New Trends in Anti-Malarial Agents

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Abstract: Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally young African children) out of 400 million cases each year (WHO world health report 2000). More than half of the world's population live in areas where they remain at risk of malaria infection. During last years, the situation has worsened in many ways, mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs. Furthermore, the control of malaria is becoming more complicated by the parallel spread of resistance of the mosquito vector to currently available insecticides. Discovering new drugs in this field is therefore a health priority. Several new molecules are under investigation. This review describes the classical treatments of malaria and the latest discoveries in antimalarial agents, especially artemisinin and its recent derivatives as well as the novel peroxidic compounds.

1. INTRODUCTION

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally young African children) out of 400 million cases each year (WHO world health report 2000). More than half of the world's population lives in areas where they remain at risk of malaria infection. In fact, more people could potentially be infected by malaria today than during the last century. During last years, the situation has worsened in many ways, mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs [1]. This resistance concerns numerous drugs, but is thought to be most serious with chloroquine, the most widely and cheapest drug used to treat malaria. Urgent efforts are therefore necessary to identify new classes of antimalarial drugs. Furthermore, the control of malaria is becoming more complicated by the parallel spread of resistance of the mosquito vector to currently available insecticides.

2. THE MALARIA PARASITE AND ITS LIFE CYCLE

Malaria is caused by Protozoa of the genus *Plasmodium.* Four species of *Plasmodium* cause the disease in humans (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) but *P. falciparum* causes most problems as a result of its prevalence, virulence and drug resistance. *P. falciparum* may cause the conditions known as cerebral malaria, which is often fatal.

The life cycle of the malaria parasite is complex [Fig. (**1**)] and involves two stages: a sexual reproductive stage with multiplication (sporogony) wich occur in the midgut of the mosquito, and an asexual reproduction phase with multiplication (schizogony), which takes place in the human host. We could distinguish, in the human part of the cycle, two important phases: the exoerythrocytic (hepatic) phase and the erythrocytic phase.

The sporozoites are transmitted to humans by a bite of an infected female mosquito of the genus *Anopheles*. The sporozoites circulate for a short time in the blood stream, then invade liver cells, where they develop into exoerythrocytic schizonts during the next 5 to 15 days. *Plasmodium vivax* and *ovale* have a dormant stage, the hypnozoite [2], that may remain in the liver for weeks or many years before the development of exoerythrocytic schizogony. This results in relapses of infection. *Plasmodium falciparum* and *P. malariae* have no persistent phase, but *P. malariae* could persist in the blood for many years if inadequately treated.

An hepatic (exoerythrocytic) schizont contains 10 000 to 30 000 merozoites, which are released and invade the red blood cells. Invasion of erythrocytes by malarial parasites involves a complex series of events that depend on receptor interactions between the surface of both the erythrocyte and merozoite [3]. The entire invasion process takes about 30 seconds. Once the merozoites invade erythrocytes, they begin to undergo their asexual reproduction which leads to the formation of erythrocytic schizonts, through ring and trophozoite (erythrocytic schizogony). The erythrocyte containing the mature schizont will then rupture and release the merozoites which could invade other red blood cells. In the course of these events, some merozoites

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Table 1. Length of the Different Stages of Life Cycle of *Plasmodium Falciparum*

1	Ookinetes formation	24 to 48 hours
2	Oocysts maturation	9 days
	Time for invasion of salivary glands (1+2)	**10 days**
3	Time of circulation of sporozoites in the blood stream	max. 1 hour
4	Hepatic shizogony	6 days
5	Hypnozoites	non existent
6	Erythrocytic schizogony	**48 hours**
7	Gametocytogony	10 days
	Complete cycle (1 to 7)	**27 days**

which have invaded erythrocytes could differentiate themselves into microgametocytes (male) and macrogametocytes (female). These gametocytes can be transmitted to an *Anopheles* mosquito during a blood meal, and then complete there their life cycle. Mature gametocytes taken into the midgut of the *Anopheles* mosquito escape from the erythrocyte to form gametes. The microgamete moves quickly to fertilize a macrogamete and forms a zygote. Within 18 to 24 hours, the zygote elongates into a slowly motile ookinete. The ookinete cross the peritrophic membrane and the epithelial cell of the midgut, and then transforms into an oocyst beneath the basement membrane of the midgut epithelium. Between 7 and 15 days postinfection, depending on the Plasmodium species and ambient temperature, a single oocyst forms more than 10000 sporozoites. The motile sporozoites migrate into the salivary glands and accumulate in the acinar cells of the salivary glands. When an infected mosquito bites a susceptible vertebrate host, the Plasmodium life-cycle begins again. The length of *P. falciparum* life cycle is mentioned in Table 1.

3. BIOLOGY OF THE MALARIAL PARASITE [FIG. (2)]

The malaria parasite exhibits a rapid growth and multiplication rate during many stages of its life cycle [4]. The intracellular location of the parasite, protecting itself against the immune system of the host, lead to a greater difficulty to obtain nutrients from the external environment. As a result of this, the parasite modifies its host cell in several ways and intertwines its metabolism with that of the host. The host erythrocyte possess a sluggish metabolism and limited transport capabilities, which poses some problems for the actively growing parasite. In reality, the infected erythrocyte exhibits a substantial increase in its permeability to low molecular weight solutes and in its transport pathways [5-7]. Metabolites also need to cross the parasitophorous vacuole membrane (PVM) and the parasite membrane. The cytostome, a structure formed by invagination of the PVM and parasite membrane, is responsible for the uptake of hemoglobin and a portion of the vacuole [8]. Projections of the PVM, called the tubulo-vesicular membrane (TVM) network and the Maurer's clefts [9], have been implicated in the acquisition of

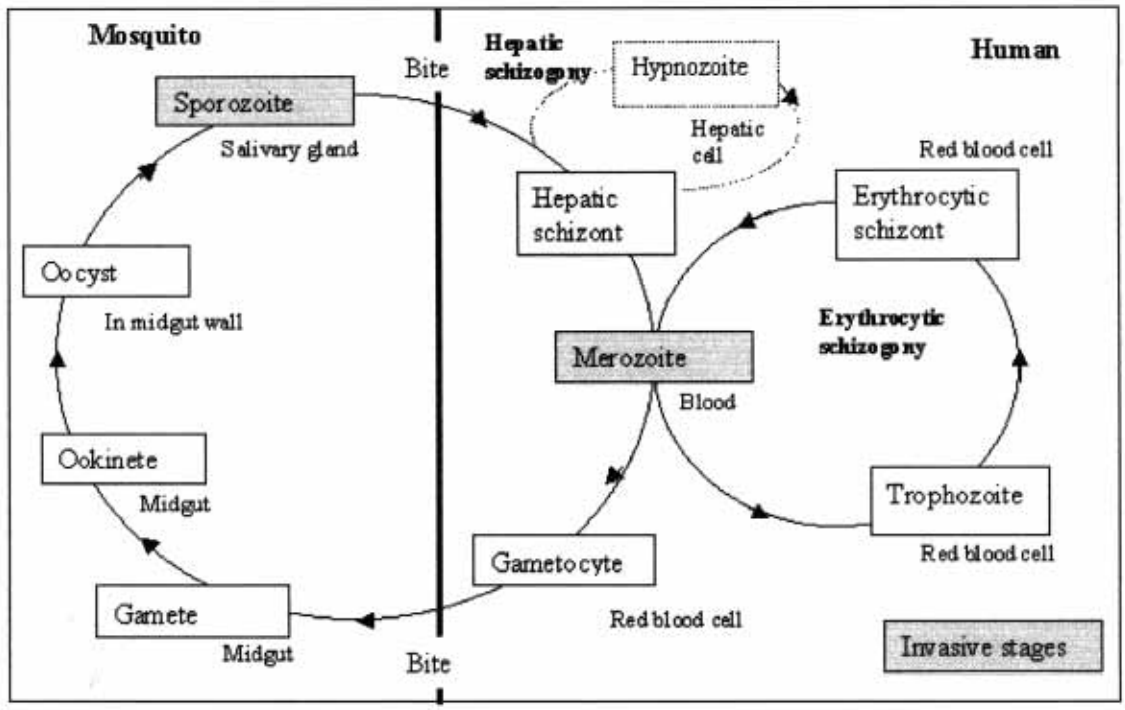


Fig. (1). Life cycle of *Plasmodium* species.

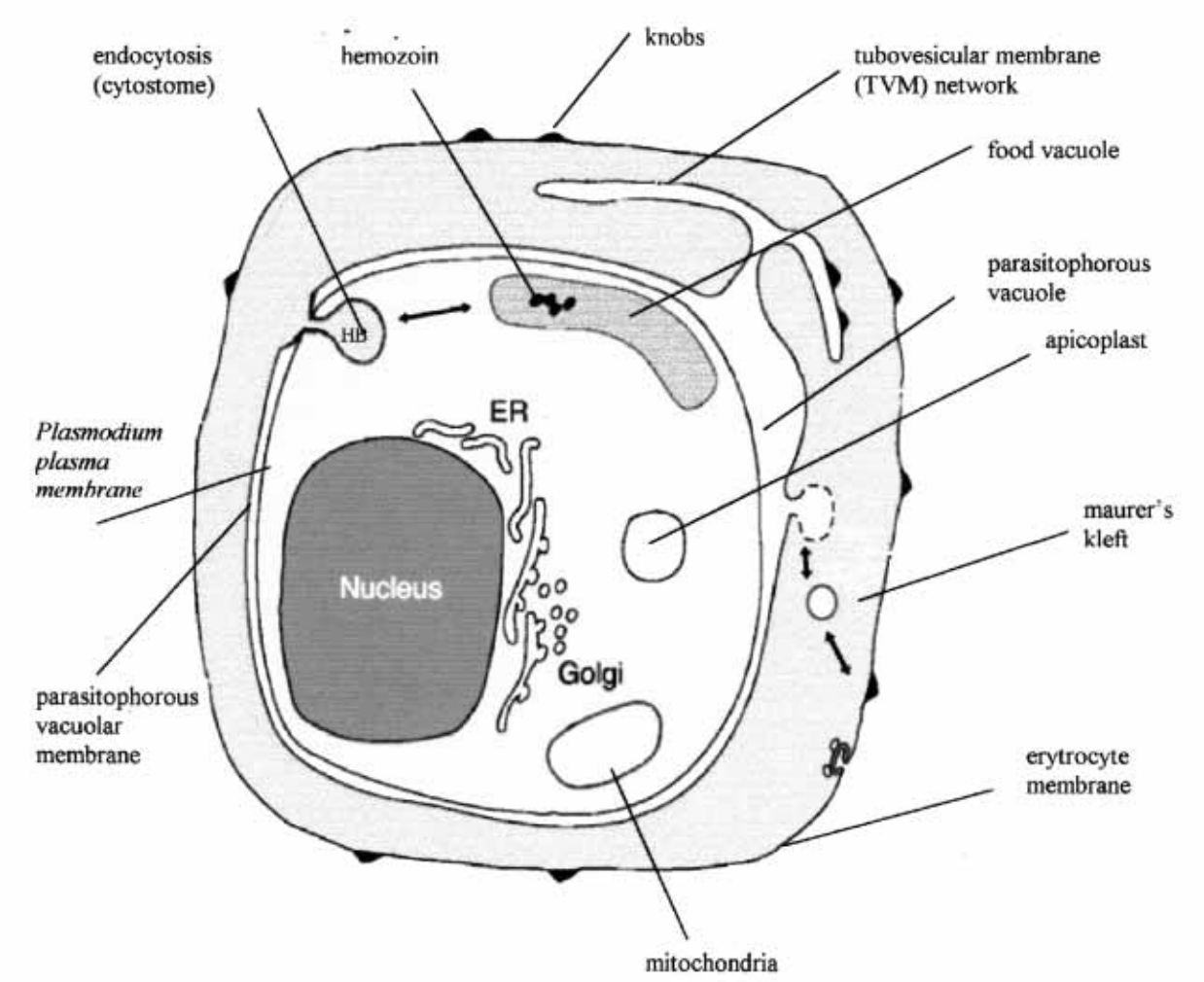


Fig. (2). Schematic overview of a plasmodium inside of an erythrocyte. HB = hemoglobin; ER = endoplasmic reticulum.

nutrients [4]. Others have proposed a direct connection to the host plasma via a "parasitophorous duct", which existence is today submitted to much controversy [10,11]. Protein transport is also responsible for the uptake of some nutrients [7,12].

