

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.

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† This article is dedicated to the achievements of the North-East Institute of Science and Technology (formerly RRL), Jorhat (CSIR), on the eve of its 50th anniversary.



Devdutt Chaturvedi

Dr Devdutt Chaturvedi obtained his PhD degree in Medicinal Chemistry, from the Dr B. R. Ambedkar University, Agra (research work was carried out at CDRI, Lucknow), in 2003. After working as a Postdoctoral Fellow at the University of Georgia, USA and University of Göttingen, Germany, he returned to India and worked shortly as a Senior Postdoc at the Department of Chemistry, IIT, Madras and as a Scientist (Fellow) at Indian Institute of Integrative

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Abhishek Goswami

Abhishek Goswami was born in Jorhat, Assam (India) in 1981. He completed his BSc (2002) degree from Science College, Jorhat, Dibrugarh University and MSc (2005) degree from Gauhati University, Assam (India) with specialization in Physical Chemistry. At present he is pursuing his PhD degree at Natural Products Chemistry Division, NEIST, Jorhat, Assam (India) under the guidance of Dr Nabin C. Barua. His area of research interest is partial

and total synthesis of natural products of biological significance and development of new synthetic methodologies for target oriented synthesis.

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.²

(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.³

(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.⁴

(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.⁵ 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.⁶

(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.⁷

(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.⁸

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.



Partha Pratim Saikia

interest are stereoselective total synthesis of natural products of biological significance and development of new synthetic methodologies for target oriented synthesis.

Partha Pratim Saikia was born in Sivasagar, Assam (India) in 1980. After obtaining his BSc (Chemistry Honors) from Science College, Jorhat, in 2002, he moved to Gauhati University, Guwahati, where he finished his MSc in 2004. He is currently working towards his PhD at Natural Products Chemistry Division, North-East Institute of Science & Technology (CSIR), Jorhat, Assam (India) under the guidance of Dr Nabin C. Barua. His areas of research



Nabin C. Barua

Division. He has trained many masters, doctoral and post-doctoral students. He has been actively involved on synthesis of biologically active natural products/drug intermediates and artemisinin chemistry as well.

Dr Nabin C. Barua obtained his PhD degree in Natural Products Chemistry in 1981, under the supervision of Dr R. P. Sharma. Later on, he worked as an AvH-Post-doctoral Fellow with Prof. Dr Richard R. Schmidt, at the University of Konstanz, Germany, where he worked on the chemistry of functionally substituted vinyl carbanions. He joined NEIST, Jorhat in 1981, where he is currently working as a Head of the Natural Products Chemistry



Paruchuri G. Rao

of chemical sciences area of NEIST, Jorhat. Dr Rao is a Fellow of Indian Institute of Chemical Engineers and was also its past President.

Dr Paruchuri Gangadhar Rao, obtained his MTech and PhD from IIT, Madras, India. He joined NEIST, Jorhat, initially in 1976 and moved to CLRI, Chennai in 1991. He returned to NEIST, Jorhat, in 2002 as Director of the institute. His research areas of interest are chemical process development of agrochemicals, process design and engineering to provide basic engineering packages and applications of ultrasound. He is presently coordinating all the divisions

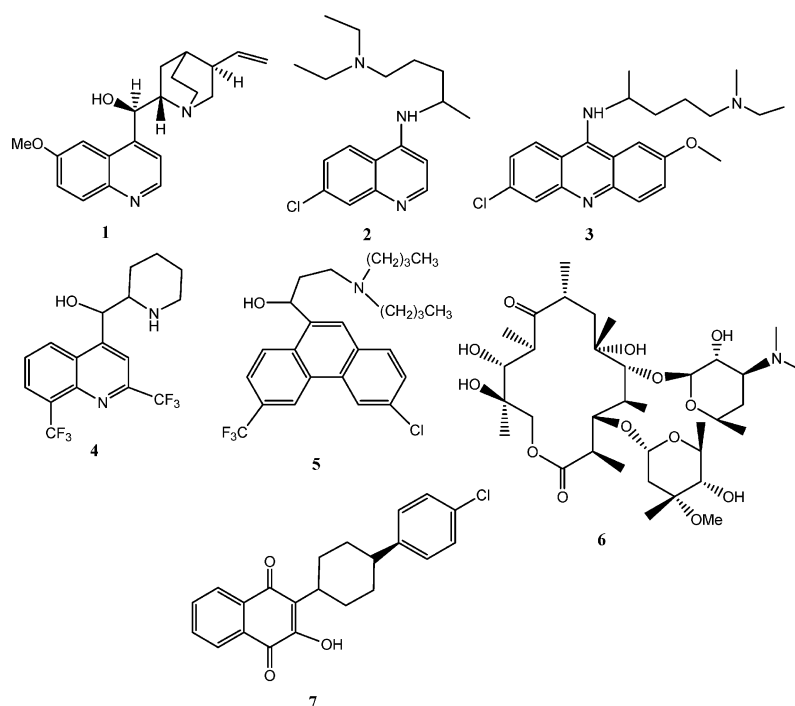


Fig. 1 Structures of commonly available anti-malarial drugs.

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 cadinene⁹ from the hexane extract of a traditional Chinese medicinal plant, *Artemisia annua* (Asteraceae) a plant which has been used for the treatment of fever and malaria since ancient times.¹⁰ 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,¹¹ (ii) the high rate of parasite recrudescence after treatment¹² and (iii) its short-plasma half life (3–5 h) and its poor oral activity.¹³ 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 published¹⁴ 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.

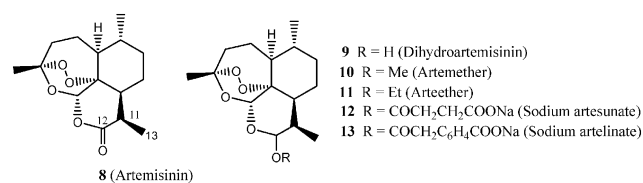


Fig. 2 Structure of artemisinin and its analogs.

4. Artemisinin derivatives

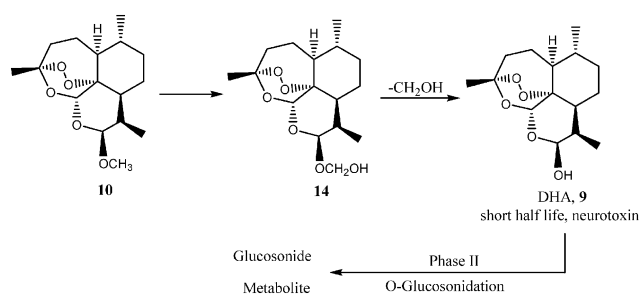
(A) C-12 ether/ester derivatives

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 acetal 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).

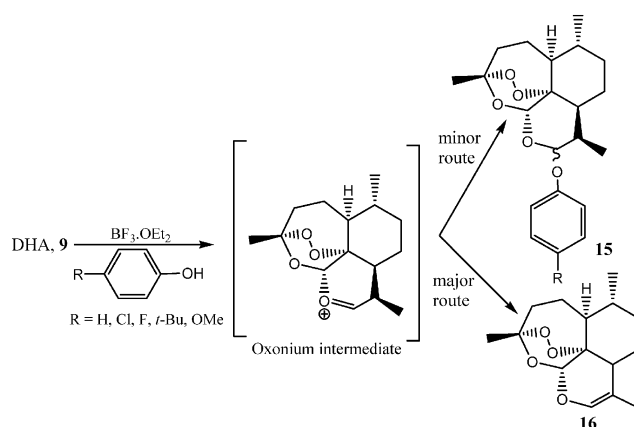
An approach to increasing metabolic stability of artemisinin derivatives involves incorporation of a phenyl group in place of alkyl group (in the ether linkage) of arteether and artemether. This modification would be expected to block oxidative metabolic formation of DHA *in vivo*. With this idea in mind, O'Neill's group synthesized¹⁶ a series of C-12 phenoxy derivatives by reacting DHA with 4 equivalents of the phenol in anhydrous ether at room temperature in presence of BF₃-etherate. This reaction is believed to proceed *via* an 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* antimalarial potency with an ED₅₀ value of 2.12 mg kg⁻¹ (*cf.* artemether = 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)dihydroartemisinin **17** with 1-hydroxypolyacetylated sugars in presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate in CH₂Cl₂ at -78 °C. Deacetylation of intermediates **18a–d**



Scheme 1



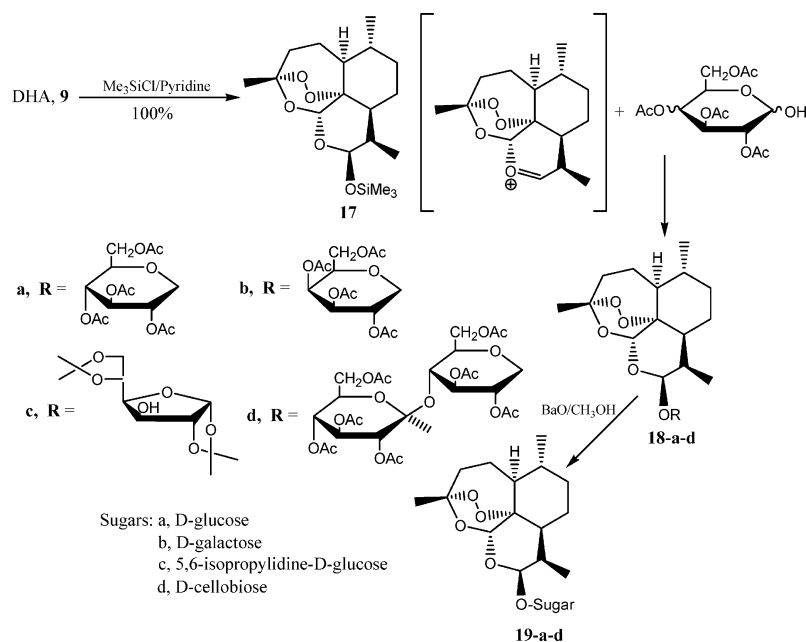
Scheme 2

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.

