Malaria: New Chemotherapeutic Peroxide Drugs

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Abstract: Chemical insights into artemisinin's biological mechanism of action have allowed rational design of some new trioxane and endoperoxide antimalarial drug candidates that are efficacious and safe. This review summarizes recent achievements in this area of peroxide drug development for malaria chemotherapy.

INTRODUCTION

Besides current research aimed at developing a vaccine to **prevent** malaria infection, new drugs to **cure** today's 300-500 million malaria-infected individuals are desperately needed. The complex life cycle of the *Plasmodium falciparum* protozoan parasite offers many opportunities for antimalarial drug design. Most efforts at novel malaria chemotherapy have been directed at killing the parasite when it has infected human erythrocytes. Starving the malaria parasite by blocking its digestion of human hemoglobin (*e.g.* using protease inhibitors) and poisoning the malaria parasite by blocking polymerization of toxic heme into innocuous hemozoin are two areas of active research. Also, killing the malaria schizonts in human erythrocytes has been achieved effectively with iron-activated peroxide drugs related to the natural herb-derived antimalarial peroxide artemisinin (**1**). Chemical insights into artemisinin's biological mechanism of action have allowed rational design of some new trioxane and endoperoxide antimalarial drug candidates that are efficacious and safe. This review summarizes recent achievements in this area of peroxide drug development for malaria chemotherapy.

BIOLOGY: INTRODUCTION

Malaria is caused by protozoa of the *Plasmodium* genus. The four species responsible for human infection are *P. vivax, P. malariae, P. ovale* and *P. falciparum*. Of the four species, infections by *P. falciparum* have the highest mortality rate. The vector for the disease is a female mosquito from the *Anopheles* genus. About 100 of the nearly 500 species of anopheline mosquitoes are vectors for the disease [1].

LIFE CYCLE

The disease is spread when the infected female mosquito feeds on the blood of the uninfected human host. Sporozoite forms of the parasites are passed from the mosquito salivary glands into the host bloodstream. Once in the vertebrate host, sporozoites travel from the bite site through the blood stream to the liver. Circulation time in the blood stream is short, less than 1 hour for *P. falciparum* or *P. vivax* [2]. In the liver, sporozoites invade hepatocytes where they round up and undergo exoerythrocytic schizogony [2]. After a set time, depending of the species, the schizont ruptures, and merozoite forms of the parasite are released [2]. The large amounts of newly released merozoites invade erythrocytes almost immediately. In erythrocytes, the merozoites first undergo asexual development. The parasite undergoes nuclear division until it reaches maturity, at which point it under goes schizogony [2]. The rupture of each schizont produces a specific number of new merozoites [2]. These new merozoites invade new healthy erythrocytes and the cycle continues. Schizogony occurs at regular intervals, every 2-3 days depending on the species. Each cycle is accompanied by the typical malarial symptoms: fever, chills, headache and fatigue [3]. After several cycles, some of the merozoites undergo sexual development and differentiate into macrogametocytes (female) and microgametocytes (male). The gametocytes remain in erythrocytes and are ingested by an *Anopheles* mosquito when it bites an infected person. In the mosquito, a male and a female malaria gametocyte join to form a zygote. The zygote eventually gives rise to new sporozoites. These sporozoites migrate to the mosquito's salivary glands and continue the cycle [4].

DISEASE & COMPLICATIONS

P. falciparum malaria has several factors that complicate the disease. Complications occur without warning and most often in individuals with poor immune systems [5]. Erythrocytes infected with *P. falciparum* malaria develop "knob-like" protrusions on their membranes, which are sites of adhesion between sequestered erythrocytes and venous endothelial cells [6]. These aggregated erythrocytes can have a mechanical effect that leads to a reduced human blood flow [7]. Reduced blood flow causes a reduction in oxygen and other nutrients to the tissues. Aggregation also leads to a concentration of toxins produced as erythrocytes rupture [8]. It has been shown that rupture of erythrocytes leads to the release off various cytokines such as tumor necrosis factor (TNF), interleukin 1 (IL1) and others. These cytokines can then lead to release of nitric oxide [9]. When sequestration occurs in the brain it often leads to cerebral malaria, the most severe complication of *P. falciparum* malaria. Cerebral

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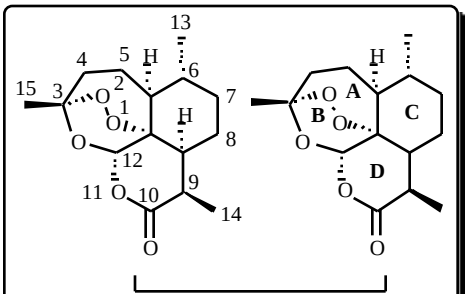
malaria is defined as an acute encephalopathy accompanied by various neurological signs including coma [5]. Cerebral malaria is the most common cause of death in malaria, with a mortality of about 20% despite the drugs available [7].

TRADITIONAL CHEMOTHERAPY

A course of chemotherapy that would target the parasite at all stages is difficult due to the parasite's complex life cycle. Most of the drugs currently used target only one phase of the life cycle. The new drug malarone (atovaquone proguanil) is an exception in that it targets both the liver and red blood cell phases [10, 11]. Not many drugs that target the liver phase are available. Such drugs would be beneficial, since eradication at the liver phase is the only way to prevent relapses in *P. vivax* and *P. ovale* [1]. The majority of drugs in use today target the human blood phase of parasite development. The drugs currently on the market in the United States are nitrogen-containing, aromatic compounds such as quinine, mefloquine, doxycycline, chloroquine, hydroxycloroquine, primaquine, pyrimethamine, sulfadoxine/pyrimethamine and malarone, with chloroquine and mefloquine being most often used [12]. Resistance to chloroquine was first reported in 1961 [13]. Resistance to chloroquine is now common in most malarial areas. Additionally, multidrug resistance is becoming increasingly common [12].

