Chapter 1

Historical Background of Antimalarial Drugs - A Review
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1.1 Introduction

Malaria, a vector born infectious disease, affects more than 500 million people per annum, causing more than one million deaths, mostly in Africa.¹ Infants, young children and pregnant women are particularly at risk. In fact, it has been estimated that a child dies of malaria every 30 seconds.² The scenario is getting worse with the rapid spread of multidrug-resistant parasites. In addition to the morbidity and mortality, malaria imparts a great economic burden on the affected regions. It has been estimated that the African continent has forgone almost $100 billion in lost GDP over the last 35 years due to malaria alone.³ Malaria ranks third among the major infectious diseases in causing deaths after pneumococcal acute respiratory infections and tuberculosis, and accounts for approximately 2.6% of the total disease burden of the world.⁴ Malaria is caused by a protozoan parasite of genus Plasmodium. Only four species, out of 100s so far known, are found to be infective to human beings, which are P. falciparum, P. vivax, P. ovale, and P. malariae. Of these four, P. falciparum and P. vivax account for 95% of all malaria infections. Nearly all severe and fatal cases are caused by P. falciparum, which is geographically the most widespread out of the four known species, and the most pernicious one, causing majority of the malaria related morbidity and mortality, while P. vivax and P. ovale cause true relapsing malaria. Malaria is distributed chiefly in tropical regions that includes sub-Saharan Africa, South-East 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, its distribution is patchy.⁵

1.2 Life cycle of malaria parasite

The human malaria parasite has a complex digenetic life cycle which requires two hosts for completion (Figure 1). The asexual phase is passed in humans by a process termed schizogony. The sexual cycle is completed in the vector, the female Anopheles mosquito, involving gametogony and sporogony. The infection starts when the infected female Anopheles mosquito injects sporozoites into the subcutaneous tissue and less frequently directly into the blood stream; from there sporozoites travel to the liver. This is followed
by a period of incubation during which the sporozoites invade the hepatocytes where each sporozoite develops into a tissue schizont, containing 10000-30000 merozoites. The schizonts rupture after one to two weeks and release the merozoites into the blood stream, which infect the erythrocytes. 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. Merozoites released into the bloodstream invade erythrocytes and start the erythrocytic cycle. In the erythrocyte, the parasite develops from a ring stage to the trophozoite stage to a blood schizont. After a time characteristic for each specific *Plasmodium* species, erythrocytes rupture and each erythrocyte releases 16-32 new merozoites into the bloodstream, which in turn invade healthy 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. 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. *P. vivax*, *P. ovale*, and *P. malariae* show distinct selectivity towards the age of the infected erythrocytes. For that reason, the degree of total parasitaemia is limited. In contrast, *P. falciparum* infects erythrocytes of all ages, leading to high parasitaemia. 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. Malaria caused by these three parasites is often called benign malaria. In contrast, *P. falciparum* malaria (also known as tropical malaria) 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. Observed complications can include coma (cerebral malaria), respiratory distress, renal failure, hypoglycemia, circulatory collapse, acidosis, and coagulation failure.

1.3 Classification of antimalarial drugs

Traditionally, antimalarial agents are classified by the stages of life cycle of malaria parasite that are targeted by the drug.

**Blood schizonticides:** These act on the asexual intraerythrocytic stages of the parasites; thereby terminate clinical attacks of malaria. Drugs belonging to this class include quinine 1, chloroquine 3, mefloquine 17, halofantrine 18, sulfadoxine 49, pyrimethamine 52, artemisinin 83 and its derivatives, sulfones and tetracycline derivatives etc.

**Tissue schizonticides:** These kill hepatic schizonts, and thus prevent the invasion of erythrocytes, acting in a causally prophylactic manner. Primaquine 22 and pyrimethamine...
52, (to a lesser extent) have activity against this stage. However, since it is impossible to predict the infection before clinical symptoms begin; this mode of therapy is more theoretical than practical.

**Hypnozoiticides:** These kill the persistent intrahepatic stages of *P. vivax* and *P. ovale*, thus preventing relapses from these dormant stages. Primaquine 22 is the only prototype drug available for this stage.

**Gametocytocides:** These destroy the intraerythrocytic sexual forms of the parasites and prevent transmission from human to mosquito. As there are no dormant liver stages in *P. falciparum* malaria (tropical malaria), 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. Chloroquine and quinine have gametocytocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum*. However, primaquine has gametocytocidal activity against all human malarial parasite species including that against *P. falciparum*.

### 1.4 Historical development of antimalarial drugs

The history of malaria chemotherapy dates back to 168 BC. The herb *Artemisia annua* (sweet wormwood) was known to the Chinese as *qing-hao* for more than 2000 years. Artemisinin, the active ingredient of *qinghao* was isolated by Chinese scientists in 1971 and since then, artemisinin and its derivatives have become the most effective weapons in our arsenal against malaria. Quinine represents another lead from the bark of Chinchona tree. Ever since the isolation of quinine, tremendous efforts have been devoted towards developing more effective quinoline based antimalarial drugs. Thus, the role of natural products as a source of novel molecular scaffolds for novel antimalarial agents has been well demonstrated in the case of quinine and artemisinin, both of which have served as templates for the development of structurally simpler analogs that either served or continue to serve as effective antimalarials. In addition, researchers have reported novel synthetic compounds with unique scaffolds as antimalarials. This review will briefly discuss about history of antimalarial chemotherapy. It mainly covers major natural products and their semisynthetic derivatives that have served either as antimalarial agents or as potential lead compounds in the further development of antimalarial drugs. It also covers structurally simple synthetic analogues of these natural products and novel synthetic compounds with
distinctive scaffolds, which have influenced or have potential to influence the course of malaria chemotherapy.

1.5 Quinoline and related antimalarials

1.5.1 Quinine

The powdered bark of the cinchona tree has been used for the treatment of malaria for over 350 years. The active antimalarial ingredients are quinine 1 and its diastereomer quinidine 2. Quinine, an arylaminoalcohol, was isolated in 1820 and has been used ever since. This makes malaria one of the first diseases to be treated by a pure substance. Quinine is still one of the most important drugs for the treatment of uncomplicated malaria. It has multiple side-effects, most of which are reversible, but some are severe in nature. Quinidine 2 is about 2-3-fold more active than quinine 1. However, it is also more prone to induce cardiac arrhythmias.

Following the early success of quinine in combating malaria, quinoline-derived compounds were extensively studied for the development of newer synthetic therapeutic agents.

