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Review Article

The future of antimalarials: artemisinins and synthetic endoperoxides

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Abstract

Malaria is one of the most widespread infectious diseases in the world. *Plasmodium falciparum*, the main strain responsible for human malaria, has developed resistance to most available antimalarial agents. Artemisinin is a sesquiterpenic lactone isolated from *Artemisia annua* L., a plant used in traditional Chinese medicine. It is very effective in treating drug-resistant malaria and appears very promising for fighting the disease. Numerous investigations are currently being performed with the aim of elucidating the mechanism of action, determining efficient clinical uses and discovering new drugs with better pharmacological properties. Most investigations revolve around the highly unusual endoperoxide present in artemisinin, which is responsible for the antimalarial activity. Several strategies have been developed for the design of semisynthetic and synthetic endoperoxides with greater metabolic and hydrolytic stability than artemisinin itself. Here we present an overview of this area.

Introduction

Malaria continues to be one of the most important infectious diseases in the world (1). Of the four human malaria parasites, *Plasmodium falciparum* is the major cause of severe clinical malaria and death. Morbidity and mortality associated with malaria are mostly attributable to the increasing resistance of malaria parasites, in particular *P. falciparum*, to the most widely prescribed antimalarial drugs (chloroquine, pyrimethamine, proguanil, halofantrine, etc.) (2). Moreover this situation is aggravated by the emergence of insecticide-resistant strains of mosquito vectors. Faced with the alarming decline in the efficacy of antimalarial drugs, a broad consensus on the need to develop new antimalarial drugs is now well established (3). Artemisinin (Fig. 1), isolated from the plant *Artemisia annua* L. and used to treat high fever for over 2,000 years in Chinese traditional medicine (4), has emerged as a promising drug. This naturally occurring sesquiterpene lactone contains an endoperoxide bridge. This highly unusual function in medicinal chemistry provides a unique molecular framework from which medicinal chemists can prepare more effective antimalarial drugs while retaining the pharmacologically essential 1,2,4-trioxane core.

Clinical importance of artemisinins

Artemisinin derivatives (artemisinins) are fast-acting and potent antimalarial drugs against all *Plasmodium* parasites, in particular multidrug-resistant *P. falciparum* (5). They act at the early stages of development of parasites once they have invaded red cells (6). Artemisinins are gametocytocidal, but they do not kill the hepatic stages of parasites (7). No clinical cases of resistance to artemisinins have been reported so far (8).

The therapeutic value of artemisinin itself is limited by its low solubility in both oil and water. To overcome these solubility problems, a first generation of semisynthetic analogues –dihydroartemisinin (DHA) (**1**), artemether, arteether and sodium artesunate– were prepared in the 1980s (Fig. 1) (9, 10). Intravenous and oral formulations of these derivatives, as well as suppositories, have been developed and are now available.

Artemisinins have had a substantial impact on the treatment of malaria (11-13). Artemisinins are the only antimalarials, along with quinine, used to treat severe and cerebral malaria. In comparative studies, artemisinins have shown an unequivocal benefit, particularly in the

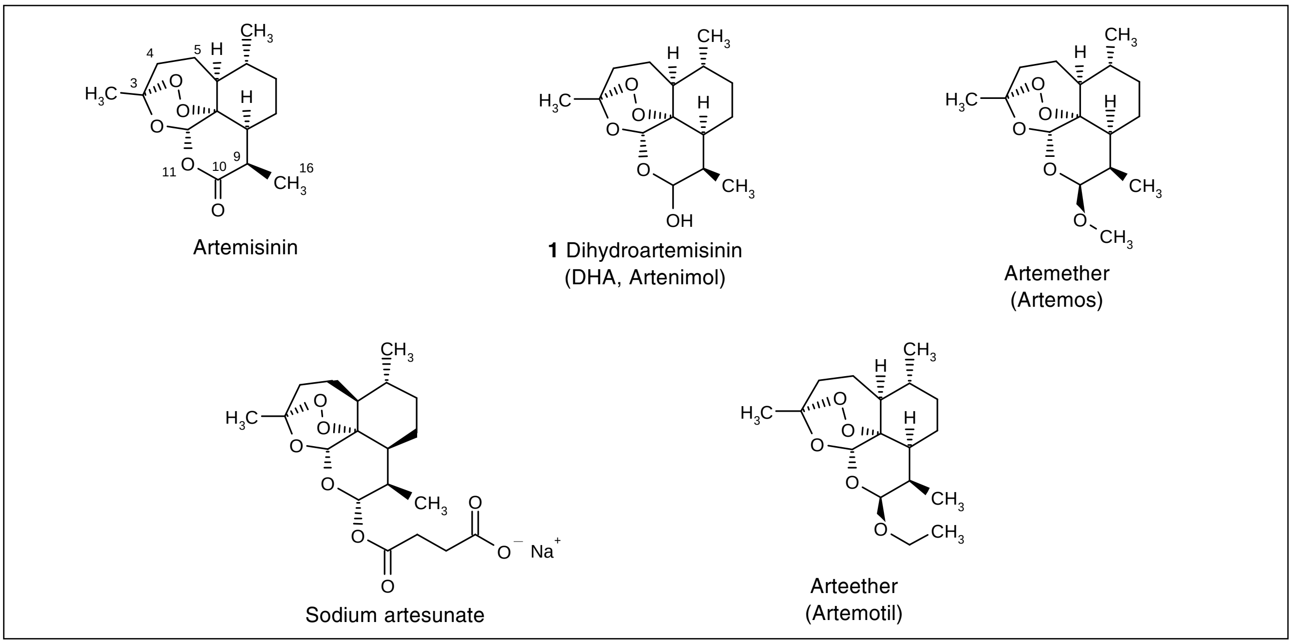


Fig. 1. First-generation artemisinins.

