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Progress in the development of peroxide-based anti-parasitic agents

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Progress made in the past decade pertaining to the development of anti-parasitic agents based on artemisinin is presented. Apart from discussions on important derivatives obtained through functionalization at the C-3, C-9, C-10 and O-11 positions of artemisinin, an outline on its seco analogs and artemisinin bundles are given for a broader perspective on structure–activity relationships. Success with synthetic peroxides, drug–hybrid approaches, broad-spectrum anti-infective properties of peroxide compounds and a survey on peroxide-containing natural products other than artemisinin with available biological data are included to highlight recent trends and avenues for future research. A supplementary material with details on the biological properties of a larger collection of molecules belonging to the above structural classes is also given for reference.

Introduction

Morbidities and mortalities associated with parasitic infections continue to threaten millions of people worldwide. Estimates show that approximately 300–500 million people are at risk from infections such as malaria, leishmaniasis and trypanosomiasis [1]. Of these, malaria alone causes approximately 1.7–2.0 million deaths each year; the majority of these being children under the age of five. In addition to the lives lost, economic development in many countries is severely hampered by continuous episodes of these diseases. Their co-occurrence with HIV is emerging as a new threat and a significant challenge to overcome [2–4].

Of those drugs available for the treatment of malaria, artemisinin (**1**, Figure 1) and its analogs have received wide attention owing to their fast action and ability to target drug resistant strains of the parasite [5]. Recent years have witnessed a growing number of reports on the chemical modification of artemisinin both to improve its therapeutic profile and to increase understanding of its biological target(s) and mode of action [5,6]. On the basis of the reasoning that the peroxide group in the molecule is responsible for anti-parasitic action, a large number of synthetic peroxides have been synthesized and studied in pursuit of new lead candidates [6]. Excitingly, new studies suggest that such natural and synthetic peroxides are active against parasites such as leishmania and toxoplasma gondii as well, thus increasing their importance as broad-spectrum anti-infective agents. This review aims to give an overview of progress in the development of peroxide-based anti-parasitic agents, with emphasis on studies carried out in the past decade.

Semisynthetic analogs of artemisinin

Although effective, artemisinin (**1**) suffers from drawbacks such as short plasma half-life, limited bioavailability and poor solubility, either in oil or water. Initial efforts to improve these properties led to the development of first generation artemisinin derivatives **2**–**5**, which are presented in Figure 1. Of these, dihydroartemisinin (**2**), which can be obtained from artemisinin by reduction with sodium borohydride, is almost six times more potent *in vitro* than the parent compound [7].

The ether derivatives **3** and **4** are oil soluble drugs and are well absorbed on intramuscular administration. Artesunate (**5**), on the contrary, is water soluble and usually given intravenously [8]. These analogs are effective against uncomplicated *Plasmodium falciparum* malaria, severe malaria, blood stage *Plasmodium vivax* infections and have gametocidal activities. Good safety profiles

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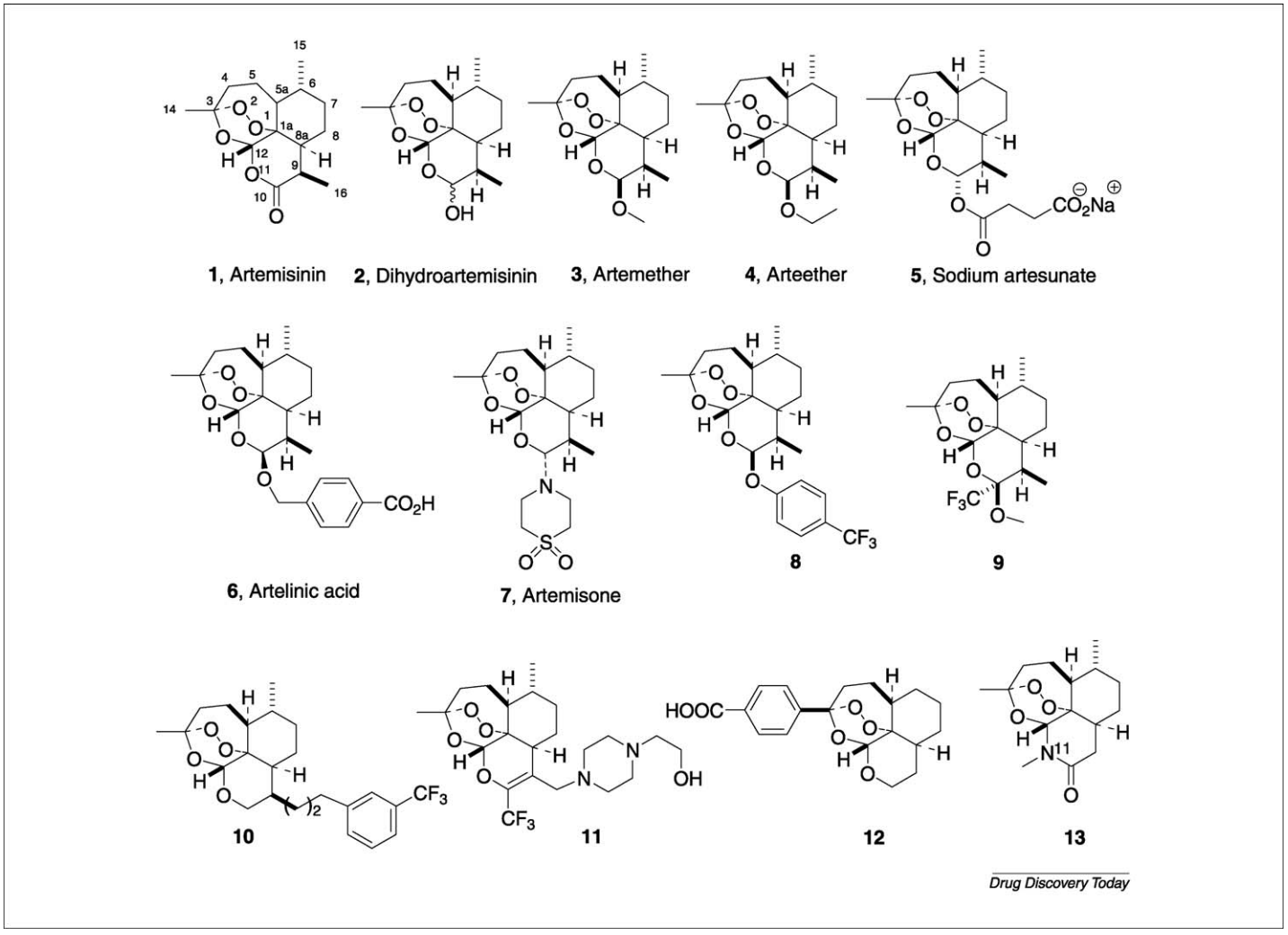

FIGURE 1

Artemisinin analogs with chemical modification at C-10, C-9, C-3 or O-11 positions.

and fast clinical response have made them extremely useful in the treatment of conditions such as cerebral malaria.

Since using artemisinin analogs as monotherapies results in significant recrudescence, they are generally given in combination with other long-acting anti-malarial agents [9]. This strategy, known as artemisinin-based combination therapy (ACT) is currently one of the highly recommended treatment option against parasitic infections [10]. The drugs in these combinations are chosen such that the component with the shorter half-life (e.g. artemisinin analogs) removes a large fraction of parasites, so that the second drug with a longer half-life (e.g. mefloquine, lumefantrine or amodiaquine) would encounter only a smaller fraction. This leads to effective parasite clearance and avoids the risk of resistance development [11].

A co-formulation of artemether with lumefantrine, commonly known as Coartem® is a very effective combination against multidrug resistant strains of *P. falciparum*. Other combinations based on artemisinin that have been recommended for oral administration are (i) artesunate + amodiaquine, (ii) artesunate + mefloquine, (iii) artesunate + sulfadoxine–pyrimethamine, (iv) artesunate + pyronaridine, and (v) chloroproguanil + dapsone + artesunate (CDA™ or Lapdap plus™) [9,12].

Although potent, the analogs **3–5** possess acid-labile acetal functions and exhibit short biological half-lives [13]. Further, they are metabolized to dihydroartemisinin (DHA), which is known to induce neurotoxicity in animal models [14,15]. Since such toxicity has not been observed in humans [16], the main focus during the past decade was to synthesize and study new artemisinin derivatives with improved chemical and metabolic stabilities. This has been approached by the introduction of chemical groups that confer stability to the acetal functionality or the use of non-acetal type linkage at C-10 that would resist metabolism to DHA. A promising lead in this group was artelinic acid (**6**, Figure 1) with better hydrolytic stability than artesunate [17]. Complexes of artesunate or artelinic acid with cyclodextrins have been found to exhibit superior stability in solution, but therapeutic efficacies of such formulations remain to be studied in detail [18].

