Artemisinin and its derivatives: a novel class of anti-malarial and anti-cancer agents

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In this tutorial review, an effort towards presentation of a comprehensive account of the recent developments on various kinds of artemisinin derivatives including artemisinin dimers, trimers and tetramers has been made and their efficacy towards malaria parasites and different cancer cells lines was compared with that of artemisinins, and various other anti-malarial and anti-cancer drugs. It is expected that this review will provide first-hand information on artemisinin chemistry to organic/medicinal chemists, and pharmacologists working on anticancer and anti-malarial drug development.

1. Introduction

Malaria still remains one of the most dangerous widespread parasitic diseases of the developing world although it is known to humankind since ancient times in different forms, and exists over 100 countries, including the United States.1 It is caused by the Plasmodium parasite and kills approximately 1–3 million people and causes disease in 300–500 million people annually. The malaria parasite is a Plasmodium protozoan species, which evolved with time differentiating into four distinct species: P. falciparum, P. vivax, P. malariae and P. ovale, specific to humans. Some other related species including P. berghii and P. yeolii are specific to other groups of the mammalian class. This disease is transmitted from person to person through the bite of female anopheles mosquito. Out of the above four species of the malarial parasites of human host, Plasmodium vivax, P. malariae and P. ovale, are the causes of intermittent high fevers making a person very ill but they are rarely fatal. The remaining species P. falciparum, is the cause of malignant tertian, falciparum malaria which has a substantial mortality if it is untreated, especially in the first or an early attack. Among the four human malaria parasites, P. falciparum has developed resistance to all of our available drugs, therefore it is an overwhelming cause of serious disease and death. In patients with severe and complicated disease, the mortality rate is between 20–50%. The increasing resistance of malaria parasites to quinoline based anti-malarial drugs is a major contributor to the re-emergence of this disease as a major public health problem and its spread to new locations and populations.
2. Defined drugs

Commonly used drugs (Fig. 1) in single drug therapy for the early diagnosed malaria are given below:

(a) Quinine (1): Originally isolated from the bark of the Cinchona tree, quinine is the only drug which over a long period of time has remained largely effective in treating the disease. A number of its derivatives are known to be good anti-malarials. However, it is now used only for treating severe *falciparum* malaria, partly because of undesirable side effects.  

(b) Chloroquine (2): This is effective in curing all forms of malaria with few side effects when taken in a prescribed dose. It is still an effective and cheap drug both from prophylactic and chemotherapeutic point of view. Unfortunately, most strains of *falciparum* malaria are now resistant to chloroquine and more recently resistance of *vivax* malaria has also been reported.  

(c) Mepacrine (Alebrine) (3): This was developed in the early 1930s and used as a prophylactic on a large scale during the Second World War (1939–45) and had a major influence in reducing the incidence of malaria among the troops serving Southeast Asia. Because of its many undesirable side effects it is no longer used in clinics.  

(d) Mefloquine (4): Structurally related to quinine it is effective against many resistant strains of *Plasmodium*. Initially it was considered as a good prophylactic because of its long half life. Widespread resistance and undesirable side effects (mainly acute brain syndrome) associated with this drug have resulted in decline of its use. Because of its structural similarity to quinine the two are not recommended together.  

(e) Halofantrin (5): This is an effective anti-malarial, however, due to its short half life of 1 to 2 days and its high cost, it is not suitable for use as a prophylactic. Unfortunately resistant forms are increasingly being reported and there is some concern about its side effects. Halofantrin has been associated with neuropsychiatric disturbances. It is contra-indicated during pregnancy and is not advised to women who are breast-feeding. Abdominal pain, diarrhoea are some of the common side effects.  

(f) Azithromycin (6): This is a macrocyclic glycosylated lactone and is mainly used for the chemoprophylaxis. It also shows limited toxicity but the studies are limited to date.  

(g) Atovaquone (7): This is an important antifolate drug for malaria treatment and used in combination with proguanil which is a prodrug and metabolically converted to cycloguanil, an anti-folate.  

To combat the rapid spread of drug resistant malaria, effective therapeutic agents are continuously being sought, especially against those strains which are resistant to conventional quinoline and acridine based drugs. Wars have many times led not only to the development of new technology but also new medicaments. The antimalarial drugs are typical examples. Chloroquine resulted from the World War II. Mefloquine resulted from the Vietnam War on the American side. However, what many do not know is that artemisinin also resulted from the Vietnam War only as a result of large-scale research launched by the Chinese Government.
3. Discovery of artemisinin

In 1972, a group of Chinese researchers isolated a new anti-malarial drug, (+)-artemisinin 8, a sesquiterpene lactone of the amorphene sub-group of cadinene 9 from the hexane extract of a traditional Chinese medicinal plants, Artemesia annua (Asteraceae) a plant which has been used for the treatment of fever and malaria since ancient times. Abstract. Artemisinin is a sesquiterpene lactone containing an endoperoxide linkage in it. This highly oxygenated sesquiterpene lactone peroxide, unlike most other anti-malarials, lacks nitrogen containing heterocyclic ring systems and was found to be a superior plasmocidal and blood schizontocidal agent compared to conventional anti-malarial drugs, such as chloroquine, quinine etc against malaria strains, without obvious adverse effects in patients.

Artemisinin is obtained from Artemisia annua in a maximum yield of 0.1%. This plant is peculiar in its behavior. Carefully grown plants may be devoid of artemisinin and in order that the plant synthesizes the product, special agricultural conditions must be adopted. Best results have been reported in plantations in North Vietnam, mainly in the vicinity of Hanoi. Highest content was found about two weeks before flowering.

Artemisinin 8 ($420 kg^{-1}$) (Fig. 2) is active at nanomolar concentrations in vitro both against chloroquine sensitive and resistant P. falciparum strains. However, the practical values of artemisinin, nevertheless, is impaired by (i) its poor solubility either in oil or water, (ii) the high rate of parasite recrudescence after treatment and (iii) its short-plasma half life (3–5 h) and its poor oral activity. However, a low level of resistance has recently been observed using artemisinin, which disappeared as soon as the drug-selection pressure has been withdrawn. However, artemisinin with an endoperoxide linkage is a sensitive molecule for large scale derivatization. Fortunately, it was found that the carbonyl group of artemisinin 8, can be easily reduced to dihydroartemisinin 9 ($3500 kg^{-1}$) in high yields using sodium borohydride, which has in turn led to the preparation of a series of semi-synthetic first-generation analogues including the oil-soluble artemether 10 and arteether 11, and water-soluble sodium artesunate 12 and sodium artelinate 13. These three analogs become very potent anti-malarial drugs effective against chloroquine-resistant strains of P. falciparum. Artemether 10 ($3600 kg^{-1}$), has been included in the WHO lists of Essential Drugs for the treatment of severe MDR malaria. In this family, the Walter Reed Institute of research has patented a stable, water-soluble derivative called artelinic acid 12 which is now being tested in animals. A key advantage of these endoperoxides containing anti-malarial agents, which have been used for nearly two decades, is the absence of drug resistance.

Although a number of excellent review articles have been published on different aspects of artemisinin, we will concentrate our discussion on the recent and most important work carried out to study the structure–activity relationship of artemisinin derivatives which, in recent years have emerged as a novel class of anti-malarial and anti-cancer agents.

Fig. 1 Structures of commonly available anti-malarial drugs.

![Fig. 1](image_url)

Fig. 2 Structure of artemisinin and its analogs.
4. Artemisinin derivatives

(A) C-12 ether/ester derivatives

Artemisinin is only sparingly soluble in water or oil and not well absorbed by the gastro-intestinal tract. Search for more potent analogues of artemisinin with better bioavailability was initiated in China focusing attention on ethers and esters of dihydro-artemisinin i.e. arteether, artemether, artesunate, artelinate etc. Although these derivatives are potential antimalarial agents *in vitro*, they have poor bioavailability, principally as a result of metabolic instability of the acetone function.15 One of the principal routes for metabolism of artemether 10, for example, involves oxidative dealkylation to give DHA 9, a compound associated with toxicity and short half-life (Scheme 1).