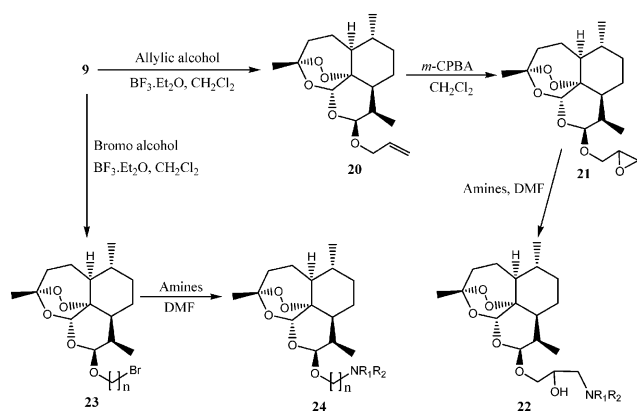
The trimethylsilyl derivative **17** was more active than derivatives **18a–d** which possess activity comparable to or better than that of artemisinin **8**. However, the deacetylated compounds **19a–d** were substantially less active than the acetylated ones **18a–d**. The anti-malarial activity results 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 allylic 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), **24h** (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 artesunic 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



Scheme 3



Scheme 4

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.* found¹⁹ that cyano artemether **25**, possessed inhibitory effect against *P. falciparum* and no cytotoxic effect

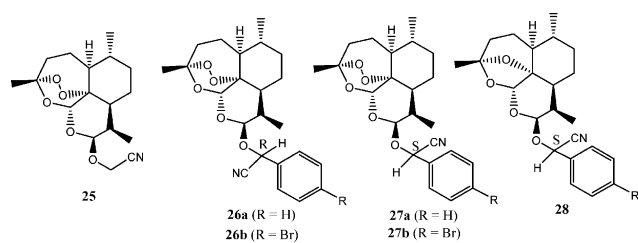
against P388 cells *in vitro*, and that a pair of isomers (compound **26a** and **27a**) in test of antiproliferative potential displayed *in vitro* cytotoxicity against P388 and L1210 murine leukemia cell lines. They prepared the compound **26b** (22% yield) and **27b** (25% yield), as a pair of isomers, in the presence of BF₃·Et₂O with 2-(4-bromophenyl)-2-hydroxyacetonitrile 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 peroxy 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 peroxy 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

<i>n</i>	NR ₁ R ₂	<i>n</i>	NR ₁ R ₂	<i>n</i>	NR ₁ R ₂	<i>n</i>	NR ₁ R ₂				
24a^a	2	NHMe	24h^a	2	NEt ₂	24v^b	2	C	24o^b	3	NH(CH ₂) ₃ NMe ₂
24b^b	2	NHMe	24i^b	2	NEt ₂	24p^b	2	A	24w^b	2	D
24c^b	2	NHEt	24j^b	3	NEt ₂	24q^b	2	A	22a^b		NMe ₂
24d^a	2	NH(<i>n</i> -C ₃ H ₇)	24k^b	2	NH(CH ₂) ₂ OH	24r^b	3	A	22b^b		NEt ₂
24e^b	2	NH(<i>i</i> -C ₃ H ₇)	24l^b	2	NH(CH ₂) ₂ NMe ₂	24s^c	3	A	22c^b		NH(<i>i</i> -C ₃ H ₇)
24f^a	2	NMe ₂	24m^b	2	NH(CH ₂) ₂ NMe ₂	24t^a	2	B	22d^b		A
24g^b	2	NMe ₂	24n^b	2	NH(CH ₂) ₂ CN	24u^b	2	B	22e^b		E

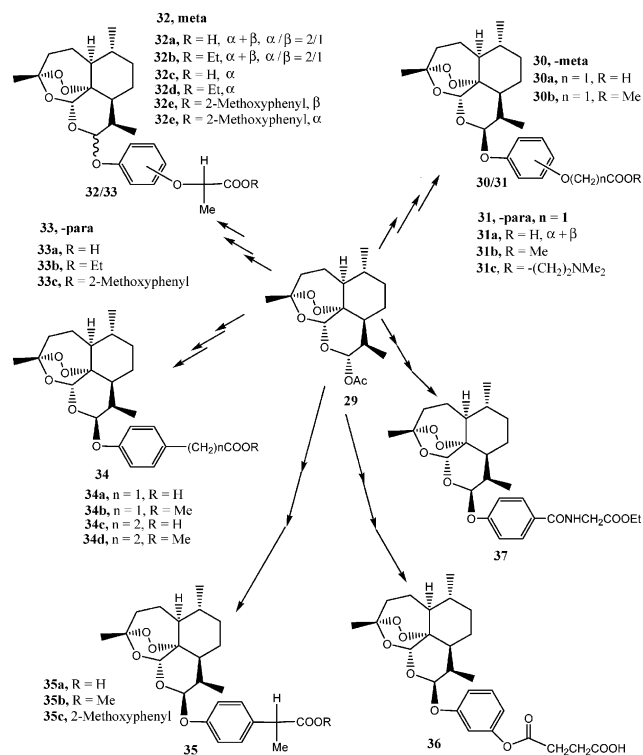
HNR₁R₂ = morpholine (A), piperazine (B), *N*-methylpiperazine (C), *N*-diphenylmethylpiperazine (D), pyrrolidine (E). ^a As oxalate salt. ^b As maleate salt. ^c As fumarate salt.



Scheme 5

possessed definite immunosuppressive activity.²⁰ In search for new potential immunosuppressive agents with much higher efficacy and lower toxicity, Yang *et al.* have synthesized²¹ a class of novel artemisinin derivatives (**30–37**) starting from dihydroartemisinin acetate **29** (Scheme 6) and found that introduction of phen(oxy)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 concanavalin 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 *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) *in vitro*. The *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.

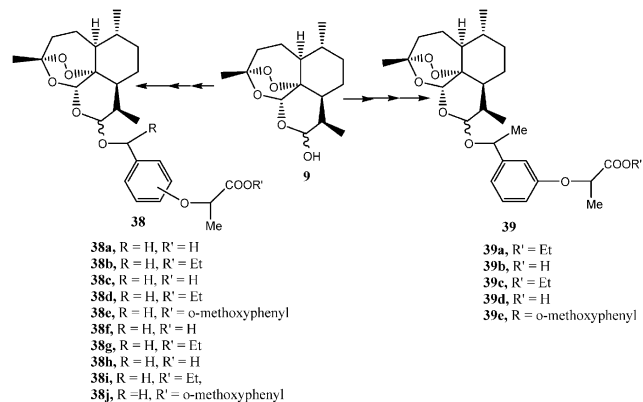
Based on their previous work, Yang *et al.* have further extended the study of immunosuppressive activity of a new series of substituted phenoxy propionic acids and ester derivatives (**38,39**).²² The synthesis of targeted compounds were achieved using dihydroartemisinin **9** (Scheme 7). These new dihydroartemisinin derivatives were tested *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 reduced cell viability to 50% (CC₅₀). The immunosuppressive activity 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% (IC₅₀) of the control value. Among the whole series of compounds, **38a** (IC₅₀ = 6.8 × 10⁻⁷), **38e** (IC₅₀ = 4.6 × 10⁻⁷), **38h** (IC₅₀ = 7.0 × 10⁻⁷), and **38j** (IC₅₀ = 8.4 × 10⁻⁷) had 5- to 9-fold higher bioactivity than artemisinin (IC₅₀ = 4.4 × 10⁻⁶), artemether (IC₅₀ = 3.8 × 10⁻⁶), artesunate (IC₅₀ = 4.8 × 10⁻⁶) in the ConA-induced T cell proliferation. In the inhibition of LPS-induced B cell proliferation **38e** (IC₅₀ = 2.8 × 10⁻⁷), **38f** (IC₅₀ = 1.6 × 10⁻⁷), **38g** (IC₅₀ = 3.0 × 10⁻⁷),



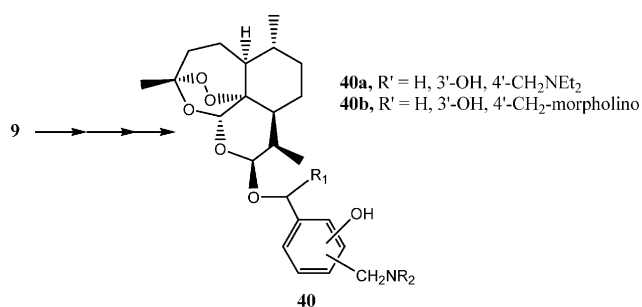
Scheme 6

38j (IC₅₀ = 2.2 × 10⁻⁷), **39a** (IC₅₀ = 5.0 × 10⁻⁷), **39b** (IC₅₀ = 1.8 × 10⁻⁷), **39c** (IC₅₀ = 1.0 × 10⁻⁷), and **39e** (IC₅₀ = 1.7 × 10⁻⁷), exhibited 30- to 88-fold higher bioactivity than artemisinin (IC₅₀ = 9.0 × 10⁻⁶), artemether (IC₅₀ = 1.8 × 10⁻⁶), and artesunate (IC₅₀ = 9.9 × 10⁻⁷) respectively.