1.5.2 Chloroquine

Chloroquine (CQ) 3, a 4-aminoquinoline, has been the most successful single drug for the treatment and prophylaxis of malaria. CQ 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. CQ is a relatively well-tolerated drug as long as it is used in therapeutic regimes.
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. The protein component of hemoglobin is digested by the successive action of various proteolytic enzymes in the food vacuole (FV). 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 hemoglobin-derived Fe(II)-heme to Fe(III)-heme promotes the formation of superoxide ions generating H$_2$O$_2$ and hydroxyl radicals. Higher concentrations of this molecule are toxic to the parasite. Therefore, free heme has to be detoxified by the *Plasmodium*. The parasite disposes this hazardous waste through the formation of an insoluble polymer called hemozoin. CQ, a dibasic compound (pKa 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.

Due to the massive use of CQ, resistant parasite strains have developed. The main cause of CQ resistance is a mutation in the pfcr1 gene that codes for a protein called the CQ resistance transporter (*PfCRT*). This 10-transmembrane-domain transport protein belongs to the drug metabolite transporter (DMT) superfamily located in the membrane of the digestive vacuole. Because there is not much else of significance inside the digestive vacuole 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. All CQ-resistant strains have a threonine residue in place of lysine at position 76 of the protein.

### 1.5.3 Amodiaquine

Enhancement of lipophilicity of the side chain of CQ by the incorporation of an aromatic structure resulted in amodiaquine (AQ). A certain degree of cross-resistance between AQ and CQ is observed. AQ is effective against low-level CQ-resistant *P. falciparum* but not against highly CQ-resistant parasites. Furthermore, its therapeutic value is significantly decreased by the biotransformation of its *p*-aminophenol moiety into a
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Quinonimine 5 (Figure 2). Quinonimine is highly susceptible to a nucleophilic attack, mainly by thiols, resulting in severe hepatotoxicity with an incidence of 1 in 15500.

![Figure 2](image)

Figure 2. AQ 4, toxicity results from oxidation of p-amino-phenol moiety to quinonimine 5, which is susceptible to a nucleophilic attack.

1.5.4 Ferroquine

Ferroquine (FQ) 6 has a diamine side-chain more lipophilic than that of chloroquine. It is an organometallic drug which bears a ferrocenyl moiety in the side-chain, a structural feature rather uncommon in potential drugs. Because of this lipophilic ferrocenyl moiety, it has been proposed that FQ does not fit into the CQ-resistance transporter (CRT). It also displays some CQ-resistance-reversing properties. FQ is active against various CQ-sensitive and CQ-resistant laboratory strains as well as field isolates. In a mouse model, it is curative at 8.4 (19 mmol) mg/kg b.w. Although efficacious, high cost is a drawback of this compound.

1.5.5 Isoquine and related compounds

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 7, displays an activity against the CQ-resistant K1 strain even superior to that of AQ. 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. It is expected that tert-butylisoquine, will soon advance to clinical trials (GlaxoSmithKline).

1.5.6 Pyronaridine
Pyronaridine is another member of the Mannich base schizonticides, although the usual quinoline heterocycle is replaced by an azaacridine. Like AQ, pyronaridine also bears the aminophenol substructure, which can be oxidized to the respective quinonimine. In contrast to AQ, pyronaridine 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 the attack of the sulfur nucleophile. Alternatively, the quinonimine group could be reduced prior to the nucleophilic attack. Nevertheless, it has been shown that pyronaridine is metabolized to a compound that is toxic to neutrophils.

1.5.7 Naphthoquine
Naphthoquine, which shares greater structural similarity with AQ, was registered in China in 1993. In a clinical trial the combination of naphthoquine and artemisinin was found to be safe and effective.
1.5.8 Dimeric 4-aminoquinolines

Another strategy to overcome CQ resistance is the connection of two 4-aminoquinoline moieties by linkers of various lengths and chemical nature. The activity of such bisquinolines against CQ-resistant strains has been explained by their steric bulk, which prevents them from fitting into the substrate binding site of \( P/JRT \). 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 11, was developed in the 1960s and heavily used in China.\(^{26}\) The combination of piperaquine and dihydroartemisinin 84 (named as Euartekin) has advanced to the phase II clinical trials. Piperaquine is reportedly well tolerated; the most important side-effect is an increase in blood pressure.

1.5.9 Chloroquine-astemizole hybrids

Astemizole 12, is known to show potent in vitro and in vivo antiplasmodial activity.\(^{27}\) Recently, Whitlock \textit{et al.} have used a strategy that combined the core portions of the two structurally distinct moieties of CQ and astemizole, each of which possesses significant antiplasmodial activity, via an appropriate linker. Various linkers were investigated to conjugate the two agents into one molecule. Many compounds possessed improved in vitro activity against a CQ-resistant strain of \( P. falciparum \), while compound 13 displayed significant activity in vivo in mouse models against \( P. berghei \).\(^{28}\)

1.5.10 Hybrids of 4-aminoquinolines and acridines with clotrimazole like scaffolds

Clotrimazole (CLT) 14, is a well-known antymycotic drug which exhibits a weak in vitro antimalarial activity against different \( P. falciparum \) strains and importantly, irrespective of their CQ sensitivity.\(^{29,30}\) Biological activities of CLT are mediated by its ability to interact with ferrirprotoporphyrin IX (Fe(III)-FP), which is present as the prosthetic group in several enzymes, such as (i) 14R-lanosteroldemethylase (14-LD), the fungal cytochrome inhibited by CLT, (ii) human P450 cytochromes, which are inhibited by CLT causing altered metabolism of both xenobiotics and endogenous chemicals, and (iii)
hemoperoxidase,\(^1\) a P. falciparum-derived enzyme, which is inhibited by CLT, in the presence of \(\text{H}_2\text{O}_2\), by a mechanism based on CLT-one electron oxidation product. Moreover, CLT is also able to form in vitro complexes with free Fe(III)-FP in which the imidazole ring behaves as a Fe(III) axial ligand and is able to inhibit in vitro, the crystallization of free Fe(III)-FP into \(\beta\)-hematin.\(^{2,3}\) Based on the aforementioned properties of CLT and considering its peculiar chemical structure, characterized by (i) an imidazole ring, known to mediate electron transfer reactions in biological systems,\(^{4,5}\) and (ii) a triphenylmethyl system, known to form and stabilize a radical intermediate, Campiani and co-workers hypothesized that CLT, in the unique FV chemical environment, could interact with hemoglobin-derived ferroprotoporphyrin IX (Fe(II)-FP) and get activated to form toxic trityl radicals able to kill the parasite by oxidative damage. Based on these facts Campiani et al. reported identification of CLT-based compound 15 with potent in vitro antimalarial activity.\(^{36}\) More recently they have reported hybrid compounds typified by 16 in which a CLT-based polyaromatic scaffold is linked with either a 4-aminoquinoline or an acridine system, with potent in vivo antimalarial activity in mice by oral route.\(^{37}\)

