a more potent antimalarial than the parent compound, with an IC_{50} value of 9.0 nM

versus 55.5 nM for LU against chloroquine-sensitive and resistant laboratory strains of *P. falciparum*. Also a mild synergy with dihydroartemisinin was observed, an indication that this metabolite could be useful for ACT combination therapies [76].

The Sulfonamides and Sulfones: Sulfadoxine, Sulfalene and Dapsone

The sulphur-based drugs belong to a large family of synthetic compounds of which many have antimicrobial properties. When some antibiotics were screened for their ability to treat other infectious diseases, including malaria, the sulfonamides sulfadoxine **11** and sulfalene **12** and the sulfone dapsone **13** were discovered (Fig. **7**). They all have a common mechanism of action: they target the parasite's folic acid biosynthetic pathway [77]. Folic acid is essential for the survival and growth of *Plasmodium* parasites (Fig. **8**) [78]. It consists of a pteridine ring, para-aminobenzoic acid and one molecule of L-glutamate [79]. One of its derivatives, tetrahydrofolic acid, plays a key role in the biosynthesis of the nucleotides thymine and purine, needed for the synthesis of DNA, RNA, and several amino acids (Met, Gly, Ser, Glu, and His). Humans biosynthesize tetrahydrofolic acid from pre-formed dihydrofolic acid which they obtain from their diet, but malaria parasites, like many bacteria, are unable to utilize dietary dihydrofolic acid, and synthesize the folic acids they need using p-aminobenzoic acid as a substrate. Nevertheless, some recent evidence suggests they may be able to use pre-formed folate [79]. Although the importance of this pathway seems to be minimal, with the *de novo* synthesis being the more efficient one, it appears that the possibility of this exogenous acquisition of folate from the host is related to the ability of the microorganism to become drug resistant [80]. The sulphur-based drugs are analogues of p-aminobenzoic acid, which is used by the enzyme dihydropteroate synthase (DHPS) to synthesize dihydrofolate, an early step in folic acid biosynthesis, and therefore they can compete with it and block the action of DHPS (Fig. **8**). By blocking folic acid biosynthesis, antifolates inhibit indirectly parasite DNA synthesis and cell growth. Since the malaria parasite has a high rate of replication, it is particularly sensitive to antifolate drugs. DHPS inhibitors are named class I antifolates. DHPS is completely absent in humans.

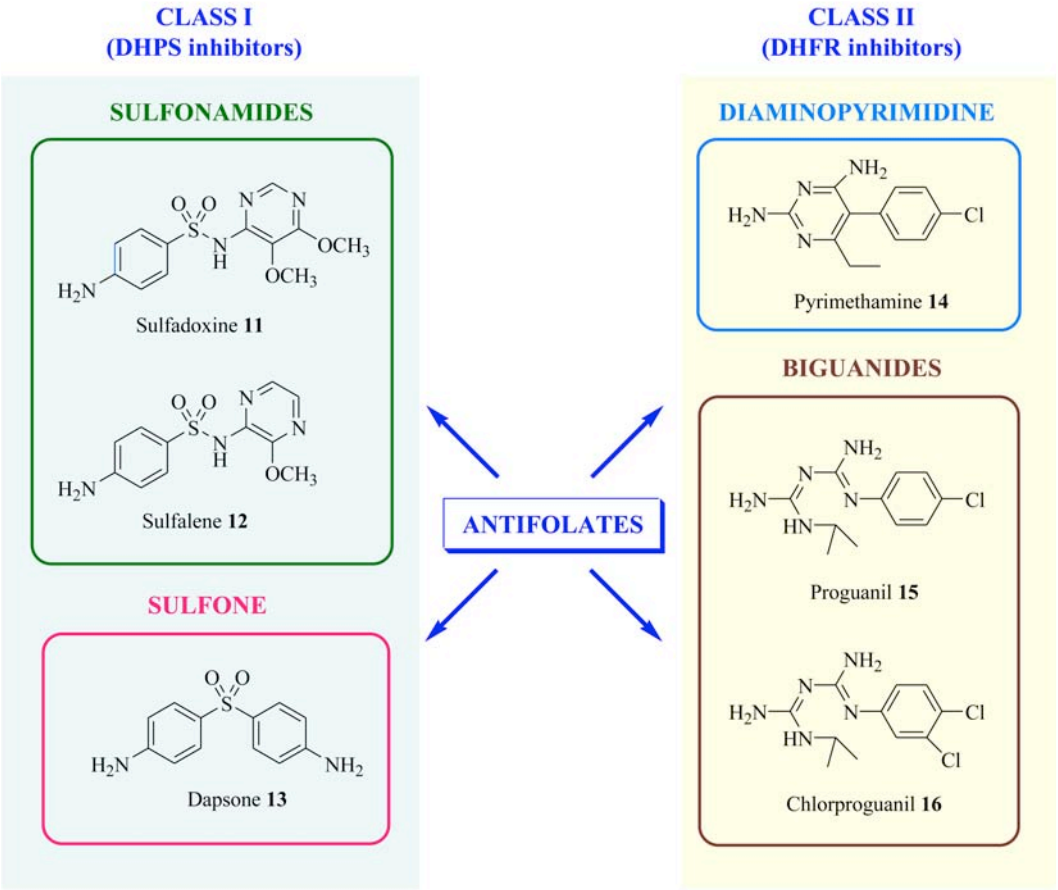


Figure 7: Antimalarial drugs that target the folic acid metabolic pathway.

These drugs have long half-lives, of between 60 and 200 h [81] and as a result they do not need to be administered several times to be effective. However, they are not very efficient in treating malaria, and for this reason they are usually used in fixed-combinations with other drugs, usually pyrimethamine or chloroguanil, which also have as target the folic acid metabolism, but inhibit a different enzyme, dihydrofolate reductase (DHFR). DHFR controls the reduction of the dihydrofolate to tetrahydrofolate, a step occurring later in the same biosynthetic route to folic acid (Fig. **8**). DHFR inhibitors are named class II antifolates. Parasitic DHFR is sufficiently different from the human enzyme to allow the development of antimalarials targeting this biosynthetic step. The sulphur drugs and the DHFR inhibitors have a synergistic effect, that is, the combined effect of

their action is greater than the sum of the effect of each compound working individually. As a result, they can be used in lower amounts, which helps to reduce the side effects of some of the sulphur drugs and the fact that they are used in combinations brings the additional benefit of reducing the probability of resistance developing.

Sulfadoxine

This is a long-acting sulfonamide (**11**, Fig. **7**), usually used in combination with other drugs to treat infectious diseases [58]. Sulfadoxine (SDX) was first used to treat malaria in combination with the antifolates pyrimethamine (PYM) **14** and proguanil (PG) **15** in the late 1950s, to increase their efficacy, and to forestall or prevent the development of resistance (Fig. **7**). The combination pyrimethamine-sulfadoxine (SP) became, like chloroquine, one of the antimalarial drugs most widely used ever [82]. Sulfadoxine and pyrimethamine are used in a fixed-dose combination of 20 parts to 1, administered orally or intramuscularly, with the trade name Fansidar. Although Fansidar is used as a co-formulation of two substances, the resulting product is considered a monotherapy because the two drugs act on the same biosynthetic pathway of the parasite, the folic acid biosynthetic pathway [3]. Their effect is synergistic.

Sulfadoxine is a class I antifolate. It is an analogue of *p*-aminobenzoic acid and a competitive inhibitor of the enzyme dihydropteroate synthetase (DHPS). Pyrimethamine inhibits dihydrofolate reductase (DHFR). SP is highly effective against asexual blood forms of *P. falciparum* and produces clinical cure [3]. It is also a suppressive agent. The combination is inactive against sporozoites and the latent exoerythrocytic stage, and it does not produce a radical cure or suppressive cure of *P. vivax*. It actually enhances gametocytogenesis, but the resulting gametocytes are unable to infect mosquitoes, so it reduces the transmission of malaria. This combination has been widely used to treat chloroquine-resistant malaria. It is also effective against wild type *P. vivax* [1]. Because of its long half-life, 4-9 days for sulfadoxine, and 4 days for pyrimethamine [3], and its low cost and safety in pregnant women and children, sulfadoxine-pyrimethamine is also used for intermittent preventive treatment of malaria in infants, children and pregnant women living in areas of high transmission. Despite widespread

development of resistance, it remains effective nowadays in areas of moderate resistance [1]. Its *in vitro* activity against *P. falciparum* has varied in time: IC_{50} (SPX, Mozambique isolates) = 0.91 μM [83]; IC_{50} (SDX, Bangladesh strains) = 40.5 μM; IC_{50} (PYM, Bangladesh strains) = 1.7 μM [84]; IC_{50} (PYM, PYM-susceptible African isolates) = 15.4 nM [85].

Malaria treatment with sulfadoxine-pyrimethamine is regarded as sufficiently safe. However, in administration for prolonged periods as in prophylaxis, the toxicity of the sulfonamide combination partner may become significant, resulting in an increased risk of agranulocytosis and toxic epidermal necrolysis (the Steven-Johnson syndrome). For these reasons prophylatic use of this combination was discontinued in some countries a few years ago [6]. Nowadays there is also the problem of resistance, and thus the more recent tendencies are to use SP in combination with artemisinins. Artesunate plus sulfadoxine-pyrimethamine is one of the currently recommended ACTs for malaria treatment as discussed later (see under Artemisinin-Based Combination Therapies) [1]. Nevertheless, the treatment failure rate of SP remained low by 2007; around 162 efficacy studies conducted in 45 countries in the period 2000-2007 showed a low failure rate of 4.7% and 5% only in South America and in the Middle East and central Asia, respectively. In Africa the situation was worse, varying from 18.7% to 52.8% from the Western to the Eastern regions [7].

Pharmacology of Sulfadoxine

Currently, sulfadoxine-pyrimethamine is the only available antimalarial effective when given as a single dose [86]. The bioavailability of sulfadoxine is 70-100%, and that of pyrimethamine 100% [20]. After oral administration, about 5% of sulfadoxine appears in the plasma as acetylated metabolite, and about 2 to 3% as the glucuronide. Pyrimethamine is transformed to several unidentified metabolites. The pharmacokinetics of Fansidar has been reviewed recently [87]. In a study with adult patients with acute falciparum malaria, who received a single dose of Fansidar (1.36 mg kg^{-1} PYM, 27.3 mg kg^{-1} SDX), a maximum concentration of sulfadoxine in the plasma of 63.2 μg mL^{-1} was reached after 24 h and the AUC was 816 d μg mL^{-1}. The half-life was 7.8 d. A maximum concentration of pyrimethamine of 278.5 ng mL^{-1} was present after 9.3 h, and the

AUC was 1,602 d ng mL^{-1}. The half-life was 3.27 d. In addition, the authors of this work recommended a revision of the doses used presently for the treatment of malaria in children, suggesting an update to the same doses as those of adults based on data obtained in this study. In a different study with children infected with *Plasmodium falciparum* and treated with SP (SDX = 25 mg kg^{-1}, PYM = 1.25 mg kg^{-1} bw), parasite clearance was achieved in 1.85 days [83].

Sulfalene

Sulfalene **12**, also known as sulfametopyrazine or sulfamethoxypyrazine, is another important synthetic sulfonamide available for antimalaria therapy. It is also an antibacterial, which is used currently for the treatment of chronic bronchitis and urinary tract infections. Sulfalene is normally used in a fixed combination with pyrimethamine, in a 20 to 1 ratio, with the trade name Metakelfin (SLP). Its efficacy is similar to that of Fansidar, but Fansidar has been much more widely used [77]. Its metabolites are not active against malaria. Like sulfadoxine, it is a class I antifolate, a competitive DHPS inhibitor. It is also used in combination with trimethoprim. It is a blood schizonticide, active against all species of *Plasmodium* but more effective against *P. falciparum.* If administered in correct doses it is well tolerated, but like all sulfonamides, it may produce side effects in certain individuals [12]. The combination of artesunate plus sulfalene-pyrimethamine is an ACT recommended by WHO as an alternative to artesunate plus sulfadoxine-pyrimethamine for the treatment of uncomplicated and severe falciparum malaria [3] (see under Artemisinin-Based Combination Therapies).

Pharmacology of Sulfalene

Sulfalene is readily absorbed, and it oral bioavailability is 70-100% [20]. Peak plasma concentrations are reached after 4-6 h [12]. The drug has a high liposolubility, and a long terminal half-life of 65 h [12]. In a trial in rural Nigeria, it was found that a single dose of SLP cleared *Plasmodium falciparum* infections in 7 days. After 3 days 95% of the patients already showed no asexual forms of *P. falciparum* in the peripheral blood [88]. Lower doses used for children (patients < 10 yrs old) had a similar effect. In a more recent trial in Northeast India, it was found to be effective treating patients with chloroquine-resistant *P. falciparum*

malaria, but resistance to this drug has already been reported in parts of India too [89].

Dapsone

Dapsone (DDS) **13** is the sulfone most widely used for the treatment of malaria (Fig. **7**). It is also active against a wide range of bacteria, but it is mainly well known as a drug for the treatment of leprosy, and as second-line treatment and prophylaxis against *Pneumocyctis carinii* pneumonia in HIV patients. It was synthesized for the first time by Fromm and Wittmann in 1908, as a result of a search for azo-dyes [77]. Its antimalarial activity was discovered in the 1930s, but it was thought to be too toxic for use due to the large amounts tried at the time. By 1949 it was discovered that it treated malaria at much smaller doses (100-200 mgs), but it proved to be unreliable in the treatment of acute falciparum malaria, and of limited efficacy. It was later introduced in synergic combinations with other antifolates for malaria prevention and chemotherapy. Dapsone is a class I antifolate, a competitive inhibitor of DHPS like sulfoxidine and sulfalene. In formulations, the most common drug partners are pyrimethamine or chlorproguanil. It is used in an 8:1 combination with pyrimethamine for prophylaxis [3], marketed as Maloprim, and in a 8:10 combination (CD) with chlorproguanil (CPG) **16**, a biguanide, for the treatment of chloroquine-resistant falciparum malaria. The latter combination, marketed as LapDap, only became available for clinical use in 2003, so it is still in post-marketing surveillance [90], but by 2006 it was already available in many African countries [77]. It is a rational alternative to sulfadoxine-pyrimethamine, particularly in areas where resistance to this antimalarial prevails, such as East Africa [20]. LapDap is however inactive against strains harbouring a quadruple mutant, abundant in Asia and South America [6]. IC_{50} values measured against a K39 strain, a PY-resistant strain of *P. falciparum* were found to be: chlorcycloguanil alone = 4.8 nM; dapsone alone = 51 μM [91].

Dapsone is highly active against the asexual blood forms of *P. falciparum.* It produces clinical cure, but it is used in combination with the other antifolates because it acts slowly. It is inactive against sporozoites and the primary exoerythrocytic stage of the parasite. It has the same effect as the sulfonamides in

increasing gametocyte production, but the resulting gametes do not infect the mosquitoes [12]. It is inactive on the latent exoerythrocytic stages and hence it does not produce a radical or suppressive cure of vivax malaria. Dapsone has two important metabolites: monoacetyldapsone, which has no activity [92], and dapsone hydroxylamine. Monoacetyldapsone can be deacetylated to give dapsone too. Dapsone hydroxylamine is probably responsible for the two most commonly reported side effects of dapsone: methemoglobinemia and haemolysis, which may occur when high doses of more than 200 mg are taken. Haemolysis can be particularly serious in patients deficient in glycose-6-phosphate (G6PD), if more than 50 mg are taken daily [3]. Another side effect that has been reported following prophylaxis with dapsone-pyrimethamine, if taken twice a week, is the development of agranulocytosis [11]. Medications with dapsone are also not recommended for lactating women, because relatively large amounts of dapsone are excreted in breast milk (14% of the adult dose) [3].

By 2004 SP was still the first choice drug for the treatment of uncomplicated falciparum malaria in most of Africa [93], although cases of resistance had been reported. A large study involving 1850 children from 5 African countries was carried out with the aim of showing the possibility of using CD as an alternative treatment in case of SP failure and to compare the efficacy of SP and CD. The results showed that the efficacy of CD was slightly better (95% to 89%, CD to SP). Haematological adverse effects were more common with CD than with SP but they were reversible [93].

Pharmacology of Dapsone

Dapsone is slowly and completely absorbed from the gastrointestinal tract. Its bioavailability following oral administration is > 90% [20]. Dapsone is eliminated quickly in comparison with sulfadoxine, with a mean half-life of about 26 h, although there is large interindividual variability. The pharmacokinetics of Maloprim (100 mg DDS + 12.5 mg PYR) was studied in healthy individuals, after weekly administration of Maloprim [94]. The studies showed that after the last dose a mean maximum plasma concentration of dapsone of 1,134 ng mL^{-1} was reached after 1.7 h, and the mean AUC was 35.0 h μ mL^{-1}. The mean elimination

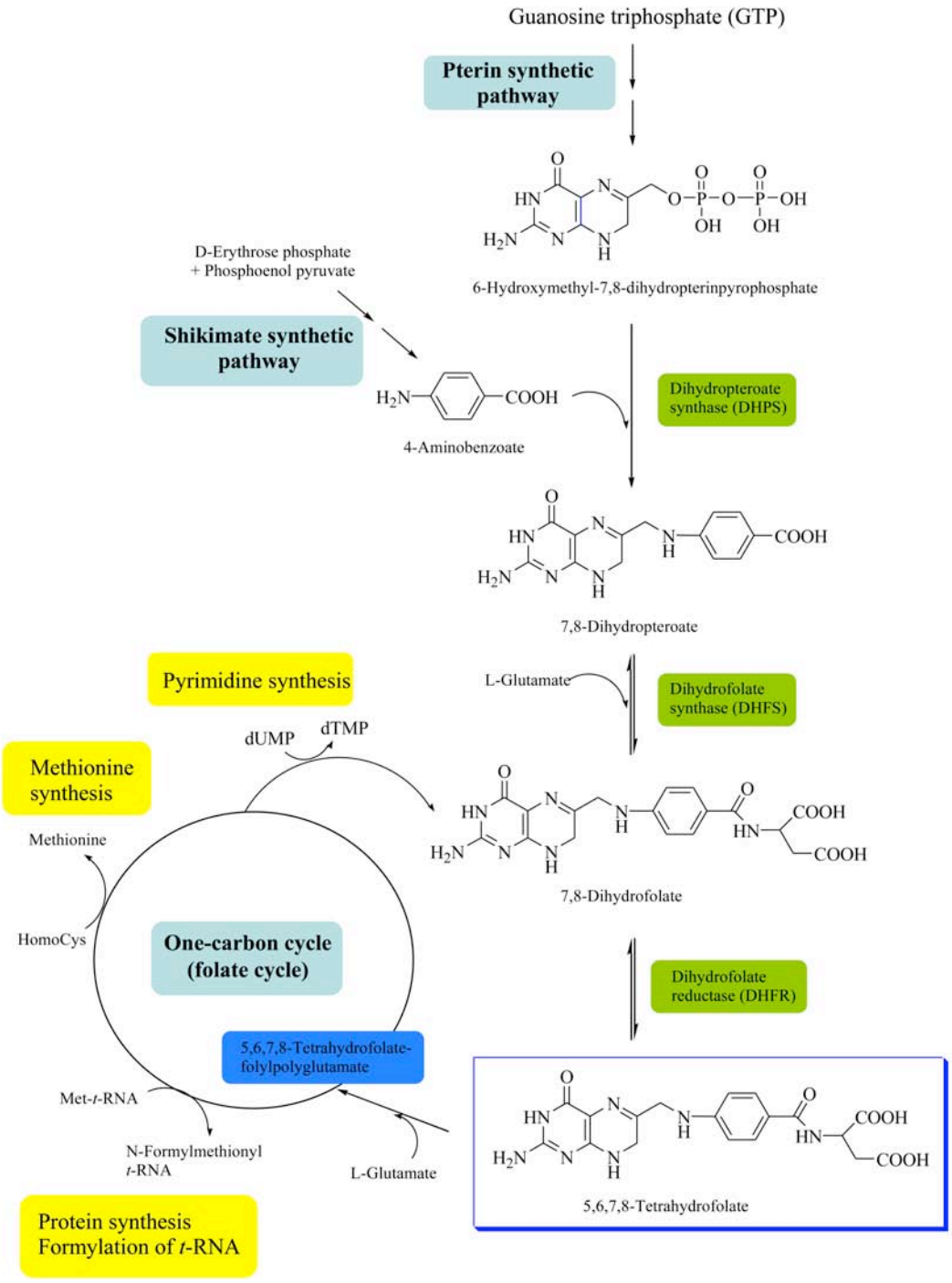


Figure 8: Folate synthesis in *Plasmodium* [78].

half-life of DDS was 22.6 h and that of PYR 104.9 h. The half-life of the major metabolite of dapsone, monoacetyldapsone, was similar, 22 h. In the case of pyrimethamine, a peak plasma concentration of 116 ng mL^{-1} was reached after 41.5 h, and the mean AUC was 104.9 h μg mL^{-1} [94].

The pharmacokinetics of the combination dapsone-proguanil (DDS+PROG) was studied after administration of a 1:20 daily dose to healthy volunteers [95]. In the case of DDS, after the last dose, the maximum concentration in the plasma was 285 ng mL^{-1}, and the elimination half-life 23.3 h. For PROG C_{max} was 151 ng mL^{-1} and $t_{½}$ was 18.3 h. The peak plasma concentration of the active metabolite of proguanil, cycloguanil was 56 ng mL^{-1} and $t_{½}$ 15.0 h. The pharmacokinetics of CD in healthy adults is similar to those in malaria patients. However, peak plasma concentrations in children are reached in half of the time needed by adults [96]. In a study with patients infected with pyrimethamine-sulfadoxine resistant falciparum malaria [97], in Tanzania, it was found that a 3-day dose regimen of chlorproguanil plus dapsone (2:2.5 mg kg^{-1} body weight) administered once a day, was enough to provide parasite clearance in 93% of all patients, suggesting that this drug combination could be a useful alternative for the treatment of malaria in areas where resistance to pyrimethamine-sulfadoxine prevails.

DIAMINOPYRIMIDINES: PYRIMETHAMINE

Nowadays pyrimethamine **14** is the only pyrimidine included in the WHO's list of currently available drugs for the treatment of malaria [7]. It was selected during the 1950's after a study made on a large number of pyrimidines and analogues to investigate the relationship between chemical structure and their participation as precursors and modifiers of nucleic acid biosynthesis [98]. Trimethoprim, another pyrimidine, has also been used in the past for malaria therapy, particularly in combination with sulfamethoxazole. It is less active than pyrimethamine or cycloguanil against *Pf*DHFR. Nowadays its use is more confined to the treatment of bacterial infections.

