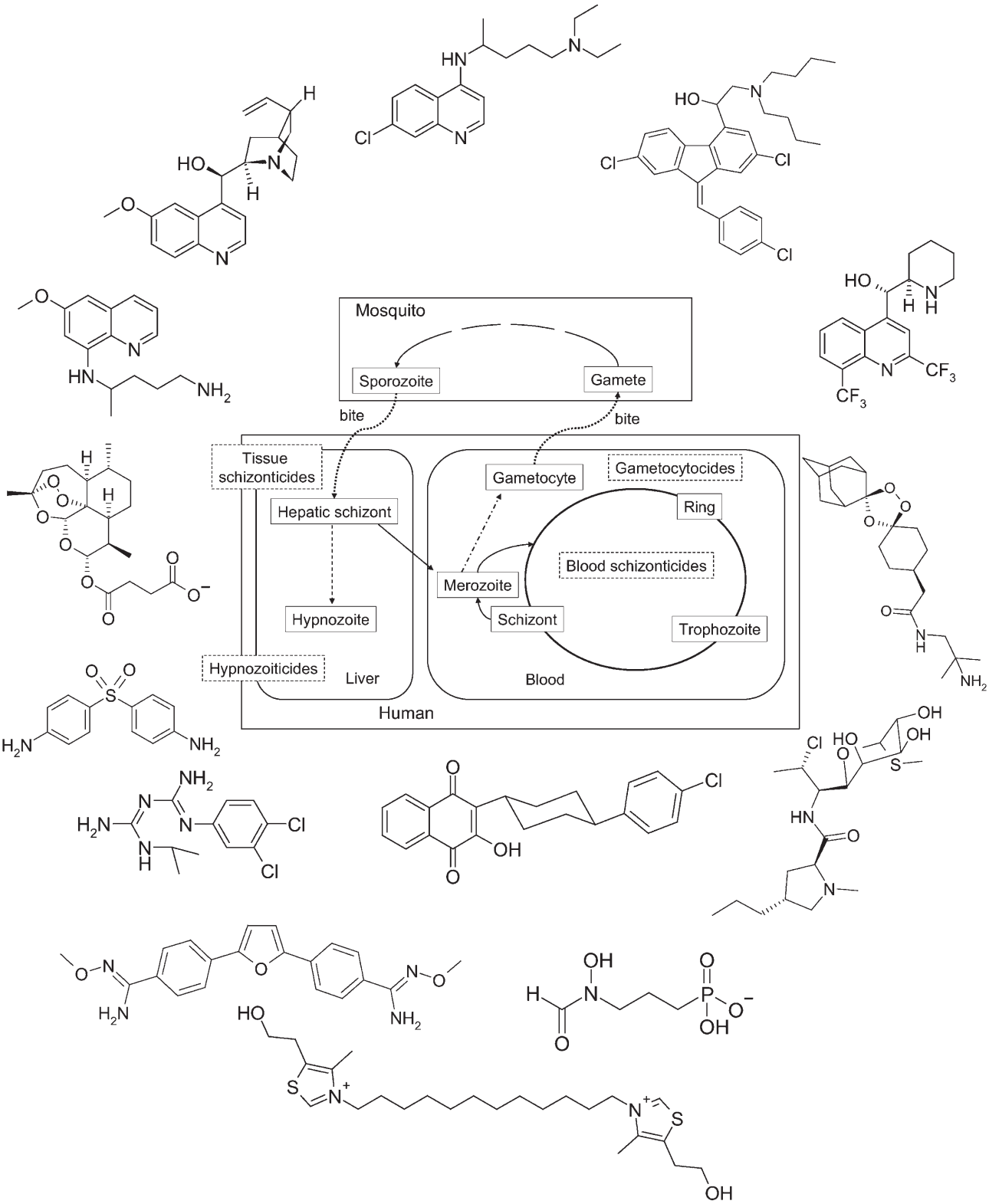
Malaria Chemotherapeutics



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Malaria Chemotherapeutics Part I: History of Antimalarial Drug Development, Currently Used Therapeutics, and Drugs in Clinical Development

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Since ancient times, humankind has had to struggle against the persistent onslaught of pathogenic microorganisms. Nowadays, malaria is still the most important infectious disease worldwide. Considerable success in gaining control over malaria was achieved in the 1950s and 60s through landscaping measures, vector control with the insecticide DDT, and the widespread administration of chloroquine, the most important antimalarial agent ever. In the late 1960s, the final victory over malaria was believed to be within reach. However, the parasites could not be eradicated because they developed resistance against the most widely used and affordable drugs of that time. Today, cases of malaria infections are on the rise and have reached record numbers. This review gives a short description of the malaria disease, briefly addresses the history of antimalarial drug development, and focuses on drugs currently available for malaria therapy. The present knowledge regarding their mode of action and the mechanisms of resistance are explained, as are the attempts made by numerous research groups to overcome the resistance problem within classes of existing drugs and in some novel classes. Finally, this review covers all classes of antimalarials for which at least one drug candidate is in clinical development. Antimalarial agents that are solely in early development stages will be addressed in a separate review.

1. Introduction

Malaria is caused by protozoal parasites of the genus *Plasmodium* (Figure 1). Four of the >100 *Plasmodium* species infect humans and cause distinct disease patterns: *P. falciparum* (malaria tropica), *P. vivax*, *P. ovale* (both malaria tertiana), and *P. malariae* (malaria quartana). *P. falciparum* and *P. vivax* account for 95% of all malaria infections. Nearly all severe and fatal cases are caused by *P. falciparum*. About 40% of the world's population lives in malaria-endangered areas. Malaria is found in tropical regions throughout sub-Saharan Africa, Southeast Asia, the Pacific Islands, India, and Central and South America. *P. falciparum* is found throughout tropical Africa, Asia, and Latin America. It is the predominant species in most areas. *P. vivax* is more common in India and South America, but is also found worldwide in tropical and some temperate zones. *P. ovale* is mainly confined to tropical West Africa, while the occurrence of *P. malariae* is worldwide, although its distribution is patchy.[1,2]

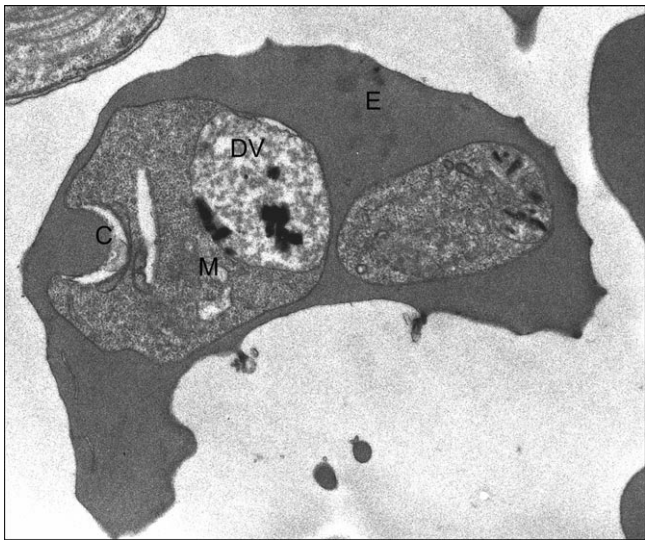

Figure 1. Electron micrograph showing the ring stage of *P. falciparum*. C: cystosome; DV: digestive vacuole; E: erythrocyte; M: mitochondrion.

"If as standard of importance is taken the greatest harm to the greatest number",[3] malaria is the most important infectious disease. It has been estimated that there were up to 660 million clinical cases of *P. falciparum* malaria in 2002.[1,4] Malaria kills between 1 and 3 million people annually, most of whom are children under the age of 5 and pregnant women. It is estimated that every 40 seconds a child dies from malaria.[5]

The infectious stages of the malaria parasite reside in the salivary glands of female *Anopheles* mosquitoes that bite humans for a blood meal. During blood extraction, the mosquito injects its saliva into the wound, thereby transferring ap-

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proximately 15–20 so-called sporozoites into the blood stream. In a matter of minutes, these sporozoites are able to conceal themselves from the host's immune system by entering into the liver cells. Each sporozoite develops into a tissue schizont, containing 10000–30000 merozoites.[6] After one to two weeks, the schizont ruptures and releases the merozoites into the blood stream, starting the erythrocytic phase of the parasite's life cycle. In the cases of *P. vivax* and *P. ovale*, some sporozoites turn into hypnozoites, a form that can remain dormant in the liver cells, causing relapses months or even years after the initial infection. *P. falciparum* and *P. malariae* lack this liver-persistent phase, but *P. malariae* can persist in the blood for many years if inadequately treated.[6] Merozoites released into the bloodstream hide again from the host's immune system by invading erythrocytes. In the erythrocyte, the parasite develops from a ring stage via a trophozoite stage into a blood schizont. After a time characteristic for each specific *Plasmodium* species, the erythrocyte ruptures and releases 16–32 new merozoites into the blood stream which in turn again invade erythrocytes, thereby starting a new erythrocytic cycle. This asexual life cycle, from invasion of the erythrocytes until the schizont ruptures, spans 48 h for *P. falciparum, P. vivax*, and *P. ovale,* and 72 h for *P. malariae*. After a number of asexual life cycles, some merozoites develop into sexual forms, the gametocytes, which are transferred to a mosquito during another blood meal. These gametocytes undergo sexual reproduction within the mosquito mid-gut producing thousands of infective sporozoites, which migrate to the salivary gland where they are ready for a new infection (Figure 2).[1,2]

With the rupture of the erythrocyte, the parasite's waste and cell debris is released into the blood stream, causing some of the clinical symptoms of malaria. The main symptom is fever, but rarely in the classical tertian (every 48 h) or quartan (every 72 h) patterns. Further symptoms include chill, headache, abdominal and back pain, nausea, and sometimes vomiting. Thus, the early stages of malaria often resemble the onset of an influenza infection. *P. vivax, P. ovale*, and *P. malariae* show distinct selectivity toward the age of the infected erythrocytes. For that reason, the degree of total parasitemia is limited. In contrast, *P. falciparum* infects erythrocytes of all ages, leading to high parasitemia. Although the symptoms of *P. vivax, P. ovale*, and *P. malariae* infections can be severe in non-immune persons, these parasites seldom cause fatal disease. Nevertheless, chronic infection with *P. malariae* can result in an (eventually fatal) nephrotic syndrome.[7] Malaria caused by these three parasites is often called benign malaria. In contrast, *P. falciparum* malaria (also known as malaria tropica) can progress within a few days from uncomplicated to severe malaria with a fatal outcome in 10–40% of all cases of severe malaria, depending on the time lag between the onset of the symptoms and effective treatment, as well as on the hospital facilities for the management of complications.[8] Observed complications can include coma (cerebral malaria), respiratory distress, renal failure, hypoglycemia, circulatory collapse, acidosis, and coagulation failure.[9]

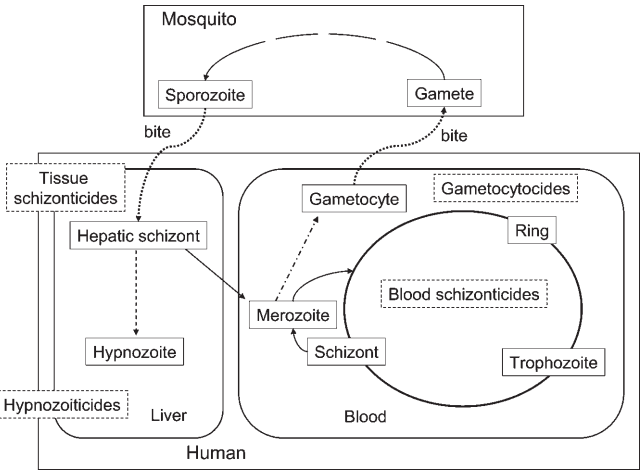

Figure 2. The life cycle of malaria parasites.

Traditionally, antimalarial agents are classified by the stages of the malaria life cycle that are targeted by the drug (Figure 2): **Blood schizonticides** acting on the asexual intraerythrocytic stages of the parasites. **Tissue schizonticides** kill hepatic schizonts, and thus prevent the invasion of erythrocytes, acting in a causally prophylactic manner. **Hypnozoiticides** kill persistent intrahepatic stages of *P. vivax* and *P. ovale*, thus preventing relapses from these dormant stages. **Gametocytocides** destroy intraerythrocytic sexual forms of the parasites and prevent transmission from human to mosquito.

As there are no dormant liver stages in *P. falciparum* malaria (malaria tropica), blood schizonticidal drugs are sufficient to cure the infection. In cases of *P. vivax* and *P. ovale*, a combination of blood schizonticides and tissue schizonticides is required.[2]

2. 4-Aminoquinolines and Arylamino alcohols

2.1. Development of synthetic antimalarials

Powdered bark from the chinchona tree containing the plasmodicidal quinoline alkaloids quinine (**1**) and quinidine (**2**) was the first medicine to be used against malaria (Figure 3). In 1856, chemist William Henry Perkins set out to synthesize quinine (**1**). His efforts resulted not in quinine (the first total synthesis was accomplished later in 1944), but rather in the first synthetic textile dye called "mauve". This sparked the development of the synthetic dye industry in Germany. Microbiologists used these novel dyes to stain and thereby enhance the visibility of microorganisms under the microscope. Paul Ehrlich no-

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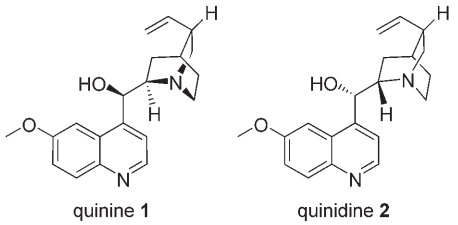

Figure 3. First antimalarial agents quinine (**1**) and quinidine (**2**).

ticed that methylene blue (**3**) was particularly effective in staining malaria parasites (Figure 4). He rationalized that this dye might also be selectively toxic to the parasite. In 1891, Ehrlich and Guttmann cured two malaria patients with methylene blue (**3**), which became the first synthetic drug ever used in therapy. Although it was not used further at that time, methylene blue constituted the basis for the development of synthetic antimalarials. In the 1920s, chemists at Bayer in Germany (at that time, part of IG Farben) started to modify the structure of methylene blue (**3**). A key modification was the replacement of one methyl group by a dialkylaminoalkyl side chain to give compound **4**. Subsequently, this side chain was connected with different heterocyclic systems such as the quinoline system, yielding the first synthetic antimalarial drug, plasmochin (**5**, also known as plasmoquine or pamaquine) in 1925. However, under clinical evaluation, this drug displayed multiple side effects, and was therefore not widely used. The congeneric primaquine (**6**), introduced in 1952, was better tolerated, making it the main representative of the class of 8-aminoquinolines (see below). Connection of the diethylaminoisopentylamino side chain with an acridine heterocycle yielded mepacrine (**7**, also known as quinacrine), which was introduced as Atebrin® in 1932. When the US was cut off from its quinine (**1**) supply as a result of the Japanese occupation of Indonesia in 1942, considerable efforts were undertaken in the US to reconstruct the synthetic pathway to mepacrine (**7**) from German patent literature.

Mepacrine became the main drug for prophylaxis and treatment of malaria for the allied troops during the Pacific war campaigns. Mepacrine (**7**) is a drug with substantial side effects, the most visible of which is a yellow staining of the skin and eyes. Nowadays, it is still used for the treatment of *Giardia lamblia*, a parasite that causes intestinal infections of variable severity. A major success with the drug-design strategy described above was achieved in 1934 with the introduction of a diethylaminoisopentylamino side chain into position 4 of a 7-chloroquinoline, yielding a compound named resochin by the German inventors (later known as chloroquine (**8**)). However, after initial trials in Germany, resochin was regarded as too toxic for use in humans. Through a pre-war license agreement between Bayer and Withrop, resochin was licensed in the US by the latter company. In 1936, the structurally closely related sontoquin (**9**, later known as nivaquine) was prepared in the Bayer laboratories and tested in Germany. From 1942 onward, sontoquin (**9**) was evaluated by French scientists in Tunis. Once a considerable supply of this compound, together with clinical data, was handed over to the Allies after German troops surrendered at Tunis in 1943, resochin was re-evaluated and later renamed to chloroquine (**8**). After the war, chloroquine became the foundation of malaria therapy for at least two decades.[10–13]

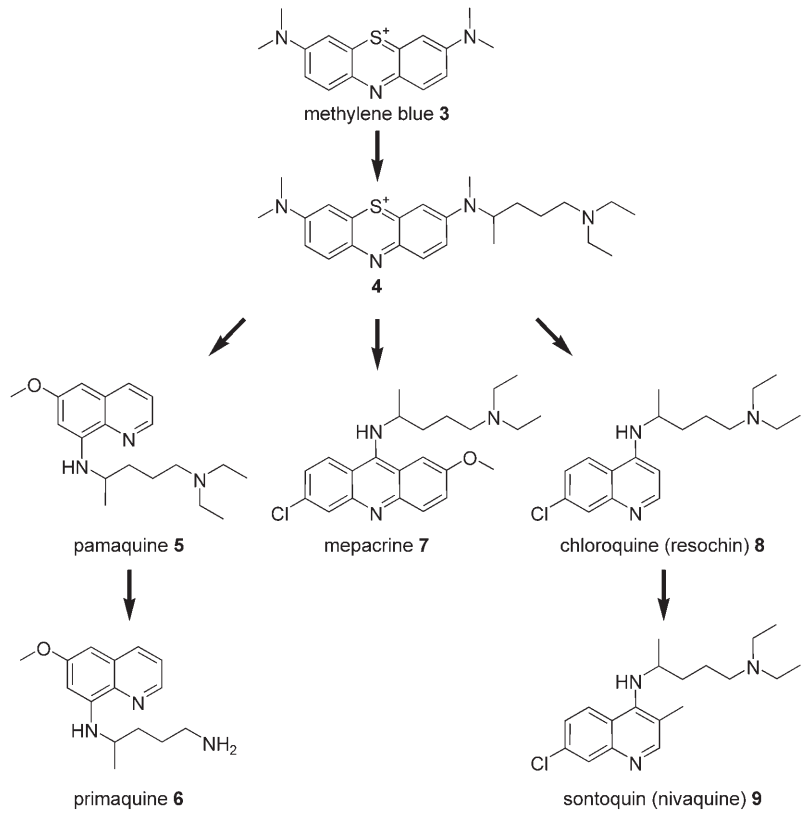

Figure 4. The dye methylene blue (**3**) is the predecessor of early synthetic antimalarial drugs.

2.2. 4-Aminoquinolines

2.2.1. Chloroquine

Chloroquine (CQ, **8**) has been the most successful single drug for the treatment and prophylaxis of malaria.[14] Chloroquine is a safe and affordable drug, and it was effective before resistant strains began to emerge in the 1960s. It was the drug of choice in the World Health Organization (WHO) Global Eradication Program. Landscaping

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measures, vector control with DDT, and the prophylactic use of CQ led to a considerable containment of malaria, which had once been endemic as far north as 64° N.[15,16]

Chloroquine is a relatively well-tolerated drug as long as it is used in therapeutic regimes. The therapeutic index is rather small, with the therapeutic dose being 10 mg kg^{-1} b.w.; a dose of 20 mg kg^{-1} causes serious toxic effects and 30 mg kg^{-1} is potentially lethal.[17,18] When CQ is used in long-term prophylaxis, serious and irreversible side effects such as neuromyopathy, retinopathy, erythema multiform, and bone-marrow toxicity may occur. However, these reactions are rare. Retinopathy has been associated with a cumulative total dose of 50–100 g.

Despite its overwhelming importance, the mechanism of action of chloroquine is still a matter of debate.[19–21] Various theories have been proposed which have been comprehensively reviewed.[19] There is common consent that CQ interacts with the parasite's ability to digest hemoglobin. During its erythrocytic stages, the parasite consumes large quantities of hemoglobin from its host cell, either for the purpose of amino acid supply, or simply to create space inside the erythrocyte. Hemoglobin is shuttled by vesicles to a specialized organelle called digestive vacuole (DV). Either there or already in the transport vesicles, the protein component of hemoglobin is digested by the successive action of various proteolytic enzymes. First in sequence are the aspartate proteases plasmepsins I–IV, followed by falcipains (cysteine proteases) and the zinc protease falcilysine. The resulting small peptides and possibly free amino acids are transported across the vacuole membrane into the cytoplasm, leaving the heme part behind. Oxidation of the central iron yields ferriprotoporphyrin IX (FPIX or FPPIX) (Figure 5).[22,23] Higher concentrations of this molecule are toxic to the parasite, yet the precise mechanism by which FPIX exerts its toxicity is not entirely clear. Membrane disruption and the generation of oxidative stress may be a factor in this context. The parasite disposes this hazardous waste through the formation of an insoluble polymer called hemozoin, which is microscopically visible in the DV as the so-called malaria pigment. In addition to the formation of hemozoin, further mechanisms for the detoxification of FPIX have been discussed. FPIX could be destroyed by hydrogen peroxide formed during the conversion of oxyhemoglobin to methemoglobin.[24] Heme leaking out of the DV is degraded by the action of the glutathione system. Chloroquine (**8**), a dibasic compound (pK_a values: 8.1 and 10.2), is trapped in the acidic digestive vacuole (pH 5.0–5.4) as a dication where it accumulates by some orders of magnitude. Similar to the other 4-aminoquinolines, CQ forms a complex with ferriprotoporphyrin IX and thereby prevents its polymerization into hemozoin. This has been recently confirmed by spinning-disc confocal microscopy of live intraerythrocytic malaria parasites.[25] Crystallographic information of the structure of the chloroquine–FP complex is not available. Most NMR and molecular modeling studies[20,26] show a face-to-face π staggering of the porphyrin and quinoline systems, although a structure showing an edge-to-face complex with the ring nitrogen atom sitting above the ring iron center has also been reported.[27] Very recently, structure determination by NMR spectroscopy showed CQ sitting in a central position over the outermost porphyrin rings of a FPIX–CQ 4:2 complex.[28] Furthermore, CQ inhibits the glutathione-mediated and hydrogen peroxide-mediated destruction of FPIX.[24,29] Most researchers assume that the buildup of noncrystalline FPIX, either in its free form or as a FPIX–CQ complex, finally kills the parasite. The precise mechanism by which this toxic effect is exerted remains to be elucidated.[19,22] According to a newer theory, the FPIX–CQ complex acts on a yet undefined membrane target, thereby either impairing the membrane function directly, or triggering the release of Ca^{2+} ions, resulting in the premature fusion of the transport vesicles shuttling hemoglobin to the DV. In these prematurely fused vesicles, hemoglobin is no longer properly degraded.[30] This hypothesis is supported by an independently conducted study[31] that demonstrated the inhibition of macromolecule endocytosis by more than 40% and the accumulation of transport vesicles in the parasite cytosol upon the addition of CQ to late ring-stage parasites.

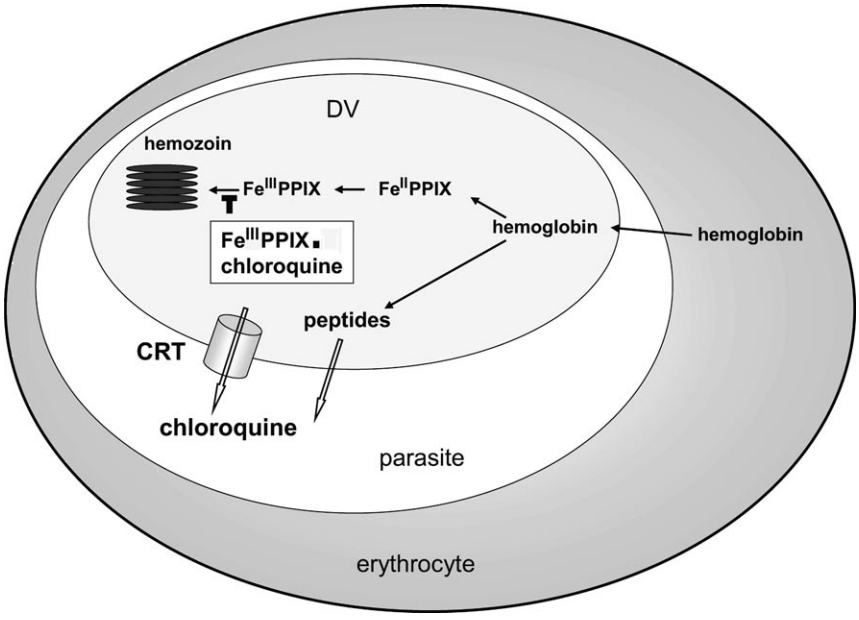

Figure 5. Large quantities of hemoglobin are degraded in the digestive vacuole (DV), yielding peptides and ferriprotoporphyrin IX (FPIX); the latter, which is deposited as the insoluble polymer hemozoin, represents a harmful waste product to the parasite. 4-Aminoquinolines form complexes with FPIX which are toxic to the parasites. In chloroquine-resistant strains, the drug is expelled from the DV by the action of a membrane-bound transporter called the chloroquine resistance transporter (CRT).