treatment of children, and present several other clinical advantages over quinine (lack of local toxicity, wider therapeutic margins, etc.) (14, 15). Moreover, rectal administration is more efficient than the parenteral administration used for quinine (15). This aspect is of great importance in the treatment of patients in remote areas, since they can receive intrarectal artemisinins prior to transportation to healthcare facilities.

Artemisinins are not recommended as prophylactic antimalarials in order to minimize the risk of selection for artemisinin-resistant parasites, but also because of their short elimination time. Due to this latter drawback, monotherapy with artemisinins for nonsevere malaria requires prolonged regimens to counter recrudescence (16). The principle of drug combination therapy, which has been successfully applied to tuberculosis, AIDS and cancer, has emerged as the best strategy for malaria control and is recommended by the WHO (18). Artemisinins, which provide a rapid reduction in the parasite biomass, are combined with an antimalarial drug with a longer half-life (17). Despite the problem of cost, great advantages are gained in suppressing both recrudescence and the selection of resistant strains (18-20). A number of fixed-dose combinations of first-generation artemisinins with standard antimalarials (amodiaquine, chloroquine, mefloquine, sulfadoxine/pyrimethamine, etc.) have been studied in randomized, controlled trials in Africa (21). Novartis markets coartemether, a combination of artemether and lumefantrine, as Riamet® in developed and nonendemic countries, and, at a preferential price, as Coartem® in malaria-endemic countries. It provides a safe treatment for *P. falciparum* malaria (22, 23). The combination of dihydroartemisinin and piperaquine is registered in China as Artekin™ (24). Several combinations of artesunate with other antimalarials are in clinical development, for example with chlorproguanil and dapsone (Lapdap™) (GlaxoSmithKline and WHO/TDR), with pyronaridine (Korea SP Pharma, WHO/TDR and MMV [Medicines for Malaria Venture]) or with amodiaquine (Drugs for Neglected Diseases Initiative [DNDi] and Sanofi-Aventis).

Mechanism of action of artemisinins

The unusual peroxide bridge of artemisinins is an absolute requirement for the antimalarial activity. When the endoperoxide is lacking, as in the reduced desoxoartemisinin, the compound is devoid of antimalarial activity. This peroxide function has become a focus for experiments aimed at understanding the mode of action of artemisinins and other trioxanes.

The digestion by several proteases (plasmepsins and falcipains) of hemoglobin from infested human erythrocytes occurs in the food vacuole of the parasite, releasing heme (protoferriprotoporphyrin IX) and amino acids required for the feeding of parasites. Heme is then detoxified by dimerization to form hemozoin, the insoluble malaria pigment. Numerous hypotheses have been proposed to explain the mechanism of action of artemisinins. A widely held view is that the specific antimalarial effect of artemisinin is due to activation by heme in the food vacuole, generating free radicals, which are able to alkylate vital proteins of the parasite or interact with the heme detoxification process, inducing in both cases parasite death (25-27). In these processes, Fe^{2+} catalyzes the reductive cleavage of the endoperoxide of artemisinin, giving rise to *O*-centered radicals, which could then isomerize to alkylating *C*-centered radicals (Fig. 2) (26-29).

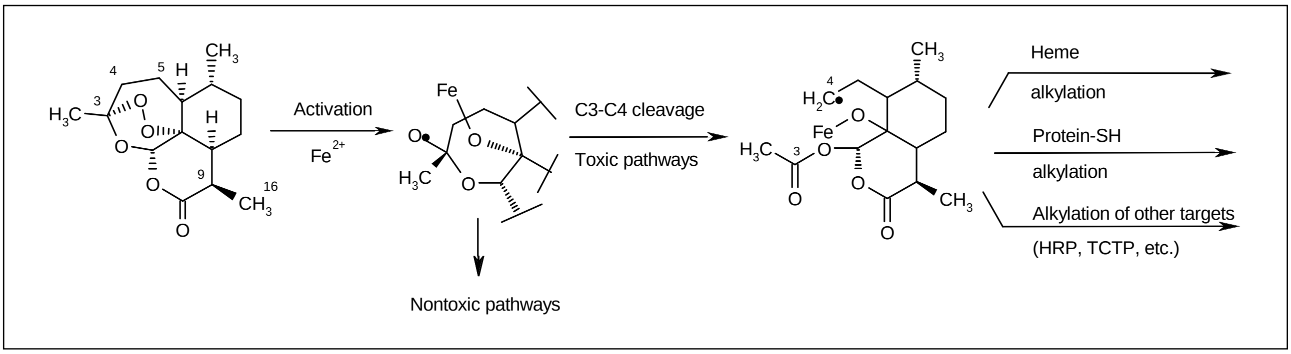


Fig. 2. Artemisinin: radical activation.