1.5.11 Mefloquine

Mefloquine 17, an arylaminoalcohol, which is used as the erythro racemate, can be regarded as structurally simplified quinine and was developed during World War II. It was put to therapeutic use in 1985 and has been widely used, especially in Asia, where a considerable degree of resistance has developed over the years. It displays high activity against most CQ-resistant Plasmodium strains (\(\text{IC}_{50} = 8.4\) nM against the CQ-sensitive laboratory strain D6; 3.4 nM against the CQ-resistant strain W2; 6.2-10.7 nM against 32 CQ-resistant isolates from Cameroon).\(^{38,39}\) 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. The mechanism of action of mefloquine and other arylaminoalcohols remains unclear. It is most likely different from the mechanism of 4-aminoquinolines. Recently, it has been proposed that aryl amino 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.\(^{40}\)

1.5.12 Halofantrine

Halofantrine 18, like mefloquine, was developed by the Walter Reed Army Institute of Research from a series of phenanthrene methanols, whose antimalarial activity was discovered during World War II. It is active against CQ-resistant *Plasmodium* strains (mean IC\(_{50}\) values: 1.2 nM against 45 CQ-resistant Cameroonian wild isolates; 1.5 nM against 22 CQ-sensitive isolates).\(^{39}\) Its mechanisms of action and resistance are most probably shared with those of mefloquine. Not surprisingly, cross-resistance is observed between these two antimalarial agents.\(^{41}\) Halofantrine is associated with a high risk of cardiac arrhythmias.\(^{42-44}\) Therefore, halofantrine has been withdrawn from the market in several countries.

1.5.13 Lumefantrine

Lumefantrine 19, also known as benflumetol, is structurally similar to halofantrine. It was developed in the 1970s by the Academy of Military Sciences in Beijing, China. It displays lower antimalarial activity than halofantrine. CQ-resistant parasites are slightly more susceptible than CQ-sensitive strains.\(^{45}\) The most significant difference from halofantrine is the absence of the dangerous cardiac side-effect.\(^{46}\) Lumefantrine displays in vitro synergism with artemether 86.\(^{47}\) This combination is currently used under the brand name Riamet.\(^{48}\)
Desbutyllumefantrine 20 is one putative metabolite, although it has not been detected in humans. It displays about four fold higher antimalarial activity than its parent drug.

### 1.5.14 Primaquine

Pamaquine 21, also known as plasmoquine or plasmochin, is an 8-aminoquinoline and 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. The terminal diethylamino moiety of pamaquine is replaced by an unsubstituted primary amine in primaquine (PQ) 22. PQ 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. PQ is still the only antimalarial drug licensed for the radical cure (or anti-relapse therapy) of *P. vivax* infections. The most serious side-effect of PQ is a potentially life-threatening hemolysis in persons deficient in glucose-6-phosphate dehydrogenase.
1.5.15 Tafenoquine
Tafenoquine 23 is a more lipophilic derivative of PQ. The main structural difference is the trifluoromethylphenoxy substituent, which confers higher activity against the blood and liver stages as well as a higher sporontocidal activity, but decreased gametocidal activity.\textsuperscript{50} It’s activity against blood stages of \textit{P. falciparum} is weaker than that of most other blood schizonticidal agents. Tafenoquine is generally regarded to be better tolerated than PQ, but it still carries some risk of causing hemolysis in glucose-6-phosphate dehydrogenase-deficient humans.

1.5.16 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 24 responsible for the antimalarial effect.\textsuperscript{51} Another 8-aminoquinoline named bulaquine or elubaquine 25 is in clinical use in India against \textit{P. vivax} infections. To block biotransformation into potentially toxic metabolites 2-\textsuperscript{tert-butyl} primaquine 26 has been prepared with an IC\textsubscript{50} value of 124 nM.\textsuperscript{52} In yet another modification of PQ, Moreira \textit{et al.} have reported, PQ dipeptide derivatives 27 bearing an imidazolidin-4-one moiety at the N-terminus as anti-transmission agents.\textsuperscript{53}

1.5.17 Quinoline and isoquinoline alkaloids
Twelve 2-substituted quinoline alkaloids were isolated from \textit{Galipea longiflora} (family Rutaceae). Among them, 2-(\textit{n}-pentyl) quinoline 28 was effective at 50 mg/kg/day against \textit{P. vinckei petteri} infected mice.\textsuperscript{54} Taking 28 as a lead molecule, synthesis and antimalarial activity of some related compounds, prototype 29 and 30 has also been reported.\textsuperscript{55}

\begin{center}
\begin{tabular}{ccc}
\textbf{28} & \textbf{29} & \textbf{30} \\
\end{tabular}
\end{center}

Naphthylisoquinoline alkaloids 31-34 have been identified as the active principles of the African antimalarial medicinal plants \textit{Ancistrocladus abbreviatus}, \textit{A. barteri} (family Ancistrocladaceae), and \textit{Triphyophyllum peltatum} (Dioncophyllaceae). These alkaloids showed in vitro activity against the K1 (multidrug-resistant) and NF-54 (CQ-sensitive) strains of \textit{P. falciparum},\textsuperscript{56} as well as \textit{P. berghei}.\textsuperscript{57} Further studies have shown that
dioncopeltine A 31 suppresses parasitaemia almost completely, while dioncophylline C 34 cured *P. berghei* infected mice completely after oral treatment at 50 mg/kg/day for 4 days without noticeable toxic effects. A more rapid clearance of parasitaemia was detected when 34 was delivered directly to the circulatory system via a mini osmotic pump.

![Chemical structures of dioncopeltine A 31 and dioncophylline C 34](image)

Four benzyl tetrahydroisoquinolines 35-38 and one benzyl isoquinoline alkaloid 39 were isolated from *Hernandia voyronii* (family Hernandiaceae). Compounds 35-38 showed moderate in vitro antimalarial activity (IC$_{50}$ = 1.68 to 3.38 μg/mL) against the CQ-resistant *P. falciparum* strain (FCM29/ cameroon). In rodent studies, 35 and 37 showed 31.8% and 15% suppression of parasitaemia, respectively against the CQ-resistant strain N67 of *P. yoelii* in mice at 10 mg/kg/day for 4 days.

![Chemical structures of compounds 35-38 and 39](image)

In a chloroquine combination in vivo study, 36 and 37 showed synergism, 35 had simple additive effects, while 38 showed antagonism.
1.6 β-Carboline alkaloids
Manzamine A 40 present in several marine sponge species, inhibits the growth of \( P. \) berghei in mice. More than 90% of the asexual erythrocytic stages of \( P. \) berghei were inhibited after a single i.p. injection of 40 (50 \( \mu \)g/mL) into infected mice.

