

From classical antimalarial drugs to new compounds based on the mechanism of action of artemisinin*

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Abstract: A short review of the antimalarial drugs currently used in human clinics is reported. The molecular aspect of the different possible mechanisms of action of artemisinin is documented, including recent data on heme alkylation. The preparation and the *in vitro* antimalarial activity of new modular molecules named “trioxaquines” are also presented.

INTRODUCTION

Malaria, a tropical disease caused by protozoan parasites of the genus *Plasmodium*, has been a real concern for centuries and is now extended to more than 40% of the world's population. *Plasmodium falciparum*, the most prevalent species across the globe, may cause cerebral malaria that is often fatal [1]. In 1897, Ronald Ross reported that the parasite *Plasmodium* could infect a female mosquito, thus showing the complete parasite cycle. He later wrote that “the discovery was, perhaps, as really important as the discovery of America” [2]. Indeed, WHO estimated that, in 1998, there were 273 million cases of malaria and more than 1 million deaths due to it. Furthermore, as international travel becomes more common, malaria is not confined to the tropical zones of the world, and imported malaria is an increasingly serious problem.

The main reason for the dramatic increase in deaths from malaria in Africa is attributed to the spread to West Africa of resistance to the mainstay antimalarial chloroquine (one of the well-known commercial names is Nivaquine[®]), and the influence of drug resistance on malaria mortality is almost certainly underestimated [3]. Actually, morbidity and mortality are rising, not because of ineffective treatment of severe malaria, but because of ineffective first-line oral treatment that causes an increasing proportion of patients who develop severe disease. In addition, the transmission of resistant strains is facilitated by unsuccessful treatment [4]. Given the inexorable spread of drug resistance, and until the development of an effective antimalarial vaccine, the search for effective, safe, and affordable drugs for falciparum malaria is one of the most pressing health priorities worldwide.

The widespread and rapid development of drug resistance requires that measures should be taken to prevent this evolution with the remaining effective compounds and with the new compound that might be produced in the future. Ideally, drugs should be used only when needed (i.e., only for treatment, not for prophylaxis) and should be given to patients who are compliant with respect to the treatment regimen, in order to avoid treatments at subtherapeutic doses. During treatment with two associated drugs, the chance of selection of a mutant resistant to both drugs can be calculated as the product of the individual per-parasite mutation rates. The choice of drug combinations is indeed necessary [5,6].

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In this report, a short review of the main drugs and combinations of drugs currently used against *Plasmodium* is presented, as well as the recent advances on new drugs named “trioxoquinines”. These new molecules have been designed after a study of the mechanism of action of artemisinin.

CURRENTLY AVAILABLE ANTIMALARIAL DRUGS

In most cases, antimalarial drugs are targeted against the asexual erythrocytic stage of the parasite. The parasite degrades hemoglobin in its acidic food vacuole [7], producing free heme able to react with molecular oxygen and thus to generate reactive oxygen species as toxic by-products. A major pathway of detoxification of heme moieties is polymerization as malaria pigment [8,9]. Majority of antimalarial drugs act by disturbing the polymerization (and/or the detoxification by any other way) of heme, thus killing the parasite with its own metabolic waste [10].

The main classes of active schizontocides are 4-aminoquinolines, aryl-alcohols including quinoline-alcohols, antifolate compounds which inhibit the synthesis of parasitic pyrimidines. The newest class of antimalarials is based on the natural endoperoxide artemisinin and its hemisynthetic derivatives and synthetic analogs. Some antibiotics are also used, generally in association with quinoline-alcohols [11].

Few compounds are active against gametocytes, and also against the intra-hepatic stages of the parasite.

8-Aminoquinolines

This is the only class of gametocytocides (see Fig. 1 for the structures).

Primaquine has been widely used for the treatment of the hypnozoites (liver reservoirs) responsible for the relapsing forms of *P. vivax* and *P. ovale*. However, primaquine was recently reconsidered for malaria chemoprophylaxis [12] to eliminate *P. falciparum* at the early stage of infection, when parasite develops in the liver, thus preventing the clinical disease. Despite its good oral absorption, this molecule has a short half-life and needs to be administered daily. Serious toxicity can be a major problem in patients with glucose-6-phosphate dehydrogenase deficiency. Primaquine is interfering with the mitochondrial function of *Plasmodium*.

Tafenoquine (WR 238605) [13], is a primaquine analog with a longer elimination half-life (14 days compared to 4 hours for primaquine). It has also a larger therapeutic index than primaquine. This molecule may be useful for chemoprophylaxis of *P. falciparum* [14] and for prevention of relapses of vivax malaria [15].

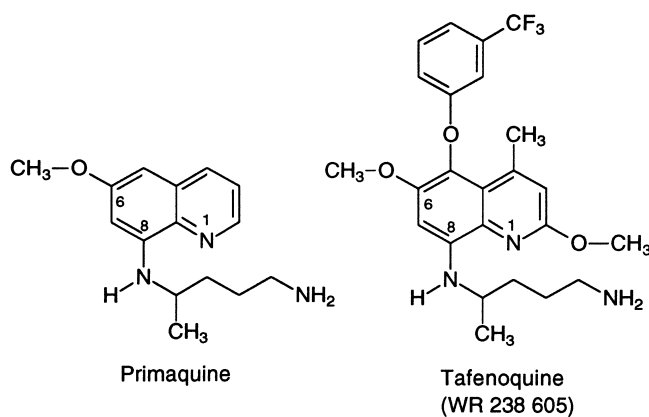


Fig. 1 The 8-aminoquinoline antimalarial drugs: primaquine and tafenoquine.