The hypothesis of artemisinin activation by ferrous heme is strongly supported by the isolation in high yields of heme adducts following *in vitro* incubation of heme with artemisinins (26, 27, 30). The adduct **2** resulting from alkylation by the *seco-C*-4-centered radical, has been isolated and characterized (Fig. 3) (26, 27). Although these processes have been clearly demonstrated *in vitro*, their relevance to the mechanism of action *in vivo* remains to be shown.

Moreover, this mechanism based on the alkylation of heme is not in agreement with a number of results, in particular the lack of correlation between reactivity towards heme and antimalarial activity (25, 31, 32). Consequently, it has been suggested that these reactive radical intermediates may react with other proteins of the parasite. Among these potential targets, cysteine proteases (falcipains II and III) present in the digestive vacuole (33), the histidine-rich protein (HRP), a protein suspected to catalyze the formation of hemozoin (31, 32), or the parasitic translationally controlled tumor protein (TCTP) (34) have been proposed. In the latter case, despite the isolation of the labeled protein after incubation of the parasite with radiolabeled artemisinins (34), more detailed studies seem to indicate that this interaction is not important for antimalarial activity (31, 32, 35).

Finally, it recently emerged that artemisinins may be activated only through specific binding to the active site of the target, and therefore they do not act directly as proradicals. This view is strongly supported by the very high activity of artemisinins (nanomolar or subnanomolar IC_{50}), and by the great sensitivity of the *in vitro* antimalarial activity to steric factors. For instance, replacement of the 3-Me by larger groups, inversion of the 9-Me configuration or introduction of a substituent at 4-C or 5-C markedly attenuates the antimalarial activity (36).

Along this line, a protein target has been recently proposed for artemisinin, based on the structural similarities of artemisinins with the core of another sesquiterpene lactone, thapsigargin. The latter is a highly specific and potent inhibitor of SERCAs, mammalian sarcoplasmic reticulum Ca^{2+}-transporting ATPases (37). These proteins reduce cytosolic free calcium by actively concentrating Ca^{2+} into membrane-bound stores. Similar to thapsigargin, artemisinins may interact specifically with the PfATP6 (a *P. falciparum* calcium-dependent ATPase), structurally very close to SERCAs (37-39). It has been demonstrated that artemisinin specifically and irreversibly binds and inhibits PfATP6 in the endoplasmic reticulum. This inhibition is Fe^{2+}-dependent. Other evidence strongly suggests that artemisinins exert their antimalarial activity by selectively targeting the PfATP6 enzyme (25, 31, 32, 37).

It can be concluded from these complementary but often contradictory experiments that the activity of artemisinin is probably multifactorial (38, 39). As frequently observed in medicinal chemistry, highly effective drugs interact simultaneously and efficiently with several targets.

The future for the treatment of malaria

While the low oral bioavailability of artemisinins is partially countered by their very high intrinsic activity, the short half-life in the body requires protracted regimens to avoid recrudescence of parasitemia. With the aim of improving the oral bioavailability of artemisinins, a great number of compounds have been synthesized from artemisinin, with two objectives: first, derivatives should be metabolically and hydrolytically more stable, and second, polar or ionizable functions must be introduced to increase water solubility. These semisynthetic artemisinins essentially result from structural transformations at the 9-, 10- and 11-positions (*cf.* Fig. 1). At the same time, based on the peroxide pharmacophore, a very large number of synthetic endoperoxides and other trioxanes have been prepared *de novo* with the hope of finding an alternative to the semisynthetic approach.

Although the toxicity of the first-generation artemisinins can be considered as very low in patients after more than 10 years of widespread use in endemic areas of the world, particular attention must be paid to their potential neurotoxicity, which has been observed both *in vitro* and at high doses in experimental animals (28, 40, 41). To avoid neurotoxicity, the novel semisynthetic or synthetic endoperoxide drugs for the treatment of nonsevere malaria should be unable to penetrate the