4. ANTIMALARIAL DRUGS AND TARGETS (FIG. (3))

Traditionally, antimalarial agents are classified as blood schizonticides, tissue schizonticides, gametocides and sporontocides, depending on the stages of the malaria life cycle which are targeted by the drug [13] (Table 2).

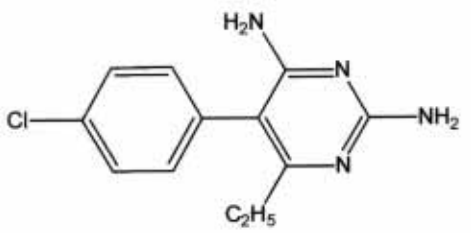
- **Blood schizonticides** are drugs acting on asexual intraerythrocytic stages of malarial parasites. They suppress the proliferation of plasmodia in the erythrocytes.
- **Tissue schizonticides** prevent the development of hepatic schizonts. They are causally prophylactic because they affect the early developmental stages of the protozoa and prevent the invasion of the erythrocytes. An **hypnozoiticide** acts on persistant intrahepatic stages of *P. vivax* and *P. ovale* in the liver.
- **Gametocides** destroy the intraerythrocytic sexual forms (gametes) of the protozoa and then prevent transmission from human to another mosquito. Antimalarials are rarely used clinically just for their gametocidal action.

Table 2. Classification of Antimalarial Agents According to their Stage of Action

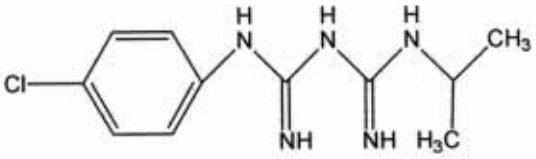
Tissue schizonticides	Primaquine, pyrimethamine, sulfonamides
Hypnozoiticides	Primaquine, tafenoquine
Blood schizonticides	*Type 1, quick onset:* Chloroquine, mefloquine, quinine, halofantrine, artemisinin *Type 2, slow onset :* Pyrimethamine, sulfonamides, sulfones, other antibiotics, atovaquone
Gametocides	Primaquine for Plasmodium falciparum Quinine for *P. vivax, P. malariae, P. ovale*.
Sporontocides	Primaquine, chloroquine.

- **Sporontocides** block the development of oocysts and sporozoites in the anopheles mosquito. They ablatethen the transmission of malaria. Nevertheless, no antimalarial agent is used clinically for this purpose.

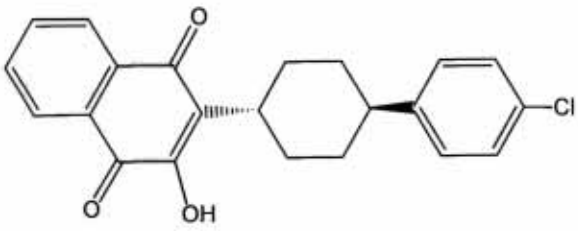
In fact, numerous drugs target several stages of *Plasmodium* life cycle, although the majority of them act on the intra-erythrocytic phases of development.



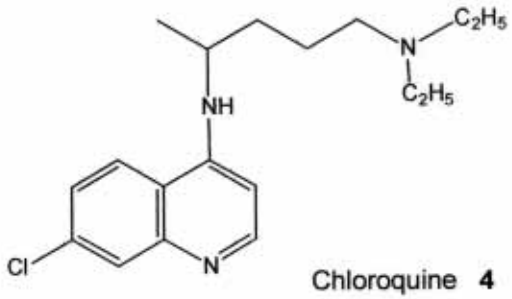
Pyrimethamine **1**



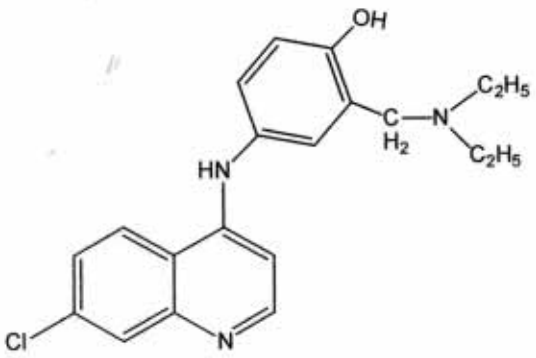
Proguanil **2**



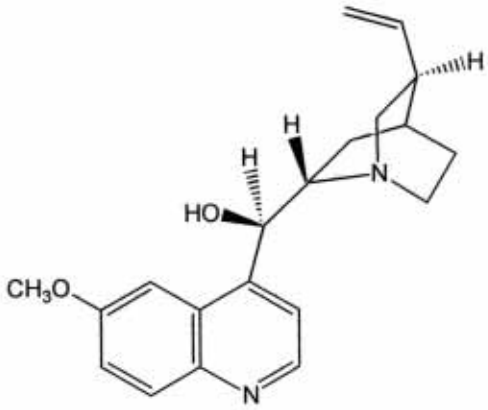
Atovaquone **3**



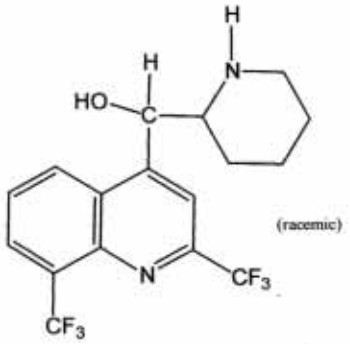
Chloroquine **4**



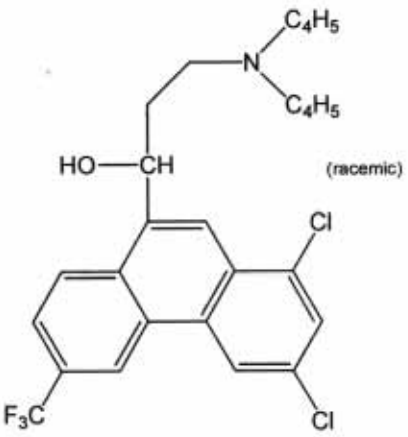
Amodiaquine **5**



Quinine **6**



Mefloquine **7**



Halofantrine **8**

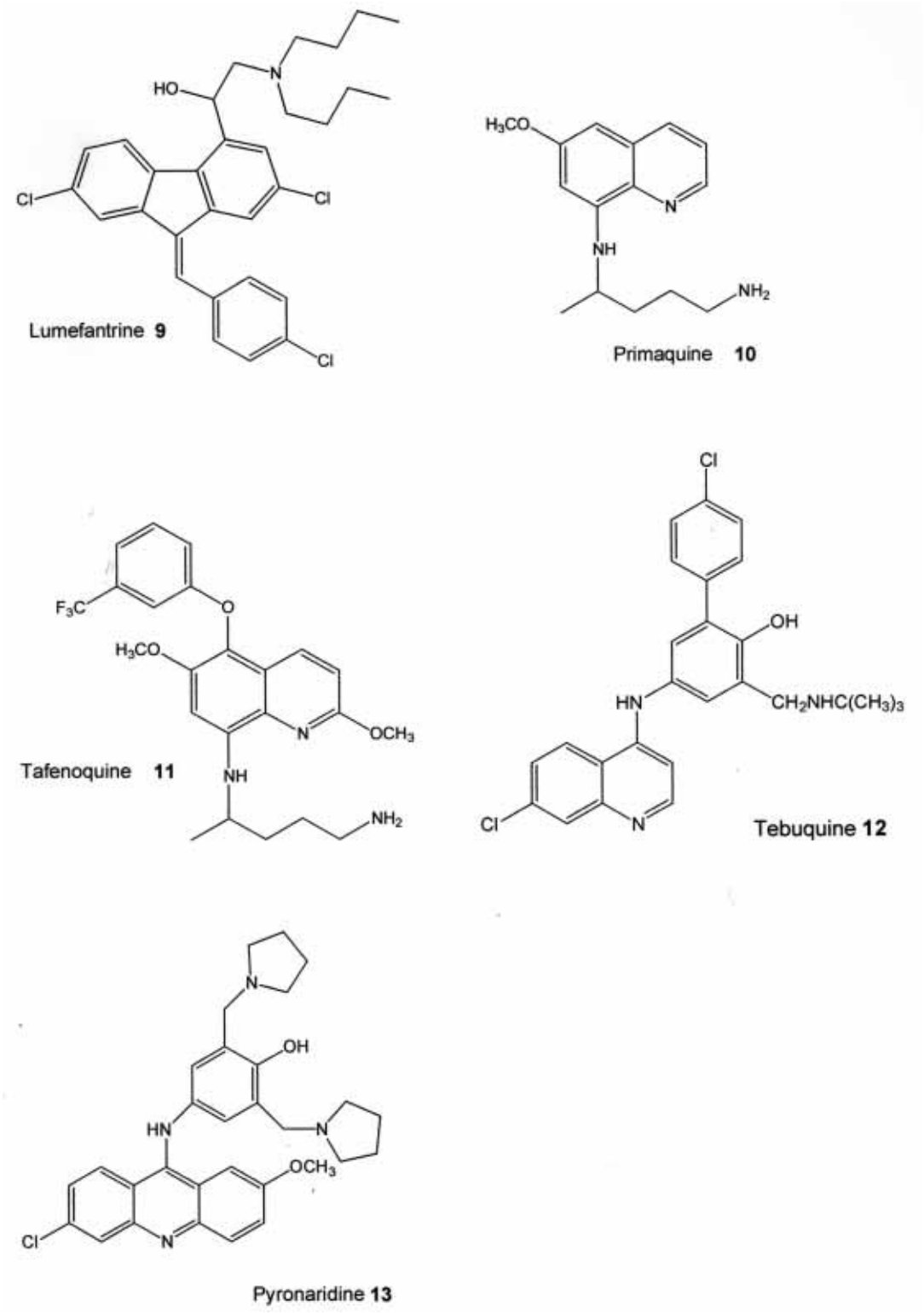


Fig. (3). Chemical structures of current antimalarial agents.