4-Aminoquinolines

The main antimalarials are the 4-aminoquinolines because they have proven to be the most highly successful class of compounds for the treatment and prophylaxis of malaria. They are easily synthesized, cheap, and generally well tolerated. These compounds, as well as the quinoline-alcohols, are active against the intra-erythrocytic stages of the parasite. The 4-aminoquinolines are able to accumulate to high concentrations within the acid food vacuole of *Plasmodium*, to kill the parasite [16].

Chloroquine (CQ) (Fig. 2) was introduced in 1944–1945 and soon became the mainstay of therapy and prevention, since this drug was cheap, non-toxic, and active against all strains of malaria parasites. In 1994, CQ was the third most widely consumed drug in the world after aspirin and paracetamol [17]. The precise mode of action of the quinoline antimalarials and the mechanism of parasite resistance are still not completely understood. Among the proposed hypotheses on the mode of action of CQ, one can cite [16]: (i) direct-heme binding, (ii) inhibition of an unidentified heme ferriprotoporphyrin-IX “polymerase”, (iii) inhibition of vacuolar phospholipase, (iv) inhibition of protein synthesis, and (v) interaction with DNA. However, the main mode of action of CQ seems to be related to the accumulation of this weak base in the acidic lysosome and binding to ferriprotoporphyrin-IX, thereby preventing its detoxification by polymerization and thus killing the parasite [11,18,19].

Chloroquine resistance was observed in Southeast Asia and South America at the end of the 1950s and in Africa in the late 1970s. In Sénégal, the emergence of CQ resistance over a 12-year period was associated with at least a doubling of the risk of death from malaria in children younger than 10 years [20]. Resistant parasites accumulate CQ less avidly than do sensitive ones. Resistance can be reversed *in vitro* using drugs known to reverse drug resistance in tumor cells, such as verapamil [21–23]. The observation that CQ resistance appeared rather late, about 10 years after its widespread use, has been considered as an argument to support the hypothesis that CQ resistance has multigenic bases. Involvement of a Na^+/H^+ exchanger *cg2* protein was suggested [24]. Single mutations of two genes, *pfprt* T76 (a gene on *P. falciparum* chromosome 7, encoding for a putative transmembrane transporter) and *pfmdr 1* Y86 (a gene located on chromosome 5, which encodes for the P-glycoprotein homologue-1, Pgh 1) may be responsible for high level of drug resistance [25–27]. The *pfmdr 1* gene seems to be also involved in the resistance to a number of other antimalarials.

In spite of its reduced efficacy, chloroquine is still the mainly used antimalarial drug in most parts of Africa, both for reason of cost and because of the widespread prevalence of partial immunity among

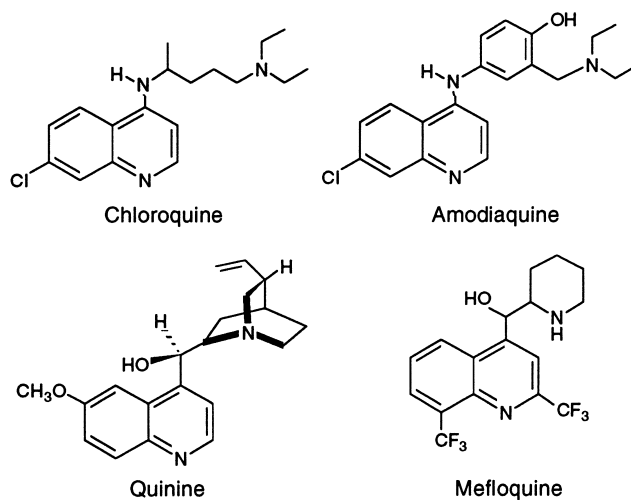


Fig. 2 The 4-aminoquinolines: chloroquine and amodiaquine, and the quinoline-alcohols: quinine and mefloquine.

symptomatic children older than five and adults. Moreover, tumor necrosis factor (TNF), a cytokine responsible for some cerebral damages which is produced by immune system during the malaria crisis, have been proven to have a synergistic effect with chloroquine, thus enhancing the effect of the drug [28].

Due to the important spread of CQ resistance, there was less CQ prescription in the last years, and then less CQ pressure. So, there is now a significant decrease in CQ resistance in Gabon, and this could indicate a possibility of reuse of CQ in the future [29].

Amodiaquine (Fig. 2) is chemically related to CQ, but is more effective than CQ for clearing parasitemia in cases of uncomplicated malaria, even against some chloroquine-resistant strains [16,30]. However, drug resistance and potential hepatic toxicity limit its use. Amodiaquine has been shown to bind to heme and to inhibit heme polymerization *in vitro*, with a similar efficiency than CQ [31]. Furthermore, amodiaquine exhibit cross-resistance with CQ suggesting that it exerts its activity by a similar mechanism [32].