Pyrimethamine

Pyrimethamine (PYR) is a 2,4-diaminopyrimidine. It was synthesized in 1951 by an American and British team during searches for analogues of folic acid for the

treatment of tumours [99]. It was found to be highly active against human malaria and it was used as a monotherapy with the trade name Daraprim in the 1950s. PYR is a class II antifolate, a highly active inhibitor of *P. falciparum* dihydrofolate reductase (DHFR) with a K_i = 2.6 nM [6], hence it blocks indirectly the synthesis of nucleic acids in the parasite (Fig. **8**). It has a higher binding affinity for DHFR than for the human enzyme and hence a good therapeutic index. It inhibits the plasmodial enzyme at a concentration 3500 times lower than the human enzyme [98]. In addition to inhibiting DHFR, PYR also appears to inhibit nuclear division in the parasite. It is also active against the other 3 human malaria parasites [3]. Comparing doses, pyrimethamine is one of the most powerful suppressive agents known against *P. falciparum* and sometimes against *P. vivax* [12]. It is active against asexual blood stages of the parasite and it produces clinical and radical cure in most cases of malaria [12]. It is not active against sporozoites, although it may have some effect on exoerythrocytic stages. In addition, although it appears to have no effect on gametocytes, it inhibits subsequent sporozoite development in the mosquito vector. PYR is inactive on latent erythrocytic stages, therefore it cannot be used for radical treatment of vivax malaria. The IC_{50} value of PYR against a wild-type *P. falciparum* strain (TM4) was found recently to be 58 nM [100].

Initially PYR was used as a monotherapy on its own, and one weekly dose could provide a completely suppressive cure of malaria. It was not recommended on its own for the treatment of acute attacks of malaria, because of its slow action. It was extensively used as a monotherapy on its own in some Asian and South American countries. Resistance appeared soon after and it is no longer used alone, but in synergistic combinations with class I antifolates, more commonly with the sulfonamides sulfoxidine or sulfalene or with the sulfone dapsone, as highlighted in their respective sections. On its own pyrimethamine is also used for the treatment of toxoplasmosis and isosporiasis and as prophylaxis against *Pneumocystis carinii* pneumonia [3]. PYR is generally a well-tolerated drug. After prolonged treatment depression of the haematopoiesis may occur due to interference with folic acid metabolism. It may also cause occasional skin rashes and hypersensitivity [3]. However, it is safe to be taken during pregnancy. In

combination with sulfoxidine it became one of the most successful antimalarials ever used [82] (see under Sulfadoxine too).

Pharmacology of Pyrimethamine

PYR is almost completely absorbed from the gastrointestinal tract and peak plasma concentrations are reached within 2-6 h after oral doses. Its bioavailability is 100% [101]. It has a half-life of 4 days. The effective concentration to inhibit blood schizogony of *P. falciparum* is 10-100 μg mL^{-1}, which can be achieved with a single 25 mg dose [12]. PYR is nowadays available in oral and parental formulations with the sulfonamides for treatment of malaria or with dapsone for prophylaxis. Its pharmacokinetics upon administration in combination with sulfadoxine is described under Pharmacology of Sulfadoxine. In addition, sulfadoxine-pyrimethamine combined with artesunate is nowadays one of the ACTs recommended by WHO for malaria treatment (see under Artemisinin-Based Combination Therapies).

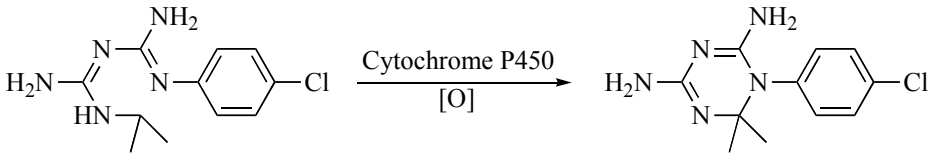
THE BIGUANIDES: PROGUANIL AND CHLORPROGUANIL

These drugs inhibit the action of dihydrofolate reductase, in the folic acid biosynthetic pathway. They are therefore class II antifolates. However, the original biguanides have almost no activity themselves, but they act as prodrugs. They are metabolized in the body to their triazine forms, cycloguanil (**17**) and chlorcycloguanil (**18**) respectively, the true DHFR inhibitors, *via* the polymorphic cytochrome P450 enzyme CYP2C19 (Fig. **9**). They have been used both for prophylaxis and treatment, either on their own or in combination with other antimalarials, often antifolates. Proguanil has been used with chloroquine, dapsone and nowadays with atovaquone, and chlorproguanil with dapsone [77].

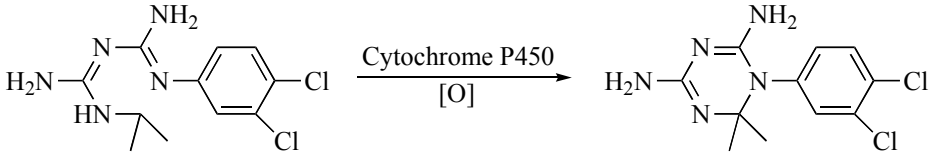
Proguanil

Proguanil (PG) was the first biguanide described as an antimalarial and also the first antimalarial known to act on the folic acid biosynthetic pathway. It was discovered in 1945 at Imperial Chemical Industries (ICI), United Kingdom, as part of an intensive research program to search for synthetic antimalarials [77]. After oral administration proguanil is rapidly metabolized in the liver, *via*

oxidative ring closure to form the triazine cycloguanil (CPG). Cycloguanil is a class II antifolate, a highly active inhibitor of DHFR, with K_i = 1.5 nM [6]. The parent compound has weak intrinsic antimalarial activity [3]. CPG also inhibits human dihydrofolate reductase, but its affinity for the parasitic enzyme is much higher, and hence it can be successfully used for therapy. Some populational groups are "poor metabolizers" of this drug, which may account for its inefficiency at times. The incidence in Caucasian and African populations is only 3%, but among Orientals it is as high as 20% [3].



Proguanil **15** Cycloguanil **17**



Chlorproguanil **16** Chlorcycloproguanil **18**

Figure 9: Proguanil and chlorproguanil have almost no antimalarial activity on their own. They act as prodrugs for cycloguanil and chlorcycloproguanil, respectively, metabolites formed by metabolic oxidative ring closure by the action of cytochrome P450. The metabolites are highly active antimalarial compounds.

Proguanil is highly active against the primary active exoerythrocytic forms of *P. falciparum* and *P. vivax* and also against the asexual blood stages of all species of human malaria parasites. PG is probably inactive against sporozoites and it has no effect on gametocytes, but when taken in appropriate doses it inhibits the development of sporogenic forms in the mosquito vector [12]. Sporogony in *P. vivax* is affected in a similar manner. PG is probably inactive on latent exoerythrocytic stages of the parasites, and hence it is not effective for radical cure of vivax malaria. The mean active plasma levels of proguanil in man are 10-20 μg L^{-1} for *P. vivax* and > 100 μg L^{-1} for a Costa strain of *P. falciparum* [3]. Against a drug-sensitive African strain, cycloguanil was found to be fully active

in vitro at 5 μg L^{-1}, and at 250 μg L^{-1} against a drug-resistant strain from S. E. Asia [3].

Proguanil was introduced on the market in the 1950s as Palundrine®. It is highly active as a causal prophylatic in falciparum malaria, and it is also a good general suppressive against *P. falciparum* and *P. vivax* malaria. PG has a marked inhibitory effect on transmission. However, it acts too slowly to treat acute attacks of malaria in nonimmune persons. It has been widely used for many years as a prophylatic, alone or with chloroquine, marketed as Savarin® [20, 77], as a result of its outstanding properties: low toxicity, wide range of action and low cost [12]. As a result of the development of resistance, its popularity for use alone has decreased.

Proguanil has few adverse effects: mild gastric intolerance, diarrhoea, occasional aphthous ulceration and hair loss. In patients with renal impairment doses should be reduced because haematological changes occur [3]. The biotransformation to cycloguanil is also reduced during pregnancy and in women taking oral contraceptives [3]. In recent years a new fixed-ratio combination has been developed, atovaquone-proguanil 2.5:1, marketed as Malorone® [102]. It is useful for the treatment and prophylaxis of multidrug resistant falciparum malaria, but because of its high cost, it is not widely used in malaria endemic countries, and it remains more for prophylaxis of those travelling to these areas [7]. The high cost of this medication is related to the complexity of the synthetic route to obtain atovaquone [60]. This combination is also regarded as a monotherapy, because the two drugs act in synergy. Atovaquone (ATO) inhibits the parasite mitochondrial electron transport. The atovaquone-proguanil combination is currently the most potent prophylatic agent known [103]. This combination is recommended by WHO as a first-line option drug for the treatment of uncomplicated falciparum malaria in travellers returning to non-endemic countries, including those in special risk groups [3].

Pharmacology of Proguanil

Proguanil is absorbed from the gastrointestinal tract rapidly after oral administration. Peak plasma concentrations occur after approximately 4 h, and

those of its active metabolite, cycloguanil, about 1 h after the peak plasma concentration of the parent drug [3]. They are eliminated slowly, and the half-life of both is approximately 20 h. The bioavailability of both is approximately 100% [20]. Proguanil must be taken daily for causal prophylaxis or suppression, and very low doses are effective. For treatment doses > 1000 mg daily are needed, but the drug is no longer used for treatment alone [12]. Cycloguanil has also been administered in the past by intramuscular injection, in the form of an embolate [3]. The use of PG in combination with dapsone is described under Pharmacology of Dapsone.

The pharmacokinetics of atovaquone-proguanil has been studied in healthy adult volunteers [104]. After a daily dose of the combination (400 mg PG, 1000 mg ATO) for 3 days, a peak plasma concentration of 509 ng mL^{-1} was found for PG after 3 h, and $AUC_{0-\infty}$ was 5,998 h ng mL^{-1}. The half-life of PG was 14.5 h. For CPG, C_{max} was 79.2 ng mL^{-1} after 6 h, and $AUC_{0-\infty}$ was 1,203 h ng mL^{-1}. The half-life was 11.8 h. In volunteers who took proguanil alone, a daily dose of 400 mg for 3 days, a peak plasma concentration of 547 ng mL^{-1} was reached after 3 h, and $AUC_{0-\infty}$ was 6,437 h ng mL^{-1}. The half-life was 13.7 h. The pharmacokinetic parameters of the metabolite were found to be C_{max} = 82.1 ng mL^{-1} after 6 h, $AUC_{0-\infty}$ = 1,355 h ng mL^{-1}, and the half-life 11.1 h. This study showed that the pharmacokinetics of proguanil is similar when it is taken alone or in combination with atovaquone. In studies with more than 500 patients showing symptoms of acute uncomplicated falciparum malaria, it was found that when the recommended dose of 4 tablets of Malarone was taken daily for 3 days, parasite clearance was achieved in 44-72 h and more than 98% of the patients were cured [105].

Chlorproguanil

The discovery of the antimalarial properties of proguanil led to the search of analogues that could have even better properties. Chlorination of the aromatic ring gave chlorproguanil (CPG), a compound with even higher antiplasmodial activity. Like proguanil, it also undergoes metabolic oxidative ring closure, catalyzed by cytochrome P450 to the triazine chlorcycloguanil, and it is the metabolite that has the high antimalarial activity. It is also a class II antifolate, acting as a DHFR inhibitor

[20]. Chlorproguanil is highly active against primary exoerythrocytic forms of *P. falciparum* and it is active against the asexual blood stages of all species of human malaria parasites [12, 20]. CPG has no apparent effect on gametocytes, but in appropriate doses it inhibits the sporogenic forms in the mosquito. The sporogony of *P. vivax* is affected in a similar manner. It has no effect on latent exoerythrocytic forms, therefore it is not effective for radical cure of vivax malaria [12].

Chloproguanil was initially marketed as Lapudrine®, and it was used for prophylaxis. Since it is more potent than proguanil, and it is retained in the body longer, it was recommended at lower doses. However it was not used as much as proguanil. The side effects that have been seen in the past are the same as those observed with proguanil [106]. In recent years it attracted attention for use as a combination partner. It was introduced in 2003 in combination with the sulfone dapsone and marketed as LapDap® [106]. By 2006 chlorproguanil was only available in combination with dapsone [106]. This antifolate combination was also regarded as a monotherapy, it was cheap and because chlorproguanil has a shorter half-life than pyrimethamine, it was thought that it had less selection pressure for resistance [20]. However it was used only for a few years, and in 2008 it was withdrawn [107] due to toxicity associated with dapsone [108].

Pharmacology of Chlorproguanil

Chlorproguanil has similar properties to proguanil. After oral administration it is metabolized in the liver to chlorocycloguanil and the maximum plasma concentration is reached within 4 h. It has a half-life ranging from 17 to 30 h. The pharmacokinetics of CPG was determined after a single oral dose of Lapudrine (320 mg) [109]. The mean maximum plasma concentration was found to be 36.7 ng mL^{-1} after 3.8 h. The elimination half-life was 17.5 h.

Naphthoquinones: Atovaquone

The antimicrobial properties of naphthoquinones have been known for more than 50 years [110]. The Fieser's research group had already prepared more than 300 naphthoquinone derivatives as potential antimalarials [6]. Atovaquone **19** was however the only naphthoquinone that eventually became approved for human

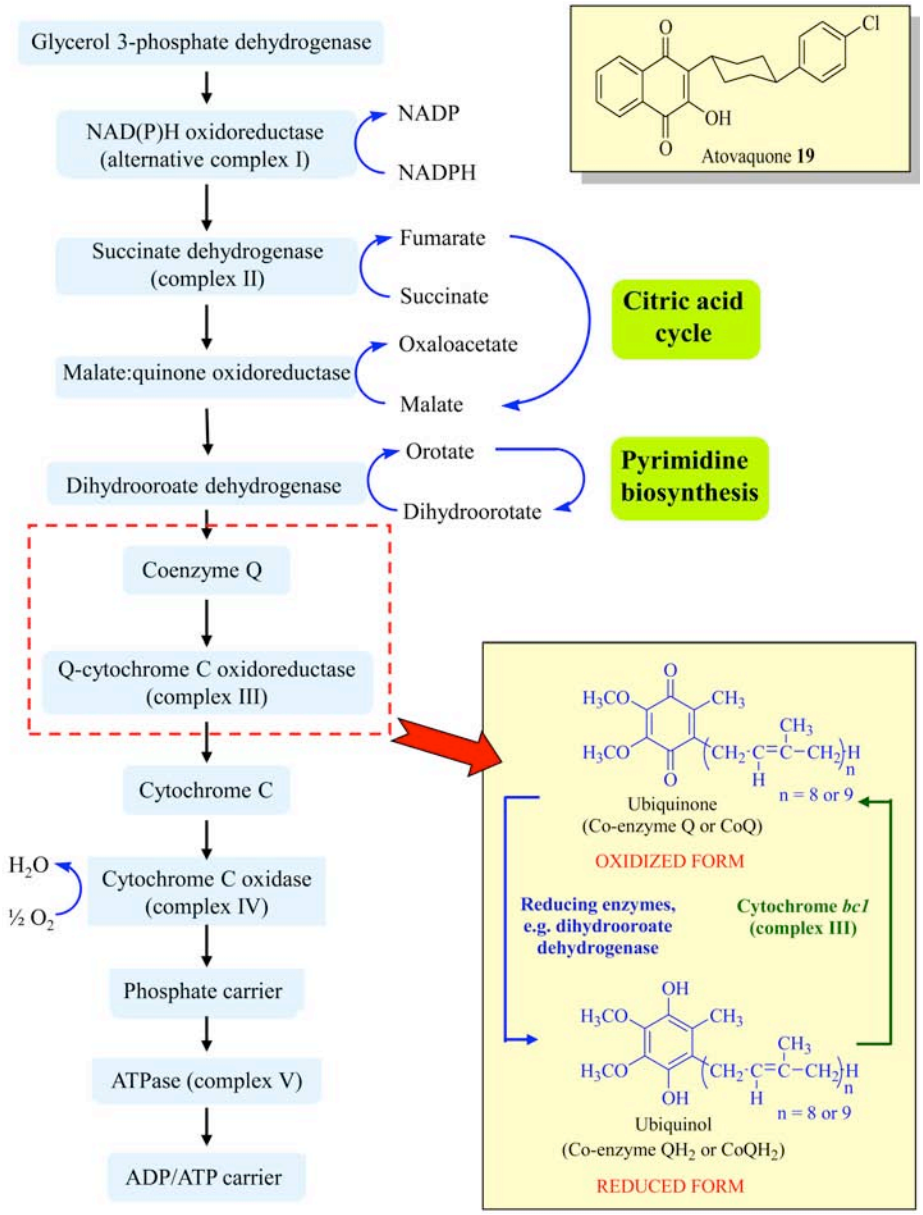


Figure 10: The mitochondrial respiratory chain in *P. falciparum* [112-116]. The glycerol 3-phosphate shuttle is illustrated. Complexes I, III and IV use the energy from electron transfer to pump electrons into the membrane space and create an electrochemical gradient. The energy is used by the ATPase complex (complex V) to produce ATP. The parasite does not depend on oxidative phosphorylation for its ATP but when Coenzyme Q is blocked, by the action of atovaquone and similar mitochondial electron transport inhibitors, pyrimidine synthesis and hence DNA synthesis are blocked. Other enzymes which transfer electrons to CoQ are also blocked.

treatment, because other options were only effective in very high doses. Nevertheless, it was only during the 1990s that atovaquone was approved by the FDA after the clinical trials undertaken in those years. The combination atovaquone-proguanil was registered throughout Europe in 1999 [111].

Atovaquone

Atovaquone (ATO) is a synthetic naphthalene derivative, a hydroxynaphthoquinone, and an analogue of ubiquinone (coenzyme Q), the electron carrier in the electron transport chain. ATO was developed by GlaxoWellcome Inc., in 1992. It is a potent selective inhibitor of mitochondrial electron transport in *Plasmodium* species, acting on the cytochrome bc_1 complex (Fig. **10**). The passage of electrons from ubiquinone to cytochrome bc_1 (complex III) requires the binding of ubiquinone to this complex. When atovaquone is present, it binds irreversible to the cytochrome *bc1* complex, preventing any further reactions and as a result the mitochondrial membrane potential drops. This inhibits any transport process across the mitochondrial membrane, it inhibits many parasitic enzymes linked to the electron transport system, including the synthesis of pyrimidine required for DNA synthesis, and eventually it results in the death of the parasite [112-116]. The mammalian cytochrome complex is up to 1000 times less sensitive than the parasite's complex [6], hence this drug can be used successfully as an antiplasmodial. ATO is active against all *Plasmodium* species [3, 117]. It inhibits pre-erythrocytic development in the liver [3], it is active on both the asexual and the sexual stages of *P. falciparum* [118] and it also inhibits oocyst development in the mosquito vector [3]. Some IC_{50} values that have been recorded are: 0.56-4.53 nM (laboratory strains) [119] and 1.1 [120], 3.56 [121] and 6.2 nM [122] (field isolates). When atovaquone is used on its own there is rapid development of resistance, due to a mutation at the mitochondrial gene that encodes cytochrome b. This causes an approximately 1000-fold increase in IC_{50} values [7]. For this reason atovaquone is not used on its own, but a combination with proguanil has been developed, which prevents the rapid development of resistance [103, 123, 124]. Marketed as Malarone, it acts in a synergic manner and hence it is considered a monotherapy. This drug has been used for treatment and prophylaxis of multidrug-resistant falciparum malaria since the mid 1990s, but it has not found widespread use due to its high cost [3]. For

prophylaxis, the activity of atovaquone-proguanil against the liver stages of the parasite is particularly important, because the parasites are killed before erythrocyte infection starts. Presently it is the most potent prophylatic agent known [77]. It is suitable for use by air crews [20]. Atovaquone is generally well tolerated and the more common side effects have been skin rashes, headache, fever, insomnia, nausea, diarrhoea, vomiting, raised liver enzymes, hyponatraemia and, very rarely, haematological disturbances, such as anaemia and neutropenia [3]. Atovaquone is licensed in many countries for the treatment of the AIDS associated disease *Pneumocyctis carinii* pneumonia and it is active against toxoplasmosis. Atovaquone-proguanil is recommended by WHO as a first-line option drug for the treatment of uncomplicated falciparum malaria in travellers returning to non-endemic countries, including those in special risk groups [3].

Pharmacology of Atovaquone

Atovaquone is poorly absorbed from the gastrointestinal tract, and it is excreted almost exclusively in an unchanged form. It is a highly lipophilic drug that has a relatively poor bioavailability of approximately 23% [20]. This value can increase to about 47% when ATO is taken concurrently with a fatty meal [58]. The bioavailability is reduced in AIDS patients and in late pregnancy [3]. ATO has a half-life of 50-70 h due to enterohepatic recycling [3]. The first peak plasma concentration occurs between 1 and 8 h, and the second at 24-96 h [58]. The pharmacokinetics of atovaquone has been studied in healthy adult individuals after 1000 mg were taken once daily for three days [104]. It was found that a maximum concentration of ATO in the plasma of 10.52 μg mL^{-1} was reached after 3 h, with the $AUC_{0\rightarrow\infty}$ being 549 h μg mL^{-1}. The mean half-life of ATO in this study was 57.1 h. Similar results were obtained when the volunteers took Malarone (ATO 1000 mg, PG 400 mg) for a similar period. This is the recommended dose for treatment of acute falciparum malaria. In this case a maximum concentration of atovaquone of 11.54 μg mL^{-1} was found in the plasma after 3 h, $AUC_{0-\infty}$ was 510 h μg mL^{-1} and the half-life was 59.0 h. The same dose of Malarone given in Thailand to patients with acute falciparum malaria once a day for 3 days, produced parasite clearance in 65 h, and 100% cure rate [110].

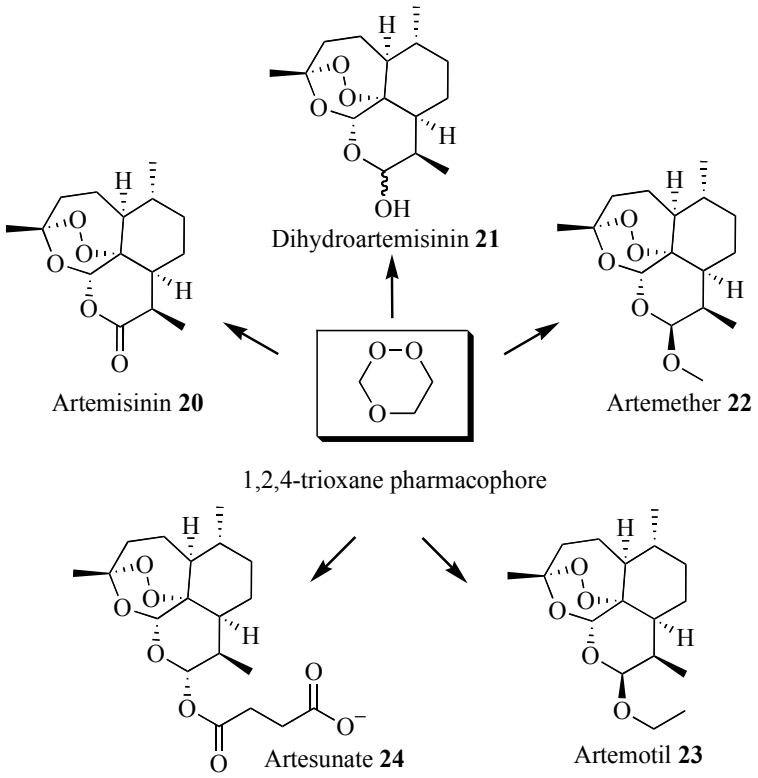


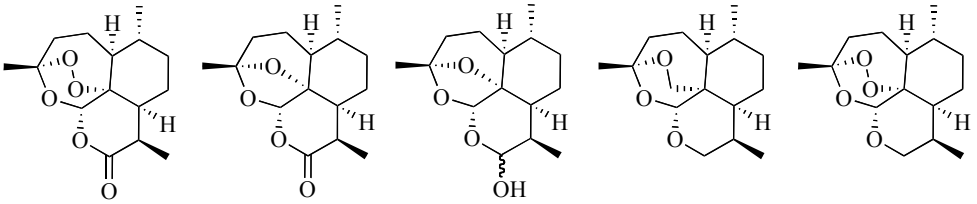
Figure 11: Artemisinin and derivatives presently in use to treat malaria. The common peroxide bridge within the 1,2,4-trioxane system is believed to be responsible for their bioactivity. It has been shown that artemisinins lacking a peroxidic oxygen atom are devoid of activity.