Yang *et al.* have also synthesized²³ a series of artemisinin derivatives bearing Mannich base groups (**40a** and **40b**) starting from dihydroartemisinin **9** and tested for their anti-malarial activity against *P. berghei* and *P. falciparum* in K₁ and NF₅₄ cells. Compound **40a** (IC₅₀ = 0.18 and 0.36 ng mL⁻¹) and **40b** (IC₅₀ = 0.25 and 0.17 ng mL⁻¹) were found to be more active in mice than artesunic acid (IC₅₀ = 1.20 and 1.20 ng mL⁻¹). These derivatives **40a** and **40b** (dose 3.16 and 10 mg kg⁻¹ day⁻¹) were also examined for their anti-malarial activity against *P. knowles* in rhesus monkeys (Scheme 8) of 7 days treatment using artesunic acid as standard drug



Scheme 7

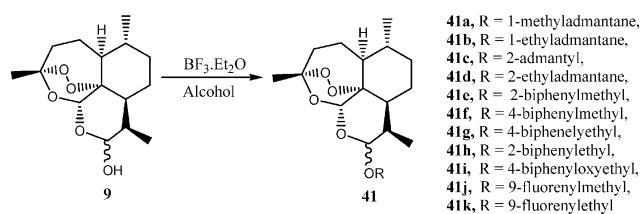


Scheme 8

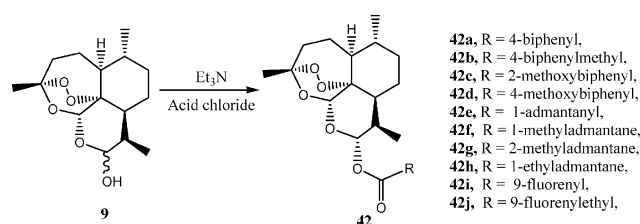
(dose 3.16 and 10 mg kg⁻¹ day⁻¹) and thus found to be better than artesunic acid.

Recently, Singh *et al.* have synthesized²⁴ 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.* BF₃·Et₂O) coupling reaction between dihydroartemisinin **9** with the corresponding alcohol in CH₂Cl₂ at subzero temperature (−10 °C to −5 °C) furnishing the corresponding ether derivative in 65–99% yields as diastereomeric mixtures of α and β -isomers, with the β -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 β -arteether (log *P* 3.84), and several of them are two- to four-fold more active than β -arteether. Among, the ether derivatives, the α -isomers are more active than the β -isomers. The α -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 β -arteether, *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 synthesized²⁵ 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 β -arteether **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.



Scheme 9

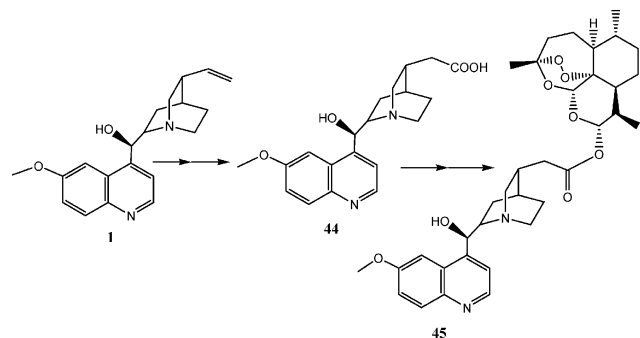


Scheme 10

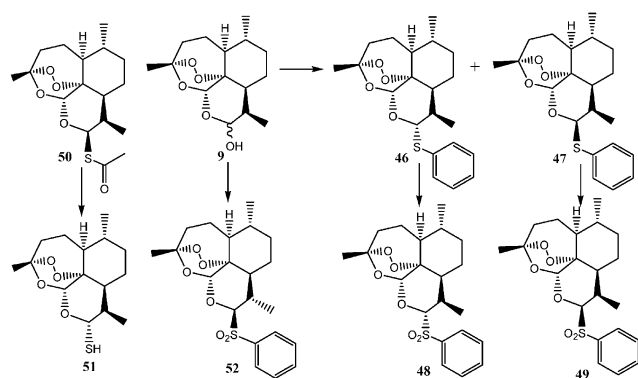
Although, the target for anti-malarial action of artemisinins is controversial, recent evidence suggest that an Fe²⁺-activated form of the drug potentially inhibits PfATP, a key parasite Ca²⁺ transporter. On the other hand, the mode of action of another anti-malarial drug quinine **1**, has been suggested as a result of interference with host hemoglobin digestion. Due to anti-malarial synergism between artemisinin and quinine, Walsh *et al.* have recently prepared²⁶ 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** (IC₅₀ = 8.95 nM) was superior to that of artemisinin **9** (IC₅₀ = 49.4 nM) or, quinine **44** (IC₅₀ = 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 sulfur 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.



Scheme 11



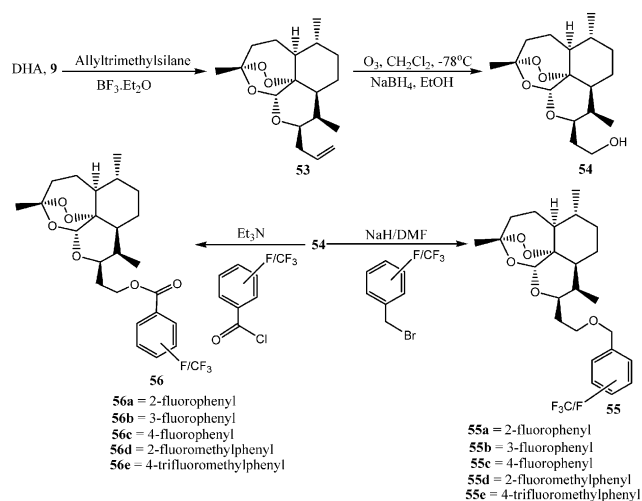
Scheme 12

Recently, Chen *et al.* have reported²⁷ that artemisinin and dihydroartemisinin and C-12 acetal type of artemisinin derivatives display anti-angiogenic-activity. Consequently, Oh *et al.* have synthesized²⁸ C-12 sulfur derivatives of artemisinin (**46–52**) starting from dihydroartemisinin **9** (Scheme 12) and tested against HUVEC proliferation at the concentration level of 1 μM using artemisinin **8**, and DHA **9**. Compounds **46** ($\text{IC}_{50} = 0.93 \mu\text{M}$), **52** ($\text{IC}_{50} = 1.74 \mu\text{M}$), and **51** ($\text{IC}_{50} = 1.29 \mu\text{M}$), have displayed potent growth inhibitory activity as compared to **8** ($\text{IC}_{50} > 50 \mu\text{M}$), and **9** ($\text{IC}_{50} = 8.91 \mu\text{M}$).

(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 **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.

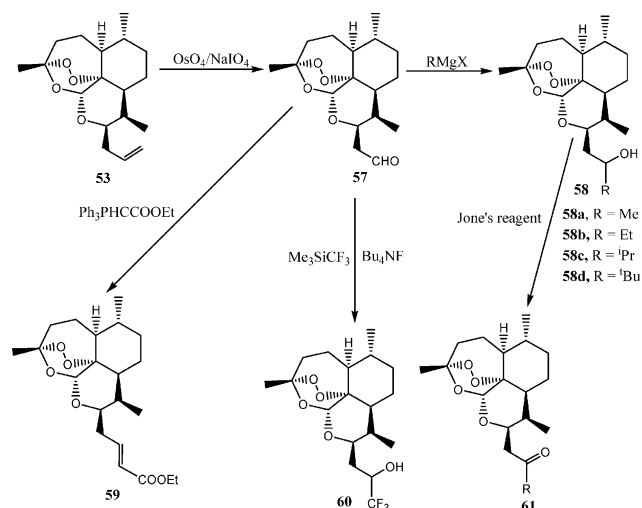
O'Neill's group has synthesized²⁹ several novel second-generation fluorinated ether and ester analogues of arteether and artemether and evaluated for their anti-malarial potency (Scheme 13). All of their derivatives demonstrated high anti-malarial potency *in vitro* against the chloroquine sensitive HB₃ and resistant K₁ strains of *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** ($\text{IC}_{50} = 0.22$) was 15 times more active than artemisinin ($\text{IC}_{50} = 3.04$) and 5 times more potent than DHA **9** ($\text{IC}_{50} = 1.04$) against HB₃ strain of *P. falciparum*. However, *in vitro* against *P. berghei* in the mouse, selected derivatives were generally less potent than DHA **9** ($\text{ED}_{50} = 1.15$) with ED_{50} values between 5 to 8 mg kg^{-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**.