Quinoline-methanols

Quinine (Fig. 2), the active ingredient of cinchona bark, introduced into Europe from South America in the 17th century, had the longest period of effective use, but there is now a decrease of the clinical response of *P. falciparum* in some areas [33,34]. Nevertheless, it remains an essential antimalarial drug for severe falciparum malaria and intravenous infusion is, in this case, the preferred route. The addition of a single dose of artemisinin enhances the parasite elimination rate and thus increases the cure rate [35]. Quinine interacts weakly with heme, but has been shown to inhibit heme polymerization *in vitro*. The mechanism of resistance to quinine is unknown, but a similar one than for mefloquine has been suggested [31]. Association of quinine and clindamycine significantly shorten the duration of treatment with respect to quinine used alone [36].

Mefloquine (Fig. 2) is structurally related to quinine, and its long half-life (14–21 days) has probably contributed to the rapid development of resistance (a commercial name of mefloquine is Lariam[®]). For this reason, mefloquine should be used in combination with other antimalarial agents. It binds with high affinity to membranes, causes morphological changes in the food vacuole of *Plasmodium*, and interacts relatively weakly with free heme. The plasmodial P-glycoprotein (Pgh 1) plays a role in mefloquine resistance and Pgh 1 may also be the target of action of this drug [31]. However even if mefloquine resistance is associated with the *pfmdr1* gene encoding for Pgh 1, some strains are resistant despite an alteration of this gene [37].

Other aryl-alcohols

Halofantrine (Fig. 3) is effective against chloroquine-resistant malaria [38]. Despite this, cardiotoxicity has limited its use as a therapeutic agent [39]. Mefloquine usage appears to lead to selection of parasites resistant also to halofantrine [40]. Furthermore, it is an expensive drug without parenteral formulation.

Pyronaridine (Fig. 3), an acridine derivative, is a synthetic drug widely used in China that may have utility for multiresistant falciparum malaria [41,42]. The current Chinese oral formulation is reported to be effective and well tolerated, but its oral bioavailability is low, and this contributes to an unacceptably high cost of the treatment. It seems likely that drug resistance would emerge rapidly if pyronaridine is used as monotherapy.

As reported above, resistance to a lot of antimalarial drugs has been observed in clinical isolates, but resistance to mefloquine, quinine, and halofantrine appears to be inversely correlated with resistance to chloroquine and amodiaquine, suggesting that the development of a high level of resistance to chloroquine makes the parasite more sensitive to the aryl-methanols [43].

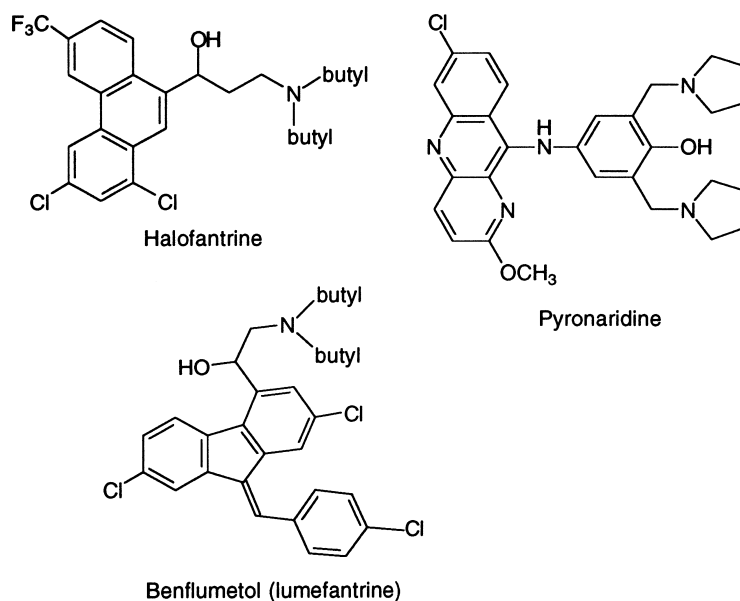


Fig. 3 Antimalarial active aryl-alcohols other than quinoline-alcohols: halofantrine, pyronaridine, and benflumetol.

Folate antagonists

These compounds (Fig. 4) inhibit the synthesis of parasitic pyrimidines, and thus of parasitic DNA. There are two groups of antifolates: (i) the dihydrofolate reductase (DHFR) inhibitors like

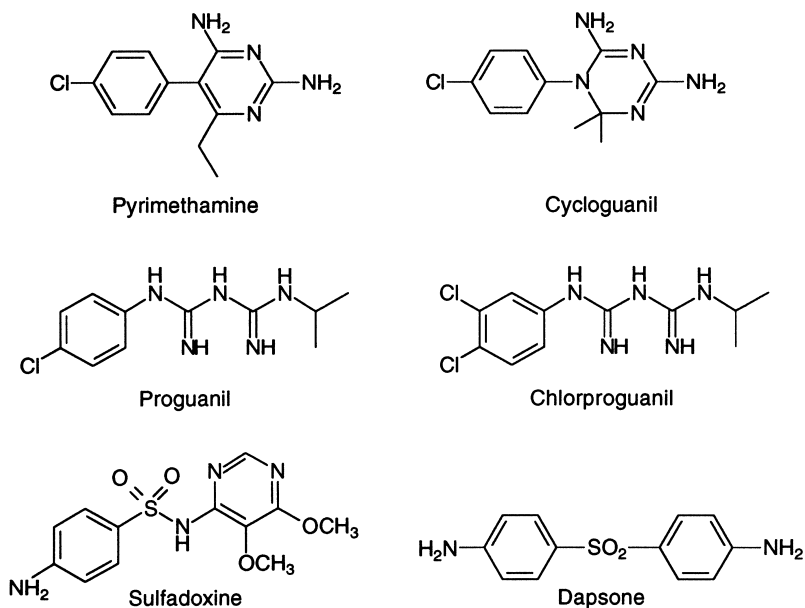


Fig. 4 The antimetabolite antimalarial drugs: DHFR and DHPS inhibitors.