An approach to increasing metabolic stability of artemisinin derivatives involves incorporation of a phenyl group in place of alkyl group (in the ether linkage) of arteether and artemether. This modification would be expected to block oxidative metabolic formation of DHA *in vivo*. With this idea in mind, O’Neill’s group synthesized16 a series of C-12 phenoxy derivatives by reacting DHA with 4 equivalents of the phenol /C06m gk gk difficulty, sugar derivatives of DHA were prepared17 (than arteether and artemether. This should have a longer half-life and potentially lower toxicity was not metabolized to dihydroartemisinin, suggesting it in vivo phenyl substituted derivative 15 CH2Cl2 at amounts of trimethylsilyl trifluoromethanesulfonate in presence of catalytic 1-hydroxypolyacetylated sugars in presence of catalytic oxonium intermediate as shown in Scheme 2.

Several C-12 phenoxy derivatives were evaluated against malaria parasites and found to possess excellent *in vitro* anti-malarial activity. On the basis of the excellent yield and stereoselectivity obtained from the p-trifluoromethyl derivative 15 (R = CF3, IC50 = 3.90 nM), this compound and the parent phenyl substituted derivative 15 (R = H) were subjected to *in vivo* biological evaluation by the authors on *P. berghei* in a mouse model and metabolism studies in rats. Compound 15 (R = CF3) demonstrated excellent *in vivo* anti-malarial potency with an ED50 value of 2.12 mg kg−1 (cf. arteether = 6 mg kg−1) vs *P. berghei*. Furthermore, from preliminary metabolic studies they have reported that this compound was not metabolized to dihydroartemisinin, suggesting it should have a longer half-life and potentially lower toxicity than arteether and artemether.

As discussed earlier, one of the major disadvantages of using artemisinins is their poor water solubility. To overcome this difficulty, sugar derivatives of DHA were prepared17 (18a–d) by condensing 12-O-(trimethylsilyl)dehydroartemisinin 17 with 1-hydroxypolyacetylated sugars in presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate in CH2Cl2 at −78 °C. Deacetylation of intermediates 18a–d afforded the desired sugar derivatives 19a–d (Scheme 3). On *in vitro* anti-malarial bioassay of the derivatives against *P. falciparum*, they were found to be more effective against W-2 and W-6 clones and were not cross-resistant with existing anti-malarials.

The trimethylsilyl derivative 17 was more active than derivatives 18a–d which possess activity comparable to or better than that of artemisinin 8. However, the deacetylated compounds 19a–d were substantially less active than the acetylated ones 18a–d. The anti-malarial activity results suggested that the *in vivo* activity of these sugar derivatives parallel those observed in *in vitro* tests and that the increase in polarity or water solubility tends to decrease anti-malarial activity.

In search of water-soluble and potent artemisinin derivatives, Li et al. have reported18 syntheses and anti-malarial activities of new 30 dihydroartemisinin derivatives (Table 1), containing an amino group (Scheme 4). Syntheses of targeted compounds were achieved by treatment of dihydroartemisinin 9, with the aliphatic alcohol in the presence of BF3·Et2O in dry CH2Cl2 solution to furnish compounds 20, 23 in quantitative yield. Compound 21 was obtained through the epoxidation of 20 using m-chloroperbenzoic acid. A series of amine derivatives 22, 24 were prepared by treating compounds 21 or 23 with various amines. Treatment of these basic compounds with organic acids (oxalic acid, maleic acid, etc.) yielded the corresponding salts. Generally, the maleates have better solubility in water than the corresponding oxalates. Compounds 24f (SD50 = 1.61 mg kg−1 day−1), 24b (SD50 = 1.74 mg kg−1 day−1), and 24r (SD50 = 1.82 mg kg−1 day−1) showed 4–5 fold higher activity against *P. berghei* infected mice by oral administration than artemisinic acid 12 (SD50 = 6.33 mg kg−1 day−1), although their activities drastically decrease (30–60 times) when administered via subcutaneous injection. Compounds 24f, 24h and 24r and artesunic acid 12 in a dose of 3.16 mg kg−1 day−1 and compounds 24f and 12 in a dose of 10.0 mg kg−1 day−1 were given orally in *P. knowlesi* infected monkeys for 7 days.

Compounds 24f, 24h and 24r reduced parasites more rapidly than artesunic acid 12, but a dose of 3.16 mg kg−1 24f did not cleanse all parasites. Compounds 24h and 24r recrudescence in 5–10 days after administration, whereas artesunic acid 12 can
cleanse parasites at a dose of 10.0 or 3.16 mg kg\(^{-1}\); no recrudescence within 105 days was observed. Its contradictory results in mice and monkeys explain that their water-soluble artemisinin derivatives have different types of absorption, excretion and metabolism in different species.

Li et al. found\(^1\) that cyano artemether 25, possessed inhibitory effect against *P. falciparum* and no cytotoxic effect against P388 cells *in vitro*, and that a pair of isomers (compound 26a and 27a) in test of antiproliferative potential displayed *in vitro* cytotoxicity against P388 and L1210 murine leukemia cell lines. They prepared the compound 26b (22% yield) and 27b (25% yield), as a pair of isomers, in the presence of BF\(_3\)-Et\(_2\)O with 2-(4-bromophenyl)-2-hydroxyacetoneitrile and KCN. These compounds were tested for the anti-proliferative effect against P388 and A549 tumor cell lines (Scheme 5). In order to test whether the peryx group is essential for anti-tumor activity, compound 28 (60% yield) was also prepared from compound 27b. Compound 26b and 27b showed the potent and similar activity to inhibit the proliferation of P388 and A549 cells, but compound 28 was not active. It is noteworthy that the peryx group appears to be essential for cytotoxicity as in the case of anti-malarial activity, and the configuration of C-16 has insignificant influence on the activity. Compounds 26b and 27b were equipotent for the inhibition of the proliferation and cell cycle progression.

The immunosuppressive action of artemisinin and its derivatives has also been studied in China for many years. Many experimental results *in vitro* and *in vivo* suggested that these new type of antimalarial drugs, such as artemisinin 8, dihydroartemisinin 9, artemether 10, and artesunic acid 12, 

### Table 1  Series of synthesized compounds

<table>
<thead>
<tr>
<th>n</th>
<th>NR(_1)R(_2)</th>
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<th>NR(_1)R(_2)</th>
<th>n</th>
<th>NR(_1)R(_2)</th>
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<th>NR(_1)R(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24a</td>
<td>NHMe</td>
<td>24b</td>
<td>NHMe</td>
<td>24c</td>
<td>NHEt</td>
<td>24d</td>
<td>NH(n-C(_3)H(_7))</td>
</tr>
<tr>
<td>24h</td>
<td>NHMe</td>
<td>24i</td>
<td>NHMe</td>
<td>24j</td>
<td>NHMe</td>
<td>24k</td>
<td>NH(CH(_2))OH</td>
</tr>
<tr>
<td>24m</td>
<td>NMe(_2)</td>
<td>24n</td>
<td>NMe(_2)</td>
<td>24o</td>
<td>NHMe</td>
<td>24p</td>
<td>NH(CH(_2))2NMMe(_2)</td>
</tr>
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</table>