In contrast, it has been shown that the FPIX–CQ complex is able to leave the DV and bind to various cytosolic enzymes. The *P. falciparum* glycer-

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aldehyde-3-phosphate dehydrogenase (*Pf*GAPDH) has shown to be particularly sensitive (K_i = 0.2 μM).[32] Quite recently, FPIX was successfully modeled into the crystal structure of *Pf*GAPDH.[33] Further studies will hopefully reveal the exact mechanism of chloroquine and other 4-aminoquinolines.

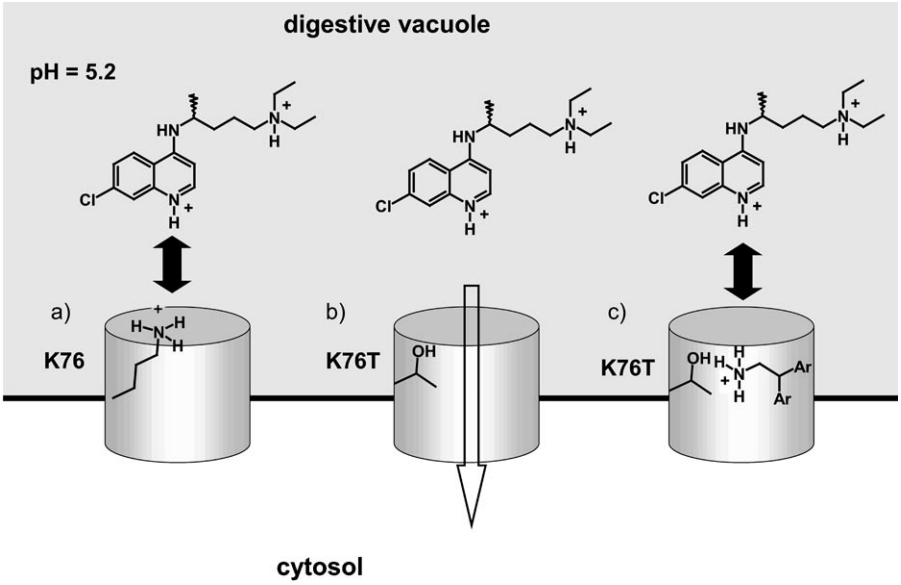

Figure 6. a) The positively charged side chain of K76 of the wild-type *Pf*CRT repels the chloroquine dication. b) The K76T mutation removes a positively charged side chain from the chloroquine resistance transporter. c) Chloroquine resistance reversers restore the positive charge.

2.2.2. Chloroquine resistance

Due to the massive use of chloroquine (**8**), resistant malaria strains have developed independently in four different regions, and have successively spread over almost the entire malaria-endangered area.[34,35] Today, more than 80% of wild isolates are resistant to CQ.[36] The molecular mechanism of resistance has been a matter of intense research and debate. In chloroquine-resistant strains, the drug is apparently removed from its putative locus of action, the digestive vacuole (Figure 5).[37,38] The main cause of chloroquine resistance is a mutation in the *pfcrt* gene that codes for a protein called the chloroquine resistance transporter (*Pf*CRT). This 10-transmembrane-domain transport protein belongs to the drug metabolite transporter (DMT) superfamily located in the membrane of the DV. Because there is not much else of significance inside the DV worthy transport, it has been proposed that the physiological role of this protein is the transport of amino acids or small peptides resulting from the degradation of hemoglobin into the cytoplasm.[39] All chloroquine-resistant strains have a threonine residue in place of lysine at position 76 of the protein. In wild-type CRT, this positively charged side chain is thought to prevent access of the dicationic form of CQ to the substrate binding area of the transporter. The K76T mutation replaces the positively charged side chain by a neutral moiety, and thereby allows access of the CQ dication to the transporter, which then decreases the concentration of CQ in the DV considerably (Figure 6). The K76T mutation is accompanied by up to 14 more amino acid replacements which are thought to restore the physiological function of the transporter, as an engineered strain carrying only the K76T mutation is not viable.[40–42]

Interestingly, a chloroquine-resistant strain kept under continuous drug pressure with halofantrine (**30**) (see below) shows a S163R mutation that renders this strain halofantrine-resistant but restores susceptibility to chloroquine (**8**), most probably through re-emergence of the cation-repelling positive charge in the substrate binding area of the transporter.[40,43] This is in agreement with the fact that chloroquine resistance can be reversed in vitro by several compounds of which verapamil (**10**) is the prototype (Figure 7). The common molecular feature of these so-called chloroquine resistance reversers are two lipophilic aromatic residues and a basic aminoalkyl side chain. It is believed that the aryl residues interact with a lipophilic pocket in the substrate binding site of the CRT, while the protonated amino group restores the positive charge that repels the CQ dication. The underlying molecular scaffold for chloroquine resistance reversers resembles a variety of molecules including certain H1-antihistaminic agents and neuroleptics.[44–47] However, only the combination of chloroquine with the histamine H1 receptor antagonist chlorpheniramine (**11**) has been tested in humans with limited success.[48] In addition to *Pf*CRT, the involvement of other transport proteins in chloroquine resistance is discussed.[49,50] A mutation (N86Y) in the *pfmdr1* (multidrug resistance) gene that codes for another membrane transporter (*Pf*MDR1) is significantly related to the *pfcrt* K76T mutation. Recent results suggest that

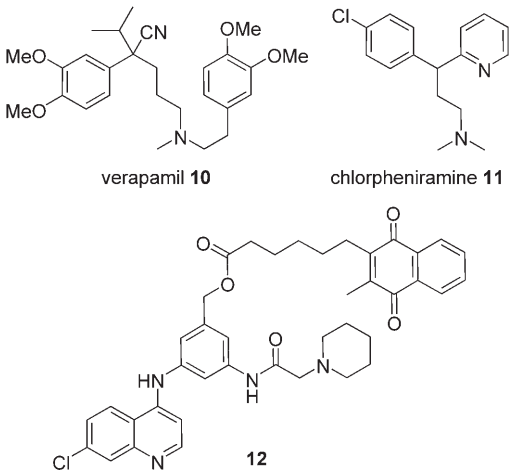

Figure 7. Chloroquine resistance reversers.

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this mutation plays a compensatory role in chloroquine-resistant isolates under CQ pressure and may also have some fine-tuning effects on the degree of chloroquine resistance.[51] The N86Y mutation is strongly associated with increased sensitivity to mefloquine (**29**), halofantrine (**30**), lumefantrine (**31**), and dihydroartemisinin (**39**) (see below).

One further explanation for CQ resistance focuses on the enzyme glutathione reductase (GR), which might be another target of the chloroquine–ferriprotoporphyrin IX complex.[19] Considerably elevated glutathione levels are found in chloroquine-resistant strains, leading to the theory that a combination of CQ (**8**) with a glutathione reductase inhibitor might overcome resistance. A dual drug consisting of a quinoline derivative[52] and a GR inhibitor (compound **12**) showed activity against various chloroquine-resistant strains that was superior to the parent quinoline, but failed to produce a radical cure in *P. berghei*-infected mice.[53] The presumed role of glutathione in chloroquine resistance could also be the rationale behind the recently renewed interest in methylene blue (**3**), which is known to inhibit GR.[54] However, very recent results showed that methylene blue and CQ are antagonistic in vitro.[55] In light of these results, it is not surprising that a clinical trial showed no advantage in using a combination of methylene blue and CQ over CQ monotherapy in an area with a high probability of chloroquine resistance.[56]

2.2.3. Molecular modifications of chloroquine

Much work has been invested in the structural modification of chloroquine (**8**), resulting in a large number of derivatives. Excellent reviews have described these efforts in depth.[14,21,57,58] Three different structural modifications are able to overcome chloroquine resistance (Figure 8): 1) the elongation, or more important, the shortening of the diaminoalkyl side chain; 2) the introduction of lipophilic aromatic moieties into the side chain; and 3) the dimerization of two 4-aminoquinolines by a linker of variable nature and length.

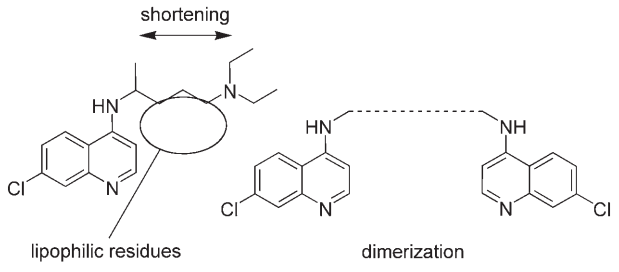

Figure 8. Modifications of chloroquine to overcome resistance.

2.2.3.1. Chloroquine analogues with shortened side chains

A chloroquine derivative with a shortened side chain is AQ13 (**13**, Figure 9). It retains activity against chloroquine-resistant parasites (IC_{50} = 59 nM versus 315 nM for CQ), but there is a clear correlation between the susceptibility of different isolates

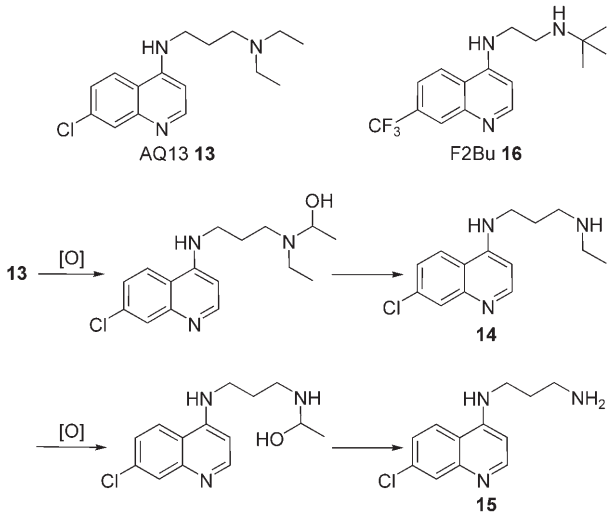

Figure 9. Chloroquine derivatives with shortened side chains.

toward AQ13 and CQ, pointing to some degree of cross-resistance. At high doses, AQ13 is described to be more toxic in rats than CQ. Furthermore, the two alkyl residues at the terminal nitrogen atom are highly susceptible to oxidative dealkylation starting with hydroxylation in the α position to the heteroatom. The resulting metabolites **14** and **15** are almost inactive.[59] A recently completed dose-ranging trial in healthy volunteers suggests that the adverse effects of AQ13 may not be different from those of CQ and that higher doses of AQ13 over CQ may be necessary to produce similar blood levels and AUC values.[60]

A possible alternative is a compound called F2Bu (**16**), in which the diethylamino residue is replaced by a *tert*-butylamino group, which is metabolically more stable because it lacks the susceptible α position.[61] Interestingly, in such short-chain quinoline derivatives, the chlorine atom at position 7, long thought to be indispensable for antimalarial activity,[62] can be replaced by a trifluoromethyl group.

2.2.3.2. Chloroquine analogues with aromatic moieties in the side chain

2.2.3.2.1. Amodiaquine

Enhancement of lipophilicity of the side chain by the incorporation of an aromatic structure resulted in amodiaquine (AQ) (**17**, Camoquin®, Figure 10). A certain degree of cross-resistance between amodiaquine (**17**) and chloroquine (**8**) is observed. Amodiaquine is effective against low-level chloroquine-resistant *P. falciparum* but not against highly chloroquine-resistant parasites.[17] An elevated rate of treatment failures (40–80%) is observed in some Asian countries.[63] Furthermore, the therapeutic value of amodiaquine is significantly decreased by the biotransformation of its *p*-aminophenol moiety into a quinonimine (compound **18**, reviewed in Ref. [57]). Quinonimine **18** is highly susceptible to nucleophilic attack, mainly by thiols, resulting in severe hepatotoxicity with an incidence of 1 in 15500. This adverse effect has been observed after prophylac-

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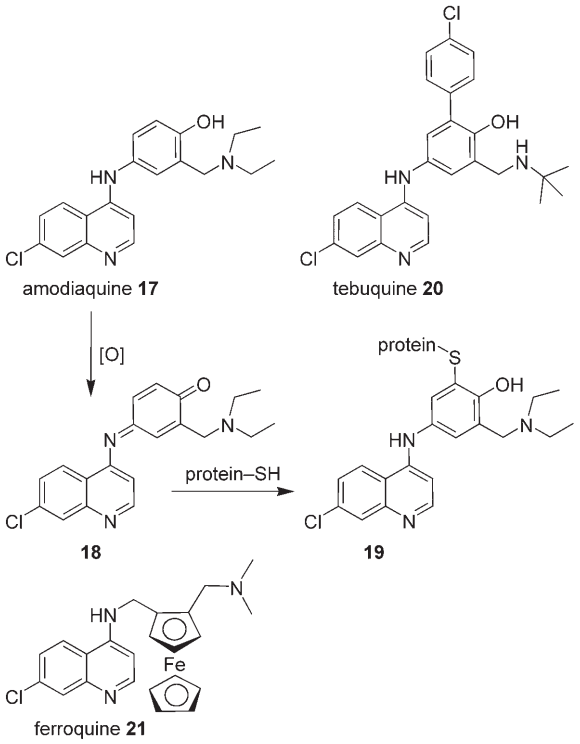

Figure 10. Amodiaquine (**17**) and other chloroquine derivatives with aromatic side chains. Amodiaquine toxicity results from oxidation of the *p*-aminophenol moiety to quinonimine **18**, which is susceptible to nucleophilic attack.

tic use of amodiaquine (**17**) for periods of three weeks up to 10 months. Moreover, similar AQ–protein complexes **19** are highly immunogenic, leading to life-threatening agranulocytosis with an incidence as high as 1 in 2100 when the drug is used prophylactically for 5–14 weeks.[17,64–67] As a result, amodiaquine is no longer on the market in western countries. It is assumed that these serious side effects occur only when AQ is used prophylactically over a prolonged period of time, and that shorter therapeutic regimes are sufficiently safe.[67] Hence, because of its activity against CQ-resistant strains and its affordability, AQ could be still used for antimalarial therapy in the developing world.[68] In a clinical study the combination of amodiaquine (**17**) with artesunate (**42**) was more efficient than amodiaquine alone.[69] Owing to some cases of neutropenia, this study stressed the need for further investigations to assess the risk-to-benefit ratio of the repeated use of amodiaquine/artesunate combinations. Despite these concerns, amodiaquine is one of the antimalarial drugs currently recommended by the WHO,[70] and many African countries have introduced amodiaquine/artesunate as a first-line therapy.[71] The triple combination of amodiaquine (**17**) with sulfadoxine (**54**) and pyrimethamine (**51**) (see section 5: Antifolates) has received a considerable amount of interest in recent years. Numerous clinical studies have been conducted with this combination (for a recent analysis of these studies, see Ref. [72])

Tebuquine (**20**) (Figure 10) is an even more lipophilic 4-aminoquinoline, which has, in addition to the Mannich base substructure, a chlorophenyl group at the aminophenol residue. The development of tebuquine has been discontinued because of its toxicity to white blood cells.

2.2.3.2.2. Ferroquine

Ferroquine (**21**, SSR97193, Figure 10) also has a diamine side chain more lipophilic than that of chloroquine. This molecule bears a ferrocenyl moiety in the side chain, a structural feature rather uncommon in potential drugs.[73] Because of this lipophilic ferrocenyl moiety, it has been proposed that ferroquine does not fit into the chloroquine-resistance transporter (CRT). It also displays some chloroquine-resistance-reversing properties.[74] Ferroquine is active against various chloroquine-sensitive and chloroquine-resistant laboratory strains (IC_{50} values: 14–42 nM)[75] as well as field isolates (IC_{50} values: 1–62 nM).[76] These activities were found to be unrelated to the mutational status of the *pfcrt* gene. The selectivity index measured against a lymphoma cell line is about 700.[77] In a mouse model, ferroquine is curative at 8.4 (19 μmol) mg kg^{-1} b.w. Reportedly, this drug is about to enter clinical development.[76]

2.2.3.2.3. Isoquine and related molecules

Two different strategies have been followed to prevent the undesirable formation of quinonimines from amodiaquine-like molecules. The exchange of the positions of the hydroxy and diethylaminomethyl groups on the phenyl ring prevents formation of the toxic quinonimine. Fortunately, this modification has no negative influence on the antimalarial activity. The resulting molecule, isoquine (**22**, ISQ1, Figure 11), displays an activity against the chloroquine-resistant K1 strain even superior to that of amodiaquine (**17**).[78] Unfortunately, as with AQ13 (**13**), the access of hydroxylating enzymes to the methylene groups in the α position to the nitrogen atom results in poor bioavailability. Again, replacement of the diethylamino moiety by a *tert*-butylamino group presumably solves the problem of rapid biotransformation.[79] It is expected that *tert*-butyliso-

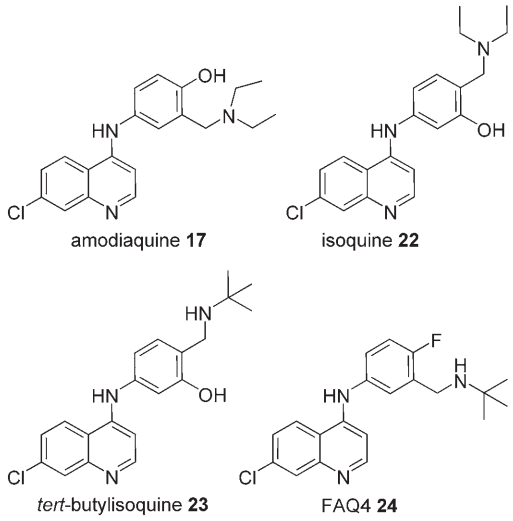

Figure 11. Amodiaquine (**17**) and congeners unable to form harmful quinonimines.

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quine (**23**) will soon advance to clinical trials (GlaxoSmithKline).[80]

A more obvious modification of the susceptible aminophenol substructure is replacement of the hydroxy function with a fluorine atom, leading to fluoroamodiaquine-4 (**24**, FAQ4).[81] The development of this type of aminoquinoline seems not to have advanced beyond preclinical stages.

2.2.3.2.4. Pyronaridine

Pyronaridine (**25**, Figure 12) is another member of the Mannich base schizonticides, although the usual quinoline heterocycle is replaced by an azaacridine. Like amodiaquine, pyronaridine also bears the aminophenol substructure, which can be oxidized to the respective quinonimine. The oxidized form has only been detected in vitro, however, when pyronaridine was incubated with liver microsomes. In rats, quinonimine or its glutathione conjugate was not detected.[82] In contrast to amodiaquine (**17**), pyronaridine (**25**) contains not one but two Mannich base side chains. It has been suggested that the second Mannich base moiety prevents formation of the hazardous thiol adducts by sterically shielding the quinonimine moiety from attack by the sulfur nucleophile.[83] Alternatively, the quinonimine group could be reduced prior to nucleophilic attack.[82] Nevertheless, it has been shown that pyronaridine is metabolized to a compound that is toxic to neutrophils.[66] Pyronaridine was developed in China in the 1980s, but has not been registered in other countries because the Chinese formulations were unable to meet western quality standards. In a clinical study performed in Thailand, high recrudescence was observed. In vitro assays revealed the presence of strains resistant to pyronaridine.[84] In Africa, where the compound has not yet been used, it showed high activity against chloroquine-resistant field isolates (IC_{50} values: 0.8–17.9 nM).[85] A significant correlation has been observed in this study between the activity of pyronaridine (**25**) and that of chloroquine (**8**), quinine (**1**), amodiaquine (**17**), and halofantrine (**30**), suggesting in vitro cross-resistance or at least cross-susceptibility. High efficiency was observed in clinical studies,[86] but efficiency dropped to 75% when the follow-up time was extended from 14 to 30 days.[87] The combination of pyronaridine (**25**) and artesunate (**42**) (acronym: PANDA) is in clinical development, having entered phase III trials in 2006 (Medicines for Malaria Venture, MMV).[88] In addition, an intravenous form for the treatment of severe malaria is to be developed.[89]

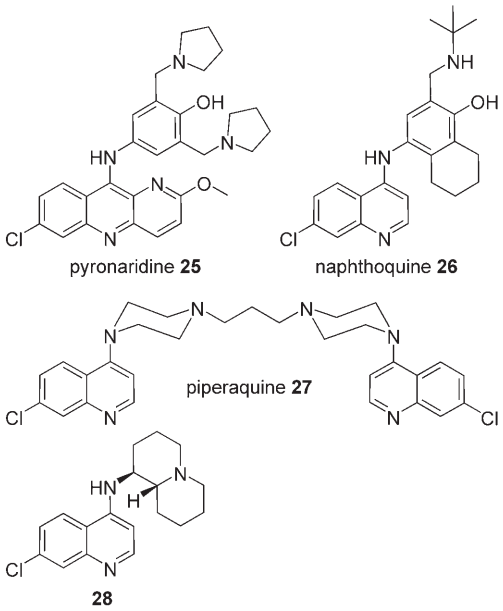

Figure 12. Pyronaridine (**25**) and more 4-aminoquinolines.

2.2.3.2.5. Naphthoquine

Naphthoquine (**26**, Figure 12), which shares greater structural similarity with amodiaquine (**17**), was registered in China in 1993. In a clinical trial the combination of naphthoquine and artemisinin (**38**) was found to be safe and effective.[90]

2.2.3.3. Dimeric 4-aminoquinolines

The third strategy to overcome chloroquine resistance is the connection of two 4-aminoquinoline moieties by linkers of various length and chemical nature. The activity of such bisquinolines against chloroquine-resistant strains has been explained by their steric bulk, which prevents them from fitting into the substrate binding site of *Pf*CRT. Alternatively, the bisquinolines may be more efficiently trapped in the acidic digestive vacuole because of their four positive charges. The most advanced representative of the bisquinolines, piperaquine (**27**, Figure 12), was developed in the 1960s and heavily used in China.[91] Widespread resistance has developed in areas where piperaquine has been extensively applied (mean IC_{50} values: 240–320 nM). In contrast, chloroquine-sensitive and chloroquine-resistant field isolates collected in Africa, where piperaquine has not yet been introduced, are still susceptible (IC_{50} values: 36 and 41 nM, respectively). Piperaquine is reportedly well tolerated; the most important side effect is an increase in blood pressure. However, there are indications of cross-resistance with chloroquine (**8**). Furthermore, cross-resistance between piperaquine (**27**) and dihydroartemisinin (**39**) can be induced in vitro. This may be a significant finding, as the combination of piperaquine and dihydroartemisinin (named Euartekin®) has entered phase II clinical trials.[88,89] In a trial conducted in Thailand, this combination showed a cure rate of 100% in a four-dose course, relative to 95% for the standard combination treatment of mefloquine (**29**) and artesunate (**42**).[92]

2.2.3.4. Further derivatives

In a more recent approach, an aminoquinolizidine moiety was connected to the 7-chloroquinoline ring to give compound **28** (Figure 12), which is highly active against the chloroquine-sensitive strain D-10 (IC_{50} = 24 nM) and the chloroquine-resistant W-2 strain (IC_{50} = 21 nM).[93]

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2.3. Arylamino alcohols

2.3.1. Quinine

As mentioned above, the bark of the cinchona tree has been used for the treatment of malaria for over 350 years.[94] The active antimalarial ingredients are quinine (**1**) and its diastereomer quinidine (**2**) (Figure 13). Quinine was isolated in 1820 and

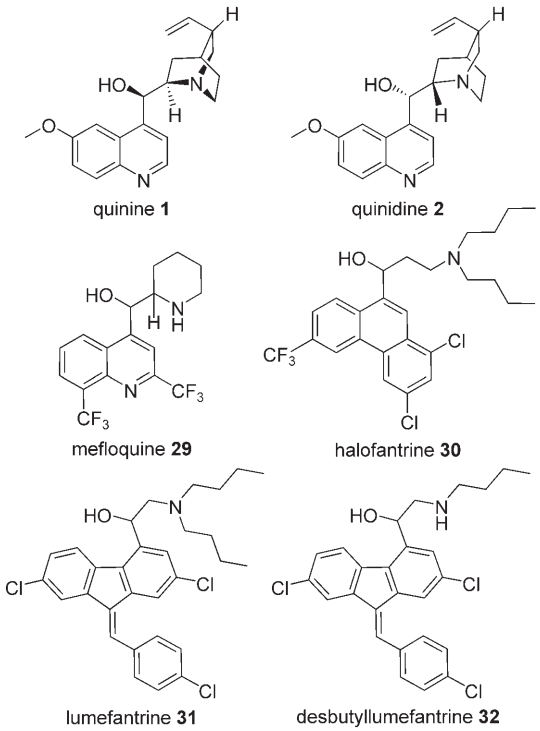

Figure 13. Arylamino alcohols.

has been used ever since. This makes malaria one of the first diseases to be treated by a pure substance. Quinine (**1**) is still one of the most important drugs for the treatment of uncomplicated malaria.[95,96] A course of seven days is required to prevent recrudescence, and because of its side effects, compliance can be low. Quinine is often the only therapeutic option for the treatment of severe malaria because preparations for intravenous applications are available.[97] An emerging alternative for this indication is intravenous artesunate (**42**) which is in clinical development (see section 3: Artemisinins). IC_{50} values between 96 and 380 nM against four different laboratory strains have been reported.[98] In clinical isolates, mean IC_{50} values vary between 136 and 286 nM.[99–101] Clinical resistance to quinine monotherapy occurs sporadically in Southeast Asia and Western Oceania. Resistance is less frequent in South America and Africa.[102] Generally, a combination of quinine (**1**) with tetracycline (**96**), doxycycline (**97**), or clindamycin (**103**) is recommended.[70,95,96] Quinine has multiple side effects, most of which are reversible, but some are severe in nature. Chinchonism, a constellation of minor but unpleasant adverse effects including nausea, headache, tinnitus, hearing impairment, dysphoria, and blurred vision is experienced by almost every patient treated with quinine (**1**). More important is its arrhythmogenic potential and the release of insulin, resulting in severe hypoglycemia. This insulin release is amplified during pregnancy, and the resulting hypoglycemia in pregnant women treated with quinine for severe malaria is particularly difficult to manage.[17]

Quinidine (**2**) is about 2–3-fold more active than quinine (**1**).[103] However, it is also more prone to induce cardiac arrhythmias. In the US and other countries, where intravenous quinine is unavailable, quinidine (**2**) is used for the treatment of severe malaria.