THE ARTEMISININS

Artemisia annua, the annual wormwood, has been used for more than 1000 years in Chinese traditional medicine for the treatment of fever [3]. In 1971, working in an army program to find a treatment for malaria, Tu Youyou [125] of China's Academy of Chinese Medical Sciences, Beijing, isolated the active ingredient from the leaves of *Artemisia annua,* and named it *qinghaosu* or artemisinin. Artemisinin (ART) **20** is a sesquiterpene lactone with a 1,2,4-trioxane substructure, the endoperoxide, which is responsible for its antimalarial activity [126]. Since artemisinin is only poorly soluble in water and in oil, there has been since then a search for derivatives that could have better physical properties and greater potency. A number of semi-synthetic derivatives are nowadays also used for therapy, resulting from modifications at the C10 position, all of which contain

the endoperoxide bridge (Fig. **11**). The most commonly used are artenimol or β-dihydroartemisinin (DHA) **21**, artemether (AM) **22**, artemotil (AE) (previously known as arteether) **23** and artesunate (AS) **24**, all of which are more potent than artemisinin [126-130]. They are used to treat uncomplicated and severe malaria in both adults and children. In fact, besides the cinchona alkaloids quinine and quinidine, the artemisinins are the only class of compounds useful to manage severe malaria [127]. The artemisinins are presently the most active and rapid-acting antimalarial drugs known [6]. Nevertheless, although they produce rapid clearance of parasites, they have a short half-life. If used as a monotherapy, a seven-day course of therapy is required to prevent recrudescence. Hence, usually they are not used as monotherapies, but in combination with longer-acting blood schizonticidal drugs. This strategy is also recommended by WHO to prevent the development of resistance.

Artemisinin and its derivatives act very efficiently against asexual erythrocytic forms of *P. falciparum* [126], and in contrast to other antimalarials, they also act against the early ring stages present in the erythrocytes a few hours after infection. Artemisinin and artemether do not act on trophozoites, however dihydroartemisinin acts very fast on all three asexual stages. The artemisinins are also active against *P. vivax.* They are inactive against extra-erythrocytic forms (sporozoites, liver schizonts, merozoites) [126], and thus are not useful for causal prophylaxis. They are also less active against gametocytes, but in areas where they are used, a decrease in the transmission of malaria has been observed, suggesting that there is some inhibitory effect [126]. In *P. falciparum* malaria, artemisinin kills stage 4 gametocytes, which are otherwise sensitive only to primaquine [4].



Artemisinin **20** **26** **27** **28** **29**

Figure 12: Artemisinin and analogues. Compounds **26-28** have no antimalarial activity, which suggests that the peroxide bridge is essential for bioactivity. In contrast compound **29** is more active than artemisinin [132].

The exact mechanism of action of the artemisinins is not known [131-133]. It has been shown that the peroxide structure is essential for the antimalarial activity (Fig. **12**), since related compounds lacking this unit are devoid of activity [134-136]. The artemisinins concentrate mainly in the food vacuole [137, 138].

Fe(II) is thought to be the main species responsible for bioactivation and this has been the subject of several studies [132]. Since the degradation of haemoglobin by the parasite produces large quantities of soluble haem in the Fe^{2+} form, haem iron is abundant. Initially it was proposed that Fe(II) cleaves the endoperoxide bridge, generating toxic oxygen radicals [141]. It was later proposed that the oxygen centred radicals generated rearrange to form carbon centred radicals [142, 143], which alkylate haem. A reductive scission model was proposed as a probable mechanism for this process (Fig. **13**) [132, 139-141] Alkylation can take place at positions α, β, γ or δ. Experiments showed that ferrous haem (not exogenous Fe^{2+}) bound to the artemisinins and promoted this process. It has also been shown by electron paramagnetic resonance spin trapping techniques [144-146] that carbon radicals alkylate haem. In a different model of haem activation, the open peroxide model [147-149], it was proposed that Fe acts as a Lewis acid, facilitating ionic rather than radical bioactivation of the artemisinins (Fig. **14**). Reaction with a water molecule leads to the formation of an unsaturated hydroxide species capable of modifying proteins irreversibly by direct oxidation. Other reactive oxygen species can also be produced in this manner, which can oxidize amino acids and this could be an important means of antimalarial action of these drugs. The alkylation of proteins by artemisinins is indeed a well established process [132].

Although intracellular iron (II) also exists in the food vacuole in equilibrium with iron (III), experiments comparing different redox forms of haem, ferrous iron, and deoxygenated and oxygenated haemoglobin under similar *in vitro* conditions showed that haem was the species that reacted with artemisinin more efficiently [150]. In humans the most abundant form of iron is oxyhaemoglobin, to which peroxide antimalarials are stable, which explains the selectivity of these drugs towards infected but not healthy erythrocytes.

Other potential targets for the artemisinins have been pointed out, such as inhibition of *Pf*ATP6, the parasite membranes and the mitochondria. It has been found that artemisinin is a highly selective inhibitor of a Ca^{2+} transporting ATP-ase (SERCA *i.e.,* sarco/endoplasmic reticulum membrane calcium ATP-ase). The role of this protein is to reduce cytosolic free calcium concentrations by concentrating Ca^{2+} into the membranes, an activity crucial for parasite survival [132]. However, *Pf*ATP6 resides in the sarcoplasmic endoplasmic reticulum, not in the food vacuole. The new mechanism was proposed [150], which assumes that endoperoxide cleavage takes place not in the food vacuole but in the cytoplasm, catalyzed by a cytoplasmic Fe (II) source, and that the radicals formed inhibit the parasite's calcium ATPase, *Pf*ATPase 6 (SERCA). A few studies have since been undertaken to test this hypothesis [132]. Parasite membrane damage by artemisinins has also been shown to occur [151], but so far there is no evidence of loss of mitochondrial functions as an initial stage of antimalarial activity with these drugs [132]. Presumably the antimalarial activity of these compounds results from more than one mechanism of action.

Artemisinin and its derivatives are active against all four species of malaria parasites that infect humans [3]. They are very well tolerated and show excellent human safety. There have been reports of mild gastrointestinal disturbances, dizziness and tinnitus [3]. The only potentially serious adverse effect reported is type 1 hypersensitivity reactions in approximately 1 in 3000 patients. Since there has yet been no evaluation of their tolerance during pregnancy, it is recommended that they should be avoided in the first trimester, because although fetotoxicity has not been reported in humans, it has been observed in animal experiments [3]. Neurotoxicity has also been observed in animals only. These adverse effects are associated with dihydroartemisinin, the main metabolite of all artemisinin semi-synthetic drugs.

Artemisinin and its derivatives are highly active antimalarial agents. In a review published in 2001, *in vitro* IC_{50} values against chloroquine-resistant *P. falciparum* strains were reported for artemisinin and derivatives as follows: 10-100 nM (artemisinin), 0.36-7.00 nM (dihydroartemisinin), 1.66-2.18 nM (artesunate), 0.57-6.10 nM (artemether), and 1.74-3.44 nM (arteether) [129].

Resistance to artemisinin has developed recently, appearing initially in the Cambodia-Thailand border area [7]. There is also early evidence of artemisinin resistance in Myanmar and Vietnam [1], which has led to greater efforts by WHO to press against the use of monotherapies for malaria treatment. In 2011 the *Global Plan for Artemisinin Resistance Containment* (GPARC) was published by WHO with recommendations for the successful management of artemisinin resistance, which include a call for increased monitoring and surveillance, improved access to diagnostics and treatment with artemisinin-based combination therapy (ACTs), and investment in research. The ACTs recommended nowadays for malaria treatment are described in the next section in this chapter.

Artemisinins pose the problem that although they are inexpensive by international standards, the derivatives are significantly more expensive than traditional antimalarials such as chloroquine and sulphadoxine/pyrimethamine [82]. A sufficient supply of artemisinin is also needed as raw material. The main cultivation areas are in China and Vietnam and the maximum yields are approximately 0.6%. Although synthetic routes do exist, production on a large-scale is not economically viable. The cost is a reflection of the complexity of the *de novo* synthetic procedures needed to obtain the unusual endoperoxide trioxane moiety.

Pharmacology of Artemisinin and its Derivatives

Artemisinin **20** is a potent antimalarial. It is converted to inactive metabolites *via* the cytochrome P450 enzyme CYP2B6 and other enzymes [3]. The derivatives artemether, artemotil and artesunate have antimalarial activity of their own, but the main therapeutic effect is due to the primary metabolite dihydroartemisinin, which forms rapidly after administration. DHA is then converted into inactive metabolites *via* hepatic cytochrome P-450 and other enzyme systems [127]. EC_{50} values depend on the compound and lie usually around or below 10 nM [126]. Recommended doses for the treatment of uncomplicated falciparum malaria in humans are 10 mg kg^{-1} day^{-1} for artemisinin and 2-5 mg kg^{-1} day^{-1} for artemisinin derivatives [126].

Artemisinin

Artemisinin **20** is a potent inducer of its own metabolism [3]. The pharmacokinetics of artemisinin has been studied a few times. For example, in two different studies [152, 153], after administration of a single oral dose of 500 mg ART to healthy individuals, a mean peak plasma concentration of 450 and 443 ng mL^{-1} was reached within 2.3 and 1.8 h respectively, and the half-life was determined to be 2 h in the first study and 0.97 h in the second. Multiple dose pharmacokinetics have also been investigated [154]. A study in Tanzania with uncomplicated falciparum malaria patients showed that when 500 mg of artemisinin are administered on the first day of treatment, followed by 2 doses of 250 mg taken daily for 4 days, and another dose of 500 mg on the last day of treatment, the mean time to parasite clearance was 31 h. On the first day a maximum mean concentration of antimalarial in the plasma of 588 ng mL^{-1} was reached after 2.4 h. On day 6, the maximum concentration was only 116 ng mL^{-1}, which was reached after 3.1 h. This decrease suggests that the metabolic capacity increases as a result of pronounced autoinduction. The area under the plasma concentration-time curve ($AUC_{0\rightarrow\infty}$) changed from 2,601 to 432 h ng mL^{-1} from day 1 to day 6. The half-life was 2.3 and 2.2 h respectively.

Dihydroartemisinin

Dihydroartemisinin **21** is the main active metabolite of the artemisinin derivatives, but it can also be used on its own. DHA has an hemiacetal substructure, obtained *via* reduction of the lactone substructure of artemisinin. It is relatively insoluble in water. When administered orally, peak plasma concentrations occur after 2.5 h, and after 4 h *via* the rectal route [3]. It is converted to inactive metabolites by the hepatic cytochrome P-450 and other enzyme systems. DHA has an elimination half-life of about 45 minutes [127]. The cure rates are similar to those of oral artesunate [3]. The pharmacokinetics of dihydroartemisinin was studied in patients with acute falciparum malaria [155], after administration of 200 mg of oral dihydroartemisinin. They were also administered 200 mg of artesunate 24 h later. A maximum mean concentration of 4,028 nmol L^{-1} of antimalarial in the blood plasma was reached after 2.63 h. $AUC_{0\rightarrow\infty}$ was 14,804 h nmol L^{-1} and the half-life 1.09 h. The treatment was completed with an additional 4 mg of artesunate and 25 mg of mefloquine per kg body weight

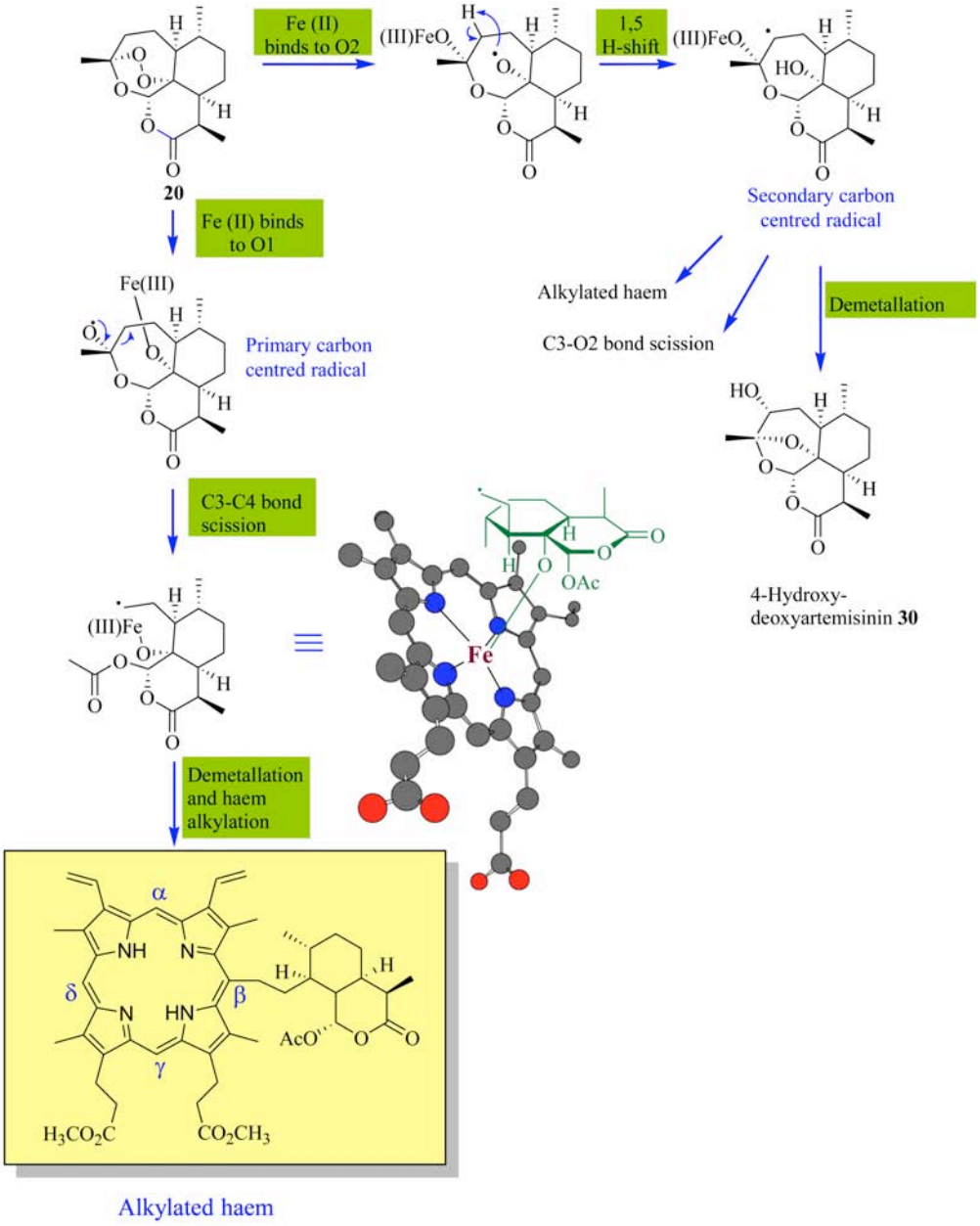


Figure 13: Haem alkylation, a probable mechanism of action of artemisinin and other peroxide antimalarials. This may prevent haem polymerization and detoxification as hemazoin. Since the peroxide bridge is unsymmetrical, more than one radical intermediate can be produced in the presence of Fe(II) ions. Both primary and secondary radicals have been spin-trapped by electron magnetic resonance spin trapping techniques. The reductive scission model is illustrated [132, 139-141].

administered after 48 h, and still 10 mg kg^{-1} of mefloquine 24 h after this. Dihydroartemisinin plus piperaquine is an ACT recommended by WHO as a first-line option treatment of uncomplicated *P. falciparum* malaria worldwide [12].

Artemether

Artemether **22** results from methylation of the hemiacetal substructure of dihydroartemisinin. It is insoluble in water, and it is more lipid-soluble than artemisinin or artesunate [3]. The peak plasma concentration occurs 2-3 h after oral administration but after intramuscular injection absorption is slow and erratic, and the peak plasma concentration occurs only 6-18 h later [3]. It has a half-life of 1 h. The pharmacokinetics of artemether was studied in patients with acute uncomplicated *P. falciparum* malaria, after oral or intramuscular administration of a 2 mg kg^{-1} body weight dose of AM [156]. A second dose was given 24 h later by the alternative route. Patient treatment was eventually concluded with the administration of 15 mg kg^{-1} body weight of mefloquine on the third day. Measurements were done by bioassays, which do not distinguish between the parent drug and the active metabolite DHA [157], and hence the values obtained and described here refer to DHA equivalents. After oral administration, a maximum concentration of 1,905 nmol L^{-1} was measured in the blood plasma after 1.0 h, with $AUC_{0\rightarrow\infty}$ = 6,045 h nmol L^{-1} and a half-life of 1.34 h. When the drug was administered intramuscularly, peak concentrations were reached in a median of 8 h, but they were only 121 nmol L^{-1}. The median $AUC_{0\rightarrow\infty}$ was then only 2,718 h nmol L^{-1}. The relative bioavailability of oral artemether during the first 24 h, clinically the most important period, was over three times that of intramuscular artemether. Artemether is recommended by WHO as a first-line option for the initial emergency 24 treatment of severe malaria, as an option to artesunate or quinine, for adults or for children. Artemether-lumefantrine is one of the first line options for the treatment of uncomplicated falciparum malaria in travellers returning to non-endemic countries [3].

Artemotil

Artemotil, also known as β-arteether **23**, is another semi-synthetic derivative of artemisinin. It is obtained by ethylation of the hemiacetal substructure of

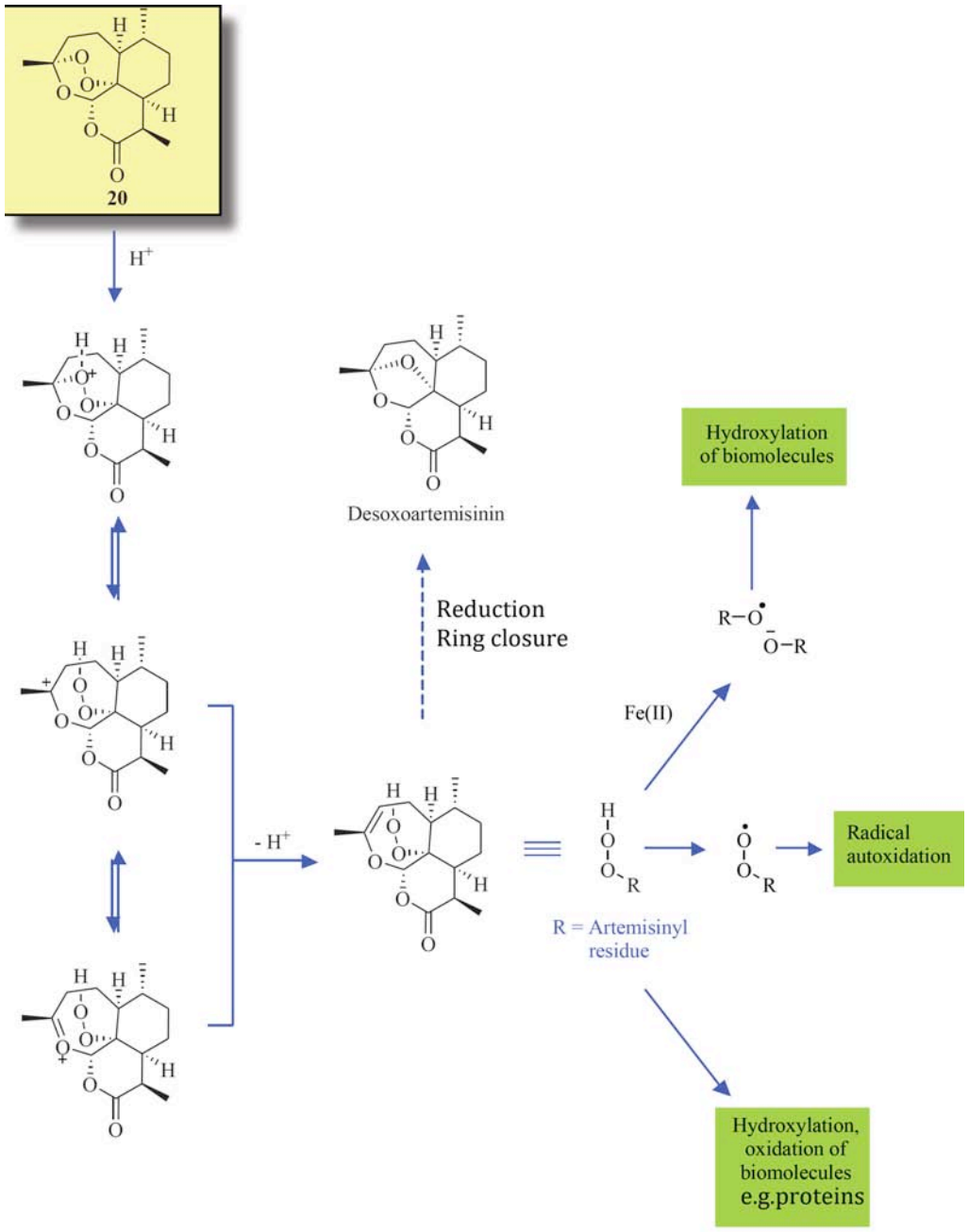


Figure 14: The open-peroxide model. The figure shows a proposal for peroxide ring opening to generate hydroperoxides, and the subsequent possibilities for decomposition. Instead of a proton, a Lewis acid like Fe(II) could activate the endoperoxide for ring opening [147, 149]. The species formed can oxidize amino acids, proteins.

dihydroartemisinin. It is water-insoluble, and it is administered by intramuscular injection only [3]. AE is absorbed slowly and more erratically than artemether, and can be detected in the plasma until more than 24 h after administration [3]. It is a fast-acting blood schizonticide specifically indicated for the treatment of chloroquine-resistant *P. falciparum* malaria and cerebral malaria [126]. The pharmacokinetics of arteether was studied on Thai patients with acute uncomplicated falciparum malaria after intramuscular administration of different doses of antimalarial to determine effective dose regimens [158]. From this study they concluded that the best regimen was one equivalent of the standard regimen used with artemether, *i.e.,* 3.2 mg kg^{-1} body weight of artemotil on the first day of treatment, followed by administration of 1 dose of 1.6 mg per kg body weight taken daily for 4 days thereafter. The pharmacokinetics after administration of the last dose were as follows: the mean peak plasma concentration was found to be 105.8 ng mL^{-1}, AUC (96,120 h) equal to 1,956 h ng mL^{-1}, and the half-life 22.4 h. Parasite clearance was achieved in a mean of 25.5 h.