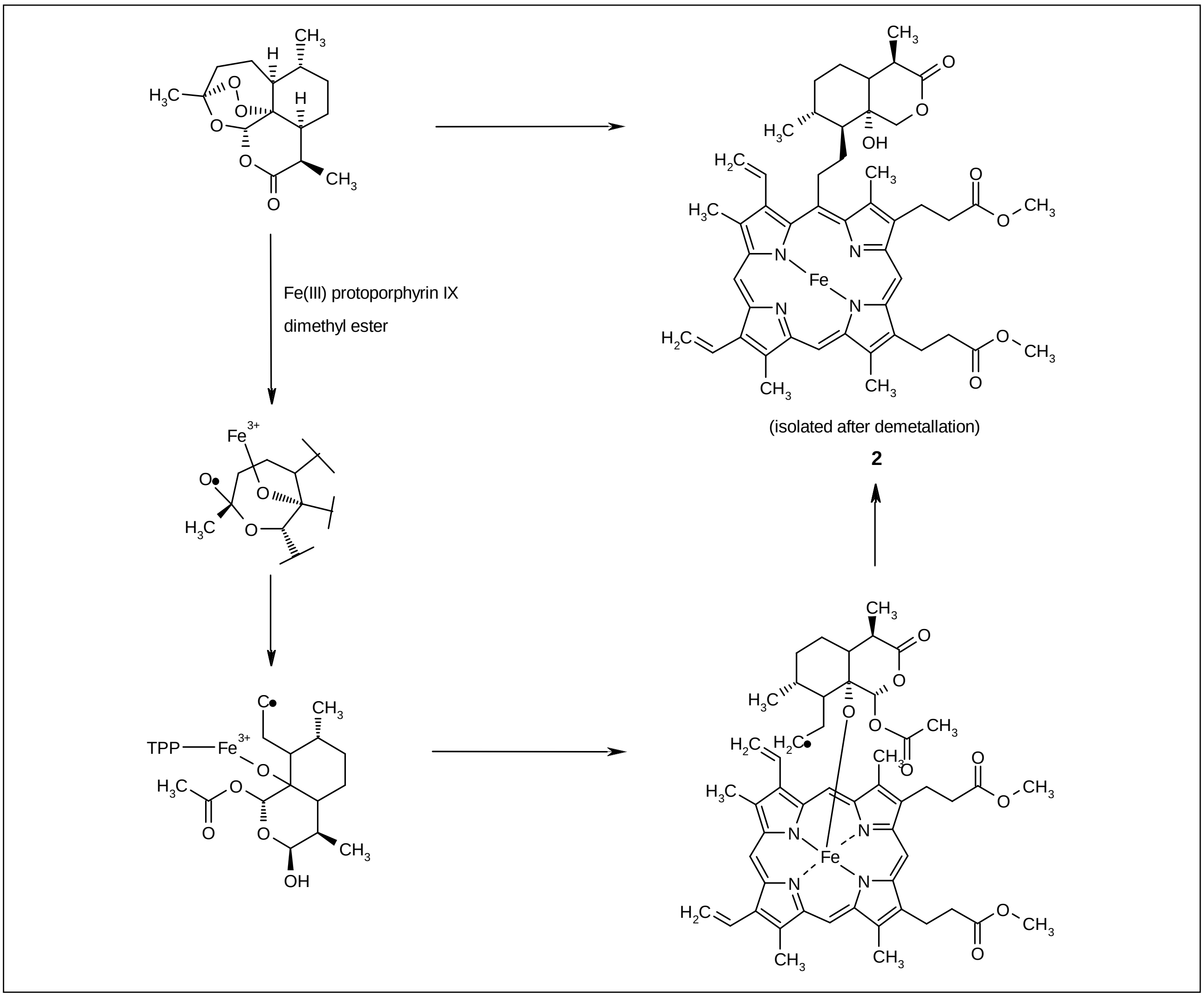


Fig. 3. Reaction of artemisinin with Fe(II) protoporphyrin IX dimethyl ester as a model of heme (29).

blood-brain barrier. It is thus desirable that the log *P* of the drug candidate be lower than the log *P* of artemether (log *P* = 3.5).

New generations of artemisinins

Artemisinin is a cheap and readily available natural material. The large-scale isolation and purification of crystallizable artemisinin from wild or cultivated *A. annua* are easy and not costly (more than 10 tons/year are produced in China and Vietnam) (25, 42, 43). In fact, availability and price should not be impediments to the development of semisynthetic artemisinins, compared to synthetic endoperoxides or many other antimalarials, which has encouraged chemists to search for new-generation artemisinins.

The numerous chemical modifications to artemisinin have been extensively reviewed (25, 44-46). Interestingly, from a chemical point of view, despite the presence of the endoperoxide bridge, the artemisinin structure tolerates a large variety of chemical reactions without damage. For instance, the endoperoxide is generally stable towards Lewis acids, which allows the generation of an oxonium ion from DHA. Further reaction with alcohols, phenols, electron-rich aromatics, silyl enol ethers, functionalized silanes, alkynes, etc., provided a series of 10-substituted analogues of artemisinin (47-50). Furthermore, nucleophilic addition can also be realized directly on the lactone function of artemisinin with several nucleophiles (51, 52). Radical chemistry is also possible without reduction of the endoperoxide bridge (53). Only reactions involving catalytic reductive conditions (Zn, Fe^{2+}, etc.) remain critical for artemisinin derivatives.