HNR\(_1\)R\(_2\) = morpholine (A), piperazine (B), N-methylpiperazine (C), N-diphenylmethylpiperazine (D), pyrrolidine (E).\(^a\) As oxalate salt.\(^b\) As maleate salt.\(^c\) As fumerate salt.
possessed definite immunosuppressive activity. In search for new potential immunosuppressive agents with much higher efficacy and lower toxicity, Yang et al. have synthesized\textsuperscript{21} a class of novel artemisinin derivatives (30–37) starting from dihydroartemisinin acetate 29 (Scheme 6) and found that introduction of phen(ox)yl aliphatic acid and ester into artemisinin nucleus enhanced their immunosuppressive activity. These compounds (30–37) were assayed in their cytotoxicity of lymphocyte, inhibition activity on cancanavalin A (ConA) induced T cell proliferation and lipopolysaccharide (LPS) induced B cell proliferation. Among them, 31b, 33b, 34d, 35b, 36, and 37 remarkably exhibited lower cytotoxicity and higher inhibition activity on the mitogen-induced T-cell and B-cell proliferation in comparison with artemisinin, artesunate, and artemether \textit{in vitro}. More significantly, compound 31b displayed reduced cytotoxicity by over 100-fold compared with cyclosporine A (CsA) and comparable inhibition activity (SI = 848) on ConA-induced T cell proliferation to CsA (SI = 963) and more than 4000 times the inhibitory effect (SI = 28473) on LPS-induced B-cell proliferation compared with CsA (SI = 7). The \textit{in vivo} experimental results showed that compound 36 could inhibit 2,4-dinitrofluorobenzene (DNFB)-induced delayed type hypersensitivity (DTH) reaction and sheep red blood cells (SRBC) induced antibody, production respectively.

Based on their previous work, Yang et al. have further extended the study of immunosuppressive activity of a new series of substituted phenoxy propionic acids and ester derivatives (38,39).\textsuperscript{22} The synthesis of targeted compounds were achieved using dihydroartemisinin 9 (Scheme 7). These new dihydroartemisinin derivatives were tested \textit{in vitro} for their cytotoxicity on murine spleen cells and inhibitory activity on ConA-induced T cell proliferation or lipopolysaccharide (LPS) induced B cell proliferation with artemisinin, artemether, and artesunate as the controls. The cytotoxicity of each compound was expressed as the concentration of compound that inhibited ConA-induced T cell proliferation and LPS-induced B cell proliferation to 50% (IC\textsubscript{50}) of the control value. Among the whole series of compounds, 38a (IC\textsubscript{50} = 6.8 × 10\textsuperscript{-7}), 38e (IC\textsubscript{50} = 4.6 × 10\textsuperscript{-7}), 38h (IC\textsubscript{50} = 7.0 × 10\textsuperscript{-7}), and 38j (IC\textsubscript{50} = 8.4 × 10\textsuperscript{-7}) had 5- to 9-fold higher bioactivity than artemisinin (IC\textsubscript{50} = 4.4 × 10\textsuperscript{-6}), artesunate (IC\textsubscript{50} = 3.8 × 10\textsuperscript{-6}), artesunate (IC\textsubscript{50} = 4.8 × 10\textsuperscript{-6}) in the ConA-induced T cell proliferation. In the inhibition of LPS-induced B cell proliferation 38e (IC\textsubscript{50} = 2.8 × 10\textsuperscript{-7}), 38f (IC\textsubscript{50} = 1.6 × 10\textsuperscript{-7}), 38g (IC\textsubscript{50} = 3.0 × 10\textsuperscript{-7}), 38j (IC\textsubscript{50} = 2.2 × 10\textsuperscript{-7}), 39a (IC\textsubscript{50} = 5.0 × 10\textsuperscript{-7}), 39b (IC\textsubscript{50} = 1.8 × 10\textsuperscript{-7}), 39c (IC\textsubscript{50} = 1.0 × 10\textsuperscript{-7}), and 39e (IC\textsubscript{50} = 1.7 × 10\textsuperscript{-7}) exhibited 30- to 88-fold higher bioactivity than artemisinin (IC\textsubscript{50} = 9.0 × 10\textsuperscript{-6}), artemether (IC\textsubscript{50} = 1.8 × 10\textsuperscript{-6}), and artesunate (IC\textsubscript{50} = 9.9 × 10\textsuperscript{-7}) respectively.

Yang et al. have also synthesized\textsuperscript{23} a series of artemisinin derivatives bearing Mannich base groups (40a and 40b) starting from dihydroartemisinin 9 and tested for their anti-malarial activity against \textit{P}. \textit{berghei} and \textit{P}. \textit{falciparum} in \textit{K}1 and NF54 cells. Compound 40a (IC\textsubscript{50} = 0.18 and 0.36 ng mL\textsuperscript{-1}) and 40b (IC\textsubscript{50} = 0.25 and 0.17 ng mL\textsuperscript{-1}) were found to be more active in mice than artesunic acid (IC\textsubscript{50} = 1.20 and 1.20 ng mL\textsuperscript{-1}). These derivatives 40a and 40b (dose 3.16 and 10 mg kg\textsuperscript{-1} day\textsuperscript{-1}) were also examined for their anti-malarial activity against \textit{P}. \textit{knowles} in rhesus monkeys (Scheme 8) of 7 days treatment using artesunic acid as standard drug.
(dose 3.16 and 10 mg kg$^{-1}$ day$^{-1}$) and thus found to be better than artesunic acid.

Recently, Singh et al. have synthesized a new series of ether derivatives (41a–k) of dihydroartemisinin and their antimalarial activity was evaluated against multidrug-resistant *P. yoelii nigeriensis* in mice. The synthesis of the targeted compounds was achieved through the Lewis acid catalyzed (i.e. BF$_3$•Et$_2$O) coupling reaction between dihydroartemisinin 9 with the corresponding alcohol in CH$_2$Cl$_2$ at subzero temperature (−10 °C to −5 °C) furnishing the corresponding ether derivative in 65–99% yields as diastereomeric mixtures of $\alpha$ and $\beta$-isomers, with the $\beta$-isomers as the major products (Scheme 9). These new derivatives are highly lipophilic (log $P$ in the range of 5.51 to 7.19) as compared with $\beta$-artether (log $P$ 3.84), and several of them are two- to four-fold more active than $\beta$-artether. Among, the ether derivatives, the $\alpha$-isomers are more active than the $\beta$-isomers. The $\alpha$-ether biphenyl derivatives 41f (log $P$ = 6.91), and 41h (log $P$ = 6.85) are most active compounds of the series, provided 100% protection to infected mice at 12 mg kg$^{-1}$ × 4 days as compared to $\beta$-artether, *i.e.* 100% and 20% protection at 48 mg kg$^{-1}$ × 4 days and 24 mg kg$^{-1}$ × 4 days, respectively.

More recently, Singh et al. have also synthesized a series (42a–j) of ester derivatives starting from DHA 9, incorporating pharmacologically privileged substructure, such as biphenyl, adamantane and fluorene (Scheme 10) and evaluated for antimalarial activity against multidrug-resistant (MDR) *P. yoelii nigeriensis* by oral route. Several of these compounds 42a (log $P$ = 6.95), 42b (log $P$ = 6.89), 42c (log $P$ = 6.53), 42d (log $P$ = 6.53), 42e (log $P$ = 6.05), 42f (log $P$ = 5.99), 42g (log $P$ = 5.85), 42h (log $P$ = 6.41), 42i (log $P$ = 6.61), 42j (log $P$ = 6.79), were found to be more active than the anti-malarial drugs $\beta$-artether 12 (log $P$ = 3.84) and artesunic acid 13 (log $P$ = 3.04). Compound 42i was found to be most active of this series, providing 100% and 80% protection to the infected mice at 24 mg kg$^{-1}$ × 4 days and 12 mg kg$^{-1}$ × 4 days, respectively.