![Image of Manzamine A]

A remarkable aspect of treatment with compound 40 is its ability to prolong the survival of highly parasitemic mice; 40% mice survived beyond 60 days after a single injection.\(^{59}\)

1.7 Chalcones
Licochalcone A 41 was isolated from Chinese licorice root,\(^{60}\) a traditional treatment for a number of disorders. It acts in vitro against CQ-sensitive and CQ-resistant strains of \( P. \) falciparum and in vivo against \( P. \) yoelii in the infected mice.\(^{61}\) An analogue of Licochalcone A, 2,4-dimethoxy-4'-butoxy chalcone 42, was active orally, and was much less toxic than 41.\(^{62}\)

![Image of Licochalcone A and 2,4-dimethoxy-4'-butoxy chalcone]

1.8 Xanthones
These are secondary plant metabolites that are found almost exclusively in the members of two families of higher plants, Guttiferae and Gentianaceae as well as certain fungi, ferns and lichens.\(^{63}\) Rufigallol 43, a hydroxylated anthraquinone, was the first compound of this class, which was found to exhibit antimalarial activity in 1995. There is some disparity in the literature regarding its activity. It is active against CQ-sensitive \( P. \) falciparum (D\(_{50}\)).\(^{64,65}\)
Exiphone 44, the structural analogue of rufigallol showed in vitro activity with an IC\textsubscript{50} of 4.1 μM against CQ-sensitive strain (D6) of *P. falciparum*. However, combination studies have shown that 43 potentiated the antimalarial activity of 44. The antimalarial activity of xanthones has been linked to their ability to inhibit heme polymerization. 66

![Chemical structures](image)

### 1.9 Tazopsine

Tazopsine 45, which was isolated from the bark of *Strychnopsis thouarsii* from Madagascar’s rainforest, constitutes a new class of antimalarial. 67 Tazopsine has specific inhibitory action against liver but not blood stages of *P. yoelii*. Its antimalarial activity was shown to be due to growth retardation of the early developmental stages of the hepatic parasite. Simple chemical modification of tazopsine led to the compound N-cyclopentyl (NCP)-tazopsine 46, which demonstrated an improved toxicity profile, and conferred full protection from malarial challenge in a mouse malaria model. IC\textsubscript{50} values and therapeutic indices did not differ substantially from primaquine, the only licensed drug with specific activity against the hepatic stage of malaria. 68

![Chemical structures](image)

However, primaquine and its derivatives are hampered significantly by toxicity and a risk of haemolysis in individuals with a glucose-6-phosphate dehydrogenase deficiency, a condition common in malaria endemic regions. Therefore, NCP-tazopsine presents an excellent lead candidate for compounds with improved pharmacological characteristics.

### 1.10 DHPS and DHFR inhibitors

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 dihydrofolate as an essential nutrient, which is then reduced to tetrahydrofolate acid. Pathogenic microorganisms including *Plasmodium* can synthesize dihydrofolate acid from simple precursors. Furthermore, *P. falciparum* is able to use exogenous dihydrofolate acid via a salvage pathway. 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 DHPS is completely absent in humans, bacterial and protozoal DHFRs are sufficiently different from the human enzyme to allow the development of 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 hydroxymethylidihydropterin pyrophosphokinase 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 and the possibility of using other enzymes along the folate biosynthetic pathway as drug targets has already been reviewed.

1.10.1 Sulfachrysoidine and sulphadoxine

The first antifolate to be used against malaria was the well-known DHPS inhibitor sulfachrysoidine. It was developed in 1932 by Domack as an antibacterial agent. Later it was found that sulfanilamide, 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 and the development of other synthetic antimalarials. Only when sulfonamides such as sulfadoxine with longer half lives and improved toxicological profiles were developed in the late 1950s, was the interest in sulfonamides renewed, especially as combination partners for the DHFR inhibitors.
1.10.2 Proguanil and pyrimethamine
After the early development of sulfachrysoidine, two DHFR inhibitors, Proguanil 50, (Palundrine) and pyrimethamine 52 (Daraprim) were introduced in close succession in the late 1940s and early 1950s for the therapy and prophylaxis of malaria. Proguanil is a prodrug that yields the active metabolite cycloguanil 51 through oxidative ring closure by a cytochrome P450 dependent reaction. Both proguanil and pyrimethamine are highly active inhibitors of \textit{P. falciparum} DHFR (PfDHFR) with Ki values of 1.5 nM and 2.6 nM, respectively.

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \\
\text{H}_2\text{N} & \quad \text{H}_2\text{N} \\
\text{Cl} & \quad \text{Cl} \\
50 & \quad 51
\end{align*}
\]

1.10.3 Combination of DHPS/DHFR inhibitors
The combination of the sulfonamide sulfadoxine 49 with the DHFR inhibitor pyrimethamine 52, known under its brand name Fansidar, became the most important antimalarial next to CQ. The combination 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. 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). For this reason, the prophylactic use of Fansidar was discontinued in most countries years ago. Despite its limited efficacy, it is still widely used in Africa in combination with CQ, AQ, or artesunate 88 because of its low price.

1.10.4 Novel DHFR inhibitors under development
1.10.4.1 Dihydrotriazines based on the cycloguanil structure
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 analogue WR99210 53, which exerts excellent activity ($IC_{50} = 2.7$ nM) against the quadruple-mutant-bearing \textit{P.
falciparum strain V1S. A crystal structure revealed the basis of the preserved activity of WR99210 even against the quadruple mutant form of PfDHFR. Several derivatives of WR99210 and its prodrug PS-15 54 have been prepared and evaluated for their antimalarial properties. JPC-2056 55 has emerged as the most promising candidate for preclinical development. 75

1.10.4.2 Trimethoprim and derivatives
Trimethoprim 56, which is widely used in combination with sulfamethoxazole for the treatment of bacterial infections, is less active against PfDHFR than pyrimethamine or cycloguanil (Ki against wild-type PfDHFR: 10.3 nM). By replacing one of the methoxy groups with a benzyloxy group (compound 57), activity against wild-type DHFR can be markedly improved (Ki = 0.4 nM). In addition, such derivatives display Ki values against the quadruple mutant form of DHFR in the range of 60-90 nM and IC_{50} values in the low micromolar range. 76

1.11 Antibiotics
1.11.1 Effect of antibiotics on malaria parasites
Antibiotics, which are known to specifically target prokaryotic structures, show significant antimalarial activity against eukaryotic malaria parasites. 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. Apart from tetracyclines, which are thought to act mainly against the mitochondrion, all other antibiotics seem to act on the apicoplast. It has been
shown that doxycycline 61 blocks the expression of apicoplast genes. 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 phenomenon is known as “delayed death phenotype” or “delayed kill effect”. According to another theory, apicoplasts inherited by parasites treated with antibiotics contain insufficient levels of apicoplast-encoded proteins, which are required for the import and processing of nuclear gene-encoded proteins needed for normal function. The in vitro activity of antibiotics depends very much on the incubation time because of the delayed kill effect. For example, if clindamycin 66 is evaluated in a conventional 72-hour growth inhibition assay, the IC_{50} value is only about 50 mM. By extending the incubation time to 120 h, the IC_{50} value drops to approximately 20 nM.