ARTEMISININ

In the 1960s Chinese chemists began to screen traditional herbal drugs in an attempt to find new antimalarial drugs. Among the herbs tested was *Artemisia annua L.*, whose use dates back to 168 B.C. [14]. In 1972 Chinese scientists reported seven sesquiterpene compounds [14]. The compound with principal antimalarial properties was named qinghaosu (artemisinin, **1**) [14]. Several total syntheses of artemisinin (**1**) have been reported since its isolation [15]. Artemisinin (**1**) was found to act on the blood phase of *P. falciparum* [14]. Artemisinin (**1**) and its derivatives are effective against both chloroquine sensitive and chloroquine resistant strains of *P. falciparum* [16]. Artemisinin derivatives have also proven useful for the treatment of severe cerebral malarial [7]. The downside of artemisinin based antimalarials is high recrudescence rates, which have been attributed to rapid metabolic clearance [17].



Artemisinin, **1**

PROTEASES

Plasmodium falciparum food vacuoles contain at least three proteases, which are likely involved in globin hydrolysis. The three known proteases are cysteine protease falcipain, and the two aspartic proteases plasmepsin I and plasmepsin II [18-20]. The precise roles of these proteases are not entirely known, but they most likely act in a concerted way to hydrolyze globin to free amino acids. Inhibitors of cysteine and aspartic proteases have been shown to have antimalarial properties [19]. Cysteine protease inhibitors, including E64, act mainly on trophozoites causing accumulation of undegraded globin in food vacuoles [19]. The antimalarial effect is directly correlated with inhibition of falcipain [19]. Aspartic protease inhibitors seem to act primarily on ring and schizont stages of parasites causing abnormal pyknotic forms [19]. In order to use protease inhibitors as antimalarials, specificity towards parasite proteins is important. Antimalarials should be able to inhibit parasite proteases but not analogous host proteases, such as cysteine proteases cathepsin L and D and aspartic protease cathepsin D. Aspartic protease inhibitors show specificity, but the cysteine proteases have not yet been rigorously studied [19]. The two classes of protease inhibitors work best synergistically to degrade hemoglobin *in vitro* and to inhibit the growth of parasites [19].

HEMOGLOBIN DEGRADATION

During the course of infection, parasites hydrolyze 25-75% of the human host erythrocyte hemoglobin. Hemoglobin appears to the major source of amino acids for parasite protein synthesis. In the course of hemoglobin degradation, large amounts of free heme, toxic to the parasites, are released [21]. Free heme is detoxified by its being polymerized into hemozoin. Hemozoin is heme polymerized in the β-hematin form. A recent crystal structure of synthetic β-hematin, which has identical chemical, spectroscopical, and crystallopgraphical properties to hemozoin, indicates that heme molecules form dimers [22,23]. These dimers are linked into chains by hydrogen bonding [23]. The new results support the beliefs that hemozoin is a noncovalent coordination complex with ferric iron of heme bound to the carboxyl side chain of adjacent heme molecule [21,23]. The mechanism by which hemozoin forms is also controversial. Hemozoin forms spontaneously under nonphysiological conditions. Formation of hemozoin under physiological conditions is sluggish and requires preformed hemozoin. Initiation of hemozoin formation appears to require other molecules [21].

HEMOZOIN AND ARTEMISININ

It was found that artemisinin taken up by parasites is concentrated in hemozoin. The reaction between heme and artemisinin appears to give the same products both *in vivo* and *in vitro* [24]. Recently, the alkylation product of heme by artemisinin *in vitro* has been characterized [25, 26]. All evidence points to a two-step mechanism of action of artemisinin [24, 27]. The first step is heme catalyzed

cleavage of the endoperoxide bridge to give free radicals. This is followed by alkylation of heme and proteins [24, 27, 28]. Strong evidence for this two step mechanism is that the endoperoxide bridge is necessary for antimalarial action [24, 27]. Also, free radical scavengers and iron chelators antagonize the action of artemisinin [19].

MECHANISM OF ACTION

As determined by structure-activity relations, the endoperoxide is crucial for antimalarial activity. Studies by Posner *et al.*, indicate that carbon radicals are involved in antimalarial activity [29]. The mode of action proceeds in two steps. The first step involves cleavage of the endoperoxide bridge, which is catalyzed by intraparasitic iron and heme [30]. Cleavage of the endoperoxide moiety leads to unstable free radical intermediates [31]. The first radical to form is an O-centered radical formed by reduction of the endoperoxide bridge by ferrous iron. This radical then rearranges to a C-centered radical probably via a 1,5-hydrogen shift [28, 29]. The loss of a high-valent iron-oxo intermediate [Fe(IV)=O] from the carbon radical results in the formation of a reactive epoxide [28]. Such epoxides are known to be strong alkylating species.

FIRST GENERATION SEMISYNTHETIC ENDOPEROXIDES: INTRODUCTION

Although natural artemisinin (**1**) shows promise as an antimalarial, its poor bioavailability prompted the search for better analogs. Artemisinin (**1**) is poorly soluble in water or oil and is not well absorbed by the gastrointestinal tract [32]. Generally, effective levels are slightly higher with an oil suspension, since it slows the distribution of the drug [12]. First generation semisynthetic endoperoxides are all simple esters and ethers of dihydroartemisinin (DHA, **2**) [30]. Dihydroartemisinin (**2**) is a lactol easily obtained from natural artemisinin by sodium borohydride reduction [32]. It is twice as active as artemisinin itself [33], but it also exhibits a high degree of neurotoxicity [12]. Although not ideal antimalarial agents because of poor oral bioavailability and a high incidence of recrudescence, these compounds are effective even against severe cerebral malaria [30].