2.3.2. Mefloquine

Mefloquine (**29**, Figure 13) was developed from analogues of quinine (**1**) initially synthesized during World War II.[104] In 1963, the Malaria Research Program was re-established at the Walter Reed Army Institute of Research. As a product of these efforts, mefloquine was selected from nearly 300 quinoline methanol derivatives.[105] Mefloquine, which is used as the *erythro* racemate, can be regarded as structurally simplified quinine. Mefloquine was put to therapeutic use in 1985 as Lariam®. It displays high activity against most chloroquine-resistant *Plasmodium* strains (IC_{50}: 8.4 nM against the chloroquine-sensitive laboratory strain D6; 3.4 nM against the chloroquine-resistant strain W2; 6.2–10.7 nM against 32 chloroquine-resistant isolates from Cameroon).[106,107] Mefloquine (**29**) has been widely used, especially in Asia, where a considerable degree of resistance has developed over the years. In 2003, the efficiency of mefloquine monotherapy was only 62% in certain areas of Thailand.[108] The mean IC_{50} value of nearly 300 isolates collected from Thailand in the late 1990s was 71 nM.[109] Therefore, a combination with artesunate (**42**) is recommended.[69] However, because the two drugs have different pharmacokinetics, their use in combination has raised concerns that long-term exposure of the parasites to low concentrations of mefloquine (elimination half-life is 21 days)[110] may lead to selection for resistant strains.[111] A recrudescence rate of 17% has been reported for a mefloquine–artesunate combination therapy.[112] Prophylactic use of mefloquine is associated with neuropsychiatric side effects such as insomnia, depression, and panic attacks. Such side effects may be experienced by 5–29% of all patients, depending on the particular study consulted. Because of these side effects, prophylactic use of mefloquine is forbidden for people such as air crew members who require unimpeded psychomotor abilities (for an in-depth discussion of this issue, see Refs. [17, 113–116]). In cases of mefloquine (**29**) prophylaxis failure, quinine (**1**) cannot be used for therapy because of the risk of unmanageable arrhythmias. The same applies for the use of halofantrine (**30**) after mefloquine prophylaxis.[117]

The mechanism of action of mefloquine and other arylamino alcohols remains unclear. It is most likely different from the mechanism of 4-aminoquinolines. Recently, it has been proposed that arylamino alcohols act on the same (unidentified) membrane target as 4-aminoquinolines, but in a manner antagonistic to 4-aminoquinolines, by inhibiting the release of Ca^{2+} ions and thus preventing the fusion of hemoglobin-shuttling vesicles with the digestive vacuole.[118]

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2.3.3. Halofantrine

Halofantrine (**30**, Halfan®, Figure 13) was, like mefloquine (**29**), developed by the Walter Reed Army Institute of Research from a series of phenanthrene methanols, whose antimalarial activity was discovered during World War II. Halofantrine was first described in 1972[119] and introduced into therapy in 1988.[105] The highly lipophilic molecule is practically insoluble in water. Gastrointestinal resorption is improved if taken with a fatty meal, but bioavailability has been judged unpredictable.[110] Halofantrine (**30**) is active against chloroquine-resistant *Plasmodium* strains (mean IC_{50} values: 1.2 nM against 45 chloroquine-resistant Cameroonian wild isolates; 1.5 nM against 22 chloroquine-sensitive isolates).[107] Its mechanisms of action and resistance are most probably shared with those of mefloquine (**29**). Not surprisingly, cross-resistance is observed between these two antimalarial agents.[120] Halofantrine is associated with a high risk of cardiac arrhythmias (prolongation of QT interval caused by an inhibition of the inward K^+ current).[17,116,121,122] Therefore, halofantrine has been withdrawn from the market in several countries. For the *N*-desbutyl metabolite, no such effect was found.[121]

2.3.4. Lumefantrine

Lumefantrine (**31**, also known as benflumetol, Figure 13) is structurally similar to halofantrine (**30**). It was developed in the 1970s by the Academy of Military Sciences in Beijing, China. It displays lower antimalarial activity than halofantrine. IC_{50} values against three different laboratory strains were 8.9–9.9 nM for halofantrine and 34–44 nM for lumefantrine.[98] In a study with parasites collected from Cameroonian patients, the median IC_{50} value was 11.9 nM for lumefantrine and 1.6 nM for halofantrine. Chloroquine-resistant parasites are slightly more susceptible than chloroquine-sensitive strains.[123] Similar to halofantrine, the oral bioavailability of lumefantrine is variable. Oral absorption of this highly lipophilic drug is enhanced 16-fold if taken with a fatty meal. The most significant difference from halofantrine is the absence of the dangerous cardiac side effect.[124] Lumefantrine (**31**) displays in vitro synergism with artemether (**40**).[125] This combination is currently used under the brand name Riamet®.[126] Desbutyllumefantrine (**32**) is one putative metabolite, although it has not been detected in humans. It displays about fourfold higher antimalarial activity than its parent drug.[127]

2.3.5. Resistance to arylamino alcohols

The membrane transport protein *Pf*MDR1, which is the *P. falciparum* analogue of the mammalian ABC multidrug-resistance transporters, plays a central role in the sensitivity of malaria parasites to mefloquine (**29**) and the other arylamino alcohols quinine (**1**), halofantrine (**30**), and lumefantrine (**31**), and to the structurally unrelated artemisinins (see below) (Figure 14). A single point mutation N86Y of the *pfmdr1* gene is co-selected with the K76T mutation in the *pfcrt* gene which confers chloroquine resistance (see above). This *pfmdr1* mutation leads to increased sensitivity of the parasites to mefloquine, halofantrine, lumefantrine, and dihydroartemisinin (**39**).[112,128] As a consequence, the median IC_{50} value for mefloquine changes from 55 to 21 nM upon selection for the N86Y mutation. Further single point mutations S1034C and N1042D of the *pfmdr1* gene are found predominantly in chloroquine-resistant strains isolated in South America. These mutations result in 2–4-fold increased sensitivity to mefloquine (**29**), halofantrine (**30**), and artemisinin (**38**), but in contrast, decrease sensitivity to quinine (**1**) by 1.3–1.5-fold.[129]

Resistance against quinine (**1**), mefloquine (**29**), halofantrine (**30**), and lumefantrine (**31**), as well as decreased sensitivity against the structurally unrelated dihydroartemisinin (**39**) is associated with an increased copy number of the wild-type *pfmdr1* gene.[49,130–134] This leads to an increase of the median IC_{50} value for mefloquine from 50 to 170 nM, for quinine from 900 to 1717 nM, for halofantrine from 6.4 to 26.4 nM, and for artesunate (**42**) from 3.5 to 6.2 nM.[112] The numbers of *pfmdr1* gene copies correlates positively with the risk of failure of mefloquine and mefloquine–artesunate therapy.[112] Accordingly, decreasing the *mdr1* copy number by genetic disruption of one of two *mdr1* copies in a laboratory strain raises the sus-

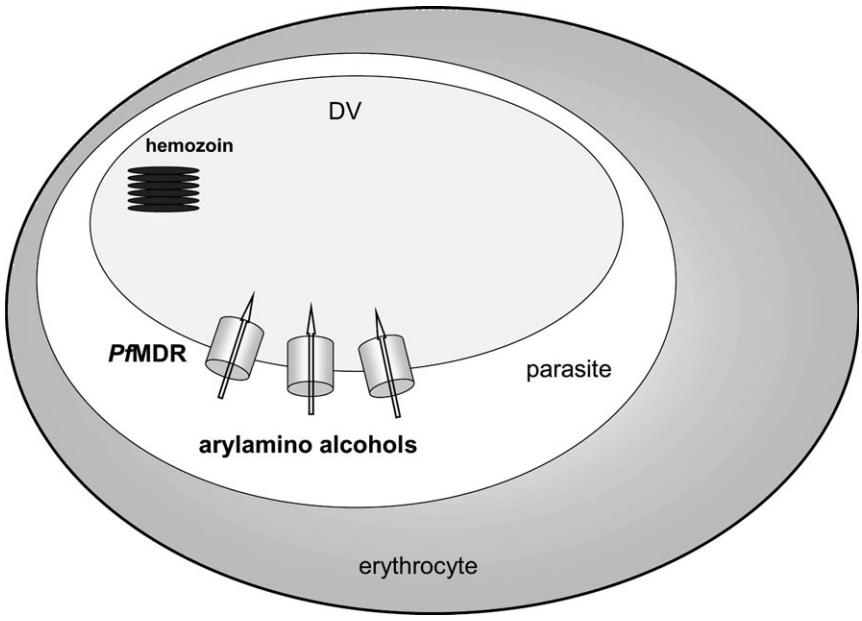

Figure 14. The main factor in the resistance against quinine (**1**), mefloquine (**29**), halofantrine (**30**), lumefantrine (**31**) and artemisinins is the increase in copy numbers of the *pfmdr1* gene coding for an ABC membrane transporter (*Pf*MDR1), which transports arylamino alcohols into the digestive vacuole (DV).

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ceptibility toward mefloquine, lumefantrine, halofantrine, quinine, and artemisinin 2–4-fold.[135] Very recent results indicate that mefloquine, halofantrine, and artemisinin are transported from the cytoplasm into the food vacuole, thereby removing these drugs from their putative targets in the cytosol.[136]

In summary, the current putative mechanism of resistance against 4-aminoquiniolines and arylamino alcohols is as follows:[134] Chloroquine resistance proceeds through *pfcrt* mutations accompanied by (compensatory?) *pfmdr1* mutations, resulting in increased sensitivity of chloroquine-resistant strains to the arylamino alcohols mefloquine (**29**), halofantrine (**30**), and lumefantrine (**31**). Under pressure by these arylamino alcohols, the *pfmdr1* wild-type is first selected for and then amplified with *pfcrt* (in particular, K76T) remaining unchanged. The *pfmdr1* amplification results in resistance against arylamino alcohols and also in slightly decreased IC_{50} values for chloroquine (**8**) which are probably of no clinical significance. Resistance to quinine (**1**) is a little more complex. Decreased sensitivity is associated with point mutations in the *pfmdr1* gene, but also in the *pfcrt* and *pfnhe-1* genes, the latter coding for a Na^+/H^+ exchange protein. The main factor in quinine resistance, however, seems to be the amplification of the *pfmdr1* gene described above.[49,112,127,137,138]

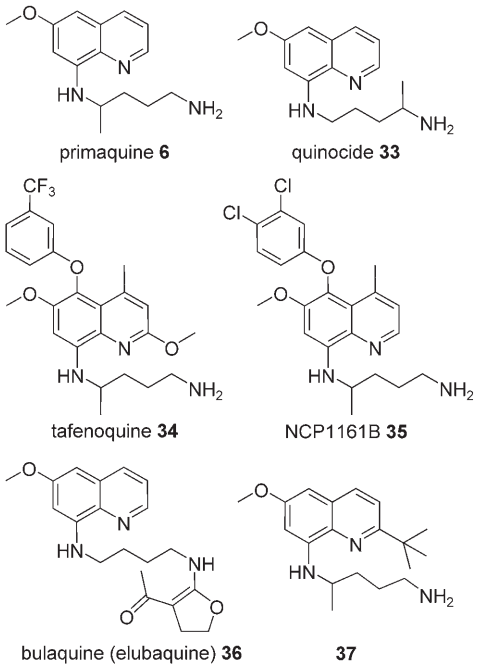

Figure 15. 8-Aminoquinolines.

2.4. 8-Aminoquinolines

2.4.1. Primaquine

As mentioned previously, pamaquine (**5**, also known as plasmoquine or plasmochin) was the first synthetic antimalarial agent that emerged from the development efforts at Bayer in the 1920s, but it was not widely used owing to its toxicity. Extensive efforts were undertaken in the US to vary the structure of pamaquine. Under the pressure of the Korean conflict, primaquine (**6**, Figure 15) was introduced in 1952. The terminal diethylamino moiety of pamaquine (**5**) is replaced by an unsubstituted primary amine in primaquine (**6**).[139] Its more toxic isomer quinocide (**33**) was used in the former USSR and Eastern Europe. Primaquine distinguishes itself from other antimalarials, as it shows activity against the liver and the sexual blood stages of different *Plasmodia*, while its activity against asexual blood stages is too low to be therapeutically significant. Primaquine is still the only antimalarial drug licensed for the radical cure (or anti-relapse therapy) of *P. vivax* infections. Because chloroquine resistance is not widely spread with these parasites, the acute stages are treated with chloroquine (**8**). However, hepatic forms (hypnozoites) of the parasite can persist for long periods of time in the liver and cause late relapses. Therefore, chloroquine therapy of *P. vivax* infections is normally followed by a second course with primaquine to eradicate the hypnozoites. Owing to its short half-life of 4–6 h, primaquine requires daily administration for 14 days to achieve a cure. In addition, a single dose of primaquine is sometimes used to sterilize sexual blood stages to prevent transmission of *P. falciparum* malaria. Although not approved for this indication, primaquine has shown to be effective in the chemoprophylaxis of *P. falciparum* infections. In clinical trials, 30 mg of primaquine per day had a protective efficacy against *P. falciparum* and *P. vivax* of 85–93% (for a review of primaquine (**6**) as a prophylactic agent, see Ref. [113]).[140,141] The mechanism of action of the 8-aminoquinolines is unclear, and there is no firm understanding of the mechanism of primaquine. A ubiquinone (**82**) antagonistic effect of a quinonimine metabolite has been suggested, leading to the inhibition of electron transport in the respiratory chain. Furthermore, these metabolites should undergo a redox cycle causing oxidative stress, thereby depleting the glutathione storage in individuals deficient in glucose-6-phosphate dehydrogenase (G6PD).[142] Interestingly, primaquine (**6**) also exhibits some activity in reversing chloroquine resistance.[143] The most serious side effect of primaquine is a potentially life-threatening hemolysis in persons deficient in G6PD, a genetic polymorphism particularly abundant in Africa and Asia.[17] The 8-amino substituent has been shown to be the main cause of antiplasmodial activity but also in the formation of methemoglobin.[144]

2.4.2. Derivatives of primaquine

Compared with the development of 4-aminoquinolines, fewer yet substantial efforts have been invested in the development of 8-aminoquinolines, especially by the Walter Reed Army Institute of Research, beginning in 1968 as a result of the US engagement in Vietnam.[139,145] At least one drug in clinical development has emerged from these efforts.

2.4.2.1. Tafenoquine

Tafenoquine (**34**, WR 238605, Figure 15) is a more lipophilic derivative of primaquine (**6**). The main structural difference is the trifluoromethylphenoxy substituent, which confers higher ac-

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tivity against the blood and liver stages as well as a higher sporontocidal activity, but decreased gametocidal activity. In a study against seven laboratory clones and isolates, tafenoquine showed a mean IC_{50} value of 436 nM in comparison with 1400 nM for primaquine and 189 nM for chloroquine.[146] Against isolates, mean IC_{50} values of 2.68 and 7.28 μM were found for tafenoquine and primaquine, respectively.[147] Therefore, its activity against blood stages of *P. falciparum* is weaker than that of most other blood schizonticidal agents. Tafenoquine (**34**) is generally regarded to be better tolerated than primaquine (**6**), but it still carries some risk of causing hemolysis in G6PD-deficient humans. As with primaquine, nothing firm is known about the mechanism of tafenoquine. In addition to a primaquine-like effect on the respiratory chain, some inhibitory activity toward heme polymerization[146] or activity similar to quinine has been postulated to explain the activity of tafenoquine against asexual blood stages.[148] Several clinical studies have shown protective efficacy between 86–100%.[144,149,150] For this reason, tafenoquine may become an important agent in chemoprophylaxis, although it is mandatory to define the G6PD status of the individual patient beforehand.[113] In a rodent malaria model, tafenoquine monotherapy rapidly resulted in the selection for resistant strains.[151] However, in the same study, resistance could also be induced against primaquine. Because clinical resistance to primaquine has not been reported despite its use for 50 years for anti-relapse therapy,[142] the rodent data may not be a significant predictive model.

2.4.2.2. Further 8-aminoquinolines

In the 2006 MMV portfolio, the preclinical development of an enantiomerically pure 8-aminoquinoline is indicated. The compound in question seems to be the (−) isomer NCP1161B (**35**) responsible for the antimalarial effect, whereas the (+) enantiomer NCP1161A appears to cause the formation of methemoglobin.[152]

Another 8-aminoquinoline named bulaquine or elubaquine (**36**) is in clinical use in India against *P. vivax* infections. To block biotransformation into potentially toxic metabolites 2-*tert*-butylprimaquine (**37**) has been prepared with an IC_{50} value of 124 nM.[153]

3. Artemisinins and Synthetic Peroxides

Extracts of the herb known as sweet wormwood have been used in China for the treatment of fever for as long as 2000 years. In 1971 the active ingredient, the sesquiterpene lactone artemisinin (**38**) was isolated, which has been used in China for the treatment of malaria since 1972.[154] Artemisinin is a highly active antimalarial agent. In assays with 40 wild isolates from northwestern Thailand, a mean IC_{50} value of 12.1 nM (8.2–17.9 nM) has been reported.[148] In a different study, a slightly higher value of 21 nM (15.5–28.3 nM) was observed.[155]

3.1. Mechanism of action, possible resistance, and activity

A key structural feature of all artemisinins is the 1,2,4-trioxane substructure or, more precisely, the endoperoxide, which is mandatory for antimalarial activity. Despite the growing importance of artemisinins, their exact mechanism of action is still unresolved and remains a matter of intense debate. It has been proposed that iron(II)-mediated cleavage of the endoperoxide leads to the formation of different C-centered radicals which may be primary or secondary in nature (Figure 16). Which, if not possibly both, of these radicals is the active species is unclear.

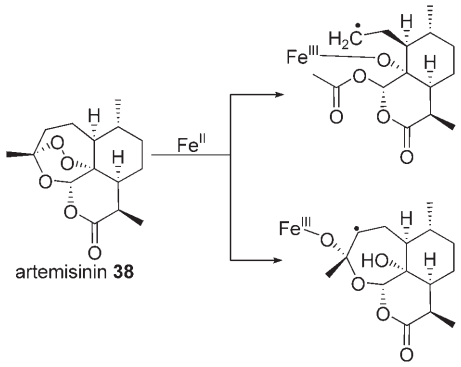

Figure 16. Iron(II)-mediated formation of primary or secondary carbon radicals from artemisinin.

For a long time it was thought that the formation of C radicals takes place in the digestive vacuole and that ferrousprotoporphyrin IX is the activating species. The reactive C radicals are thought to subsequently react more or less indiscriminately with different protein targets as well as with ferriprotoporphyrin IX itself, thus preventing heme detoxification and inhibiting a multitude of enzymes.[23,156–159] O'Neill and Posner formulated the mechanism of artemisinins as "iron-triggered cluster bombs" (Figure 17).[160] Although very attractive—the development of resistance against a drug that acts nonspecifically against multiple targets is unlikely—this concept has been questioned owing to some contradictory findings: artemisinins act against all developmental parasite stages, including those which do not produce hemozoin. Several experiments (reviewed in Ref. [163]) detected labeled artemisinin derivatives localized not within but only outside the digestive vacuole, and there are some highly active artemisinin derivatives that are more or less insensitive to Fe^{II}-mediated cleavage.[207] Recently, Krishna and co-workers put forward another theory: endoperoxide cleavage should take place in the cytoplasm catalyzed by a cytoplasmic iron(II) source. The resulting reactive species then very specifically inhibits an ATP-dependent Ca^{2+} pump located on the endoplasmic reticulum (Figure 18). The pump, called *Pf*ATP6, is a homologue of a mammalian sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA).[161] A homology model of *Pf*ATP6 has been prepared, and a number of artemisinin derivatives have been docked computationally into the thapsigargin binding site of this model. The main binding

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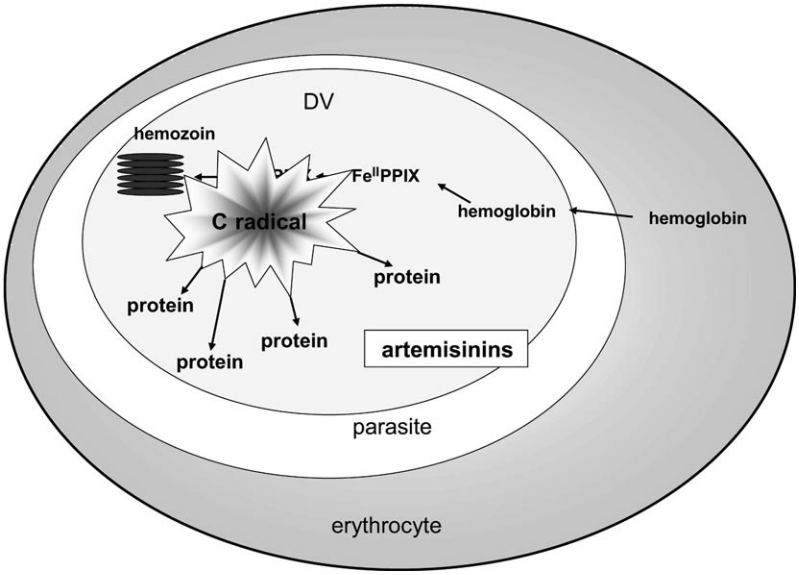

Figure 17. The "iron-triggered cluster bomb": According to the former theory about the mechanism of action of artemisinins, Fe^{II}FPIX catalyzes the formation of carbon-based radicals in the digestive vacuole, deactivating proteins more or less indiscriminately.