Scheme 13

Latter, Ziffer's group has synthesized³⁰ 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 **60 α** and **60 β** .

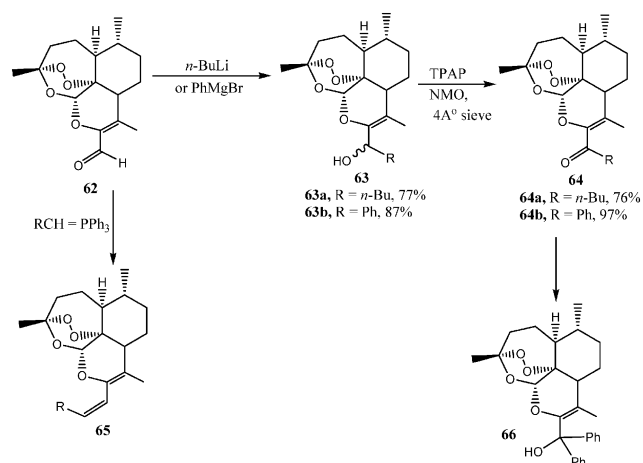
The *in vitro* anti-malarial activities of all the synthesized compounds (**58a–d**, **59**, **60**, and **61**) were determined against two drug resistant clones (W-2 is chloroquine resistant, mefloquine sensitive while D-6 is mefloquine resistant and chloroquine sensitive) of *P. falciparum*. Out of all the synthesized compounds tested, compounds **58b β** ($\text{IC}_{50} = 4.8$ and 5.8) and **58d β** ($\text{IC}_{50} = 5.4$ and 6.8) were 5–7 times more potent than artemisinin **8**.



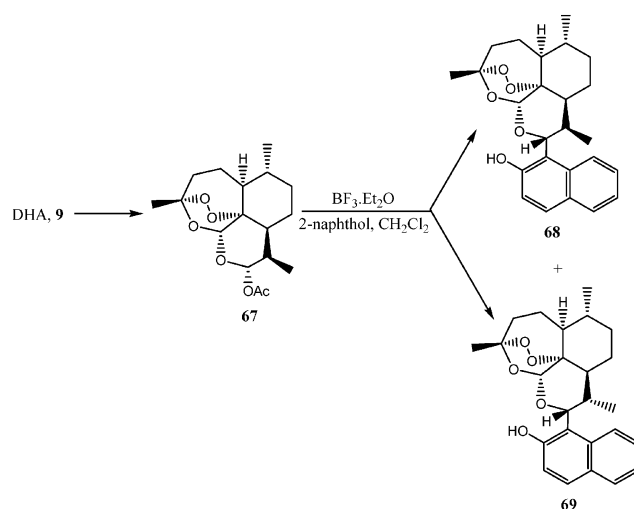
Scheme 14

Posner *et al.* reported³¹ a series of C-12 carba analogues by treating organometallic reagents with aldehyde **62** under controlled conditions (Scheme 15). Treatment of aldehyde **62** with organometallic reagents produces the allylic alcohol **63** while aldehyde **62** reacts with Wittig reagent to form a mixture of geometric isomers of exocyclic alkene **65** 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 **64** ($IC_{50} = 4.3$ nM where R = *n*-Bu and 4.6 nM where R = Ph), tertiary alcohol **66** ($IC_{50} = 4.5$ nM) and exocyclic alkene **65** [$IC_{50} = 28$ nM (R = CH=CH₂), 16 nM (R = (*E*)-CH=CHPh), 8.1 nM (R = (*Z*)-CH=CHPh), 11 (R = (*E*)-CH=CHPhNO₂-*p*)] are all similar to clinically used natural artemisinin ($IC_{50} = 10.1 \pm 1.3$ 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.*³² 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 **68** 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 **69** ($ED_{50} = 7.08$ mg kg⁻¹, $ED_{90} = 60.99$ mg kg⁻¹) is obviously much less potent than **68** ($ED_{50} = 0.58$ mg kg⁻¹, $ED_{90} = 1.73$ mg kg⁻¹) against *P. berghei* K173 strain administered orally to mice as suspensions in Tween 80 as compared to artemether **10** ($ED_{50} = 1.00$ mg kg⁻¹, $ED_{90} = 3.10$ mg kg⁻¹). The marked difference in the anti-malarial potencies of **68** and **69**, due to their difference in stereochemistry only at C-12 in the structures, has been claimed by these workers as an entirely new observation and according to them the ease with which



Scheme 15

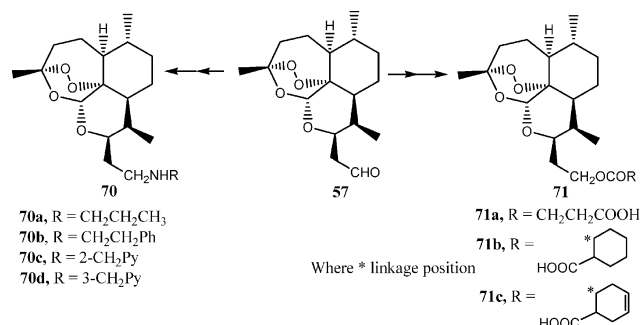


Scheme 16

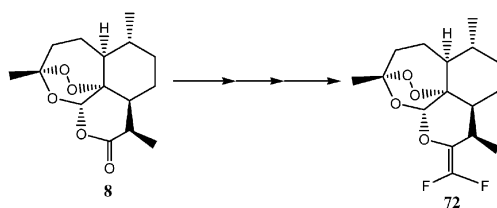
the peroxide bond is cleaved may directly be related to the anti-malarial potency of trioxane.

Recently, Khac *et al.* have reported³³ the synthesis of C-12 amino and ester/acid analogues using Ziffer's key intermediate aldehyde **57** (Scheme 17). The key intermediate **57** was subjected to condensation with a variety of aliphatic, substituted aromatic and substituted heterocyclic amines to afford the corresponding Schiff base derivatives, which upon borohydride reduction afforded corresponding C-12 amine derivatives **70a-d**. For the synthesis of C-12 esters/acid derivatives, aldehyde **57** was reduced to alcohol, and further treated with a variety of carboxylic acid anhydrides affording the corresponding ester/acid derivative **71a-c**. Among the synthesized compounds, **71a** ($IC_{50} = 0.4$ and 0.5 nM), **71b** ($IC_{50} = 0.4$ and 0.5 nM), and **71c** ($IC_{50} = 0.4$ 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 ($IC_{50} = 10$ and 9 nM). In addition, other derivatives containing amino-alkyl and heterocycles were also highly potent against *P. falciparum*. Compound **70d** ($IC_{50} = 1.0$ and 1.0 nM) is about 10 times more potent than artemisinin.

Recently, Magueur *et al.* have reported³⁴ that introduction of *gem*-difluoromethylene group at the C-12 position of the artemisinin resulted in better *in vitro* antimalarial activity. They



Scheme 17

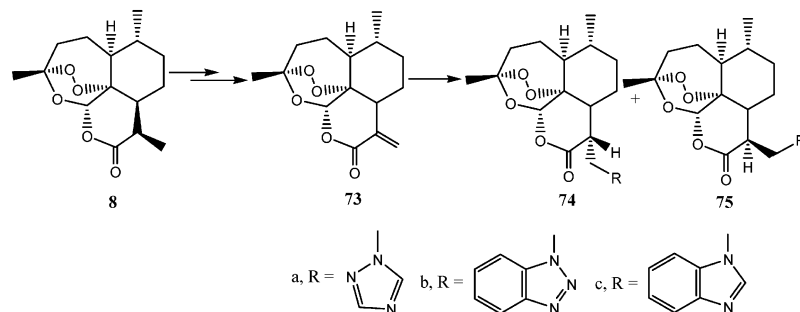


Scheme 18

have synthesized *gem*-difluoromethylene deoxy-artemisinin **72** through artemisinin **8** in three steps (Scheme 18). The *in vitro* antimalarial activity of artemisinin **8** ($IC_{50} = 8.9$) and of compound **72** ($IC_{50} = 4.6$) 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.³⁵ Artemisitene **73** exists in the same plant in much lower yield and has less anti-malarial activity than artemisinin **8**.³⁶ 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.* prepared³⁷ **74a–c** and **75a–c** with 1,2,4-triazole, benzotriazole or benzimidazole moiety under different conditions (Scheme 19), heating 1,2,4-triazole as its salts and artemisitene **73** in CH_3CN at 60 °C gave a mixture of **74a** and **75a** (40% yield), refluxing benzotriazole and artemisitene **73** in aqueous ethanol gave **74b** and **75b** (40 and 29% yield), reacting benzimidazole and artemisitene **73** in THF in the presence of $KF-Al_2O_3$ gave **74c** and **75c** (26 and 38% yield). In *in vivo* anti-malarial tests against K173 strain of *P. berghei*, the mixture of **74a** and **75a** showed nine times ($SD_{50} = 2.38 \text{ mg kg}^{-1} \text{ day}^{-1}$, $SD_{90} = 6.52 \text{ mg kg}^{-1} \text{ day}^{-1}$) more potency than artemisinin **8** ($SD_{50} = 5.13 \text{ mg kg}^{-1} \text{ day}^{-1}$, $SD_{90} = 11.50 \text{ mg kg}^{-1} \text{ day}^{-1}$). Compound **74b** ($SD_{50} = 5.48 \text{ mg kg}^{-1} \text{ day}^{-1}$, $SD_{90} = 11.41 \text{ mg kg}^{-1} \text{ day}^{-1}$) showed activity comparable to that of the artemisinin **8**. Compound **74c** ($SD_{50} = 3.94 \text{ mg kg}^{-1} \text{ day}^{-1}$, $SD_{90} = 10.05 \text{ mg kg}^{-1} \text{ day}^{-1}$) and **75c** ($SD_{50} = 1.59 \text{ mg kg}^{-1} \text{ day}^{-1}$, $SD_{90} = 56.03 \text{ mg kg}^{-1} \text{ day}^{-1}$) were more active than artemisinin **8**. Compound **74c** had higher activity than its isomer **75c**.