Although, the target for anti-malarial action of artemisinins is controversial, recent evidence suggest that an Fe$^{2+}$-activated form of the drug potentially inhibits PfATP, a key parasite Ca$^{2+}$ transporter. On the other hand, the mode of action of another anti-malarial drug quinine 1, has been suggested as a result of interference with host hemoglobin digestion. Due to anti-malarial synergism between artemisinin and quinine, Walsh et al. have recently prepared a covalently linked novel artemisinin-quinine hybrid compound 45 through the coupling of dihydroartemisinin 9 with a carboxylic acid derivative of quinine 44 via an ester linkage (Scheme 11). This hybrid molecule had potent activity against the 3D7 and (drug-resistant) FcB1 strains of *P. falciparum* in culture. The activity of this hybrid molecule 45 (IC$_{50}$ = 8.95 nM) was superior to that of artemisinin 8 (IC$_{50}$ = 49.4 nM) or, quinine 44 (IC$_{50}$ = 149 nM) alone, or a 1 : 1 mixture of artemisinin and quinine. Hybrid molecules (known as reversed chloroquine) of chloroquine was earlier made by Peyton et al. and found to be effective against chloroquine resistant parasites.

(B) C-12 sulfar derivatives

Angiogenesis, the formation of new blood vessels from existing host capillaries stimulated by biochemical stimulators, in normal vascular systems is involved in wound healing, embryonic development, and the female reproductive cycle under elaborate regulations. In particular, tumor angiogenesis is caused by angiogenic inducers playing a key role in the growth of the solid tumors, their invasion, and metastasis. Therefore, the control of angiogenesis may be a promising therapeutic strategy for the related disease. Strategies for regulating angiogenesis have been carried out mainly in molecular biology. However, in spite of the settlement of bioavailability, biostability, and effectiveness, it has been insufficiently carried out to develop small molecule anti-angiogenic agents. Therefore, it is important to discover anti-angiogenic small molecules that might be suitable as clinical therapies.
Recently, Chen et al. have reported\textsuperscript{27} that artemisinin and dihydroartemisinin and C-12 acetal type of artemisinin derivatives display anti-angiogenic-activity. Consequently, Oh et al. have synthesized\textsuperscript{28} C-12 sulfur derivatives of artemisinin (46–52) starting from dihydroartemisinin\textsuperscript{9} (Scheme 12) and tested against HUVEC proliferation at the concentration level of 1 \(\mu\)M using artemisinin\textsuperscript{8}, and DHA\textsuperscript{9}. Compounds 46 (IC\textsubscript{50} = 0.93 \(\mu\)M), 52 (IC\textsubscript{50} = 1.74 \(\mu\)M), and 51 (IC\textsubscript{50} = 1.29 \(\mu\)M), have displayed potent growth inhibitory activity as compared to 8, 9 (IC\textsubscript{50} 4.50 \(\mu\)M), and 9 (IC\textsubscript{50} = 8.91 \(\mu\)M).

(C) C-12 carbon analogues

The poor bioavailability and rapid clearance observed with first-generation analogues of DHA is due to the acetal function present in these derivatives. As discussed earlier in this article, one of the principle routes of metabolism of artemether, for example, involves oxidative dealkylation to DHA\textsuperscript{9}, a compound associated with toxicity and short half life (Scheme 1). Replacement of the oxygen at the C-12 position with carbon would be expected to produce compounds not only with greater hydrolytic stability but also with a longer half-life and potentially lower toxicity. Consequently, several groups have developed synthetic and semisynthetic approaches to C-12 carba-analogues.

O’Neill’s group has synthesized\textsuperscript{29} several novel second-generation fluorinated ether and ester analogues of arteether and artemether and evaluated for their anti-malarial potency (Scheme 13). All of their derivatives demonstrated high anti-malarial potency in vitro against the chloroquine sensitive HB\textsubscript{3} and resistant K\textsubscript{1} strains of \textit{P. falciparum}. The fluorinated aromatic ring systems selected were linked to alcohol 54 by either an ester linkage, 56 or an ether linkage 55, starting from the key intermediate allyl deoxo-derivative 53. In vitro, the most potent derivative 55a (IC\textsubscript{50} = 0.22) was 15 times more active than artemisinin (IC\textsubscript{50} = 3.04) and 5 times more potent than DHA 9 (IC\textsubscript{50} = 1.04) against HB\textsubscript{3} strain of \textit{P. falciparum}. However, in vitro against \textit{P. berghei} in the mouse, selected derivatives were generally less potent than DHA 9 (ED\textsubscript{50} = 1.15) with ED\textsubscript{50} values between 5 to 8 mg kg\textsuperscript{-1}. On the basis of the products obtained from the in vitro biomimetic Fe(II)-mediated decomposition of 55a, they believe that the radical mediator of biological activity of this series may be different from that of the parent drug artemisinin 8.

Latter, Ziffer’s group has synthesized\textsuperscript{30} another series of C-12 carba analogues (58a–60 and 61) using key intermediate aldehyde 57, prepared through the O’Neill’s compound 53 (Scheme 14). The aldehyde 57 was then reacted with a variety of Grignard reagents to produce 58a–d. These Grignard products (58a–d) were oxidized to their corresponding ketones (61) using Jones’ reagent. Aldehyde 57 was also reacted with a Wittig reagent to afford 59. They observed that the peroxide moiety essential for anti-malarial activity was not altered under the reaction conditions of the Wittig or Grignard reactions. The reaction of 57 with trimethyl(trifluoromethyl)silane yielded a pair of isomeric alcohols 60a and 60b.

The in vitro anti-malarial activities of all the synthesized compounds (58a–d, 59, 60, and 61) were determined against two drug resistant clones (W-2 is chloroquine resistant, mefloquine sensitive while D-6 is mefloquine resistant and chloroquine sensitive) of \textit{P. falciparum}. Out of all the synthesized compounds tested, compounds 58b (IC\textsubscript{50} = 4.8 and 5.8) and 58d (IC\textsubscript{50} = 5.4 and 6.8) were 5–7 times more potent than artemisinin 8.
Posner et al. reported a series of C-12 carba analogues by treating organometallic reagents with aldehyde under controlled conditions (Scheme 15). Treatment of aldehyde with organometallic reagents produces the allylic alcohol while aldehyde reacts with Wittig reagent to form a mixture of geometric isomers of exocyclic alkene without cleaving the endoperoxide linkage. Anti-malarial testing of these analogues in vitro against P. falciparum NF54 malaria parasites, showed that C-9,10 unsaturated, C-10 carbon substituted heteroaryl artemisinin analogues ketones (IC50 = 4.3 nM where R = n-Bu and 4.6 nM where R = Ph), tertiary alcohol (IC50 = 4.5 nM) and exocyclic alkene (IC50 = 28 nM (R = CH═CH2), 16 nM (R = (E)-CH═CPh), 8.1 nM (R = (Z)-CH═CPh), 11 (R = (E)-CH═CPhNO2-p)] are all similar to clinically used natural artemisinin (IC50 = 10.1 ± 1.3 nM) analogues.

It is generally accepted now that the carbon centered free radicals generated in the course of degradation-rearrangement of artemisinin and the like may play a major role in the killing of malaria parasites. Thus, Wu et al. have reported their findings on the Fe(II) induced cleavage of the peroxide bond in artemisinin and its derivatives and the DNA damage associated with this process. In order to afford a sounder basis for probing the chemical and biochemical processes that artemisinin derived compounds may participate in, they designed a few C-12 carba-derivatives that carry a UV chromophore through a C-C σ bond (Scheme 16). The isomer which has the normal configuration (i.e. the same configuration as in artemisinin) at C-12 showed high anti-malarial activity in the preliminary in vivo test on mice against P. berghei IC173 strain. The abnormal isomer (ED50 = 7.08 mg kg\(^{-1}\), ED90 = 60.99 mg kg\(^{-1}\)) is obviously much less potent than (ED50 = 0.58 mg kg\(^{-1}\), ED90 = 1.73 mg kg\(^{-1}\)) against P. berghei K173 strain administered orally to mice as suspensions in Tween 80 as compared to arteether (ED50 = 1.00 mg kg\(^{-1}\), ED90 = 3.10 mg kg\(^{-1}\)).