ARTEMETHER AND ARTEETHER

Artemether (**3**) is the β-methyl ether of DHA (**2**). The methyl ether was formed to increase solubility of the compound in oil relative to that of natural artemisinin (**1**) [33]. Arteether (**4**) is the β-ethyl ether isomer of DHA (**2**). It was investigated as a potential drug for several reasons. The ethyl group makes arteether (**4**) more lipophilic than artemether (**3**) [32]. Also, after cleavage into DHA (**2**), arteether (**4**) gives rise to ethanol as opposed to toxic methanol from artemether (**3**). This is an important consideration since a relatively large dosage of the drug needs to be administered [32]. In addition, the β-ether isomer is highly crystalline while the α-isomer is not, which is an advantage in large scale preparation and purification [32]. In the case of artemether (**3**), both isomers are low melting solids.

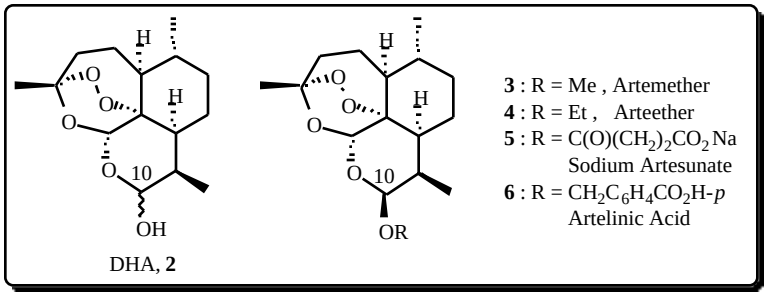
Both arteether (**4**) and artemether (**3**) are fast acting and are active towards drug-resistant strains. Arteether (**4**) was chosen by the World Health Organization (WHO) and by the US Army as emergency treatment of individuals with severe malaria, including cerebral malaria [34]. It was developed as a sesame oil solution for intramuscular injection. Toxicity in clinical use and in toxicological studies appears to be low [34]. Animal studies show artemisinin (**1**) and its derivatives to be considerably less toxic than quinoline compounds [14,35]. The first toxicity studies of artemisinin (**1**) showed a high therapeutic index, causing researchers to believe the drug is without toxicity [12, 35]. Artemether (**3**) proved to be more toxic than artemisinin (**1**) [35, 36]. Some toxicology studies reported unexplained deaths in animals receiving high doses of artemisinin and its derivatives [34, 35]. At first the cause was presumed to be of cardiovascular origin. Later it was shown that the deaths were in fact due to neurotoxicity of these drugs [34]. Studies show neurotoxicity when artemether (**3**) and arteether (**4**) are administered daily in high doses to dogs or rats [34]. The effects are specific to neuroanatomical areas in the caudal brain-stem area [34]. The toxicities of arteether (**4**) and artemether (**3**) are comparable. It is believed that a metabolite of the drug, most likely DHA (**2**), is responsible for toxicity, since the drugs are rapidly and extensively metabolized [12, 37]. In contrast, however, recent studies have shown that lower doses are not responsible for neurotoxicity [12, 38, 39]. Neurotoxicity becomes a problem when the administered dose is at least five times greater than the recommended dose [38].

ARTESUNATE

For treatment of advanced cases of *Plasmodium falciparum* malaria, a water-soluble derivative of artemisinin (**1**) is desired. A water-soluble derivative can be injected intravenously, and thus the drug can be delivered faster than by intramuscular injection [40]. The sodium salt of artesunic acid (**5**) is such a water soluble derivative, capable of rapidly diminishing parasitemia and restoring consciousness of comatose cerebral malaria patients [40]. Due to the high recrudescence rate, however, sodium artesunate (**5**) is normally administered in combination therapy, most often with mefloquine [41]. The toxicity of artesunate (**5**) is higher than that of artemisinin (**1**), but less than that of arteether (**4**) or artemether (**3**) [35]. Unfortunately, artesunate (**5**) has low stability in aqueous solution [41]. Artesunate (**5**) is dissolved in a solution of sodium bicarbonate in dextrose or saline and thus solutions must be prepared immediately before injection because of artesunate's relatively rapid hydrolysis into DHA (**2**) [41]. Artesunate (**5**) can be, and now generally is, given also in pill form.

ARTELINIC ACID

Although artemether (**3**), arteether (**4**) and sodium artesunate (**5**) show much improvement over artemisinin (**1**) in terms of efficacy and increased solubility, new analogs are



needed due to the short plasma half-life and central nervous system (CNS) toxicity of these trioxanes in rats and dogs [34]. The usefulness of sodium artesunate (**5**) is also offset by problems associated with its susceptibility to hydrolysis in aqueous solution [42], the high rate of recrudescence, and the short plasma half-life (20-30min) [43]. Artelinic acid (**6**), which contains an ether rather than an ester linkage at C-10, was designed to overcome the instability of sodium artesunate (**5**) in aqueous solution. It was found that artelinic acid (**6**) is not only very stable in aqueous solution, but also has a longer plasma half-life (1.5 – 3h) [42]. Artelinic acid (**6**) showed superior *in vivo* activity against *Plasmodium berghei* compared to that of artemisinin (**1**) or sodium artesunate (**5**), [44] as well as lower toxicity [45]. In recent pharmacokinetic studies of artelinic acid (**6**), it showed the highest plasma concentration, the highest binding capacities in red blood cells, the highest oral bioavailability, and the lowest toxicity among the first generation drug candidates [46].