C-10-alkylamino artemisinin derivatives form another interesting group of anti-malarials that were studied to identify new leads with superior metabolic stabilities and oral bioavailabilities. After preclinical pharmacokinetic and toxicity evaluations, compound **7** from this group, named artemisone, was selected as the lead candidate. Although 'Medicines for Malaria Venture' (MMV) has ended 'active support' to this project in 2005 [19], the latest report

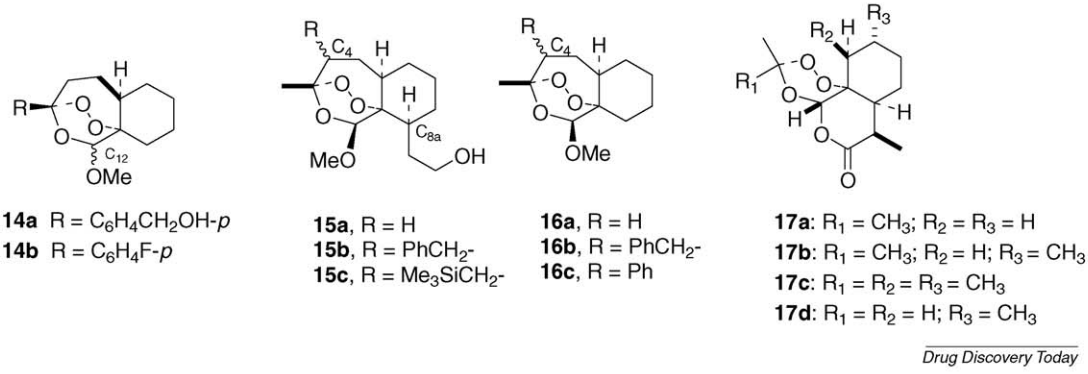

FIGURE 2
Seco artemisinin analogs.

on safety, tolerability and pharmacokinetics assessments conducted in human subjects shows that artemisone has better oral efficacy than artesunate and is devoid of any neurotoxic side effects. Owing to possible recrudescence, artemisone need to be used in conjunction with a long-acting anti-malarial such as mefloquine and is a promising candidate for artemisinin-based combination therapy [20–22].

Phenoxy derivative **8** was designed to thwart phase I oxidative metabolism that is a limitation in first generation ether derivatives **3** and **4.** When studied in mice, **8** showed an ED_{50} (*Plasmodium berghei*, oral route) value of 2.7 mg/kg (*cf.* sodium artesunate = 4.0 mg/kg) and there was no indication of metabolism to dihydroartemisinin [23]. In a related approach Magueur *et al*. have explored the possibility of using a C-10-trifluoromethyl group to improve hydrolytic stability of acetal functionality. Impressively, the lead candidate **9** from this group was ~33 times more stable than artemether in simulated stomach acid, and showed an ED_{50} value of 1.25 mg/kg when tested intraperitonially in mice infected with *P. berghei* (*cf.* artemether = 2.5 mg/kg) [24]. Considering their chemical and metabolic stabilities, compounds **8** and **9** merit further attention and are ideal for advanced studies.

In addition to derivatization at C-10, there have been a number of approaches to identify new artemisinin analogs with superior therapeutic profiles through chemical derivatization at positions such as C-3, C-9 and O-11 [25]. Of these, derivatization at C-9 involved conversion of artemisinin to artemisitene, followed by Michael addition of various donor synthons. An important analog to emerge from this study is the compound **10** (Figure 1) that showed an ED_{50} value of 1.25 mg/kg (*cf.* sodium artesunate = 2.4 mg/kg) when tested orally in mice infected with *P. berghei* (N) [26]. A number of C-9β-phenethyl analogs of artemisinin in this series were remarkably active, but their solubility characteristics need to be optimized to address issues associated with formulation and drug absorption [26]. From a related study, Grellepois *et al*. have reported the preparation of a number of C-9 analogs through direct derivatization of 16-bromo-10-trifluoromethyl anhydrodihydroartemisinin, accessible easily from artemisinin [27]. Of these, compound **11** gave 100% reduction in parasitemia by day 4 (on oral as well as subcutaneous administration with 10 mg/kg dose) when tested in mice infected with *P. berghei*. Impressively, this was more potent than artesunate, had an ED_{90} value below 10 mg/kg, and showed ~25 times more oral bioavailability than artemether.

Artemisinin analogs with C-3 substitution were accessed mainly through total synthesis. The derivative **12** from this group possessed good thermal stability and aqueous solubility, and showed an ED_{50} value of 15 mg/kg (*cf.* artelinic acid = 9.6 mg/kg) when tested orally in *P. berghei* infected mice [28]. In comparison with lead candidates from C-10 functionalization, the C-3 modified analogs studied thus far were not good contenders to reach the stage of clinical development. A similar activity profile was seen with aza analogs prepared by modifying the O-11 position of artemisinin. The compound **13** from this class was ~5 times more potent than artemisinin when tested *in vitro*. Short aliphatic carbon chains (C-1–C-3), benzyl substitution or phenethyl substitution at N-11 appeared to marginally improve or retain the activity relative to artemisinin, but no considerable improvement in activity was achieved by other N-11 derivatizations [29].

Compounds presented in Figure 2 were designed to see whether the unique structural framework in artemisinin is really essential for its biological activity. While a few compounds in this series possess activities comparable to that of artemisinin, structural perturbations seem, in general, adversely to affect their potencies. Among compounds of the type **14**, the $C_{12\beta}$ isomers exhibited either comparable or better anti-malarial activities *in vitro* relative to their $C_{12\alpha}$ analogs. Of these, **14a** ($C_{12\beta}$) and **14b** ($C_{12\beta}$) respectively showed ED_{50} values of 5.5 and 10 mg/kg when tested orally against *P. berghei* (N) in a mouse model (*cf.* artemisinin = 8.5 mg/kg) [30]. Compounds **15** and **16** were synthesized primarily to study the role of C-4 radicals in the anti-parasitic action of artemisinins. The important conclusions that can be derived from this study, reported by Posner and co-workers, are (i) groups favoring the formation of radicals at C-4 (e.g. **15b** and **16b**) are better anti-malarials than the unsubstituted analogs **15a** and **16a**; (ii) substituents that stabilize the C-4 radical to a larger extent than simple methyl or benzyl groups (e.g. **15c** and **16c**) decrease potency; (iii) $C_{4\beta}$ epimers, where 1,5-H shift is more favorable, exhibit better potencies than their $C_{4\alpha}$ analogs and (iv) the presence of a hydroxyethyl group at the C-8a position enhances anti-malarial potencies (compounds **15** vs. **16**). When *R* in **15** and **16** are

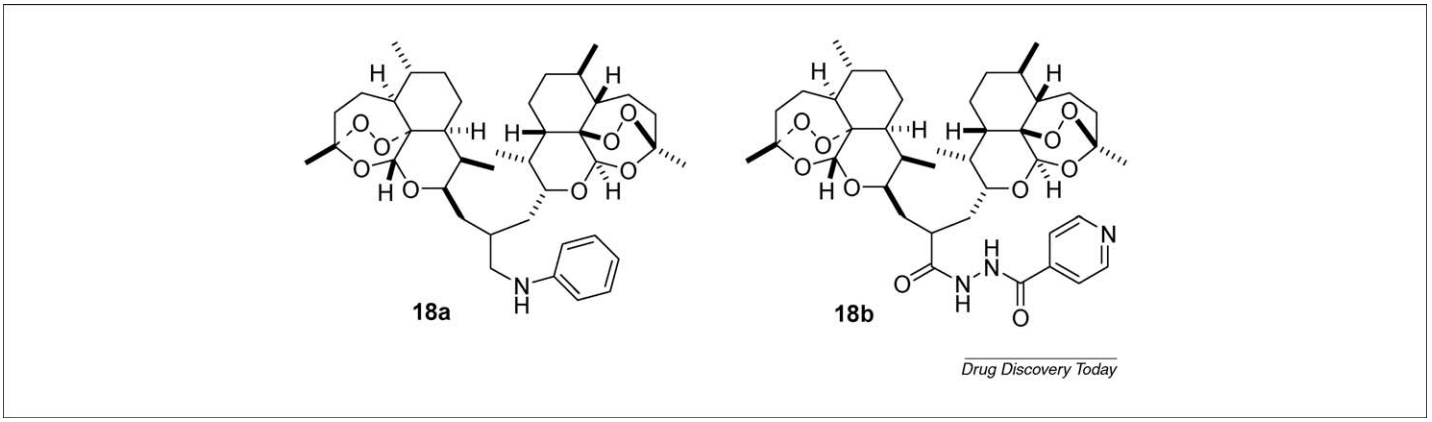

FIGURE 3

Highly potent artemisinin dimers reported by Posner and co-workers.