1.1.2 Quinolones
Among the quinolones commonly used in antibacterial therapy, ciprofloxacin 58 displays the highest activity against cultured *P. falciparum* parasites. IC_{50} values between 38 mM and 1.4 mM, depending on the parasite strain and incubation time, have been reported.

1.1.3 Rifampicin
Rifampicin 59 (also known as Rifampin) is a well known inhibitor of bacterial RNA polymerase. Rifampicin inhibits the growth of various laboratory strains in a 48-hour assay with IC_{50} values between 3.2 and 1.3 mM. With the W2 clone, the IC_{50} value dropped from 1.3 mM at 48 h to 0.09 mM at 144 h incubation time. In a clinical trial, rifampicin alone displayed insufficient activity against *P. vivax* malaria.

1.1.4 Protein biosynthesis inhibitors
A variety of antibiotics such as tetracyclines, macrolides, lincosamides, thiostrepton, 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.

Tetracycline 60 has been administered in combination with quinine, and minocycline 62 is sporadically used in the clinic. Doxycycline 61 has successfully been combined with artesunate 68 in a clinical study. Doxycycline is also combined with mefloquine to tackle mefloquine resistance. 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.

Azithromycin 64 was more active than erythromycin 63 in vitro, with IC₅₀ values of 6.5 versus 68 mM against a CQ-resistant strain, and 3.0 versus 6.3 mM against a CQ-sensitive strain. In clinical trials, azithromycin 64 was well tolerated as a prophylactic agent, but less effective than doxycycline 61 against \textit{P. falciparum} malaria. In a comparative trial for the treatment of multidrug-resistant \textit{P. falciparum} malaria, the combination of azithromycin and dihydroartemisinin was less effective than mefloquine/dihydroartemisinin.

Lincosamides are two closely related antibiotics, the naturally occurring lincomycin 65 and its semisynthetic derivative clindamycin 66. In a clinical trial, artesunate/clindamycin was as effective as the quinine/clindamycin combination. 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. Thiostrepton 67
and several structurally related micrococcin, amythiamycin antibiotics inhibit prokaryotic protein biosynthesis at different stages. They bind to plastid 23S rRNA, inhibiting plastid protein synthesis and \( P. falciparum \) growth in culture.\(^8\) Fusidic acid 68 has been shown to display moderate antimalarial activity, with IC\(_{50}\) values in the range of 29-66 mM against four different strains.\(^7\) Despite this high activity, it seems unlikely that these antibiotics will be developed as antimalarials.

![Fusidic acid 68](image)

1.12 Febrifugine and isofebrifugine

The roots of \( Dichroa febrifuga \) (family Saxifragaceae) have been used for centuries in China to treat malaria fevers. Febrifugine 69 and isofebrifugine 70 were isolated from \( D. febrifuga \), and have attracted considerable attention as antimalarial agents.\(^8\) In studies in mice, febrifugine 69 significantly reduced mortality. Febrifugine acts by impairing hemoglobin formation required for maturation of the parasite at the trophozoite stage.\(^9\) The use of febrifugine as an antimalarial agent was initially appealing not only because of its rapid effect and no drug resistance, but also because of its availability. Subsequent pre-clinical researches have found that febrifugine possesses adverse side effects.

![Compounds 69 and 70](image)

Strong liver toxicity has precluded febrifugine as a clinical drug.\(^9\)
1.12.1 Synthetic analogs of febrifugine
Recently, Zhu et al. have reported synthesis of a large number of analogs of febrifugine typified by 71.94 Synthesized compounds were tested against \textit{P. falciparum} clones W-2, a CQ-resistant cell line for in vitro and against \textit{P. berghei} for in vivo efficacy in mice. All the compounds were also tested for their in vivo toxicity in mice. Most compounds have exhibited comparable or superior in vitro and in vivo antimalarial activity, as compared to the parent natural product febrifugine and at the same time were found to be much less toxic than the parent compound.

1.13 Polyethers
Some specific polyether antibiotics have been reported to have potent antimalarial activity.95-98 Though rich in oxygen atoms, these molecules are lipophilic in nature. Since the parasite infected cell membrane is vulnerable to binding with lipophilic compounds, the putative mechanism of action for these polyethers is via transfer of ions through the membrane. This transport is potentially done after complexation with a cation (mobile carrier: true ionophores) or by formation of trans-membrane channels (quasi-ionophores). Bacteria, especially \textit{Streptomyces} sp. (isolated from soil samples) are reported as the primary producers of such molecules.99 Very little in formation is available about isolation of these compounds from marine \textit{Streptomyces} sp.100 Recently, an antimalarial screening effort of marine microorganisms from Hawaiian sediments yielded a \textit{Streptomyces} sp. designated strain H668 with highly potent in vitro activity against \textit{P. falciparum} without significant cytotoxicity to Vero cells. Bioassay-guided fractionation of the EtOAc-soluble fraction of the H668 culture led to the isolation of a new polyether metabolite 72.
The antimalarial activity of the new metabolite was evaluated against both the CQ-susceptible (D6) and -resistant (W2) clones of *P. falciparum*, and their toxicity was tested against Vero cells. Compound 72 showed antiprotozoal activity against both the D6 and W2 clones, with IC₅₀ values ranging from 100 to 200 ng/mL.

1.14 Gallinamide A

The organic extract of a *Schizothrix* species of cyanobacteria from a tropical reef near Piedras Gallinas (Caribbean coast of Panama) showed potent initial antimalarial activity against the W2 CQ-resistant strain of *P. falciparum*. Bioassay guided fractionation afforded a new and highly functionalized linear peptide, gallinamide A 73. Pure gallinamide A was evaluated in the *P. falciparum* assay and was shown to possess moderate antimalarial activity (IC₅₀ = 8.4 μM).

1.15 Coccinone A and related compounds

From the trun latex of *Moronobea coccinea* Aubl. (Clusiaceae), eleven new polycyclic polyprenylated acylphloroglucinols (PPAPs) typified by coccinone A 74, have been isolated and tested for antiplasmodial activity against the CQ-resistant strain of *P. falciparum* FcB1 and for cytotoxicity on the human MRC-5 cell line. Compounds have shown IC₅₀ values between 3.3 and 9.0 μM.