To illustrate current work in this field, the following effective approaches are highlighted:

—The first concerns the preparation of 10-alkyloxy, 10-aryloxy (48) or 10-fluoroalkyloxy DHA, eventually functionalized (54). Some of these DHA ethers have been

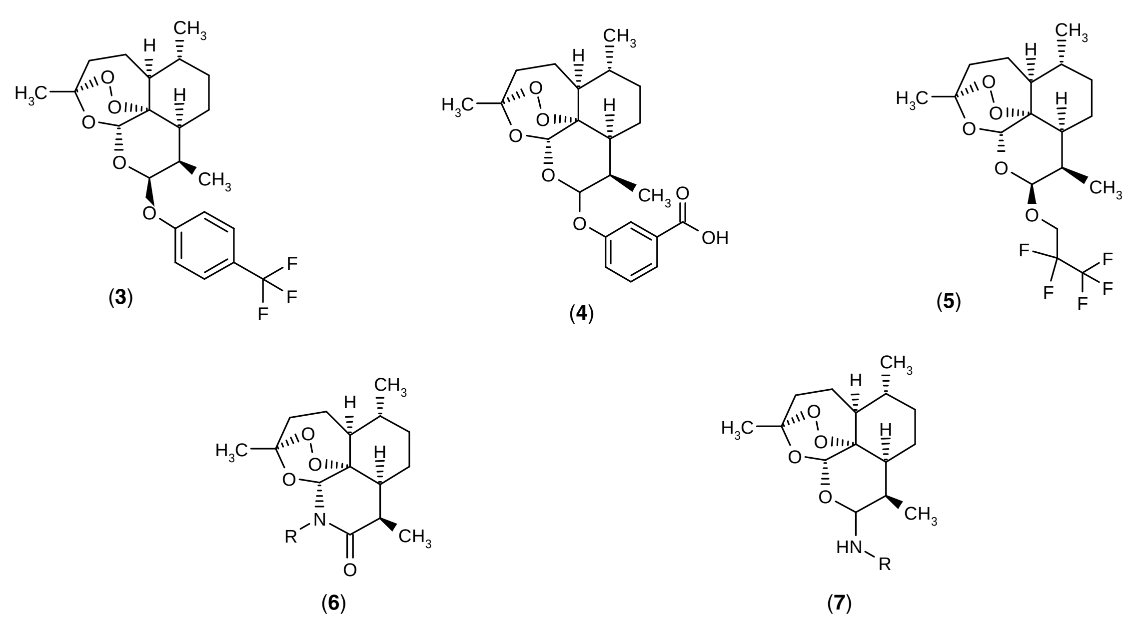


Fig. 4. Second-generation ethers and (*N,O*)-acetals of DHA.

designed to be more resistant to oxidative metabolism. Ethers **3**, **4** and **5** are representative of this approach (Fig. 4) (55, 56). However, since they still contain the 10-acetal functionality, they may be acid-sensitive and they also have a very high log *P* (log *P* > 6 for **5**).

—The replacement of the (*O,O*)-acetal function at C-10 by the (*N,O*)-acetal, as in compounds **6** or **7**, is also effective and very useful (57, 58), as demonstrated by the current clinical development by Bayer and MMV of artemisone, a 10-amino artemisinin of type **7** (58).

—The suppression of the acetal function at C-10 provides 10-carba analogues (types **8**, **9** and **10**) which exhibit high antimalarial activity and are much more robust than DHA ethers towards acidic conditions found in the stomach (25). Current efforts are focused on 10-carba analogues containing polar or ionizable groups with the aim of increasing water solubility and decreasing the log *P*. A good example of this approach is compound **11** (TDR-40292), a potent *in vitro* and *in vivo* antimalarial (Fig. 5) (59).

—All the structural modifications described above concern the C-10 site. Artemisinin derivatives functionalized at C-16 are less easy to prepare. One approach is a Michael addition of various nucleophiles on artemisitene, which is itself easily prepared from artemisinin by photodeshydrogenation (60). This provides compounds of type **12**, which maintain the lactone function (61). Another approach consists in starting from another naturally occurring precursor, artemisinic acid, although it is less available than artemisinin. A multistep synthesis from this acid provides 16-functionalized derivatives of type **13** with no substituent at C-10 (Fig. 5) (62).

—A novel approach to design more metabolically stable artemisinins has been developed by introducing a fluoroalkyl substituent at C-10. Due to its electron-withdrawing character, this substituent was expected to efficiently protect artemisinins from oxidative and hydrolytic cleavage, and from glucuronidation when a hydroxyl is present at C-10. The hypothesis has been clearly validated by the *in vitro* and *in vivo* antimalarial activities of glycals **14a** and **14b** (Fig. 6) (63). Additionally, 10-CF_3 analogues of DHA, artemether, arteether and artesunate have been prepared and found to exhibit very interesting *in vivo* antimalarial properties, better stability under acidic conditions and a prolonged plasma half-life (64). This approach has led to the preclinical development of the orally active 10-trifluoromethyldihydroartemisinins **15** (TDR-9706) and **16** (TDR-22706) (Fig. 7) (56, 65, 66).