In this review, we will classify antimalarial agents according to their mode of action. Despite many years of study, the precise mode of action of main antimalarials is still not completely understood. Nevertheless, we could reasonably accept four major modes of action for the main actual antimalarial agents: drugs which inhibit the nucleic acid synthesis (i.e. sulfadoxine), drugs which interfere with protein synthesis (i.e. doxycycline), drugs which interfere in the heme detoxification pathway (i.e chloroquine) and the compounds of the artemisinin family, which would act by oxidative stress.

4.1. Nucleic acid Synthesis Inhibitors

The activity of these compounds is exerted at all stages of intraerythrocytic development and also at early stages of gametocyte development.

Folate Antagonists

We find in this group pyrimethamine, **1**, a 2,4-diaminopyrimidine, and proguanil, **2**, (formerly chloroguanide), a biguanide derivative, which inhibit the dihydrofolate reductase (DHFR), causing a depletion of tetrahydrofolate and an inhibition of DNA synthesis [13,14]. As resistance to chloroquine **4** spreaded inexorably from Southeast Asia and South America to almost all regions where malaria is a problem, pyrimethamine, associated to sulfadoxine (SP association) were increasingly used as a first line treatment for falciparum malaria. This has led inevitably to the selection of populations of *P. falciparum* resistant to this drug combination. This resistance is now widespread in South America, East Asia and West Africa [15,16]. Proguanil is a pro-drug that, in order to inhibit parasite DHFR, needs to be converted to cycloguanil by cytochrome P450. Resistance of both *P. falciparum* and *P. vivax* to proguanil developed rapidly too after the drug was introduced in malaria endemic areas[17], and this drug is no longer used alone in treatment or prophylaxis. Nevertheless, associations of proguanil with chloroquine or atovaquone **3** are still of wide use [18]. Resistance to DHFR inhibitors is conferred by simple single mutations of the gene encoding for the enzyme [14].

Sulfonamides

Shortly after their introduction as antibacterials, sulfonamides were found to possess antimalarial properties. This activity has been extensively used during world war II. The sulfonamides (and sulfones) mimic the *p*-aminobenzoic acid (PABA). They prevent the formation of dihydropteroate from hydroxymethyldihydropterin catalysed by dihydropteroate synthase (DHPS) by competing for the active site of DHPS [14]. This result in decreased pyrimidine synthesis, and then DNA, serine and methionine formation. As for pyrimethamine and proguanil, resistance to sulfonamides is today widespread all around the world. The association sulfadoxine – pyrimethamine (SP) is however still in use in some regions of Africa [19]. As for DHFR inhibitors, resistance to sulfonamides (DHPS inhibitors) is caused by single mutations on the corresponding gene [20].

Atovaquone

Atovaquone **3** is an hydroxy naphtoquinone with a cyclohexyl ring at the 2-position. The antimalarial potential of naphtoquinones is known since, in 1936, the plant derived product hydrolapachol (2-hydroxy-1,4-naphtoquinone) was shown to have activity against *Plasmodium lophurae* in ducks. This observation provided the stimulus for the synthesis of hundreds of analogues (2-hydroxy-3-alkyl-1,4-naphtoquinones). Some of these analogues showed high activities in experimental models but required high doses and caused pharmacological problems (poor absorption and rapid metabolism) in patients. Then the interest in naphtoquinones faded until, in the late 1970s and 1980s, a series of hydroxynaphtoquinones was synthesized at the Wellcome laboratories (UK) that overcame the problems of poor oral absorption, rapid metabolism and protein binding associated with previous series. This work resulted in the development of atovaquone for treatment of malaria [21,22]. Single-agent atovaquone regimens resulted in numerous treatment failures with recrudescent isolates exhibiting a marked reduction in the susceptibility to atovaquone in vitro. This has prompted its fixed ratio combination with proguanil (Malarone®). This combination is significantly more effective than either component alone, is effective against strains that are resistant to a variety of other antimalarial drugs and has a favourable safety profile [23]. Recent studies have also shown the effectiveness of this combination in chemoprophylaxis of malaria in chloroquine-resistant area [18]. This combination is highly effective, both in patients with a polymorphism in the gene-encoding cytochrome P 450 2C19 (the main metabolising enzyme) who do not convert proguanil to cycloguanil and against highly cycloguanil resistant parasites. Thus it is deduced that the observed synergy is due to proguanil itself [24]. The synergy is specific to proguanil and atovaquone (not observed with cycloguanil, pyrimethamine, and other mitochondrial electron transfer inhibitors) [14]. Whilst known to act primarily on mitochondrial functions, its mode of action and synergy with proguanil is not completely understood. It is generally agreed that atovaquone acts on the mitochondrial electron transfer chain, although more recently, its activity and synergy with proguanil has been ascribed to its interference with mitochondrial membrane potential [14,25]. Resistance to atovaquone could be conferred by mutations in the cytochrome B gene, resulting in modifications in the coenzyme Q binding site [26].

4.2. Drugs Acting on Heme Detoxification (Quinoline-Containing Drugs)

This class includes the most common antimalarial drugs: the chloroquine-type 4-aminoquinolines (chloroquine, **4**, amodiaquine, **5**, pyronaridine, **13**) and the quinine-type aryl-amino alcohols (quinine, **6**, quinidine, mefloquine, **7**, halofantrine, **8**, lumefantrine, **9**).

Chloroquine Type (4-Aminoquinolines)

Chloroquine (**4**) is the result of an intensive cooperative program of antimalarial research in the United States during World War II. When hostilities ceased, it was discovered that the compound had been synthesized and studied under the name of resochin by the Germans as early as 1934. Chloroquine is one of

the most successful chemotherapeutic agents ever synthesised. This success was based on its safety, affordability and (prior to resistance development) its great efficacy. Amodiaquine (**5**) is a congener of chloroquine, which is essentially equivalent to chloroquine and which is no longer recommended because its use is associated with hepatic toxicity and agranulocytosis. Since 1959, resistance of *P. falciparum* to chloroquine has spread progressively from Colombia and eastern Thailand to envelop most of the tropical world. Few areas are now unaffected: these are central America, Haiti and some areas of the middle east.

Quinine Type (Aryl-Amino-Alcohols, Quinolinemethanols)

The medicinal use of quinine (**6**) dates back over 350 years. It is first in 1633 that an Augustinian monk first noted the use of a powder of *Cinchona* to treat fevers. By 1640, *Cinchona* was used to treat fevers in Europe, imported mainly by the Jesuit fathers, hence the name Jesuit's bark. In 1820, Pelletier and Caventou isolated quinine and cinchonine from the bark and, in 1944, quinine was synthesized. The process is however complex and then *Cinchona* alkaloids are still obtained from natural sources [27]. Quinidine, diastereoisomer of quinine at the secondary alcohol group, is both more potent and more toxic than quinine and is preferentially used as an antiarythmic. Despite over 350 years of use, quinine still retains excellent activity against all species of human malaria parasites. Altough quinine resistance was first reported at the beginning of the 20th century, it has developed slowly, particularly in south America and south east Asia, and there are still no well documented cases of high grade resistance to quinine treatments. So, in south east Asia, quinine is used associated with doxycycline to treat multidrug resistant strains of *P. falciparum*.

In the 1960s, the Walter Reed Army Institute established a malaria research program to develop new compounds to combat chloroquine resistant strains of *P. falciparum*. Mefloquine (**7**) (4-quinoline methanol derivative) and halofantrine (**8**) (phenanthrene methanol derivative) emerged from this program in the 1980s as derivatives of quinine [28,29]. Resistance to mefloquine becomes more and more common, principally in south east Asia and south America.

Lumefantrine (**9**) (formerly benflumetol) is a 2,4,7,9-substituted fluorene that has been synthesized in the 1970s by the Institute of Military Medical Sciences, Beijing and registered for use as an antimalarial drug in China in 1987. The compound is an active blood schizonticide and show synergy with artemether [30]. The association lumefantrine – artemether (co-artemether) is currently registered in a growing number of countries (including Switzerland).

Mode of Action of Chloroquine

Despite years of use and study, the mechanisms of action and resistance of quinoline drugs remains not completely resolved. Chloroquine is thought to be selectively accumulated (at least 1000-fold) in the parasite food vacuole, where digestion of haemoglobin takes place. This accumulation in the food vacuole is generally explained by the weak base properties of chloroquine: at neutral pH, chloroquine could diffuse freely through membranes, and at the acidic pH of the food vacuole, chloroquine is protonated and is trapped inside the food vacuole [31-33]. Nevertheless, the accumulation of chloroquine in the food vacuole requires metabolic energy [34]; it is presumed that this energy is used for the maintenance of the pH gradient, although the binding to an intracellular receptor [35] or the involvement of the plasmodial Na^+/H^+ exchanger [36] were proposed. Several mechanisms have been suggested to explain chloroquine mode of action: inhibition of protein synthesis [37], inhibition of DNA and RNA synthesis [38], inhibition of food vacuole lipase [33], but none of these options is regarded as convincing, because these proposed mechanisms require higher drug concentrations than those that can be reached *in vivo*. A clue to the mechanism of action of chloroquine came from the observation that chloroquine is active only against the blood stages of the *Plasmodium* parasite, and particularly against the stages of the intraerythrocytic cycle during which the parasite is actively degrading haemoglobin. The actual commonly accepted hypothesis is an interaction with the haemoglobin digestion process in the food vacuole of the parasite. Chloroquine would interfere with heme polymerisation process, leading to liberation of toxic heme. However, the presence of free heme does not seem to be fatal for the parasite, as the excess could presumably be disposed by diffusion out of the cell or by degradation by reduced glutathione [39]. The toxic event, then, would be formation of a toxic heme–chloroquine complex (which protect heme from degradation by gluthathione) [40].

It is likely that chloroquine inhibits parasite growth by a number of additive or synergistic effects. Nonetheless, it seems very probable that heme-chloroquine interactions play a very important role in the mechanism of chloroquine action [41].

Mode of Action of Quinolinemethanols

Like chloroquine, the quinolinemethanols act mainly on the intraerythrocytic asexual stages of *Plasmodium*. Recent experiments using proteinase inhibitors show that interaction with heme is central to the activity of quinoline containing antimalarials [42]. However, these compounds are weaker bases than chloroquine, then their uptake must be enhanced by the action of a specific transport system [43] or will be much less efficient than this of chloroquine. Actually, there is conflicting data about which step of heme disposal is affected by quinolinemethanols [41].

Mechanisms of Resistance

Chloroquine-resistant parasites accumulate chloroquine in their acidic food vacuoles much less efficiently than chloroquine-sensitive strains. This suggests that drug resistance results from exclusion of the drug from its site of action [32]. Three mechanisms have been proposed to explain this phenomenon: expulsion (by a plasmodial P-glycoprotein (pgh-1) or by an other protein), alcalinisation of the food vacuole resulting from modifications in H^+ATPase, alterations in the Na^+/H^+-exchanger

which result in decrease in chloroquine uptake [41]. Chloroquine resistance is probably conferred by multiple gene mutations. Initially, chloroquine resistance was attributed to *pfmdr-1* gene (coding for Pgh-1 protein), but the association of *pfmdr-1* and chloroquine resistance remains unsubstantiated. Recently, the determinant of chloroquine resistance would have been located on a 36kb segment of chromosome 7 (*pfcg2* gene) [44]. However, association of *pfcg2* with chloroquine resistance in field isolates is incomplete [45] and a nearby gene (*pfcrt*) is now proposed [46].