SECOND GENERATION SEMISYNTHETIC ENDOPEROXIDES: C-10 SUBSTITUTED ANALOGS

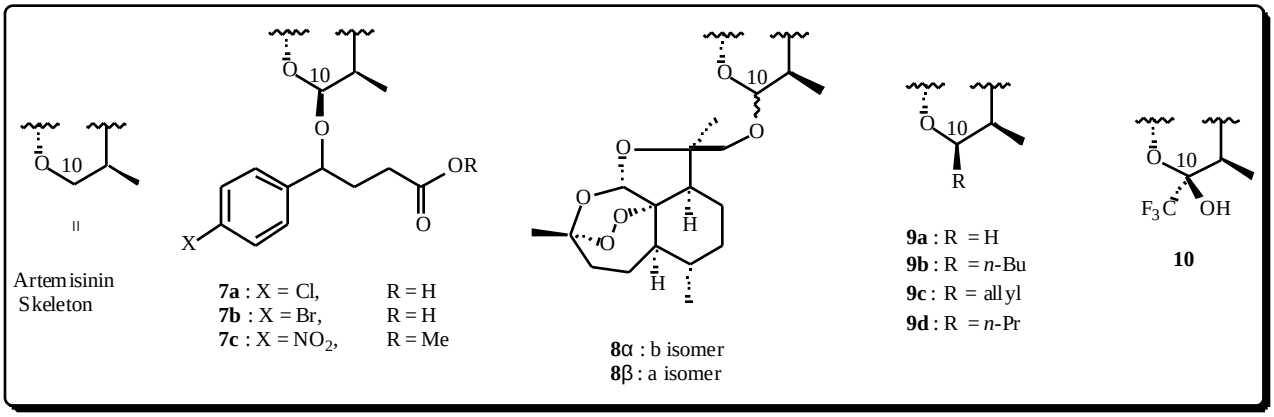
Knowledge based on the first generation drugs described above can be used to develop a new class of second generation semisynthetic antimalarials with even more desirable properties. Much effort has been directed at obtaining analogs with improved bioavailability by modification of artelinic acid (**6**) [47]. Manipulation of the electron density of the aromatic ring of artelinic acid (**6**) and the steric effects of the benzylic carbon atom have resulted in modified analogs **7** of artelinic acid (**6**). Compounds with electron withdrawing functional groups in their aromatic ring showed a substantial increase in antimalarial activity. Compounds with a small substituent group at the benzylic carbon atom show weaker activity than compounds with a larger substituent, indicating that lipophilicity and steric aspects of the molecule are also factors [47]. Among this modified artelinic acid series, carboxylic acid **7a** showed superior results vs. artelinic acid (**6**) in *in vivo* oral antimalarial activity against *P. berghei* [48].

Other C-10 substituted highly active ether analogs are the bis-trioxane analogs **8a** and **8b** [49]. Their *in vivo* activity is comparable to that of arteether (**4**) [49].

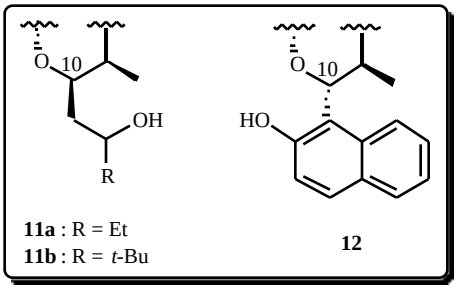
Another class of C-10 substituted analogs is deoxoartemisinin (**9a**), which lacks the carbonyl group at C-10. It is therefore presumed to be more stable in the body [50]. It is slightly more active than arteether (**4**) *in vitro*, but not as active as DHA (**2**) *in vivo* [50].

Fluorinated compounds often exhibit interesting biological properties. Some previously synthesized fluorinated analogs of artemisinin have shown higher antimalarial activities than their nonfluorinated precursors [51, 52]. A C-10 fluorinated compound, 10α-trifluoromethylhydroartemisinin **10**, was synthesized [51]. Analog **10** has higher *in vivo* activity than artemisinin (**1**) [51]. Unfortunately, it has a short plasma half-life.

Artemisinin (**1**) and all other first generation drugs are believed to act as prodrugs for DHA (**2**). Metabolic studies indicate that arteether is rapidly converted into DHA (**2**) [53, 54]. In order to produce derivatives that would not be a prodrug for toxic DHA (**2**), 10β-alkylartemisinin derivatives were prepared. The compound 10β-*n*-butyldeoxoartemisinin (**9b**) has *in vivo* activity approximately equal to that of artemisinin (**1**) [55]. Other 10β-alkyldeoxoartemisinin derivatives were prepared [54, 56]. 10β-Allyldeoxoartemisinin (**9c**) was converted into several



promising derivatives, of which 10β-*n*-propyldeoxoartemisinin (**9d**) was the most promising. The *in vivo* results with 10-*n*-propylartemisinin (**9d**) indicate that its activity and toxicity are comparable to those of arteether (**4**) [54]. In another series of 10β-alkyl series, the most promising analogs are alcohols **11a** and **11b** [57]. They are 5-7 times more active *in vitro* than artemisinin (**1**). In a series of aromatic C-10 substituted analogs, some show high activity *in vitro* [58]. The C-10 naphthyl substituted derivative **12** exhibited antimalarial activity similar to that of artemether (**3**) *in vivo* [59]. Jung also used the C-10 substituted series to add a water-soluble group [60]. The preliminary *in vitro* data suggest the compounds are comparably active to artemisinin (**1**) [60].