Artesunate

Artesunate (AS) **24** is also obtained *via* modification of dihydroartemisinin. The hemiacetal hydroxyl group is acylated with succinic acid, and AS is used as the sodium salt. It is water-soluble and it can be administered orally, by intravenous or by intramuscular injection, or as suppositories [3]. It is absorbed rapidly, and the peak plasma concentration occurs 1.5 h after oral administration, 0.5 h after intramuscular administration and 2 h after rectal administration. It is eliminated rapidly and it has a half-life of 45 min [3]. The water solubility is important since it means that it can be administered intravenously which may be critical in cases of severe malaria when the patients are too ill and due to their condition administration *via* another route is not possible. The absolute bioavailability after a single dose of oral artesunate in adults with uncomplicated falciparum malaria is about 60% [159]. After intramuscular administration, DHA bioavailability is greater than 80% [160]. The pharmacokinetics of artesunate has been reviewed [161]. It was studied in patients with acute falciparum malaria [162], after administration of 200 mg of oral artesunate. They were also administered 200 mg of dihydroartemisinin 24 h later. Measurements were done by bioassays, which do not distinguish between the parent drug and the active metabolite DHA [157], and hence the values obtained and described here refer to

DHA equivalents. A maximum mean concentration of 3,889 nmol/L^{-1} of antimalarial in the plasma was reached after 1.55 h. $AUC_{0\rightarrow\infty}$ was 12,664 h nmol L^{-1} and the half-life 1.17 h. The treatment was completed with an additional 4 mg of artesunate and 25 mg of mefloquine per kg body weight administered after 48 h, and still 10 mg kg^{-1} of mefloquine 24 h after this. These results were similar to some obtained in a different study with Vietnamese patients [163]. The results highlighted here were obtained in the study referred to above in which the pharmacokinetics of dihydroartemisinin was also determined. A comparison of the data obtained for the two antimalarials led the authors of the study to conclude that since the pharmacokinetics of the two drugs are similar, and DHA is potentially cheaper and easier to manufacture than artesunate, that DHA could be a satisfactory alternative to artesunate in combination therapies to treat uncomplicated falciparum malaria. The big advantage of artesunate is its water-solubility, which allows for intravenous use. A few studies have been carried out on the pharmacokinetics of artesunate administered intravenously [161]. The AS concentration is initially high. Artesunate metabolises rapidly through esterase-catalyzed hydrolysis to dihydroartemisinin. The average AS half-life is less than 5 minutes. For example, administration of 120 mg artesunate over 2 min, leads to peak plasma concentrations ranging from 13,685 to 16,530 ng mL^{-1}. AUC values ranging from 846-1,269 h ng mL^{-1} were obtained. In all studies, T_{max} of DHA following intravenous administration was less than 25 min. The maximum concentration of DHA in the plasma was in the range 2,192 to 2,758 ng mL^{-1}. AUC ranged from 1,845 to 3,298 h ng mL^{-1}.

Artesunate is the first line antimalarial recommended by WHO for the treatment of severe malaria, for adults or children. Artemether or quinine are alternatives if artesunate is not available [3]. After the initial emergency 24 h treatment, artesunate plus clindamycin or doxycycline is one of the alternatives recommended for follow-up treatment.

ANTIBIOTICS: TETRACYCLINE, DOXYCYCLINE, CLINDAMYCIN AND AZITHROMYCIN

After the discovery of penicillin by Fleming in 1928, many antibiotics were isolated from fungal broths and characterized. By the 1950's some were already known to have antiplasmodial activity [164], including the tetracyclines, used

nowadays in malaria therapy and chemoprophylaxis. Besides tetracycline and its derivative doxycycline, clindamycin and azithromycin are other non-sulfur antibiotics included in the WHO's list of principle antimalarial drugs available nowadays (Fig. **15**) [7].

All these antibiotics are thought to have a similar mode of action. They are translation inhibitors in prokaryotic synthesis and they are thought to inhibit protein synthesis inside the mitochondrion and the bacterial apicoplast [6]. The apicoplast is a non-photosynthetic plastid organelle, probably a remnant of endosymbiotic red algae, unique to parasites of the phylum Apicomplexa that includes protozoa like *Plasmodium* [165]. Many metabolic functions take place in the apicoplast [166], including fatty acid and haem biosynthesis [167] and hence this organelle is essential to the life of the parasite. The apicoplast has its own genome and it expresses about 30 genes, although the vast majority of the proteome is encoded in the nuclear genome [168]. Antibiotics disrupt translation during the first replication cycle of the parasite [169]. The apicoplasts metabolic functions are not disrupted in this first cycle, especially since many are catalyzed by nuclear-encoded proteins, and the parasite continues to grow normally in this first cycle [170]. However, during the second cycle, when the progeny of antibiotic-treated parasites invade a new host cell, the apicoplast cannot perform its normal functions and the new schizonts will not mature properly, erythrocyte rupture does not take place, leading to parasite death. This effect is called "the delayed-death effect" and as a result of this phenomenon even when antibiotics have potent antimalarial effects, they are slow to act.

Antibiotics have been used alone for the treatment of malaria, *e.g.,* clindamycin [81], but usually they are used in combination with other antimalarials. In fact the use of quinine or artesunate with an antibiotic is recommended as a first line treatment in severe malaria after the initial 24 h emergency treatment, and as second-line combination for the treatment of uncomplicated falciparum malaria [3]. Antibiotics are also used for chemoprophylaxis, as for instance doxycycline [3]. The use of antibiotics is important not only because of their antimalarial action, but also because septicaemia and severe malaria are associated and there is a diagnostic overlap, particularly in children [4]. Complications may also arise in malaria from bacterial infections.

The concept of antibiotic-based combination therapy (ABC) was introduced in 2009 [171], suggesting that a standardized (possibly co-formulated) combination of an antibiotic with antimalarial activity with a traditional antimalarial like quinine or artesunate should become standard treatment for severe malaria in the same way that ACTs are recommended for the treatment of malaria. Although the use of antibiotics in the treatment of malaria may give rise to fears that resistance could develop against this class of drugs which are used for the treatment of other illnesses, it has been suggested that the well-controlled environment in which intravenous therapy takes place, for example in severe malaria, in which patients are likely to receive the full course of treatment, would make this less probable to happen. Tetracyclines are one of the cheapest classes of antibiotics available [172].

Tetracycline

Tetracycline (TC) **32** was discovered in 1953 [172]. It consists of a linear system of four fused rings to which a variety of functional groups are attached (Fig. **15**). Originally it was obtained from certain species of *Streptomyces* bacteria, namely *S. aureofaciens*, *S. rimosus* and *S. viridofaciens* [172], but nowadays it is produced mainly synthetically. TC is amphoteric, with low solubility. It is used in therapy as the hydrochloride or phosphate salts, which are soluble in water. However, the intravenous preparations are stable for a few hours only [12]. Tetracycline is a broad-spectrum antibiotic, effective against Gram-positive and Gram-negative bacteria. In fact, besides the penicillins, the tetracyclines are the most widely prescribed antibiotics [173]. In malaria, TC is effective against primary erythrocytic forms of *P. falciparum* [12]. It is a blood schizonticide, which is also active against chloroquine-resistant strains and those resistant to dehydrofolate reductase inhibitors. It has no effect on gametocytes, and it is also not effective for radical cure of vivax malaria, since it has no effect on exoerythrocytic forms of the parasite. The effect on sporozoites is unknown [12]. In 2001 IC_{50} values measured against 5 strains of *P. falciparum* from Africa, Indochina, Uganda, Gambia and Brazil were found to vary from 33.8 to 88.9 μM [174].

When tetracycline is used alone for the treatment of malaria, parasite and fever clearance are slow. Hence tetracycline is usually used together with another antimalarial like quinine. In the USA, for example, tetracycline is recommended

for the treatment of *P. falciparum* or *P. vivax* malaria from chloroquine-resistant areas in combination with quinine or quinidine [175]. There are no major side effects from tetracycline use, although a few adverse reactions have been reported such as nausea, vomiting, diarrhoea, and sometimes there are adverse effects on the skin, mucous membranes and the gastrointestinal tract. Tetracycline complexes may deposit in teeth and bones, so it should not be given to women after the fourth month of pregnancy or to infants and young children.

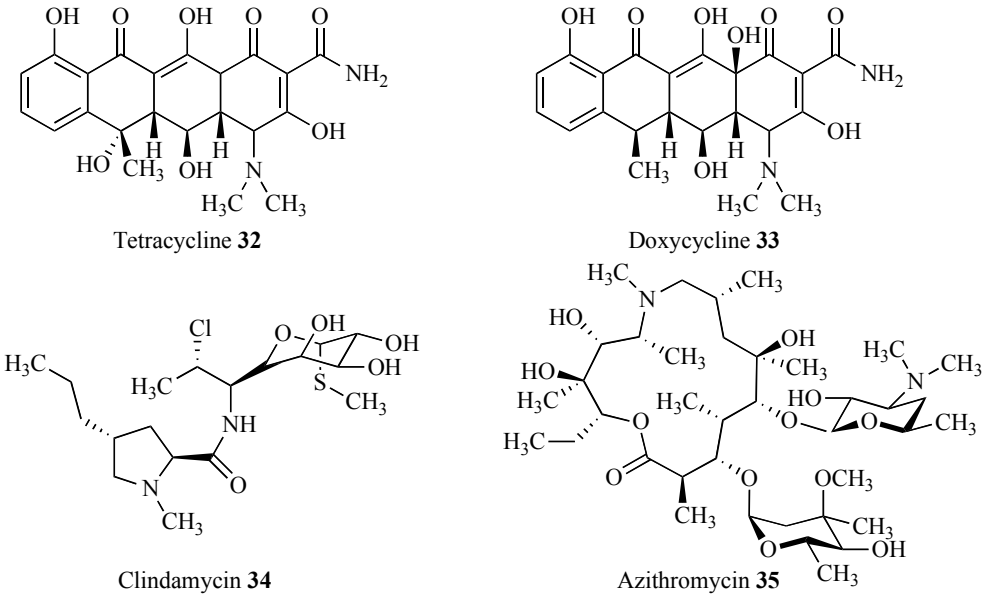


Figure 15: The antibiotic antimalarials.

In contrast to other antibiotics, it is thought that tetracyclines act on plasmodial mitochondria. Nevertheless, recent publications support the idea that they also target the apicoplast [176]. The tetracyclines inhibit protein synthesis by binding to the 30S subunit of ribosomes and preventing aminoacyl-*t*-RNA from binding. As a result, amino acid addition to the growing protein chain is impeded. Protein release is also inhibited [173].

Pharmacology of Tetracycline

Tetracycline is absorbed from the gastrointestinal tract following oral administration. About 5% of tetracycline is metabolised and excreted as

D-epitetracycline [177]. In a study on the pharmacokinetics in healthy volunteers [178], it was found that after oral intake of 500 mg of tetracycline by fasted subjects, a maximum concentration of 4.1 μg mL^{-1} was reached in the plasma after 4.2 h, and the mean $AUC_{0\rightarrow\infty}$ was 74.7 h μg mL^{-1}. The mean elimination half-life was 8.1 h. With meals the serum levels are reduced by about 50%, and the elimination half-life also decreases [178].

A study was carried out in Thailand, a country in which P. falciparum malaria is resistant to many antimalarials, to see the effectiveness of oral quinine plus tetracycline in treating patients with acute uncomplicated falciparum malaria [179]. Patients were administered quinine (600 mg quinine sulphate at 8 h intervals) and tetracycline (250 mg at 6 h intervals) for a period of 7 days. The mean PCT was 73.2 h and the cure rate was 100%. At the same time in other patients to whom oral artesunate was administered, the parasite clearance took place in half the time, with a similar cure success rate. Studies undertaken between 2002 and 2007 in six countries showed that quinine-tetracycline was 100% effective in Pakistan and in Cambodia [7], another area where resistance to antimalarials is high. In Cambodia, 4 patients out of 60 treated at the same time with artesunate showed falciparum resistance to this drug.

Doxycycline

Besides tetracycline, there are other naturally produced compounds with a basic tetracycline skeleton that have useful antibiotic properties. Chlortetracycline and oxytetracycline are examples. However, soon after their discovery, there were attempts to produce synthetic or semi-synthetic derivatives with better properties, such as improved water solubility or greater oral absorption. Structure-activity studies showed that the linearly fused tetracycline ring system should be kept to preserve activity, as well as the stereochemical configuration at the 4a, 12a (A-B ring junction) and the 4 (dimethylamino group) positions, and the keto-enol system (positions 11, 12, and 12a) near the phenolic ring D [172]. Doxycycline (DX) **33** was invented and developed for medicinal applications by Pfizer Inc. in the 1960s, as a semi-synthetic derivative of oxytetracycline. It was marketed as Vibramycin. The first report of its effectiveness to treat malaria dates back to 1971 [180]. DX has the advantage over tetracycline of having a longer half-life,

which implies that it needs fewer administrations to be effective. It is relatively water insoluble, but highly lipophilic. It is a broad spectrum antibiotic, also used to treat anthrax infections and acne. It also active against certain species of *Plasmodium*. DX is active against primary exo-erythrocytic forms of *Plasmodium falciparum* so it can be used for malaria chemoprophylaxis. In chemoprophylaxis, destruction of the pre-erythrocytic forms, the tissue schizonts, prevents the development of merozoites and parasite release into the blood stream, which leads to the clinical symptoms of malaria that are observed. Nowadays doxycycline is a first line drug for chemoprophylaxis of malaria [181, 182]. It is the drug of choice in cases in which mefloquine cannot be administered because of its neuropsychiatric side effects, for example for aircrews and divers. It is also a blood schizonticide [182] and it can be used for the treatment of various types of malaria, *i.e.,* uncomplicated *P. falciparum, P. vivax and P. ovale* malaria, but because it acts slowly, it is used only in conjunction with fast acting blood schizonticidals like quinine. In the case of the last two, primaquine must also be administered. It is not recommended for pregnant women after the first trimester and for children under 8 years of age [3]. In the case of severe malaria, as a first line option treatment, doxycycline or clindamycin used in conjunction with quinine or artesunate are recommended as a follow-up on the initial emergency 24 h treatment with artesunate, quinine or artemether, as alternatives to ACTs [3]. The side effects of DX are similar to those of tetracycline [3], but it has fewer gastrointestinal effects. It may be used on patients with renal impairment, in which the use of tetracycline is not recommended, because it may accumulate and lead to renal failure [3].

IC_{50} values of doxycycline were measured against three *P. falciparum* strains, including a multi-drug resistant strain, and the mean values observed were in the range 2.7-4.4 μM [183]. Against 71 isolates in Senegal, the mean values were 11.3 μM, with no significant differences in activity between CQ-susceptible and CQ-resistant isolates [184]. IC_{50} values measured against 5 strains of *P. falciparum* from Africa, Indochina, Uganda, Gambia and Brazil were found to vary from 7.7 to 14.9 μM [174]. There have been no confirmed reports of resistance against doxycycline [6]. Studies undertaken during 2000-2007 showed

that quinine-doxycycline was 100% effective in the treatment of uncomplicated falciparum malaria in Pakistan and Cambodia [7].

Pharmacology of Doxycycline

After oral administration doxycycline is quickly absorbed, mostly from the duodenum, and its bioavailability is 95% [185]. Like other tetracyclines, it is relatively metabolically inert, except for minor amounts of 4-epidoxycycline that have been detected in faeces and urine [186]. In a study on the pharmacokinetics in healthy volunteers [178], it was found that after oral intake of 200 mg of doxycycline by fasted subjects, a maximum concentration of 5.4 μg mL^{-1} was reached in the blood plasma after 4.3 h, and the mean $AUC_{0\rightarrow\infty}$ was 108.4 h μg mL^{-1}. The mean elimination half-life was 8.8 h. With meals the serum levels are reduced by about 20%, and the elimination half-life increases [178].

The pharmacokinetics of doxycycline was studied recently on patients recovering from severe falciparum malaria and receiving combination treatment [177]. After an initial treatment with intravenous artesunate, when the patients could take oral treatment, doxycycline was administered in combination with artesunate or quinine sulphate. The doxycycline treatment taken concurrently with the other drugs lasted for seven days, at a dose of 200 mg every 24 h. On day zero, after 200 mg of doxycycline intake, a maximum concentration of 3.17 μg mL^{-1} was found in the blood plasma after 2 h, and $AUC_{0\rightarrow\infty}$ was 49.6 h μg mL^{-1} [187]. The elimination half-life was 10.5 h. On day 7, the pharmacokinetics determined on the convalescent group was as follows: the peak plasma concentration was 4.44 μg mL^{-1}, reached after 3 h, and $AUC_{0\rightarrow\infty}$ was 73.0 h μg mL^{-1}. The elimination half-life was 11.6 h. From this study the authors concluded that the recommended dose of doxycycline of 3.5 mg kg^{-1} body weight may not be optimal for treatment. Doxycycline is known to have no effects on the pharmacokinetics of quinine [20].

For prophylaxis, doxycycline is taken once a day beginning 1-2 days before travel, while in malarious areas, and for 4 weeks after leaving. The daily dose for adults is 100 mg and for children 8 years of age is 2.2 mg kg^{-1} [182]. Adherence to the recommended doses for the periods stated is vital for its effectiveness [182]. For example, in a multinational military deployment in East Timor, consisting of

11,000 people who were present in this malaria endemic country from September 1999 to February 2000, it was found that in troops taking mefloquine there were no cases of malaria, but in the case of doxycycline, there were 11 cases per 1000 [188]. This was attributed to lack of compliance to the prescribed dose by some troops, since in the Italian Defence Forces there were no cases of malaria. Doxycycline has been reported to be as effective as mefloquine for malaria prophylaxis in the surrounding areas [188]. Since resistance to doxycycline in malaria has not been documented yet, DX can be used by travellers to all malaria-endemic countries [182].

Clindamycin

Clindamycin (CL) **34** is a lincosamide antibiotic, a derivative of lincomycin, which was first isolated from the actinomycete *Streptomyces lincolnensis* in a soil sample from Lincoln, Nebraska. Lincosamides have the carbohydrate 6-amino-6,8-dideoxy-D-erythro-D-galacto-octose as basic structural unit. Lincomycin is nowadays produced in fermentations of *Streptomyces lincolnensis*, and clindamycin is a chlorinated semi-synthetic derivative of lincomycin that exhibits better antibacterial activity and is also active against protozoa [189]. It is used as the phosphate ester, and it is very soluble in water. CL was introduced as an antibiotic in 1960. It is often used to treat infections with anaerobic bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) infections, and also as a topical treatment for acne. It has virtually no activity against aerobic Gram-negative bacteria, but excellent activity against both Gram-positive cocci and Gram-positive or -negative anaerobes [190]. The antimalarial properties of clindamycin have been known for a while. In one study, the IC_{50} mean values measured against three *P. falciparum* strains, including a multi-drug resistant one, were in the range 18-28 μg mL^{-1} [183]. It was also observed that in the presence of the newer antibiotic fosmidomycin, there was good synergy and the IC_{50} values dropped considerably. CL also increases the activity of quinine. CL is effective not only against *P. falciparum* but also *P. vivax* [3]. It is not active against the exo-erythrocytic stages of the parasites [189].

The first report of the successful treatment of falciparum malaria with clindamycin alone dates back to 1975 [191]. Clindamycin is effective as a

monotherapy for the treatment of malaria, as long as it is administered for at least 5 days, twice daily [192]. However, parasite clearance is slow, taking about 4-6 days, and hence it is not recommended for use as a monotherapy, but only in conjunction with a faster acting antimalarial [192], like quinine or chloroquine. CL is well tolerated. The adverse side effects are mild [192], and include gastrointestinal side effects like diarrhoea and perioral rashes. However, in some patients pseudomembranous colitis may develop during or after treatment, which can be fatal [3].

An advantage of clindamycin over the tetracyclines is that it may be used by pregnant women safely [193] and also by children [192]. The pharmacokinetic properties remain unchanged during pregnancy [194]. Small amounts are secreted in human milk.

The lincosamides block protein synthesis in bacteria by inhibiting the peptidyl transferase reaction on the 50S ribosomal subunit [195, 196]. Clindamycin inhibits bacterial protein synthesis and acts specifically on the 50S subunit of the bacterial ribosome, probably interfering with the process of peptide chain initiation [189]. It may also stimulate dissociation of peptidyl-tRNA from ribosomes [195, 196]. In *Plasmodium* it disrupts the apicoplast translation machinery during the first replication cycle, in the same way as the tetracyclines and the macrolides, provoking a "delayed-death effect" [168, 197]. Clindamycin is recommended as second-line treatment in combination with artesunate or quinine for the treatment of uncomplicated falciparum malaria. CL is recommended as the first choice of treatment together with quinine or artesunate throughout pregnancy. In the case of severe malaria, as first-line treatment options, antibiotics like clindamycin or doxycycline in conjunction with quinine or artesunate are recommended as a follow-up on the initial emergency 24 h treatment with artesunate, quinine or artemether, as alternatives to ACTs [3].

Pharmacology of Clindamycin

After intramuscular or intravenous administration, clindamycin phosphate is rapidly hydrolyzed in plasma to active clindamycin. The ester is not active. When administered orally, the hydrochloride is rapidly absorbed from the

gastrointestinal tract. Clindamycin is metabolized into three major, biologically active derivatives (clindamycin sulfoxide, de-*N*-methyl clindamycin, and de-N-methyl clindamycin sulfoxide) and is excreted mainly into the bile, and about 20% is excreted by the kidneys [192].

The pharmacokinetics studied in a group of healthy volunteers, after oral administration of 600 mg of clindamycin hydrochloride, were as follows [198]: the mean absolute bioavailability F was 0.53, a peak plasma concentration of 5.3 mg L^{-1} was reached after 0.76 h, $AUC_{0\rightarrow\infty}$ was 16.9 h mg L^{-1}, and the mean half life was 2.4 h.

Several studies have been carried out on the use of quinine-clindamycin to treat uncomplicated falciparum malaria [199]. A study in Thailand on adult patients with multi-drug resistant falciparum malaria [200] showed that quinine in combination with clindamycin was 100% effective in treating the disease. Patients were treated with an oral dose of quinine sulphate (10 mg salt/bw thrice daily) plus clindamycin (5 mg base kg^{-1} body weight 4 x daily) for a total of 7 days, and parasite clearance was achieved after a mean of 78 h.