Mefloquine, quinine and halofantrine resistance have been associated with the amplification of the *pfmdr1* gene and overexpression of its protein product Pgh-1 [47,48], but it is likely that mefloquine resistance can arise by more than one mechanism. It is not clear how Pgh-1 should modulate resistance to mefloquine, halofantrine and quinine but not to chloroquine (generally, selection for mefloquine resistance is associated with an increase in chloroquine sensitivity and vice versa [47]).

Recently, it has been shown that mutations in Pgh1 confer resistance to mefloquine, quinine and halofantrine, and that they also influence parasite resistance to chloroquine in a strain-specific manner, as well to the artemisinin compounds [49]. This work has shown direct evidence of the involvement of Pgh1 in conferring high levels of chloroquine resistance through decreased chloroquine accumulation. It is nevertheless clear that chloroquine resistance cannot be conferred by Pgh1 alone and requires the presence of mutations in other genes (*pfcg2, pfcrt,...*).

4.3. 8-AMINOQUINOLINES

Primaquine **10** has been developed during World War II among a series of quinoline derivatives, further to the observation (in 1891!) that methylene blue possess weak plasmodicidal properties. Primaquine is normally not used for falciparum malaria, although it is more and more suggested for *P. vivax* and *P. falciparum* prophylaxy (while it might induce a rise of *P.vivax* resistance to this drug) [50,51]. Hypnozoites (persistent hepatic stages) of *P.vivax* and *P.ovale* are not eradicated by any of the blood schizonticides, and late relapse could occur. Primaquine is the only available drug for this purpose, although a derivative, tafenoquine **11** is being developed [52]. Primaquine is thought to be converted in the liver to an active quinone metabolite. It has been suggested that primaquine exerts its activity by interfering with mitochondrial function [53,54].

4.4. Protein Synthesis Inhibitors (Antibiotics)

The use of antibiotics in the treatment of malaria is old. However, renewed interest in this drug class emerged with the appearance of chloroquine- and multidrug-resistant strains of *P. falciparum*. Tetracyclines (usually doxycycline or tetracycline) inhibit parasite protein synthesis in the mitochondria and/or in the apicoplast [55]. Their relative slowness of action makes concurrent treatment with quinine mandatory for rapid control of parasitemia. Doxycycline is also used in prophylaxy, particularly in areas where resistance to mefloquine is usual, as in Thailand and Cambodia. Clindamycin and azithromycin could also be used in association with fast-acting antimalarials (quinine, artemisinin) to treat malaria. Azithromycin could be used as a prophylactic agent [56,57].

4.5. Oxidative Stress (Artemisinin Derivatives)

The commonly accepted hypothesis for the artemisinin derivatives mode of action is the generation of an oxidative stress. These compounds will be developed more particularly below.

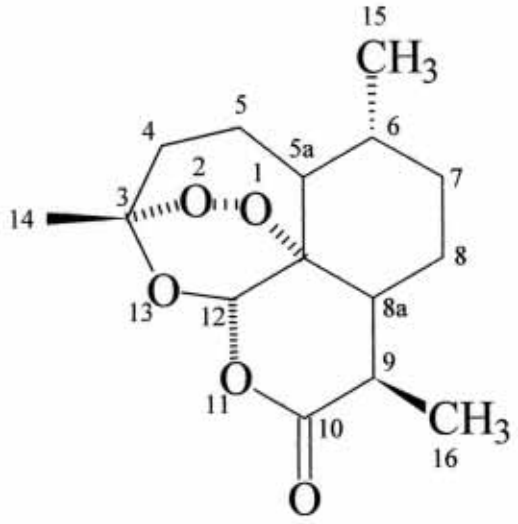
4.6. New Quinoline Antimalarials

An extensive review of prospects for novel quinoline antimalarials has been conducted by Foley and Tilley [41]. Among these, we could name tebuquine **12** and pyronaridine **13**, amodiaquine related compounds [35,58]. Pyronaridine has been used clinically in China since the 1970s and is actually marketed in this country.

5. ARTEMISININ DERIVATIVES

5.1. Introduction

Artemisia annua L. (*Asteraceae*) is an annual herb native to China and actually growing in many regions such as Australia, centre and north Europa and United States. The plant has been used in traditional Chinese medicine as a remedy for chills and fevers for more than 2000 years (a mention was found in the grave of the *Mawangdui Han* dynasty, constructed 2000 years ago). In the middle of the 4th century, its efficacy for malaria treatment



(14)

Fig. (4). Chemical structure of artemisinin.

was mentioned in *Zhouhou Bei Ji Fang* (Handbook of emergency medicine) [59]. Artemisinin **14** (formerly qinghaosu, arteannuin) is a sesquiterpene trioxane lactone containing a peroxide bridge and has been isolated by Chinese researchers in 1972 [Fig. **(4)**]. Its structure has been elucidated in 1979 by X-ray analysis [60] and its total synthesis has been realised in 1983 [61]. The main suppliers of artemisinin are however China and Vietnam where *A. annua* is cultivated in plantations.

Artemisinin posses very little solubility in water and lipids. That's why Chinese researchers tried to modify its structure [Fig. (**5**)]. The lactone can easily be reduced (with sodium borohydride), resulting in the formation of dihydroartemisinin (**15**) (artenimol), which is even more active *in vitro* than artemisinin itself. A series of derivatives of this lactol where then synthesized. These derivatives are also generally more potent than artemisinin itself *in vitro* and *in vivo* with animal studies. Artemether (**16**) and arteether (**17**) (artemotil) are the methyl and ethyl ether of dihydroartemisinin (*b* epimer), respectively.

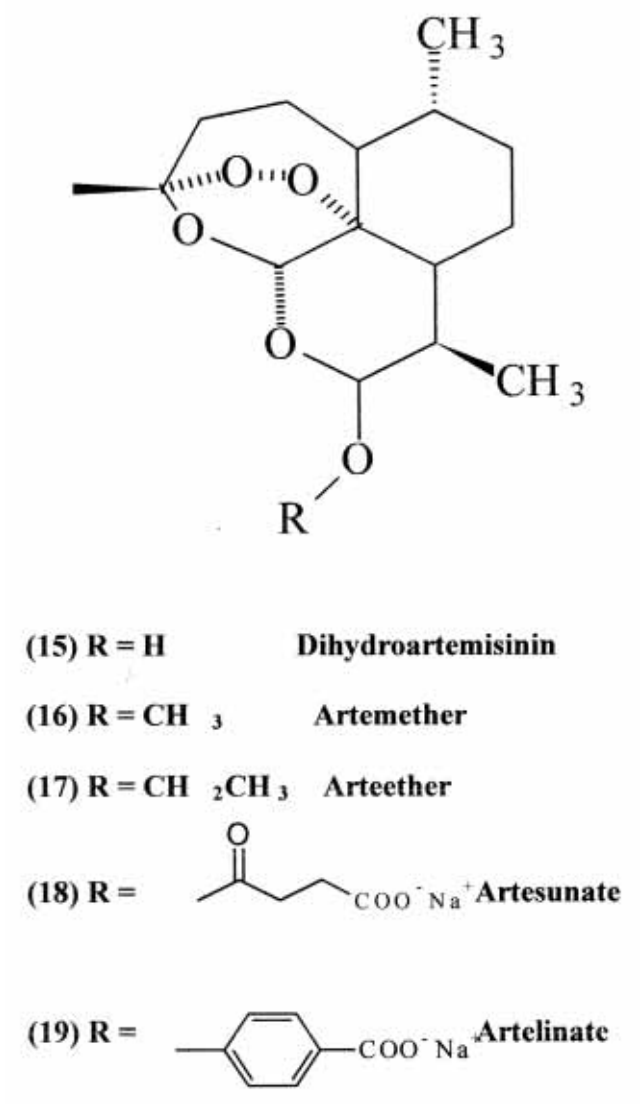


Fig. (5). Chemical structures of dihydroartemisinin, artemether, arteether, artesunate and artelinate.

Sodium artesunate (**18**) and sodium artelinate (**19**) are the dihydroartemisinin-*b*-O-hemisuccinate sodium salt and the dihydroartemisinin-*b*-O-*p*-carboxybenzyl ether sodium salt, respectively [62]. Artesunate and artelinate are formulated as sodium salts in salines for intravenous or intramuscular administration. Arteether, artemether and dihydroartemisinin are currently administered intramuscularly in various oil formulations, though cremophore formulations have been used experimentally for intravenous administration [63]. Artemether is the most widely used derivative and was, current 2000, registered in 27 countries as oral tablets or oily solution for IM injection (Paluther®, Artenam®, Artemos®). Sodium artesunate was registered in six countries current 2000 as tablets, IM or IV injections (Arsumax®). Artemisinin and dihydroartemisinin are essentially used in China and Vietnam as tablets or suppositories [64]. Dihydroartemisinin appears to be the principal metabolite and the active form of all the artemisinin derivatives, with varying degrees of conversion [65].

Artemisinin and its derivatives are fast-acting drugs and should be administered preferably in combination with another "long-acting" effective antimalarial drug in order to reduce recrudescences and to prevent or slow the development of resistances. At present, the "drug of choice" for combined therapy is mefloquine, but recently, the association artemether – lumefantrine (co-artemether, Coartem®, Riamet®) has been registered in numerous countries and has been shown to be as active and less toxic than the mefloquine – artesunate association, the current standard treatment in the multidrug-resistant *P. falciparum* malaria area [66,67].

5.2. Chemical Structures and Synthesis of Artemisinin and its Main Derivatives

From a chemical point of view, artemisinin is an unstable compound characterized by a peroxide bridge (responsible for its antimalarial properties) incorporated in a sesquiterpene lactone structure [64]. Artemisinin is commonly extracted and purified from *Artemisia annua*. Because of the limited availability of artemisinin, different synthetic preparations have been reported [68-71]. Nevertheless, these syntheses are complex, uneconomical and give low yields. The other alternative of complex synthetic reactions is the hemi-synthetic preparation of artemisinin from artemisinic acid, a closely related biosynthetic precursor. It is the most abundant sesquiterpene in *Artemisia annua* which occurs at concentrations as much as 10-fold higher than artemisinin [72,73]. Recently, Vonwiller *et al*. reported an extraction method which makes possible the extraction of both compounds from the same plant material, thus increasing the final production of artemisinin. [74]. Indeed, artemisinin can be easily obtained from artemisinic acid. For example, in 1991, Roth and Acton proposed a conversion of artemisinic acid to artemisinin with a 20% yield [75]. In this synthetic pathway, artemisinic acid is converted into artemisinin in 2 steps. In the first step, artemisinic acid is reduced with $NaBH_4$ into dihydroartemisinic acid. The second step consists in a photooxidation of dihydroartemisinic acid to generate artemisinin. Thus, oxygen is passed through a –78°C CH_2Cl_2 solution of dihydroartemisinic acid containing methylene blue while irradiating with a street lamp. After completion of the photooxidation, CH_2Cl_2 is replaced with petroleum ether and the mixture left at room temperature for four days. Removal of

solvent followed by triturating with petroleum ether affords crystalline artemisinin which is recrystallized from cyclohexane to give material identical in all respects with artemisinin isolated directly from A. annua. Dihydroartemisinin (mixture of epimers) is then obtained by borohydride reduction of artemisinin. The mixture is treated with methanol or ethanol in the presence of Lewis acid (and separation of epimers) to give artemether or arteether [Fig. (**6**)].