$(Me)_3SnCH_2$–, the analogs were inactive. On treatment with Fe(II), elimination of $Me_3Sn^{\bullet}$ took place, leaving an exo-methylene at C-4 as expected [31].

Among tricyclic seco analogs **17a–d**, the compound **17b** possessed *in vitro* potency comparable to that of artemisinin. Their percentage relative activities against *P. falciparum* (D6) were 20:108:7:0 respectively (*cf.* artemisinin = 100). Although the peroxide group in **17b** is in a different chemical environment than artemisinin, this is capable of producing a methyl-centered, secondary carbon-centered or oxygen-centered radicals after interacting with Fe(II) (*vide infra*), and could be responsible for the parasiticidal effect. Relatively low activities of **17a**, **17c** and **17d**, however, show that small structural changes around the peroxide group in such systems can have a significant effect on their biological activities [32].

Of equal importance is the class where more than one artemisinin is present in the molecule as a dimer, trimer or tetramer. While increased oxidative stress due to larger peroxide content can be expected, the overall anti-parasitic effect of such systems is likely to be influenced by their ADME properties, the degree of activation and target interactions—factors that almost certainly differ from the mono-analogs listed above. Compounds in this group were mainly synthesized by dimerizing artemisinin through reactive functionalities at C-9 or C-10 positions (see supplementary material for examples). Dimers **18a** and **18b** (Figure 3) from this group, reported by Posner *et al.*, are highly efficacious and could cure *P. berghei* infected mice with just a single subcutaneous dose (30 mg/kg) on day one, post infection [33].

Although structural modification of artemisinin at the C-3, C-9, C-10 and O-11 positions have generated a number of analogs with interesting biological activities [25,34], derivatization at positions ranging from C-4 to C-8 remained a challenge owing to the difficulty in introducing functional groups by common synthetic methods. An alternate strategy involving microbial fermentation technique has, however, recently given access to these hitherto inaccessible analogs. New artemisinin derivatives (**19**–**24**) obtained through this route are presented in Figure 4 [35–37]. Chemical derivatization of these analogs could lead to a new

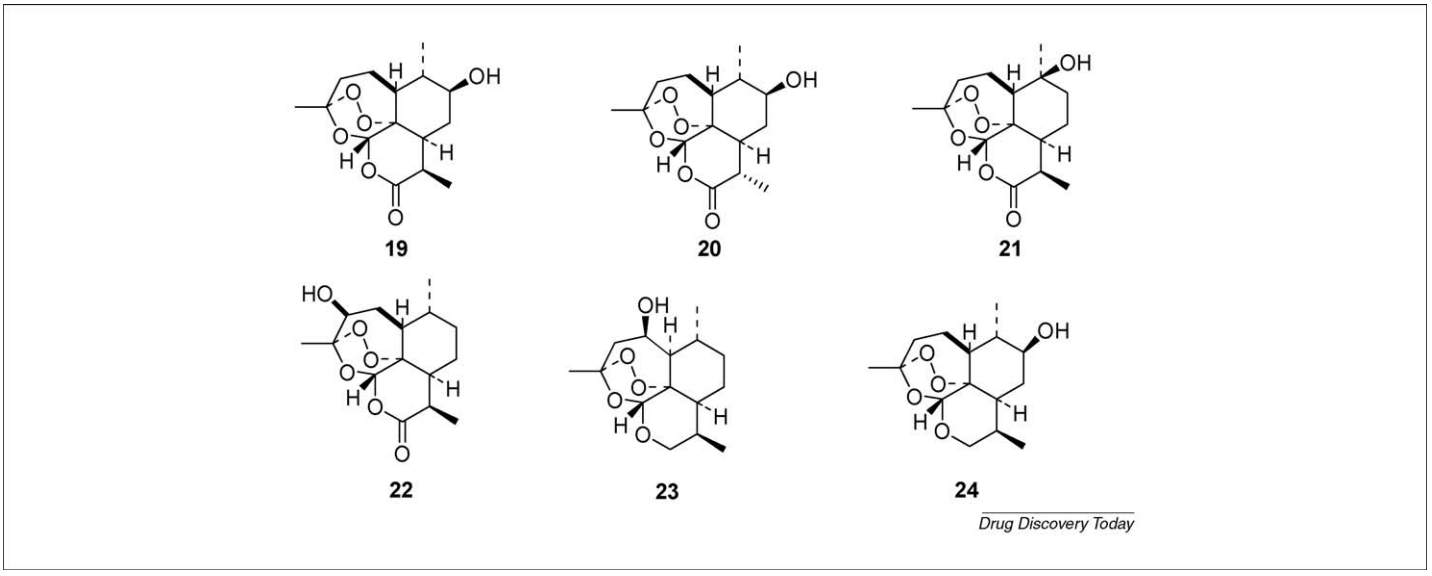

FIGURE 4

Artemisinin derivatives prepared through microbial fermentation methods.

generation of artemisinin analogs that would help in understanding structure–activity relationships, and in the design of new anti-parasitic drugs.

New synthetic peroxides based on the mode of action of artemisinin

As mentioned, the endoperoxide group is responsible for the anti-parasitic activity of artemisinin class of compounds. Heme, generated as a result of hemoglobin degradation in the food vacuole of the parasite, is believed to interact with the peroxide bridge, initiating a cascade of reactions that result in the generation of cytotoxic intermediates. Activation of artemisinin by heme from undigested hemoglobin has also been observed under experimental conditions [38,39]. Key steps involved in this process are schematically represented in Figure 5. Existing evidence suggests that oxygen or carbon centered radicals, high-valent iron-oxo species or electrophilic alkylating agents such as epoxides (e.g. species A–F) are likely contributors to the anti-parasitic effect. In order to find out whether the chemical reactivity of artemisinins do correlate with their biological potencies, Haynes *et al.* have performed an *in vitro* study involving some highly potent C-10-alkylaminoartemisinin derivatives. As no correlation was seen under the experimental conditions employed, the authors have proposed that artemisinin analogs could be eliciting anti-parasitic effect by binding to a specific therapeutic target [40,41]. Possible generation of reactive species akin to A–F during or after the binding event was however not completed ruled out [40].

A study by Stocks *et al.* suggests that common mechanisms of accumulation in infected cells and subsequent activation by 'non-heme chelatable-iron' from parasites are, in general, responsible for selective anti-malarial activity of peroxidic anti-malarials [42]. Some of the artemisinin targets identified thus far through *in vitro* experiments include translationally controlled tumor protein (TCTP) [43], heme [44], reduced glutathione [45] and PfATP6 – a SERCA-type Ca^{2+} – ATPase [46].

Identification of artemisinin–heme adducts in the spleen of infected mice [47], and a report by Jambou *et al.* using field isolates showing a correlation between S769N PfATPase-6 mutation and their reduced susceptibility to artemether [48], are evidences indicative of more than one type of drug–target interaction contributing to the parasiticidal effect of artemisinin analogs. The latter observation needs to be taken seriously as artemisinins are the only class of compounds still active against drug resistant strains of *P. falciparum*. Any potential resistance development needs to be avoided by using appropriate drug combinations effectively.

Can simple synthetic peroxides exhibit anti-parasitic activities like that of artemisinin? If so, will their mode of action be similar to that of this natural product? These are the key questions that medicinal chemists tried to address in parallel with their studies involving artemisinin analogs. Progress in this area has been reviewed by Tang *et al.* [49] and Jefford [6,50]. Important structural types belonging to this class include 1,2-dioxanes (endoperoxides), 1,2,4-trioxanes, 1,2,4-trioxalanes, and tetraoxanes representative examples of which are presented in Figure 6 (also see the supplementary material). The activities of these synthetic peroxides clearly indicate that an artemisinin core is not really essential for anti-parasitic action. Fenozan B07 (**25**) is a lead candidate identified after evaluating a series of *cis*-fused cyclopentano-1,2,4-trioxanes. This was equally active, both orally and subcutaneously, when tested in mice infected with *P. berghei*