Scheme 19

(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.

I. 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.

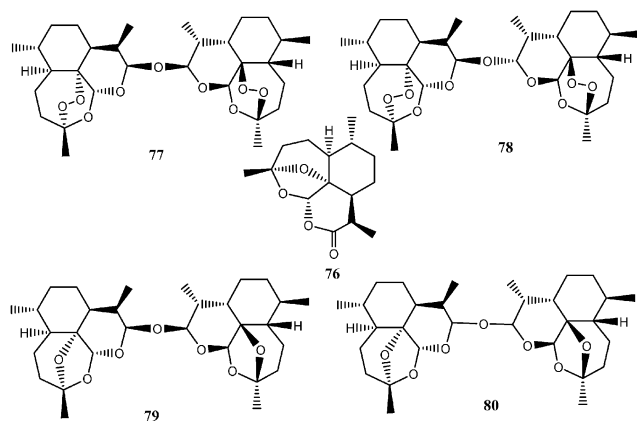
(b) *C-12 carba dimers*: Wherein two artemisinin monomers units are linked through the C-12 non-acetal (*i.e.* carbon–carbon linkage) linker.

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

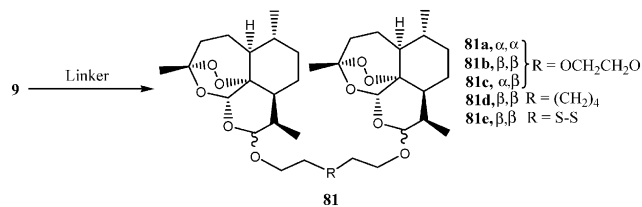
(a) **C-12 oxa dimers**. In search for new potential artemisinin derivatives, several new C-12 oxygen derivatives have been reported by various groups. Thus, Beekman *et al.* have reported³⁸ the anti-cancer activity of C-12 oxa-dimers where two artemisinin units were connected through an ether-linkage (Scheme 20). The cytotoxicity of these derivatives were determined against EN2 tumor cells using the MTT assay. They realized that artemisinin **8** ($IC_{50} = 0.98$) was more cytotoxic than the corresponding deoxyartemisinin **76** ($IC_{50} = 111$), 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** ($IC_{50} = 0.11$) was more cytotoxic than the symmetrical dimer **78** ($IC_{50} = 2.0$). Similarly, the symmetrical dimer **80** ($IC_{50} = 99.8$) was less effective than **79** ($IC_{50} = 8.9$).

In order to study the role of linker in affecting the biological activity of dimers, Posner *et al.* have synthesized³⁹ 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 anti-tumor activity in various cancer cells *in vitro*. Of the dihydroartemisinin polyethylene-glycol dimers **81a-c**, the one with β -stereochemistry at both of the lactol acetal positions (*i.e.* **81b**, $IC_{50} = 1.9$ nM) is more active than artemisinin **8** ($IC_{50} = 10$ nM). Likewise, the β,β -dimers **81d** and **81e** are highly antiproliferative.

Recently, Grellepois *et al.* have synthesized⁴⁰ another C-12 oxa dimer **82** through olefinic metathesis reaction using compound **20** (Scheme 22). Preliminary growth inhibitory activities were evaluated *in vitro* using a diverse panel of 60 human cancer cell lines. This dimer **82** was efficient in cancer cell growth inhibition with a GI_{50} less than 10 nM. In particular, TGI (total growth inhibition) data shows the selectivity and potency of dimer **82** against a few cancer cell



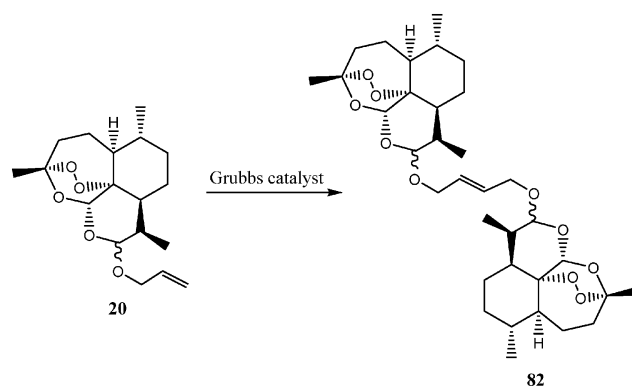
Scheme 20



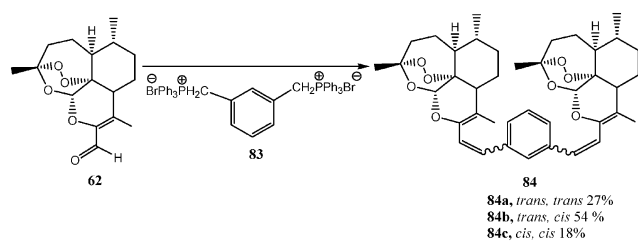
Scheme 21

lines (*e.g.* leukemia, HL-60, non-small cell lung cancer NCI-H226, colon cancer COLO 205, and KM-12, CNS cancer SF-295).

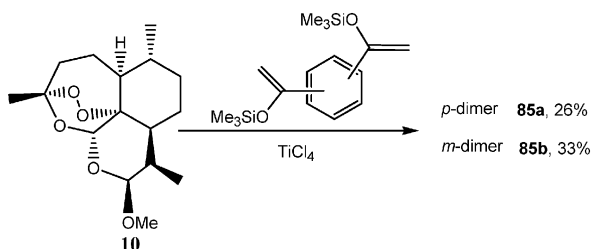
(b) **C-12 carba dimers**. Although C-12 oxa dimers have displayed high antimalarial, antiproliferative and anti-tumor activities *in vitro*, often they are hydrolytically unstable. In order to enhance the hydrolytic stability, researchers have directed their efforts to synthesize a variety of C-12 olefinic carba dimers. Thus, Posner *et al.* have first reported⁴¹ 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 **84a-c** (Scheme 23). The *in vitro* anti-malarial potencies of *m*-xylene dimers **84a-c** against chloroquine sensitive *P. falciparum* (NF54) parasites was measured, wherein **84a** ($IC_{50} = 77$ nM), **84b** ($IC_{50} = 35$ nM), **84c** ($IC_{50} = 18$ nM) were found less potent than artemisinin **8** ($IC_{50} = 9.7$ nM). They have further extended the study and synthesized C-12 non-acetal saturated dimers **85a,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 acetylde condensations (Scheme 25). Although benzoylmethylene dimers **85a** and **85b** and acetylenic dimers **89a** and **89b** 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-acetylene dimers **89a** and **89b**, aryl dimer **87** and furan dimer **88** are considerably more potent anti-malarial agents ($IC_{50} = 1.3-3.2$ nM) than natural artemisinin ($IC_{50} = 9.7$ nM) as mentioned in Table 2. Compounds **85a-c**, **87**, **88**, **89a-c** showed good to excellent antiproliferative activity. Dimers **85a**, **87** and **89a** were specially potent and selective in



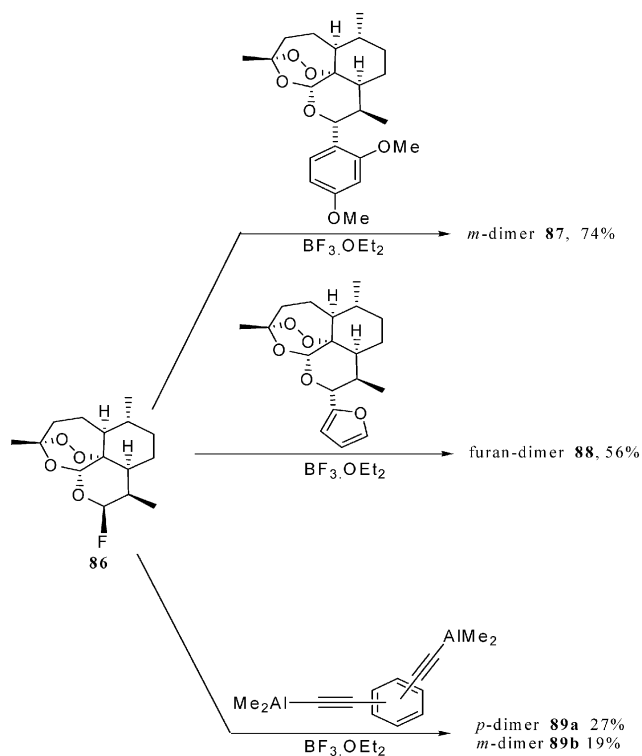
Scheme 22



Scheme 23



Scheme 24



Scheme 25

inhibition of growth of some human cancer cell lines in the NCI *in vitro* 60-cell line assay.