The marked difference in the anti-malarial potencies of (IC50 = 0.4 and 0.5 nM), (IC50 = 0.4 and 0.5 nM), and (IC50 = 0.4 and 0.5 nM) have shown very strong anti-malarial potency against strains (W-2 and Ghana) of P. falciparum. These compounds (71a, 71b, 71c) are about 25 times more potent to the resistant clone (W-2) and 20 times to the sensitive clone (Ghana) than artemisinin (IC50 = 10 and 9 nM). In addition, other derivatives containing amino-alkyl and heterocycles were also highly potent against P. falciparum. Compound (IC50 = 1.0 and 1.0 nM) is about 10 times more potent than artemisinin.

Recently, Magueur et al. have reported that introduction of gem-difluoromethylene group at the C-12 position of the artemisinin resulted in better in vitro antimalarial activity. They
have synthesized gem-difluoromethylene deoxy-artemisinin 72 through artemisinin 8 in three steps (Scheme 18). The in vitro antimalarial activity of artemisinin 8 (IC₅₀ = 8.9) and of compound 72 (IC₅₀ = 4.6) were determined using the chloroquine resistant FCB1 strain of P. falciparum.

(D) C-13 substituted derivatives from artemisitene

The majority of derivatives of artemisinin prepared so far were derivatized through C-12 either as acetal and non-acetal type, and only a few C-13 derivatives were reported. Artemisitene 73 exists in the same plant in much lower yield and has less anti-malarial activity than artemisinin 8. However, artemisitene 73 can be easily prepared from artemisinin 8 in a single-step reaction in 73% yield. It contains an α,β-unsaturated lactone moiety, which can be used as a Michael receptor for derivatization. Thus, Li *et al*. prepared 37 74a-c and 75a-c with 1,2,4-triazole, benzotriazole or benzamidazole moiety under different conditions (Scheme 19), heating 1,2,4-triazole as its salts and artemisitene or benzamidazole moiety under different conditions

(E) Artemisinin dimers, trimers and tetramers: novel leads in drug discovery

It has been established through the structure–activity relationship (SAR) studies of artemisinin and its various kinds of C-12/C-13 ether/ester derivatives that only peroxide-linkage affects the anti-malarial and anti-cancer activity. Furthermore, several drawbacks associated with these compounds viz solubility, thermal and hydrolytic stability, bioavailability, and short half life etc., have led to development of second generation C-12/C-13 trioxane-derivatives. Furthermore, it was thought worthwhile that the extent of anti-malarial activity depends upon the extent of the number of peroxide units, which can be increased by adding of additional artemisinin moiety through careful chemical manipulations. Thus, researchers have directed their efforts for the synthesis of various kinds of artemisinin dimers, trimers and tetramers of various length and flexibility. Artemisinin dimers reported till date, have displayed structural diversity, separated through artemisinin monomer units with or without linkers of various length and flexibility with diverse stereochemistry. Several of these C-12/C-13 carbon artemisinin dimers have shown outstanding anti-malarial and anti-cancer activity and are better than C-12 ether/ester dimers. Artemisinin trimers and tetramers of C-12/C-13 non-acetal derivatives have also been reported in recent years, wherein artemisinin units are connected through linkers of various kinds with diverse length and stereochemistry. However, the number of artemisinin dimers synthesized so far is far-ahead of the number of artemisinin trimers and tetramers. Many of these dimers, trimers and tetramers have shown outstanding anti-malarial and anti-cancer activities compared to artemisinin and related compounds, and are in various phases of clinical trials. These compounds may become future potential leads in anti-malarial and anti-cancer chemotherapy.

1. Artemisinin dimers

Based on the C-12/C-13 linkage between artemisinin units through the linker, whether acetal or non-acetal, artemisinin dimers have been classified as follows:

(a) C-12 *oxa dimers*: Wherein two artemisinin monomers units are linked through the C-12 acetal (i.e. ether–ester linkage) linker.

(b) C-12 *carba dimers*: Wherein two artemisinin monomers units are linked through the C-12 non-acetal (i.e. carbon–carbon linkage) linker.
(c) C-13 carba dimers: Wherein two artemisinin monomers units are linked through the C-13 non-acetal (i.e. carbon–carbon linkage) linker.

(a) C-12 oxa dimers. In search for new potential artemisinin derivatives, several new C-12 oxygen derivatives have been reported by various groups. Thus, Beekman et al. have reported\textsuperscript{38} the anti-cancer activity of C-12 oxa-dimers where two artemisinin units were connected through an ether-linkage (Scheme 20). The cytotoxicity of these derivatives were determined against EN2 tumor cells using the MTT assay. They realized that artemisinin 8 (IC\textsubscript{50} = 0.98) was more cytotoxic than the corresponding deoxyartemisinin 76 (IC\textsubscript{50} = 111), which lacks an endo-peroxide linkage. Ether–linked dimers of deoxyartemisinin with defined stereochemistry were found to differ in extent of cytotoxic effect on EN2 cells. The non-symmetrical dimer 77 (IC\textsubscript{50} = 0.11) was more cytotoxic than the symmetrical dimer 78 (IC\textsubscript{50} = 2.0). Similarly, the symmetrical dimer 80 (IC\textsubscript{50} = 99.8) was less effective than 79 (IC\textsubscript{50} = 8.9).

In order to study the role of linker in affecting the biological activity of dimers, Posner et al. have synthesized\textsuperscript{39} a series of artemisinin C-12 oxa dimers linked through two artemisinin units either by a polyethylene glycol or carbon chain link or disulfide linker, with varying length and flexibility (Scheme 21). The syntheses of targeted dimers were achieved starting from dihydroartemisinin 9 using the required linker. They have tested the anti-proliferative activities in normal murine keratinocytes using calcitriol as a standard drug and found several new C-12 oxygen derivatives have been synthesized. Thus, Posner et al. have first reported\textsuperscript{41} syntheses of C-12 olefinic carba dimers of m-xylene by reacting their previously prepared aldehyde 62, with Wittig reagent 83 to afford three isomers with different stereochemistry 84\textsubscript{a–c} (Scheme 23). The in vitro anti-malarial potencies of m-xylene dimers 84\textsubscript{a–c} against chloroquine sensitive P. falciparum (NF54) parasites was measured, wherein 84\textsubscript{a} (IC\textsubscript{50} = 77 nM), 84\textsubscript{b} (IC\textsubscript{50} = 35 nM), 84\textsubscript{c} (C\textsubscript{50} = 18 nM) were found less potent than artemisinin 8 (IC\textsubscript{50} = 9.7 nM). They have further extended the study and synthesized C-12 non-acetal saturated dimers 85\textsubscript{a,b} starting from artemether 10 via novel titanium-promoted condensation (Scheme 24). They have further synthesized the C-12 non-acetal saturated C-12 dimers (87–89) by coupling of recently prepared artemisinin derived fluoride 86 via Friedel–Crafts or aluminium acetylide condensations (Scheme 25). Although benzoylmethylene dimers 85\textsubscript{a} and 85\textsubscript{b} and acetylenic dimers 89\textsubscript{a} and 89\textsubscript{b} are stereochemically β-linked to C-12 of the artemisinin, aryl dimers 87 and 88 are α-linked; the basis for this difference in stereochemistry of attachment is not fully understood. Unlike the bis-acetylenic dimers 89\textsubscript{a} and 89\textsubscript{b}, aryl dimer 87 and furan dimer 88 are considerably more potent anti-malarial agents (IC\textsubscript{50} = 1.3–3.2 nM) than natural artemisinin (IC\textsubscript{50} = 9.7 nM) as mentioned in Table 2. Compounds 85\textsubscript{a–c}, 87, 88, 89\textsubscript{a–c} showed good to excellent antiproliferative activity. Dimers 85\textsubscript{a}, 87 and 89\textsubscript{a} were specially potent and selective in