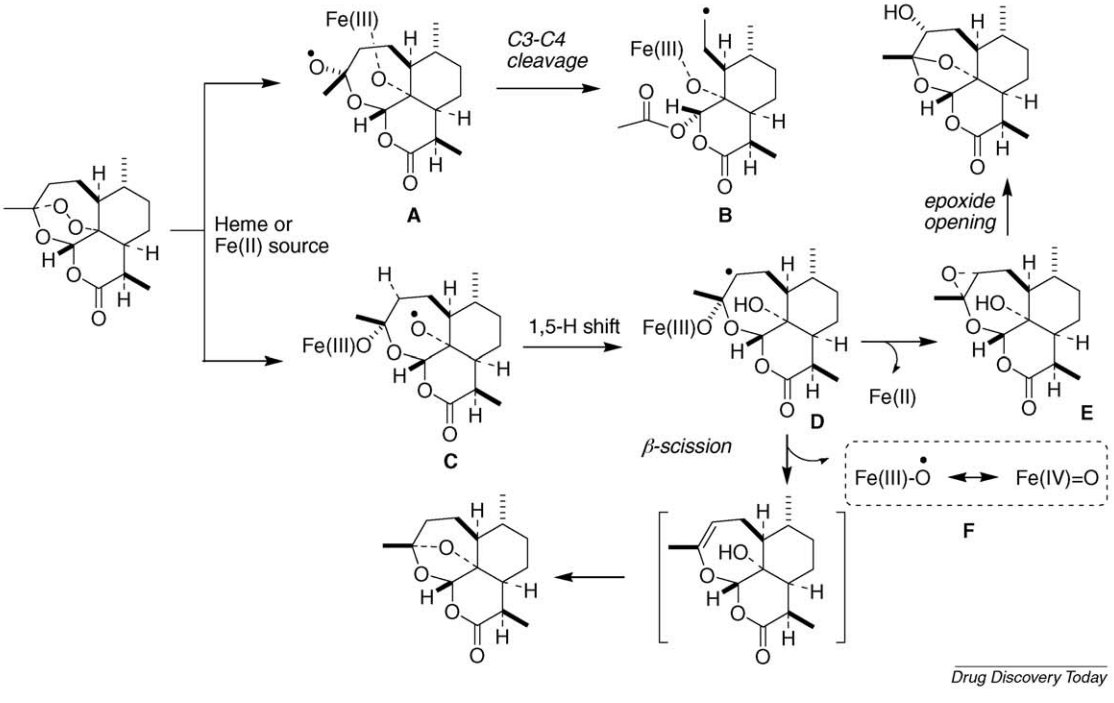

FIGURE 5

Fe(II) induced activation of artemisinin.

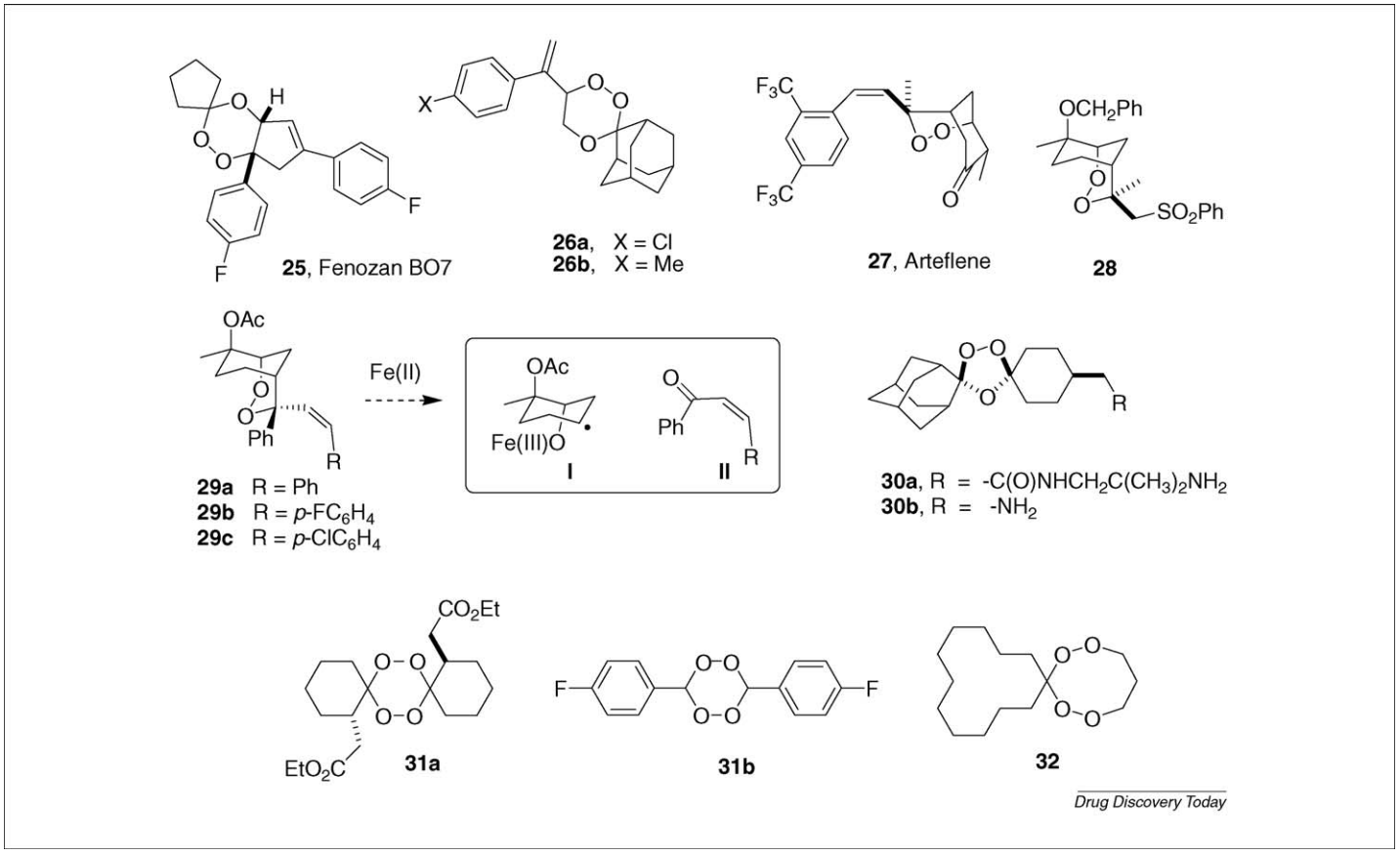

FIGURE 6

Representative examples of highly potent synthetic peroxides.

(ED_{50} = 2.5 mg/kg; *cf.* artemisinin, po = 5.0 mg/kg) [50,51]. Despite having such a promising therapeutic profile, this has not yet advanced to the level of clinical development. Adamantane-based 1,2,4-trioxanes **26a** and **26b**, reported by Tripathi *et al.*, are lead candidates from compounds this series. They were able to give 100% protection and cure when tested in rhesus monkeys infected with *P. knowlesi* (W1) with a dose of 80 mg/kg × 5 days, and are promising candidates for preclinical studies [52].

The lead candidates **27–29** are structurally related to Yingzhaosu A—a natural product isolated from the roots of *Artabotrys uncinatus* (*vide infra*). Of these, arteflene **(27)**, which showed excellent stability and safety profiles, was in the developmental phase [50,53]. Recent reports, however, indicate that studies with this as a drug candidate have been discontinued after phase III trials [6]. The related analog **28**, showed an ED_{50} value of. 4.2 mg/kg (*cf.* artemether = 3.1 mg/kg, *P. berghei*, N, po) and exhibited good safety profiles [54].

Pro-drugs **29a–c**, with a peroxide group as part of a latent chalcone were designed to function like a 'Trojan horse' and deliver both a carbon radical and an enone (**I** and **II**, Figure 6) on activation. The parasiticidal effect due to artemisinin-like action of **I** in these cases was expected to get augmented by simultaneous interaction of **II** with targets such as cysteine proteases in the parasite. *In vitro* studies against K1 strain of *P. falciparum* showed that these compounds are more potent than arteflene, but less effective than artemisinin. Their IC_{50} values were in the range of 23–34 nM (*cf.* arteflene = 47 ± 8 nM; artemisinin = 15 ± 4 nM) [6,55].

Identification of the ozonide **30a** (OZ277 or RBx-11160), by Vennerstrom *et al.* was a significant breakthrough in anti-malarial drug development efforts during the past decade [56]. In preliminary studies, **30a** showed an ED_{50} (*P. berghei*, po) value of 0.78 mg/kg (*cf.* ED_{50} artesunate/artelinate/artemether = 4.7/4.8/2.2 mg/kg) and exhibited prolonged duration of action than traditional drugs such as artesunate and artemether. When $R = NH_2$ (**30b**), the compound exhibited prophylactic activity against malaria parasites [56,57]. Clinical development of RBx-11160 began in 2003 through collaboration between MMV and Ranbaxy Laboratories Ltd., India. A combination of this compound with piperaquine has shown an additive therapeutic effect [58]. Interestingly, MMV, after reviewing the data on RBx-11160 seems to have stopped funding the project in 2006, and is currently supporting the development of another candidate called OZ439 from this series [19]. However, Ranbaxy has continued the efforts, and RBx-11160 is advancing through phase IIb dose-range finding studies in India, Africa and Thailand [59]. One of the latest reports suggests that phase III clinical trials involving RBx-11160 (arterolane) in combination with piperaquine will be commenced shortly [60].