Azithromycin

Azithromycin (AZ) **35** belongs to the macrolide family of antibiotics, which have as characteristic structural feature a macrocyclic 12-, 14-, or 16-membered lactone ring to which sugar molecules are attached [201]. The widely used antibiotic erythromycin is also a member of this family. It is produced from a strain of the actinomycete *Streptomyces erythreus*, later reclassified as *Saccharopolyspora erythrea.* Azithromycin is a semi-synthetic derivative of erythromycin with an extra atom in the ring, a methyl-substituted nitrogen atom, and it is therefore a 15-membered azalide. It has 10 asymmetric centres in the macrocyclic ring, which make it difficult to produce synthetically. AZ was discovered in 1980 by a team of researchers working at the Croatian pharmaceutical company Pliva [202, 203]. The presence of the extra nitrogen atom in the ring causes increased bioavailability, increased tissue penetration and longer elimination half-life in comparison with erythromycin [204]. Nowadays it is one of the world's best selling antibiotics, with a broad spectrum of activity. It is often used to treat

middle ear infections, strep throat, pneumonia, typhoid, sinusitis and certain sexually transmitted infections. Its antimalarial potential was discovered at the Walter Reed Army Institute of Research, USA [205-207]. The *in vitro* activity against multidrug-resistant *P. falciparum* K1 strain was determined by measuring the ^{3}H-hypoxanthine incorporation, and it was found to inhibit parasite growth with IC_{50} value of 8.4±1.2 μM [208].

The macrolides have a similar mechanism of action to that of the lincosamides, despite the fact that their chemical structures are very different [189]. Against bacteria, AZ acts by binding reversibly to the 50S ribosomal subunit, thus interfering with microbial protein synthesis [209]. Nucleic acid synthesis is not affected. It has been shown that in plasmodia, the antimalarial properties of AZ are a result of its binding to the apicoplast 50S ribosomal subunit and inhibiting protein synthesis in the apicoplast [209].

Azithromycin is active against pre-erythrocytic forms of *Plasmodium* [210], but it also causes the "delayed-death effect" since it does not prevent maturation of liver-stage merozoites, but these are incapable of causing the subsequent blood-stage infection. It is therefore slow to act [211], but it has been combined with other drugs like quinine or chloroquine for effective treatment [168]. Clinical trials have shown that azithromycin is a good chemoprophylatic agent, less effective than doxycycline against falciparum malaria [212, 213], but as effective as doxycycline against vivax malaria [213]. It has no apparent effect on *P. vivax* hypnozoites [214]. AZ is also active against blood schizonts [215, 216], but it is a weak antimalarial with a slow parasite clearance rate, and it was concluded in a recent review, that it offers no equivalence or superiority either as monotherapy or combination therapy for the treatment of *P. falciparum*- or *P. vivax*-induced malaria, compared with other existing antimalarials [214].

AZ is well tolerated. Reported adverse effects are nausea, vomiting, diarrhoea, pruritis and dizziness, but they usually disappeared when drug intake stopped [217]. Some studies show that it is safe for use in children and pregnant women [207, 218]. Presently azithromycin is not one of the drugs recommended by WHO for the treatment or prophylaxis of malaria [3], although it is already listed as an

available antimalarial in the recent "Global report on antimalarial drug efficacy and drug resistance: 2000-2010" [7].

Pharmacology of Azithromycin

After oral administration, azithromycin is rapidly adsorbed from the gastrointestinal tract. AZ is eliminated mostly as an unchanged drug *via* biliary excretion [219]. The absolute oral bioavailability is approximately 34-52% with single doses of 500 mg to 1.2 g, probably due to incomplete absorption [219]. The standard dose to treat bacterial infections in adults is 250 to 500 mg orally every six hours or 0.5 to 1 g every 12 hours for seven days or more [214]. The half-life of azithromycin is approximately 68 h [220]. There have been a few studies of the pharmacokinetics of AZ as highlighted in a recent review [214]. In healthy Bangladeshi individuals, a single 500 mg tablet was administered under fasting conditions followed by a period of 3 weeks in which no drug was taken. The following data were determined: a maximum plasma concentration of 382.4 ng mL^{-1} was reached after 4.83 h, $AUC_{0\rightarrow\infty}$ was 6.307.5 h ng mL^{-1} and the mean half-life was 41.44 h [221].

A study carried out in India showed that when AZ was used alone in adults with acute uncomplicated falciparum malaria, clearance of parasites was incomplete [32]. In a study in Bangladesh, azithromycin-artesunate administered once daily for 3 days (30 mg kg^{-1} per day of AZ plus 4 mg kg^{-1} per day of ART) to patients with uncomplicated falciparum malaria, parasite clearance was obtained in a mean of 25.1 h [216].

ARTEMISININ-BASED COMBINATION THERAPIES (ACTs)

The appealing properties of artemisinin and its derivatives, *i.e.,* strong and fast initial activity, favourable pharmacokinetics and apparently excellent human safety and tolerability [220, 222] have made them become favourites for the treatment of malaria. During the 1990s WHO promoted a series of trials in different countries through its Roll Back Malaria Programme to test the efficacy of artemisinins in combination with other antimalarials to treat uncomplicated falciparum malaria [223, 224]. These combinations became known as artemisinin-

based combination therapy (ACT). One of the reasons to try them as combinations was to prevent the development of resistance. The fact that artemisinins have very short half-lives of 2 to 5 h implies that they have to be administered for more than 5 days to be fully effective, which led to high failure rates. When they are combined with other antimalarials with longer half-lives, preferably of more than 24 h, they can be used for shorter periods, and the replicating parasites are not exposed to sub-therapeutical drug levels that favour the development of resistant parasites [223]. The three-day course recommended nowadays by WHO gives 90% reduction of parasites, which are then completely eliminated by the longer-acting drug partner, normally a schizonticide, leading to radical cure. In addition, the gametocytocidal activity of the artemisinins helps to reduce transmission. There are nowadays 5 ACTs recommended for use [1], and even formulations as syrups, powders, and granules to make them appealing to children [223]:

- artemether plus lumefantrine,
- artesunate plus amodiaquine,
- artesunate plus mefloquine,
- artesunate plus sulfadoxine-pyrimethamine, and
- dihydroartemisinin plus piperaquine.

The choice of ACT to use will depend on the resistance patterns in the area or country of intended use. All these drugs are now available as fixed-combinations except for artesunate-sulfadoxine-pyrimethamine [223]. One advantage of fixed-dose combination formulations is that they promote adherence to the treatment. WHO started to recommend the use of combination therapy in 2003, after the first signs of resistance to mefloquine-artesunate were observed in the Pailin province in Cambodia in 2002, particularly for areas where resistance to many antimalarials is common [225, 226]. The Thailand-Cambodia border has been historically the centre of emergence of resistance. However, by 2011 resistance had spread to other areas in East Asia. WHO launched then its Global Plan for Artemisinin Resistance Containment (GPARC) [7], a high-level plan to protect ACTs as an effective treatment for *P. falciparum* malaria. Nowadays ACTs are

recommended by WHO as first line options for malaria treatment [3]. Nevertheless their use is not as widespread as it could be, due to cost and poor access, which are still enormous obstacles [227].

The dose and frequency of administration of the antimalarial drug have to be adequate so as to provide drug concentrations sufficiently high for a certain period of time to kill all the parasites [228]. The drug-concentration time profile is determined from the pharmacokinetics. The pharmacokinetic parameters of the drugs when administered alone may differ from those observed when they are used in a combination. Optimal designs for population pharmacokinetics are being developed [228] to optimize sampling schedules that can become very tiresome for weakened malaria patients, and also to make economical use of resources [228]. Experience has shown that the ACTs are more effective than the individual drugs when administered alone. In the last decade the pharmacokinetic parameters of most ACTs were determined, but the data is still incomplete especially for particularly vulnerable groups such as pregnant women and children. The spread of resistance may also create the need for dose readjustment [57]. Table **3** summarises the pharmacokinetic data obtained in some studies on the five ACTs presently recommended by WHO for the treatment of malaria. The data presented is illustrative, not exhaustive.

The pharmacokinetics of AS and DHA, administered singly or as a combination therapy, have been reviewed recently [160]. For the artesunate-amodiaquine (1:2.5) combination, pharmacokinetics has been studied in healthy volunteers [230]. It was found that the total drug exposure of both drugs was reduced considerably when they were administered as ACTs. AUC for the main active metabolites, DHA and DSA, decreased considerably, from 2044 to 1410 h ng mL^{-1} and from 12,041 to 8,437 h ng mL^{-1}, respectively. AUC is the key parameter for parasite killing and it is a useful predictor of therapeutic response. Since it is known that the ACT is more effective clearing parasitaemia and preventing recrudescence than AQ alone [231], it appears that the effectiveness is related to an additive or possibly synergistic effect. However, this decrease in AUC may become important in areas of high resistance, resulting in reduced parasite killing.

Table 3: Pharmacokinetic Parameters of the ACTs Currently Approved for Malaria Treatment[a]

Antimalarial	Dosage	Drug	C_{max}, ng mL^{-1}	T_{max}, h	AUC, h ng mL^{-1}	$T_{½}$, h	PCT, h	Ref.
Artesunate plus Amodiaquine	AQ 10 mg kg^{-1}, AS: 4 mg kg^{-1}; single dose (healthy volunteers)	AS	141.6	0.86	183.3	NA	72[b]	[229, 230]
		DHA	446.2	2.08	1410.5	2.20		
		AQ	22.7	2.18	108.5	3.9		
		DSA	301.4	2.18	8437	137		
	Artesunate alone: 4 mg kg^{-1} (healthy vol.)	AS	231.8	0.62	206.4	NA		
		DHA	844.5	1.10	2044.4	1.5		
	Amodiaquine alone: 10 mg kg^{-1} (healthy vol.)	AQ	29.2	2.32	162.4	5.3		
		DSA	268.7	3.68	12,041	241		
Artesunate plus Mefloquine	Standard dose: AS: 4 mg^{-1}/kg for 3 d, MQ: 15 mg kg^{-1} on day 1, 10 mg kg^{-1} on day 2 (in malaria)	MQ	3,239	70.9	1095,421	322	40	[232]
		AS	451	0.93	NA	NA		
		DHA	2,043	1.4	3,745	0.8		
	Artesunate: 4 mg kg^{-1} for 3 d; after the 3rd day, MQ administered (in malaria)	AS^1 AS^2	201 316	0.50 1.00	237 338	0.58 0.29	48 72	[233]
		DHA^1 DHA^2	1088 1452	1.01 1.50	2701 3766	0.85 0.77		[235]
	Alternative: 200 mg AS, 750, 500 mg MQ, 6 and 12 h later (in malaria)	MQ	1623	15.0	12.8*	264	47.5	[236]
	MQ alone: 750 mg, 500 mg 6 h later (in malaria)	MQ	2212	20.3	17.2*	288	82.3	[236]
Artesunate plus Sulphadoxinea and Pyrime-thamine	400 mg AS plus 1500 mg SDX, 75 mg PYR, on day 1, then 100 mg AS once a day for 4 d. (healthy volunteers)	SDX	98900	8.0	18876,700	180	72[c]	[237,238]
		PYR	790	3.0	112,000	177		
	SP alone: 1500 mg SDX, 75 mg PYR (healthy volunteers)	SDX	92900	10.0	15840,500	165		
		PYR	860	4.0	124,600	158		
Artmether plus Lumefantrine	20 mg AM, 120 mg LU, twice daily for 3 d Coartem® (in malaria)	AM	66.2	2	211,000	2.2	29	[241]
		DHA	205	2-3	604,000	1.6		[241]
		LU	9000	3-4	561,000	72-96		[242]
	80 mg AM, 480 mg LU at 0, 8, 24, 48 h; (in malaria) Co-artemether	AM	133	2.13	2035	0.86	30	[243]
		DHA	137	2.44	3135	1.06		
	80 mg AM at 0, 8, 24, 48 h (in malaria)	AM	157	1.73	1834	1.16	30	
		DHA	161	1.86	2926	0.95		

(Table 3) contd…..

Antimalarial	Dosage	Drug	C_{max}, ng mL^{-1}	T_{max}, h	AUC, h ng mL^{-1}	$T_{½}$, h	PCT, h	Ref.
Dihydroarte-misinin plus Piperaquine	DHA alone: 2 mg kg^{-1} bw (±50 kg bw); Single dose (healthy vol.)	DHA	181	1.5	377	0.96		[247]
	640 mg PPQ phosphate, 80 mg DHA, Artekin® Single dose (healthy vol.)	PPQ	586.9	2.1	46419	303		[248]
		DHA	244	1.6	450	1.25		[249]
	960 mg PPQ phosphate, 120 mg DHA Single dose (healthy vol.)	DHA	159	1.5	370	1.01	28[d]	[250,251]
	PPQ alone: 640 mg; Single dose (healthy vol.)	PPQ	578.5	2.3	44293	317		[248]
	Dihydroartemisinin alone: 120 mg (healthy vol.)	DHA	442	2.5	858	1.02		[252]

[a]units are µg d mL^{-1}; [b]three-day course in uncomplicated falciparum malaria; [c]AS (4 mg kg^{-1} d^{-1}) for 3 days + SP (25 mg kg^{-1} SDX+1.25 mg kg^{-1}) single dose on day 1, in uncomplicated falciparum malaria; [d]DHA (2.4 mg kg^{-1} d^{-1}) + PPQ (19.2 mg kg^{-1} d^{-1}) together for 3 days in uncomplicated falciparum malaria.

The standard dose prescribed for the artesunate-mefloquine combination is 4 mg kg^{-1} AS per day, with a total MQ dose of 25 mg kg^{-1} split into 2 days [232]. This ACT was introduced as an inexpensive combination therapy directed especially at Sub-Saharan Africa [233], and has now been adopted by many countries in Africa, *i.e.,* Burundi, Liberia, Sudan and Sierra Leone as the first-line therapy, and it has also been recommended for use in Central Angola, for example, where high levels of chloroquine resistance were found to occur [234].

Pharmacokinetic studies in malaria patients treated with the artesunate-mefloquine combination showed significant interactions between the two drugs. Parameters for the standard dose of 4 mg kg^{-1} bw of artesunate were obtained in two locations: Wang Pha in Thailand and Pailin in Cambodia. They were found to be similar, although the areas under the concentration-time curve were slightly greater in Pailin [235]. However, here parasite clearance took an average of 72 h, markedly slower than in Wang Pha, where this was achieved in 48 h. The

difference between the two sites corresponds to the development of some resistance by *P. falciparum* to artesunate in Cambodia. In a different study, in which the same dose of artesunate was administered at the same time as mefloquine, it was found that the pharmacokinetics were similar to those observed in Wang Pha, the area where greater sensitivity to artesunate is observed [232], showing that the presence of mefloquine does not affect the artesunate or dihydroartemisinin disposition kinetics. However, the opposite is not true. The presence of artesunate causes significant changes in mefloquine pharmacokinetics. Mefloquine concentrations are lowered, with C_{max} being reached faster and changing from 2,212 ng mL^{-1} to 1,623 ng mL^{-1} [236]. Consequently AUC also drops. A larger volume of distribution and faster clearance was also observed in patients receiving the combination treatment. Despite these differences, the time for parasite clearance is nearly halved when combination therapy is used.

The artesunate-sulfadoxine-pyrimethamine combination was also studied in healthy volunteers [237]. It was found that artesunate had no effect on the pharmacokinetics of SP, although it has been observed in the past that the combination is more effective to treat malaria than the individual component drugs when administered alone [228]. In malaria, the repeated administration of AS causes a drastic reduction in parasitaemia, which is assisted by a continuous inhibition of folic acid biosynthesis in the parasite for more than 14 days by SP [238]. Cure rates improve, transmission decreases, and the development of resistance is delayed with the ACT. This ACT is being used nowadays in parts of South America, the Middle East, and South Asia where SP susceptibility is still high [227].

The artemether-lumefantrine (1:6) combination is referred to as co-artemether. It was originally developed in China, and in 2007 it was the only ACT registered internationally [239]. Presently it is the only ACT registered in Europe [127]. Lumefantrine is active against all human malaria parasites [227]. The two components have been shown to be synergistic in their action against *P. falciparum in vitro* [240]. The pharmacokinetics of the two drugs given in combination is not much different from those used when they are administered individually [20]. T_{max} of artemether and DHA increase by almost 0.5 h when

artemether is used in combination with lumefantrine, but this is not statistically significant [241-243]. There is also a slight delay in absorption and the absorption time also increases, but the total AUC does not change [239, 244, 245]. Since lumefantrine is highly lipophilic, the bioavailability of this antimalarial increases up to 2-fold when it is administered together with food, which is therefore recommended [239]. Recent research has shown that desbutyl-lumefantrine (DBL), a metabolite of lumefantrine, is a more potent antimalarial than the parent compound with which it acts in synergy against *P. falciparum* and *P. vivax* [246]. It is also synergistic with DHA.

The pharmacokinetics of the dihydroartemisinin-piperaquine (1:8) combination studied in healthy volunteers showed that when a single dose of piperaquine (640 mg) was administered in the presence of artesunate there was no effect on piperaquine pharmacokinetics [247, 248]. Administration of dihydroartemisinin (2 mg kg^{-1} body weight) to volunteers with an average body weight of 50 kg had the same effect as when the drug was administered in combination with piperaquine [249]. However it was found that when twice this amount was administered, C_{max} doubled and AUC was 2.4 times higher. In a different study [250-252] a significant difference was found between single and combined administration of dihydroartemisinin. The oral bioavailability of piperaquine is also increased when it is administered with fat. A fixed-dose combination is now commercially available in many countries in Asia, and also more recently in Africa [227].

THE PIPELINE OF ANTIMALARIALS IN DEVELOPMENT

In the past 40 years antimalarial drug resistance has been increasing in frequency and intensity and it has also been spreading [253]. As a result, the efficacy of some of the more commonly used antimalarials has dropped in the affected areas. Resistance is one of the major obstacles in the fight against malaria. It develops mainly as a result of the extensive use of some antimalarial drugs. It can develop during treatment, when the concentration of a drug falls below subtherapeutic levels, before all the parasites are completely eliminated. It causes selection. It allows the few parasites present that were less susceptible to the drug to be transmitted to the vector and multiply in the next life cycle [254]. Since the concentration of the drug in the body at a given time depends on the

pharmacokinetics of the drug, pharmacokinetics is the one of the most important aspects influencing the onset of resistance [255]. If the drug has a short half-life it has to be administered more frequently. Lack of compliance implies that plasma concentrations drop below the values required to eliminate the parasites. If the drug has a long half-life, problems may arise during prolonged periods at which the concentration of the drug has fallen to low levels. Combinations of drugs with suitable pharmacokinetics can help to solve this problem. Genetic mutations that confer resistance can also occur spontaneously, but they are rare [7, 256]. Point mutations in the genes encoding for a drug target in the parasite will result in less affinity of the target for the drug, and hence the drug will be less effective [7]. Various aspects related to resistance are focused in a few recent reviews [257, 258].

Resistance has been reported to occur with *P. falciparum, P. vivax* and *P. malariae.* The resistance of *P. falciparum* to two of the most widely used antimalarial drugs, chloroquine and sulfadoxine-pyrimethamine occurs now on a global scale [82], but resistance has also been reported to almost all other antimalarials being currently used, *e.g.,* amodiaquine, mefloquine, quinine, although it varies considerably from region to region [3]. Recently there have also been reports of resistance to the artemisinins in a number of countries in South-East Asia, particularly on the Cambodia-Thailand border, Myanmar and Vietnam [1]. *P. vivax* shows resistance to sulfadoxine-pyrimethamine in many areas, and also to chloroquine, but in this case it is confined largely to Indonesia, East Timor, Papua New Guinea and other parts of Oceania [6]. It has also been reported in Brazil and Peru. Immunity is reduced in pregnancy and also when individuals move out of the transmission areas. The increase in the numbers of international travellers has also contributed to an increase in imported malaria [259].

Resistance can be prevented or at least be delayed by combining drugs or through the use of antimalarials with different modes of action. The chance of the parasite developing resistance against both simultaneously is much lower. The use of combinations of drugs is common practice in the treatment of other diseases such as tuberculosis, most HIV and cancer [253]. In malaria the situation can also be complicated by cross-resistance, which can occur among drugs that belong to the same chemical family or which have similar modes of action [3]. Multidrug

resistance also occurs, which implies that the parasite is resistant to more than two antimalarial compounds of different chemical classes and modes of action. Table **4** summarizes the main target molecules or processes aimed at by antimalarials presently in use or under development [260-262].

Table 4: Main Drug Targets for Malaria Chemotherapy [260-262]

Target Location	Pathway/Mechanism	Target Molecule or Process	Examples
Cytosol	Folate metabolism	Dihydrofolate reductase	**Pyrimethamine, proguanil, cycloguanil, chlorproguanil, trimethoprim, P218**
		Dihydropteroate synthase	**Sulfadoxine, sulfalene, dapsone**
	Purine biosynthesis (Salvage pathway)	Purine nucleoside phosphorylase	**BCX4945**
	Pyrimidine biosynthesis	Dihydrooroate dehydrogenase	**DSM265**
Parasite membrane	Phospholipid metabolism		**Albitiazolium**
	Membrane transport	*Pf*CRT	**Piperaquine**
Food vacuole	Haemoglobin metabolism	Hemozoin formation	**Chloroquine, quinine, quinidine, amodiaquine, halofantrine, lumefantrine, mefloquine, 8-aminoquinolines, ferroquine**
		Haem iron as activator of protein alkylation	**Artemisinins**
		Free radical generation	**Artemisinins**
Mitochondrion	Electron transport	Ubiquinol-cytochrome C oxidoreductase	**Atovaquone**
Apicoplast	Translation		**Tetracyclines, clindamycin, azythromycin, spiroindole**
	Isoprenoid biosynthesis	DOXP reductoisomerase	**Fosmidomycin**
Endoplasmic reticulum	**Ca^{2+} level regulation (ATP-dependant Ca^{2+} pump)**	**ATPase 6 (SERCA)**	**Artemisinins**

Resistance is the main factor determining the life span of a drug [253]. It is therefore clear that there is a basic need for sustainable development and discovery of new drugs for the future, to fill in any gaps that may arise. This also applies to the need to develop new insecticides, for use in nets, since insecticide resistance to the pyrethroids, the agents that are most commonly used for this

purpose, has already been reported in 27 countries in Africa and 41 countries worldwide [1]. New research and development in this area is needed for improved antimalarial drugs, vector control tools, diagnostics and vaccines. Ideally new antimalarials should be cheap, well tolerated, require the administration of fewer doses, and be useful for many types of malaria [253, 263].

Although antimalarials are needed in many parts of the world, the market is small in terms of profit, and by 1999 the pipeline for new antimalarials was almost empty [259]. The United Nations resolution to eradicate malaria from the planet, implied in one of its Goals for the Millennium, brought a new impetus to research in this area.