Simplified synthetic 1,2,4-trioxanes

Parallel to the semisynthetic approaches from naturally occurring artemisinin, the search for synthetic drugs focused on the endoperoxide pharmacophore has aroused a huge amount of interest from chemists. More than 12 structural classes of endoperoxides have been synthesized and biologically evaluated: mono-, bi- and tricyclic peroxides, spiroendoperoxides, 1,2,4-trioxanes, spiro-1,2,4-ring-fused trioxanes, tetraoxanes and ozonides (1,2,4-trioxolanes) (67). However, only a few drug candidates have emerged from these molecules. Arteflene (**17**) (68), fenozan B07 (**18**) (69) and tricyclic compounds such as **19** (a D-*nor*-artemisinin) (70) (Fig. 8) have acceptable pharmacological properties. However,

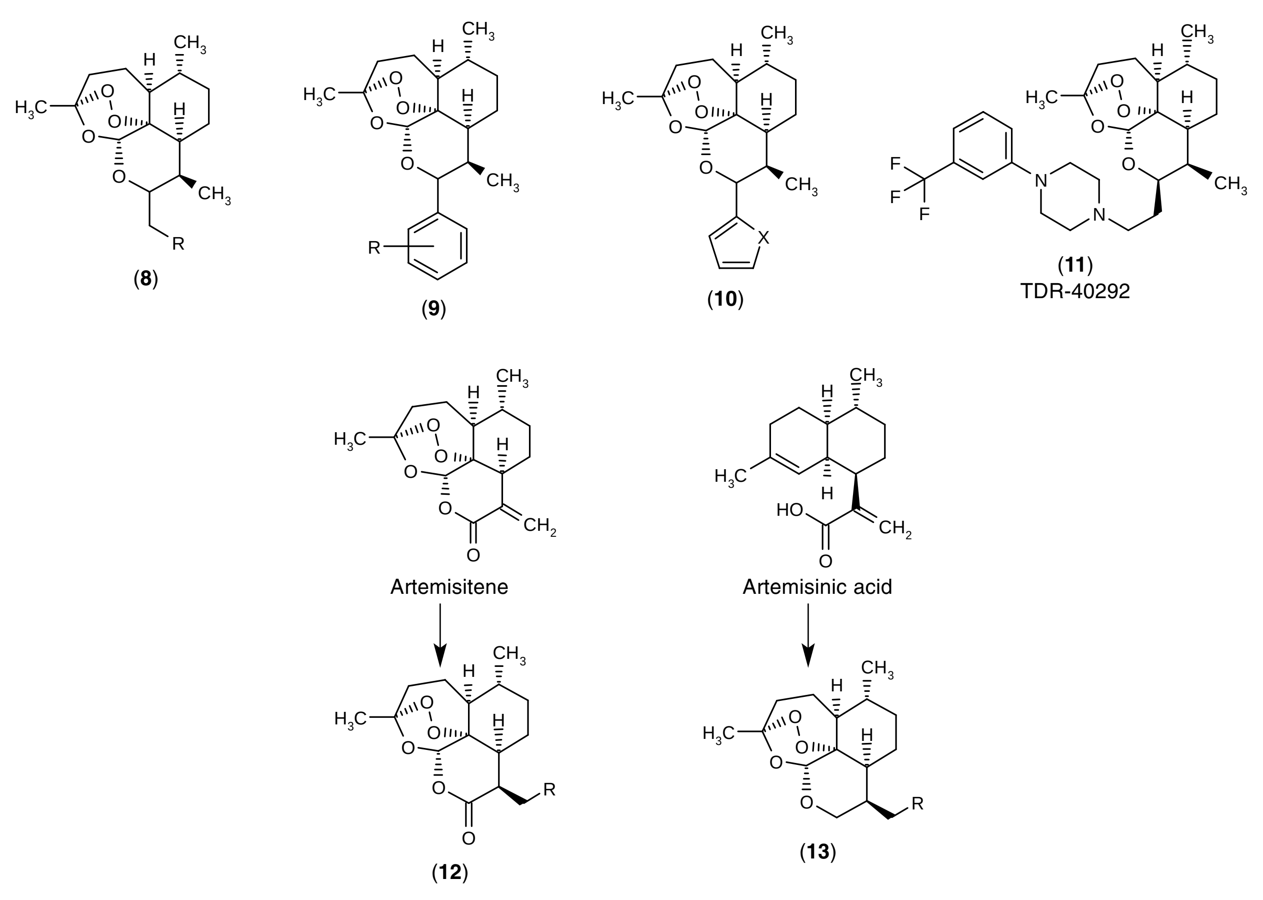


Fig. 5. Main structural variations of artemisinin skeleton for antimalarial drug discovery.