(b) C-12 carba dimers. Although C-12 oxa dimers have displayed high antimalarial, antiproliferative and anti-tumor activities in vitro, often they are hydrolytically unstable. In order to enhance the hydrolytic stability, researchers have directed their efforts to synthesize a variety of C-12 olefinic carba dimers. Thus, Posner et al. have subsequently reported\textsuperscript{38} syntheses of C-12 olefinic carba dimers of m-xylene by reacting their previously prepared aldehyde 62, with Wittig reagent 83 to afford three isomers with different stereochemistry 84\textsubscript{a–c} (Scheme 23). The in vitro anti-malarial potencies of m-xylene dimers 84\textsubscript{a–c} against chloroquine sensitive P. falciparum (NF54) parasites was measured, wherein 84\textsubscript{a} (IC\textsubscript{50} = 77 nM), 84\textsubscript{b} (IC\textsubscript{50} = 35 nM), 84\textsubscript{c} (C\textsubscript{50} = 18 nM) were found less potent than artemisinin 8 (IC\textsubscript{50} = 9.7 nM). They have further extended the study and synthesized C-12 non-acetal saturated dimers 85\textsubscript{a,b} starting from artemether 10 via novel titanium-promoted condensation (Scheme 24). They have further synthesized the C-12 non-acetal saturated C-12 dimers (87–89) by coupling of recently prepared artemisinin derived fluoride 86 via Friedel–Crafts or aluminium acetylide condensations (Scheme 25). Although benzoylmethylene dimers 85\textsubscript{a} and 85\textsubscript{b} and acetylenic dimers 89\textsubscript{a} and 89\textsubscript{b} are stereochemically β-linked to C-12 of the artemisinin, aryl dimers 87 and 88 are α-linked; the basis for this difference in stereochemistry of attachment is not fully understood. Unlike the bis-acetylenic dimers 89\textsubscript{a} and 89\textsubscript{b}, aryl dimer 87 and furan dimer 88 are considerably more potent anti-malarial agents (IC\textsubscript{50} = 1.3–3.2 nM) than natural artemisinin (IC\textsubscript{50} = 9.7 nM) as mentioned in Table 2. Compounds 85\textsubscript{a–c}, 87, 88, 89\textsubscript{a–c} showed good to excellent antiproliferative activity. Dimers 85\textsubscript{a}, 87 and 89\textsubscript{a} were specially potent and selective in

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**Scheme 20**

**Scheme 21**

**Scheme 22**
inhibition of growth of some human cancer cell lines in the NCI in vitro 60-cell line assay. Later on, Posner’s group has also synthesized 42 C-12 non-acetal dimers \(90a–c\) starting from dihydroartemisinin acetate \(29\) and their ketone functionality was converted into difluoro derivative \(91a,b\) using bis(2-methoxyethyl)amino sulfur trifluoride (BAST) (Scheme 26). The peroxide group of C-12 \(90a\) dimer was reduced using Zn/AcOH to afford \(92\). Anti-malarial activity of these compounds were tested against chloroquine-sensitive NF54 strain of \(P. falciparum\). Dicarbonyl dimers \(90a–c\) are 2–5 times more potent (\(IC_{50} = 1.9 \text{nM}\) for \(90a\), \(IC_{50} = 1.7 \text{nM}\) for \(90b\), \(IC_{50} = 3.9 \text{nM}\) for \(90c\)) than artemisinin 8 (\(IC_{50} = 7.6 \text{nM}\)), whereas tetrafluorinated dimers \(91a–b\) are 2–4 times less potent (\(91a, IC_{50} = 28 \text{nM}\); \(91b, IC_{50} = 15 \text{nM}\)) than artemisinin 8. Dimer \(92\) (\(IC_{50} = 1030 \text{nM}\)) without peroxide linkage possesses very weak or no antimalarial activity.

Antiproliferative activities were measured in vitro using murine keratinocytes for new non-fluorinated dimers \(90b\) and \(90c\) and for new fluorinated dimers \(91a\) and \(91b\). It is noteworthy that these dimers are more effective at 1 \(\mu\)M concentration than calcitriol used as a standard drug. Growth inhibitory activities at nanomolar to micromolar concentrations, measured in vitro using a diverse panel of 60 human cancer cell lines, indicates that non-fluorinated dimers \(90b\) and \(90c\) are particularly inhibitory to leukemia cells, and these dimers are very selectively potent in a few other cancer cell lines (e.g. colon 205, ovarian cancer OVCAR-4, non-small cell lung EKVX). The highly selective and powerful anticancer activities of dimers \(90b\) and \(90c\), coupled with lack of cytotoxicity, make these promising lead compounds for further preclinical study in dual action chemotherapy of both malaria and cancer.

Later on, Jung et al. reported a series of C-12 non-acetal amido (\(97a,b\), \(100, 101\)) and sulfide/sulfoxide (\(102, 103, 104, 105\)) dimers (Scheme 27). Synthesis of target compounds were achieved from the key intermediates \(94\) and \(96\), which were easily synthesized from compound \(93\). The syntheses of \(97a,b\) were achieved through the direct coupling of acid \(94\) with amino compound \(96\) using EDC–HOBt system. Synthesis of dimers \(100\) and \(101\) was achieved by coupling of compound \(96a\) with protected glutarate \(98\) using the EDC–HOBt system to afford compound \(99\), which upon further coupling with \(96a\) using EDC–HOBt afforded \(t\)-Boc protected amido-dimer \(88\). Compound \(88\) upon treatment with TFA afforded

<table>
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<th>Dimer</th>
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<th>Anti-malarial activity, (IC_{50}/\text{nM})</th>
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<td>(85a)</td>
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<tr>
<td>(85b)</td>
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<td>(89b)</td>
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<td>36</td>
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<td>(8)</td>
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IC \(50 = 1.7 \text{nM}; 90c, IC_{50} = 3.9 \text{nM}\) than artemisinin 8 (\(IC_{50} = 7.6 \text{nM}\)), whereas tetrafluorinated dimers \(91a–b\) are 2–4 times less potent (\(91a, IC_{50} = 28 \text{nM}; 91b, IC_{50} = 15 \text{nM}\)) than artemisinin 8. Dimer \(92\) (\(IC_{50} = 1030 \text{nM}\)) without peroxide linkage possesses very weak or no antimalarial activity.

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unprotected amido dimer 101. Compound 95a on treatment with Na₂S afforded compound 102 which upon further treatment with m-CPBA afforded compound 103. Compound 95a on treatment with 1,3-thiol afforded dimer 104, which upon further treatment with m-CPBA afforded compound 105.

The in vitro cytotoxicity of artemisinin and related dimers against murine and human cancer cells. Sulfide dimer 102 (IC₅₀ = 0.40 μg mL⁻¹) is active comparable to aldriamycin (IC₅₀ = 0.39 μg mL⁻¹) and four times more active than mitomycin (IC₅₀ = 1.50 μg mL⁻¹) against mouse fibroblast leukemia (P388). Sulfone-linked dimer 103 (IC₅₀ = 1.04 μg mL⁻¹) is active comparable to mitomycin (IC₅₀ = 0.85 μg mL⁻¹) and six times more active than adriamycin (IC₅₀ = 6.24 μg mL⁻¹) and taxol (IC₅₀ = 7.39 μg mL⁻¹) against human placental choriocarcinoma cells (Bewo). Dimer 97a (IC₅₀ = 0.005 μg mL⁻¹), particularly, is 24 times more active than aldriamycin (IC₅₀ = 0.12 μg mL⁻¹) and 200 times more active than mitomycin (IC₅₀ = 0.93 μg mL⁻¹), but 50 times less active than taxol (IC₅₀ = 0.0001 μg mL⁻¹).