1.16 Diamidines

Over the last few years more diamidine derivatives have been described that display greater in vitro activity than furamidine 75 (IC₅₀ = 15.5 nM), such as the diaza analogue of
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furamidine 76 (IC\textsubscript{50} = 3.9 nM),\textsuperscript{104} 1,4-diamidinophenylpiperazine 77 (IC\textsubscript{50} = 4 nM),\textsuperscript{105} and the biphenylbenzimidazole derivative 78.\textsuperscript{106} The latter displays the highest in vitro activity of this compound class against \textit{P. falciparum}, with an IC\textsubscript{50} value of 0.5 nM. The bisguanidinofluorene derivative 79 (IC\textsubscript{50} = 2.3 nM)\textsuperscript{107} as well as two linear diamidines (compounds 80 and 81) with high activity (IC\textsubscript{50} = 1.0 and 0.5 nM, respectively) have been described recently. Owing to their high in vitro activity against multidrug-resistant strains, the oral bioavailability of alkylamidoxime prodrugs, and the efficiency and tolerability of DB289 82 in a phase II clinical trial, diamidines can be considered a highly promising class of compounds for malaria chemotherapy.\textsuperscript{108}

1.17 Artemisinin and semisynthetic peroxides

1.17.1 Artemisinin: A lead in malaria chemotherapy

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 endoperoxide lactone artemisinin 83 was isolated, which has been used in China for the treatment of malaria since 1972.\textsuperscript{109} Artemisinin is very effective and safe against CQ-sensitive and CQ-resistant strains of \textit{P. falciparum},\textsuperscript{110} but has certain limitations like poor oil and water solubility, and high rate of recrudescence. The limited availability of artemisinin and that too from natural source was another lagging factor that led to the development of various synthetic methodologies for the synthesis of artemisinin, but none of them was commercially viable.\textsuperscript{111} Hence, a lot of efforts have been put only to develop semisynthetic derivatives. Structure activity relationship studies of artemisinin and its
deoxy derivative have revealed that it is actually the endoperoxide linkage of artemisinin in the form of 1,2,4-trioxane which is responsible for its activity.\textsuperscript{112}

\includegraphics[width=0.5\textwidth]{artemisinin_diagram}

1.17.2 Mechanism of action of artemisinin and related peroxides
Efforts to elucidate the antimalarial action of artemisinin started in the 1970s, and in the last three decades large number of papers has been published by various workers regarding the mode of action of artemisinin and related peroxides.\textsuperscript{113-120} 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 Fe\textsuperscript{2+} mediated cleavage of the endoperoxide leads to the formation of different C-centered radicals which may be primary or secondary in nature (Figure 3). Which of these two radicals is the active species is unclear. For a long time it was thought that the formation of C-centered radicals takes place in the digestive vacuole and that ferriprotoporphyrin IX is the activating species.

\textbf{Figure 3.} Fe(II)-mediated formation of primary and secondary carbon radicals from artemisinin.

The reactive C-centered 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.\textsuperscript{121-124}
O’Neill and Posner formulated the mechanism of artemisinins as “iron-triggered cluster bombs” (Figure 4).125

Figure 4. The “iron-triggered cluster bomb”: According to former theory about the mechanism of action of artemisinin, Fe(II)FPIX catalysed the formation of carbon-based radicals in the digestive vacuole, deactivating proteins more or less indiscriminately.

Figure 5. Recent results suggest that Fe(II)-mediated radical formation take place in cytosol; these radicals specifically inhibit a sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) called as PfATP6

Although very attractive, the development of resistance against a drug that acts nonspecifically against multiple targets is unlikely, so 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 detected
labelled 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 the Fe$^{2+}$ mediated cleavage.\textsuperscript{126}

Recently, Krishna and co-workers have put forward another theory which says that the endoperoxide cleavage should take place in the cytoplasm catalyzed by a cytoplasmic Fe$^{2+}$ source. The resulting reactive species then very specifically inhibits an ATP-dependent Ca$^{2+}$ pump located on the endoplasmic reticulum (Figure 5). The pump, called PfATP6, is a homologue of a mammalian sarcoplasmic/endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA).\textsuperscript{127}

Artemisinins are highly active, decreasing the parasite biomass 10000-fold in a single asexual cycle.\textsuperscript{128} This makes artemisinins the most active and rapidly acting antimalarial drugs known today.

\textbf{1.17.3 First generation artemisinin derivatives}

\textbf{1.17.3.1 Artemether & Arteether}

To overcome the poor bioavailability of artemisinin, Chinese workers reduced the lactone moiety of parent molecule to hemiacetal to synthesize dihydroartemisinin \textsuperscript{84}.\textsuperscript{129} This compound was having better oil and water solubility, but suffered from neurotoxicity and relative instability under acidic conditions. In order to reduce its toxicity and increase its stability, it was converted into its corresponding ethyl and methyl ether derivatives arteether \textsuperscript{85} \textsuperscript{130} and artemether \textsuperscript{86} \textsuperscript{131} respectively, the first generation analogs of artemisinin. Both of these compounds were found several times more active both, \textit{in vitro} and \textit{in vivo} against multidrug-resistant malaria in comparison to artemisinin and are at present the drugs of choice for the treatment of complicated malaria. Arteether is chiefly used in India (Emal) and in Netherlands (Artemotil) but the more prevalent substance is artemether (Paluther, Artenam, Artemos).\textsuperscript{132} Currently, the application of artemether \textsuperscript{86} with lumefantrine (Coartem or Riamet) is the only artemisinin-based combination therapy available, manufactured under Good Manufacturing Practice (GMP) standards.
1.17.3.2 Artesunate

Artesunic acid 87 is another first generation artemisinin derivative, in which the hemiacetal OH group is acetylated with succinic acid. Sodium artisunate 88 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 in which the condition of the patients prohibits any other route of administration. Artesunic acid and sodium artesunate are unstable, as the succinic ester linkage gets rapidly cleaved, releasing dihydroartemisinin as the active agent.

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 was at least as effective as i.m. applied artemether and thus may be useful in settings in which parenteral therapy can not be given. The utility of sodium artesunate, however, is impaired by its poor stability in aqueous solution due to the facile hydrolysis of the ester linkage and short plasma half-life (20-30 min).