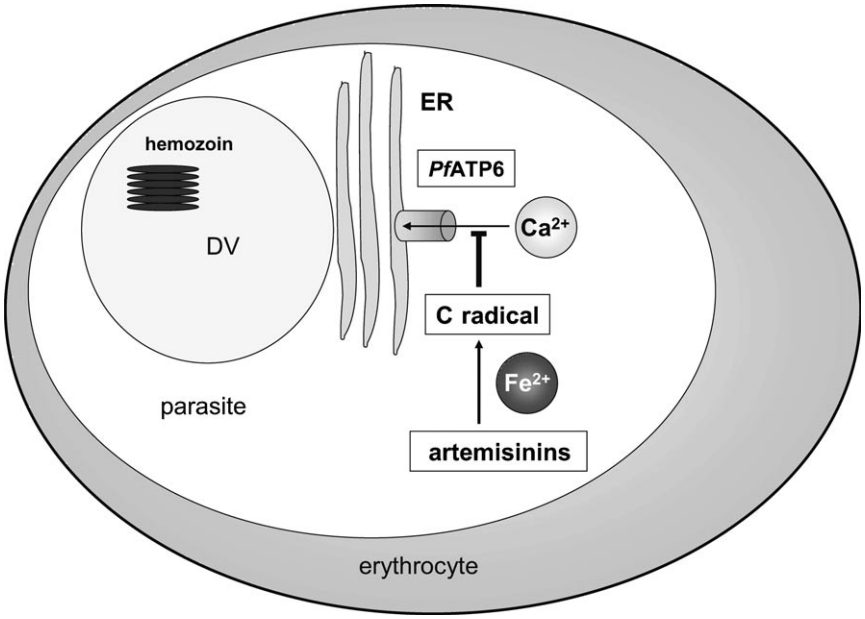

Figure 18. Recent results suggest that Fe^{II}-mediated radical formation takes place in the cytosol; these radicals specifically inhibit a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) called *Pf*ATP6.

interaction of artemisinins to *Pf*ATP6 seems to be mostly of a hydrophobic nature. The endoperoxide is exposed to the solvent. Therefore, it has been suggested that binding of artemisinins to the target protein may precede iron(II)-mediated peroxide activation.[162] It is still unknown how the inhibition of *Pf*ATP6 leads to the rapid killing of the parasite. However, these findings raise another important issue: because it has been thought so far that artemisinins act more or less indiscriminately against multiple protein targets, the risk of resistance has been regarded as low. This needs to be re-evaluated,[134,163] as it was possible to create a *Pf*ATP6 mutant that is resistant to artemisinins by the exchange of just one amino acid (L263E).[164] Indeed, *Pf*ATP6 mutations were found in isolates displaying significantly decreased susceptibility toward artemether (**40**).[165] Furthermore, stable resistance to artemisinins could be introduced in rodent parasites lacking mutations in *atp6* and *mdr1* genes.[166]

Artemisinins act predominantly against the late ring stages at which the metabolic activity is highest, but in contrast to other antimalarials, they also act against the small ring stages present in the erythrocytes a few hours after infection. In addition, artemisinins are active against sexual blood stages, thereby reducing transmission of the parasites.[167] However, in a recent study this effect was judged to be only moderate.[168] Artemisinins are highly active, decreasing the parasite biomass 10000-fold in a single asexual cycle.[169,170] This makes artemisinins the most active and rapid-acting antimalarial drugs known today.

3.2. First-generation semisynthetic artemisinins

3.2.1. Artemether

Because artemisinin (**38**) is only poorly soluble in water and in oil, semisynthetic derivatives have been developed. The reduction of the lactone substructure of artemisinin leads to the hemiacetal-containing compound dihydroartemisinin (**39**). Alkylation of the hemiacetal yields artemether (**40**) and arteether (**41**), both characterized by an acetal moiety (and not by an ether as the names might indicate) (Figure 19). Arteether is used in India and the Netherlands (Artemotil®) but the more prevalent substance is artemether (Paluther®, Artenam®, Artemos®).[170] Artemether displays high antimalarial activity, with reported mean IC_{50} values against wild isolates of 1.54, 2.5, 5.3, and 16.2 nM.[107,171,172] Oxidative demethylation starting with the well-known hydroxylation in the α position to the exocyclic acetal oxygen atom

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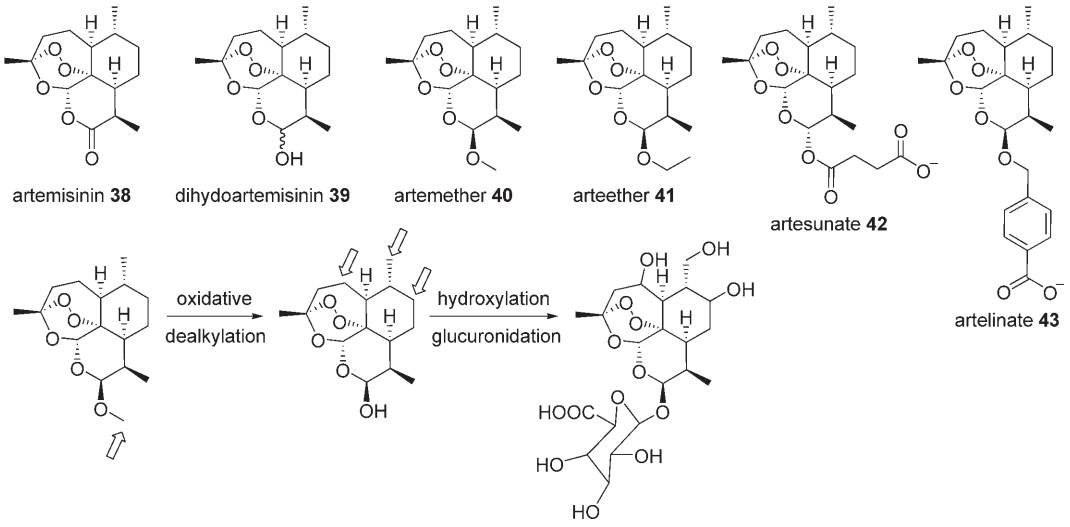

Figure 19. Artemisinin and "first-generation semisynthetic artemisinins" as well as the biotransformation of artemether (arrows indicate sites of hydroxylation).

rapidly leads to dihydroartemisinin, which contributes approximately 50% of the effect of artemether (**40**). Dihydroartemisinin (**39**) itself undergoes rapid hydroxylation at positions 5, 7, and 14 (indicated in Figure 19), and glucuronidation at the hemiacetal OH group to yield highly water-soluble metabolites, resulting in an elimination half-life of 40–60 min (reviewed in Ref. [156]).[173] Because of this high clearance rate, artemisinins have to be administered over a period of 5–7 days, which leads to poor compliance and ultimately to recrudescence.[1] Therefore, artemisinins are combined with antimalaria drugs that have prolonged half-lives (for a recent review, see Ref. [174]).[111,175,176]

The modification of dihydroartemisinin to artemether (Figure 19) led to a more lipophilic molecule, which is better absorbed from the GI tract, thus allowing oral administration. Blood levels after i.m. application of an oily solution have shown to be unpredictable and sometimes undetectable.[170] Currently, the application of artemether (**40**) with lumefantrine (**31**) (Coartem® or Riamet®) is the only artemisinin-based combination therapy available manufactured under Good Manufacturing Practice (GMP) standards. In addition, a formulation for small children (Pediatric Coartem) is in clinical development.[89] Although expensive and for most malaria patients unaffordable, this combination is generally thought to be effective and well tolerated.[177–179] Conversely, therapy failure rates of 13–30% using this combination have been reported in Cambodia.[63] A recent study reported selection for the N86 wild-type of *Pf*MDR1, which is associated with decreased sensitivity to both lumefantrine and artemisinins (see section 2.3: Arylamino alcohols).[180] For this reason, concerns have been expressed that fast development of resistance against artemether/lumefantrine may be possible.[181] In another study, it was demonstrated that the presence of multiple copies of the *pfmdr1* gene leads to a twofold increase of the IC_{50} value of lumefantrine (**31**), and an elevated risk (13%) of failure of the four-dose artemether/lumefantrine regime. However, the six-dose administration has been shown to result in a lower failure rate of only 3.2%.[182]

3.2.2. Artesunate

Another modification of dihydroartemisinin is artesunate (**42**), in which the hemiacetal OH group is acylated with succinic acid (Figure 19). Artesunate is an unstable drug; the succinic ester is rapidly (nonenzymatically?) cleaved, releasing dihydroartemisinin (**39**) as the active agent. Because of the free carboxylate, artesunate is a water-soluble drug that can be administered via the i.v. route. This is of particular importance for the treatment of severe malaria tropica in which the condition of the patients prohibits any other route of administration. A study on 80 children with complicated malaria conducted in India showed the superiority of artesunate (**42**) over quinine (**1**).[183] In a recent study conducted in various regions of Asia, intravenous artesunate was significantly superior to the standard i.v. regime with quinine in the treatment of adult severe malaria.[184] However, this study has also gathered some criticism.[185,186] Currently available artesunate preparations for parenteral application originate from China or Vietnam and are unable to meet western quality standards. Phase II and III studies were to commence in 2006 in a joint project by the University of Tübingen in Germany (P. G. Kremsner), an industrial partner, and the Walter Reed Army Institute of Research, with the aim of bringing an intravenous artesunate preparation to the market in 2009, to be manufactured according to western drug regulations.[187]

In addition to i.v. application, artesunate can also be administered via the i.m., rectal, or oral routes. In a recent study of severe malaria in children, rectally administered artesunate (**42**) was at least as effective as i.m.-applied artemether (**40**) and thus may be useful in settings in which parenteral therapy cannot be given.[188] Artesunate is the main artemisinin combination partner in artemisinin-based combination therapy (ACT),

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which is now used as the standard therapy in many countries. Combinations with numerous antimalarials are used, most of which are questionable because of unmatched pharmacokinetic profiles or widespread resistance against the non-artemisinin component of the combination. In particular, the combination of artesunate (**42**) with mefloquine (**29**) is widely used in Asia.[189–191] The combination of drugs with unmatched pharmacokinetic profiles has been especially questioned because of the risk that low concentrations of the longer-lasting drug unprotected by the more rapidly excreted artemisinin (**38**) will select for parasites that are resistant against that longer-lasting drug.[111] Recently, the decreased efficacy of the combination mefloquine and artesunate has been reported from areas of Thailand.[108]

3.2.3. Artelinate

Artelinate (**43**, Figure 19) has been developed as a potential successor of artesunate (**42**), as it bears the metabolically more stable acetal substructure and a polar carboxylate group, making the drug water-soluble. In an animal model, intravenous artelinate was shown to be superior to artesunate.[192] However, further development of artelinate has been discontinued in favor of artesunate[170] because of the higher neurotoxicity of artelinate.[193, 194]

3.2.4. Toxicity

Neurotoxicity is a major concern with all artemisinin derivatives owing to their biotransformation into dihydroartemisinin (**39**), which is believed to be the final neurotoxic agent. Specific brain-stem toxicity has been observed in animal experiments.[195] In contrast to these findings, no neurotoxicity has been observed in humans despite the widespread use of artemisinins in China for 30 years (for reviews, see Refs. [17, 116]). There are conflicting reports about the loss of hearing under combination therapy with artemether (**40**) and lumefantrine (**31**).[196, 197] A further concern is the use of artemisinins in pregnancy, as fetotoxicity has been observed in animal experiments.[198] However, another clinical study indicates that artemisinins might be safe in the second and third trimesters.[174]

3.2.5. Availability

Semisynthetic artemisinins of the first and second generations (discussed below) all rely on a sufficient supply of artemisinin isolated from plants. Maximum extracted yields of artemisinin are commonly about 0.6%.[199] Until now, the herb *Artemisia annua* is cultivated in China and Vietnam. Due to the growing need resulting from the increased adaptation of ACT by more and more countries, the raw material is already in short supply.[200] Despite current efforts to cultivate *Artemisia* in Africa as well, it remains a question as to whether increasing future needs will be met.

3.3. Second-generation semisynthetic artemisinins

A common problem of the so-called first-generation semisynthetic artemisinins is their rapid biotransformation that results in a short half-life and the formation of the neurotoxic dihydroartemisinin (**39**). Much work has been invested in the development of second-generation artemisinins (Figures 20 and 21). Methyl and ethyl residues of the first-generation semisynthetic artemisinins artemether (**40**) and arteether (**41**) have been replaced by numerous other residues, some of them carrying polar groups, as is the case with artelinate (**43**, see above), to decrease the lipophilicity and enhance water solubility. Most variations have been carried out at position 10, where the exocyclic oxygen atom is replaced by carbon substituents to remove the metabolically sensitive acetal substructure. Alkyl, aryl, and heteroaryl residues have been placed at this position. Some substituents have been used for the formation of dimers that carry two dihydroartemisinin (**39**) substructures.

Modifications have also been carried out at position 16. Several reviews cover this issue in depth.[160, 201–205] Representative examples are shown in Figures 20 and 21. Compounds with in vitro activity superior to that of the first-generation artemisinins were obtained, sometimes with promising in vivo activity,

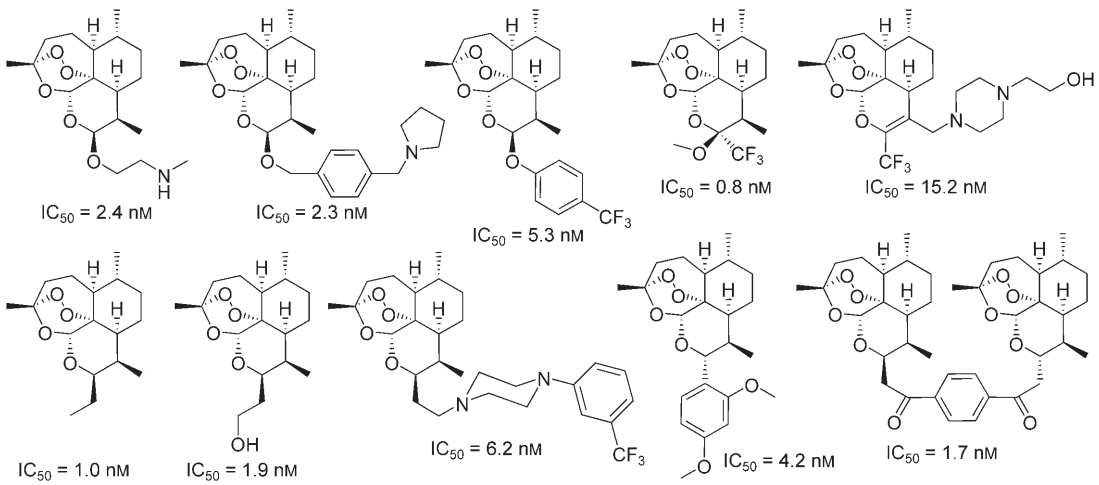

Figure 20. Second-generation semisynthetic artemisinins.

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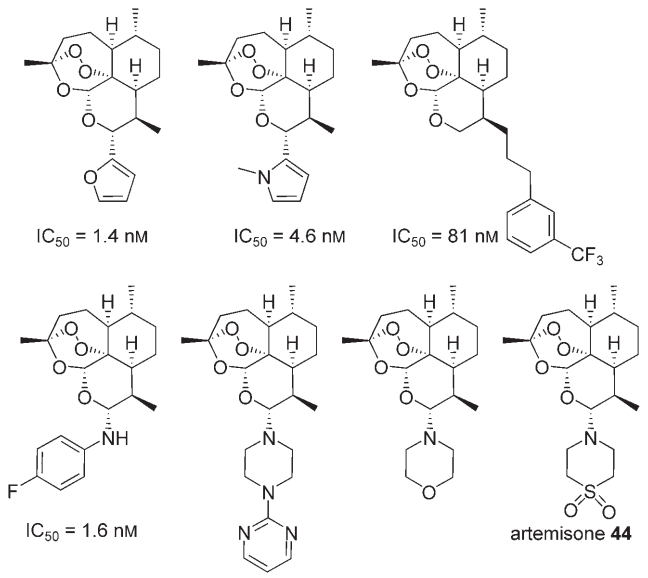

Figure 21. From the large number of "second-generation semisynthetic artemisinins" only artemisone (**44**) has made it close to the clinical development stage.

but none of these compounds have made it to the clinical stages of development.

3.3.1. Artemisone

A particularly successful series of second-generation semisynthetic artemisinins has been developed by Haynes (Hong Kong University of Science and Technology) applying the principles of ADME.[206,207] These compounds carry a nitrogen substituent at position 10, thus forming a class of *N,O*-acetal artemisinin derivatives. Although not the most active compound in this series of 10-alkylamino artemisinins, the thiomorpholino-*S,S*-dioxide derivative artemisone (**44**) has emerged as the most promising candidate for further development (Figure 21). Artemisone inhibits *Pf*ATP6 with a K_i value of 1.7 nM (artesunate (**42**): $K_i = 167$ nM).[164] The introduction of the polar heterocycle was seemingly guided by the idea to adjust the polarity of artemisone to a desirable value in order to improve pharmacokinetic properties as well as to eliminate neurotoxicity. Indeed, artemisone did not show any neuro- or cytotoxicity. In animal experiments, artemisone was about 2–5-fold more efficient than artesunate.[208] Artemisone had been in clinical studies, but at present, further development of this promising drug is allegedly uncertain.

3.4. Synthetic endoperoxides

As soon as it became clear that the antimalarial activity of artemisinin derivatives is based on the endoperoxide substructure, several groups embarked on the development of fully synthetic endoperoxides as antimalarials, resulting in numerous candidates of varying complexity. The development of antimalarial endoperoxides has been comprehensively reviewed recently.[160,201–204,209] Therefore, only representative examples are shown in Figure 22.

It has been concluded that all these compounds bear certain limitations. The complicated synthesis, which makes them unsuitable for upscale to production level, often results in racemic products and poor pharmacokinetic properties. Therefore, none of the endoperoxides described so far have made it into clinical trials.

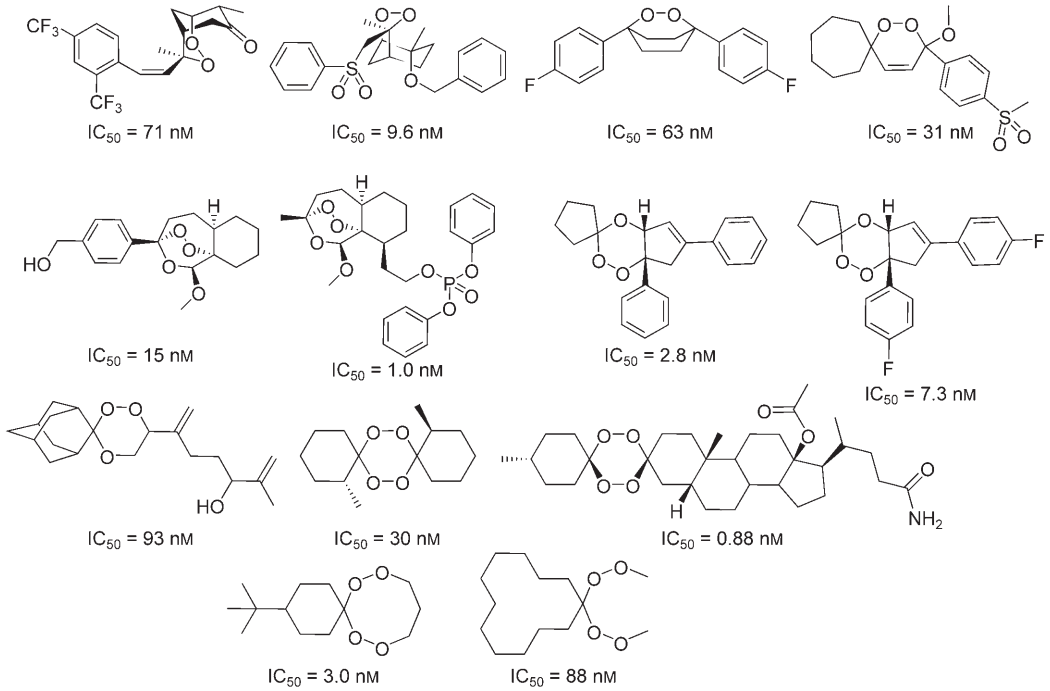

Figure 22. Despite high activity in some cases, none of these synthetic peroxides has advanced beyond preclinical development.

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3.4.1. OZ-277

In contrast to the efforts described above, the work of Vennerstrom and co-workers on 1,2,4-trioxolans has resulted in the first antimalarial endoperoxide OZ-277 (**45**, also known as RBx11160, Figure 23), which has recently entered into clinical

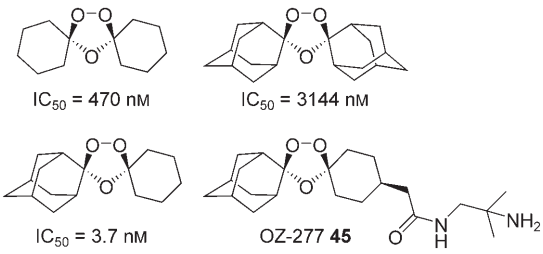

Figure 23. Development of OZ-277, the most advanced synthetic peroxide.

trials.[89,210] The 1,2,4-trioxolane system is well known to organic chemists as secondary ozonide, a highly reactive intermediate of the ozonolysis reaction. The key issue in the development was to balance stability against reactivity through the selection of appropriate residues on both sides of the trioxolane system. Whereas two cyclohexane rings clearly did not provide enough protection for the sensitive heterocycle, resulting in rapid compound breakdown, two adamantane rings sterically shielded the trioxolane too much, resulting in a stable compound albeit one with insufficient activity against *Plasmodia*. However, by decorating the trioxolane ring with an adamantane residue on one side and a cyclohexane group on the other, the critical balance between stability and reactivity could be obtained.[211,212]

Finally, the addition of an aminoacyl residue provided the correct polarity and solubility, resulting in the desired pharmacological properties. OZ-277 displayed high activity against field isolates from Gabon (median $IC_{50} = 0.47$ nM; range: 0.13–2.23 nM).[213] Its stage specificity is similar to that of artemisinins.[214] The activity against *P. vivax* is in the same range as the activity against *P. falciparum*.[215] If the outcome of phase II studies confirm the promising preclinical results, OZ-277 could become an easily accessible alternative to artesunate (**42**) owing to its short and straightforward synthesis.[216] Development of a pediatric formulation and an intravenous formulation is in progress. Piperaquine (**27**) has been selected as a first combination partner.[89] A second-generation ozonide, which should provide a single-dose oral cure of uncomplicated malaria, is in development. The lead ozonide (no structure disclosed) has an extended half-life and a higher oral bioavailability relative to the parent OZ-277. It displayed a cure rate of 100% at a single dose of 30 mg kg^{-1} b.w. in a murine model and was as effective as mefloquine (**29**) as a prophylactic agent.[89]

4. Chimeric Molecules

Based on the assumption that both 4-aminoquinolines and trioxanes act on heme, chimeric molecules have been designed to contain the 4-aminoquinoline moiety of chloroquine (**8**) and a trioxane moiety (Figure 24).[217,218] The most active compound of this series, **46**, inhibits the growth of various laboratory strains, with IC_{50} values between 5 and 19 nM. It displays ED_{50} values in *P. vinckei*-infected mice of 5.8 μmol (5 mg) kg^{-1} day^{-1}

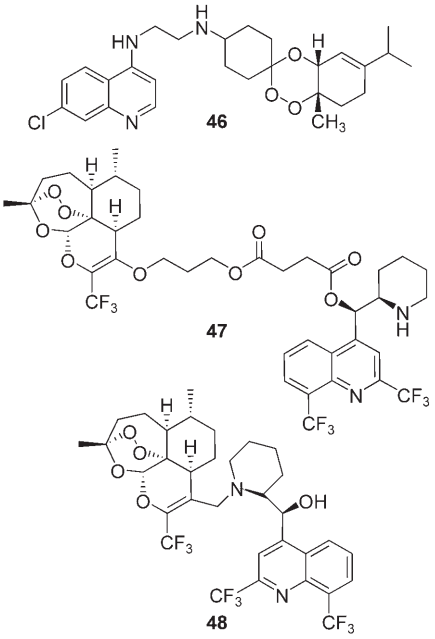

Figure 24. Chimeric molecules that comprise two different antimalarial moieties.

and 21 μmol (18 mg) kg^{-1} day^{-1} under intraperitoneal and oral administration, respectively.[219] This compound has been shown to alkylate heme in vitro.[220] In a related approach, biologically cleavable (compound **47**) and noncleavable (compound **48**) chimeras of mefloquine (**29**) and a 10-trifluoromethylartemisinin were prepared. Against four laboratory strains the interchangeable chimera **47** performed slightly better than the non-interchangeable **48**, with IC_{50} values of 2.4–6.6 nM versus 10.6–17.2 nM, respectively.[221]

5. Antifolates

In most species, tetrahydrofolic acid plays a key role in the biosynthesis of thymine, purine nucleotides, and several amino acids (Met, Gly, Ser, Glu, and His). Whereas humans depend on dietary intake of pre-formed dihydrofolic acid as an essential nutrient, which is then reduced to tetrahydrofolic acid, pathogenic microorganisms including *Plasmodia* can synthesize dihydrofolic acid from simple precursors (Figure 25). Furthermore, *P. falciparum* is able to use exogenous dihydrofolic acid via a salvage pathway.[222] Inhibitors of two key enzymes of the folate biosynthetic pathway, dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR), have long been used in the treatment of bacterial and protozoal infections. Whereas dihydropteroate synthase is completely absent in humans, bacterial and protozoal dihydrofolate reductases are sufficiently different from the human enzyme to allow the development of

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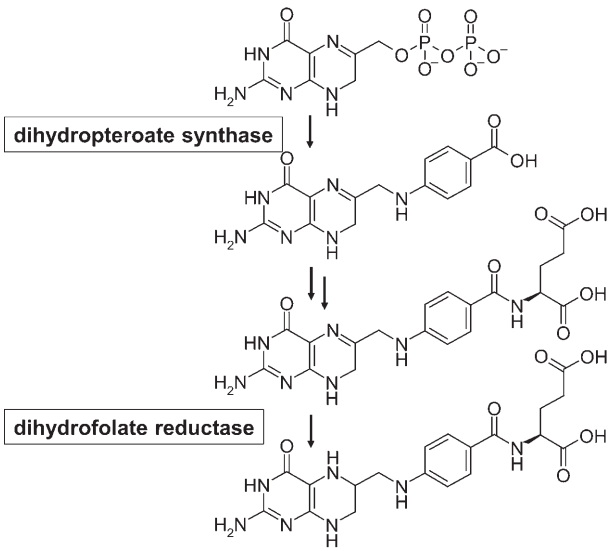

Figure 25. The folate pathway (simplified) showing the targets of the antifolates.

selective inhibitors. In *P. falciparum*, both enzymes are present not as monofunctional proteins, but the DHPS and DHFR activities are present on specific domains of bifunctional proteins. In the case of DHPS, the preceding enzymatic activity of hydroxymethyldihydropterin pyrophosphokinase[223] is located on the same polypeptide. DHFR, in turn, is collocated with the subsequent thymidylate synthase activity on a single protein. The use of antifolates against malaria[224–228] and the possibility of using other enzymes along the folate biosynthetic pathway as drug targets has already been reviewed.[229,230]

The first antifolate to be used against malaria was the well-known DHPS inhibitor sulfachrysoidine (**49**, Prontosil®, Figure 26). It was developed in 1932 by Domack as an antibacterial agent. Later it was found that sulfanilamide (**50**), arising from the reductive cleavage of the azo substructure, is the active component. In 1937, sulfachrysoidine was successfully used in a trial against malaria, but interest in sulfonamides diminished because of the continuing effectiveness of quinine (**1**) and the development of other synthetic antimalarials. Only when sulfonamides such as sulfadoxine (**54**) with longer half-lives and improved toxicological profiles were developed in the late 1950s, was interest in sulfonamides renewed, especially as combination partners for the DHFR inhibitors proguanil (**53**, Palundrine®) and pyrimethamine (**51**, Daraprim®), which were introduced in close succession in the late 1940s and early 1950s for the therapy and prophylaxis of malaria. Proguanil (**53**) is a prodrug that yields the active metabolite cycloguanil through oxidative ring closure. Both DHFR inhibitors **51** and **52** are structurally closely related; the main difference is the tetrahedral geometry at C6 of cycloguanil (**52**), which removes the heterocyclic planarity of pyrimethamine (**51**). Both drugs are highly active inhibitors of *P. falciparum* dihydrofolate reductase (*Pf*DHFR) with K_i values of 1.5 nM and 2.6 nM, respectively.