The most commonly used artemisinin derivatives are dihydroartemisinin, artemether, arteether, artesunate and artelinate (Table 3).

Artemether and arteether are the methyl and the ethyl ether derivatives of dihydroartemisinin (the β-epimer). Their preparation from artemisinin is realized as follow : artemisinin is reduced with sodium borohydride to produce dihydroartemisinin as a mixture of epimers. The mixture is treated with methanol and an acid catalyst to provide artemether [76] or etherified with ethanol in the presence of Lewis acid to provide arteether. The β-epimers are then chromatographically separated from their slower moving α-epimers [77].

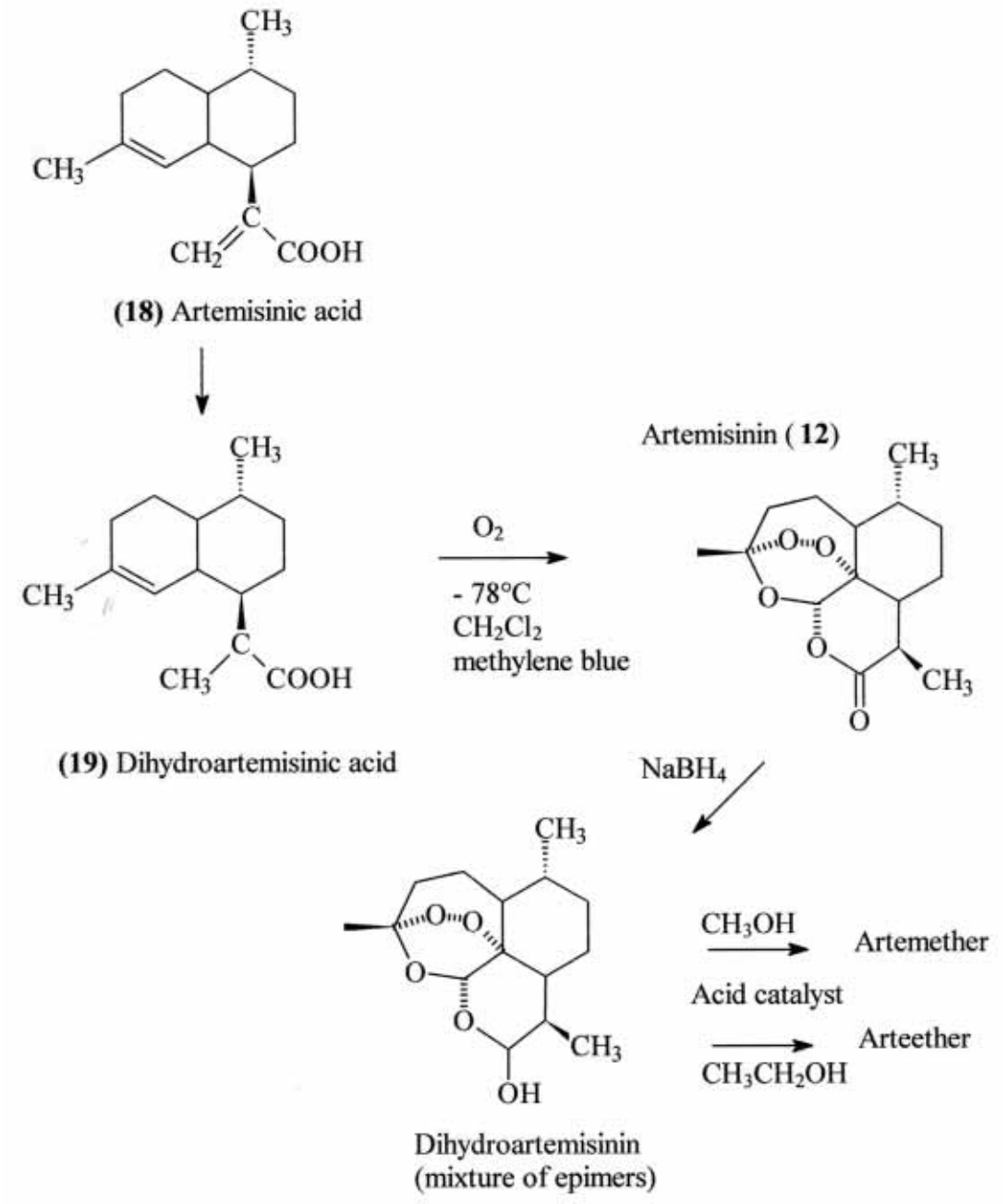


Fig. (6). Hemi-synthesis of artemisinic and its derivatives dihydroartemisinin, artemether and arteether from artemisinic acid. Artemisinic acid is converted into artemisinin in two steps via reduction of the exocyclic methylene group and photooxidation of the resulting dihydroartemisinic acid as described by Roth and Actron.

Table 3. Description of Artemisinin Derivatives

Artemisinin derivatives	Description
Dihydroartemisinin (15)	Active metabolite of artemisinin and all currently used derivatives.
Artemether (16)	Liposoluble methyl ether derivative of dihydroartemisinin (the β-epimer).
Arteether (17)	Liposoluble ethyl ether derivative of dihydroartemisinin (the β-epimer).
Artesunate (18)	Hydrozoluble hemisuccinate ester salt of dihydroartemisinin (the β-epimer).
Artelinate (19)	Hydrosoluble benzylcarboxylate ester salt of dihydroartemisinin (the β-epimer).

5.3. Mechanism of Action

The mode of action of artemisinin based compounds, in spite of intense scientific activity, is not yet completely understood. Artemisinin derivatives are toxic to malarial parasite at nanomolar concentrations and their peroxide bridge is essential for the expression of antimalarial activity. They cause structural changes in the erythrocyte stage of parasite by affecting the membranes surrounding the food vacuole, the nucleus, the mitochondria, endoplasmic reticulum and nucleoplasm. These changes lead to the total disorganization of the parasites [78]. For the first time, in 1991, the reaction between heme and artemisinin has been pointed out [79]. Following these statement, the generation of free radicals from artemisinin by an heme-iron mediated mechanism has been evidenced by cyclic voltammetry [80] and by electron paramagnetic resonance spectroscopy [81]. The importance of oxidative stress to explain artemisinin mechanism of action has then been substantiated by observations of synergism with oxidant drugs and antagonism with agents that lower oxidative stress [82]. However, peroxide-bridge compounds do not usually induce oxidative stress, an "activation" step is then necessary. The first step, the "cleavage" of the peroxide bond, occurs probably in the presence of the haeminic iron. It gives rise to the formation of an oxygen-centred and then a carbon-centred radical [83], followed by an iron-oxo reactive intermediate and finally an epoxide, which is a highly active alkylating agent (C-centred radical theory) [81-86] [Fig. (**7**)]. This iron-oxo intermediate has recently been observed by spectroscopic methods [87]. There is then alkylation of (specific?) antimalarial proteins which lead to the death of the parasite [84]. The fact that the activity of artemisinin appears to be depressed by iron chelators [81] suggests that non-heminic iron (II) is required for bioactivation of artemisinin, because the iron chelators would be unable to sequester iron from the ferrous heme. Although there is disagreements over details of the mode of action [88,89], the overall mechanism involving iron-catalysed generation of free radicals has actually world-wide support.

5.4. Mechanisms of Resistance

Although *P. falciparum* isolates may vary two- to fourfold in their *in vitro* sensitivity to artemisinin [90,91] and although

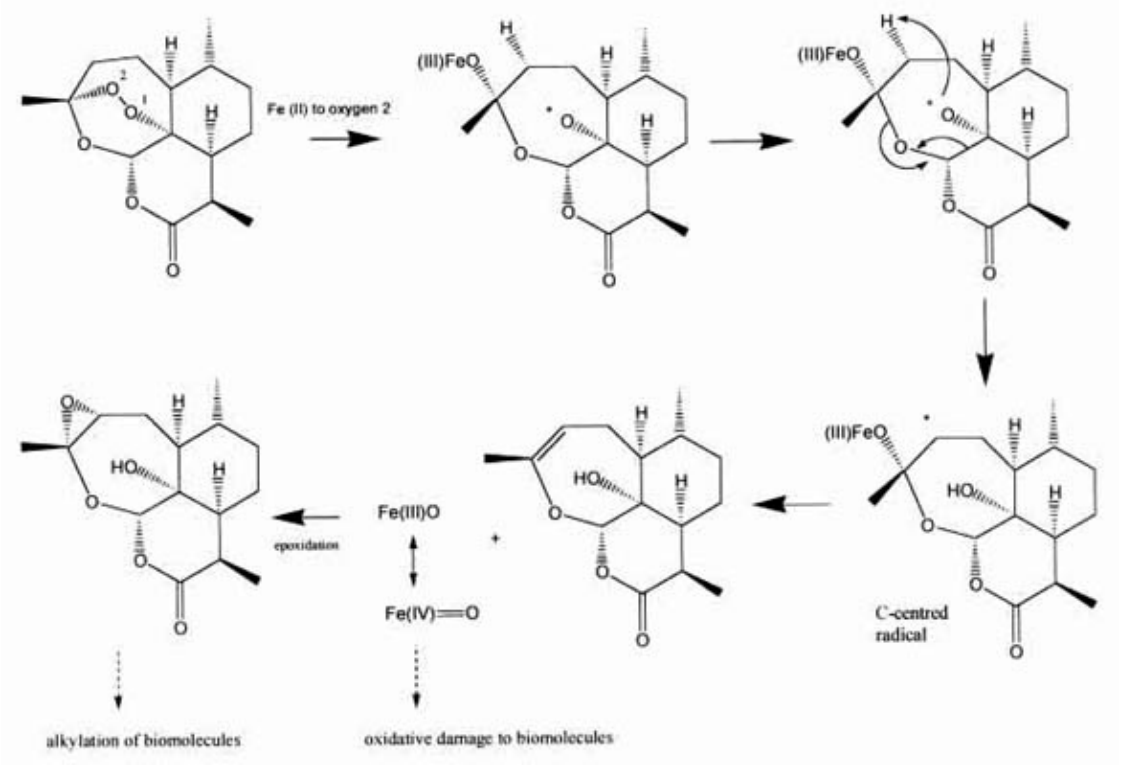


Fig. (7). Proposed molecular mechanism of action of artemisinin (carbon centred radicals theory). Fe (II) is either iron (II) in ferrous haem, or exogenous iron (II).

small decreases in sensitivity can be induced *in vitro*, true stable resistance to this group of compounds has not yet been described, either in the laboratory or in clinical practice [1]. Resistance against artemisinin could be developed to a moderate level in mouse in both *P. berghei* or *P. yoelii* parasites but resistant parasites readily lost resistance once drug-selection pressure is withdrawn [92]. However, since the drugs are being widely used, artemisinin resistance is likely to occur in the near future.