Nowadays there are a few international organizations committed to the fight against malaria [264]:

- Foundation for Innovative New Diagnostics (FIND)
- Global Fund to Fight AIDS, Tuberculosis and Malaria
- Medicines for Malaria Venture (MMV)
- Multilateral Initiative on Malaria (MIM)
- PATH Malaria Vaccine Initiative (MVI)
- Roll Back Malaria
- Special Programme for Research and Training in Tropical Diseases (TDR)

The global malaria medicine pipeline is now more extensive than in the past [265]. Many of the products being developed have the support of the Medicines for Malaria Venture, an organization created in 1999. Built on experiences from the Special Program for Research and Training in Tropical Diseases [82], it operates in concert with the Roll Back Malaria Program of the WHO, bringing together teams of scientists from the academia and industry, through suitable finance mechanisms, to develop projects leading to the development of novel

antimalarial products. Nowadays it has partnerships with more than 260 research institutions and countries across the world.

Fig. (**16**) shows the pipeline of antimalarial drugs being developed worldwide at the first quarter of 2012, according to the Medicines for Malaria Venture [266]. It includes medicines that have been already approved and are now in Phase IV of development, *i.e.,* they are undergoing post-marketing real world surveillance studies, as opposed to those previously carried out in a controlled environment [267]. The properties of these antimalarials have been discussed previously in this chapter. Table **5** shows endoperoxide ring nomenclature. Tables **6** and **7** summarize some of the properties of the prospective antimalarials.

Drugs Waiting Registration (See Table 6)

An ACT, in the form of one tablet consisting of a fixed-dose combination of artesunate **23** and mefloquine **7** is one of the new options awaiting registration. The artesunate-mefloquine combination is already recommended by WHO for the treatment of uncomplicated and severe *P. falciparum* and also for *P. vivax* malaria, but it has been administered so far as a combination of two separate tablets of mefloquine and artesunate. At this stage is also a new formulation of artesunate for the treatment of acute malaria, in the form of suppositories.

Drugs in Phases IIB and III (See Table 6)

Two new antibiotic combinations are being developed. One is a fixed-dose of the antibiotic azithromycin **35** in combination with chloroquine **3**, being developed as intermittent preventive treatment in pregnant women, as an alternative to sulphadoxine-pyrimethamine, against which resistance has developed in some parts of the world. The other is Co-trimoxazole, a combination of trimethoprim **37** and sulphamethoxazole **38**. This combination is already recommended as part of the standard treatment for people infected with the human immunodeficiency virus (HIV) and those with acquired immunodeficiency syndrome, and also for integrated management of childhood illness in Africa [268]. In malaria Co-trimoxazole acts as an antifolate, and it is another possible successor to sulphadoxine-pyrimethamine.

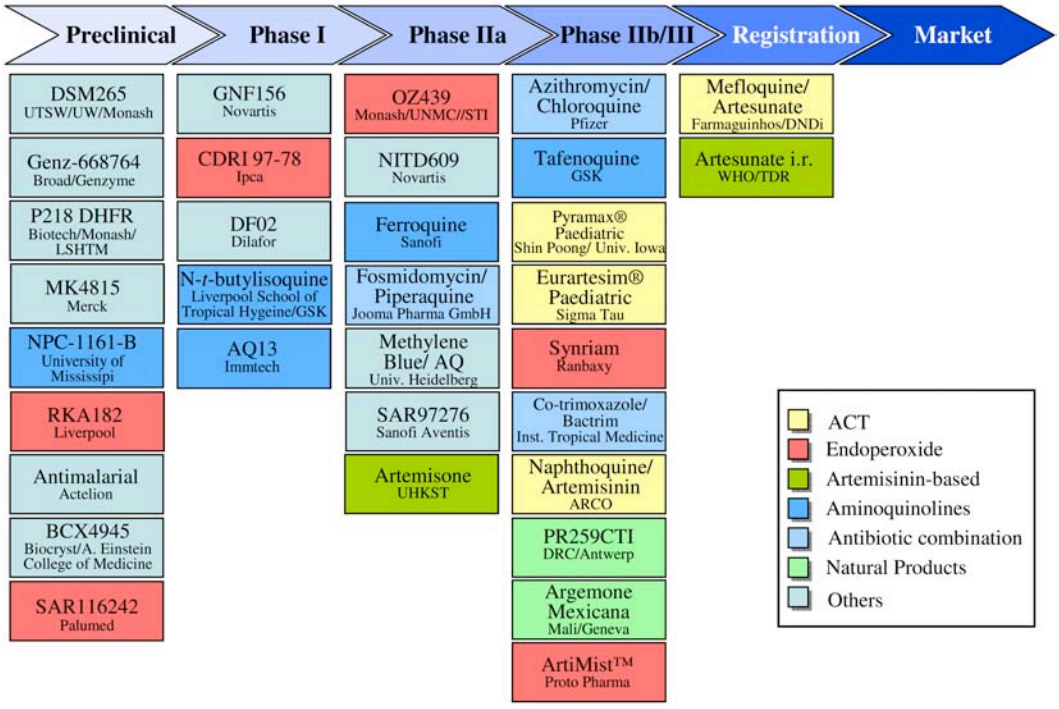


Figure 16: The world pipeline of antimalarials in development according to the Medicines for Malaria Venture [266].

ACTs in this stage of development include Eurartesim® Paediatric, a child friendly dispersible formulation consisting of a fixed dose of dihydroartemisinin **21** and piperaquine **4**, already available as tablets for adults and recommended by WHO for the treatment of uncomplicated falciparum malaria. Pyramax® Paediatric is an ACT consisting of a fixed combination of pyronaridine **39** and artesunate **23**, which is being developed as a fixed combination for children. Pyronaridine is a blood schizonticide, a Mannich base which instead of the usual quinoline heterocycle possesses an azaacridine ring system. It is effective against *P. falciparum* and *P. vivax* malaria. The tablet formulation received a positive scientific opinion from the European Medicines Agency. A fixed-dose combination of artemisinin **20** and naphthoquine **40** is also being developed as an alternative for the treatment of all forms of malaria.

A completely new fixed-dose combination is Synriam, already launched in India as an alternative for the treatment of *P. falciparum* malaria. This non-artemisinin

based combination therapy (NACT) combines a synthetic fast-acting blood schizonticide, arterolane **41**, with a half-life of approximately 1 to 3 h, with a longer acting one, piperaquine **4**, with a half-life of approximately 23 days [242].

Monotherapies in phases IIB and III of development include tafenoquine **42**, an alternative to primaquine **6**. PQ is the only drug available at present that is active against hypnozoites of the relapsing parasites *P. vivax* and *P. ovale*. Tafenoquine is the only molecule in the pipeline with activity against *P. vivax* hypnozoites. ArTiMist, a sublingual spray formulation of artemether **22** is another monotherapy being developed to treat uncomplicated *P. falciparum* malaria in young children and infants. It is also effective in severe malaria.

Included in this group are also two herbal medicines. One is *Argemone mexicana*, a weed found in many tropical areas, used by traditional healers in Mali as a decoction for the treatment of malaria. IC_{50} values of the aqueous decoction and methanol extracts of the aerial parts of the plant, against *P. falciparum* (chloroquine-resistant K1 strain) were found to be 5.89 and 1.00 μg mL^{-1} respectively [270]. In a trial conducted in Mali, its performance was compared with that of artesunate-amodiaquine ACT [271]. Both treatments were well-tolerated. Second-line treatment was not required for 89% of patients treated with *Argemone mexicana versus* 95% treated with the ACT. In cases of severe malaria it was found to perform as well as the artesunate-amodiaquine treatment.

The other antimalarial herbal medicine is PR259 CTI, a quantified 80% ethanol extract from the stem bark of *Nauclea pobeguinii*, containing strictosamide **43** as the major alkaloid [272]. It has moderate *in vitro* activity against *P. falciparum*, with IC_{50} values in the range 32-44 μg mL^{-1}.

Drugs in Phase IIA (see Table 6)

There are two NACTs in this stage of development: fosmidomycin **44**-piperaquine **4** and methylene blue **45**-amodiaquine **5**. Fosmidomycin (FOS) is an antibiotic that has potent activity against Gram-negative bacteria. Initially it was isolated from cultures of *Streptomyces lavendulae*, but nowadays it is produced synthetically. It is also active against *Plasmodium* parasites, being at present the

most potent and rapid acting antibiotic against malaria [167]. It targets isoprenoid biosynthesis in the apicoplast of the parasite, more particularly the action of 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, the second enzyme of the pathway [273]. Since in mammals isoprenoids are derived from an alternative pathway, the mevalonate pathway, FOS can be used safely to treat malaria. It is a blood schizonticide, but in contrast to other antibiotics with antimalarial properties it does not exert a delayed death effect [274], and hence it acts more rapidly. The combination dihydroartemisinin-piperaquine is an ACT currently recommended by WHO for the treatment of malaria. A synergic effect has been observed *in vitro* against *P. falciparum* drug-sensitive 3D7 and CQ-resistant K1 strains when fosmidomycin was added to piperaquine, which led to the identification of these drugs as therapeutic partners [275]. Phase II trials started at the end of 2011 in Thailand.

The second NACT in this stage of development is a combination of the first compound ever used to treat malaria, methylene blue (MB) and amodiaquine. Methylene blue as a monotherapy is not recommended for malaria treatment nowadays. It is however used in medicine as a component of a drug prescribed frequently, Prosed®, which is a urinary analgesic/anti-infective/anti-spasmodic medicine. MB is a specific inhibitor of the enzyme glutathione reductase (GR) and it inhibits haem polymerization within the parasite's food vacuole, preventing haem detoxification [276]. This inhibitory effect of GR is one of the probable reasons for the renewed interest in this substance for malaria therapy [6]. Since high levels of glutathione are usually found in the digestive vacuole of resistant strains, it is thought that one of the mechanisms of resistance is the fact that GSH reduces the level of free haem available for CQ binding. Hence PfCRT mutant strains are less susceptible to CQ. This has led to the theory that a combination of CQ with a glutathione reductase inhibitor might help overcome resistance [6]. However, initial studies of MB in combination with CQ showed that they are antagonistic *in vitro.* The combination artesunate-amodiaquine is nowadays one of the ACTs recommended by WHO for the treatment of malaria. MB-AQ could provide an alternative medicine if resistance developed. In trials in Burkina-Faso to treat uncomplicated falciparum malaria in children, a 95% efficacy at day 28 was obtained with the combination AQ-MB [277]. Methylene blue also has the

property of preventing methemoglobinemia, a serious complication that may arise in malarial anaemia [276]. MB is also capable of inhibiting gametocyte development at all stages [278], and the combination AQ-MB, two gametocytocidal drugs, could be a potent agent to prevent transmission of malaria and even lead to its eradication in areas where it is used.

Monotherapies in Phase IIA of development include OZ439, a synthetic peroxide **46**. Its IC_{50} values are comparable to those of artemisinin derivatives presently in clinical use [279]. However, due to the fact that this synthetic molecule has a more stable peroxide bond, which, as it happens with the artemisinins, is the unit responsible for activity, the half-life of OZ439 is much longer, approximately 32 h. Clearance is also reduced and the drug shows a much longer blood *versus* time profile [280].

Also at this stage of development is a novel type of antimalarial **47**, spiroindole NITD609. This synthetic molecule, prepared as a single active enantiomer, is as effective as artesunate. Against *P. falciparum* and *P. vivax* strains from the Thai-Burmese border, it showed $IC_{50s} < 10$nM. It is a blood schizonticide, and it kills both mature trophozoite and immature *P. vivax* ring stages, in contrast to the trophozoite stage-specific activity of chloroquine [281]. Its mechanism of action is different from those of existing antimalarial drugs, suppressing protein synthesis in the parasite. Its pharmacokinetic properties are compatible with once a day oral dosing, it has prophylatic activity, and it is a potent inhibitor of gametocytogenesis, blocking transmission to *P. falciparum* [282].

The fact that it was observed in the past that chloroquine analogues with shortened side-chains are good schizonticides, led to the development of another prospective antimalarial, ferroquine **48** [283], a derivative of chloroquine. In this drug, a ferrocene unit replaces the carbon side-chain of chloroquine. Ferroquine has potent activity against *P. vivax* schizont maturation (IC_{50} = 15 nM) and it is also effective against *P. falciparum* [284]. Its mechanism of action is probably similar to that of chloroquine, involving hematin as the drug target and inhibition of hemozoin formation. Since chloroquine is still the most common first-line treatment for *P. vivax* malaria, ferroquine could provide another alternative [285, 286].

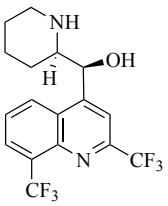
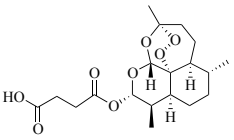
A new semi-synthetic second-generation derivative of artemisinin **20**, artemisone **49**, is also being developed. Artemisone and analogues, with a nitrogen substituent at position 10, were designed to have improved bioavailability and metabolic stability over the first generation artemisinin derivatives [287]. These N,O-acetals cannot be metabolised to dihydroartemisinin, and it was thought that in this way the neurotoxicity associated with artemisinin derivatives could be eliminated. In fact, artemisone has a slightly longer half-life than other artemisinin derivatives ($t_{½}$ 2.8 h), better bioavailability [288] and it shows no neurotoxicity [289].

Table 5: Endoperoxide Nomenclature [293]

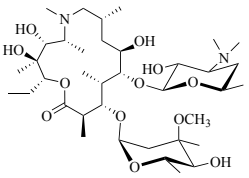
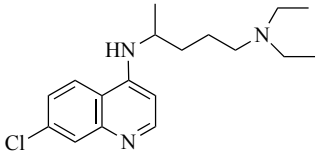
Nomenclature	Structure	Number of Oxygens	Ring Size
1,2-Dioxolane		2	**5**
1,2,4-Trioxolane		3	**5**
1,2-Dioxane		2	**6**
1,2,4-Trioxane		3	**6**
1,2,4,5-Tetraoxane		4	**6**
1,2-Dioxepane		**2**	**7**

An antimalarial with an entirely different structure is SAR97276, now named Albitiazolium, a bis-thiazolium compound **50** [263]. This choline analogue inhibits phospholipid metabolism, interfering with plasmodial

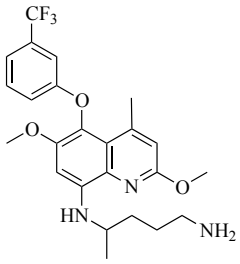
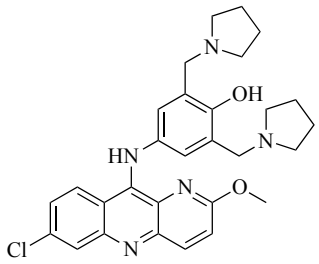
Table 6: New Antimalarial Medicines Under Development in the First Quarter of 2012 [266]

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Waiting registration	A 2.2:1 fixed-dose combination of mefloquine **7** (MQ) and artesunate **23** (AS) [Farmanguinhos/ Drugs for Neglected Diseases Initiative, (DNDi)]	MQ: Quinoline, aryl amino alcohol CAS = 53230-10-7  AS: Endoperoxide CAS = 88495-63-0  DHA, 21	ACT for the treatment of uncomplicated *P. falciparum* malaria, *P. vivax* malaria, severe malaria. MQ: Synthetic; used as a racemic mixture; it acts as a blood schizonticide effective against erythrocyte stages of *P. falciparum* and *P. vivax.* It inhibits hemozoin formation and hence haem detoxification in the parasite; IC_{50} = 27 nM (*P. falciparum*); $t_{½}$ 2-4 weeks; F (tablet *vs* oral solution) > 85%. AS: Semi-synthetic derivative of artemisinin; blood schizonticide, active on all 4 species of Plasmodium; it has been proposed that it generates fatal free radicals in the lysosomal food vacuole, which damage the parasite, but this may not be the only mechanism of action; IC_{50} = 1.62 nM (*P. falciparum*); $t_{½}$ 1 h; F(oral) = 61%. ACT pharmacokinetics: [8:4 mg kg^{-1}/day MQ:AS for 3 d]: For MQ: C_{max} 2,202 ng mL^{-1}, T_{max} 2.4 d, $AUC_{0-\infty}$ 31,395 d ng mL^{-1}, $t_{½}$ = 10.5 d; PCT = 48 h.	[1, 58, 159, 293-295]
Waiting registration	Artesunate **23** intrarectal (i.r.) [WHO/ The special Programme for Research and Training in Tropical Diseases (TDR)]	AS: Structure as above in this table.	For the treatment of acute malaria. AS is quickly converted in the body to dihydroartemisinin (DHA), the active metabolite, by blood esterases and the hepatic metabolism. Pharmacokinetics of the suppositories (DHA): 10 mg kg^{-1} suppositories: F = 58%, PCT = 20 h; 20 mg kg^{-1} suppositories: F = 23%, PCT = 22 h. For both (mean): C_{max} 4.5 µM, T_{max} 0.2 h, AUC = 4.1 h µmol L^{-1}, $t_{½}$ 0.3 h.	[296-298]

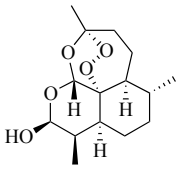
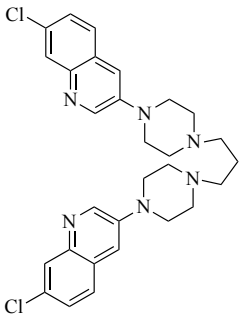
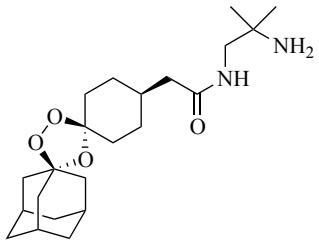
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	A fixed-dose combination of azithromycin **35** (AZ) and chloroquine **3** (CQ) (Pfizer)	AZ: macrolide antibiotic, of the azalide class CAS = 83905-01-5  PPQ, 4 CQ: 4-aminoquinoline CAS = 54-05-7 	To be adopted as Intermittent Preventative Treatment in Pregnant Women. The combination is designed to be synergistic against CQ-resistant strains of *P. falciparum*. It may simultaneously have activity against bacterial sexually-transmitted diseases. AZ: Semi-synthetic widely used broad-spectrum antibiotic; it is also an efficient blood schizonticide; it inhibits protein biosynthesis inside the apicoplast; destruction of this plastid prevents the parasite from entering a new cell; IC_{50} (laboratory strains) = 3.0-6.5 µM, IC_{50} (wild isolates) = 29.3 µM (*P. falciparum*); $t_{½}$ 68 h; F = 37%. CQ: Synthetic, highly effective against erythrocytic forms of *P. vivax*, *P. ovale* and *P. malariae*, sensitive strains of *P. falciparum* and gametocytes of *P. vivax;* it is thought to inhibit heme polymerase activity, resulting in accumulation of free heme, toxic to the parasites; IC_{50} = 49.8 ng mL^{-1} (*P. vivax*); IC_{50} = 167 nM (*P. falciparum*); $t_{½}$ 1-2 months F = 89% (as phosphate, in tablets). AZ+CQ: the pharmacokinetics of the two drugs when used alone or in combination are very similar. Together, they are effective against CQ-resistant *P. falciparum in vivo*.	[58, 299-305]

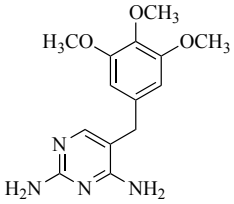
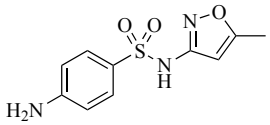
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	Tafenoquine **42** (WR-238605) (GlaxoSmith-Kline)	8-aminoquinoline CAS = 106635-81-8 	For the treatment of *P. vivax* (relapsing) malaria. Synthetic; active against the dormant liver form of *P. vivax* (the hypnozoites) *in vitro* and in patients. It is thought to act by inhibiting electron transport in the respiratory chain and to inhibit heme polymerization. This is the only molecule in the pipeline with activity against *P. vivax* hypnozoites. IC_{50} = 9.7 µM (wild strains of *P. vivax*); $t_{½}$ 2 weeks. It is also active against all stages of *P. falciparum*: IC_{50} = 4.43 µM (wild strains in Central, West and East Africa); IC_{50} = 0.06-0.3 µM in MDR *P. falciparum* lines.	[6, 53, 306-308]
Phase IIb/III	Pyramax® Paediatric: a 3:1 fixed-dose combination of pyronaridine **39** (PYR) and artesunate **23** (AS) (Shin Poong Pharmaceuticals/ University of Iowa)	PYR: 9-anilinoazaacridine CAS = 74847-35-1  AS: Endoperoxide Structure as above in this table	For uncomplicated *P. falciparum* malaria and for the blood stage of *P. vivax* malaria. A child-friendly granule formulation developed for use in paediatric patients from 3 months. PYR: Synthetic drug that inhibits the formation of β-haematin, preventing the malarial parasite from neutralising haem, thus being toxic to it. IC_{50} = 1.92 nM (*P. falciparum*); IC_{50} = 2.58 (*P. vivax*). AS: Information as above in this table. Pharmacokinetics of the granules (9:3 mg kg^{-1} dose of ACT): PYR: C_{max} 168 ng mL^{-1}, T_{max} 2.4 h, $AUC_{0-\infty}$ 25,325 h ng mL^{-1}, $t_{½}$ = 6.7 d; AS: C_{max} 171 ng mL^{-1}, T_{max} 0.5 h, $AUC_{0-\infty}$ 179 h ng mL^{-1}, $t_{½}$ = 1.2 h; PCT = 8 h.	[58, 293, 309-311]