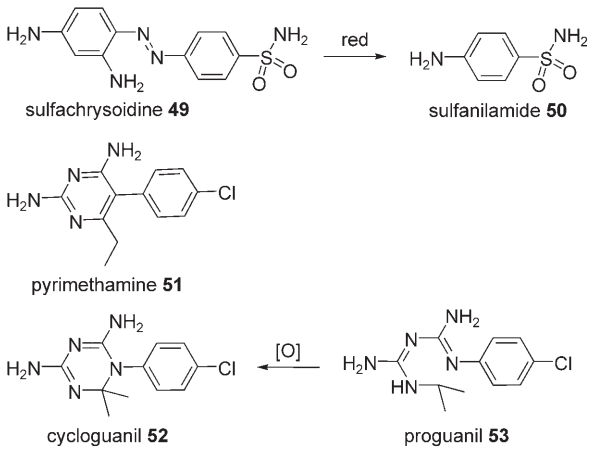

Figure 26. Bioactivation of sulfachrysoidine (**49**) yields the first dihydropteroate synthase inhibitor sulfanilamide (**50**). Dihydrofolate reductase inhibitors pyrimethamine (**51**) and cycloguanil (**52**) have been widely used. Cycloguanil is formed through oxidative cyclization of its prodrug proguanil (**53**).

5.1. Binding mechanism of DHFR inhibitors and development of resistance

In contrast to many other antimalarials, the interaction of DHFR inhibitors with their target is known at the molecular level.[231–234] The main interactions are illustrated in Figure 27 with pyrimethamine (**51**) as an example. The negatively charged carboxylate group of Asp 54 interacts with the positively charged NH moiety of the pyrimidine ring and the NH_2 group at position 2, whereas the NH_2 hydrogen atoms at position 4 form hydrogen bonds with the backbone carbonyl groups of Ile 14 and Ile 164. Another hydrogen bond is formed between the hydroxy group of the Ser 108 side chain and NADPH. The dihydropyridine ring undergoes a charge-transfer interaction with the chlorophenyl residue of pyrimethamine.

The widespread use of these compounds has led to the selection for resistant strains. Resistance against DHFR inhibitors developed through consecutive accumulation of mutations in the *dhfr* gene. The key mutation to resistance is the replacement of serine 108 by asparagine (S108N), which decreases the sensitivity of *Pf*DHFR 10-fold toward pyrimethamine (**51**) and cycloguanil (**52**). A crystal structure revealed a repulsive interaction between the *p*-chloro substituent on the phenyl ring of both inhibitors and the terminal aminocarbonyl moiety of asparagine 108 as the structural basis of resistance.[231] Subsequent mutations restore some of the catalytic activity of the enzyme and cause some delocalization of the amino acids that line the inhibitor binding site, enlarging the binding site, thereby further decreasing inhibitor binding affinity. The triple mutant S108N/N51I/C59R and the quadruple mutant S108N/N51I/C59R/I164L are respectively 100- and 500-fold less sensitive than wild-type DHFR to pyrimethamine (**51**) and cycloguanil (**52**) (reviewed in Ref. [225]). The quadruple mutant is currently present in multiple locations in Southeast Asia and South America, whereas the triple mutant is abundant in Africa. There, 90% of all isolates show the triple mutant genotype leading to therapy failures of more than 60% (assessed at day 7; assessment at day 28 would probably result in even

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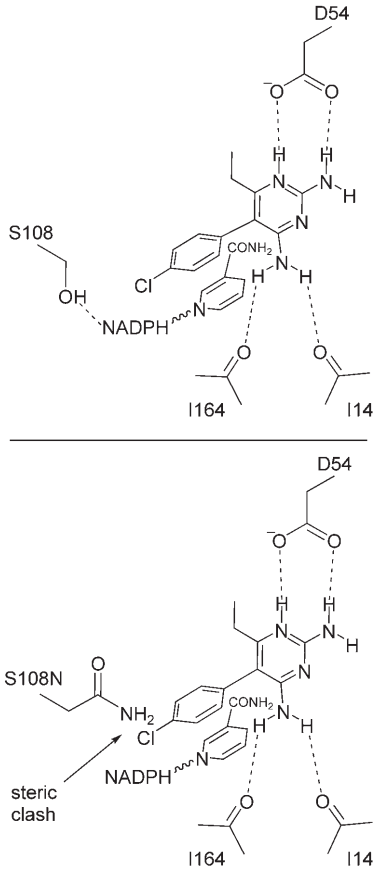

Figure 27. Binding of pyrimethamine (**51**) to *Pf*DHFR. The S108N mutation (bottom) results in a steric clash between the arginine side chain and the terminal chloro substituent of the DHFR inhibitor.

higher numbers).[235] Another genotype, A16V/S108T, independently selected under drug pressure from cycloguanil (**52**), confers high-grade resistance against this drug, but only moderately reduces sensitivity against pyrimethamine (**51**).[236] This is caused by an interaction between methyl groups from both Val 16 and cycloguanil that is not possible with the planar pyrimethamine.

5.2. Combination of DHPS/DHFR inhibitors

In the late 1950s and early 1960s several studies revealed the synergistic effect of combining sulfonamides that inhibit DHPS activity with a DHFR inhibitor. This synergism strongly depends on functional DHPS; however, the precise mechanism is not completely understood (for a discussion of current theories see Refs. [222, 225, 237]). Sulfonamides act as competitive antagonists of *p*-aminobenzoic acid, which is condensed to hydroxymethyldihydropteridine diphosphate to form dihydropteroate. In addition, sulfonamides react with hydroxymethyldihydropteridine diphosphate as false substrates to form covalent adducts, commonly called sulfa-dihydropteroates. While it has been shown that these adducts inhibit the growth of *P. falciparum*,[238] their intracellular targets are unclear. There is certain evidence that sulfa-dihydropteroates inhibit DHFR.[239, 240]

The combination of the sulfonamide sulfadoxine (**54**) (Figure 28) with the DHFR inhibitor pyrimethamine (**51**), known under its brand name Fansidar®, became the most important antimalarial next to chloroquine (**8**). The combination

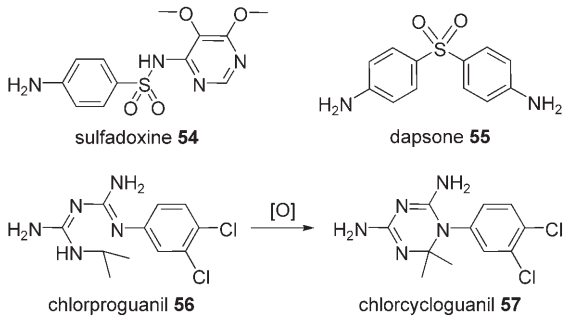

Figure 28. Antifolates.

of DHPS and DHFR inhibitors shows little effect during the first 24 h of the parasite's life cycle because the combination inhibits parasite DNA synthesis. This event peaks in the late erythrocytic schizont stage, at which antifolates exert their toxic effect (reviewed in Ref. [225]). Treatment regimes have generally been regarded as sufficiently safe, but with prolonged prophylactic use, toxicity of the sulfonamide combination partner becomes significant, resulting in an increased risk of agranulocytosis and toxic epidermal necrolysis (Stevens–Johnson syndrome).[17] For this reason, the prophylactic use of Fansidar® was discontinued in most countries years ago. The spread of strains with mutated *dhfr* and *dhps* genes has more or less terminated the useful lifespan of Fansidar® in many regions. Despite its limited efficacy, it is still widely used in Africa in combination with chloroquine (**8**), amodiaquine (**17**), or artesunate (**42**) because of its low price.

5.2.1. Resistance to DHPS inhibitors

Similar to the situation with DHFR, the selection for strains that have accumulated several mutations in the *dhps* gene have led to considerable resistance.[241] Although no crystal structure of *Pf*DHPS is available, homology modeling based on the crystal structures of different species of DHPS have provided insight into how mutations in the *dhps* gene affect sulfonamide binding.[242–245] The S436A mutation prevents the formation of an important hydrogen bond between the *p*-amino group of the sulfonamide and the hydroxy moiety of the serine side chain. Replacement of Ser 436 by a bulkier phenylalanine (S436F mutation) causes steric blockage of the active site, preventing the access of the larger sulfonamides while still allowing the binding of the smaller *p*-aminobenzoic acid. The mutations A437G, K540E, A581G, and A613S lead to an altered active site topology, allowing more rotational freedom of the inhibitor and thus decreasing the binding affinity.

Antifolate combinations display inherently lower efficacy against *P. vivax* malaria. According to homology modeling studies, the active sites of the dihydrofolate reductases of

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P. falciparum and *P. vivax* are relatively similar.[246] *Pv*DHPS, on the other hand, has a bulkier valine (Val 585) at a position equivalent to that of Ala 613 in *Pf*DHPS, which causes a major displacement of sulfadoxine (**54**) in the active site. This leads to the disruption of a crucial hydrogen bond to Ser 382 (equivalent to Ser 436 in *Pf*DHPS), which explains the relative inactivity of sulfadoxine (**54**) against *P. vivax*.[242]

5.3. Circumvention of antifolate resistance

5.3.1. Chlorproguanil/dapsone (LapDap®)

Efforts are now directed toward the circumvention of resistance. A novel combination consists of the well-known leprosy therapeutic dapsone (**55**) (chemically a sulfone, not a sulfonamide, but also an inhibitor of DHPS) and chlorproguanil (**56**, Lapudrine®, Figure 28). Like proguanil (**53**), chlorproguanil is metabolized into the active component chlorcycloguanil (**57**) by an oxidative ring closure. Chlorcycloguanil retains inhibitory activity against the triple mutant DHFR. Pyrimethamine (**51**) displayed a mean IC_{50} value of 815 nM against field isolates from Kenya harboring the triple mutant DHFR, in contrast to 10.8 nM for chlorcycloguanil (**57**). In comparison with isolates harboring wild-type DHFR, sensitivity decreased 225-fold for pyrimethamine but only 50-fold for chlorcycloguanil.[247] However, the additional I164L mutation found in the quadruple mutant reduces DHFR sensitivity by another 10-fold for chlorcycloguanil (**57**),[248] rendering the combination ineffective against parasites carrying this mutation.[249,250] The key structural feature for the activity against the triple mutant DHFR is the *m*-chloro substituent on the phenyl ring. Homology modeling predicts dapsone (**55**) to be less affected by *dhps* mutations[242] which makes it one of the most active antimalarial agents in its class of sulfonamides and sulfones (approximately 10-fold more active than sulfadoxine (**54**) in vitro).[251] However, there is some degree of cross-resistance in the group of sulfonamides. The combination of dapsone and chlorproguanil (**56**) was evaluated in different clinical studies,[252–254] and was recently introduced as LapDap® to the market. LapDap® is active against strains carrying the triple mutant DHFR which are predominant in Africa, but it is inactive against strains harboring the quadruple mutant, abundant in Asia and South America. However, the I164L mutation has already been found in various locations in Africa as well.[255–257] There are concerns that the quadruple mutation may spread further once LapDap® is used extensively.[235] In addition, random mutagenesis has yielded laboratory strains displaying high levels of resistance against chlorcycloguanil (**57**).[258,259] To expand its useful lifespan, a fixed triple combination with artesunate (**42**) (CDA) named LapDap+ is in clinical development. A dose-ranging study was completed to establish the optimum ratio of artesunate and the fixed combination of chlorproguanil/dapsone.[89] In a phase II clinical study, CDA had a significantly shorter parasite clearance time than chlorproguanil/dapsone alone.[260]

5.3.2. Novel DHFR inhibitors under development

5.3.2.1. Dihydrotriazines based on the cycloguanil structure

5.3.2.1.1. WR99210 and derivatives

In the 1970s, considerable work was invested at the Walter Reed Army Institute of Research in the development of novel DHFR inhibitors. One of the most promising results of these efforts was the development of the cycloguanil (**52**) analogue WR99210 (**58**, Figure 29), which exerts excellent activity (IC_{50} = 2.7 nM) against the quadruple-mutant-bearing *P. falciparum* strain V1S. A crystal structure revealed the basis of the pre-

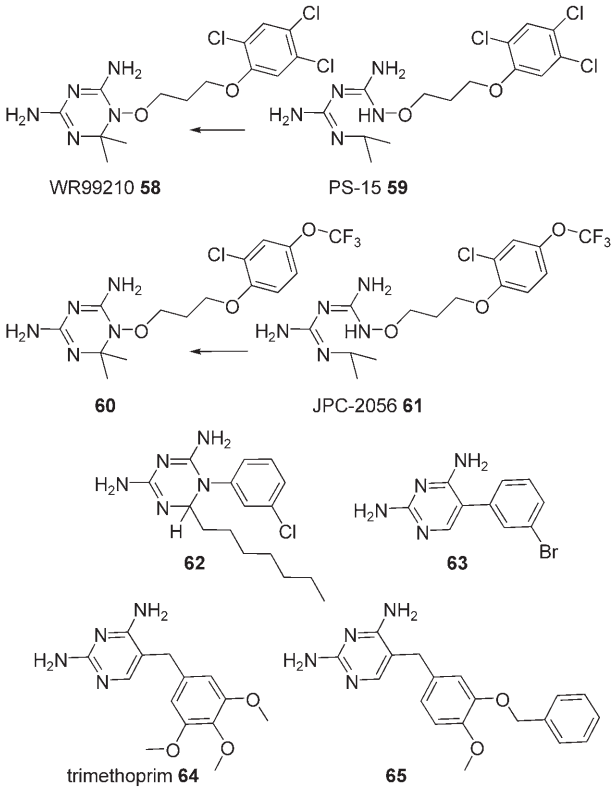

Figure 29. Antifolates in development.

served activity of WR99210 even against the quadruple mutant form of *Pf*DHFR. The key structural feature is the flexible dioxypropylene linker that connects the two rings, allowing the compound to adopt a high-affinity conformation while avoiding any repulsive interaction with the Asn 108 side chain.[231] However, mutagenesis experiments yielded a strain with 188-fold increased resistance against WR99210.[261] In addition, parasites with 15–20-fold decreased sensitivity to this drug were isolated in Africa.[262] Low bioavailability and gastrointestinal intolerance prevented further development of WR99210 (**58**). Similar to cycloguanil (**52**), a biguanide prodrug PS-15 (**59**) was designed for WR99210 that shows better absorption and tolerability. The synthesis requires the use of 2,4,5-trichlorophenol, which is strictly regulated owing to the potential formation of

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the extremely toxic tetrachlorodibenzodioxine (TCDD) during its preparation. Therefore, the development of PS-15 is no longer pursued.[263]

Several derivatives of WR99210 (**58**) and its prodrug PS-15 (**59**) have been prepared and evaluated for their antimalarial properties. JPC-2056 (**61**) has emerged as the most promising candidate for preclinical development. It is structurally closely related to PS-15, forming the active compound **60** via the well-known oxidative ring closure. It preserves the important flexible dioxypropylene linker but has different substituents on the phenyl ring.[264,265]

5.3.2.1.2 Other dihydrotriazines

In contrast to the original cycloguanil (**52**), some novel cycloguanil derivatives such as compound **62** (Figure 29) bear only one substituent at position 6. This modification was particularly designed to circumvent another mutation, A16V. This mutation causes a repulsive interaction between one of the two 6-methyl groups of cycloguanil and the larger valine side chain. These particular strains are resistant against cycloguanil, but are still sensitive to the monosubstituted pyrimethamine (**51**).[266,267] The modified cycloguanil compound **62** displays a K_i value of 3.8 nM against the A16V/S108T mutant form of DHFR which is much lower than that of cycloguanil (K_i = 1314 nM). It also inhibits the growth of parasites harboring this mutant DHFR with an IC_{50} value of 4 nM. An even lower K_i value of 0.8 nM is observed against the quadruple mutant DHFR. Only activity against cultured parasites is low (IC_{50} = 2.5 μM).[268]

5.3.2.2. Pyrimidines based on the pyrimethamine structure

The critical repulsive interaction caused by the Asn108 side chain can be avoided by relocation of the chloro substituent to the meta position.[269] The *m*-bromo derivative **63** of pyrimethamine (**51**) inhibits the quadruple mutant DHFR with a K_i value of 5.1 nM (pyrimethamine: K_i = 859 nM) and inhibits the growth of the corresponding parasites with an IC_{50} value of 37 nM (pyrimethamine: IC_{50} > 5 μM).[270]

5.3.2.3. Trimethoprim and derivatives

Trimethoprim (**64**), which is widely used in combination with sulfamethoxazole for the treatment of bacterial infections, is less active against *Pf*DHFR than pyrimethamine (**51**) or cycloguanil (**52**) (K_i against wild-type *Pf*DHFR: 10.3 nM). By replacing one of the methoxy groups with a benzyloxy group (compound **65**), activity against wild-type DHFR can be markedly improved (K_i = 0.4 nM). In addition, such derivatives display K_i values against the quadruple mutant form of DHFR in the range of 60–90 nM and IC_{50} values in the low micromolar range.[271]

5.3.2.4. Structurally different compounds

Database searches and computational docking experiments revealed several DHFR inhibitors **66**–**69** that are structurally different from previously known DHFR inhibitors (Figure 30). Al-

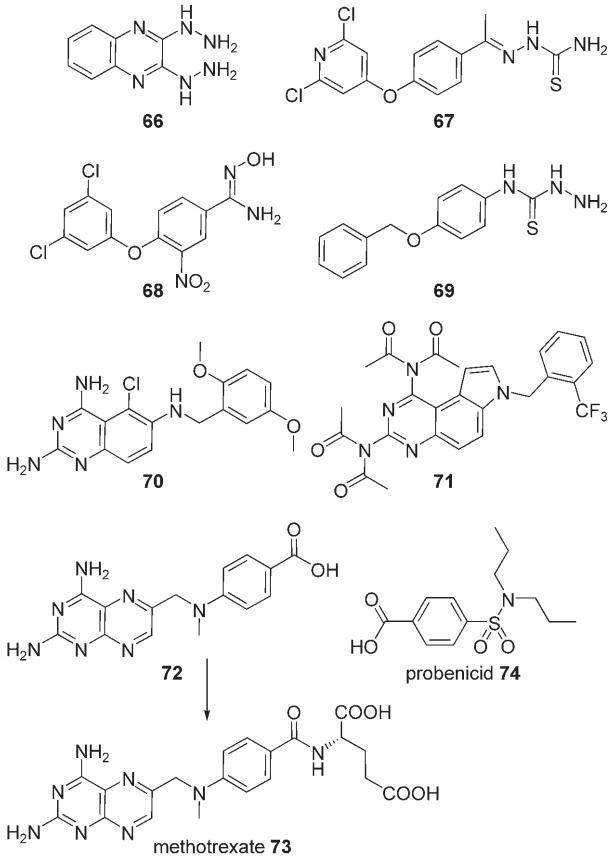

Figure 30. Structurally different antifolates and probenicid (**74**).

though the activities of the compounds are low (K_i values between 1.5 and 32.6 μM), these hits can serve as novel lead structures for further development.[272] A structurally different class of antifolates was recently reported. The diaminoquinazoline derivative **70** inhibits the V1S strain (quadruple mutant DHFR) with an IC_{50} value of 8.9 nM. Furthermore, this compound showed strong synergy with dapsone (**55**).[273] Pyrroloquinazolidinediamine derivatives were prepared in the early 1970s as folate antagonists and were found to display high antimalarial activity,[274,275] although pronounced toxicity prevented further development. Recently, the tetraacetyl prodrug **71** showed markedly superior efficacy and safety in a mouse model of severe malaria relative to artesunate (**42**).[276]

5.3.2.5. Methotrexate precursors

Methotrexate (**73**, Figure 30) is an inhibitor of human and plasmodial DHFR, but its toxicity (it is used as an anticancer agent) preclude its use against malaria. Nevertheless, because the folate biosynthesis pathway is completely absent in humans, it

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has been speculated that the application of an appropriate precursor like **72**, which would be converted only in parasites to methotrexate (**73**), could yield a selective agent. Compound **72** inhibits the growth of the quadruple mutant resistant strain V1S with an IC_{50} value of 2.5 μM and was proven nontoxic against human cells and in an animal model.[277]

5.3.3. Folate uptake inhibition

Another approach is based on the fact that resistance against antifolates is not exclusively caused by mutations in the *dhfr* and *dhps* genes, as described above. In addition to de novo synthesis, parasites can also use folate taken from the host via an active transport system. Probenicid (**74**), normally used as a uricosuricum (a drug that increases the rate of excretion of uric acid, used for the therapy of chronic gout), inhibits this transport system and shows antifolate-resistance-reversing properties in vitro.[278,279] In a clinical study, probenicid in combination with pyrimethamine–sulfadoxine proved to be more effective than the antifolate combination alone.[280]

5.4 Thymidylate synthase inhibitors

In *P. falciparum*, thymidylate synthase (*Pf*TS) activity is collocated with DHFR activity on the same bifunctional enzyme. Human thymidylate synthase is an important target in anticancer drug therapy and drug design.[281] However, only few TS inhibitors have been evaluated as potential antimalarials. In the early 1990s, high antimalarial activity (IC_{50} = 5 nM) was described for 5-fluoroorotate (**75**, Figure 31).[282] It is converted into 5-fluoro-2′-deoxyuridine-5′-monophosphate (**76**), which is a potent inhibitor of *Pf*TS (K_i = 2 nM).[283] However, it turned out that serum concentrations of 1–10 μM, which are dangerously close to toxic levels, are needed to prevent recrudescence.[284] Later on, in vitro and in vivo synergism was demonstrated for the combination of 5-flouroorotate with sulfamonomethoxine (**77**).[285] The quinazoline derivative ICI D1694 (**78**), which is a potent inhibitor of human cell growth (IC_{50} = 0.2–5 nM, depending on the particular cell line), displayed only weak activity against *P. falciparum* (IC_{50} = 20 μM).[286] This is most probably due to poor transport into the parasites or poor polyglutamylation, because the polyglutamylated analogue D1694-$(glu)_4$ (**79**) is a potent inhibitor of *Pf*TS (K_i = 1.5 nM).[283] Furthermore, a novel *Pf*TS inhibitor, 1843U89 (**80**), has been reported which inhibits *Pf*TS with a K_i value of 1.0 nM and the growth of several *P. falciparum* strains with an IC_{50} value of 70 nM.[287]

6. Compounds Acting on the Respiratory Chain

6.1. Mitochondrial electron transport of *Plasmodium falciparum*

In contrast to higher eukaryotic organisms, the mitochondrial electron transport of *P. falciparum* seems not to be coupled with the synthesis of ATP. The mean source of this high-energy compound for *P. falciparum* is anaerobic glycolysis. The enzymes of the citric acid cycle are incomplete. Portions of this pathway contribute only to a small extent or not at all to the electron flow through the respiratory chain. In addition, in *P. falciparum* the conventional rotenone-sensitive NADH dehydrogenase complex I is completely missing. Only very recently an alternative complex I that is not involved in proton pumping was characterized in *P. falciparum* mitochondria.[288] Different dehydrogenases are the main electron source, of which dihydroorotate dehydrogenase is the most important member. It catalyzes the reduction of dihydroorotate to orotate, the key intermediate of pyrimidine biosynthesis. Providing an electron sink for this reaction seems to be the most important function of mitochondrial electron transport. Parasites depend on this pathway because they are unable to scavenge used pyrimidine bases.