Tetraoxanes **31** and **32** are from a related class of synthetic peroxides that have undergone detailed preliminary evaluations. The dispiro–tetraoxane **31a**, despite being more potent than artemisinin *in vitro*, was inactive when tested orally (IC_{50}, *P. falciparum*, K1 strain = 6.2 nM; *cf.* artemisinin = 10 nM) [61]. Steric crowding on cyclohexane rings in such systems, in general, was found to have an adverse effect on their potencies. A closely related

example is compound **31b** that contains tetraoxane unit in between two substituted phenyl rings. This showed a selectivity of >200 over mouse mammary tumor FM3A cells (as a control for mammalian cell cytotoxicity) but gave only 90% inhibition of parasitemia in mice infected with *P. berghei* when tested intraperitonially with dose of 50 mg/kg × 4 (artemisinin ED_{50} = 5.4 mg/kg) [62]. Tetraoxacycloalkane **32**, reported by Kim *et al.*, contains peroxide units as part of a ring structure. This was moderately active in preliminary studies and showed an ED_{50} value of 20 mg/kg (*cf.* artemisinin = 13 mg/kg) when tested orally against *P. berghei* in mice model. Considering the safety profile and low cost of production, **32** and its analogs can be considered for detailed studies [63].

Hybrid drugs based on peroxidic anti-malarials

In hybrid drug approach, pharmacophores of known modes of action are linked covalently to create a new drug candidate with the expectation of achieving a net increase in therapeutic efficacy. Important strategies pursued in this direction include (i) linking peroxy compounds to carriers such as cholic acid and (ii) designing hybrid systems with other anti-malarial drugs such as quinine. The former class, mainly based on the uptake and absorption characteristics of bile acids [64], has given interesting results. Important conclusions from recent reports in this area by Šolaja *et al.* are (i) amide derivatives of *bis* and mixed cholic acid-based tetraoxanes generally exhibit better potencies and safety profiles than corresponding acids, (ii) of those mixed tetraoxanes with different spiro–cycloalkane ring sizes, those substituted with a cyclohexane ring exhibit best potency and (iii) mixed tetraoxanes are generally more active against the W2 strain of *P. falciparum* than D6 [65,66]. The lead candidate from this group – compound **33** (C4″R isomer, Figure 7) – was ~6 times more potent than artelinic acid and ~2.5 times potent than arteether in preliminary *in vitro* assays.

Compounds **34**–**36** were synthesized to study synergistic effects when anti-malarial pharmacophores with different half-lives and mode of action are assembled in a single molecule. Enhanced accumulation in food vacuole of the parasites due to 'weak base ion trapping' effect from quinoline moiety is an additional factor that could augment anti-parasitic activity in such systems. The quinine–artemisinin adduct **34** was approximately three times potent than a 1:1 mixture of artemisinin and quinine (*in vitro* IC_{50} against *P. falciparum*, 3D7 = 8.95 nM; *cf.* artemisinin = 49.4 nM, quinine = 149 nM, 1:1 quinine + artemisinin = 31.8 nM) [67]. It is probable in this case that the increased potency is due to additive or synergistic effects from quinine and dihydroartemisinin (**2**) that is formed through ester hydrolysis. Trioxaquines represented by structures **35** and **36** belong to a related class of hybrid molecules with inbuilt peroxide and quinoline groups. A recent study by Loup *et al.* has shown that hybrids, structurally related to **35** and **36**, are very good inhibitors of β-hematin formation *in vitro* [68]. Compound **35** showed an ED_{50} value of 18 mg/kg/day (po) when tested in mice infected with *P. vinckei*, and a dose of 20 mg/kg/day (ip) was found to clear parasitemia completely for over 60 days. Further, no toxic response was observed in mice when this compound was tested orally in mice with a dose of 120 mg/kg/day × 4 [6,69]. *In vitro* potency of **36** – the covalent adduct of primaquine with trioxane – was much lower relative to artemisinin or **35**. In comparison with primaquine, there was, however, a noticeable increase in anti-malarial activities against both CQ-sensitive and resistant strains (IC_{50} value against *P. falciparum* FcM29-Cameroon, highly CQ resistant strain = 108 nM; *cf.* artemisinin = 8 nM, primaquine = 1400 nM). As primaquine mainly targets hepatic stages of plasmodium parasites, **36** holds lot of promise as a prophylactic agent against malaria.

Broad-spectrum anti-infective properties of artemisinin analogs

Apart from activities against malaria parasites, studies involving artemisinin class of compounds have unraveled their potential to

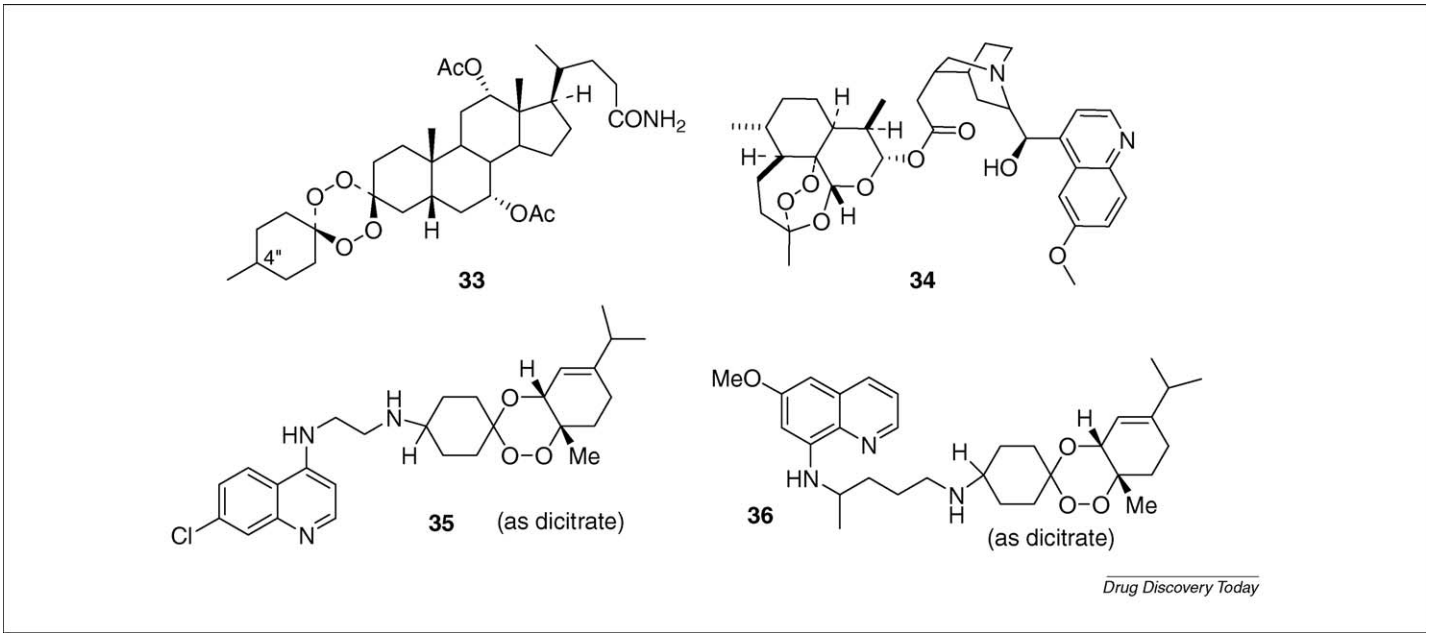

FIGURE 7

Peroxide-based hybrid drug candidates.

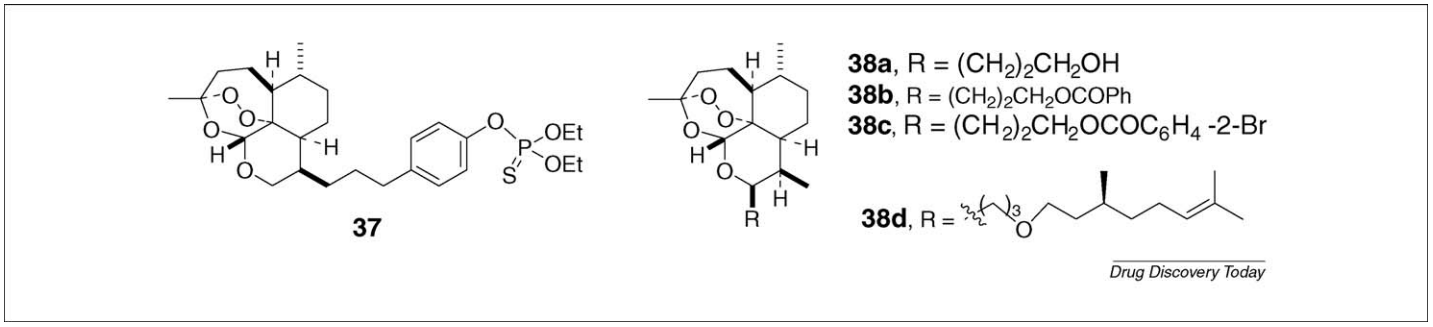

FIGURE 8

Artemisinin derivatives with broad-spectrum anti-infective properties.