pyrimethamine and proguanil and (ii) the dihydropteroate synthase (DHPS) inhibitors that are sulfones and sulphonamides like sulfadoxine and dapson, respectively. Due to a marked synergistic effect, a drug of the first group is usually used in combination with a drug of the second one. Unfortunately, resistance is widespread in Asia, India, and now in Africa [44,45]. Pyrimethamine–sulfadoxine (SP) is the most widely used combination. It is cheap, practicable (only one dose is needed because of the slow elimination from the body), and currently efficient in many parts of Africa. However, it is poorly active against highly chloroquine-resistant strains. SP is also particularly prone to rapid emergence of resistance. The mechanism of resistance has been shown to be due to mutations in the genes of DHFR [46] and DHPS [47]. Proguanil has also been associated with chloroquine in an oral formulation called Savarine[®].

It should be mentioned that proguanil is a prodrug, its P-450 metabolite cycloguanil being the active compound. Chlorproguanil (Fig. 4) is a chlorinated analog of proguanil that is also metabolized as an active triazine compound. It is more efficient and has a larger therapeutic index than proguanil, and its combination with dapson is eliminated more rapidly than SP, offering the possibility of lowering the selection pressure for resistance [48].

Finally, the development of the combination of proguanil and atovaquone (Malarone[®]) will provide another useful way for the treatment [49,50]. Atovaquone, a hydroxynaphthoquinone derivative (structure not shown), is an analog of ubiquinone, a parasite mitochondrial electron-carrier which is the cofactor of the dihydroorotate deshydrogenase. Atovaquone acts by inhibiting parasite mitochondrial electron-transport. However, the mechanism of synergy of proguanil with atovaquone is complex [51,52]. This combination is well tolerated and more effective than CQ alone, CQ-SP [53], or mefloquine [54], against acute uncomplicated multidrug resistant *P. falciparum*. It is also effective in regions where proguanil alone is ineffective due to resistance [51]. Unfortunately, atovaquone is expensive and not easily affordable in most African countries.

Artemisinin derivatives

Artemisinin derivatives are the fastest active antimalarial drugs [55] (Fig. 5). Four compounds have been used, the parent one, artemisinin, extracted from *Artemisia annua* [56] and three derivatives that are actually more active than artemisinin itself [55,57]. One of them is a water-soluble hemisuccinate: artesunate; two others are oil-soluble ethers: artemether and arteether. All of them are readily metabolized to the biologically active metabolite, dihydroartemisinin. Artemisinin is active at nanomolar concentrations *in vitro* on both CQ-sensitive or -resistant *P. falciparum* strains. The treatment of several million patients with artemisinin derivatives for acute malaria failed to detect any significant toxicity [5,58,59], even for pregnant women [60], despite the fact that neurotoxicity was observed in animals with higher doses than used clinically [61]. Artemisinin and its derivatives appear to be the best alternative for the treatment of severe malaria [62,63], and artemether has been included in the WHO List of Essential Drugs for the treatment of severe multiresistant malaria. In this family, the Walter Reed Institute of Research has patented a stable, water-soluble derivative called artelinic acid (Fig. 5) that is now being tested in animals [64].

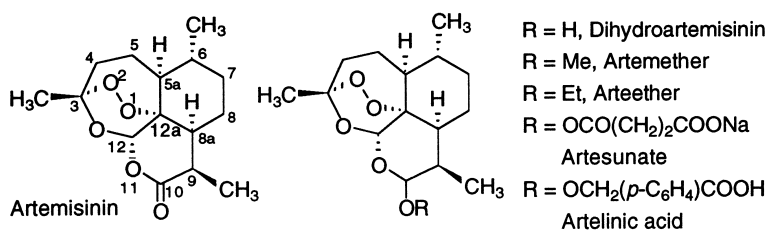


Fig. 5 Artemisinin and its hemisynthetic derivatives.

A key advantage of these endoperoxide-containing antimalarial agents, which have been used for nearly two decades, is the absence of any drug resistance [6]. When several strains of *P. berghei* or *P. yoelii* were exposed to a selection pressure by artemisinin or synthetic analogs within infected mice, resistance proved very hard to induce. A low level of resistance has been observed, which disappeared as soon as the drug-selection pressure has been withdrawn. Furthermore, with a synthetic analog of artemisinin, BO7, while resistance to the drug was lost when drug pressure was removed, it was not regained once drug pressure was re-applied [65]. Remarkably, the introduction of artemisinin derivatives in routine treatment in some areas of Southeast Asia was associated with a significant reduction of falciparum malaria. In fact, artemisinin derivatives prevent gametocyte development and, therefore, reduce the transmission [66].

The major drawback of artemisinin derivatives is their short half-life (3–5 h). When used in monotherapy, a treatment as long as 5 days is required for complete elimination of the parasites. They are then preferentially used in combination with other antimalarial agents such as sulfadoxine-pyrimethamine [67], benflumetol [68,69], or mefloquine [70,71] to increase cure rates and to shorten the duration of therapy in order to minimize the emergence of resistant parasites [6].