Later on, Posner’s group has synthesized a series of artemisinin dimers (106–118) starting from dihydroartemisinin acetate 29 (Scheme 28). Compound 29 upon treatment with allylic bis-silane using tin chloride afforded dimer 106, which was used as a key intermediate for the transformation to a diverse series of dimer derivatives (107–116). Anti-malarial activity of these dimer derivatives were carried out in vitro using chloroquine-sensitive P. falciparum (NF 54) parasites. In sharp contrast to the potencies of the natural trioxane artemisinin 8 (IC₅₀ = 9.0 nM) and of the initial olefinic dimer 106 (IC₅₀ = 24 nM), alcohol and diol dimers 107 (IC₅₀ = 0.87 nM) and 110 (IC₅₀ = 0.59 nM) and ketone dimer 112 (IC₅₀ = 0.91 nM), all have substantially enhanced potencies, with IC₅₀ values below 1 nM. Also, water-soluble carboxylic acid dimers 108 (IC₅₀ = 2.0 nM), 109 (IC₅₀ = 1.7 nM), 111 (IC₅₀ = 3.0 nM), 114 (IC₅₀ = 2.1 nM), and 118 (IC₅₀ = 2.4 nM) all are several times more potent than artemisinin 8. Anti-cancer growth inhibitory activities of these dimers were measured in vitro using a diverse panel of human cancer cell lines, indicates that alcohol and diol dimers 107 and 110 are strongly growth inhibitory but not cytotoxic towards several human cancer cell lines. Moreover, water-soluble dimers 108, 111 and
are also potent inhibitors of cancer cell growth without being cytotoxic. These semi-synthetic new chemical entities, and especially the easily synthesized dimer carboxylic acids, therefore, deserve further preclinical evaluation as potential drug candidates for chemotherapy of malaria and cancer.

Later on, Posner and O’Neill’s group synthesized a new series of artemisinin dimers starting from their previously prepared key intermediate (Scheme 29). All of these dimers prepared displayed potent low nanomolar anti-malarial activity vs. K1 and HB3 strains of *P. falciparum*. The most potent compound assayed was phosphate dimer (IC₅₀ = 0.2 nM), which was greater than 50 times more potent than the parent drug artemisinin (IC₅₀ = 12.3 nM), and about 15 times more potent than the clinically used acetal artemether. All of the dimers expressed poor anticancer activity apart from the trioxane phosphate ester dimer and which expressed nanomolar growth inhibitory (GI₅₀) values vs. a range of cancer cell lines in the NCI 60 human cell line screen. Furthermore, detailed studies on these dimers in vitro in HL60 cells demonstrate that both phosphate ester dimers (IC₅₀ = 0.14 μM) and (IC₅₀ = 0.24 μM) are more potent than the anti-cancer agent doxorubicin (IC₅₀ = 0.51 μM).

Posner’s group has further synthesized isobutyric acid dimer and isonicotinate *N*-oxide dimer starting from alcohol dimer (Scheme 30). Antimalarial potencies of dimer (ED₅₀ = 0.53 nM) and isobutyric acid dimer (ED₅₀ = 2.4 nM) were considerably more antimalaria efficacious than clinically used sodium artesunate (ED₅₀ = 1.5 nM) via both oral and intravenous administration. Both alcohol dimer and N-oxide dimer, but not carboxylic acid dimer, very strongly inhibit the growth of prostate cancer cells.

Recently, Posner et al. have also reported the syntheses of another new series of artemisinin bis-benzylic dimers (starting from conjugated dimer, obtained directly from dihydro-artemisinin acetate (Scheme 31). Thus, conjugated new dimer undergoes reaction to afford compound. Dimer through a series of reactions...
of other synthetic-transformations affords dimer 128–131. Anti-malarial activities of these dimers (126–131) were determined in vitro against chloroquine-sensitive *P. falciparum* (NF 54) parasites. Except for the water-soluble phthalic acid dimer 128 (IC$_{50}$ = 360 nM), all of other dimers are considerably more potent anti-malarials [(126, IC$_{50}$ = 2.9 nM), (127, IC$_{50}$ = 1.6 nM), (129, IC$_{50}$ = 0.77 nM), (130, IC$_{50}$ = 3.0 nM), (131, IC$_{50}$ = 3.7 nM)] than artemisinin 8 (IC$_{50}$ = 6.6 nM).

Preliminary growth inhibitory activities at nanomolar to micromolar concentrations were measured in vitro using a diverse panel of 60 human cancer cell lines. It has been realized that trioxane phthalate dimer 127 (IC$_{50}$ = 500 nM) is approximately 10–20 times more potent than trioxane-monomer DHA 9 and that trioxane diol dimer 129 (IC$_{50}$ = 46.5 nM) is approximately 110–220 times more potent than DHA 9, without being toxic to primary normal cervical cells. The most potent and selective two dimers 127 and 129 deserve further preclinical evaluation as potential drug candidates for effective chemotherapy of malaria and cancer.

Later on, Posner’s group synthesized$^{48}$ C-12 dimers (132–134) starting from his previously reported alcohol dimer 107 (Scheme 32). The mechanism of action of these dimers were examined in human (LNCaP) and mouse (TRAMP-C1A
and -C2H) prostate cancer cell lines. All these dimers (132–134) inhibited cell growth with the 134 being most potent in C1A (GI50 = 18.0 nM), C2H (GI50 = 17 nM) and LNCaP (GI50 = 17.9 nM) cells in comparison to the standard drug doxorubicin (GI50 = 45.3 nM). These trioxane dimers induced G0/G1 cell cycle arrest in LNCAp cells and decreased cells in S phase. These changes correlated with modulation of G1 phase cycle proteins, including decreased cyclin D1, cyclin E and cdk2, and increased P21waf1 and p27kip. These dimers (132–134) also promoted apoptosis in LNCaP cells with increased expression of proapoptotic BAX. These results demonstrate that these dimers (132–134) are potentially useful agents that warrant further preclinical development for the treatment of prostate cancer.

Simultaneously, Jung et al. have also reported49 a new series of trioxane-artemisinin dimers (136, 137) starting from their previously prepared key intermediate alcohol 135. Thus, the alcohol 135 was first converted into a malonate dimer 136, obtained by treatment of alcohol 135 with malonyl chloride. This malonate dimer 136 was further converted to Bingel adduct trioxane dimer 137 (i.e. fullerene conjugate dimer) by treatment with fullerene C60 (Scheme 33). They have tested the in vivo antiangiogenesis activity of these dimers (136, 137) along with the standard drug fumagillin, thalidomide and artemisinin 8. It has been realized that the anti-angiogenic effect of fullerene dimer 137 is similar to fumagillin and thalidomide and double in comparison with artemisinin 8.

Recently, Posner’s group has synthesized50 another new series of trioxane dimers (138–140) starting from their previously prepared dimer compounds 106, 107, 110, 124 as mentioned in references (Scheme 34). Four of these dimers (134, 138, 139, 140a) cure malaria-infected mice after only a single subcutaneous dose, and two other dimers (140b, 140c) cure after three oral doses in P. Berghei infected mice. These dimer compounds have become lead drug candidates to enter in advanced preclinical evaluation and would ultimately be used in human studies.