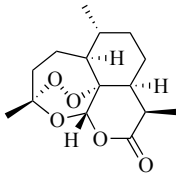
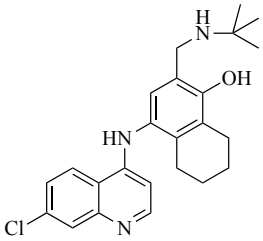
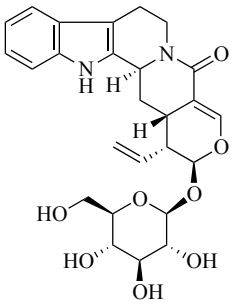
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	Euratesim® Paediatric: a 1:8 fixed-dose combination of dihydroartemi-sinin **21** (DHA) and piperaquine **4** (PPQ) Sigma-Tau Pharmaceuticals Inc./MMV	DHA: Endoperoxide CAS = 71939-50-9  PPQ: 4-aminoquinoline; bisquinolone CAS = 4085-31-8; 85547-56-4 (phosphate) 	Child-friendly dispersible formulation of the ACT to treat uncomplicated *P. falciparum* malaria in infants and children. The adult formulation is recommended for *P. vivax* too. DHA: Semi-synthetic derivative of artemisinin, potent blood schizonticide; it has been proposed that it generates fatal free radicals in the lysosomal food vacuole, which damage the parasite, but this may not be the only mechanism of action; IC_{50} = 1.22 nM; $t_{½}$ = 0.36 h. PPQ: Synthetic drug; it inhibits the parasite by binding to toxic haem (derived from the patient's haemoglobin) within the malaria parasite, and preventing its detoxification *via* a polymerisation step. IC_{50} = 36-41 nM (field isolates in Africa); $t_{½}$ = 23 days. Pharmokinetics for children (DHA+PPQ tetraphosphate, three daily 2.5:20 mg kg^{-1} doses as tablets): PPQ: $AUC_{0-\infty}$ = 44,556 µg h^{-1} L^{-1}; $t_{½}$ (PPQ) 20 d; $t_{½}$(DHA) > 1.2 h. PCT (standard dosage of 2.25 mg kg^{-1} and 18 mg kg^{-1} per dose of DHA and PPQ, for uncomplicated malaria) = 2 days.	[6, 312-317]
Phase IIb/III	Synriam®: a 1.5:7.5 fixed-dose combination of arterolane **41** (AR) (OZ277, RBX 11160) and piperaquine **4** (PPQ) (Ranbaxy Laboratories)	AR: Endoperoxide CAS = 664338-39-0  PPQ: aminoquinoline; bis-quinolone, as phosphate Structure as above in this table	For the treatment of uncomplicated *P. falciparum* malaria. AR: synthetic 1,2,4-trioxolane; a blood schizonticide. IC_{50} = 1.5 nM (*P. falciparum*), $t_{½}$ = 0.8-4.2 h. Pharmacokinetics of arterolane (once a day for 7 days, in uncomplicated falciparum malaria): for a 100 mg dose, on day 1, $AUC_{0-,8}$ = 105 h ng mL^{-1}; for a 200 mg dose, 239 h ng mL^{-1}. Median PCT (both doses) 32 h. PPQ: data as above in this table. Pharmacokinetics (AR:PPQ 150 mg AR maleate and 750 mg PPQ phosphate): PCT = 30 hours;	[318 - 320]

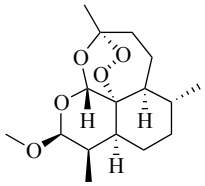
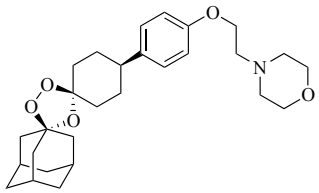
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	Co-trimoxazole (Bactrim): a 1:5 fixed-dose combination of trimethoprim **37** (TMP) and sulphamethoxazole **38** (SMZ) (Institute of Tropical Medicine)	TMP: Diaminopyrimidine CAS = 738-70-5  SMZ: Anilinosulphonamide CAS = 723-46-6 	The combination is an antibiotic used to treat various bacterial infections, but also effective in malaria treatment and prophylaxis. It inhibits successive steps in folate synthesis, an essential part of the thymidine synthesis pathway, thus preventing the synthesis of nucleotides necessary for DNA replication. Together they have a synergistic effect. Recommended as part of the treatment for people infected with HIV and with AIDS. TMP: synthetic; it blocks folic acid synthesis in the microorganism by inhibiting dihydrofolate reductase; EC_{50} = 2.5×10^{-8} M (*in vitro*); $t_{½}$ 8-11 h ; F (oral): 85-90%. SMZ: Synthetic anilinosulphonamide; it inhibits dihydropteroate synthetase, an enzyme involved further upstream in the thymidine synthesis pathway; EC_{50} = 1.2×10^{-7} M (*in vitro*); $t_{½}$ 10 h. Pharmacokinetics of TMP+SMZ [TMP 20 mg kg^{-1}, SMZ 100 mg kg^{-1}, administered orally for 3 days, divided in 3 portions taken every 6 h]: TMP: C_{max} 13.6 µgmL^{-1}, T_{max} 1.9 h, $AUC_{0-\infty}$ 70.9 h mgL^{-1}; $t_{½}$ = 13.6 h; SMZ: C_{max} 372 µg mL^{-1}, T_{max} 2.6 h, $AUC_{0-\infty}$ 2,069 h mg L^{-1}, $t_{½}$ = 14.0 h. PCT (acute uncomplicated malaria) = 2.9 days in adults; 2.6 days in children.	[58, 321-326]

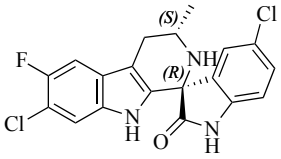
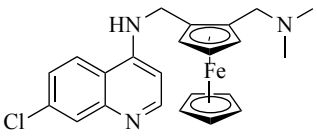
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	ARCO™: a 2.5:1 fixed-dose combination of artemisinin **20** (ART) and naphthoquine **40** (NQ) Kunming Pharmaceutical Corporation (KPC)	ART: Endoperoxide CAS = 63968-64-9  NQ: 4-aminoquinoline CAS = 663215-06-3 	ACT indicated for the treatment of all forms of malaria, including multi-drug resistant malaria. ART: Isolated from the plant *Artemisia annua*. It has been proposed that it generates fatal free radicals in the lysosomal food vacuole, which damage the parasite, but this may not be the only mechanism of action; Fast schizonticidal action. IC_{50} = 12.1 nm-21nM; $t_{½}$ = 1.9-2.61 h. NQ: Synthetic, registered in China in 1993; a *P. falciparum* schizonticide; IC_{50} = 7.0 nM (*P. falciparum*); $t_{½}$ 276 h. Pharmacokinetics (ART+NQ) [single dose 1,000 mg ART/400 mg NQ]: The combination increases $AUC_{(0\rightarrow t)}$ and C_{max} of both components relative to their monotherapies. The increases are: ART: $AUC_{(0\rightarrow t)}$ 71%, C_{max} 49%; NQ: $AUC_{(0\rightarrow t)}$ 135%, C_{max} 104%. With various doses, $t_{½}$ = 255 h; PCT = 21-28 h. ED_{50} (combination) = 8.5 mg kg^{-1}.	[6, 126, 300, 327-332]
Phase IIb/III	Argemone Mexicana (Mali/Geneva)		Mexican poppy or prickly poppy, of the *Papaveraceae* family. A decoction made from extracts of the aerial parts of the plant is used by traditional healers in Mali to cure malaria. IC_{50} = 1.00-5.89 µg mL^{-1} (*P. falciparum* chloroquine-resistant K1 strain)	[270-271]
Phase IIb/III	PR 259 CT1 (DRC/Antwerp)	CAS = 23141-25-5 	An antimalarial herbal medicinal consisting of a quantified 80% ethanol extract from the stem bark of *Nauclea pobeguinii*, a plant of the Rubiaceae family. It has been shown to display moderate *in vitro* activity against *P. falciparum*; IC_{50} = 32-44 µg mL^{-1}. Strictosamide **43** is the major alkaloid present.	[272]

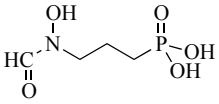
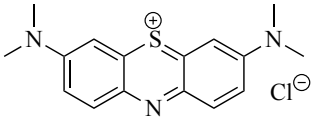
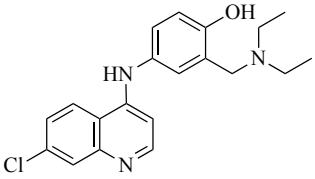
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIb/III	ArTiMist: a sublingual formulation of artemether **22** (AM) (Proto Pharma Ltd)	AM: Endoperoxide CAS = 71963-77-4 	A sublingual spray to treat *P. falciparum* uncomplicated malaria in young children and infants. Also effective for severe malaria. AM is a semi-synthetic derivative of artemisinin. It has properties and a mode of action similar to those of artemisinin, including gametocytocidal activity. IC_{50} = 1.54-16.2 nM (wild isolates). Pharmacokinetics [6× 3 mg kg^{-1} doses of ArTiMist at 0, 8, 24, 36, 48, 60 h]: for AM: C_{max} = 271 ng mL^{-1}, T_{max} = 1.6 h, $AUC_{0-\infty}$ 1,004 h ng mL^{-1}, $t_{½}$ = 4.5 h; for DHA: C_{max} = 106.5 ng mL^{-1}, T_{max} = 1.75 h. F = 2.5 × F (oral artemether). PCT_{90} = 17.6 h.	[6, 53, 333]
Phase IIa	OZ439 Monash Institute of Pharmaceutical Sciences/University of Nebraska Medical Centre/ Swiss Tropical and Public Health Institute (Monash/ UNMC/STI)	Endoperoxide **46** CAS = 1029939-86-3 	Synthetic peroxide, a 1,2,4-trioxolane, with similar efficacy as artemisinin against both *P. falciparum* and *P. vivax.* It inhibits gametocyte maturation/gamete formation and impact sporogony. It is thought to act by alkylating haem and other molecules and degrading membrane phospholipids. IC_{50} = 1.6-1.9 ng mL^{-1} (*P. falciparum* strains); it has *in vitro* activity against all asexual erythrocytic stages: IC_{50} = 1.9, 3.0, and 2.8 ng mL^{-1} for rings, trophozoites, and schizonts. Pharmacokinetics (single dose, 800 mg): C_{max} 315 ng mL^{-1}, T_{max} 3.0 h, $AUC_{0-\infty}$ 4,690 h ng mL^{-1}, $t_{½}$ = 27.9 h; (single dose, 1,200 mg): C_{max} 701 ng mL^{-1}, T_{max} 3.0 h, $AUC_{0-\infty}$ 9,130 h ng mL^{-1}, $t_{½}$ = 31.6 h; the PK parameters were approximately dose proportional.	[280, 293, 334]

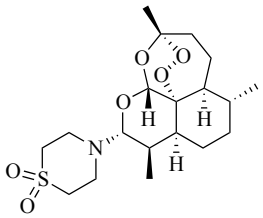
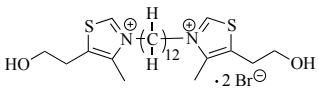
(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIa	NITD609 (Novartis Institute for Tropical Diseases)	Spiroindole **47** CAS = 1193314-23-6 	Synthetic molecule; potent against the intraerythrocytic stages of *P. falciparum* and *P. vivax*. It inhibits protein synthesis in *P. falciparum*. It targets both liver and blood stages of the parasite. One of only a handful of molecules capable of completely curing a *P. berghei* model of blood-stage malaria. At a high concentration (≈100 × IC_{50} value), all stages of the parasite (rings, trophozoites, and schizonts) are similarly sensitive. At low concentrations (≈1 or 10 × IC_{50} value), schizonts are the most susceptible. It also inhibits gametocytogenesis. IC_{50} = 0.5-1.4 nM (*P. falciparum* laboratory strains); IC_{50} < 10nM (*P. falciparum* and *P. vivax* blood isolates) Human phase II trials begin in 2012.	[281, 335]
Phase IIa	Ferroquine **48** (Sanofi-Aventis)	4-Aminoquinoline CAS = 185055-67-8 	A ferrocenic derivative of chloroquine being developed to treat *P. falciparum* malaria. It may be useful for the treatment of drug-resistant *P. vivax* malaria too. The mechanism of action is probably similar to that of chloroquine, with hematin as the drug target. IC_{50} = 14-42 nM (laboratory strains of *P. falciparum*); IC_{50} = 1-62 nM (field isolates); IC_{50} (*ex vivo P. vivax* schizont maturation) = 15 nM. Pharmacokinetics in asymptomatic carriers of *P. falciparum* infections (400-1200 mg dose): C_{max} = 82-270 ng mL^{-1}, T_{max} = 6.00-5.52 h; (400-1,600 mg dose): AUC = 13,100-49,200 h ng mL^{-1}; $t_{½}$ 16 d.	[6, 336-338]

(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIa	A fixed-dose combination of fosmidomycin **44** (FOS) and piperaquine **4** (PPQ) (Jomaa Pharma GMbH) (and MMV)	FOS: N-Hydroxyformamide phosphonic acid CAS = 185055-67-8  PPQ: structure as above in the table	For the treatment of acute *P. falciparum* malaria. FOS: It was initially developed as an antibacterial agent. In malaria it is an effective blood schizonticide. It acts on *P. falciparum* by inhibiting 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a key enzyme in the non-mevalonate pathway of isoprenoid biosynthesis. It blocks biosynthesis of isoprenoids, essential for cellular functions in *P. falciparum*. The pathway is different in humans, which are therefore not affected. IC_{50} = 400 nM. It is the most potent and rapid acting antibiotic against malaria. Fosmidomycin pharmokinetics [1200 mg dose every 8 h for 7 days]: in patients with acute uncomplicated *P. falciparum* malaria, C_{max} = 4.58 μg mL^{-1}, T_{max} 2 h, $AUC_{0-\infty}$ = 29.7 h μg mL^{-1}, $t_{½}$ 3.4 h; PCT = 44 h. PPQ: as above in the table. Fosmidomycin + PQ: trials will start at the end of 2011 in Thailand.	[6, 274, 339]
Phase IIa	A fixed-dose combination of methylene blue **45** (MB) and amodiaquine **5** (AQ) (University of Heidelberg)	MB: Phenothiazinium derivative CAS = 61-73-4  AQ: 4-Aminoquinoline CAS = 86-42-0 	MB was one of the first synthetic drugs used against malaria. Not recommended for this purpose nowadays. The combination is being studied for this purpose. It has a gametocytocidal effect. MB: A gametocytocide; it acts by inhibiting disulfide reductases and heme detoxification. For European Pharmacopoeia-grade MB, Proveblue, mean IC_{50} = 3.62 nM (against 23 *Plasmodium falciparum* strains from several countries). AQ: Aminoquinoline with anti-inflammatory properties. It is indicated for the treatment of acute malaria. The combination: In a study MB-AQ gave adequate clinical and parasitological response after 14 days.	[53, 58, 340]

(Table 6) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase IIa	Artemisone **49** (AMS) Hong Kong University of Science and Technology (UHKST)	Endoperoxide CAS = 255730-18-8 	For the treatment of *P. falciparum* malaria. Semi-synthetic derivative of artemisinin, with no neurotoxicity. It is a non-lipophilic drug. The probable drug target is the parasite's Ca^{2+} pump *Pf*ATP6. IC_{50} < 1 nM (*P. falciparum* strains); EC_{50}= 0.14 nM (*P. falciparum* isolates). Pharmacokinetics (single 80 mg oral dose): C_{max} = 140.2 ng mL^{-1}, T_{max} = 0.875 h, AUC = 281.6 h ng mL^{-1}, $t_{½}$ 2.79 h. In a phase IIa trial with patients infected with uncomplicated malaria, after oral AMS (2 mg kg^{-1}/d) for 2 days, MFQ (25 mg kg^{-1}) on day 2: PCT = 26 h.	[287, 341-343]
Phase IIa	Albitiazolium **50** (SAR97276, T3/SAR97276) (Sanofi Aventis)	Bis-thiazolium compound CAS = 321915-72-4 	For the treatment of *P. falciparum* malaria. It targets the phospholipid metabolism of *P. falciparum.* *P. falciparum* asexual blood stages: IC_{50} = 3 nM;	[344, 345]

phosphatidylcholine biosynthesis in infected erythrocytes [290, 291]. This potent antimalarial, with an IC_{50} of 3 nM, has low bioavailability because of its two permanent cationic charges, and the development of prodrugs, which overcome this drawback, is the subject of active research [292].

Drugs in Phase I and in a Stage of Preclinical Development (see Table 7)

Two potent new 4-aminoquinolines are now in Phase I of development: GSK369796, N-*tert*-butyl isoquine **51**, structurally analogous to amodiaquine, and AQ13, CQ-analogue **52** with a shorter side-chain. GSK369796 is a fluorinated derivative of amodiaquine, designed to be a cheap antimalarial to overcome CQ-resistance [346]. In addition it was hoped that it would not be hepatotoxic or promote agranulocytosis, two conditions that may arise with prolonged administration of amodiaquine, and have led to some restrictions on its use. It turned out to be more resistant to metabolic cleavage than amodiaquine, and when

tested, it did not cause gene mutations or chromosomal damage *in vitro* in a bacterial mutation assay and mouse lymphoma assay or in an *in vivo* mouse micronucleus assay [346].

AQ13 is predicted to have a similar mode of action to chloroquine, but the presence of the shorter side chain might enable the compound to overcome the parasite's resistance mechanism to chloroquine. It is active against multi-drug resistant strains of *P. falciparum* [347]. A Phase I trial has already been carried out with humans [348]. CQ was compared with chloroquine, and no hematologic, hepatic, renal, or other organ toxicity was observed with AQ-13 or with chloroquine at the doses tested. AQ-13 was cleared more rapidly than CQ and QTc prolongation was greater with CQ than AQ-13. There were minimal differences in toxicity between the two compounds and the pharmacokinetics were similar. AQ-13 has a shorter half-life of 14.3 days *versus* 23.3 days for CQ, but a slightly lower bioavailability too: the mean AUC was 140.8 μM for AQ-13 *versus* 241.2 μM for CQ, which implies that a slightly higher dose of AQ-13 may be necessary in treatment. The results indicated that this drug should proceed to further trials to determine its efficiency treating malaria [349].

NPC-1161-B is an 8-aminoquinoline in a phase of preclinical development. It has potent antimalarial action against the blood and tissue stages of *Plasmodium*. This primaquine analogue **53** was prepared originally as a racemic mixture, NPC-1161-C. When the two enantiomers were separated, it was found that the (-)-enantiomer, NPC-1161-B, was several times more active than the (+)-enantiomer in its antiparasitic activity *in vivo* [350]. When the racemic compound was tested in animal models, administered orally in multiple doses at 1 mg kg^{-1} of body weight for 3 days, it gave 100% cure rate and did not show any toxicity at the highest dose tested (64 mg kg^{-1} for 3 days). It also showed promising antimalarial efficacy for radical cure [349]. NPC-1161-B is also active against other protozoa, like the *Leishmania.*

New synthetic endoperoxides are also at these two stages of development [351]: CDRI 97-98, RKA 182 and SAR116242. CDRI, 1,2,4-trioxane **54**, was developed to be used against multidrug-resistant *P. falciparum* and to treat cerebral malaria. When *Anopheles stephenses* mosquitoes were fed on blood from gametocyte

carrying *P. cytnomolei* and *P. yoelii* models [352], the oocysts which developed had no infectivity if this trioxane had been previously administered to the hosts, which showed the gametocytocidal activity of CDRI 97-98, and its potential transmission blocking activity.

RKA 182, diaspiro compound **55**, was selected out from a series of 150 novel 1,2,4,5-tetraoxanes during a study on the antimalarial properties of these molecules [353]. Since it was known that fusion of an adamantane moiety to the 1,2,4-trioxolane system was essential for good antimalarial activity in the ozonide series, this moiety was also incorporated in the new diaspiro peroxide. It was found that the diaspiro compounds were more stable than their 1,2,4-trioxolane or 1,2,4-trioxane counterparts. RKA 182 has superior properties to the semi-synthetic artemisinins in current use [353]. It is water soluble, having solubility greater than 40 mg mL^{-1}, and it has IC_{50s} less than 6 nM against CQ-sensitive and CQ-resistant *P. falciparum* strains. Its half-life is between that of OZ277 and OZ439 [354]. It was also demonstrated that it forms adducts resulting from the formation of covalent bonds between carbon atoms of the haem porphyrin ring and carbon radicals generated from the tetraoxane peroxide ring system [353]. The demonstration that RKA 182 can alkylate haem is in line with what is expected to occur with peroxide-containing antimalarials, on the basis of current knowledge on the mechanism of action of these compounds. Although the exact mechanism of action artemisinin and its derivatives is not entirely clear yet, and more than one target has been suggested (see under The Artemisinins), haem alkylation is thought to be one of the ways in which these and other peroxide antimalarials interact with *Plasmodia* leading to their destruction. As the parasite degrades the host's haemoglobin, toxic free haem is produced which, to eliminate, the parasite converts into the redox-inactive iron (III) polymer, hemozoin. When peroxide drugs interact with iron (II)-haem, short-lived alkoxy radicals are produced, which quickly rearrange to C-centered primary radicals. These carbon radicals can alkylate haem *via* an intramolecular process to produce covalent haem-drug adducts [355], resulting in the accumulation of redox-active haem derivatives, thought to be toxic to the parasite. The ability of synthetic trioxanes or trioxolanes to alkylate haem correlates well with their antimalarial activity [355]. Although RKA contains two peroxide bonds, and scale-up of the chemistry

related to its synthesis could be potentially dangerous, a safe method to produce it on industrial kilogram-scale was developed using the Dussault's protocol, in which Re_2O_7 is used as a mild Lewis acid to generate the key tetraoxane ring, without the need for isolation of the required intermediate gem-dihydroperoxide [353].

SAR116242 is a hybrid molecule consisting of a 1,2,4-trioxane moiety and an aminoquinoline moiety, and hence it is named a trioxaquine. It is expected that by having two distinct pharmacophores, molecule **56** will have the activity characteristic of both [356]. In fact SAR116242 is highly active *in vitro* against several sensitive and resistant strains of *P. falciparum* at nanomolar concentrations. This drug was selected from a collection of 120 hybrid molecules. Its dual mode of action was shown: not only was SAR116242 able to inhibit the polymerization of β-hematin as CQ does, but it also promoted haem alkylation *via* the production of free radicals, indicated by the formation of several haem adducts [356]. This drug showed nearly the same transepithelial permeability as chloroquine (P_{tot} between 10 and 20 x 10^{-7} cm s^{-1}), which suggests that oral absorption will be efficient. Tests to show its toxicological profile revealed no mutagenic or clastogenic activity [357]. Once it was demonstrated that the two diastereoisomers have the same *in vitro* activities, subsequent tests were carried out using a mixture of both diastereoisomers. Recent reports indicate, however, that further development of this drug may have been discontinued [354].

A new substance is also being developed for adjunctive therapy, DF-02, or sevuparin sodium **57**. DF-02 is derived from heparin, a naturally occurring polysaccharide, after depolymerization [354]. Heparin is used in medicine, as an anticoagulant, to prevent thrombosis. It has been used in malaria in the past, but its use has been discontinued due to the occurrence of haemostatic side effects such as bleedings. Adjunctive therapy is an additional or secondary therapy given in malaria together with a schizonticide to reduce the effects of malaria. DF-02 has much lower anti-coagulation activity than heparin [358]. In malaria it prevents rosetting, a condition that develops in severe malaria, when *P. falciparum*-parasitized erythrocytes bind to uninfected erythrocytes blocking the flow of blood in the capillaries of the brain and other organs, and hence causing severe complications [359]. DF-02 has been shown to be safe for used in humans [358].

A few nitrogen-containing heterocycles are at these stages of development. The most advanced is GNF156, in Phase I, synthetic imidazolopiperazine **58**. Imidazolopiperazines are novel scaffolds to the antimalarial arsenal [360]. GNF156 resulted from optimization after SAR studies on a series of analogues found to have good antimalarial activity [361]. It is active against the blood, liver and gametocyte stages of Plasmodium but not against the hypnozoites [354].