Combination of artemether or artesunate and mefloquine has been early used in Thailand [72]. It is the current standard treatment in areas of multidrug resistance in Southeast Asia. Furthermore, it appeared that this association was able to halt the progression of mefloquine resistance in *P. falciparum* [73].

When associated with lumefantrine (benflumetol, a slow eliminated oral drug, Fig. 3) artemether is as effective as the artesunate–mefloquine combination, and better tolerated [68]. Artemether clears most of the infection, and the lumefantrine concentration that remains at the end of the 3- to 5-day treatment course is responsible for eliminating the residual parasites [74]. This association is safe in patients with uncomplicated falciparum malaria, even children [75]. It clears parasites rapidly and results in fewer gametocytes carriers [76].

However, the relatively high cost and erratic supply of the natural parent compound artemisinin make necessary the development of new synthetic and cheap endoperoxide-based antimalarials. In order to develop synthetic artemisinin-like derivatives, it is necessary to understand the mechanism of action of artemisinin at the molecular level.

MECHANISM OF ACTION OF ANTIMALARIAL ENDOPEROXIDES

Artemisinin and its derivatives are toxic to malaria parasites *in vitro* at nanomolar concentrations, whereas micromolar concentrations are required for toxicity to mammalian cells. One reason for this selectivity is the enhanced uptake of the drug by the parasite: *P. falciparum*-infected erythrocytes concentrate [³H]-dihydroartemisinin and [¹⁴C]-artemisinin to more than 100-fold higher concentration than do uninfected erythrocytes. Furthermore, artemisinin is hydrophobic and localized in specific parasite membranes [55]. Once inside the parasite, how do artemisinin derivatives act? Since an artemisinin derivative lacking the endoperoxide bridge (deoxyartemisinin) is devoid of antimalarial activity [77], the possible reactivity of this peroxide function within the parasite is the key factor of the pharmacological activity of these molecules.

Target(s) of artemisinin

When artemisinin or other active trioxanes were incubated at pharmacologically relevant concentrations within human red blood cells infected by *P. falciparum*, the heme-catalyzed cleavage of the peroxide bond was reported to be responsible for alkylation of heme [78] and a small number of specific parasite proteins, one of which has similar weight than a histidine-rich protein (42 kDa) [79]. Another possible target protein is the *P. falciparum* translationally controlled tumor protein (TCTP) [80]. It was also recently reported that artemisinin may be involved in specific inhibition of malarial cysteine protease activity responsible for *ca.* 30% of hemoglobin degradation by *P. yoelii*. Furthermore, the incu-

bation of purified hemozoin with artemisinin under acidic conditions (pH 5), close to the pH of the parasite vacuole, resulted in the loss of hemozoin content as compared with control, indicating that hemozoin may be dismantled by interaction with the endoperoxide-drug, and resulting in a build-up of the pool of free heme [81].

Alkylation ability of artemisinin

The importance of alkylating species generated by the homolytic cleavage of the endoperoxide function, in particular, the alkyl radical centered at position C4 of artemisinin or related trioxanes, has been early suspected [82]. Alkylation of heme by artemisinin was reported by Meshnick *et al.* after identification of heme-drug adducts by mass spectrometry, but no structure was proposed for the resulting covalent adducts [78]. We therefore investigated this crucial feature of the reactivity of artemisinin [83]. Because of the variety of possible alkylation sites on protoporphyrin-IX, we first decided to investigate the alkylating activity of artemisinin with manganese(II) tetraphenylporphyrin, a synthetic metalloporphyrin having a fourth-order symmetry and only the eight equivalent β -pyrrolic positions as possible alkylation targets. Manganese was chosen as central metal instead of iron because of easier removal from the porphyrin ligand for NMR characterization of an expected alkylated derivative. By reacting Mn^{II} TPP with artemisinin, artemether, or several synthetic trioxanes, chlorin-type adducts were formed by reaction of the macrocycle with an alkyl radical generated by reductive activation of the peroxide of the drug [84]. The structures and mechanism of formation of the artemisinin- and artemether-TPP adduct are reported in Fig. 6. It should be noted that the reduction of the porphyrin macrocycle to a chlo-