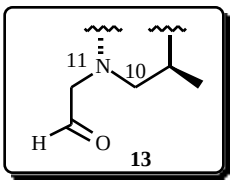


11a : R = Et
11b : R = *t*-Bu

12

11-AZAARTEMISININ ANALOGS

Most artemisinin analogs are derivatvies of DHA (**2**), which all leave the D ring intact. Since lactams are more stable than lactones towards acidic conditions, such as those found in the stomach, replacing the lactone with the lactam should decrease the hydrolysis of the drug in the stomach [61]. Avery and coworkers also reported one azaartemisinin analog to be 50% more potent than artemisinin [61, 62]. The results of the 11-azaartemisinin series correspond to the finding of Avery and coworkers that the antimalarial activities of lactams are as high or higher than that of artemisinin (**1**) both in *in vitro* and in *in vivo* tests [56, 61, 62]. The most promising compound, **13**, is approximately as active as arteether (**4**) *in vivo* [61]. Further analogs have been prepared with electronegative substituents on the alkyl chain of the lactam substituent [63, 64]. Again, although some of the new 11-azaartemsinin analogs show promise, their *in vivo* testing results have not yet been reported.



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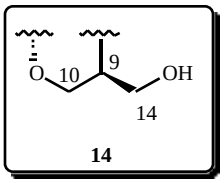
C-14 MODIFIED ANALOGS

Another class of semisynthetic artemisinin analogs is the C-14 modified analogs. The results of this series confirm previous findings of Avery *et al* that substitution at C-14 leads to a several-fold increase in antimalarial activity [65]. These compounds have not yet been tested *in vivo*. Jung *et al*. also synthesized several analogs in this series [66]. Most of these analogs incorporate functionalities that would help increase solubility in water. Of the analogs synthesized, the most promising analog is the alcohol **14**, which is 15 times more potent than artemisinin (**1**) *in vitro* [66].



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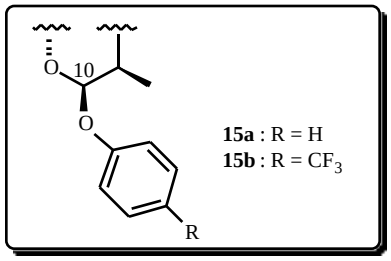
C-10 ARYLOXY ANALOGS

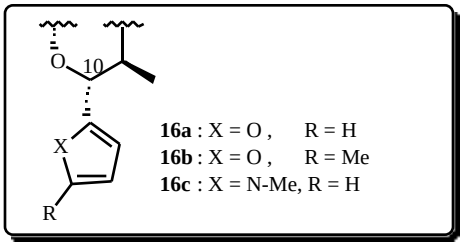
Another way of inhibiting the formation of DHA (**2**) via metabolism by P450 enzymes is to form an ether linkage with a phenyl group rather than with an alkyl group [67]. Therefore, a series of C-10 aryloxy derivatives was synthesized and was found to have high antimalarial activity, comparable to that of artemether (**3**) [67, 68]. The lead compounds, C-10 phenoxy **15a** and C-10 fluorophenoxy **15b**, show the most promising results *in vitro*. The *in vivo* results with the fluorinated compound **15b** indicate that its activity is equal to that of DHA (**2**) and thus higher than that of artemether (**3**) [68]. Further tests *in vivo* by WHO also showed that it is a more potent antimalarial than sodium artesunate (**5**) [68].



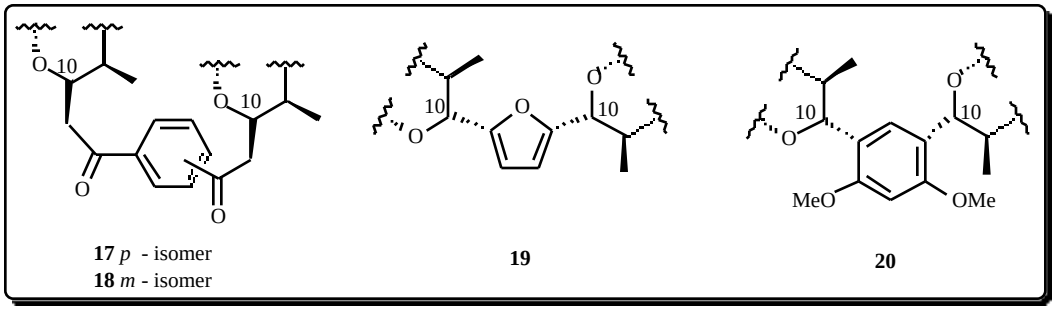
15a : R = H
15b : R = CF_3

MONOMERS AND DIMERS

C-10 aryl and C-10 heteroaryl monomeric and dimeric artemsinin derivatives **16a-20** were also synthesized. Most of the monomeric C-10 aryl analogs are as active as artemisinin *in vitro* [69]. The most promising monomers were tested for *in vivo* activity. The *in vivo* results show that all three monomers **16a-16c** are more active than artemisinin (**1**) or chloroquine, with pyrrole **16c** comparable to artemether (**3**) [69]. The furan derivatives **16a** and **16b** were also submitted for toxicity studies. Their safety is comparable to that of artemether (**3**) [69].