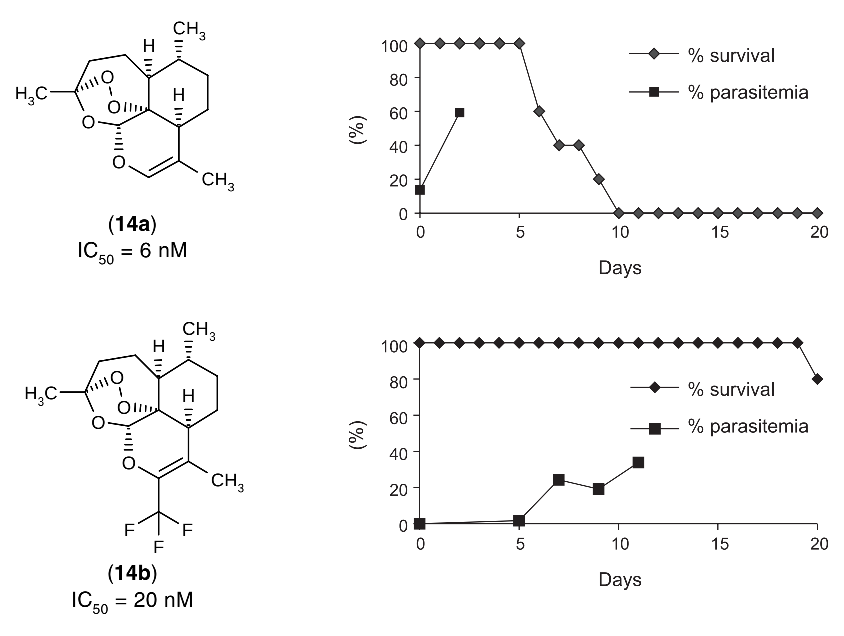
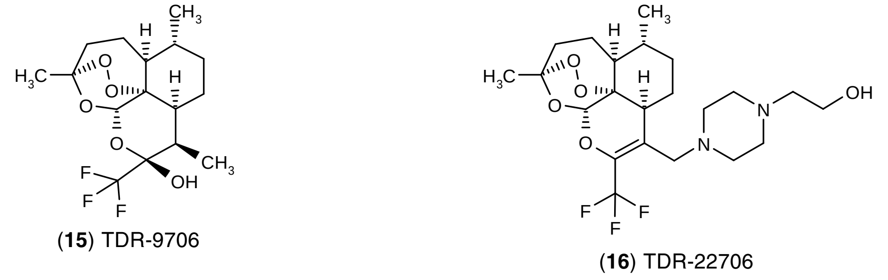


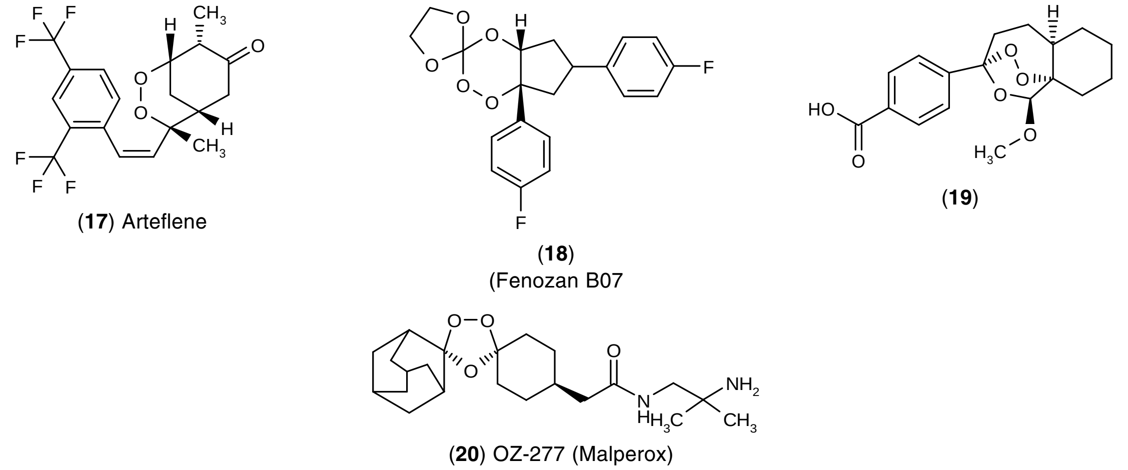
Fig. 6. Effect of fluorine substitution at C-10 on antimalarial activity of artemisinins **14a** and **14b**.



(**15**) TDR-9706

(**16**) TDR-22706

Fig. 7. Fluoroartemisinins in preclinical development.



(**17**) Arteflene

(**18**) (Fenozan B07

(**19**)

(**20**) OZ-277 (Malperox)

Fig. 8. Synthetic endoperoxides.

they have not been developed or were abandoned, mainly because of a lack of definite advantages over semisynthetic artemisinins in terms of activity, synthetic difficulty or cost. Nevertheless, very recently, a synthetic ozonide, **20** (Malperox, OZ-277) (Fig. 8), emerged as a very promising drug candidate and with the support of the MMV it has now entered phase II clinical testing (71). Interestingly the adamantane system protects the unstable ozonide moiety, while the β-amino amide is a key substituent for water solubility and a good pharmaceutical profile.

Hybrid molecules

To complete this overview on semisynthetic and synthetic antimalarial endoperoxides, it is interesting to touch on current research on chimeric molecules, which, by delivering combination therapy via a single chemical entity, are expected to fight the parasite by two distinct mechanisms. Hybrid drugs combine, via a linker, an endoperoxide-containing molecule and an aminoquinoline, most frequently chloroquine or mefloquine (72-74). Chimeric molecules with trioxaquines, such as **21** (72), **22** (73) or **23** (25, 74) (Fig. 9) and the artemisinin-mefloquine hybrid molecules **24** and **25** (75) (Fig. 10) illustrate this approach. In the latter case, the two moieties have been bound either with a nonscissile linker (drug) or with a linker cleavable by esterases (prodrug).