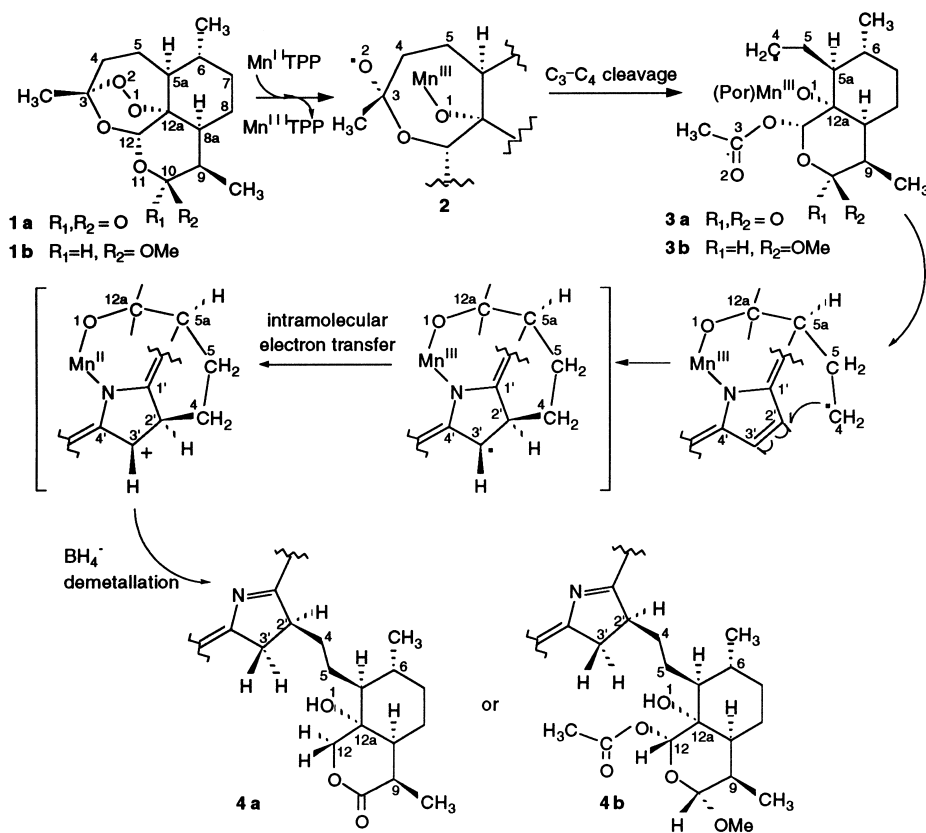


Fig. 6 Alkylation of manganese(II) tetraphenylporphyrin by artemisinin or artemether.

rin involved the borohydride initially used to generate $\text{Mn}^{\text{II}}\text{TPP}$ from $\text{Mn}^{\text{III}}(\text{TPP})\text{Cl}$. However, in the absence of a hydride able to trap the cation generated at C3', a proton release at C2' can regenerate the porphyrin ring. This rearomatization of the macrocycle is competitive with a hydride-mediated reduction, and a ratio chlorin-/porphyrin-adduct of 85/15 to 75/25 was obtained in the activation of trioxane BO7 by $\text{Mn}^{\text{II}}\text{TPP}$ [84b].

By studying a large series of trioxanes [84–86], it has been possible to correlate the alkylating ability toward $\text{Mn}^{\text{II}}\text{TPP}$ and the pharmacological activity, the inactive drugs being unable to efficiently alkylate the porphyrin ring. Furthermore, it appeared that trioxanes bearing a bulky substituent in α orientation (i.e., on the same side of the endoperoxide with respect to the mean plane of the drug) were at the same time inactive and unable to alkylate the macrocycle (Fig. 7, for the synthesis of trioxanes depicted in Fig. 7, see ref. 57b). These data confirmed that (i) a close interaction between the metal center and the peroxide bond is required, suggesting that this activation occurred through an inner-sphere electron transfer; (ii) the alkylation ability which is considered as crucial for the antimalarial activity of artemisinin is not limited to this natural compound, but is probably a general feature required for the biological activity of endoperoxide-containing antimalarial drugs.

It was of particular interest to investigate the reactivity of artemisinin toward iron^{II}-heme itself, the biological activator and an expected target of this antimalarial drug. For this purpose, iron(III) protoporphyrin-IX dimethylester was reacted with artemisinin in the presence of a hydroquinone derivative (the same result was obtained with a thiol) used as mild reducing agent to generate the iron(II)

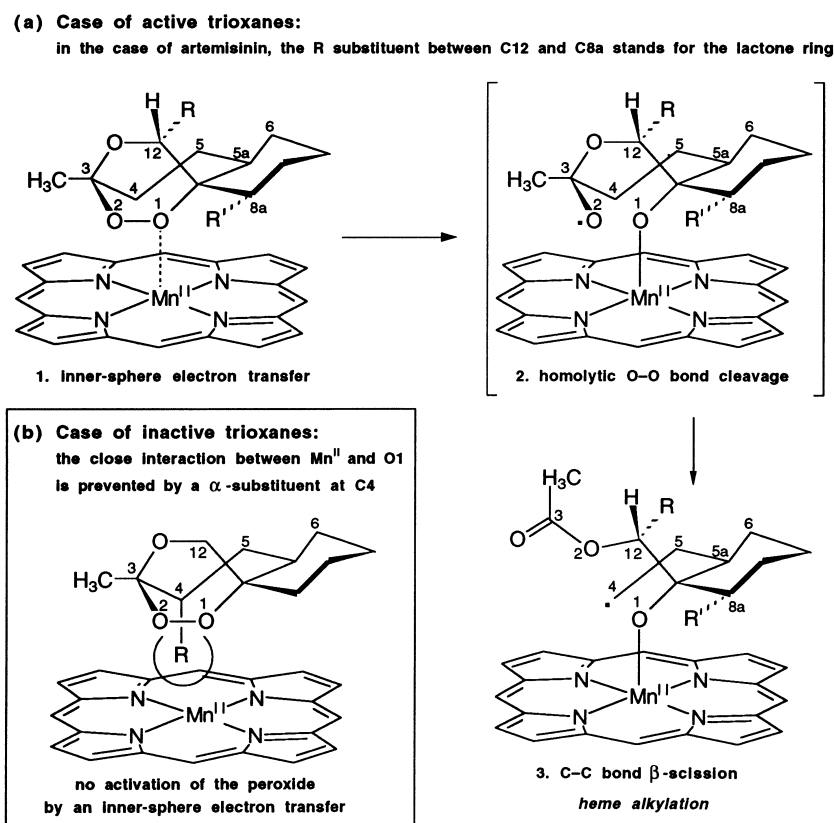


Fig. 7 Activation of trioxanes by inner-sphere electron transfer: correlation between pharmacological activity and alkylating properties toward a heme model.

heme. Heme was readily converted to heme-artemisinin adducts in high yield [87a]. The demetallation of this mixture of three adducts followed by NMR characterization indicated that the α -, β -, and δ -*meso* carbons were alkylated without particular regioselectivity (Fig. 8) [87b]. Artemether reacted as artemisinin, giving rise to covalent adducts between the *meso*-carbons of the heme and a C-centered radical derived from the drug [87a].