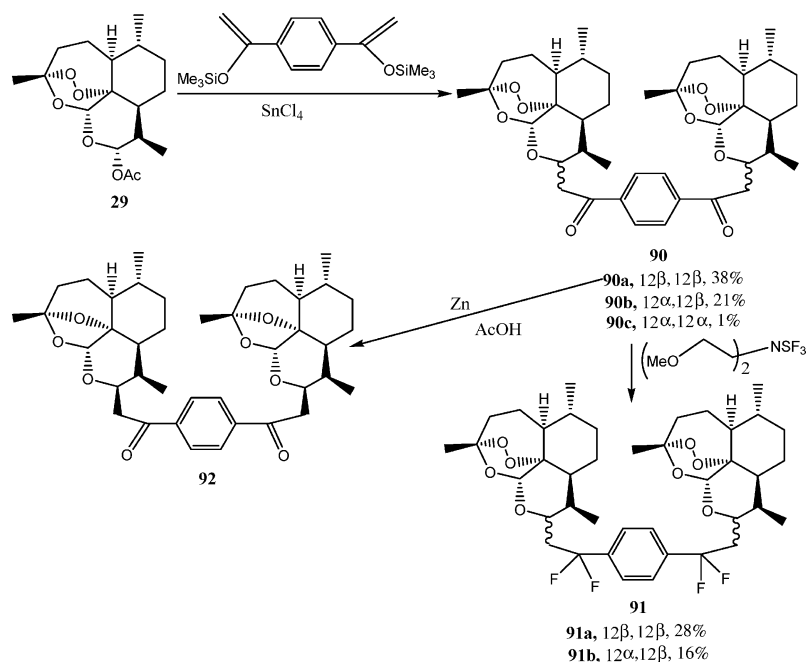
Later on, Posner's group has also synthesized⁴² C-12 non-acetal dimers **90a–c** starting from dihydroartemisinin acetate **29** and their ketone functionality was converted into difluoro derivative **91a,b** using bis(2-methoxyethyl)amino sulfur trifluoride (BAST) (Scheme 26). The peroxide linkage of 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*. Dicarboxyl dimers **90a–c** are 2–5 times more potent (**90a**, IC₅₀ = 1.9 nM; **90b**,

Table 2 Antimalarial activity of C-12 deoxyartemisinin dimers **85–89**

Dimer	Linker	Antimalarial activity, IC ₅₀ /nM
85a	12β 12β	1.9
85b	12β 12β	1.9
87	12α 12α	1.3
88	12α 12α	3.2
89a	12β 12β	30
89b	12β 12β	36
8		9.7 ± 1.8

IC₅₀ = 1.7 nM; **90c**, IC₅₀ = 3.9 nM) than artemisinin **8** (IC₅₀ = 7.6 nM), whereas tetrafluorinated dimers **91a–b** are 2–4 times less potent (**91a**, IC₅₀ = 28 nM; **91b**, IC₅₀ = 15 nM) than artemisinin **8**. Dimer **92** (IC₅₀ = 1030 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 μM 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 EK VX). 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.

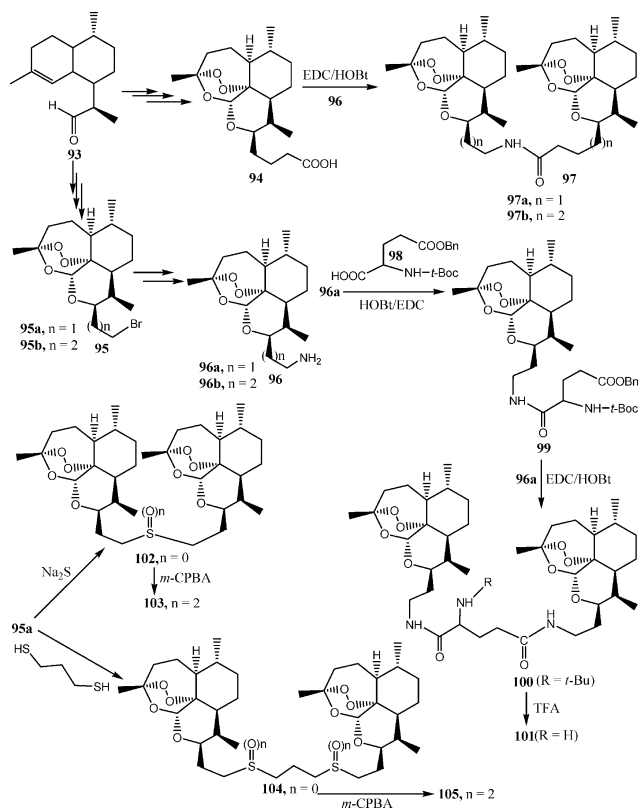
Later on, Jung *et al.* reported⁴³ a series of C-12 non-acetal amido (**97a–b**, **100**, **101**) and sulfide/sulfoxide (**102**, **103**, **104**, **105**) dimers (Scheme 27). Synthesis of target compounds were achieved from the key intermediates **94** and **96**, which were easily synthesized from 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 **100**. Compound **100** upon treatment with TFA afforded



Scheme 26

unprotected amido dimer **101**. Compound **95a** on treatment with Na_2S 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** ($\text{IC}_{50} = 0.40 \mu\text{g mL}^{-1}$) is active comparable to adriamycin ($\text{IC}_{50} = 0.39 \mu\text{g mL}^{-1}$) and four times more active than mitomycin ($\text{IC}_{50} = 1.50 \mu\text{g mL}^{-1}$) against-mouse fibroblast leukemia (P388). Sulfone-linked dimer **103** ($\text{IC}_{50} = 1.04 \mu\text{g mL}^{-1}$) is active comparable to mitomycin ($\text{IC}_{50} = 0.85 \mu\text{g mL}^{-1}$) and six times more active than adriamycin ($\text{IC}_{50} = 6.24 \mu\text{g mL}^{-1}$) and taxol ($\text{IC}_{50} = 7.39 \mu\text{g mL}^{-1}$) against human placental chorocarcinoma cells (Bewo). Dimer **97a** ($\text{IC}_{50} = 0.005 \mu\text{g mL}^{-1}$), particularly, is 24 times more active than adriamycin ($\text{IC}_{50} = 0.12 \mu\text{g mL}^{-1}$) and 200 times more active than mitomycin ($\text{IC}_{50} = 0.93 \mu\text{g mL}^{-1}$), but 50 times less active than taxol ($\text{IC}_{50} = 0.0001 \mu\text{g mL}^{-1}$).

Later on, Posner's group has synthesized⁴⁴ 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** ($\text{IC}_{50} = 9.0 \text{ nM}$) and of the initial olefinic dimer **106** ($\text{IC}_{50} = 24 \text{ nM}$), alcohol and diol dimers **107** ($\text{IC}_{50} = 0.87 \text{ nM}$) and **110** ($\text{IC}_{50} = 0.59 \text{ nM}$) and ketone dimer **112** ($\text{IC}_{50} = 0.91 \text{ nM}$), all have substantially enhanced potencies, with IC_{50} values below 1 nM. Also, water-soluble carboxylic acid dimers **108** ($\text{IC}_{50} = 2.0 \text{ nM}$), **109** ($\text{IC}_{50} = 1.7 \text{ nM}$), **111** ($\text{IC}_{50} = 3.0 \text{ nM}$), **114** ($\text{IC}_{50} = 2.1 \text{ nM}$), and **118** ($\text{IC}_{50} = 2.4 \text{ nM}$)