16a : X = O , R = H
16b : X = O , R = Me
16c : X = N-Me, R = H



17 *p* - isomer
18 *m* - isomer
19
20

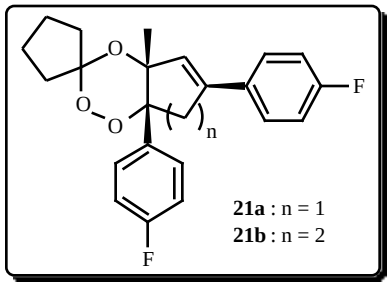
Some dimeric structures have high biological activities. It has already been shown that some trioxanes have high antimalarial, and even antiproliferative and antitumor activities [70]. Unfortunately most of the previously synthesized dimers have only moderate stability. As with other derivatives, in an attempt to improve stability, the C-10 acetal functionality was replaced with a C-10 non-acetal linkage. The new C-10 non-acetal linkage improved the stability of this series of dimers [70]. Some of these dimers possess high antimalarial, antiproliferative, and antitumor activities. The dimers with the highest activites are **17-20** [70]. Although dimers exhibit higher activity than monomers, activity of the dimers is not twice as high as that of the monomers. Dimer **17** also has desirable antiproliferative and antitumor properties, including high *in vivo* antitumor potency [70].

SIMPLIFIED SYNTHETIC ENDOPEROXIDES: INTRODUCTION

Investigations of structure-activity relationships (SAR's) identified the 1,2,4-trioxene as the critical pharmacophore and suggested that neither the peroxide function, nor the 1,2,4-trioxane ring alone, are sufficient for maximum efficacy [71]. However, with the notion that ring A and lactone ring D of artemisinin are not essential [72] for antimalarial activity, much effort was made to design and synthesize simpler drug candidates that could be prepared from inexpensive and commercial starting materials that are readily available in bulk quantities. Representative compounds include the cis-fused bicyclic 1,2,4-trioxanes synthesized by Jefford and coworkers, the sulfone endoperoxides synthesized by Bachi and coworkers, the dispiro-1,2,4,5-tetraoxanes synthesized by Vennerstrom and coworkers, the 1,2,4,5-tetraoxacycloalkanes synthesized by Nojima , McCullough and Wataya, and the tricyclic simplified 1,2,4-trioxanes synthesized by Posner and coworkers.

FENOZANS

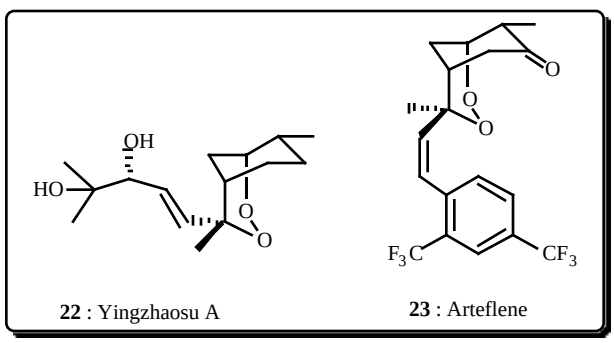
Various *cis*-fused cyclopenteno-1,2,4-trioxanes **21a** and *cis*-fused cyclohexeno-1,2,4-trioxanes **21b** were synthesized and examined against chloroquine-resistant malaria parasites *in vivo*. Among them Fenozan B07 (**21a**) proved to have the most potent blood schizontocide effect when adminstered orally in animals [73, 74, 75]. Recently many modifications of *cis*-fused cyclopenteno-1,2,4-trioxanes, such as epoxidation, aziridination, and dimerization were performed in order to increase efficacy. However, no enhancement of antimalarial activity was seen [76].



21a : n = 1
21b : n = 2

ARTEFLENE AND SULFONE ENDOPEROXIDES

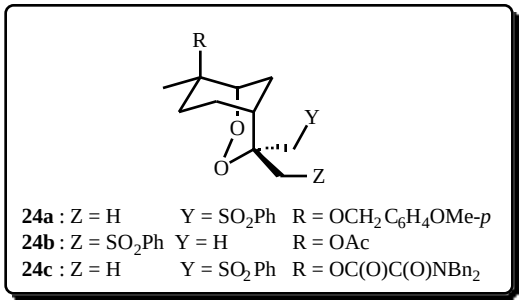
Yingzhaosu A (**22**), a structurally simpler endoperoxide than artemisinin (**1**), was isolated from a traditional Chinese herb. Due to the limitations imposed by a minute supply of yingzhaosu A (**22**) and the long tedious total synthesis, structurally related but simplified analogs containing the 2,3-dioxabicyclo [3.3.1] nonanes were synthesized. Successful analog arteflene (Ro 42-1611, **23**) is a highly active, synthetic antimalarial endoperoxide. This ring system is chemically more stable than that of artemisinin (**1**) and its first generation derivatives [77]. Arteflene (**23**) is an effective agent for treating mild cases of *P. falciparum* malaria [17, 77]. Arteflene (**23**) has a lower rate of recrudescence, longer lasting therapeutic effects, and a longer half life than that of artemisinin (**1**) and its derivatives [78]. Although arteflene (**23**) passed sucessfully through clinical trials, Hoffman La-Roche discontinued its production in part since its long and tedious synthesis was not suitable for large-scale production [79].



22 : Yingzhaosu A
23 : Arteflene

Significant advances in 1998 enabled a simpler, easier synthesis of yingzhaosu A analogs [80, 81]. A series of

endoperoxides **24** containing a sulfide or a sulfone group were synthesized. Antimalarial activity was determined against chloloquine-sensitive *P. falciparum* (NF54) *in vitro*. Sulfone analogs show superior activity to sulfide analogs and exhibit *in vitro* antimalarial activity comparable to that of arteflene (**23**) [82]. Some members of this class of sulfone endoperoxides have a good *in vivo* therapeutic index (efficacy/toxicity) (unpublished data of M.D. Bachi *et al*.).