It is remarkable that no *bis*-alkylated adducts could be obtained either with heme or with the heme model $\text{Mn}^{\text{II}}\text{TPP}$, even in the presence of a drug excess, although the metal center keeps redox properties after the first alkylation. This means that a bulky drug-derived substituent at the periphery of the macrocycle precludes the close interaction of a second drug molecule required for *bis*-alkylation. This phenomenon may be similar to the *in vivo* sterically hindered stacking of heme-drug adducts leading to accumulation of nonpolymerizable redox active heme adducts.

In fact, the artemisinin chemistry generated by the mono-electronic reduction of the peroxide bridge in the presence of iron hemin or models can probably be considered as a radical chemistry occurring in a cage and, therefore, with a relatively high selectivity. Such reported results suggest that, *in vivo*, the low and transient concentration of free heme generated by hemoglobin catabolism is probably able to perform the reductive activation of the peroxide bond of artemisinin and active trioxanes. This pathway will generate alkylating species such as radical **6** (Fig. 8) and is likely to disrupt vital biochemical processes of the parasite via alkylation of biomolecules. The alkylating properties of the rad-

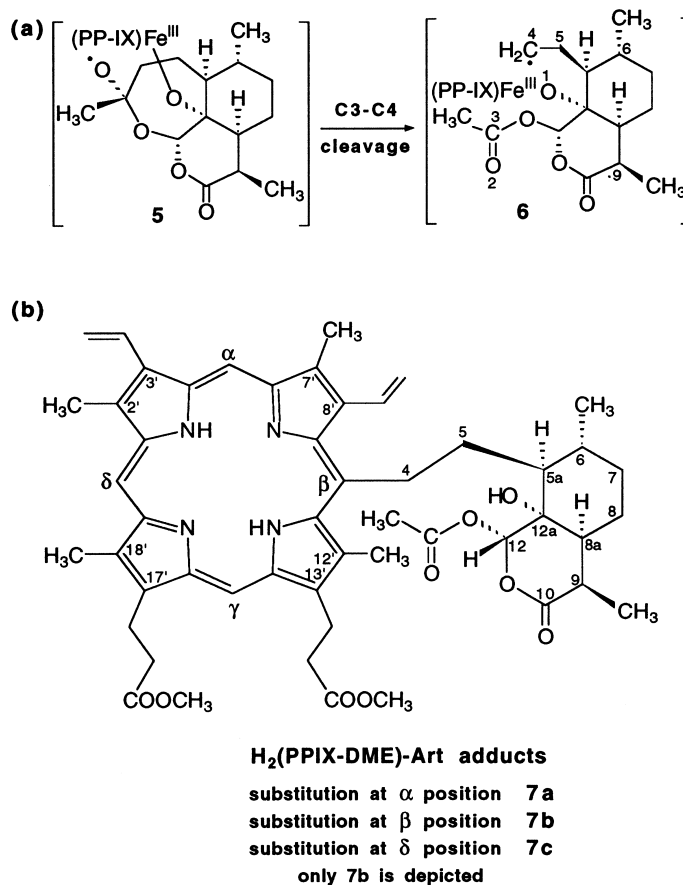


Fig. 8 (a) Formation of a C4-centered radical after reductive activation of the endoperoxide function, leading to (b) alkylation of heme-dimethylester.

ical **6** has also been evidenced by isolation of covalent adducts with cysteine [88] or glutathione [89] via a thioether linkage, indicating the possible alkylation of protein side chains.

The retention of configuration observed with drug-derived chiral C-centered radicals [85] suggests that alkylation reactions are very fast, occurring within few nanoseconds. One should keep in mind that, within 1 to 3 nanoseconds, a small organic molecule can move over a distance of 8 to 14 Å in a water solution at room temperature. Such displacement is enough for a C-centered radical generated by a free-heme molecule to alkylate a parasite protein located in the close proximity of the heme. Several protein targets may be considered, such as *P. falciparum* proteases [90] or PfHRP2. (The molecular role that PfHRP2 plays in heme polymerization is currently unknown. However, it has more than 50 heme binding sites, and formation of a PfHRP2-heme complex is required for the formation of hemozoin [91]).

NEW ANTIMALARIAL DRUGS

A lot of efforts have been made for the elaboration of malaria vaccines without success until now [92,93]. The near availability of the complete sequencing of *P. falciparum* genome should improve the chances of existing of a vaccine [94]. However, a positive result is not likely to be next door. The genome sequencing will allow the identification of new parasite proteins to be inhibited. But time is long from discovering targets to develop new therapeutic means, and high costs are likely for this approach.

Old targets, new compounds

An alternative strategy is the exploitation of known targets such as the phospholipid (PL) metabolism of infected erythrocytes [95].