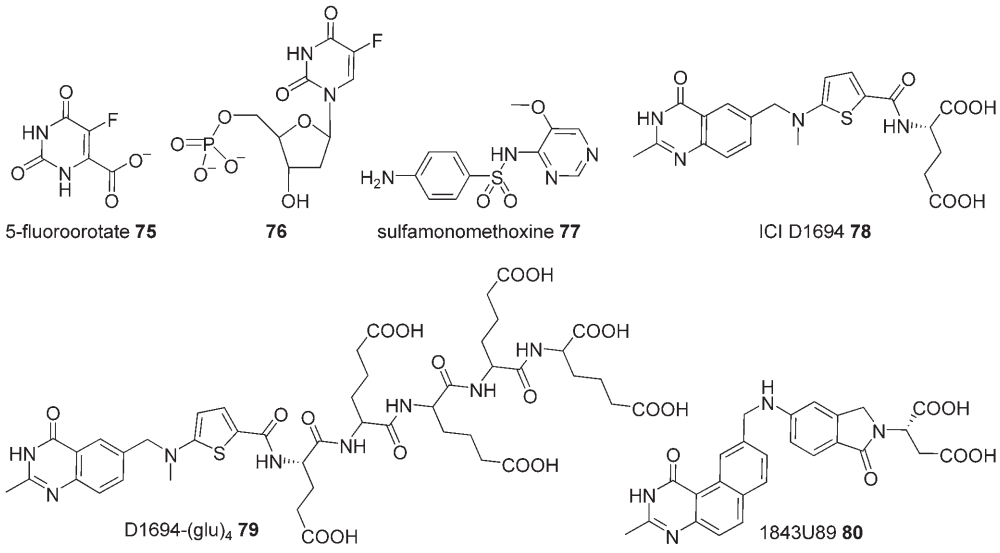

Figure 31. Thymidylate synthase inhibitors.

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Electrons are transferred from different dehydrogenases to ubiquinone (**82**, coenzyme Q). Its reduced form, ubiquinol (**81**), binds to the so-called Q_O binding site of the cytochrome bc_1 complex, where it is oxidized to ubiquinone (Figure 32). The two electrons then take different paths. One electron is transferred to the iron–sulfur center of the so-called Rieske protein. Movement of the whole protein domain transfers the electron to a *c*-type heme of cytochrome c_1. Subsequently, cytochrome c_1 is oxidized by the cytochrome oxidase complex similar to the process in higher eukaryotic organisms. The second electron is transferred by two *b*-type heme molecules to the Q_i site of cytochrome *b*, in which ubiquinone (**82**) is reduced via a semiquinone intermediate in a two-step process to ubiquinol (**81**).[289,290]

6.2. 2-Hydroxynaphthoquinones

6.2.1. Development of 2-hydroxynaphthoquinones

Compounds with quinone substructures have different biological activities. The antiplasmodial activity of naphthoquinones such as hydrolapachol (**83**, Figure 33) has been known since the 1940s. Fieser's research group has prepared more than 300 naphthoquinone derivatives as potential malaria therapeutics. These early studies resulted in a compound named lapinone (**84**). *P. vivax* infections could be cured in single patients, but only by parenteral application of high doses.[291] The availability of the cheap and effective chloroquine (**8**) during that time resulted in a lack of interest in this class of compounds. With the occurrence of chloroquine resistance, interest in hydroxynaphthoquinones was renewed in the 1960s. Menoctone (**85**) already shows the two structural features essential for antiplasmodial activity, the 2-hydroxynaphthoquinone substructure and the cyclohexyl residue in the side chain. Because it undergoes extensive biotransformation, therapeutic effects are obtained only with very high doses. Consequently, menoctone was no longer pursued as an antimalarial drug.[292] Interestingly, activity against *Theileria parva* infections in cattle was observed which led to extensive structural variations, resulting in parvaquone (**86**), a highly efficient therapeutic of the *Theileria parva* infection.[293] Subsequently, the cyclohexyl residue of parvaquone was widely explored, leading to the compound BW58C80 (**87**), which has broad antiprotozoal activity.[294] In humans, the *tert*-butyl residue is rapidly hydroxylated to the 1000-fold less active metabolite. Further development of this compound has been discontinued. Variation at the position 4 of the cyclohexyl residue resulted in the metabolically stable compound 566C80 (**88**), which shows equal antiplasmodial activity and which was later introduced into therapy as atovaquone.[295]

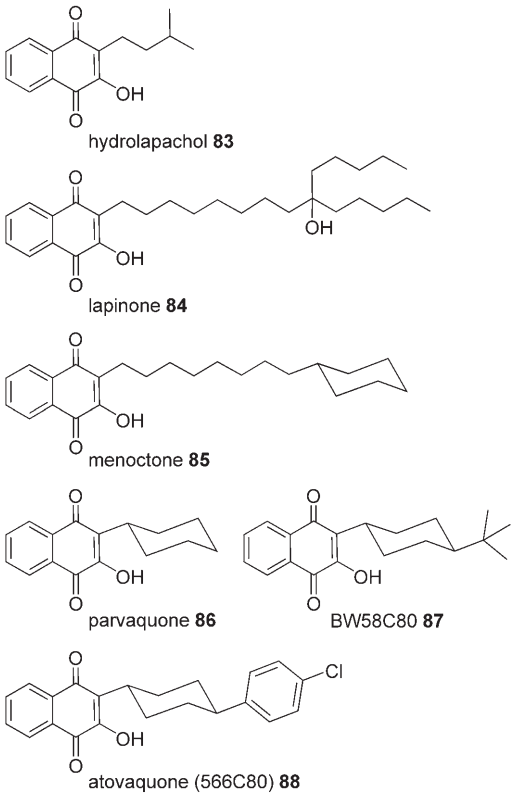

Figure 33. Naphthoquinones with antiparasitic activity.

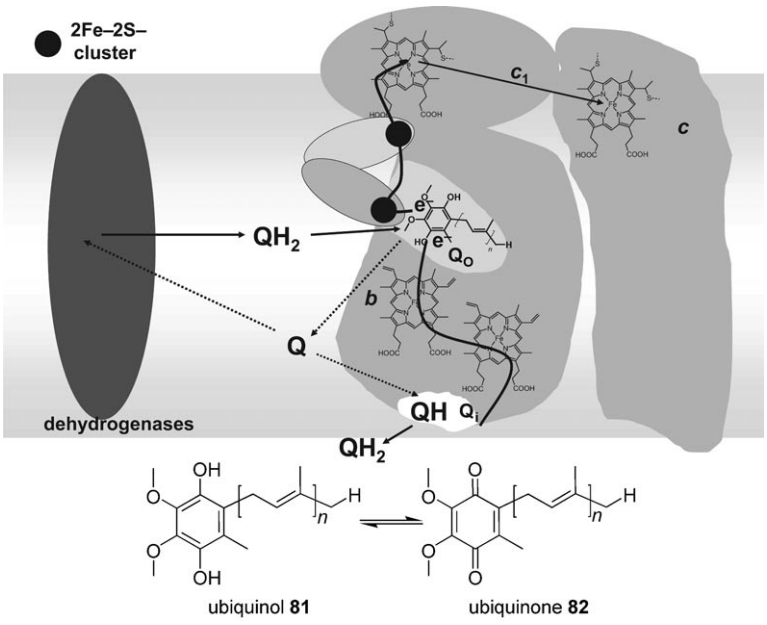

Figure 32. Representation of the electron flow through the cytochrome bc_1 complex in the parasite's respiratory chain.

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6.2.2. Mechanism of action and resistance

Atovaquone (**88**) binds to the Q_O site of the cytochrome bc_1 complex and prevents translocation of the iron–sulfur domain. Docking studies using the structure of the cytochrome bc_1 complex from yeast provided a reasonable explanation of this atovaquone effect at the molecular level.[296] The 2-hydroxy group forms a hydrogen bond to the imidazole residue of His 181 (yeast numbering) that coordinates the iron–sulfur complex, while on the opposite side of the molecule a water-mediated hydrogen bond is formed between the 4-carbonyl oxygen atom and the side chain of Glu 272 (Figure 34).

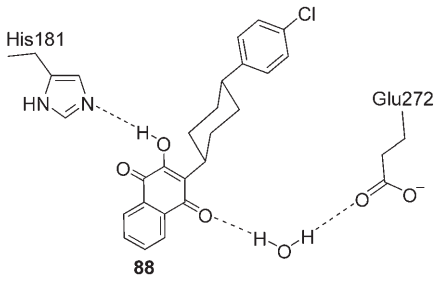

Figure 34. Atovaquone (**88**) forms simultaneous hydrogen bonds to the iron–sulfur domain and cytochrome *b*, thereby preventing the movement of the iron–sulfur domain.

This blockade of electron transport leads to a rapid collapse of the mitochondrial membrane potential ($ED_{50} = 15$ nM),[297] which causes a complete shutdown of mitochondrial metabolism (including dihydroorotate dehydrogenase-dependent pyrimidine synthesis) as well as any transport process across the mitochondrial membrane. This ultimately results in the death of the parasite. This effect has been observed in laboratory strains with IC_{50} values between 0.56 and 4.53 nM.[298] In different studies with field isolates, mean IC_{50} values of 1.1,[172] 3.56,[299] and 6.2 nM[49] were obtained. The mammalian cytochrome complex is up to 1000-fold less sensitive against atovaquone (**88**). Application of atovaquone as single agent showed rapid selection for resistant strains, resulting in therapy failure rates of 30%.[300] Such resistant strains show cytochrome bc_1 complexes in which the Q_O site has been altered by the exchange of one amino acid. These mutations (L271V, K272R, I258M, F267I, Y268S/C; all *P. falciparum* numbering) decrease the sensitivity of the cytochrome bc_1 complex to atovaquone more than 1000-fold (ED_{50} values between 10000 and 25000 nM).[297] Again, using the known crystal structure of the yeast cytochrome bc_1 complex, the consequences of these changes in the amino acid sequence can be elucidated at the molecular level.[301] The exchange of Ile 258 for methionine decreases the space available for the naphthoquinone ring of atovaquone. The replacement of Phe 267 with isoleucine takes away the central aromatic residue of an array of three aromatic side chains accommodating the terminal chlorophenyl residue in the wild-type. Exchanging the aromatic side chain of Tyr 268 with the more or less nucleophilic serine or cysteine leads to a decreased hydrophobic binding of the cyclohexyl residue. Finally, replacement of Leu 271 by the less bulky valine results in a delocalization of several side chains, among them Tyr 268, leading to similar consequences as described above. Another affected amino acid is the histidine residue of the Rieske protein that forms the decisive hydrogen bond with atovaquone (**88**). It is interesting to note that some of these amino acid sequence differences occur naturally in mammals which explains the selective activity of atovaquone against the parasite cytochrome bc_1 complex. In contrast to the mammalian counterpart, these amino acid replacements markedly decrease the efficiency of electron transport in the parasites.[302]

6.2.3. Atovaquone

Atovaquone (**88**) displays broad-spectrum antiprotozoal activity. It is used against *Pneumocystis jiroveci* (formerly termed *Pneumocystis carinii*) pneumoniae, toxoplasmosis, and in the therapy of human barbesiosis in combination with azithromycin (**100**). Because of the rapid development of resistance as described above, atovaquone as a single agent is unsuitable for the therapy and prophylaxis of malaria.[303]

Fortunately, there is a strong synergism with proguanil (**53**, Figure 35) in vitro (and also, but to a lesser extent, with doxycycline (**97**)). In many publications, this synergism is *wrongly* attributed to the biotransformation of proguanil (**53**) into the DHFR inhibitor cycloguanil (**52**). This synergism is observed in strains that are unable to form cycloguanil as well. Furthermore, there is no synergism of atovaquone (**88**) with other inhibitors of DHFR such as pyrimethamine (**51**). The *non-metabolized* proguanil is responsible for the synergism with atovaquone. Proguanil itself has no measurable effect on the mitochondrial membrane potential, but it decreases the concentration of atovaquone necessary for the collapse of the membrane potential. The mechanism behind this effect is unknown.[304,305] Through this synergism the selection for resistant strains during therapy is diminished, but once a strain has become resistant to atovaquone it is also resistant to the combination with proguanil.[298] The same synergism as with proguanil is also observed with chlorproguanil (**56**).[306]

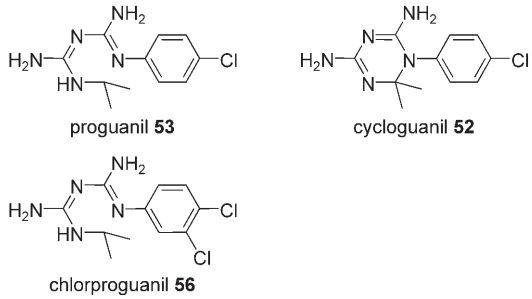

Figure 35. Proguanil and chlorproguanil, but not cycloguanil, show a strong synergism with atovaquone.

Since the mid 1990s the combination of atovaquone (**88**) and proguanil (**53**) has been used under the brand name Malarone® for prophylaxis and therapy of uncomplicated malaria

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tropica.[300,303,307] It is also effective against liver stages which also allows a so-called causal prophylaxis with this combination. Because of the low water solubility of atovaquone, application together with a fatty meal is recommended. In general, tolerability seems to be good, with no severe side effects reported. The elimination half-life is between 51 and 77 h.[303] The combination is also suited for prophylactic use by air crews.[308] The widespread administration of Malarone® is limited due to its high price. At present, resistance does not seem to be a problem, although there are some reports on single treatment failures with wild-type cytochrome *b* in the primary and single mutations in the recrudescent isolates.[309–311] However, the prevalence of mutations of the cytochrome *b* gene that confer resistance to this combination in primary isolates seems to be lower than 1%.[312–314] To shield the atovaquone/proguanil combination from emerging resistance, it has been successfully combined with artesunate (**42**) for the treatment of uncomplicated malaria.[315] This combination was also used for the treatment of uncomplicated as well as recrudescent multiple-resistant malaria during pregnancy.[316,317]

6.2.4. Other naphthoquinones

Buparvaquone (**89**, Figure 36) is another 2-hydroxynaphthoquinone that is structurally related to atovaquone (**88**), albeit markedly less effective ($IC_{50} = 550$ nM). In contrast, in the form of its copper complex, $[Cu(buparvaquone)_2(EtOH)_2]$, an IC_{50} value of 0.2 nM against the 3D7 strain was described.[318] Buparvaquone has also been evaluated in the local therapy of cutaneous leishmaniasis.[319]

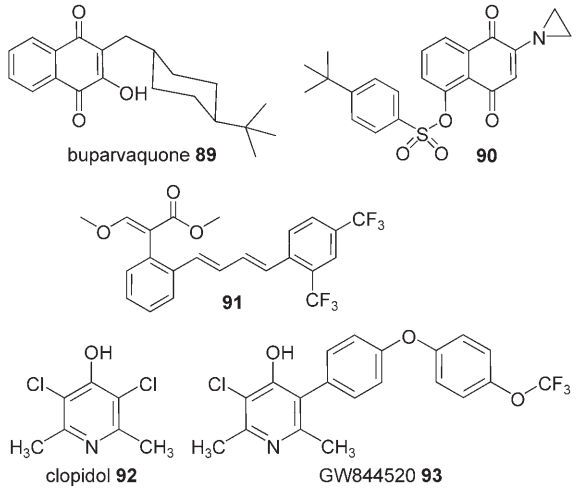

Figure 36. More naphthoquinones and electron-transport inhibitors.

There are relatively few reports on naphthoquinone derivates with activity against *P. falciparum* in the recent literature. Activities are generally not promising. Considerable activity has only been reported for some 2-aziridylnaphthoquinones such as compound **90**, which has an IC_{50} value of 24 nM.[320]

6.3. Other electron-transport inhibitors

Derivatives of 3-methoxyacrylic acid are a well-known class of inhibitors of mitochondrial electron transport. The natural products azoxystrobin and myxothiazol as well as a number of synthetic fungicides belong to this class. Like the 2-hydroxynaphthoquinones, they bind to the Q_O site, but their interactions with the amino acid side chains are different from those of the naphthoquinones. They enhance the mobility of the Rieske protein, whereas naphthoquinones act to decrease mobility, as described above.[321] The most active compound of this class against *P. falciparum* is the phenylmethoxyacrylate **91** (Figure 36) with an IC_{50} value of 0.28 nM and an ED_{90} value $< 22\ \mu mol\ kg^{-1}$.[322]

A novel class of compounds, the 4-(1*H*)-pyridones, is based on the natural product clopidol (**92**, Figure 36). Its antimalarial activity (curative at 833 μmol (160 mg) kg^{-1} in *P. cynomologi*-infected monkeys) was described in 1972.[323] The synthetic derivative GW844520 (**93**) was in preclinical development. The compound acts on the cytochrome bc_1 complex with an IC_{50} value of 2 nM, 10-fold higher than that of atovaquone (**88**). However, atovaquone-resistant parasites are inhibited with IC_{50} values of 2.5–7.6 nM. In contrast to atovaquone, GW844520 exhibits no synergism with proguanil (**53**).[324] According to the MMV annual report of 2005, the development of GW844520 has been discontinued. The most recent MMV portfolio discloses another pyridone, GW308678, which is in preclinical development.

7. Antibiotics

7.1. Effect of antibiotics on malaria parasites

At first sight, it is surprising that several antibacterial agents display considerable activity against the eukaryotic malaria parasites, as antibiotics are known to specifically target prokaryotic structures. This apparent contradiction can be explained by the presence of two organelles, the mitochondrion and the apicoplast. Both organelles have their own DNA and bacteria-like machinery for replication, transcription, and translation. The mitochondrial genome is relatively small (6 kb) and encodes only three proteins.[289] All other proteins and rRNAs have to be imported from the cytosol. The apicoplast is most probably the remnant of an endosymbiotic red algae. Although the apicoplast genome is considerably larger (35 kb), most proteins involved in the metabolic pathways are encoded in the nucleus and are imported. The DNA of the organelle includes housekeeping genes responsible for the maintenance of the organelle.[325–327] Apart from tetracyclines, which are thought to act mainly against the mitochondrion,[328] all other antibiotics seem to act on the apicoplast.[289] Only very recent results do not support the theory of the mitochondrion as the site of tetracycline action. It has been shown that doxycycline (**97**) blocks the expression of apicoplast genes.[329] Characteristically, most antibiotics do not exert any visible effect in the first intracellular cycle, but during the second cycle the parasites are killed after the invasion of the new host cell. This phenom-

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enon is known as "delayed death phenotype" or "delayed kill effect". At present, this effect is not fully understood, but several theories have been offered. It has been proposed that antibiotic-mediated inhibition of the apicoplast may cause liponic acid starvation, which increases oxidative stress and mitochondrial injury during the subsequent asexual reproductive cycle.[330] According to another theory, apicoplasts inherited by parasites treated with antibiotics contain insufficient levels of apicolast-encoded proteins, which are required for the import and processing of nuclear gene-encoded proteins needed for normal function.[329] The apicoplast may be required for the formation of daughter cell plasma membranes, as fatty acid biosynthesis is a likely function of apicoplasts, and ultrastructural studies indicate that these structures are missing following treatment with doxycycline (**97**).[329]

As a result of the delayed kill effect, fever and parasite clearance times are significantly longer than they are with classical antimalarials (approximately 4 versus 2 days) when antibiotics are administered as single agents. Because this delay may be fatal in non-immune patients, antibiotics are used only in combination with a faster-acting drug for the therapy of acute malaria. However, antibiotics, especially doxycycline (**97**), can be used prophylactically as single agents, although they are not registered for this indication in most countries.

The in vitro activity of antibiotics depends very much on the incubation time because of the delayed kill effect.[331,332,333] For example, if clindamycin (**103**) is evaluated in a conventional 72-hour growth inhibition assay, the IC_{50} value is only about 50 μM. By extending the incubation time to 120 h, the IC_{50} value drops to approximately 20 nM.[334] The same behavior has been observed with other antibiotics. In general, there is a much greater fluctuation in reported IC_{50} values for a single antibiotic between different literature references than there is for other antimalarials. There are only few references in which a greater number of distinct antibiotics have been assayed consecutively.[331–333] Because there is no reference covering all antibiotics of interest, caution is advised when comparing the IC_{50} values cited in the following section.

7.2. Quinolones

Quinolones bind to one subunit (GyrA) of prokaryotic topoisomerase II (gyrase), thus preventing the re-ligation of DNA cut in the gyrase-mediated process of controlling the amount of negative supercoiling present.[335] Ciprofloxacin (**94**, Figure 37) has been shown to induce cleavage of the plastid DNA.[336] Among the quinolones commonly used in antibacterial therapy, ciprofloxacin displays the highest activity against cultured *P. falciparum* parasites. IC_{50} values between 38 and 1.4 μM, depending on the parasite strain and incubation time, have been reported.[337,338] As with other antibiotics, the in vitro activity of ciprofloxacin considerably improved with prolonged exposure. The MIC value obtained with the K1 strain dropped from 82 μM at 48 h to 300 nM at 144 h observation time.[339] In clinical studies, ciprofloxacin monotherapy showed insufficient activity. The IC_{50} values for parasites obtained from the patients were significantly higher than the plasma concentrations that were measured in these patients.[340,341] However, ciprofloxacin treatment was stopped due to the deteriorating conditions of the patients before the expected onset of the delayed kill effect. Therefore, further studies are needed to clarify the possible value of ciprofloxacin (**94**) as part of a combination therapy or as a prophylactic agent.

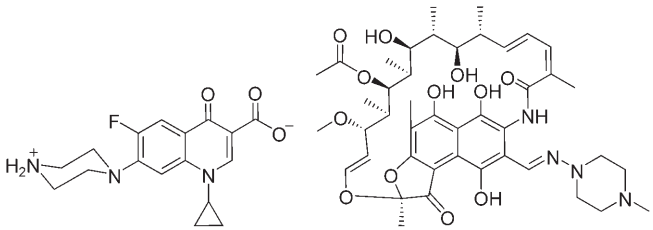

Figure 37. Antibiotics **94** and **95** that inhibit DNA and RNA replication, respectively.

7.3. Rifampicin

Rifampicin (**95**, also known as Rifampin, Figure 37) is a well-known inhibitor of bacterial RNA polymerase. A similar RNA polymerase was shown to be encoded on the plastid genome.[342] Rifampicin inhibits the growth of various laboratory strains in a 48-hour assay with IC_{50} values between 3.2 and 1.3 μM.[333] With the W2 clone, the IC_{50} value dropped from 1.3 μM at 48 h to 0.09 μM at 144 h incubation time.[333] In a clinical trial, rifampicin alone displayed insufficient activity against *P. vivax* malaria.[343] A fixed combination of rifampicin, sulfamethoxazole, trimethoprim (**64**), and isoniazid (Cotrifazid®) was effective in the treatment of malaria tropica.[344] In this combination, isoniazid has no antimalarial activity, but was shown to protect mice from endotoxin lethality.[345] In another clinical trial, rifampicin was added to a quinine (**1**) regime. An adverse side effect was revealed due to enzyme induction caused by rifampicin, resulting in an increased metabolism of quinine into the less active 3-hydroxyquinine.[346]

7.4. Protein biosynthesis inhibitors

A variety of antibiotics such as tetracyclines, macrolides, lincosamides, chloramphenicol (**104**), spectinomycin, fusidic acid, and various peptide, polyketide, and polyene antibiotics, which are all translation inhibitors in prokaryotic systems, are also considered to inhibit protein synthesis inside the apicoplast (reviewed in Ref. [326]).