target other diseases as well. A report by Yang and Liew have previously shown the efficiency of artemisinin class of compounds against cutaneous leishmaniasis under *in vivo* conditions [70]. Significant reduction in lesion size was observed with 50 mg/kg/day doses of artemether when administered via the intravenous, intralesional, intramuscular or oral routes [70,71]. On the basis of this, Avery *et al.* have performed a detailed structure–activity relationship study to understand the stereo-electronic requirements for optimal anti-leishmanial action. This study not only highlighted the importance of the peroxide group in eliciting anti-leishmanial response, but also suggested that derivatization at C-9 with suitably substituted aryl–alkyl groups could in fact enhance their potencies [34]. Fine-tuning of structural features later led to the identification of compound **37** (Figure 8) with an IC_{50} value of 0.26 μM (*cf.* pentamidine isethionate, 2.45 μM; amphotericin B, 0.097 μM), when tested *in vitro* against *L. donovani* promastigotes [72]. Recent mechanistic studies by Sen *et al.* have shown that leishmanicidal effect of artemisinin occurs primarily via apoptosis as demonstrated by the externalization of phosphatidyl serine, loss of mitochondrial membrane potential and degradation of nuclear DNA [73]. Factors responsible for its activation and the actual molecular targets of artemisinin in leishmania parasites, however, remain to be elucidated.

The effect of artemisinin analogs on the growth of parasites belonging to the genus toxoplasma, reported by Jones-Brando *et al.* and Ou-Yang *et al.* further supports the notion that artemisinin could be a promising starting point for drug development efforts against parasitic infections in general [74,75]. Of the C-10 derivatives analyzed by Jones-Brando *et al.* against *T. gondii*, artemether (**3**, Figure 1) showed an ID_{50} value of 0.7 μM (*cf.* artemisinin = 8.0 μM; trimethoprim = 17.90 μM). Except for compound **38a** with a terminal hydroxyl group, all of the other C-10 derivatives analyzed (**38b–d**) exhibited more or less comparable potencies (ID_{50} = 1.1–1.4 μM) [74]. A similar range of anti-parasitic activity was observed when artemisinin and related compounds were tested against trypanosoma parasites. Although the inhibitory concentrations of analogs studied here (artemisinin, artemisone and dihydroartemisinin) against *Trypanosoma cruzi* fall in the range of 13–23 μM, refinement of structure and optimization of activities could give rise to better leads for further development [71].

Anti-shistosomial activity of artemisinin was first reported by Chen *et al.* in 1980 [76]. This and subsequent experiments have unequivocally demonstrated the potential of this class of compounds to reduce worm burden in infected animals [77,78]. Shaohong *et al.* have recently evaluated the effect of artesunate (**5**) in mice infected with *S. mansoni*. Results suggest that sexual maturation of the parasite is most affected on artesunate treatment and four successive oral doses of 300 mg/kg at two-week intervals can confer almost complete protection from disease progression [79]. In strong support of the usefulness of artemisinin analogs against shistosomiasis, Li *et al.* have recently reported their findings from a randomized controlled trial involving 783 individuals in the Poyang Lake region of southern China [80]. After a single oral dose of praziquantel (50 mg/kg), the group was randomized and given either oral artemether (6 mg/kg) or placebo once every two weeks for 9–11 doses until the end of transmission season. Stool examination a month after the final dose showed that only 0.8% (3/373) of the artemether group was egg-positive for *S. Japonicum*, whereas that in the placebo group was as high as 15.5% (56/361), indicating the protective effect of artemether.

Examination of anti-parasitic activities shows a clear difference in concentration ranges in which artemisinin analogs act against plasmodium and other parasites such as leishmania, trypanosoma and toxoplasma. Their *in vitro* IC_{50} values against the former generally fall in the nanomolar range where as micromolar IC_{50} values are generally observed in assays against the latter group. This could be most probably due to the difference in their modes and extent of uptake and degree of activation in the target cells. Peroxide compounds are also receiving wide attention owing to their promising anticancer [81–83], and anti-fungal activities [84,85]. Considering their proven safety profiles, lead optimization and drug development efforts in this direction need to be continued to identify new broad-spectrum anti-parasitic agents.

Peroxide-containing natural products other than artemisinin

Having the medicinal potential of artemisinin and related peroxides unequivocally proven, it seems worthwhile to look at other natural products that possess peroxide groups. An excellent review by Casteel on this subject is available and gives a detailed account of medicinally useful natural peroxides [86]. Some selected compounds in this category are presented in Figure 9. A larger collection of natural products with inbuilt peroxide group is presented in the supplementary material for reference.

Yingzhaosu A (**39a**) is a sesquiterpene peroxide isolated from the roots of *Artabotrys uncinatus*. As mentioned, synthetic peroxide such as Arteflene (**26**) was designed on the basis of the structure of

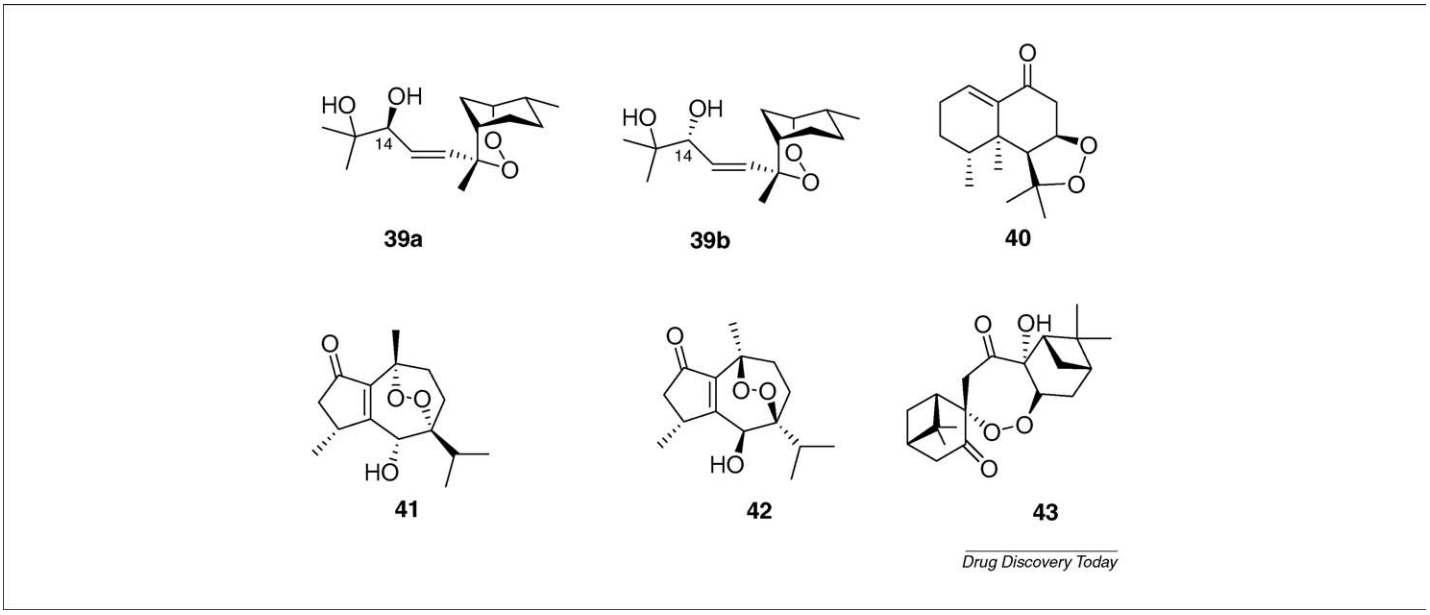

FIGURE 9

Representative examples of peroxidic natural products with promising anti-parasitic activities.

this natural product. Recently, Szpilman *et al.* have reported the total synthesis of **39a** and its C-14 epimer (**39b)**. They respectively showed ED_{50} values of 250 and 90 mg/kg when tested in mice infected with chloroquine-sensitive NY strain of *P. berghei* (*cf.* artesunate = 4.2 mg/kg) [87]. Compounds **40–42** isolated from the extracts of *Nurdostachys chinensis* roots [88,89], and **43**, isolated from *Amomum krervanh* fruits [90] are active against plasmodium parasites with submicromolar EC_{50} values. Synthesis of new analogs based on such natural products (see supplementary material) and detailed structure–activity relationship studies need to be carried out to identify new peroxide-based anti-infective agents for clinical development.