The free heme liberated in the parasite food vacuole is also an “old” but always attractive pharmacological target: it is the most specific target that can be exploited since it comes from the hemoglobin digestion by the parasite, that occurs only in infected erythrocytes. Many chemical entities are directed toward this well-known target, among them chloroquine derivatives and artemisinin derivatives. Chloroquine is a cheap and easy-to-prepare molecule that has proved to be a highly effective, safe, and well-tolerated drug for treatment and prophylaxis. However, the spread of resistance has resulted in a huge reduction in the utility of chloroquine. Many chemical modifications have been attempted to obtain a molecule as affordable as chloroquine and active on resistant strains [16]: substitutions in the quinoline nucleus, variations in the side chain, synthesis of bisquinolines, and, more recently, the introduction of a ferrocenyl moiety [96]. A little modification seems to be enough to make a compound that is active on resistant strains, but the wait for a safe and effective chloroquine alternative continues. Artemisinin and its derivatives (artemether, arteether, artesunate) are used increasingly in Asia and Africa where multidrug-resistant *P. falciparum* is prevalent. They are rapidly effective and well-tolerated treatments, but the total synthesis is too complex to be exploited and the yield of extraction from the plant is still low, despite the investigation toward the enhancement of artemisinin or one of its precursors production in *A. annua* [56b]. As a result, they remain expensive treatments that are hardly accessible to people in endemic area. The cost will also be limitative for sophisticated artemisinin derivatives [97]. Synthetic trioxanes, simplified analogs of artemisinin retaining the crucial endoperoxyde bridge, have been developed, but none of them has entered successfully clinical trials [5].

New modular drugs: “trioxaquines”

Keeping in mind that heme liberated in the parasite food vacuole is a target of special interest, there are good arguments for combining an artemisinin derivative to all newly introduced antimalarial drug to delay or even prevent the development of resistance [6]. We adopted this therapy approach by prepar-

ing new chimeric molecules by covalently attaching a trioxane moiety to a 4-aminoquinoline entity [98]. These molecules, named trioxaquinines (Fig. 9a) were designed according to what we know about the mechanism of action of artemisinin derivatives: They combine a peroxidic entity acting as a potential alkylating agent and an aminoquinoline known to easily penetrate within infected erythrocytes [10]. The aim of this strategy is to dispose of two active entities, that of chloroquine and that of artemisinin, in a single molecule. Better than a simple combination, this “covalent bitherapy” is supposed to reduce considerably the risk of drug resistance. For obvious reasons, these molecules must be cheap to prepare and easily accessible; thus, we used a convergent synthesis based on classical reactions. Many simple modulations (quinoline, diamine, diene, diketone) are possible, leading to a large family of new potential antimalarial compounds. The first synthesized trioxaquinines (Fig. 9b) have been tested *in vitro* on laboratory strains (chloroquine-sensitive and chloroquine-resistant ones) of *P. falciparum*: all IC_{50} values obtained were below 30 nM, without any significant difference between sensitive and resistant strains (IC_{50} is the concentration of drug inhibiting 50% of the growth of the parasite culture). DU-1102 has also been tested *in vitro* on human isolates in Yaoundé (Cameroon) and was found to be highly active, with IC_{50} values ranging from 11 to 70 nM, without any significant difference between chloroquine- and pyrimethamine-sensitive and -resistant strains. No cross-resistance was observed with chloroquine and pyrimethamine [99].

Trioxaquinines have the peculiarity to be modular molecules: the trioxane moiety and its substituents, the linker, and the aminoquinoline may be modified in order to increase the efficacy of these compounds. Then, a large family of new compounds have already been synthesized. One of these trioxaquinines was shown to be active *by oral route* in mice (*P. berghei*) with $ED_{50} = 18 \mu\text{mol/kg}$ (ED_{50} for artemisinin = $30 \mu\text{mol/kg}$). These encouraging results validate this strategy of “covalent bitherapy” and indicate a promising future for this approach. We are currently working on the *in vivo* evaluation of these drug candidates that can be developed for clinical trials in a reasonable future in collaboration with a newly created company Palumed [100].

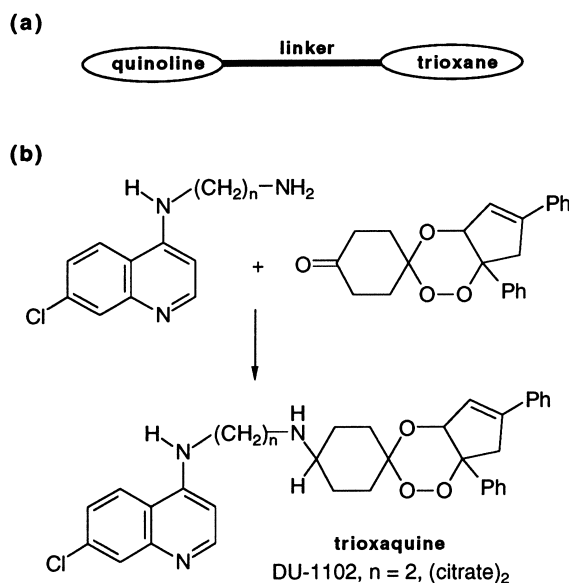


Fig. 9 (a) General structure of trioxaquinines. (b) Structure of trioxaquine DU-1102.

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100. For information on the development of antimalarial trioxaquines, see <http://www.palumed.com>.