Conclusions

To overcome the rapid and widespread resistance to most standard antimalarial drugs, new antimalarial therapies are emerging. Ideal antimalarial drugs must be efficient, with no recrudescence of parasitemia, well tolerated, orally available and cheap. While awaiting these drugs of tomorrow, the development of drugs based on artemisinins has brought huge progress to the fight against malaria, in particular for the treatment of severe malaria and drug-resistant malaria. The efforts of medicinal chemists are needed to provide more and more efficient and convenient antimalarials with the aim of eventually discovering the “ideal” drug.

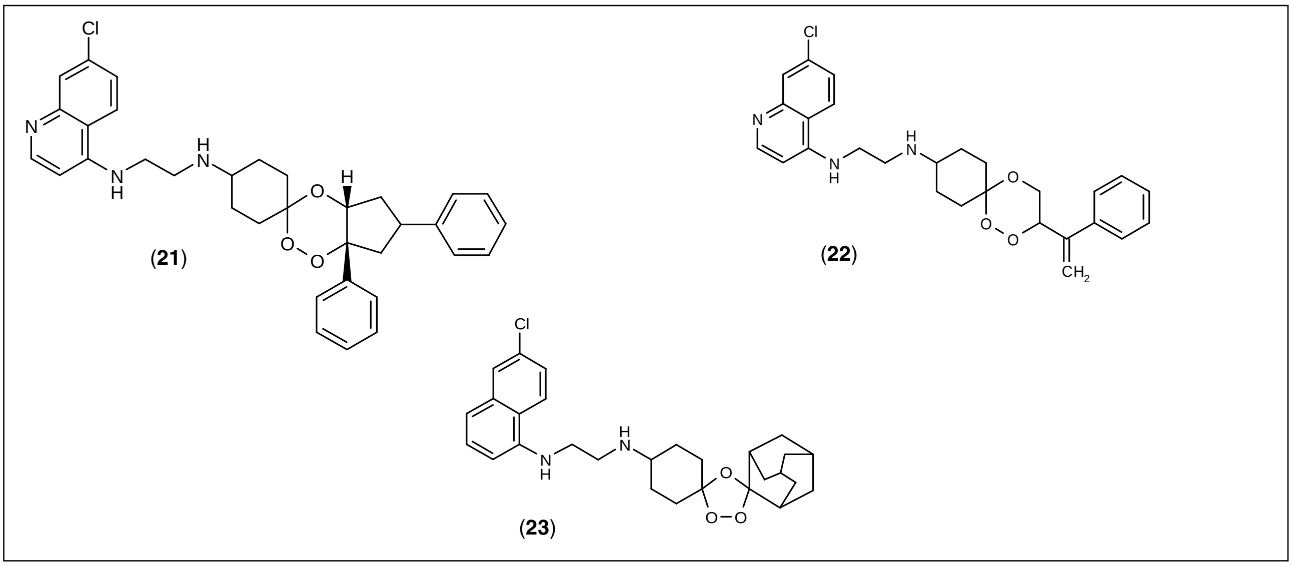


Fig. 9. Hybrid antimalarial endoperoxides

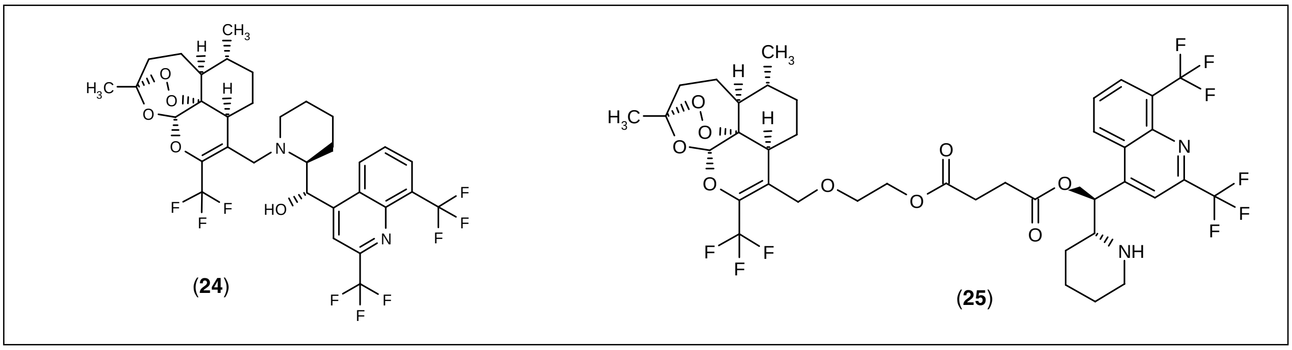


Fig. 10. Hybrid antimalarial artemisinins.

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