More recently, Posner’s group has also reported51 another series of trioxane dimers (141–163) starting from their previously reported dimers 106, 107, 110, 112, 124 as a key intermediates (Scheme 35). Thus, hydrazone dimer 141 was obtained from the corresponding keto-compound 112, ketal dimers (142–149) were synthesized from the diol dimer 110, various alkylated ether/esters dimers (150, 152) were synthesized by selective alkylation of diol dimer 110, various amide dimers were synthesized (156–161), and oxadiazoles dimers (162, 163) from the corresponding acid dimer 124, which was obtained through the oxidation of alcohol dimer 107. This alcohol dimer 107 was further converted to trioxane dimers (153–155). Out of 23 dimers synthesized, 11 of these new trioxane dimers (142–145, 147–150, 153, 155 and 156) cure malaria-infected mice via oral dosing at 3 x 30 mg kg⁻¹. These trioxane dimers are stable both thermally and hydrolytically. Furthermore, chemical structure–biological activity relationship (SAR) is ongoing, aimed at developing trioxane dimers able to achieve single oral dose cure.

(c) C-13 carba dimers. In order to search for more hydrolytically stable and potent compounds, researchers became interested to synthesize the C-13 dimers of artemisinin 8, starting from the key intermediate artemisitene 73.

Ekthawatchai et al. have reported52 synthesis of C-13 carba-dimers (164, 166–168) starting from the key intermediate artemisitene 73 (Scheme 36). The synthesis of dimers 164a–d was achieved through the base-catalyzed coupling of 73 with the disulfide linkers of different length. However, compounds 164a–d were not found to be very stable and spontaneously decomposed in solutions or upon storage at room temperature giving complex mixtures. Synthesis of C-13 dimers 167 and 168 was achieved through Grignard reagent of suitable length with the key intermediate 73. Dimer 166 was achieved through the nucleophilic addition of 165 with 73. Anti-malarial activities of dimers 164a–d, 167 and 168 were tested in an in vitro malaria screening system against P. falciparum (K1, multidrug
resistant strain). It has been observed that these dimers (164a–d, 167 and 168) do not show promising anti-malarial activity, perhaps since the stereochemical orientations at C-13 and C-13' of the two artemisinin molecules in these dimers might play almost insignificant roles with regard to their biological activities. Anticancer activities of artemisinin dimers 166a–g show high potency against vero cells only.

Recently, Grellepois et al. have reported synthesis of C-13 carba dimer 170 (Scheme 37) starting from C-13 allylic ether compound 169 using Grubbs metathesis reaction. They have further synthesized C-13 olefinic dimer containing hydroxy groups 172 through a metathesis approach, starting from C-13 allylic alcohol 171.

Preliminary growth-inhibitory activity of these dimers (170, 172) was evaluated in vitro using a diverse panel of 60 human cancer cell lines. Compound 170 was efficient in cancer cell growth inhibition with a GI₅₀ less than 10 nM in many cases. Particularly, TGI data shows the selectivity and potency of dimer 170 against a few cancer cell lines (e.g. leukemia HL-60, non-small cell lung cancer NCI-H-226, colon cancer COLO 205, and KM-12, CNS cancer SF-295).

Scheme 35

(II) Artemisinin trimers and tetramers

In order to search for more potent, more bioavailable, hydrolytically stable, and less toxic compounds of artemisinin derivatives, researchers have directed their efforts towards the synthesis of trimer and tetramer derivatives of artemisinin.

Ekthawatchai et al. have first reported synthesis of trimers 173a,b and tetramers 174a,b of artemisinin, starting from their own key intermediate artemisitene 73 (Scheme 38). Anti-malarial activities of these trimers [(173a, EC₅₀ = 2.4 nM), (173b, EC₅₀ = 3.1 nM)] and tetramers [(174a, EC₅₀ = 5.8 nM), (174b, EC₅₀ = 20 nM)] are quite impressive and higher (except 174b) in comparison to artemisinin 8 (EC₅₀ = 12.1 nM).

Later on, Jung et al. synthesized another artemisinin trimer 175 through the coupling of their previously synthesized key intermediates 94 and 96a (Scheme 39). The in vitro cytotoxicity of this trimer 175 was tested against murine and human cancer cells. The trimer 175 (IC₅₀ = 0.12 μg mL⁻¹) is three times more active than adriamycin (IC₅₀ = 0.39 μg mL⁻¹), 12 times more active than mitomycin ((IC₅₀ = 1.50 μg mL⁻¹), and 20 times more active than taxol (IC₅₀ = 2.27 μg mL⁻¹) against P388. Furthermore, it has been realized by these researchers that this trimer 175 is most potent in almost all the human cancer cell lines tested, and should receive more attention as a possible anti-cancer drug candidate.

Mechanism of action of artemisinins

(A) Mode of anti-malarial activity

The entry of the malaria parasites into their human host is through a mosquito bite. They first enter the liver and replicate there for two weeks, before beginning a cycle of red blood cell invasion, followed by growth, replication and red cell destruction that leads to the symptoms of the disease. The artemisinin drugs are known to act specifically during this blood stage. Although the mechanism of action of artemisinins is still not conclusive, there are strong evidences to suggest that an
endoperoxide linkage of artemisinins and a heme iron play critical roles in their mechanism of action, which is comprised of two distinct steps. In the first step (activation step), the heme iron attacks and breaks the endoperoxide linkage of artemisinin to produce an oxy free radical, which is then rearranged to give a carbon free radical. In the second step, the carbon free radical produced from the first step will alkylate specific malarial proteins causing lethal damage to malarial parasites (Scheme 40).

For the activation step, there are two possible pathways 1 and 2 (Scheme 40). In pathway 1, the heme iron attacks the
endoperoxide moiety at the O₃ position, giving the free radical at the O₁ position (176). This process is followed by an intramolecular 1,5-H shift and the C₄ free radical (178) is obtained. In pathway 2, the heme iron, on the other hand, attacks the endoperoxide moiety at the O₁ position, giving the free radical at the O₂ position (177). This process is followed by a hemolytic cleavage of the C₃–C₄ bond, also resulting in the C₄ free radical (179). Hence, it could be concluded that the C₄ free radical product is very critical for antimalarial activity of artemisinins.

(B) Mode of anti-cancer activity

In the mid-1990s selective cytotoxicity of artemisinin-derived peroxides towards cancer cells also became known. Cancer cells require much iron to assist their rapid proliferation and indeed, human cancer cells are known to be richer than normal human cells in receptors for transferrin, an iron transporting protein. Most cancer cells express higher cell surface concentration of transferrin receptors than normal cells and have high rates of iron intake via transferrin receptors. Efforts to explore the molecular mechanism of action of these monomeric 1,2,4-trioxanes towards tumor cells have established a correlation between a trioxane’s potency and m-RNA gene expression, cell doubling time and the portion of cells in different cell cycle phases. According to Moore and his co-workers, a unique structure bearing endo-peroxide could be a trigger for the generation of active oxygen radicals via homolytic cleavage of the weak oxygen peroxide bond accelerated by higher ferrous iron concentration of the cancer cells which may cause selective and preferential damage to vital cellular structure of the relatively active cancer cells. While the anticancer mode of action of artemisinins is relatively little studied and known, recent studies have revealed a radical alkylation and inhibition of the G₁ cycle for anticancer activity.

Conclusions

In this review, we have attempted to give a comprehensive overview on the recent developments of artemisinin and its derivatives as potential anti-malarial and anti-cancer agents. Comparative anti-malarial and anticancer activities of these derivatives were discussed and a study on the efforts towards the development of various artemisinin dimers, trimers and tetramers as potential ‘leads’ for anti-malarial and anticancer drugs have been carried out, where the activities have been correlated with artemisinin and various other standard anti-malarial and anti-cancer drugs. From the current degree of development, it is understandable that several new potent dimers and trimer ‘lead’ molecules have been discovered in recent years which are in the various phases of clinical trials. Such drug candidates include compounds 90b, 90c, 107, 110, 108, 111, 127, 129, 132–134, 138–140 and 175 as novel leads in anti-malarial and anti-cancer drug discovery. In view of development of new ‘lead’ compounds, it is hoped that interest in this rapidly growing area will continue further to yield exciting results in the coming years.

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