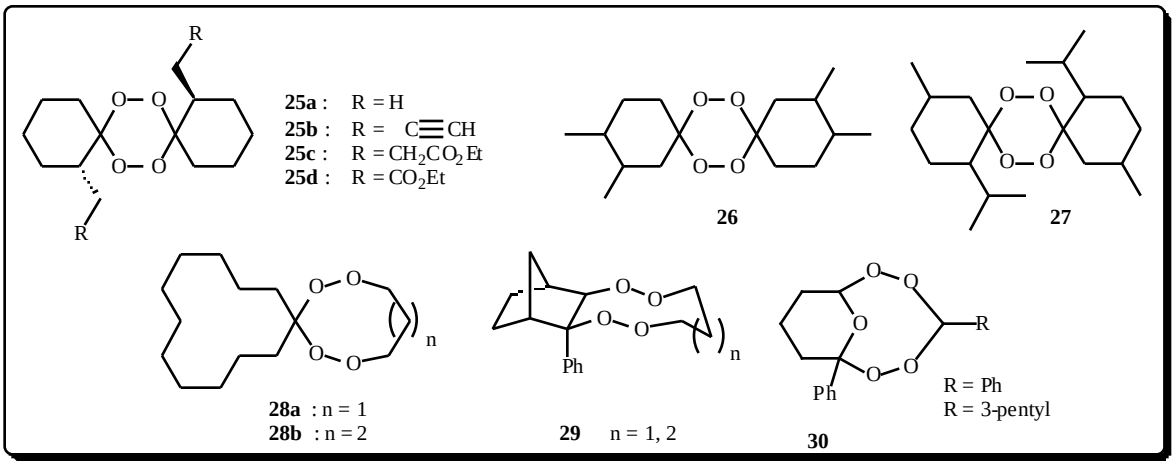


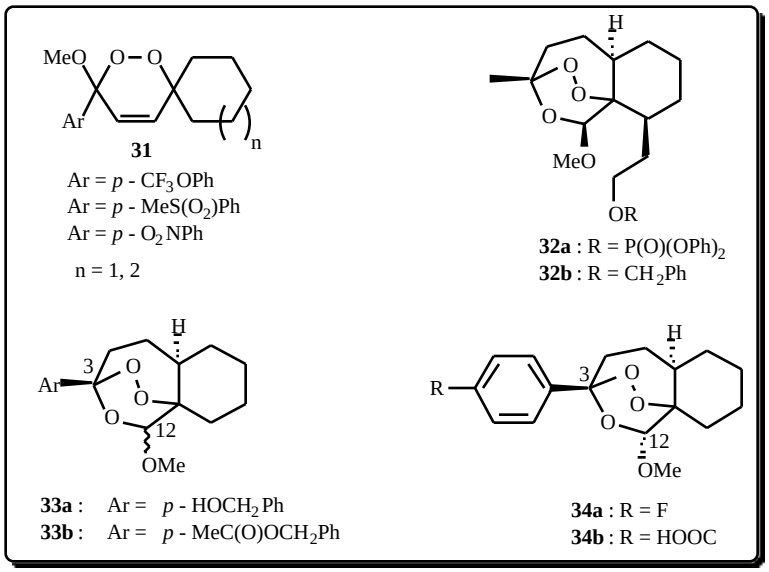
24a : Z = H Y = SO_2Ph R = $OCH_2C_6H_4OMe$-*p*
24b : Z = SO_2Ph Y = H R = OAc
24c : Z = H Y = SO_2Ph R = $OC(O)C(O)NBn_2$

TETRAOXANES

Since the endoperoxide is an important functional group for antimalarial activity, Vennerstrom and coworkers synthesized molecules with two endoperoxide groups, dispiro-1,2,4,5-tetraoxanes **25**, via acid-catalyzed peroxy ketalization. The tetraoxanes represent a new class of potent, inexpensive peroxide antimalarial agents including WR148999 (**25a**), which can be readily synthesized in one step from inexpensive materials. Teraoxane **25a** has an IC_{50} comparable to that of artemisinin against *P. falciparum in vitro*, and an ED_{50} equivalent to that of artemisinin against *P. berghei in vivo* [83]. In order to maximize antimalarial activity and elucidate the mechanism of action for tetraoxane **25a**, many structurally diverse dispiro-1,2,4,5-tetraoxanes were synthesized. The ozonolysis of *O*-methyl oximes was introduced for the preparation of dispiro-1,2,4,5-tetraoxanes, some of which are inaccessible by the acid-catalyzed peroxidation method [84]. Various tetraoxane analogs **25b-d** bearing unsaturated and polar functional groups, which were installed for better oral administration, showed enhanced antimalarial activity against K1 and NF54 strains of *P. falciparum* compared to the corresponding IC_{50} value of the prototype **25a**. However, none of the analogs that were active *in vitro* were as effective as **25a** or artemisinin (**1**) *in vivo* [85]. In SAR studies of sixteen alkyl-substituted analogs of the prototype tetraoxane **25a**, the tetramethyl substituted tetraoxane **26** and diisopropyl-dimethyl substituted tetraoxane **27** showed a better IC_{50} against chloroquine-sensitive D6 and chloroquine-resistant W2 clones of *P. falciparum* compared to the tetraoxane **25a**. These tetraoxanes, together with artemisinin (**1**) as control, were administrated (po) to *P. berghei*-infected mice. Tetraoxanes **26** and **27** cured between 40% and 60% of the infected animals. In comparison, artemisinin (**1**) and the prototype tetraoxane **25a** produced no cure [86]. Recently, tetraoxane **25a** was shown to be synergistic with chloroquine, mefloquine, and artemisinin (**1**) against both D6 and W2 clones of *P. faciparum*, and research is proceeding to elucidate the mechanism of action along with design of better analogs [87].