Summary

Although unmatchable in their fast clinical response, first generation artemisinin analogs have short biological half-lives, a drawback that results in recrudescence. Efforts during the past decade have identified a number of new artemisinin derivatives with superior therapeutic profiles. As parasitic infections are more prevalent in underdeveloped nations, cost effectiveness during large-scale production of drug candidates remains as the key factor that can affect their success. Apart from compounds that retain an artemisinin core, information pertaining to the mode of action of this natural product has been helpful in the development of new synthetic peroxides with promising biological activities. A commendable success in this area is the compound OZ277 (or RBx-11160)—an orally active synthetic ozonide. A combination of this drug with piperaquine has been selected for phase III clinical trials. Related studies have shown that parasites belonging to genera such as leishmania, schistosoma, trypanosoma and toxoplasma are also susceptible to the artemisinin class of compounds, showing their potential as broad-spectrum anti-infective agents. In addition to artemisinin, a large number of peroxide-containing natural products with interesting biological properties have been isolated from plant and marine sources. Structure-optimization and structure–activity relationship studies involving these new skeletons hold lot of promise in the area of peroxide-based anti-infective agents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2009.05.008.

References

1 Renslo, A.R. and McKerrow, J.H. (2006) Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.* 2, 701–710

2 Abu-Raddad, L.J. *et al.* (2006) Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science* 314, 1603–1606

3 Wolday, D. *et al.* (1999) Leishmania-HIV interaction: immunopathogenic mechanisms. *Parasitol. Today* 15, 182–187

4 Pacheco, R.S. *et al.* (1998) Chagas' disease and HIV co-infection: genotypic characterization of the *Trypanosoma cruzi* strain. *Memórias do Instituto Oswaldo Cruz* 93, 165–169

5 O'Neill, P.M. and Posner, G.H. (2004) A medicinal chemistry perspective on artemisinin and related endoperoxides. *J. Med. Chem.* 47, 2945–2964

6 Jefford, C.W. (2007) New developments in synthetic peroxidic drugs as artemisinin mimics. *Drug Discov. Today* 12, 487–495

7 Janse, C.J. *et al.* (1994) Comparison of *in vivo* and *in vitro* antimalarial activity of artemisinin, dihydroartemisinin and sodium artesunate in the *Plasmodium berghei*-rodent model. *Int. J. Parasitol.* 24, 589–594

8 Balint, G.A. (2001) Artemisinin and its derivatives: an important new class of antimalarial agents. *Pharmacol. Ther.* 90, 261–265

9 Guidelines for the treatment of malaria—a publication by World Health Organization (http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf)

10 Bukirwa, H. *et al.* (2006) Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. *PLoS Clin. Trials* 1, e7

11 Danis, M. and Bricaire, F. (2003) The new drug combinations: their place in the treatment of uncomplicated *Plasmodium falciparum* malaria. *Fundam. Clin. Pharmacol.* 17, 155–160

12 Poole S., (text editor) (2001) The use of anti-malarial drugs—a report by WHO (http://rbm.who.int/cmc_upload/0/000/014/923/use_of_antimalarials.pdf)
13 Baker, J.K. *et al.* (1993) Decomposition of arteether in simulated stomach acid yielding compounds retaining antimalarial activity. *Pharm. Res.* 10, 662–666
14 Brewer, T.G. *et al.* (1994) Fatal neurotoxicity of arteether and artemether. *Am. J. Trop. Med. Hyg.* 51, 251–259
15 Brewer, T.G. *et al.* (1994) Neurotoxicity in animals due to arteether and artemether. *Trans. R. Soc. Trop. Med. Hyg.* 88 (Suppl. 1), 33–36
16 Gordi, T. and Lepist, E.-I. (2004) Artemisinin derivatives: toxic for laboratory animals, safe for humans? *Toxicol. Lett.* 147, 99–107
17 Lin, A.J. *et al.* (1987) Antimalarial activity of new water-soluble dihydroartemisinin derivatives. *J. Med. Chem.* 30, 2147–2150
18 Hartell, M.G. *et al.* (2003) Artemisinins with improved stability and bioavailability for therapeutic drug development and application. US patent applications 7084132 B2 and 6951846 B2
19 Information from 'Medicines for Malaria Venture' web site (http://www.mmv.org)
20 Haynes, R.K. *et al.* (2006) Artemisone—a highly active antimalarial drug of the artemisinin class. *Angew. Chem.* 45, 2082–2088
21 Vivas, L. *et al.* (2007) Antimalarial efficacy and drug interactions of the novel semi-synthetic endoperoxide artemisone *in vitro* and *in vivo*. *J. Antimicrob. Chemother.* 59, 658–665
22 Nagelschmitz, J. *et al.* (2008) First assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone. *Antimicrob. Agents Chemother.* 52, 3085–3091
23 O'Neill, P.M. *et al.* (2001) Synthesis, antimalarial activity, biomimetic iron(II) chemistry, and *in vivo* metabolism of novel, potent C-10-phenoxy derivatives of dihydroartemisinin. *J. Med. Chem.* 44, 58–68
24 Magueur, G. *et al.* (2004) Fluoroartemisinin: trifluoromethyl analogues of artemether and artesunate. *J. Med. Chem.* 47, 2694–2699
25 Avery, M.A. *et al.* (2002) Structure–activity relationships of the antimalarial agent artemisinin, 6. The development of predictive *in vitro* potency models using CoMFA and HQSAR methodologies. *J. Med. Chem.* 45, 292–303 and references therein
26 Avery, M.A. *et al.* (2002) Structure–activity relationships of the antimalarial agent artemisinin. 7. Direct modification of (+)-artemisinin and *in vivo* antimalarial screening of new, potential preclinical antimalarial candidates. *J. Med. Chem.* 45, 4321–4335
27 Grellepois, F. *et al.* (2004) Orally active antimalarials: hydrolytically stable derivatives of 10-trifluoromethyl anhydrodihydroartemisinin. *J. Med. Chem.* 47, 1423–1433
28 Posner, G.H. *et al.* (2002) Orally active, water-soluble antimalarial 3-aryltrioxanes: short synthesis and preclinical efficacy testing in rodents. *J. Med. Chem.* 45, 3824–3828
29 Avery, M.A. *et al.* (1995) Structure–activity relationships of the antimalarial agent artemisinin, 2. Effect of heteroatom substitution at O-11: synthesis and bioassay of N-alkyl-11-aza-9-desmethylartemisinins. *J. Med. Chem.* 38, 5038–5044
30 Posner, G.H. *et al.* (1998) Orally active antimalarial 3-substituted trioxanes: new synthetic methodology and biological evaluation. *J. Med. Chem.* 41, 940–951
31 Cumming, J.N. *et al.* (1998) Design, synthesis, derivatization, and structure–activity relationships of simplified, tricyclic, 1,2,4-trioxane alcohol analogs of the antimalarial artemisinin. *J. Med. Chem.* 41, 952–964
32 Avery, M.A. *et al.* (1994) Synthesis, conformational analysis, and antimalarial activity of tricyclic analogs of artemisinin. *Tetrahedron* 50, 957–972
33 Posner, G.H. *et al.* (2007) Malaria-infected mice are cured by a single dose of novel artemisinin derivatives. *J. Med. Chem.* 50, 2516–2519
34 Avery, M.A. *et al.* (2003) Structure–activity relationships of the antimalarial agent artemisinin. 8. Design, synthesis, and CoMFA studies toward the development of artemisinin-based drugs against leishmaniasis and malaria. *J. Med. Chem.* 46, 4244–4258
35 Parshikov, I.A. *et al.* (2004) Hydroxylation of 10-deoxoartemisinin by *Cunninghamella elegans*. *J. Nat. Prod.* 67, 1595–1597
36 Parshikov, I.A. *et al.* (2004) Transformation of artemisinin by *Cunninghamella elegans*. *Appl. Microbiol. Biotechnol.* 64, 782–786
37 Zhan, J.-X. *et al.* (2002) Microbial metabolism of artemisinin by *Mucor polymorphosporus* and *Aspergillus niger*. *J. Nat. Prod.* 65, 1693–1695
38 Selmeczi, K. *et al.* (2004) Alkylation of human hemoglobin A0 by the antimalarial drug artemisinin. *FEBS Lett.* 556, 245–248
39 Robert, A. *et al.* (2002) From mechanistic studies on artemisinin derivatives to new modular antimalarial drugs. *Acc. Chem. Res.* 35, 167–174
40 Haynes, R.K. *et al.* (2004) Highly antimalaria-active artemisinin derivatives: biological activity does not correlate with chemical reactivity. *Angew. Chem.* 43, 1381–1385
41 Haynes, R.K. (2005) Reply to comments on 'Highly antimalaria-active artemisinin derivatives: biological activity does not correlate with chemical reactivity'. *Angew. Chem.* 44, 2064–2065
42 Stocks, P.A. *et al.* (2007) Evidence for a common non-heme chelatable-iron-dependent activation mechanism for semisynthetic and synthetic endoperoxide antimalarial drugs. *Angew. Chem.* 46, 6278–6283
43 Bhisutthibhan, J. *et al.* (1998) The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. *J. Biol. Chem.* 273, 16192–16198
44 Kannan, R. *et al.* (2002) Heme-artemisinin adducts are crucial mediators of the ability of artemisinin to inhibit heme polymerization. *Chem. Biol.* 9, 321–332
45 Wang, D.-Y. and Wu, Y.-L. (2000) A possible antimalarial action mode of qinghaosu (artemisinin) series compounds. Alkylation of reduced glutathione by C-centered primary radicals produced from antimalarial compound qinghaosu and 12-(2,4-dimethoxyphenyl)-12-deoxoqinghaosu. *Chem. Commun.* 22, 2193–2194
46 Eckstein-Ludwig, U. *et al.* (2003) Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature* 424, 957–961
47 Robert, A. *et al.* (2005) The antimalarial drug artemisinin alkylates heme in infected mice. *Proc. Natl. Acad. Sci. U.S.A.* 102, 13676–13680
48 Jambou, R. *et al.* (2005) Resistance of *Plasmodium falciparum* field isolates to *in vitro* artemether and point mutations of the SERCA-type PfATPase6. *Lancet* 366, 1960–1963
49 Tang, Y. *et al.* (2004) Synthetic peroxides as antimalarials. *Med. Res. Rev.* 24, 425–448
50 Jefford, C.W. (2001) Why artemisinin and certain synthetic peroxides are potent antimalarials. Implications for the mode of action. *Curr. Med. Chem.* 8, 1803–1826
51 Peters, W. *et al.* (1993) The chemotherapy of rodent malaria. XLIX. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 2: structure–activity studies on *cis*-fused cyclopenteno-1,2,4-trioxanes (fenozans) against drug-sensitive and drug-resistant lines of *Plasmodium berghei* and *P. yoelii* ssp. NS *in vivo*. *Ann. Trop. Med. Parasitol.* 87, 9–16
52 Tripathi, R. *et al.* (2007) Evaluation of some adamantane-based synthetic trioxanes against *Plasmodium knowlesi* in rhesus monkeys. *Life Sci.* 81, 1544–1548
53 Hofheinz, W. *et al.* (1994) Ro 42-1611 (arteflene), a new effective antimalarial: chemical structure and biological activity. *Trop. Med. Parasitol.* 45, 261–265
54 Bachi, M.D. *et al.* (2003) A short synthesis and biological evaluation of potent and nontoxic antimalarial bridged bicyclic β-sulfonyl-endoperoxides. *J. Med. Chem.* 46, 2516–2533
55 O'Neill, P.M. *et al.* (2004) Design and synthesis of endoperoxide antimalarial prodrug models. *Angew. Chem.* 43, 4193–4197
56 Vennerstrom, J.L. *et al.* (2004) Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature* 430, 900–904
57 Vennerstrom, J.L. *et al.* (2005) Spiro and dispiro 1,2,4-trioxolane antimalarials, and their preparation, pharmaceutical compositions, and use in the treatment of malaria, cancer, and schistosomiasis. US patent applications: 6825230B2 (2004); 6486199 B1 (2002) and 6906205 B2
58 Snyder, C. *et al.* (2007) *In vitro* and *in vivo* interaction of synthetic peroxide RBx11160 (OZ277) with piperaquine in Plasmodium models. *Exp. Parasitol.* 115, 296–300
59 Information from Ranbaxy's website (http://www.ranbaxy.com)
60 Press release in the web (http://www.bseindia.com/xml-data/corpfiling/announcement/Ranbaxy_Laboratories_Ltd_160908.pdf)
61 Dong, Y. (2002) Synthesis and antimalarial activity of 1,2,4,5-tetraoxanes. *Mini-Rev. Med. Chem.* 2, 113–123
62 Kim, H.-S. *et al.* (1999) Synthesis and antimalarial activity of cyclic peroxides, 1,2,4,5,7-pentoxocanes and 1,2,4,5-tetroxanes. *J. Med. Chem.* 42, 2604–2609
63 Kim, H.-S. *et al.* (2001) Synthesis and antimalarial activity of novel medium-sized 1,2,4,5-tetraoxacycloalkanes. *J. Med. Chem.* 44, 2357–2361
64 Sievänen, E. (2007) Exploitation of bile acid transport systems in prodrug design. *Molecules* 12, 1859–1889
65 Opsenica, D. *et al.* (2000) Cholic acid derivatives as 1,2,4,5-tetraoxane carriers: structure and antimalarial and antiproliferative activity. *J. Med. Chem.* 43, 3274–3282
66 Šolaja, B.A. *et al.* (2002) Mixed steroidal 1,2,4,5-tetraoxanes: antimalarial and antimycobacterial activity. *J. Med. Chem.* 45, 3331–3336
67 Walsh, J.J. *et al.* (2007) A novel artemisinin–quinine hybrid with potent antimalarial activity. *Bioorg. Med. Chem. Lett.* 17, 3599–3602
68 Loup, C. *et al.* (2007) Trioxaquines and heme-artemisinin adducts inhibit the *in vitro* formation of hemozoin better than chloroquine. *Antimicrob. Agents Chemother.* 51, 3768–3770
69 Dechy-Cabaret, O. *et al.* (2004) Synthesis and antimalarial activity of trioxaquine derivatives. *Chem. Eur. J.* 10, 1625–1636
70 Yang, D.M. and Liew, F.Y. (1993) Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. *Parasitology* 106, 7–11