1.17.3.3 Artelinate

Lin et al. have reported a new series of water-soluble derivatives in which the solubilizing group, carboxylate, is on a moiety that is joined to dihydroartemisinin by ether, rather than an ester, linkage. One of these derivatives, artelinic acid 89, is not only considerably more stable than artesunic acid in weakly alkaline solution but is also more active against *P. berghei* in mice. Its sodium salt, sodium artelinate 90 possesses comparable antimalarial
activity both in vivo as well as in vitro to artemether or arteether. However, further development of sodium artelinate has been discontinued in favor of sodium artesunate because of the higher neurotoxicity of the former.\textsuperscript{136,137}

1.17.3.4 Toxicity

Neurotoxicity is a major concern with all artemisinin derivatives owing to their biotransformation into dihydroartemisinin, which is believed to be the final neurotoxic agent. Specific brain-stem toxicity has been observed in animal experiments.\textsuperscript{138} In contrast to these findings, no neurotoxicity has been observed in humans despite the widespread use of artemisinins in China for 30 years. There are conflicting reports about the loss of hearing under combination therapy with artemether and lumifantrine.\textsuperscript{139,140} A further concern is the use of artemisinins in pregnancy, as fetotoxicity has been observed in animal experiments. However, another clinical study indicates that artemisinins might be safe in the second and third trimesters.\textsuperscript{141}

1.17.4 Second generation artemisinin derivatives

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. Much work has been invested in the development of second-generation artemisinins. Methyl and ethyl residues of the first-generation semisynthetic artemisinins, arteether and artemether, have been replaced by numerous other residues, some of them carrying polar groups, as is the case with artelinate, 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 substructures. Several reviews cover this issue in depth.\textsuperscript{142-144} Representative examples are shown in Figure 6. Compounds with in vitro activity superior to that of the first generation artemisinins were obtained, sometimes with promising in vivo activity but none of these compounds have made it to the clinical stages of development except artemisone (91).
Figure 6. From the large number of "second-generation semisynthetic artemisinins" only artemisone (91) has made it close to the clinical development stage.
1.18 Synthetic peroxides as potential antimalarial agents

1.18.1 1,2-Dioxanes

Yingzhaosu A 92, a natural product endoperoxide with antimalarial properties was isolated from Chinese herb, Yingzhao, Artabotrys uncinatus, but its scarcity in nature and difficult total synthesis had led to the development of its various structurally simpler synthetic analogs.

Roche's group, reported preparation of variety of analogs (93a-93g) of 92 containing its 2,3 dioxabicyclo[3.3.1]nonane core from the enantiomers of carvone.

A short and efficient synthesis of 4,8-dimethyl-4-phenylsulfonylmethyl-2,3 dioxabicyclo[3.3.1]nonanes from the enantiomers of limonene or R(-)-carveol afforded a new series of bicyclic analogs of yingzhaosu represented by 94a and 94b, with a variety of substituents at C-8. All the synthetic analogs of Yingzhaosu A have weak antimalarial activity in vivo except 93a (arteflene). Although arteflene is an order of magnitude less potent than the semisynthetic artemisinins in vitro, it is only 3-fold less active than artemether in vivo. Other attractive properties of arteflene include a chemically more stable 1,2-dioxane (endoperoxide) versus the 1,2,4-trioxane in artemisinin, a lower rate of recrudescence and a longer plasma half-life than either artemether or arteether. From these data, arteflene was selected as the clinical candidate, and it progressed to Phase II clinical trials in semi-immune African patients with mild P. falciparum malaria. In these trials, the
drug was given orally as a lipid suspension, but the results were inconsistent and the compound was abandoned.\(^{149}\)

Posner's group reported the mechanism-based design of a series of easily prepared symmetrical bicycle[3.2.2]nonane \(95\) and bicyclo[2.2.2]octane \(96\) endoperoxides. As illustrated by the sulfone \(95b\), heterocyclic analogs of \(95a\) containing sulfur, oxygen or nitrogen atoms were synthesized; however, these were all an order of magnitude less potent than their carbocyclic analog \(95a\) even though they are reduced by ferrous iron to form reactive carbon centered radicals and epoxides.\(^{150}\)

Varieties of dioxanes have been prepared so far and have been assessed for their antimalarial activity but none of them has shown potent antimalarial activity.

1.18.2 1,2,4-Trioxanes

This class of compounds has been known in the literature since 1957, when Payne and Smith synthesized first synthetic trioxane.\(^{151}\) Later on several researchers developed various methodologies for the synthesis of different types of trioxanes only from synthetic point of view.\(^{152}\) It was only after the disclosure of the fact that it is actually the endoperoxide linkage of artemisinin in the form of 1,2,4-trioxane, which is responsible for its antimalarial activity, large emphasis has been made towards the synthesis and bioevaluation of various types of synthetic trioxanes.

The bicyclic trioxanone\(^{152c}\) \(97\) was prepared from 2-methyl-2-cyclopenten-1-ol in six steps. Bicyclic trioxane\(^{153}\) \(98\) (2,3,5-trioxabicyclo[2.2.3]nonane), easily recognizable as the pharmacophoric core of artemisinin, was prepared from 6-tetrahydrooxepanol as starting material. However, these bicyclic trioxanes had only marginal antimalarial activity.

The epimeric 1,2,4-trioxanes \(98a\) and \(98b\) were synthesized by the photooxygenation reaction. Compound \(98a\) was just an order of magnitude less potent than artemisinin, whereas \(98b\) was quite less potent than artemisinin. Jefford \textit{et al.} showed that replacement of the bridgehead C-3 methyl group by C-3 phenyl group in \(98a\) improved antimalarial potency by 6-fold.\(^{154}\) Based on these facts Posner \textit{et al.}\(^{155}\) synthesized various substituted C-3 phenyl analogs of prototype \(99\). Some of these compounds \(99a-e\) have shown
promising in vivo activity. Trioxane alcohol 99b and acetate trioxane 99c were more potent than artemisinin whereas water soluble carboxylic acid derivative 99d was less active than artemisinin.

In continuation of their work, Posner et al. prepared carboxyphenyl trioxanes 100a and 100b which were more soluble in water at pH 7.4 than artesunate.

A large number of derivatives of artemisinin like 1,2,4-trioxanes, including ethers, carboxylate esters, phosphate esters, carbamates and sulfonates have been prepared by Posner and co-workers. Some of the compounds found active in vitro were also tested in vivo in mice model. Based on their antimalarial potency in mice, two trioxanes 101 and 102 were selected for biological evaluation in Aotus monkeys infected with multidrug-resistant P. falciparum. The activity data revealed that both 101 and 102 are as effective as arteether against multidrug-resistant P. falciparum in Aotus monkeys.

Spiro ring-fused trioxane 103 was synthesized starting with (-)-isopulegol. This trioxane was only slightly less potent than artemisinin. The analog in which the spirocyclopentane ring was replaced with geminal methyl substituents was 9-fold less potent than 103. 1,4-Endoperoxides, formed by photooxygenation of 1,4-diaryl-1,3-cyclopentadienes, reacted with aldehydes or ketones in reactions catalyzed by Me3SiOTf to produce a large series of cis-fused 1,2,4-trioxanes, exemplified by 104a (Fenozan B07). Several such analogs 104b-f were synthesized and assessed for antimalarial activity. Among the cis-fused cyclopenteno-1,2,4-trioxanes, 104a (Fenozan B07) had the most promising activity profile and was chosen for further development.
Spiro trioxanes 105 and 106 and their analogs were prepared by photooxygenation of the corresponding allylic alcohols followed by peroxyacetalization reactions with aldehydes or ketones. Griesbeck et al.\textsuperscript{162} reported the synthesis of antimalarial 1,2,4-trioxanes via photooxygenation of chiral allylic alcohol, 4-methyl-3-penten-2-ol, followed by subsequent BF\textsubscript{3} catalyzed peroxyacetalization with aldehydes or ketones to afford four monocyclic and spirobicyclic 1,2,4-trioxanes, of which 105 was the most potent one. O’Neill et al.\textsuperscript{163} reported Co(II)-mediated regioselective Mukaiyama hydroperoxy silylation of 2-alkyl- or 2-aryl-prop-2-en-1-ols to furnish peroxysilyl alcohols which were treated with aldehydes or ketones to provide various spiro trioxanes. Trioxane 106, the best of these, was only an order of magnitude less potent than artemisinin.