Recently, it has been shown that mutations in Pgh-1 (*pfmdr* gene) can influence the level of sensibility of a strain to artemisinin [49]. The effect of *pfmdr-1* on artemisinin susceptibility seems to be comparable to its effect on mefloquine and halofantrine. This could be explained by a common mechanism whereby *pfmdr-1* can influence the accumulation of mefloquine, halofantrine and artemisinin [49]. This hypothesis is consistent with the field studies which show a positive correlation between the IC_{50} for artemisinin and that of mefloquine and halofantrine [93]. Artemisinin resistant *P. yoelii* strain seems also to accumulate less artemisinin than a sensitive strain. The phenotype of artemisinin resistance appears actually to be multifactorial [94].

5.5. Artemisinin Derivatives: Current Developments

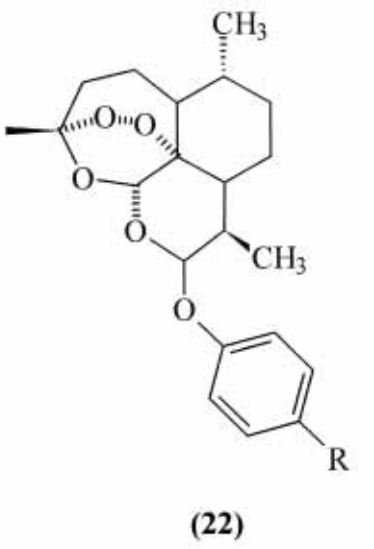
5.5.1. New Artemisinin Derivatives

Early efforts at improved artemisinin analogues preserved the entire artemisinin skeleton. A variety of new artemisinin derivatives have been developed and tested to identify new chemical entities that would possess high antimalarial activity associated with a good chemical and metabolic stability and low toxicity. In this chapter, we will describe some of the most promising novel compounds characterized by a good antimalarial activity.

Hemi-Synthetic Artemisinin Derivatives

Reduction of artemisinin to dihydroartemisinin has led to the preparation of a series of hemi-synthetic first-generation analogues which include artemether and arteether described above. However, poor availability and rapid clearances were observed with these compounds. This is principally due to the poor chemical and metabolic stability of their C-10 acetal function. Indeed, one of the principal routes of metabolism of these derivatives involves oxidative dealkylation to dihydroartemisinin which is associated with toxicity and short half-life. Thereby, first efforts to prepare chemically more robust hemi-synthetic artemisinin derivatives have involved replacing the C-10 acetal functionality by less hydrolytically functional groups.

Incorporation of a phenyl group in place of the alkyl groups (methyl, ethyl) of artemether and arteether was predicted to block oxidative dealkylation to form dihydroartemisinin [95,96]. Consequently, a series of new C-10 phenoxy derivatives of dihydroartemisinin (**22**) were investigated [Fig. (**8**)]. All of the new compounds showed potent *in vitro* antimalarial activity with IC_{50} values < 10 nM (Table 4). Compound **22F** emerged as the most interesting drug. Indeed, in contrast to **22B** which underwent unexpected P450 catalyzed dephenylation (to form dihydroartemisinin), **22F** was stable to metabolic dearylation. Moreover, **22F** was shown to be orally active as antimalarial agent with a level of activity superior to artesunate [97,98].



(22)

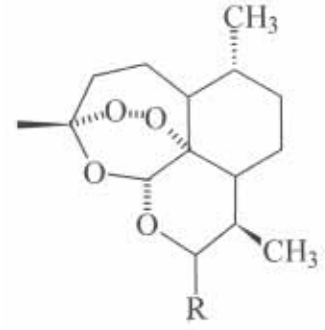
Fig. (8). Chemical structure of C-10 phenoxy dihydroartemisinin derivatives.

Table 4. ***In Vitro*** **Antimalarial Activity Against Chloroquine-Sensitive P. Falciparum (HB3) and Chloroquine-Resistant P. Falciparum (K1) of C-10 Phenoxy Dihydroartemisinin Derivatives**

Compound	R	IC_{50}(nM)	
		HB3	K1
Artemisinin	-	9.7	11
Artemether	-	3.4	4.6
22A (α-epimer)	H	2.6	3.0
22B (β-epimer)	H	3.2	3.7
22C (α -epimer)	CH_3	2.9	3.2
22D (β -epimer)	CH_3	3.2	3.9
22E (α -epimer)	CF_3	3.4	4.6
22F (β -epimer)	CF_3	3.9	5.3
22G (α -epimer)	OCH_3	3.3	3.9
22H (β -epimer)	OCH_3	NA	4.6
22I (α -epimer)	F	4.0	5.7
22J (β -epimer)	F	2.9	4.6

Replacement of the oxygen at the C-10 position of artemether or arteether with a carbon is an attractive strategy since this approach would produce more hydrolytically stable compounds with a longer half-life and potentially lower toxicity. Indeed, Jung *et al*. have shown that several deoxoartemisinin derivatives are

more resistant to hydrolyse in simulated mixtures of stomach acid than the dihydroartemisinin compounds currently in use [99]. Thereby, different 10-substituted deoxoartemisinin derivatives have also been developed and it remains an active area of research [100-103]. For example, Ma and collaborators recently reported the synthesis and the antimalarial activities of a series of 10-substituted deoxoartemisinin compounds (**23**) of which **23a** and **23b** were 5-7 times more active than artemisinin against Indochina *P. falciparum* clones (W-2, chloroquine-resistant and mefloquine-sensitive) and Sierra Leone *P. falciparum* clones (D-6, chloroquine-sensitive and mefloquine-resistant) while 10-(*n-propyl*)deoxoartemisinin **23c** was only 2-3 times more active [Fig. (**9**)] [104]. Electron-rich aromatics and heteroaromatics can also be introduced at the C-10 position of deoxoartemisinin [105]. For example, the hemisynthetic analogue **23d** is hydrolytically stable and retains an IC_{50} value of 1.4 nM against chloroquine sensitive *P. falciparum* (IC_{50} of 9.9 nM for artemisinin). It is orally active and it is more potent than artemisinin [97,105].



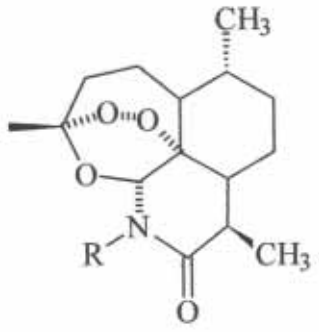
(21)

(21a) R = $CH_2CHOHCH_2CH_3$
(21b) R = $CH_2CHOHC(CH_3)_3$
(21c) R= $CH_2CH_2CH_3$
(21d) R = 2-furanyl

Fig. (9). Chemical structures of some 10-substituted deoxoartemisinin compounds.

The O-11 position of artemisinin is another reactive site at which chemical modifications can be performed to improve antimalarial activity and pharmacokinetic profile. Different 11-N substituted artemisinin lactams (or 11-azaartemisinin) (**24**) have been synthesized [Fig. (**10**)]. Indeed, the greater stability of lactams to acidic conditions should reduce the destruction of the drug that occurs in the stomach and therefore lead to an increase in the drug's bioavailability.

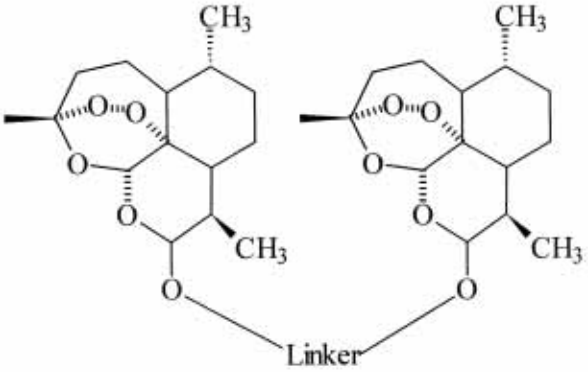
Torok and Ziffer described in 1995 a chemical method of conversion of artemisinin into 11-azaartemisinin (**24a**) in two step reaction sequence between artemisinin and methanolic ammonia followed by treatment with Amberlyst with a yield of 65%. The authors also described the synthesis and



(24)

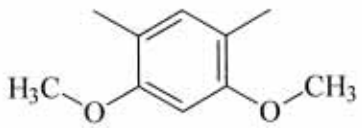
(24a) R = H
(24b) R = CH_2CHO

Fig. (10). Chemical structure of 11-azaartemisinin and one N-substituted 11-azaartemisinin.



(23)

(23a)



(23b)

Fig. (11). Chemical structures of dimeric trioxanes.

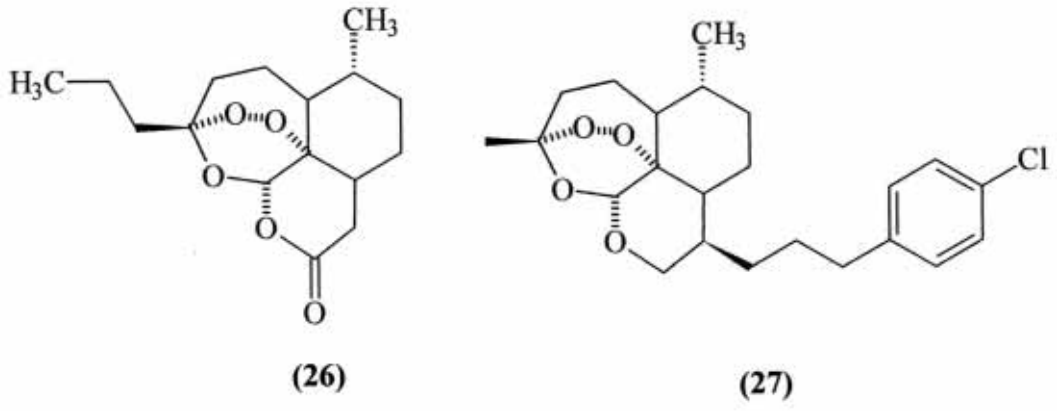


Fig. (12). Chemical structures of two synthetic artemisinin derivatives.

the antimalarial properties of a series of N-substituted azaartemisinin derivatives. The most active derivative obtained was **24b** which was much more active (26-times) than artemisinin *in vitro* (against chloroquine-resistant *P. falciparum* FCR3). **24b** was also at least 4-times more active than artemisinin *in vivo* in reducing the number of dead mice after a 60 days infection by *P. berghei* [106].

With the aim of preparing more chemically stable hemi-synthetic derivatives of artemisinin, several dimeric trioxanes with a carbon linkage between the two G10 positions (**25**) were synthesized [Fig. (**11**)]. Dimers such as **25a** and **25b** showed an excellent antimalarial activity *in vitro* (IC_{50}: 1.9 nM and 1.3 nM, respectively) against cultured chloroquine-sensitive *P. falciparum* (NF54) [107].

Synthetic Artemisinin Derivatives [Fig. (**12**)].

Avery *et al*. described the complete synthesis of 3-substituted and 9-substituted artemisinin derivatives (and both in the same derivatives). The replacement of the 3-methyl group with an n-propyl moiety and the absence of a methyl group at position 9, as in **26**, produced 7-fold and 21-fold increases in activity with respect to artemisinin against Indochina (W-2) and Sierra Leone (D-6) *P. falciparum* clones, respectively. Moreover, Avery also demonstrated that C-9 substitution led to compounds that exhibited excellent *in vitro* antimalarial activities. Thus, the 10-deoxoartemisinin derivative **27** was shown 70-fold more active than artemisinin *in vitro* [108].