There are also other piperazine-based antimalarials now a preclinical phase of development, by Actelion Pharmaceuticals. The structures of 14 compounds with general structure **59**, with IC_{50s} of less than 20 nM against P. falciparum have been revealed [362].

There are also two pyrimidine derivatives at this stage of development, DSM265 and P218. DSM265, synthetic triazolopyrimidine **60**, was found to be a *P. falciparum* dihydroorotate dehydrogenase (DHODH) inhibitor, potent against CQ-sensitive and resistant *P. falciparum* strains. The enzyme DHODH catalyzes the rate-limiting step in the pyrimidine biosynthetic pathway (Fig. **10**). Since *P. falciparum* is unable to salvage pyrimidines and must rely on *de novo* biosynthesis for survival, DSM265 leads to parasite death. Human studies are planned for early 2013 [363]. P218 or **61** is a dihydrofolate reductase inhibitor, and hence it prevents folic acid synthesis by the parasite [364].

BCX4945 is another DNA synthesis blocker currently under development. *P. falciparum* is a purine auxotroph. It can't synthesize the purines it requires for synthesis of nucleotides, co-factors, and nucleic acids, but it uses the enzyme purine nucleoside phosphorylase (PNP) to synthesize hypoxanthine. Hypoxanthine is then converted to inosine monophosphate (IMP), a precursor for all the purines, by the action of hypoxanthine-guanine-xanthine phosphoribosyl-transferase (PfHGXPRT). This pyrimidine **62** is a transition state analogue and inhibitor of PNP [365]. When the action of PNP is blocked, the parasites are killed in culture by purine starvation. PNP inhibition is an attractive option, since it is a new target for malaria treatment.

In a screen of approximately 70,000 compounds in the Broad Institute's small molecule library and the ICCB-L compound collection at Harvard Medical

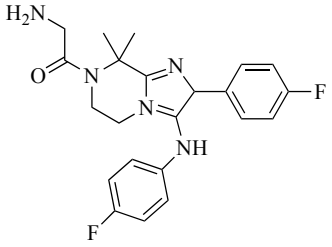
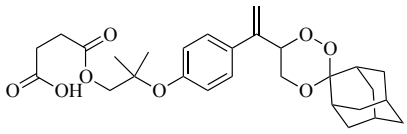
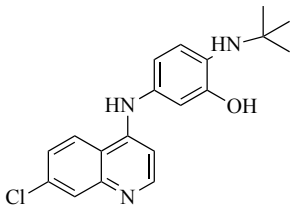
School, Genz-644442, an aminoindole, was selected as a novel lead compound for malaria treatment [366]. Based on its structure, a library of 321 aminoindoles was selected for screening, which led finally to Genz-668764 being selected for further studies in representation of this novel class of antimalarials. Single enantiomer **63** has IC_{50} values in the range 28-65 nM against *P. falciparum in vitro*.

MK4815, aminoalkylphenol **64**, is an inhibitor of the electron transport chain in the mitochondrion, which when administered concentrates in infected erythrocytes. It is active against *P. falciparum* drug-resistant strains in the low nanomolar range, and at present it is in a preclinical stage of development [367]. Tests showed that this antimalarial is most effective against the metabolically active late trophozoite and early schizont stages of the parasite's life cycle. Since it is a small molecule readily synthesized from cheap starting materials, it is an attractive option for an antimalarial.

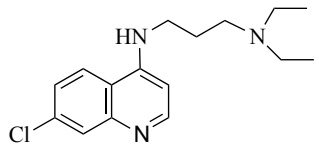
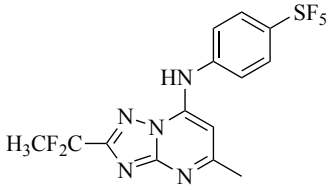
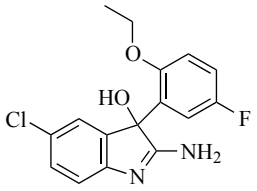
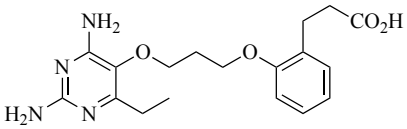
THE PRESENT DAY PANORAMA, MARKET SHORTCOMINGS AND CONCLUSIONS

When the UN adopted in 2000, as one of its goals for the Millennium, to halt and begin to reverse the incidence of malaria by the year 2015, several initiatives were set in motion to achieve this (see also The Pipeline of Antimalarials in Development). To prevent transmission at community level greater efforts at vector control were adopted. In the African region alone the percentage of indoor residual spraying rose from less than 5% in 2005 to 11% in 2010 and the number of households owing at least one insecticide-treated net (ITN) increased from 3% in 2000 to 53% in 2011 in the sub-Sahara [2]. By 2011, 32 countries in the African region and 78 other countries where malaria is endemic had adopted WHO's recommendation to provide ITNs to persons at risk of malaria and many distributed them free of charge [2]. Sub-Saharan Africa has the greatest burden of malaria, with 90% of the worldwide number of malaria-related deaths occurring in this region. The economic consequences are severe too, and malaria uses up to 2% of the gross domestic product of countries in this region. Since pregnant women and children are the most vulnerable to the disease, besides vector control

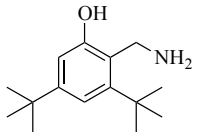
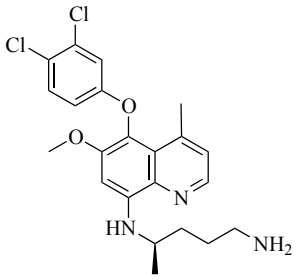
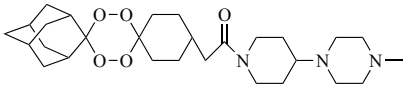
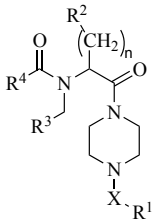
Table 7: New Antimalarial Medicines in Phase I or Preclinical Development in the First Quarter of 2012 [266]

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase I	GNF156 (Novartis)	Imidazolopiperazine **58** 	Imidazole-based compound, synthetic. *In vitro* activity: IC_{50} = 6 and 10 nM respectively against W2 and 3D7 *P. falciparum* multidrug resistant strains. Active against the blood, liver and gametocyte stages of *Plasmodium*.	[361, 368]
Phase I	CDRI 97-78 (IPCA Pharmaceuticals Ltd.)	1,2,4-Trioxane **54** 	Synthetic. To be used against drug resistant *P. falciparum* and cerebral malaria.	[352]
Phase I	DF02 (Sevuparin sodium) (Dilafor)	Depolymerized heparin **57**	Semi-synthetic; obtained from the glycosaminoglycan heparin. Developed to be used as an adjunct treatment in severe malaria. Mode of action: it prevents rosetting, a complication in malaria, by reducing capillary blocking and releasing blood cells.	[358, 359]
Phase I	N-*Tert*-butyl isoquine (GSK369796) [Liverpool School of Tropical Hygiene/GlaxoSmith-Klyne (GSK)]	4-Aminoquinoline **51** 	Amodiaquine analogue, synthetic. *In vitro* activity: IC_{50} = 11.2, 12.6 nM (CQ-sensitive 3D7, HB3 *P. falciparum* strains); IC_{50} = 13.2 nM (CQ-resistant K1 *P. falciparum* strain). Mode of action: hemozoin formation is inhibited and hence haem detoxification.	[346]

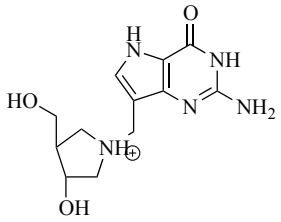
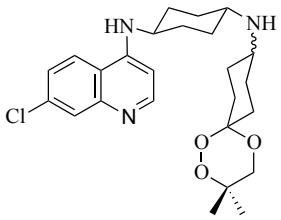
(Table 7) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Phase I	AQ13 (Immtech Pharmaceuticals, Inc.)	4-Aminoquinoline **52** 	Synthetic, CQ-analogue with a shorter side chain. *In vitro* activity: IC_{50} = 18 nM (drug susceptible NF54 *P. falciparum* strain), 59 nM (K1 CQ-resistant *P. falciparum* strain) Mode of action: hemozoin formation is inhibited and hence haem detoxification.	[347, 348]
Preclinical	DSM265 (UTSW/UW/ Monash)	Triazolopyrimidine **60** 	Triazolopyrimidine-based synthetic antimalarial. *In vitro* activity: IC_{50} = 33 nM (*Pf* DHODH), 2.5 μM (*Pb*DHODH), >100 μM (*human* DHODH) and 46 nM (*P. falciparum* CQ-sensitive 3D7 cell) Mode of action: selective inhibitor of the *P. falciparum* enzyme dihydroorotate dehydrogenase (DHODH). It blocks pyrimidine synthesis.	[363]
Preclinical	Genz-668764 (Broad/ Genzyme)	Aminoindole **63** 	Synthetic indole derivative. *In vitro* activity: IC_{50} = 28-65 nM (*P. falciparum*) A single enantiomer.	[366]
Preclinical	P218 (Biotec/ Monash/ LSHTM)	2,4-Diaminopyrimidine **61** 	Synthetic. *In vitro* activity: IC_{50} => 0.01 µM (wild type); Mode of action: inhibitor of the dihydrofolate reductase (DHFR) enzyme in *P. falciparum*. It prevents folic acid synthesis.	[364]

(Table 7) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Preclinical	MK4815 (Merck)	Aminoalkylphenol **64** 	Synthetic. *In vitro* activity: IC_{50} = 0.004-0.030 μg mL^{-1} (a variety of drug-resistant *P. falciparum* isolates) Mode of action: inhibition of haem polymerization is a possibility.	[354, 367]
Preclinical	NPC-1161-B (University of Mississippi)	8-Aminoquinoline **53** 	A dichlorophenoxy analogue of primaquine. The (-)-enantiomer shows promising antimalarial efficacy for radical cure and significant activity against the blood stages of *Plasmodium*. *In vitro* activity: IC_{50} = 50-500 nM (*P. falciparum* laboratory strains) It inhibits sporogony	[350]
Preclinical	RKA182 (Liverpool)	1,2,4,5-Tetraoxane **55** CAS = 	Synthetic endoperoxide. Dispiro-tetraoxane compound with two peroxide moieties. *In vitro* activity: IC_{50} = 1.5-6.0 nM (laboratory strains of *P. falciparum*), 0.87 nM (*P.f.* 3D7 strain) and 1.1 nM (*P.f.* K1 strain). Mode of action: it induces formation of free radicals, which damage the parasite	[353]
Preclinical	Antimalarial (Actelion Pharmaceuticals Ltd)	Piperazine **59** 	Synthetic. The exact structure of the antimlarial is unknown. Fourteen piperazine derivatives with IC_{50s} < 20 nM (*P. falciparum* K1 strain) have been disclosed.	[354, 362]

(Table 7) contd…..

Phase of Development	Drug	Active Ingredient and Chemical Class	Properties	Ref.
Preclinical	BCX4945 (Byocryst/Albert Einstein College of Medicine)	Deazaguanine **62** 	Deazaguanine derivative, an analogue of Immucillin-G. Mode of action: purine nucleoside phosphorylase (PNP) inhibitor. It blocks purine synthesis from hypoxanthine, and hence DNA synthesis.	[365]
Preclinical	SAR116242 (PA1103/ SAR116242) (Palumed)	Trioxaquine **56** CAS = 1096519-66-2 	Hybrid combination of a synthetic peroxide, which mimics the 1,2,4-trioxane group of artemisinins and an aminoquinoline. Gametocytocidal. *In vitro* activity: IC_{50} = 7-24 nM (*P. falciparum* laboratory strains) Mode of action: dual, reactive free radicals are formed, which can alkylate haem, and polymerization to β-hematin and detoxification are inhibited.	[53, 357]

programmes, chemoprevention through the adoption of Intermittent Preventive Treatment for pregnant women became part of the national policy in 36 of 45 sub-Saharan African countries by the end of 2011 and also in Papua New Guinea in 2009 [2]. For infants and children the adoption of these measures has been slower to implement. Presently increased vector control and prevention, increased diagnostics and treatment with quality assured antimalarials have led to what appears to be a situation in which the incidence of malaria is in fact retreating [1, 2, 369]. These short-term massive targets will however need a follow-up by long term strategies [370].

The scientific community has also responded to the appeal for increased efforts to fight malaria. A search through the databases of Web of Knowledge (Thompson Corporation), revealed a steep increase in the number of publications on malaria

control in later years (Fig. **17**). From 2000 to 2011 the number of publications with the key-words "antimalarial" OR "anti-malarial" OR "anti-malaria drug" has almost tripled. However, the arsenal of antimalarials for prevention and control of the disease is still small. There have been no new classes of antimalarials introduced into clinical practice since 1996 [371]. This situation is due not only to the intrinsic difficulties in discovering and developing new drugs, but also due to low financial resources [372]. Worst of all, many existing drugs may have to be discarded as a result of the fast development of resistance.

Studies involving large sets of compounds have been a very useful recent contribution. In one such study, undertaken at St. Jude Children's Research Hospital, approximately 310,000 compounds were screened for their ability to inhibit growth of *P. falciparum* in asexual blood stages. More than 1,100 were found to inhibit parasite growth by more than 80% at a concentration of 7 μM, and 560 at concentrations lower than 2 μL [372]. A closer look at a selection of 172 of these, with novel structures for antimalarials, revealed that at least 80% seemed to act on targets different from those of the antimalarials used commonly nowadays. The researchers predicted that the targets of many of these new compounds were kinase enzymes of *P. falciparum*, *i.e.,* the enzymes that transfer phosphate from high energy donor molecules like ATP to specific substrates in the phosphorylation process, or that they were targets related to interactions between the pathogen and its host. This being true, they represent new classes of targets for antimalarials. The pharmacokinetics studied also suggested that many of these compounds were suitable for further development. At Novartis, another large compound study involving the in-house library of 12,000 pure compounds, yielded 275 prospective antimalarials, of which 17 were selected for further studies due to their favourable potency and low toxicity [373, 374]. Phenotypic screening like this, which in its simplest variation may employ cell lines and monitor the influence of the drug on a single parameter (the phenotype), like cellular death or the influence on a certain enzyme-catalyzed process, has been used historically as the basis for the discovery of new drugs. These new studies, however, distinguish themselves by the large numbers of samples covered, and they are referred to as Global phenotypic screening. However, the process of determining the relevant target or target molecules subsequently is usually slow

and difficult [375]. Phenotypic screening is an alternative to target-based screening, in which the effect of a drug on a purified target protein is observed.

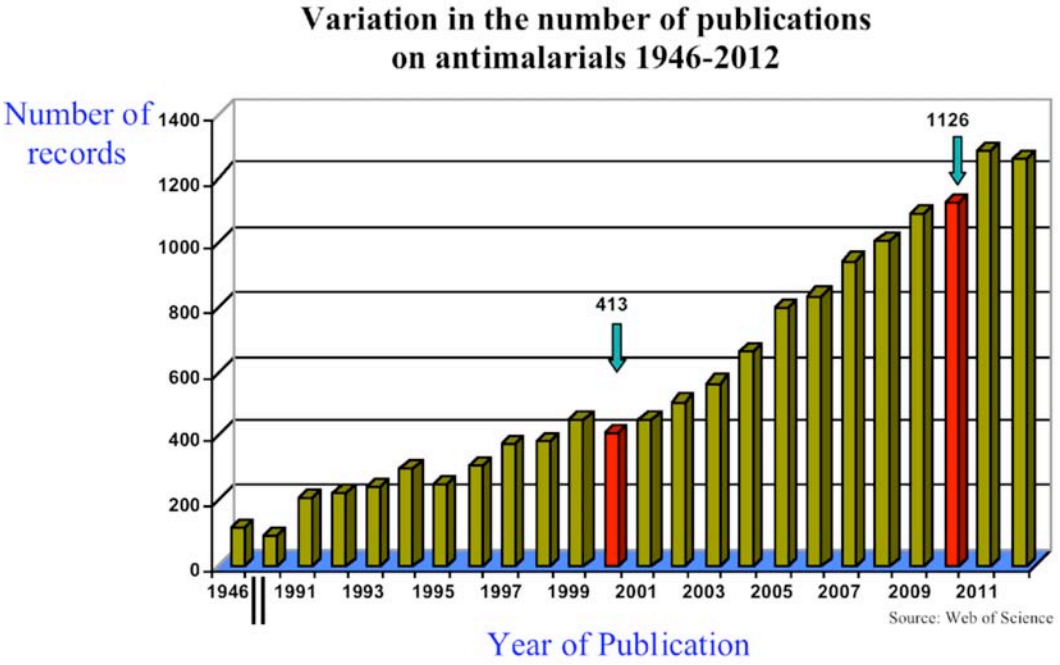


Figure 17: Evolution in the numbers of publications on malaria control since 1990. The number published in 1946 is representative of the number of records found for the previous decades.

In a separate study at GlaxoSmithKline [376], the in-house database was screened for compounds possessing activity against the blood stages of *P. falciparum*. More than 2 million compounds were included in this search. Approximately 13,500 were found to be active in concentrations of up to 2 μM. The data on more than 11,000 of these compounds as well as from the studies described above, was subsequently made available to the general research community at the European Bioinformatics Institute's CHEMI database at https//www.ebi.ac.uk/chemblntd/. The studies described so far did not present the discovery of novel antimalarials, but the information available provides a collection of novel chemical structures, which may be the basis for future research in this area [377]. These databases are nowadays being the subject of several screening studies, in different attempts to prioritize target antimalarials [378, 379].

Genomics is also being used to identify appropriate targets in the parasite. Another area where genome information can be useful, is in the identification of targets that have already been validated for another organism [167]. This has been of importance in the area of drug repositioning [167]. For example, the malaria genome information revealed that bacterial and malaria parasites utilize the same non-mevalonic pathway to synthesize isoprene [380]. Based on this knowledge, the antibiotic fosmidomycin, known to target this pathway, was selected for testing, and not only was its antimalarial activity discovered, but it was also afterwards selected as a prospective antimalarial drug, and it is nowadays undergoing clinical trials (see also under Drugs in Phase IIA).

At present there are no vaccines approved for malaria. Several attempts have been made, but the road to discovery has proven to be difficult. There are at present a few undergoing testing [381]. The most advanced is RTS, S/AS01, which is being developed by PATH, GlaxoSmithKline and the Bill & Melinda Gates Foundation. Large clinical trials are underway in 7 countries in Africa. The final results are expected at the end of 2014 [381].

As mentioned under The Pipeline of Antimalarials Under Development, one aspect of anti-malarial drug development that may help overcome parasite resistance is to have new drug targets. This may be achieved through an increased understanding of the parasite's biology and the mode of action of current antimalarials. Many studies are underway in this area. Table **8** lists possible life cycle processes that appear not to be affected by present-day antimalarials and which could be future targets for antimalarial development [369].

Recent plans at malaria control aim to reduce the parasite's basic reproduction rate [369]. In terms of parasite numbers, there are two critical events in the parasite's life cycle: the transmission of sporozoites from the parasite to the human host and the transmission of gametocytes from the host to the parasite. Only a few dozen to a few hundred parasites are transmitted in these events, compared to the billions which are formed during asexual blood replication. Nevertheless medicines targeting the blood stage are essential because it is during this stage that the disease manifests itself in the host. During a blood meal, 15-123

Table 8: *Plasmodium* Cell Components or Life-Cycle Processes that Could be Future Drug Targets. Until Now, no Antimalarials have been Designed which Aim Specifically at these Targets [369]

Parasite Cell Components or Life-Cycle Processes	Enzyme (Life Stage)	Enzyme Function	Ref.
Cytoplasm	**Serine hydroxymethyl transferase (BS/LS)**	It acts on the pyrimidine biosynthetic pathway; it promotes the conversion of serine and tetrahydrofolate to glycine and methylenetetrahydrofolate.	**[382]**
Endoplasmic reticulum	**Plasmepsin V (BS/LS)**	Enzyme which cleaves the export element (protease recognition sequence) PEXEL;RxLxE/Q/D from parasite proteins which are to be transferred to the erythrocyte.	**[383]**
Food vacuole	**Falcipains (BS)**	Haemoglobin degradation proteases.	**[384]**
	Plasmepsins (BS)	Haemoglobin degradation proteases.	**[384]**
Parasitophorus vacuole (PVM)	**Putative translocon (BS/LS)**	Complex that exports proteins across the Parasitophorus vacuole membrane into the human host cell.	**[385]**
Merozoite invasion	**Rhomboid 4 (BS)**	Merozoites interact with the erythrocytes before invasion *via* parasite ligands, transmembrane adhesins. Romboid 4 releases the adhesins so that the parasite can subsequently enter the cell.	**[386]**
	Subtilisin 2	It is involved in processing and shedding proteins on the merozoite surface during invasion.	**[387]**
Merozoite egress	**DPAP3 (BS/LS)**	*Pf* protease that regulates erythrocyte rupture.	**[388]**
	SERA5 (BS/LS)	Enzyme which mediates PVM and erythrocytic membrane breakdown to allow merozoite invasion.	**[389]**
	Subtilisin 1 (BS/LS)	**Enzyme which mediates PVM and erythrocytic membrane breakdown to allow merozoite invasion.**	**[390]**

sporozoites are deposited under the skin of the host [391]. They move through the dermis until they find a blood vessel, and then migrate to the liver, invading the liver parenchyma cells. Here thousands of merozoites form. Ideally this would be a good point to control the disease, or even the hepatic stage, but these stages are asymptomatic, and hence they are not targets for therapy. The liver stage is appealing for vaccine development, and some of the current research is aimed at this stage of the disease. Later, as a result of gametocytogenesis, a small proportion of parasites are transmitted and reproduce asexually in the middle gut of the mosquito. The numbers are also low: 10-103 [369]. Artemisinin and primaquine are the only antimalarials currently available which kill stage 4 gametocytes, although a few others, which do not kill them, have the effect of

rendering them incapable replicating in the mosquito, and hence also allow for transmission control (see under Currently Available Antimalarial Drugs).

Research on *P. vivax* has been slower, because this species is difficult to grow in the laboratory. Even nowadays it is not known how hypnozoite dormancy is induced.

According to a few sources, some of needs for the future of malaria control, besides efficient drugs to replace existing ones in case they fall to resistance [371, 377], are the following [392]:

1. A drug with potential to act at several stages of the parasite's life cycle, which could be taken in one dose.

2. Assays to identify drugs having the capacity to eliminate *P. vivax* hypnozoites from the liver.

3. The development of *P. vivax* and *P. falciparum* culture systems for every stage of the parasite life cycle

4. The implementation and validation of *in vitro* and *in vivo* G6PD deficiency-dependent haemolysis assays.

5. New molecules to block the development of mature gametocytes and hence prevent transmission.

6. To develop and validate high throughput screening assays, to test new drugs against *P. falciparum* and *P. vivax* gametocytes and parasite transmission.

7. An efficacious vaccine.

8. Improved diagnostic tests for malaria.

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CONFLICT OF INTEREST

The author confirms that this chapter content has no conflict of interest.

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