The observation that dispiro-1,2,4,5-tetraoxanes **25** exhibit remarkable antimalarial activities *in vitro* and *in vivo* [85] gave rise to speculation that other types of cyclic peroxide systems might be valuable. Various cyclic peroxide systems having two geminal peroxide units within the same ring (e.g. 1,2,4,5-tetraoxacycloalkanes **28**) were prepared [88]. Preliminary studies of antimalarial activity of the tetraoxacycloalkane **28a** against *P. falciparum* showed an IC_{50} value as low as 3.0 nM compared with 7.8 nM for artemisinin (**1**). Other systems, such as 1,2,5,6-tetraoxacycloalkanes **29**, 1,2,4,5,7-pentaoxacycloalkanes **30** were also synthesized and a preliminary study of antimalarial activity against *P. falciparum* was performed [89, 90]. Subsequent evaluation of antimalarial activity of various tetraoxacycloalkanes showed that tetraoxacycloalkane **28a** has considerable potential as a new antimalarial drug candidate. Administrating **28a**, prepared in various concentrations in olive oil, to infected mice by either by intraperitonial injection (ip) or oral administration (po) resulted in no parasites in their blood after a 4 day suppressive study. Consistent with this result, all infected mice were cured and experienced no cytotoxic effects for more than 60 days. A similar treatment of infected mice with artemisinin (**1**) resulted in death during 18 days due to *P. berghei*. infection [91].





CYCLIC PEROXY KETALS AND SIMPLIFIED TRIOXANES

Various structurally simplified cyclic peroxy ketals **31** were synthesized. The SAR studies revealed the importance of the cycloalkane ring size and the electronic effect in the aromatic ring. Feedback from *in vitro* antimalarial testing has allowed rational design and short synthesis of several analogs which have antimalarial potencies between 1/4 to 1/10 that of artemisinin (**1**) [92]. However, the high antimalarial activity of these peroxy ketals was offset by their short plasma half-life in vivo (unpublished results of G.H. Posner).

A series of ester and ether derivatives of trioxane alcohol **32**, R = H, were synthesized [93]. Many of these compounds were highly efficacious *in vivo.* In preclinical studies, phosphate ester **32a** and benzyl ethers **32b** were shown to be as efficacious as arteether (**4**) against multidrug-resistant *P. falciparum* in *Aotus* monkeys. This result supported the idea that the lactone ring of artemisnin (**1**) is not essential for antimalarial activity. Based on a detailed understanding at the molecular level of the chemical cascade leading from simplified trioxanes to various cytotoxic chemical intermediates [94], a number of 3-aryltrioxanes **33** and **34** were synthesized [95]. 3-Fluorophenyltrioxane **34a**, which had promising *in vitro* activity, showed *in vivo* antimalarial activity comparable to that of artemisnin (**1**) in rodents against chloroquine sensitive *P. berghei*. Preclinical toxicity evaluation of 3-fluorophenyltrioxane **34a** revealed that it is also safer than arteether (**4**) [96]. In order to install a desirable characteristic for oral administration, a water-soluble, thermally stable simplified trioxane containing a phenyl group at C-3 was designed [96]. 3-*p*-Carboxyphenyltrioxane **34b** was prepared, with practical use of air (rather than pure O_2). This water-soluble 3-carboxyphenyltrioxane **34b** was evaluated for preclinical efficacy and toxicity against *P. berghei*, in comparison with water-soluble artelinic acid (**6**). In mice, trioxane **34b** was about four-fold less efficacious than artelinic acid (**6**) via sc and iv administration. In rats, a dose of trioxane **34b** six-fold higher than that of artelinic acid (**6**) showed no toxicity, whereas artelinic acid (**6**) showed significant toxicity. Thus, the therapeutic index for the synthetic trioxane **34b** is at least as good as that of artelinic acid [96].

CONCLUSION

Endoperoxides have shown much promise in antimalarial chemotherapy. First generation drugs are already widely used in the treatment of patients having malaria in areas of the world where chloroquine and antifolate resistance is widespread and where resistance to quinine and to mefloquine is spreading. Based on structure-activity relations, many research groups have designed and synthesized semi-synthetic and synthetic endoperoxides. Hundreds of analogs have been synthesized and tested against antimalarial activity *in vitro*. Although some analogs show great promise *in vitro*, their *in vivo* results often are not as good as hoped. Therefore, *in vivo* data are crucial for selecting new potential antimalarial agents. Relatively few compounds have been tested for *in vivo* efficacy. A very small portion of those have been further tested for *in vivo* toxicity. Except for semi-synthetic artelinic acid (**6**), all of the new analogs tested for *in vivo* toxicity are synthetic. Two of the older compounds are arteflene (**23**) fenozan B07 (**21a**). The two newer compounds are tetraoxane **28a** and 3-carboxyphenyltrioxane **34b**. These leading candidates will be subjected to further *in vivo* biological evaluation, leading hopefully to a new antimalarial peroxide drug that can be prepared inexpensively and that can be used either alone or, more likely, in combination therapy.

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