5.5.2. Other Peroxidic Antimalarial Agents

A number of novel chemical structures including synthetic bicyclic peroxides, spiro-1,2,4,5-tetraoxacycloalkanes, 1,2,4-trioxanes and trioxaquines characterized by at least one peroxide moiety have been synthesized as new antimalarial agents.

Endoperoxide Derivatives [Fig. (**13**)]

Yingzhaosu A (**28**) was isolated from a herbal extract used in China (Chinese plant *Artabotrys uncinatus*) as a folk remedy against malaria and was subsequently obtained by total synthesis. This discovery has stimulated the development of new strategies for the synthesis of cyclic peroxides and the investigation of the chemical transformations and properties of such compounds. The sesquiterpene peroxide Ro 42-1611 (Arteflene) (**29**) was the first synthetic derivative of yingzhaosu A to be developed [109-111]. It was evaluated extensively against various drug-sensitive and drug-resistant lines of *P. falciparum in vitro* and *P. berghei in vivo* in mice. Experimentally arteflene proved to be a highly effective antimalarial drug. *In vivo* it is active at low doses against blood stages of *P. berghei* in mice after oral or parenteral administration. It has a rapid onset of drug action and a long lasting suppressive effect when given after infection, as well as a good potential for prophylactic activity when given before infection. The suppressive and prophylactic properties are comparable to chloroquine and superior to artemisinin, artemether. *In vitro* the compound showed no signs of cross-resistance with existing antimalarials. It was consistently rather more active against drug-resistant than against drug-sensitive strains of *P. falciparum*. Drug interaction studies *in vitro* and *in vivo* with chloroquine, mefloquine and quinine revealed an additive to synergistic effect with arteflene. Compared with standard antimalarials the activity of arteflene *in vitro* is lower than would be expected from the *in vivo* results [109]. Two clinical trials were reported with this antimalarial agent. It was first evaluated for safety and efficacy in an open, non-comparative study of patients with mild malaria in the south of Cameroon. Thirty male patients aged 12 to 42 years, with an initial *P. falciparum* count of > 5000 parasites/microliters and a body temperature of 37.7% to 39.8 degrees C, were selected to receive a single dose of arteflene, corresponding to 25 +/- 2.5 mg/kg bodyweight. Mean body temperature was reduced from 38.9 degrees C at baseline to 37.3 degrees C 12 hours after arteflene administration, and by this time 80% of patients had a normal temperature. Clinical cure rates were also high, with 70% of patients free of all signs and symptoms after 24 hours. However, by day 7, 6/30 (20%) presented with smears positive for *P. falciparum*. There were no adverse events considered to be related to treatment [112]. Nevertheless, in a second clinical trial (phase III), single dose monotherapy with arteflene was not effective in curing children suffering from uncomplicated *P. falciparum* malaria in Gabon, while mefloquine proved to be highly effective for this purpose [113].

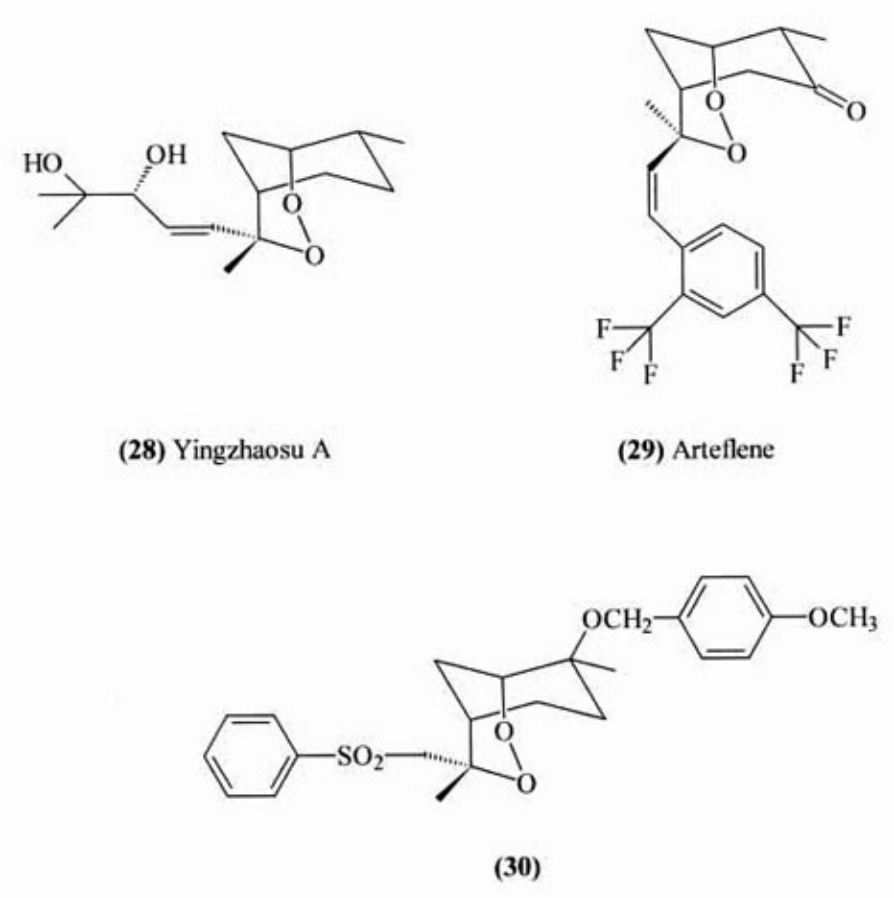


Fig. (13). Chemical structures of endoperoxide derivatives.

Based on the bicyclic peroxide yingzhaosu and arteflene, structurally related endoperoxide **30** was designed and synthesized by a short and efficient method from R-(+)-limonene. **30** was found to exhibit an *in vitro* antimalarial activity comparable to that of artemisinin and superior to that of arteflene [114,115]. *In vivo* oral experiments are needed to assess the viability of this class of endoperoxides.

Spiro-1,2,4,5-Tetraoxacycloalkane Derivatives [Fig. (**14**)]

The promising antimalarial activity of artemisinin prompted the evaluation of dispiro-1,2,4,5-tetraoxanes such as WR148999 (**31**). Indeed, **31** possesses antimalarial activity comparable to artemisinin, but unfortunately, it also shares the characteristics of being hydrolytically unstable and poorly available by oral administration [116,117]. Like the antimalarial WR148999, spiro-1,2,4,5-tetraoxacycloalkanes characterized by two geminal peroxide units in the same ring appeared as a new chemical class of antimalarial agents. one of the new compounds, **32** emerged with an *in vitro* activity against chloroquine-sensitive *P. falciparum* (FCR-3) 2 times superior to that of artemisinin [118,119]. Unfortunatly, no *in vivo* activity has been reported to date.

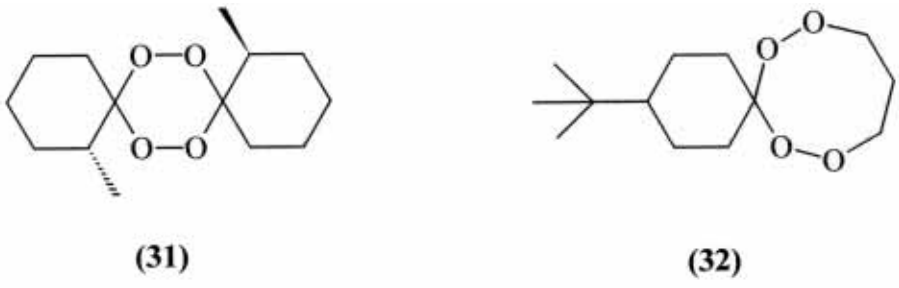


Fig. (14). Chemical structures of spiro-1,2,4,5-tetraoxacycloalkanes.

Synthetic 1,2,4-Trioxane Derivatives [Fig. (**15**)]

A series of structurally simple 3-aryl-1,2,4-trioxanes **33** featuring the essential tricyclic unit (pharmacophore) [120] of tetracyclic artemisinin were synthesized and evaluated for antimalarial activity [97]. Compound **33a** was 2.5 times less active than artemisinin *in vitro* against chloroquine-sensitive *P. falciparum* (NF54) but was 2 times more potent *in vivo* when administrated to rodents [121]. Nevertheless, **33a** appeared to be poorly soluble in water. Thereby, relatively polar sulfone **33b** has been synthesized and reported to be a potent antimalarial agent *in vitro* [122].

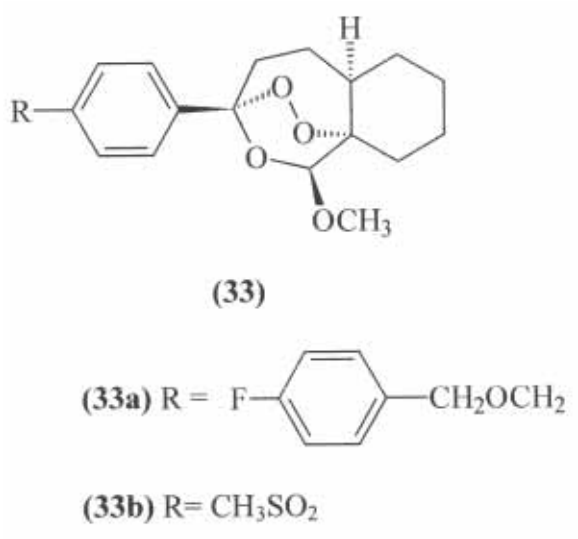


Fig. (15). Chemical structures of 1,2,4-trioxane derivatives.

Trioxaquine Derivatives [Fig. (**16**)]

Combination treatments of fast-acting artemisinin-type drugs and quinoline antimalarial agents have produced encouraging results [123]. Thereby, a combination therapy within a single molecule has been proposed. The hybrid trioxaquine **34** that combine a fenozan-type peroxide and a 4-aminoquinoline in a single molecule has been developed with the aim of overcoming drug resistance. This strategy is encouraging since **34** exhibits a potent *in vitro* antimalarial activity against chloroquine-sensitive *P. falciparum* (Nigerian), chloroquine-resistant *P. falciparum* (FcB1) and highly chloroquine-resistant *P. falciparum* (FcM29) [124]. No *in vivo* data for these compounds are currently available.

6. NEW DRUG TARGETS

Increase in resistance of Plasmodium falciparum to conventional treatments is a world-wide problem, and few alternative drugs are under development, necessitating urgent efforts to identify new classes of antimalarial drugs. Paradoxically, the actual problem is, more and more since 20 years, the disengagement of pharmaceutical companies in the search for new antimalarial agents. Actually, the major part of efforts are conducted outside the industry [125].

Among actual principal ways of research, the development of new artemisinin derivatives will be described below. We could however name some other ways which seems to be promising.