7.4.1. Tetracyclines

Tetracyclines (Figure 38) bind to the 16S RNA of the 30S ribosomal subunit touching the aminoacyl (A) site. Through direct steric interactions, tetracyclines prevent the rotation of aminoacyl-tRNA into the correct position, resulting in unproductive GTP hydrolysis by the elongation factor Tu (EF-Tu).[347] In contrast to other antibiotics, tetracyclines are believed to act on the plasmodial mitochondrion (see above); however, more

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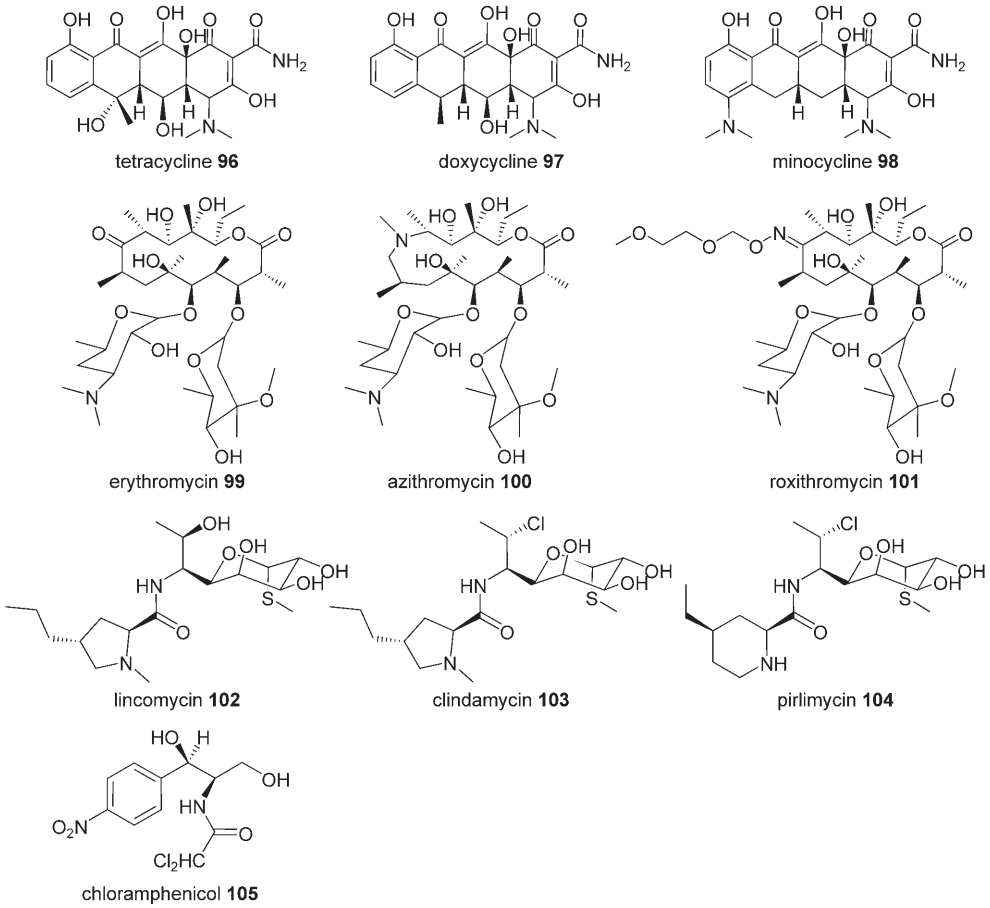

Figure 38. Antibiotics (tetracyclines, macrolides, lincomycins and chloramphenicol) that inhibit protein biosynthesis.

recent work has demonstrated some activity of minocycline (**98**)[348] and doxycycline (**97**)[329] against the apicoplast. Among different tetracyclines assayed against five laboratory strains, doxycycline (IC_{50}: 7.7–14.9 μM) and minocycline (IC_{50}: 10.2–16.0 μM) were significantly more active than tetracycline (**96**) (IC_{50}: 33.8–88.1 μM) in a 48-hour assay. After an exposure time of 144 h, differences in activity were nearly absent (IC_{50} values (W2 strain): 0.5, 0.3, and 0.8 μM for **97**, **98**, and **96**, respectively).[333] Against 71 isolates, doxycycline (**97**) displayed a mean IC_{50} value of 11.3 μM (range: 0.7–108 μM).[349] Tetracycline (**96**) has been administered in combination with quinine (**1**), and minocycline (**98**) is sporadically used in the clinic.[350] Doxycycline (**97**) is used in combination with quinine against severe malaria. For both drugs, intravenous formulations are available. With the same indication, doxycycline has successfully been combined with artesunate (**42**) in a clinical study.[174] Doxycycline is also combined with mefloquine (**29**) to tackle mefloquine resistance. Synergistic effects have also been reported with atovaquone (**88**),[351] but proguanil (**53**) is the preferred combination partner. Doxycycline has also become the mainstay in malaria prophylaxis for cases (for example, aircrews or divers) in which mefloquine cannot be administered due to neuropsychiatric side effects.[113] Doxycycline is as effective as mefloquine in this indication.[352] No confirmed resistance has been reported so far. A problem common to all tetracyclines is the formation of tetracycline–calcium phosphate complexes, which are deposited in calcifying areas of bones and teeth. This prohibits the use of all tetracycline derivatives during pregnancy and for children under the age of 8, which are unfortunately two of the most important populations affected by malaria. In addition, phototoxic skin reactions can occur under sun exposure. Novel tetracycline derivatives especially designed for improved antimalarial activity had been under development (now discontinued), but no further details on the structures or activities have been disclosed.[88]

7.4.2. Macrolides

Macrolides (Figure 38) bind to the 50S (large) ribosomal subunit in a binding site adjacent to and partly overlapping those of lincosamides and streptogramin B, at the so-called MLS_B site. They bind inside the tunnel that runs from the peptidyl transferase center to the back side of the ribosome and that the growing peptide must pass on its way out of the ribosome. By blocking this tunnel, macrolides terminate peptide growth at chain lengths that vary between two and eight residues, depending on the actual size of the macrolide.[353] Azithromycin (**100**) was more active than erythromycin (**99**) in vitro, with IC_{50} values of 6.5 versus 68 μM against a chloroquine-resistant strain, and 3.0 versus 6.3 μM against a chloroquine-

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sensitive strain.[354] The mean IC_{50} value of azithromycin against 39 wild isolates was 29.3 μM.[355] An in vitro study revealed synergistic activity of azithromycin with chloroquine (**8**) (chloroquine-resistant strains only), quinine (**1**), primaquine (**6**), and tafenoquine (**34**).[356] In a different study using the chloroquine-resistant K1 laboratory strain, IC_{50} values of 8.4 and 58.2 μM were obtained for azithromycin and erythromycin, respectively (96-hour assay).[357] Synergy was observed in this study with chloroquine and quinine, while the combination of azithromycin with mefloquine (**29**) or pyronaridine (**25**) was additive, and that with artesunate (**42**) was antagonistic. In mouse models, azithromycin was 31-fold more effective than erythromycin,[358] and curative in combination with artemether (**40**).[359] Erythromycin has failed to improve chloroquine treatment in humans.[360] Roxithromycin (**101**) displays an IC_{50} value of 18.6 μM in a 48-hour assay against the chloroquine-resistant laboratory strain W2, which drops to 1.9 μM in a 144-hour assay.[333] Roxithromycin shows synergy with chloroquine and, to a lesser extent, with mefloquine as well. A resistance-reversing effect due to the inhibition of the MDR1 transporter could be a factor in this synergy.[361]

In clinical trials, azithromycin (**100**) was well tolerated as a prophylactic agent,[362] but less effective than doxycycline (**97**) against *P. falciparum* malaria.[363,364] Against *P. vivax* malaria, both were equally effective.[363] A more recent study revealed a similar protective efficacy of 98% against *P. vivax* malaria.[365] In a comparative trial for the treatment of multidrug-resistant *P. falciparum* malaria, the combination of azithromycin and dihydroartemisinin (**39**) was less effective than mefloquine/dihydroartemisinin.[366] In another trial, azithromycin proved ineffective as a single agent against uncomplicated malaria, but effective in combination with chloroquine.[367] Higher doses of azithromycin in combination with quinine were equally effective as quinine/doxycycline in the treatment of uncomplicated malaria.[368] Cure rates of 92% were obtained with 1500 mg day^{-1} azithromycin in combination with quinine or artesunate.[369] Azithromycin is used in combination with atovaquone (**88**) for the treatment of human babesiosis.[370] Furthermore, it is used in combinations against toxoplasmosis[371] and *Pneumocystis jiroveci* infections.[372]

7.4.3. Lincosamides

Lincosamides (Figure 38) are two closely related antibiotics, the naturally occurring lincomycin (**102**) and its semisynthetic derivative clindamycin (**103**). These also bind to the large ribosomal subunit with their binding site partially overlapping that of the macrolides (see above). Clindamycin binds to the aminoacyl (A) and to the peptidyl (P) binding site, interfering with substrate binding at both sites and blocking the progression of the nascent peptide toward the tunnel.[373] In a conventional 72-hour assay against three laboratory strains, clindamycin was more active (IC_{50} values: 43–66 μM) than lincomycin (IC_{50} values: 80–110 μM).[98] As mentioned above, extension of the observation time to 120 h decreased the IC_{50} value to 20 nM. Clindamycin has been extensively evaluated in various clinical trials.[374] Although clindamyin is effective as a single agent when given for at least 5 days, this regime is not recommended. The slow onset of action makes it unsafe for non-immune adults and children in which fast parasite clearance is necessary. Clindamycin (**103**) shows a synergistic or additive effect in vitro with quinine (**1**), depending on the strain under investigation. In several clinical studies, this combination was equally effective as the combination of quinine and doxycycline (**97**), and superior to quinine monotherapy in terms of therapy duration and tolerability. This holds true for the treatment of uncomplicated as well as severe malaria. Additive activity has also been demonstrated in vitro for the combination of clindamycin with dihydroartemisinin (**39**).[375] In a clinical trial, artesunate/clindamycin was as effective as the quinine/clindamycin combination.[376] These clinical studies strongly suggest clindamycin as an alternative to doxycycline because, in contrast to doxycycline, it is considered safe in pregnancy and can even be used in small children. However, owing to its relatively short elimination half-life of 2–4 h, it is unsuited for prophylactic use. Clindamycin is associated with the potentially fatal pseudomembranous colitis caused by *Clostridium difficile*. But this condition is not observed more frequently than with the extensively used cephalosporins and broad-spectrum penicillins.[377] Furthermore, the risk is considered relatively low under the regimes applied in antimalarial therapy.[374] Clindamycin is also used in combination with quinine against human babesiosis[370] and as a single agent against toxoplasmosis.[371] More than 20 years ago, the clindamycin analogue pirlimycin (**104**) was shown to be about threefold more active in *P. berghei*-infected mice,[378] but no further studies with this compound have been reported.

7.4.4. Chloramphenicol

Chloramphenicol (**105**) targets mainly the A binding site of the large ribosomal subunit, where it directly interferes with substrate binding. The binding site also overlaps partially with the lincosamide binding site.[373] Chloramphenicol inhibits the growth of cultured parasites with an IC_{50} value of 3.2 μM,[331] but due to its high toxicity it is not used for antimalarial chemotherapy.

7.4.5. Thiazole antibiotics

Thiostrepton (**106**) and several structurally related antibiotics (Figure 39) inhibit prokaryotic protein biosynthesis at different stages.[326] They bind to plastid 23S rRNA,[379] inhibiting plastid protein synthesis and *P. falciparum* growth in culture.[380] Thiostrepton displays an IC_{50} value of 1.8 μM[380] and was effective in *P. berghei*-infected mice at 292 μmol (500 mg) kg^{-1} (i.p.).[381] Micrococcin (**107**) and amythiamicin A (**108**) were considerably more active, with IC_{50} values of 35 and 10 nM, respectively.[382] Despite this high activity, it seems unlikely that these antibiotics will be developed as antimalarials.

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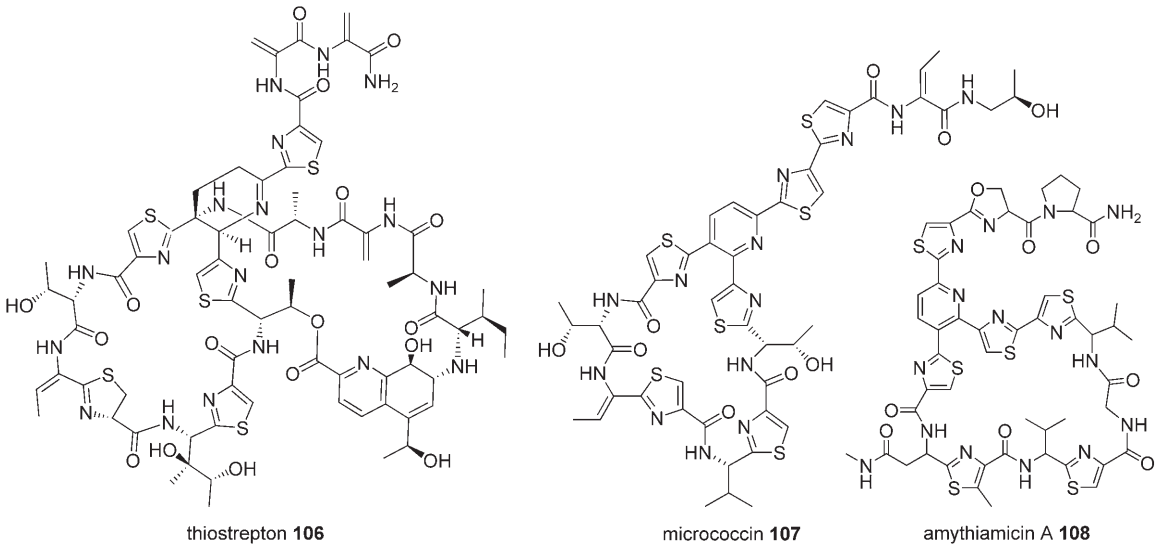

Figure 39. Thiazole antibiotics.

7.5. Miscellaneous antibiotics

Fusidic acid (**109**, Figure 40) has been shown to display moderate antimalarial activity, with IC_{50} values in the range of 29–66 μM against four different strains.[383] The polyene antibiotic amphotericin B (**110**), which is normally used for the therapy of systemic mycoses and visceral leishmaniasis, selectively lyses trophozoide-infected erythrocytes with an IC_{50} value of 188 nM. The better-tolerated lipsomal form of amphotericin B is considerably less active (IC_{50} = 5.4 μM).[384] Surprisingly, the β-lactam antibiotic azlocillin (**111**) has been shown to inhibit the growth of the chloroquine-sensitive 3D7 strain with an IC_{50} value of 1.5 μM, and that of the chloroquine-resistant W2 strain with an IC_{50} of 2.5 μM.[385] Several anthracycline antibiotics display considerable activity against culture parasites, for example doxorubicin (**112**, IC_{50} = 1.5 μM),[386] aclarubicin (**113**, IC_{50} = 0.4 μM), and mitoxantrone (**114**, IC_{50} = 0.1 μM).[384] It is unlikely that these antitumor antibiotics will be used as malaria therapeutics.

8. Diamidines

Diamidines have a long history as antiprotozoal agents. Their activity against trypanosomiasis and leishmaniasis has been described since the 1930s. Today, more than 60 years after its introduction in 1945, pentamidine (**115**, Figure 41) is still one of the most important drugs for the therapy of the early stage of African sleeping sickness (causative agent: *Trypanosoma brucei*) and antimony-resistant visceral leishmaniasis (causative agent: various *Leishmania* spp.). Pentamidine is also used against *Pneumocystis jiroveci* pneumonia and barbesiosis.[387,388] Shortly after the activity of this class of compounds against *Trypanosoma* and *Leishmania* was discovered, the curative effect of undecane bisamidine **116**, stilbamidine (**117**), and pentamidine (**115**) against *Plasmodia* was demonstrated in an animal model. As with other classes of antimalarial agents, interest in diamidines declined because of the availability of better drugs. Only 50 years later was the development of diami-

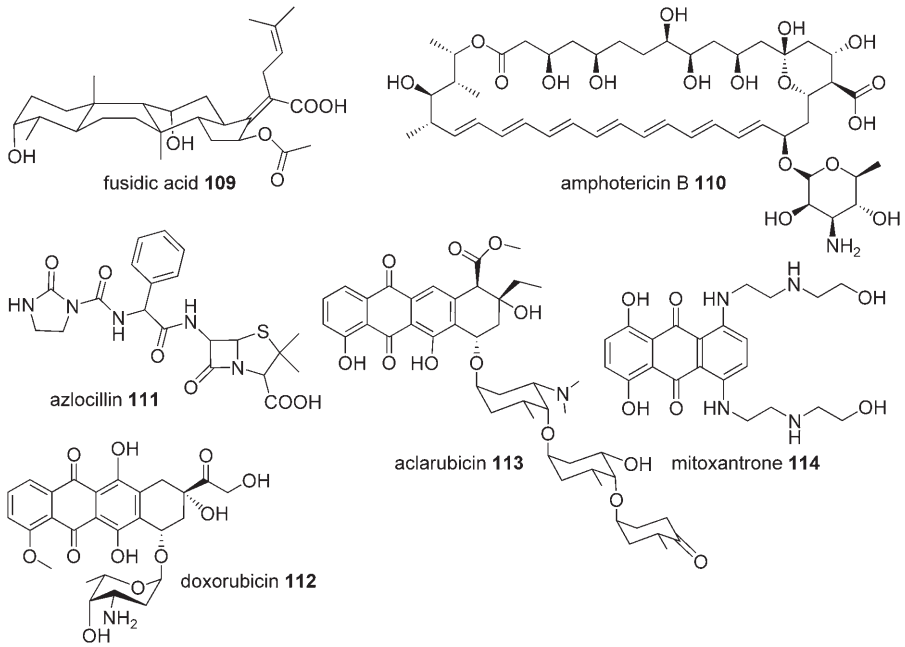

Figure 40. Miscellaneous antibiotics with antimalarial activity.

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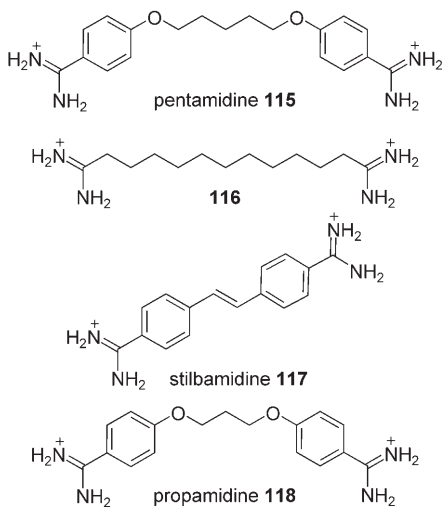

Figure 41. Pentamidine (**115**) and some diamidines of more historical interest.

dines as antimalarial agents revived.[387] In 1990 Tidwell and co-workers investigated the structure–activity relationship of diamidines extensively. The most active compound of this series, in which the ether oxygen atoms of pentamidine were replaced by amino groups, was only slightly more active than pentamidine.[389] In 2001 Ward and co-workers demonstrated propamidine (**118**, $IC_{50} = 5.6$ nM) to be significantly more active than pentamidine ($IC_{50} = 66$ nM). There was no cross-resistance toward chloroquine (**8**), quinine (**1**), or pyrimethamine (**51**).[390]

8.1. Uptake and possible mechanism of action

In the same study[390] it was also demonstrated that pentamidine (**115**) is unable to enter uninfected erythrocytes but is able to enter infected erythrocytes via the so-called "new permeability pathways" (NPPs). The same mechanism also takes place most likely for other analogues diamidines. Transport across the parasite's plasma membrane is facilitated by a proton-driven choline transporter, which displays a significantly higher capacity than the NPPs. Furthermore, approximately 25% of the total pentamidine uptake is facilitated through endocytosis together with hemoglobin (Figure 42).[391]

Inside the parasites, pentamidine accumulates at a concentration up to 500-fold greater than that in the plasma due to the formation of complexes with protoporphyrin IX in the parasite's digestive vacuole. Molecular modeling studies suggest an intercalation of the porphyrin ring system into the preferred conformation of pentamidine, in which the porphyrin system is sandwiched by the staggered aromatic rings of pentamidine.[389] Despite the fact that the two aryl residues of other diamidines (described below) are unable to arrange in a conformation similar to the FPIX binding conformation of pentamidine,[392] it was also demonstrated for those compounds.[393] This FPIX binding can be one explanation for the mechanism of action of diamidines, as the activity of pentamidine is antagonized by the inhibition of hemoglobin degradation.[391] Furthermore, diamidines bind to the minor groove, especially along AT-rich DNA sequences.[394] Although there is no correlation between DNA affinity and antiparasitic activity,[393,395,396] it has been postulated that there must be at least some threshold DNA binding affinity for antiparasitic activity.[396] Furthermore, pentamidine and DB75 (**119**, see below) have been demonstrated to cause a collapse of the mitochondrial membrane potential at least with yeast as a model organism.[397] Recently, it was proposed that diamidines act against *Plasmodium* spp. through FPIX binding and the inhibition of mitochondrial functions, and against *Trypanosoma* and *Leishmania* spp. through binding kinetoplast DNA.[398] In summary, the mechanism of action of diamidines is all but clear, even though this class of compounds has been in therapeutic use against parasitic infections for more than 60 years.

8.2. Furamidine

Today, pentamidine (**115**) is the only diamidine in clinical use. Numerous diamidine derivatives have been described which were nearly exclusively developed against *Trypanosoma*, *Leishmania*, and *Pneumocystis* infections. Furamidine (**119**, DB75), in which the flexible chain of pentamidine has been replaced by a rigid 2,5-furylene residue, is the most advanced com-

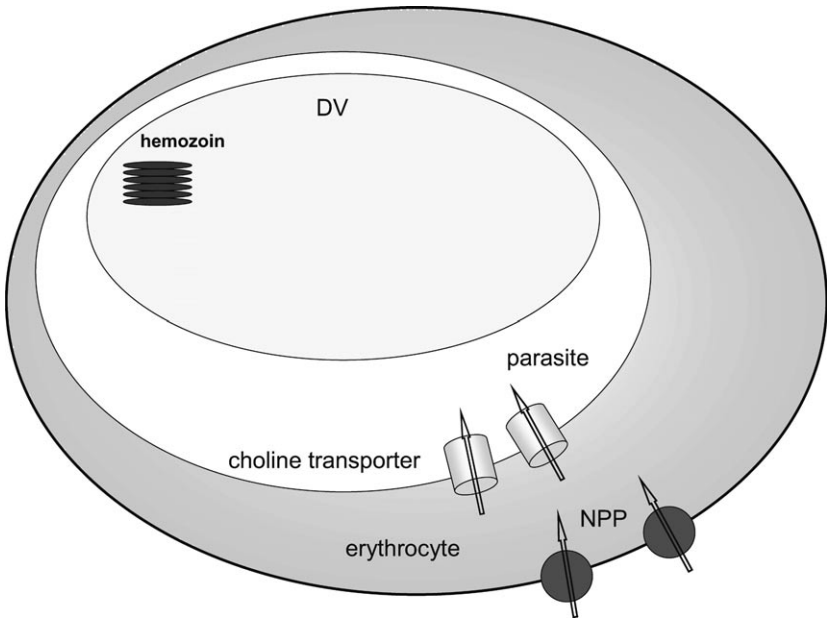

Figure 42. Diamidines and dicationic compounds are thought to enter the infected parasite through so-called "new permeability pathways (NPP)" and the parasite, through the choline carrier.

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pound.[399] It displays an IC_{50} value of 15.5 nM against *P. falciparum* and slightly lower activity against *P. vivax*.[400] Like pentamidine, furamidine is insufficiently resorbed from the gastrointestinal tract after oral administration due to its double positive charge at physiological pH. This problem could be overcome by the *O*-methylamidoxim prodrug pafuramidine (**120**, DB289). The neutral prodrug DB289 is taken up from the gastrointestinal tract and metabolized to furamidine inside liver cells. The first step is a cytochrome P450-catalyzed hydroxylation of the methyl groups, followed by spontaneous decomposition of the hemiacetals to give **121**, resulting in the generation of formaldehyde and the diamidoxim **122**. The latter is reduced to furamidine (**119**) in a cytochrome b_5-mediated reaction (Figure 43).[401] In principle, this strategy should be applicable to most diamidine derivatives.