Scheme 27

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

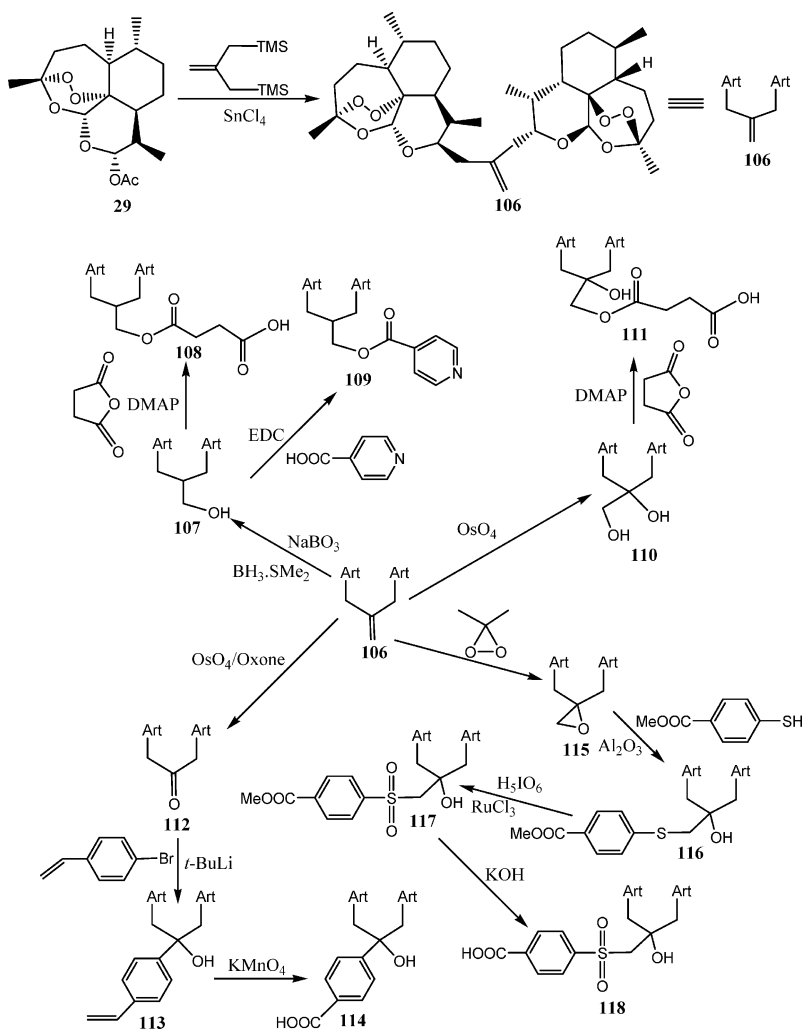
118 are also potent inhibitors of cancer cell growth without being cytotoxic. These semi-synthetic new chemical entities **107** and **110**, and especially the easily synthesized dimer carboxylic acids, **108** and **111**, therefore, deserve further preclinical evaluation as potential drug candidates for chemotherapy of malaria and cancer.

Later on, Posner and O'Neill's group synthesized⁴⁵ a new series of artemisinin dimers (**119**, **121–123**) starting from their previously prepared key intermediate **54** (Scheme 29). All of these dimers prepared displayed potent low nanomolar anti-malarial activity *vs.* the K1 and HB3 strains of *P. falciparum*. The most potent compound assayed was phosphate dimer **122a** ($IC_{50} = 0.2 \text{ nM}$), which was greater than 50 times more potent than the parent drug artemisinin **8** ($IC_{50} = 12.3 \text{ nM}$), and about 15 times more potent than the clinically used acetal artemether 10. All of the dimers expressed poor anticancer activity apart from the trioxane phosphate ester dimer **122a** and **122b**, which expressed nanomolar growth inhibitory (GI_{50}) 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

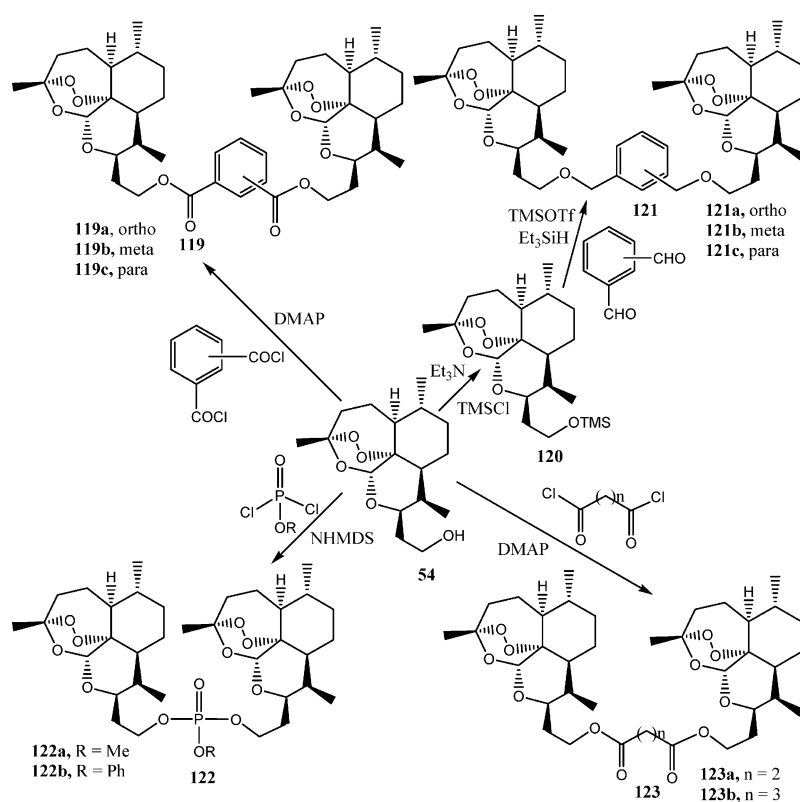
that both phosphate ester dimers **122a** ($IC_{50} = 0.14 \mu\text{M}$) and **122b** ($IC_{50} = 0.24 \mu\text{M}$) are more potent than the anti-cancer agent doxorubicin ($IC_{50} = 0.51 \mu\text{M}$).

Posner's group has further synthesized⁴⁶ isobutyric acid dimer **124** and isonicotinate *N*-oxide dimer starting from alcohol dimer **107** (Scheme 30). Antimalarial potencies of dimer **124** and **125** against chloroquine-sensitive *P. falciparum* (NF 54) parasites were determined. Both isonicotinate *N*-oxide dimer **125** ($ED_{50} = 0.53 \text{ nM}$) and isobutyric acid dimer **124** ($ED_{50} = 2.4 \text{ nM}$) were considerably more antimalarially efficacious than clinically used sodium artesunate **12** ($ED_{50} = 1.5 \text{ nM}$) *via* both oral and intravenous administration. Both alcohol dimer **107** and *N*-oxide dimer **125**, but not carboxylic acid dimer **124**, very strongly inhibit the growth of prostate cancer cells.

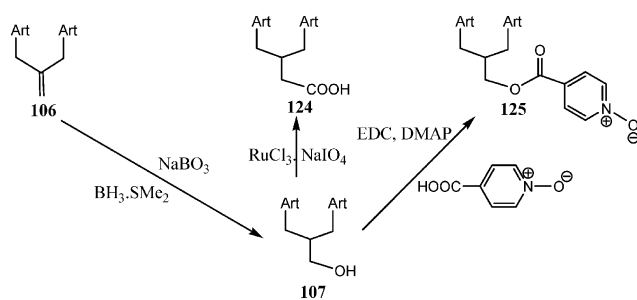
Recently, Posner *et al.* have also reported⁴⁷ the syntheses of another new series of artemisinin bis-benzylic dimers (**127–131**) starting from conjugated dimer **126**, obtained directly from dihydro-artemisinin acetate **29** (Scheme 31). Thus, conjugated new dimer **126** undergoes 4 + 2-cycloaddition reaction to afford compound **127**. Dimer **127** through a series



Scheme 28



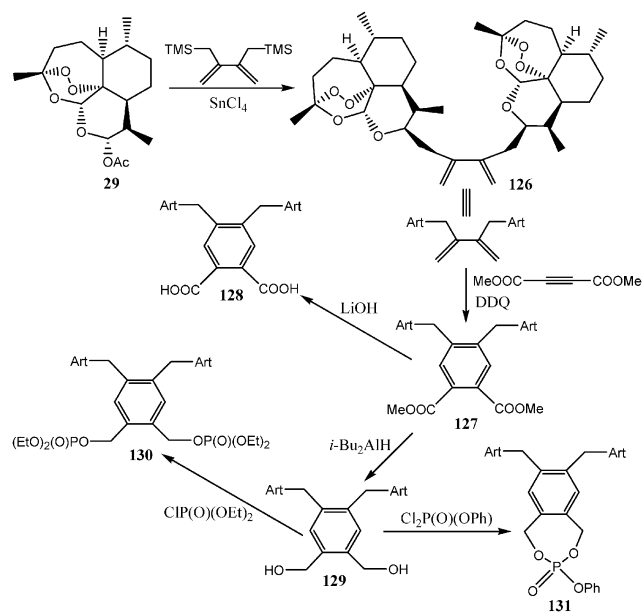
Scheme 29



Scheme 30

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).

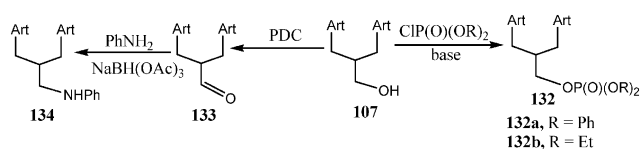
Preliminary growth inhibitory activities at nanomolar to micromolar concentrations were measured *in vitro* using a diverse panel of 60 human cancer cell lines. It has been realized that trioxane phthalate dimer **127** ($IC_{50} = 500$ nM) is approximately 10–20 times more potent than trioxane-monomer 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



Scheme 31

preclinical evaluation as potential drug candidates for effective chemotherapy of malaria and cancer.