Reversal of Chloroquine Resistance

Research into chloroquine resistance in *Plasmodium falciparum* has revealed a widespread range of functionally and structurally diverse chloroquine resistance reversors (i.e. verapamil **35** and desipramine) [126,127]. However, nearly all of these chemosensitizers reverse resistance optimally only at concentrations that are toxic to humans. Very recently, however, another resistance modulator, the antihistaminic chlorpheniramine **36**, associated to chloroquine has been shown to be equivalent to sulfadoxine-pyrimethamine for the treatment of acute, uncomplicated *P. falciparum* malaria in children [128]. An other option would be the use of combinations of chemosensitizers at concentrations not toxic to humans, which could effectively reverse chloroquine resistance without the marked toxicity from the use of a single agent at high concentrations [129]. Moreover, some natural monoindolic alkaloids as malagashanine **37** from the Malagashanian plant *Strychnos myrtoïdes* and icajine **38** from the African plant *Strychnos icaja* are able to reverse resistance

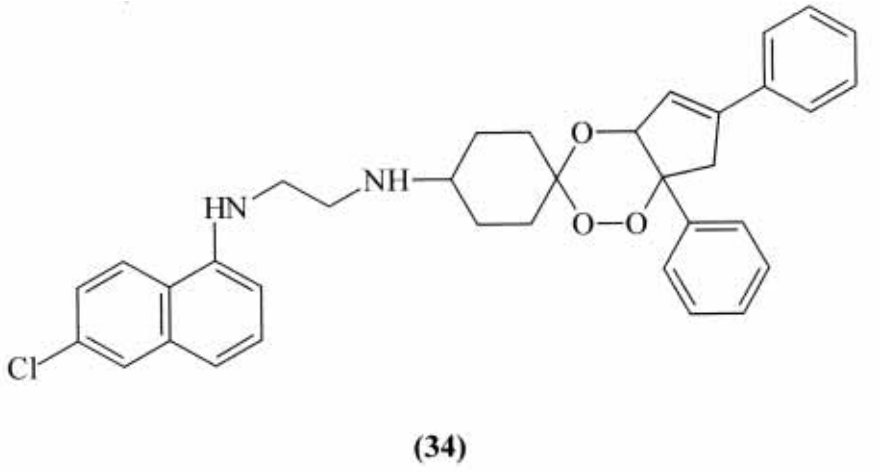
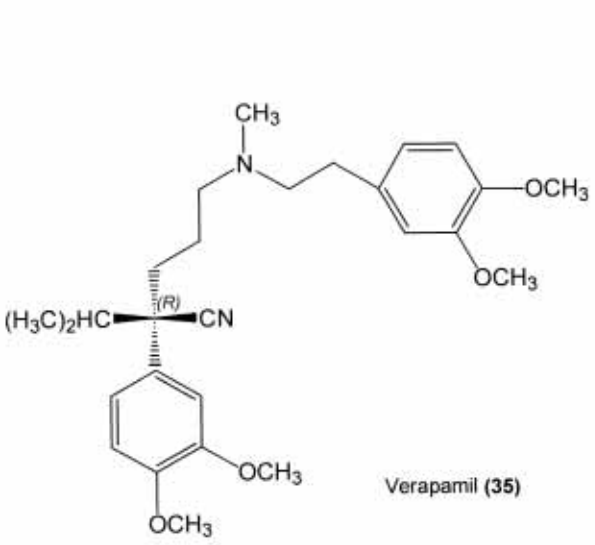
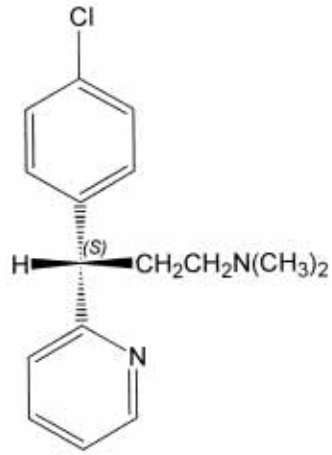


Fig. (16). Chemical structure of one trioxaquine derivative.

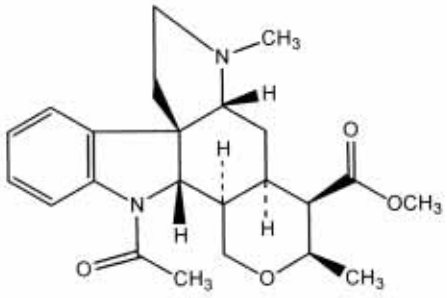
to chloroquine, but also to quinine and mefloquine [130,131]. Malagashanine, which is used in Malagashanian traditional medicine to combat malaria in association with chloroquine is at present under first clinical investigations in Madagascar [130]. The mechanism of action of these compounds, first attributed to a blocking of chloroquine efflux from the food vacuole, is



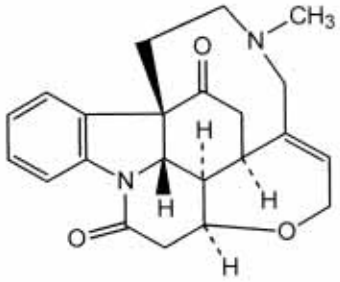
Verapamil **(35)**



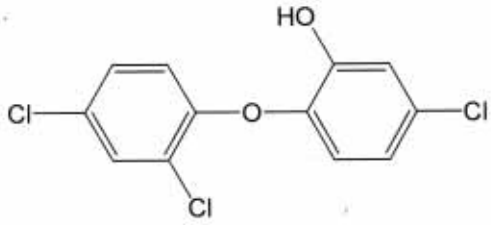
Chlorpheniramine **(36)**



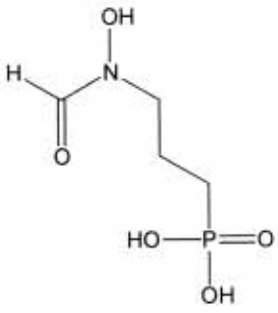
Malagashanine **(37)**



Icajine **(38)**



Triclosan **(39)**



Fosmidomycin **(40)**

(Fig. 17) contd.....

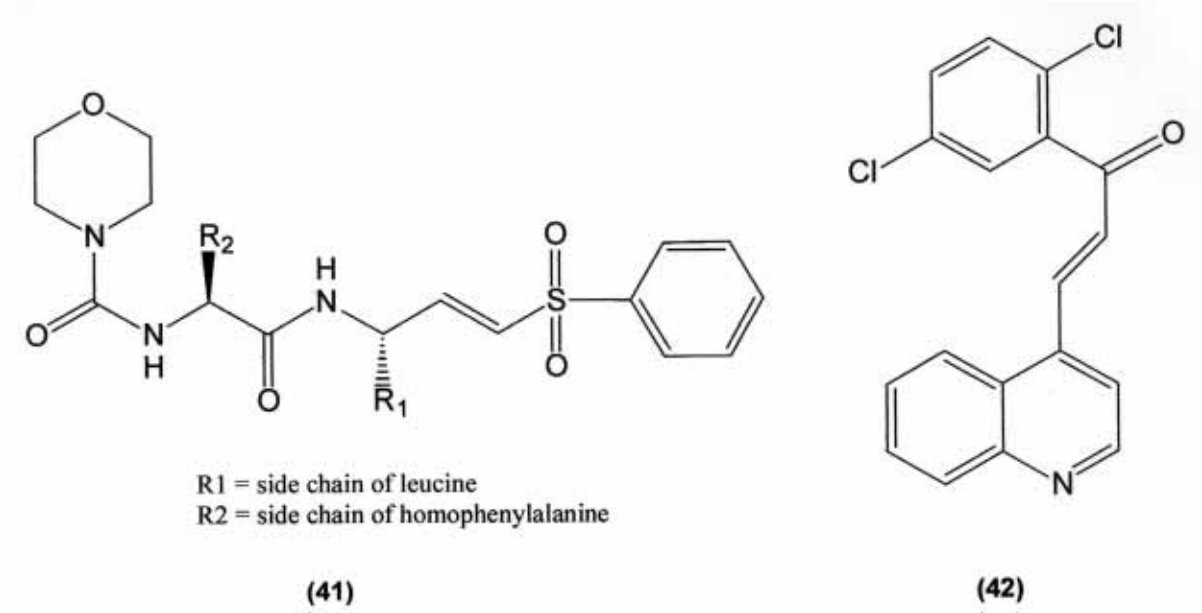


Fig. (17). Chemical structure of new potential antimalarial compounds.

not yet known with certainty and could be different for each type of compound.

Inhibition of the *de Novo* Fatty Acid Biosynthetic Pathway

The currently-used antimicrobial biocide triclosan inhibits the growth of *P. falciparum in vitro* and *in vivo* (mouse model). Triclosan **39** interfere with the fatty acid biosynthetic pathway (inhibition of enoyl-ACP-reductase) in the parasite and then could be lead for development of new antimalarial agents [132].

Phospholipid Biosynthesis Inhibitors

A range of compounds which interfere with phospholipid metabolism of *Plasmodium* have been recently identified. The candidate molecules, cationic choline analogues consisting of mono-, bis-, and triquaternary ammonium salts with distinct substituents of increasing lipophilicity, are potent and effective in *in vivo* models. These compounds are, as yet, in the preclinical stage of development, but hold great promise [133,134].

Inhibitors of the Isoprenoid Biosynthesis

The apicoplast is a recently discovered organelle of apicomplexan parasites harbouring its own genome of probably chloroplast origin. This organelle, essential to parasite survival, is associated with enzymes that are found in plant and bacterial (but not animal) metabolic pathways. Recently, Jomaa *et al*. have shown the existence of an apicoplast metabolic pathway not found in animals: the mevalonate-independent pathway of isoprenoid synthesis. They proposed that enzymes in this pathway could be valuable targets for new antimalarial drugs and they identified two compounds that block the pathway and cure malaria in a mouse model of the disease [135]. These compounds are analogues of the antibiotic fosmidomycin **40.** Other apicoplast metabolic pathway could probably be targets for new antimalarial agents.

Protease Inhibitors

Malarial proteases appear to mediate processes within the erythrocytic malarial life cycle, including the rupture and invasion of infected erythrocytes and the degradation of hemoglobin by trophozoites. Cysteine and aspartic protease inhibitors are now under study as potential antimalarials [136]. Lead compounds have blocked in vitro parasite development at nanomolar concentrations and cured malaria-infected mice [137]. The most potent of these compounds are peptidic vinyl-sulfones derivatives, as **41** and chalcones derivatives, as **42.**

7. CONCLUSION

Malaria is one of the most deadly infectious diseases known to humankind. Unfortunately, *Plasmodium falciparum*, the most dangerous of the four species responsible for malaria, has developed resistances to chloroquine (the most widely used antimalarial agent) and multiresistances to other current agents. Despite the million people at risk (especially in Africa), malaria is economically unattractive to the pharmaceutical industry, and few new drugs have been developed recently. Among these, Malarone® is unaffordable for the majority of people at risk. On the other hand, artemisinin and its derivatives have proven to be effective antimalarial drugs, active against all the *Plasmodium* of humans, including multidrug resistant *Plasmodium falciparum*. No resistance to these drugs has been identified so far. These compounds then become the most important drugs for malaria control, and are accessible to developing tropical countries. The number of other drugs currently at an advanced stage of preclinical or clinical development is limited and it may take several years before some can be made available to malaria treatment. In this

context, the development of new artemisinin analogs, direct artemisinin derivatives or other peroxydic compounds, appears to be ways of choice.

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