71 Mishina, Y.V. *et al.* (2007) Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense in vitro* growth. *Antimicrob. Agents Chemother.* 51, 1852–1854
72 Menon, R.B. *et al.* (2006) A new library of C-16 modified artemisinin analogs and evaluation of their anti-parasitic activities. *Comb. Chem. High Throughput Screen.* 9, 729–741
73 Sen, R. *et al.* (2007) Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. *J. Med. Microbiol.* 56, 1213–1218
74 Jones-Brando, L. *et al.* (2006) *In vitro* inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. *Antimicrob. Agents Chemother.* 50, 4206–4208
75 Ke, O.Y. *et al.* (1990) Inhibition of growth of Toxoplasma gondii by qinghaosu and derivatives. *Antimicrob. Agents Chemother.* 34, 1961–1965
76 Chen, D.J. *et al.* (1980) *Chin. Med. J.* 80, 422–428
77 Utzinger, J. *et al.* (2001) Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. *Curr. Med. Chem.* 8, 1841–1859
78 Utzinger, J. *et al.* (2001) The potential of artemether for the control of schistosomiasis. *Int. J. Parasitol.* 31, 1549–1562
79 Lu, S. *et al.* (2006) Evaluation of the anthelmintic effects of artesunate against experimental *Schistosoma mansoni* infection in mice using different treatment protocols. *Parasitol. Int.* 55, 63–68
80 Li, Y.-S. *et al.* (2005) A double-blind field trial on the effects of artemether on *Schistosoma japonicum* infection in a highly endemic focus in southern China. *Acta Tropica* 96, 184–190
81 Ikuhiko, N. *et al.* (2008) Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation. *Int. J. Pharm.* 354, 28–33
82 Posner, G.H. *et al.* (2006) Anticancer activity of artemisinin-derived trioxanes. *Expert Opin. Ther. Pat.* 16, 1665–1672
83 Posner, G.H. *et al.* (2007) Trioxane dimers (artemisinin derivatives) having anticancer and antimalarial activities, processes for preparing them, and pharmaceutical compositions containing them. US patent application 6586464 B2
84 Macreadie, P. *et al.* (2006) Novel endoperoxides: synthesis and activity against Candida species. *Bioorg. Med. Chem. Lett.* 16, 920–922
85 Galal, A.M. *et al.* (2005) Antifungal activity of artemisinin derivatives. *J. Nat. Prod.* 68, 1274–1276
86 Casteel, D.A. (1999) Peroxy natural products. *Nat. Prod. Rep.* 16, 55–73
87 Szpilman, A.M. *et al.* (2005) Total syntheses of Yingzhaosu A and of its C(14)-epimer including the first evaluation of their antimalarial and cytotoxic activities. *J. Org. Chem.* 70 (9), 3618–3632; see Liang, X. T. *et al.* (1979) Acta Chim. Sin., 37, 215–230 for details on its isolation
88 Takaya, Y. *et al.* (1998) Novel antimalarial guaiane-type sesquiterpenoids from *Nardostachys chinensis* roots. *Tetrahedron Lett.* 39, 1361–1364
89 Takaya, Y. *et al.* (2000) Novel Guaiane endoperoxides, Nardoguaianone A–D, from Nardostachys chinensis roots and their antinociceptive and antimalarial activities. *Tetrahedron* 56, 7673–7678
90 Kamchonwongpaisan, S. *et al.* (1995) An antimalarial peroxide from Amomum krervanh Pierre. *Tetrahedron Lett.* 36, 1821–1824