Later on, Posner's group synthesized⁴⁸ 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

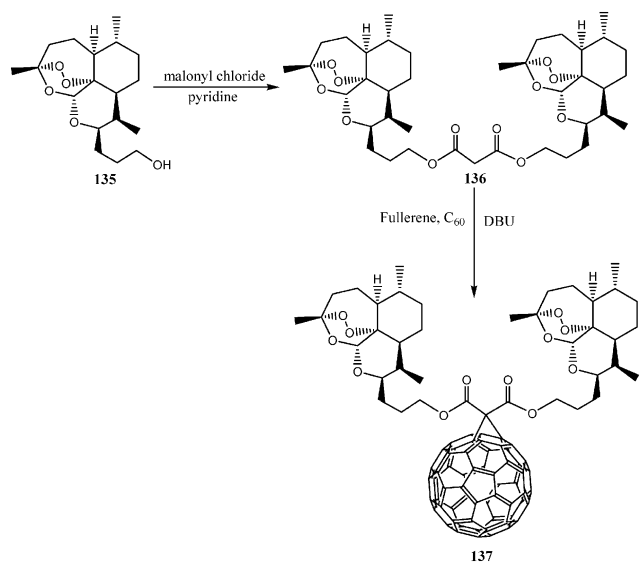


Scheme 32

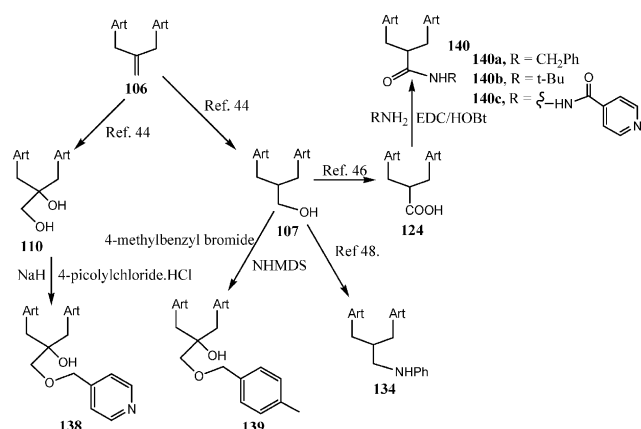
and -C2H) prostate cancer cell lines. All these dimers (**132–134**) inhibited cell growth with the **134** being most potent in C1A ($GI_{50} = 18.0$ nM), C2H ($GI_{50} = 17$ nM) and LNCaP ($GI_{50} = 17.9$ nM) cells in comparison to the standard drug doxorubicin ($GI_{50} = 45.3$ nM). These trioxane 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 P21^{waf1} and p27^{kip1}. 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



Scheme 33



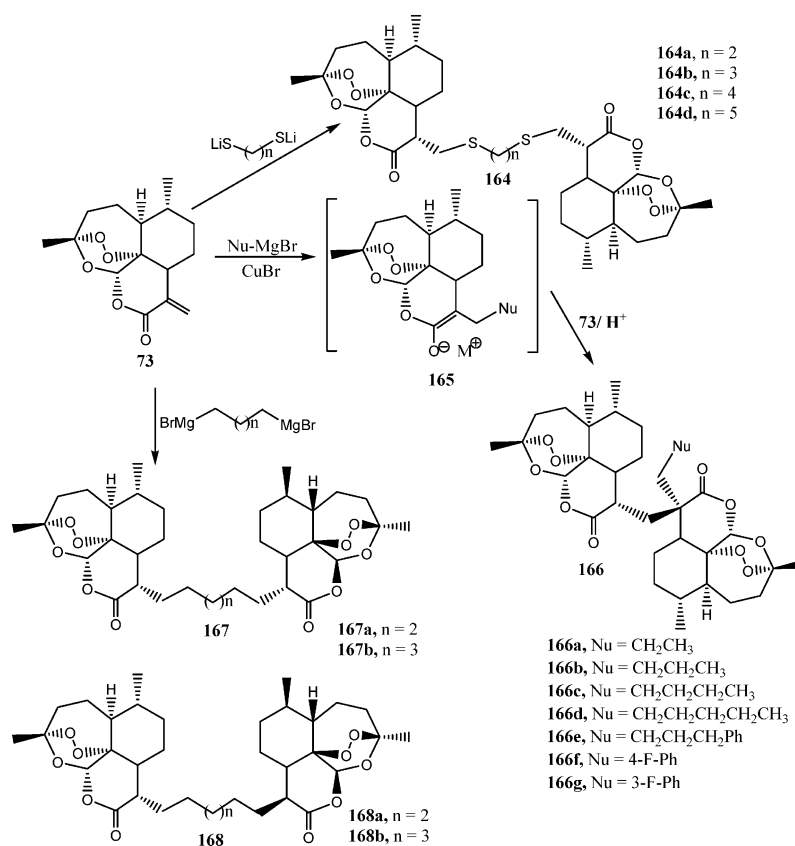
Scheme 34

(**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.

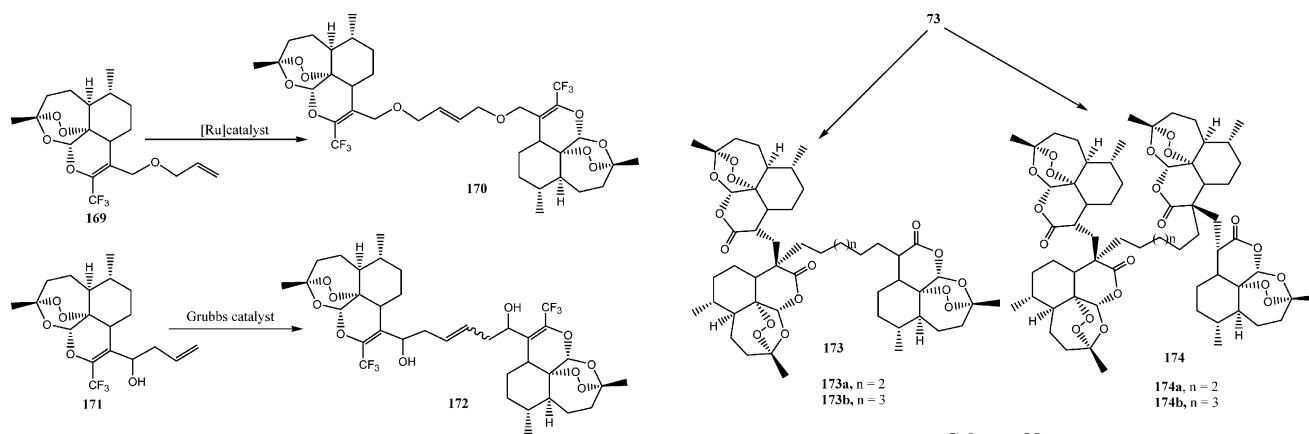
More recently, Posner's group has also reported⁵¹ another series of trioxane dimers (**141–163**) starting from their previously reported dimers **106**, **107**, **110**, **112**, **124** as a key intermediates (Scheme 35). Thus, hydrazone dimer **141** was obtained from the corresponding keto-compound **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 3×30 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 artemistene **73**.

Ektawatchai *et al.* have reported⁵² synthesis of C-13 carba-dimers (**164**, **166–168**) starting from the key intermediate artemistene **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



Scheme 36

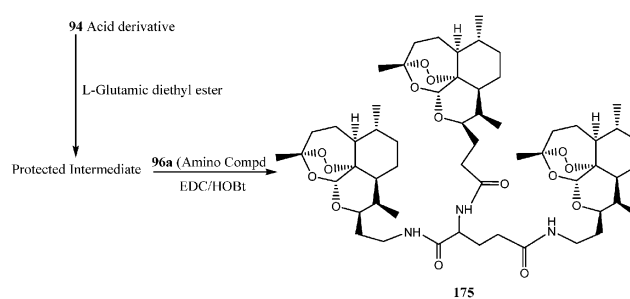


Scheme 37

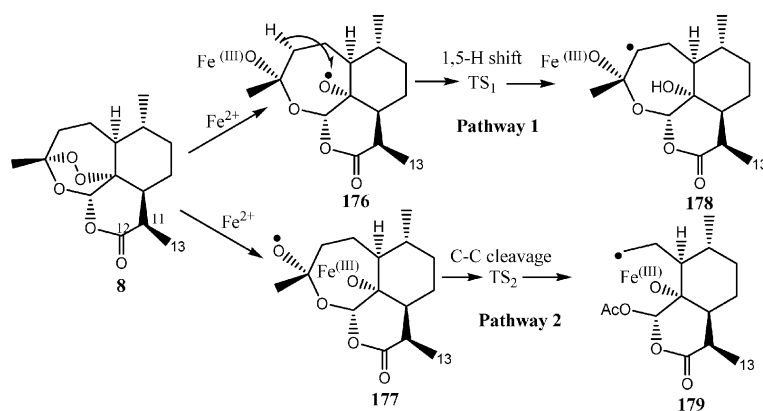
Scheme 38

endoperoxide linkage of artemisinins and a heme iron play critical roles in their mechanism of action, which is comprised of two distinct steps.⁵³ 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



Scheme 39



Scheme 40 Proposed mechanism of action of artemisinins

endoperoxide moiety at the O₂ 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 established⁵⁴ 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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