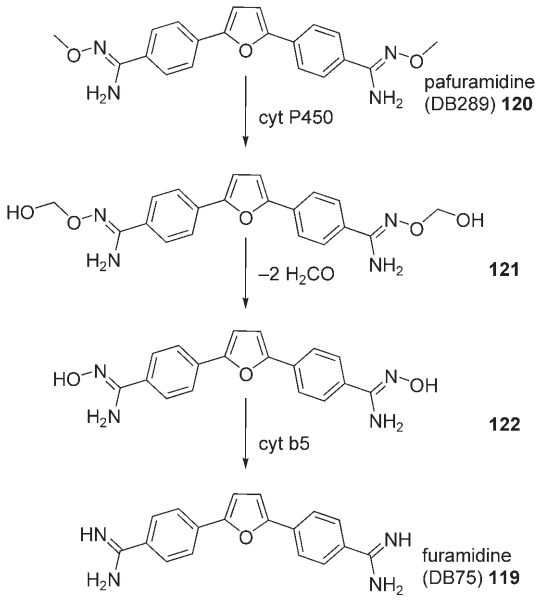

Figure 43. DB289 (**120**), the orally bioavailable prodrug of furamidine (**119**), is transformed in the liver to the antiparasitically active diamidine.

DB289 (**120**) has been evaluated against African sleeping sickness. In addition, it was tested against *P. vivax* and uncomplicated *P. falciparum* infections in a phase II clinical trial. This trial demonstrated high efficacy and good tolerability of DB289 (**120**).[402]

8.3. Novel diamidines under preclinical development

Over the last few years more diamidine derivatives have been described that display greater in vitro activity than furamidine (**119**, IC_{50} = 15.5 nM), such as the diaza analogue of furamidine **123** (IC_{50} = 3.9 nM),[395] 1,4-diamidinophenylpiperazine **124** (IC_{50} = 4 nM),[393] and the biphenylbenzimidazole derivative **125** (Figure 44).[392] The latter displays the highest in vitro activity of this compound class against *P. falciparum*, with an IC_{50} value of 0.5 nM. The bisguanidinofluorene derivative **126** (IC_{50} = 2.3 nM)[396] as well as two linear diamidines (compounds **127** and **128**) with high activity (IC_{50}: 1.0 and 0.5 nM, respectively) have been described recently.[403] Owing to their high in vitro activity against multidrug-resistant strains, the oral bioavailability of alkylamidoxime prodrugs, and the efficiency and tolerability of DB289 (**120**) in an initial clinical trial, diamidines can be considered a highly promising class of compounds for malaria therapy.

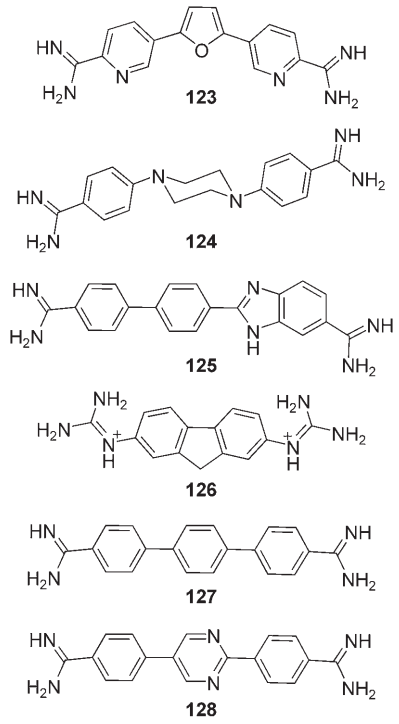

Figure 44. Diamidines in preclinical development.

9. Inhibitors of Phospholipid Metabolism

During the intraerythrocytic stages of their life cycle, parasites produce large quantities of membrane constituents through phospholipid metabolism.[404] Vial and co-workers investigated this phospholipid metabolism as a target for antimalarial drug development (reviewed in Refs. [405,406]). A considerable number of quaternary ammonium compounds have been prepared as choline analogues and evaluated against *Plasmodia*.[407–409] Important points learned from these early investigations are that two quaternary ammonium groups connected by a flexible chain lead to the most active compounds. The bis(triethylammonium) derivative **129** turned out to be the most active compound, with a remarkable IC_{50} value of 3 pM (Figure 45).[410] The lead structure of those first-generation bis(-quaternary ammonium) compounds was G25 (**130**). It inhibits the growth of cultured parasites with an IC_{50} value of 0.64 nM. Several months of exposure to this compound did not lead to the development of resistance.[411] A complete cure was achieved in *P. falciparum*-infected *Aotus* monkeys with a dose of 0.054 μmol (0.03 mg) kg^{-1}.[412] Despite excellent activities, development of this structural class was discontinued due to

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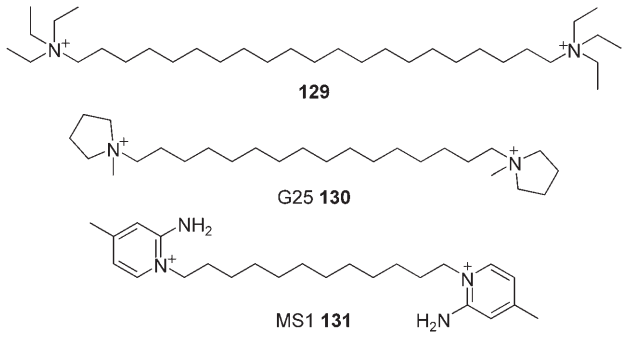

Figure 45. First- and second-generation dicationic compounds.

toxic side effects and the lack of oral bioavailability. In an attempt to improve oral bioavailability, the quaternary ammonium groups were replaced with bioisosteric amidino and guanidino groups. The lead structure of these second-generation compounds, MS1 (**131**), still carries formal charges due to its aromatic amidinium salt. It also displays high in vitro activity (IC_{50} = 0.3 nM), although the desired oral activity could not be obtained.

Success came with the bis-thiazolium compounds of the third generation for which neutral and therefore orally bioavailable prodrugs could be developed. The bis-thiazolium salts T3 (**132**) and T4 (**133**) are the lead structures of this generation (Figure 46). With IC_{50} values of 2.25 and 0.65 nM, respectively, they display in vitro activities similar to those of their predecessors. By following a prodrug concept that was successfully applied to thiamine and DOPA derivatives, the thioester prodrugs TE3 (**134**) and TE4a (**135**) were obtained.[405] These neutral prodrugs display sufficient stability to be resorbed from the gastrointestinal tract, but are rapidly cleaved by plasma esterases, with a half-life of 5 min. This enzymatic thioester hydrolysis liberates a thiol which reacts with the *N*-formyl group to form the thiazolium ring. The oral bioavailability of TE3 was determined to be 16% in rats.

Oral ED_{50} values in a mouse model were 9.2 μmol (5 mg) kg^{-1} for TE3 (**134**) and 16.7 μmol (11 mg) kg^{-1} for TE4a (**135**). There is still a considerable difference in the values obtained for i.p. application (0.46 μmol kg^{-1} for TE3 and 0.18 μmol kg^{-1} for TE4a), although they fall into the range of other effective drugs. No toxic effects were observed in mice at 37 μmol (20 mg) kg^{-1} i.p. and 1850 μmol (1000 mg) kg^{-1} oral. A complete cure without recrudescence (ED_{100}) was obtained in *P. cynomogli*-infected rhesus monkeys given 5.55 μmol (3 mg) kg^{-1} TE3 (**134**).[411,413] Analogous to the bis-amidines, bis-ammonium compounds enter the infected erythrocyte by the parasite-induced NPPs and accumulate inside the erythrocyte at concentrations up to 270–310-fold that in the plasma. From the amount of drug taken up by the erythrocyte, 60% was found in the parasite. Apparently, the compound enters the parasite via the same choline transporter also responsible for the uptake of the bis-amidines.[391] Inside the parasites, de novo phosphatidylcholine synthesis is inhibited (IC_{50} = 0.9 μM), either through the inhibition of choline uptake, the inhibition of enzymes of this pathway, or a combination of both effects.[414] The precise mechanism of action remains unclear. In addition to their effect on de novo phosphatidylcholine synthesis, bis-ammonium compounds have shown to bind to ferriprotoporphyrin IX. This may be important for the observed intraparasitic accumulation and for antimalarial activity.[415] Therefore, the bis-ammonium compounds can be regarded as dual drugs that act on phosphatidylcholine synthesis and heme detoxification. Hopefully, clinical trials with T3 (**132**), scheduled to start in 2007,[416] will confirm the promising results of this compound's preclinical development.

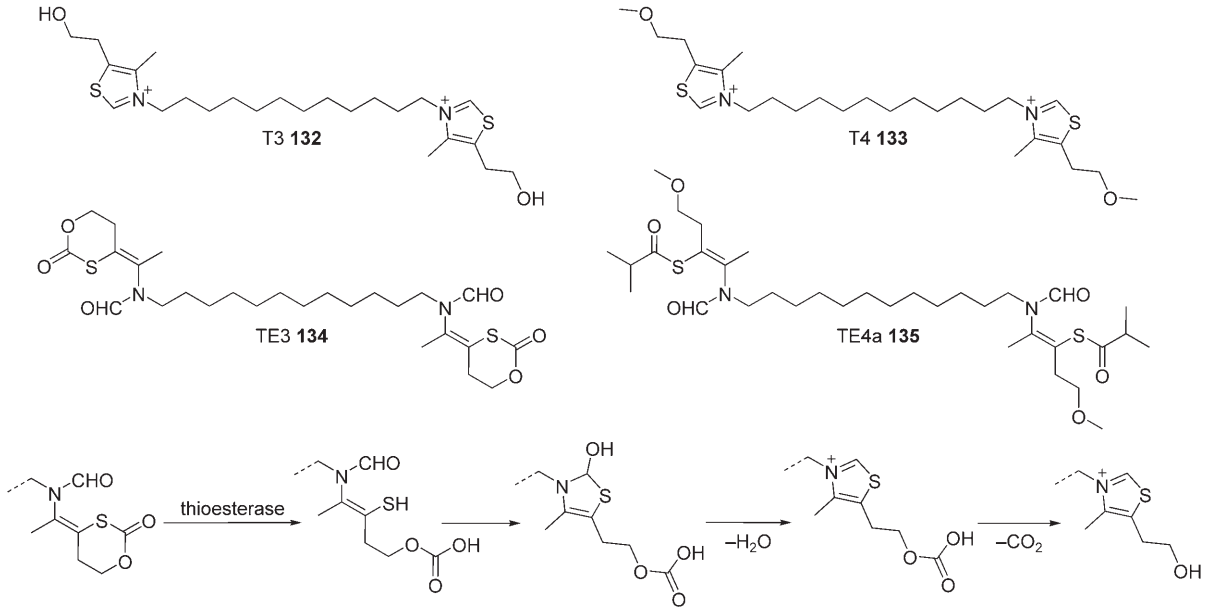

Figure 46. Third-generation dicationic compounds and their prodrugs. Bioactivation of TE3 (**134**) is initiated by a thioesterase-catalyzed cleavage of the thiocarbonic acid diester followed by spontaneous formation of the thiazolium ring.

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10. Inhibitors of Isoprenoid Biosynthesis

There are two completely unrelated pathways leading to isopentenyl diphosphate (IPP) and the isomeric dimethylallyl diphosphate (DMAPP), the common precursors of all isoprenoids. In humans, isopentenyl diphosphate is synthesized by the well-known mevalonate pathway. In contrast, many pathogenic microorganisms, including *Plasmodium* spp., use a completely unrelated mevalonate-independent pathway. It is called the 1-desoxy-D-xylulose-5-phosphate (DOXP) pathway, which is also known as the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway, the non-mevalonate pathway, or the Rohmer pathway. The enzymes of this apicoplast-localized pathway present valuable targets for the development of specific antimalarial drugs because their targets are absent in the human host system.[417–420]

In the 1970s fosmidomycin (FR31564) was isolated from *Streptomyces lavendulae* and evaluated as an antibacterial agent against urogenital tract infections by Fujisawa Pharmaceutical Company. Efforts to develop fosmidomycin as an antibacterial drug were discontinued after phase II studies, most likely because of the availability of drugs better suited for this particular application.

In 1998 fosmidomycin (**136**, Figure 48) was rediscovered as an inhibitor of 1-desoxy-D-xylulose-5-phosphate reductoisomerase (DXR; IspC). DXR is the second enzyme in the DOXP pathway, and catalyzes the isomerization and reduction of DOXP to MEP (Figure 47).[421] A crystal structure of DXR complexed with fosmidomycin shows the *N*-formyl-*N*-hydroxyamino group in a *Z* conformation, providing two oxygen ligands to the enzyme-bound manganese ion (or possibly magnesium in the native state), while the phosphonate forms several hydrogen bonds in an adjacent pocket.[422] Superposition of the substrate DOXP onto fosmidomycin (**136**) indicates the inhibitor as a substrate analogue. Fosmidomycin inhibits *Pf*DXR with an IC_{50} value of 35 nM. The growth of cultured parasites of four laboratory strains is inhibited with IC_{50} values of 390–940 nM.[98] Cross-resistance with other antimalarials and the delayed kill effect common with other antibiotics is not observed. In *P. vinckeii*-infected mice, the oral ED_{50} is 98 μmol (20 mg) kg^{-1} (i.p.: ED_{50} = 25 μmol (5 mg) kg^{-1}).[423] These activities are relatively low in comparison with other antimalarials, but sufficiently high plasma levels can be achieved due to the low toxicity of the drug (LD_{50} = 8000 mg kg^{-1} (i.p.) and 12500 mg kg^{-1} (p.o.)).[424] Using preclinical and clinical data obtained by Fujisawa, fosmidomycin could be progressed rapidly into clinical evaluation as an antimalarial. In a pilot study, rapid parasite clearance (PC) times (PC_{50} = 21 h and PC_{90} = 28 h) and a cure rate of 100% at day 7 were observed, but the recrudescence rate in non-immune patients was unacceptably high.[425] Extensive in vitro combination studies revealed indifferent effects with most antimalarials, and additive effects with quinine (**1**), doxycycline (**97**), azithromycin (**100**), and ciprofloxacin (**94**).[98] Synergy was observed only with clindamycin (**103**) and lincomycin (**102**). Based on these in vitro results, a combination of fosmidomycin (**136**) with clindamycin (**103**) was evaluated in two different clinical studies, revealing high antimalarial activity (100% cure rate in a 4-day course) combined with only mild gastrointestinal side effects.[426] For children under the age of two, efficiency is significantly lower (66%), possibly due to the difficulty in administrating the current capsule formulation to very young children.[427] A 100% cure rate was obtained in a 3-day regime of a fosmidomycin/artesunate combination, which was well tolerated.[428]

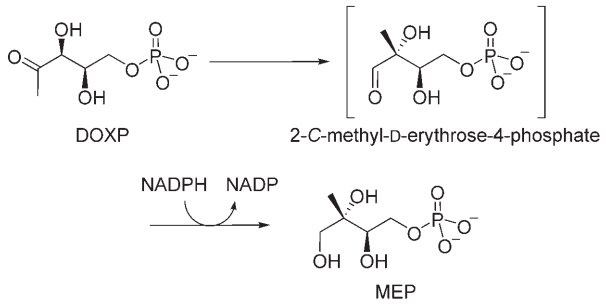

Figure 47. 1-Desoxy-D-xylulose-5-phosphate reductoisomerase (DXR) catalyzes the isomerization and reduction of 1-desoxy-D-xylulose-5-phosphate (DOXP) to 2-*C*-methyl-D-erythritol-4-phosphate (MEP).

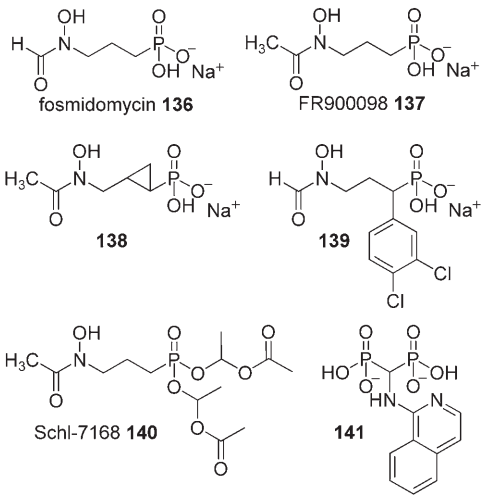

Figure 48. DXR inhibitors.

FR900098 (**137**) is structurally closely related to fosmidomycin (**136**), where the formyl hydrogen atom is replaced by a methyl group. Whereas FR900098 is less active than fosmidomycin against bacteria, it is significantly more active against *P. falciparum* (IC_{50} (*Pf*DXR) = 17 nM; IC_{50} (*P. fal.*) = 570 nM; ED_{50} (p.o.) = 36 μmol (8 mg) kg^{-1}).

Considerable effort has been invested in the structural variation of FR900098, addressing the acyl residue, the hydroxamate substructure, the 1,3-propylen linker, and the phosphonate group. Despite these efforts, none of the derivatives have yet displayed superior properties as an antimalarial (unpublished results). From the bulk of fosmidomycin derivatives, only few can be highlighted. The cyclopropane derivative **138** with limited conformational flexibility in the backbone was as active

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as fosmidomycin (IC_{50} = 480 nM, both in this particular study) and slightly less active than FR900098.[429] Two series of fosmidomycin and FR900098 derivatives with an additional residue in the α position to the phosphonate group yielded comparatively active compounds, with the dichlorophenyl derivative **139** as the most active.[430,431]

By temporarily masking the polar phosphonate group as so-called double ester cleavable by the esterase, the prodrug Schl-7168 (**140**) was obtained which was threefold more active in the mouse model than FR900098 (**137**) (ED_{50} (p.o.) = 10.6 μmol (4 mg) kg^{-1}).[432,433]

In addition to the compounds mentioned above, several more DXR inhibitors have been reported. However, these compounds have only been evaluated against the isolated enzyme (most of which are less active than fosmidomycin (**136**)), but not against *P. falciparum*. Some bisphosphonates, known as inhibitors of farnesyl diphosphate synthase, have been shown to have antiplasmodial activity, with the lowest IC_{50} values around 1 μM.[434] Compound **141** inhibits DXR with an IC_{50} value of 4 μM, and the growth of *P. falciparum* with an IC_{50} value of 50.4 μM. It has also been co-crystallized with DXR.[435] Recently, the same compounds were reported to inhibit hexokinase as well,[436] which raises the question if DXR inhibition is a major factor in their antimalarial activity. Furthermore, owing to their highly polar structure and poor oral bioavailability, bisphosphonates do not appear to be suitable candidates for antimalarial drug development.

Some of the other enzymes of the DOXP pathway are also targets of inhibitor development. Recently, a fluorescence probe for YgbP (IspF) was described.[437] A methyl viologen based colorimetric assay was developed for the iron–sulfur-cluster-containing enzyme LytB (IspH), which catalyzes the last step of the synthesis.[438] Lead discovery is in progress for this enzyme.

11. Summary

Malaria has been a scourge of humankind throughout history. It is one of the earliest reported infectious diseases in humans. At the same time, it was the first disease to be treated with a pure substance (quinine) and a synthetic drug (methylene blue). At the beginning of the last century, malaria was endemic as far north as Southern Norway. Shortly before World War II, chloroquine was invented in Germany, and became something of a wonder drug after the war. Effective, safe, and affordable, it cured billions of clinical episodes and saved millions of lives. When victory in the war against malaria, declared by the World Health Organization, seemed within reach, chloroquine-resistant strains developed and spread over nearly all malaria-endangered regions. Chloroquine had been replaced by the antifolate combination sulfadoxine/pyrimethamine with a useful lifespan shorter than that of chloroquine. Today, there are only a handful of established antimalarial drugs:

- **Quinine**, still effective in most areas and still the only drug available in many countries for intravenous treatment of severe malaria (combination with doxycycline or clindamycin is recommended).
- The 4-aminoquinoline derivative **amodiaquine**, still effective against many chloroquine-resistant strains, but there is also much resistance in Asia and the potential of life-threatening side effects.
- The arylamino alcohol **mefloquine**, with declining efficiency in Southeast Asia, which makes a combination with artesunate necessary, and some problems in tolerability when used prophylactically.
- The combination of **lumefantrine**, another arylamino alcohol, with **artemether**. This relatively new combination seems highly effective in general, but there are some alarming reports of declining efficacy from Southeast Asia.
- The combination of the respiratory chain inhibitor **atovaquone** with **proguanil**, which is also a relatively new remedy, effective in therapy and prophylaxis, but expensive.
- **Artesunate**, the main combination partner in the artemisinin-based combination therapies now recommended by the WHO, the most effective and rapidly acting antimalarial today. However, there is the unresolved issue of pharmaceutical quality of intravenous preparations used for the therapy of severe malaria (also in combination with doxycycline or clindamycin). Primary concerns are availability, as it is made from the natural product artemisinin, and the possibility of resistance development.
- **Primaquine**, an 8-aminoquinoline, still the only anti-relapse drug against dormant stages of *P. vivax* and *P. ovale*. Primaquine has also been effective in clinical studies as a prophylactic against malaria tropica, but can cause potentially life-threatening side effects in glucose-6-phosphate-dehydrogenase-deficient patients.
- The antifolate combination of **dapsone**, used for decades as a leprosis medicine, and **chlorproguanil** has been recently introduced. This combination is affordable and effective against strains predominant in Africa, but ineffective against Asian strains. Thus, there are concerns that in a short time, resistant strains could also spread in Africa.

Few novel drugs or combinations are in advanced stages of clinical studies:

- The old Chinese bis-4-aminoquinoline **piperaquine** in combination with dihydroartemisinin (Euartekin®). Piperaquine is well tolerated and was effective in clinical studies in Africa, but resistance in Southeast Asia is widespread. Furthermore, both combination partners have unmatched pharmacokinetic profiles.
- Another old Chinese drug **pyronaridine** in combination with **artesunate** (PANDA). Like piperaquine, pyronaridine was effective in clinical trials in Africa, but resistance has been found in Southeast Asia.
- The triple combination of **dapsone**/**chlorproguanil** with **artesunate** (CDA; LapDap+) with the intention to expand the useful lifespan of the antifolate combination.
- **Tafenoquine**, an 8-aminoquinoline with activity also against erythrocytic stages of the parasites, a longer half-life, and

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apparently lower risk of severe side effects. Tafenoquine may possibly become an important prophylactic.

Several more drugs are in early stages of clinical development, or are about to be evaluated in initial clinical trials:

- The 4-aminoquinolines ***tert*-butyl isoquine**, a modified structural isomer of amodiaquine unable to form the hazardous quinonimine, the short-chain 4-aminoquinoline **AQ13**, and **ferroquine**, bearing an unusual ferrocene moiety.
- **OZ-277**, a readily available, structurally simple synthetic peroxide which could, if clinical studies go well, become the successor of artesunate and other artemisinins.
- **Pafuramidine** (DB289), the orally bioavailable prodrug of furamidine (DB75), a diamidine derivative with promising results in an initial clinical study.
- **TE3**, the prodrug of a bis-ammonium compound, probably inhibiting choline biosynthesis as well heme detoxification, with promising preclinical results.
- **Fosmidomycin**, an inhibitor of 1-desoxy-D-xylulose-5-phosphate reductoisomerase (mevalonate-independent isopentenyl diphosphate synthesis), showed high efficiency and good tolerability in combination with clindamycin and artesunate in several clinical studies.

From all these novel drugs, only furamidine, TE3, and fosmidomycin act against targets hitherto unexploited in antimalarial chemotherapy.

12. Outlook

The development of antimalarial chemotherapeutics has long been neglected in industrialized countries. In close succession, the most widespread administered and affordable antimalarial drugs have lost efficacy, thus enabling malaria to cause more clinical cases than ever. However, over the past few years, considerable efforts have been made through public–private partnerships, resulting in the recent introduction of LapDap and the progression of several drugs and drug combinations to various stages of clinical development. These new drugs will hopefully augment the armory in the fight against malaria and will help to keep this ancient plague in check.

Currently, mainly academic groups have identified a large number of potentially new targets, and target verification, drug discovery, and lead optimization is in progress. Another Review will deal with this issue.

Notes Added in Proof

The clinical development of OZ 277 has been discontinued because areas under the curve (AUC) in malaria patients were less than 50% of those recorded in healthy volunteers (W. Gutteridge, personal communication).

Recently, a theory regarding the mechanism of synergy between atovaquone and proguanil was offered: When electron transport, which is normally dominant in establishing the mitochondrial membrane potential, is inhibited by atovaquone, an alternative pathway involving ATP hydrolysis and exchange of generated ADP^{3-} against ATP^{4-} by the ATP/ADP transporter becomes apparent. This pathway is inhibited by proguanil, resulting in a rapid breakdown of membrane potential (H. J. Painter, J. M. Morrisey, M. W. Mather, A. B. Vaidya, *Nature* **2007**, *446*, 88–91).

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Keywords: chemotherapy · drug development · malaria · medicinal chemistry

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