Singh reported a new and convenient \textsuperscript{1}O\textsubscript{2}-mediated synthesis of 6-arylvinyl-1,2,4-trioxanes.\textsuperscript{164} The key steps of this method are the preparation of \(\beta\)-hydroxyhydroperoxides by photooxygenation of suitably substituted allylic alcohols and then elaboration of these \(\beta\)-hydroxyhydroperoxides into 1,2,4-trioxanes by acid catalyzed condensation with various ketones or aldehydes. This method is safe and has been used for the preparation of trioxanes on multigram scale.
Singh et al. have prepared several highly lipophilic synthetic trioxanes 107a-g and trioxane quinoline hybrids (trioxaquines) 108a-f. Compounds 107a and 107b showed 100% survival at 12 mg/kg × 4 days and 24 mg/kg × 4 days dose respectively, by oral route against multidrug-resistant *P. yoelii* in Swiss mice. The trioxaquines 108a-f were found to have poor activity.

Meunier et al. have also synthesized several trioxane-quinoline hybrids (trioxaquines), some of which have shown promising activity profile in vitro and in vivo. Ascaridole-derived, trioxaquine 109 was the best compound of the series. It exhibited *ED₅₀* values of 5 mg/kg/day and 18 mg/kg/day by i.p. and p.o. routes respectively against *P. vinckei* in mice. This compound completely cleared parasitaemia in *P. vinckei* infected mice, without recrudescence, at an i.p. dose of 20 mg/kg/day.

### 1.18.3 1,2,4,5-Tetraoxanes

Symmetrical meso dispiro 1,2,4,5-tetraoxide 110, readily obtained by reaction of 2-methylcyclohexanone with acidified hydrogen peroxide, was found to be only 6-fold less active than artemisinin. Solaja and co-workers have developed several bile acid derived highly potent tetraoxanes in the past few years. Mixed tetraoxide 111 possessing...
spirocycloalkane and spirocholic acid-derived steroid substructures was found to be 6-fold more potent than artemisinin.

Mixed tetraoxanes with a spirocyclohexane were more potent than the corresponding spirocyclopentane and spirocyclooctane analogs. Several diester and diamide cholic acid-derived tetraoxanes were synthesized, best one of these, cis diamide tetraoxane 112, was only 4-fold less potent than artemisinin. More recently, chimeric compounds with tetraoxane moiety linked to a 4-aminoquinoline moiety have been reported. Chimeric compound 113 showed IC₉₀ of 3.75 nM against P. falciparum African D6 clone, IC₉₀ of 2.26 nM against P. falciparum Indochina W2 clone and IC₉₀ of 76.38 nM against Multidrug-resistant P. falciparum Thailand TM91C235 clone. It also cured mice in a modified Thompson test for antimalarial blood stage activity, with a minimum curative dose of 80 mg/kg, a minimum active dose of 20 mg/kg/day, and a maximum tolerated dose of >960 mg/kg.

1.18.4 1,2,4-Trioxolanes

Vennerstrom and co-workers have synthesized new 1,2,4-trioxolanes 114-117 as antimalarials. 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 114 clearly did not provide enough protection for the sensitive heterocycle, resulting in rapid compound breakdown, two adamantane rings 115 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, as in 116, the critical balance between stability and reactivity could be obtained. Finally, the
addition of an aminoacyl residue provided the correct polarity and solubility, resulting in the desired pharmacological properties. OZ-277 117 displayed high activity against field isolates from Gabon (median IC\textsubscript{50} = 0.47 nM; range: 0.13-2.23 nM).\textsuperscript{172} Its stage specificity is similar to that of artemisinin.\textsuperscript{174} The activity against \textit{P. vivax} is in the same range as the activity against \textit{P. falciparum}.\textsuperscript{175} OZ-277, has recently entered the clinical trials.

Trioxolanes have been successfully linked with the 4-aminoquinoline and acridine scaffolds. Compound 118 prepared by combining a trioxolane and a 4-aminoquinoline moiety showed IC\textsubscript{50} value of 12.61 nM against CQ-sensitive strain 3D7 and 26.21 nM against CQ-sensitive strain K1.\textsuperscript{176}

1.8.5 1,2,4-Trioxepanes, 1,2,4,5-Tetraoxepanes, 1,2,4,5-Tetraoxocanes & 1,2,5,6-Tetraoxonanes
There are only few methods\textsuperscript{177} reported in the literature for the synthesis of 1,2,4-trioxepanes, the next higher homolog of 1,2,4-trioxanes and there are only two reports of their antimalarial activity. O’Neill\textsuperscript{178} and co-workers first of all reported in vitro activity of 1,2,4-trioxepanes having prototype 120 and 121. Singh\textsuperscript{179} et al. have also reported in vivo assessment of new class of aryl vinyl 1,2,4-trioxepanes 122a-d. Tricyclic 1,2,4,5-tetraoxepane 123 and 1,2,5,6-tetraoxonane 124 were 35 to 40-fold less potent than artemisinin, but 124 had notably better in vivo activity (ip). Both 123 and 124 however, were completely inactive when they were administered orally.\textsuperscript{180} 1,2,4,5-tetraoxocanes
125a and 125b exhibited excellent in vitro potency, however, both were less effective than artemisinin in vivo.\textsuperscript{181}

1.19 Drugs in clinical trials

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 South-East 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 South-East 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 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 artemesunate and other artemisinins.

• CDRI-97/78 has been selected for clinical trials on account of its better pharmacokinetic profile.

• 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 artemesunate in several clinical studies.

1.20 Conclusion

History of malaria chemotherapy is full of efforts towards eradication of this ancient plague. But even after these worldwide efforts malaria continues to challenge the prowess and intellect of scientists all over the world working in the field of malaria chemotherapy. Parasites resistant to the newly discovered antimalarial drugs have been reported in various parts of the world. The combination therapy can be better in the area where resistance against single drug is reported. Another strategy is to discover antimalarial agents whose mechanism of action is completely different from those already available. Thus, there is an urgent need to screen a large number of plants and marine samples to find new potential antimalarial agents with novel structures and different modes of action.

1.21 References

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