Chapter 1

HISTORICAL DEVELOPMENT & CURRENT SCENARIO OF ARTEMISININ AND RELATED ANTIMALARIALS
1.1 Introduction

Malaria is a vector borne parasitic disease caused by the genus *Plasmodium*, affecting over 100 countries of the tropical and subtropical regions of the world.\(^1\) Although malaria has been known to man for centuries, the parasite for malaria was discovered in the nineteenth century. Four different *Plasmodium* species infect humans and cause distinct disease patterns: *P. falciparum* (malaria tropica), *P. vivax* (malaria tertiana), *P. malariae* (malaria tertiana) and *P. ovale* (malaria quartana). The latter two species are less common. It is transmitted to humans by female mosquitoes of the genus *Anopheles*.\(^2\) In India five species of *Anopheles* are known to be transmitters of the parasites: (1) *A. culicifacies*, (2) *A. stephensi*, (3) *A. philippinensis*, (4) *A. sundaicus*, and (5) *A. fluviatilis*. Endemic maps indicate that *P. falciparum* and *P. vivax* account for 95% of malaria infections.\(^3\) *P. falciparum* is found throughout tropical Africa, Asia and Latin America while *P. vivax* is worldwide in tropical and some temperate zones.\(^4\),\(^5\) Of these two parasites, *P. falciparum* is the most deadly one, causing cerebral malaria which, if remain untreated, leads to coma and ultimately death of the patient.

Approximately 40% of the world population live in areas with the risk of malaria.\(^2\) Around 300-500 million clinical cases of malaria are reported every year, of which more than a million die of severe and complicated cases of malaria.\(^6\) Malaria is known to kill one child every 30 sec, 3000 children per day under the age of 5 years.\(^7\),\(^8\) The economic toll of malaria is tremendous. It has been estimated that the African continent has forgone almost $100 billion in GDP over the last 35 years due to malaria alone.\(^9\)

Malaria ranks third among the major infectious diseases in causing deaths after pneumococcal acute respiratory infections and tuberculosis, and accounts for approximately 2.6% of the total disease burden of the world.\(^2\) Although malaria has been widely eradicated in many parts of the world, the global number of cases continues to
rise. The most important reason for this alarming situation is the rapid spread of malaria parasites that are resistant to antimalarial drugs. This review chapter accommodates some of the most significant historical achievement and development observed during the past 35 years in the discovery of antimalarial drug artemisinin and its related antimalarial peroxides. This review also highlights about the current scenario of malaria chemotherapy.

1.2 Life Cycle of Malarial Parasite

The human malaria parasite has a complex life cycle. Being digenetic, it is completed in two hosts. The asexual cycle is passed in humans by a process termed schizogony. The sexual cycle is completed in the final host or vector, the female *Anopheles* mosquito, involving gametogony and sporogony. The infection starts when the infected female *Anopheles* mosquito injects sporozoites into the subcutaneous tissue (1) and less frequently directly into the blood stream; from there sporozoites travel to the liver. This is followed by a period of incubation during which the sporozoites invade the hepatocytes where they grow and multiply (2). Towards the end of the incubation period, tissue schizonts rupture to release merozoites (3), which enter into the blood stream and infect the erythrocytes (4). Within the erythrocyte parasite grows from “ring” to mature trophozoite, then to schizont, and finally to merozoites. These merozoites are released into the blood stream by the rupture of erythrocytes (5). This is accompanied by the manifestation of the clinical symptoms of the disease such as fever, shivering and anaemia. Merozoites reinfect the healthy erythrocytes. Some of the merozoites are changed into gametocytes (6) that are ingested by female *Anopheles* when it bites an infected person. In female mosquito these gametocytes undergo sexual reproduction and produce sporozoites. These sporozoites migrate in the salivary glands; from there they are
injected into the bloodstream of a healthy person. In this way the infective cycle of the malaria parasite continues.

Life Cycle of Malaria Parasite

1.3 Classification of Antimalarial agents

The currently available antimalarial agents can be classified according to their biological activity and chemical structure (Figure 1).

A. Blood Schizontocides

These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria. The drugs belonging to this class include quinine (1), chloroquine (2), mefloquine (3), halofantrine (4), pyrimethamine (5), sulfadoxine (7), sulfones and tetracyclines.
B. Tissue Schizontocides for Causal Prophylaxis

These drugs act on the primary tissue forms of the *Plasmodium*, which after growth within the liver initiate the erythrocytic stage. By blocking this stage, further development of the infection can be prevented. Primaquine (5) and pyrimethamine (6) (to a lesser extent) have activity against this stage. However, since it is impossible to predict the infection before clinical symptoms begin, this mode of therapy is more theoretical than practical.

![Molecules](image)

**Figure 1.** Quinolines, a diaminopyrimidine, and a sulfonamide used in classical chemotherapy.

C. Tissue Schizontocides for Preventing Relapse

These drugs act on hypnozoites of *P. vivax* and *P. ovale* in the liver which cause relapse of symptoms on reactivation. Primaquine (5) is the only prototype drug available for this stage.

D. Gametocytocides

These drugs destroy the sexual forms of the parasite in the blood, and prevent transmission of the infection to the mosquito. Quinine (1) and chloroquine (2) have gametocytocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum*.
However, Primaquine (5) has gametocytocidal activity against all human malarial parasite species including *P. falciparum*.

**E. Sporontocides**

These drugs prevent the development of oocyst in the mosquito and thus ablate the transmission. Primaquine (5) and chloroguanidine are known to have activity against this stage. The two important concepts in the treatment of malaria include suppressive and radical treatment of the infection. Suppressing the erythrocytic stage of the parasitic development can alleviate the symptoms of malaria and it involves administration of appropriate blood-schizontocidal drugs. In all cases of *P. vivax* malaria and in most cases of *P. falciparum* malaria, it involves administration of chloroquine along with tissue-schizontocidal drugs.

**1.4 Classical Chemotherapy**

Unfortunately, the extremely complex life cycle of malaria parasites in the human host makes comprehensive treatment difficult. They have varying profile of drug sensitivity at different phases of their development, and current health care for malaria patients uses drugs (figure 1) selectively toxic to these specific stages. There are few agents that combat the parasites while in the liver, even though eradication at this stage is necessary to prevent relapses of *P. vivax* and *P. ovale* malaria. The synthetic 8-aminoquinoline Primaquine 5 is the main drug used to kill parasites during this phase of their development, although it does have the potential to cause deleterious side effects in certain individuals.

The most common focus of malaria chemotherapy is against blood phase parasites, which cause the clinical symptoms of the disease. Quinine (1), the principle quinoline alkaloid from the bark of the Cinchona tree, has been used to treat malaria for
several hundreds years. Quinine \( (1) \) rapidly kills all four species of *Plasmodium* while in erythrocytes, as well as gametocytes of *P. vivax* and *P. ovale*.\(^{11}\)

### 1.5 Drug Resistance

Malaria was nearly eradicated by the early 60s, owing largely to concerted antimalarial campaigns under the guidance of WHO.\(^{14,15}\) However, the disease has made a dramatic comeback, due to laxity in antimalarial campaigns, emergence of resistance by the parasite to majority of commonly used antimalarial drugs and by the vector against the insecticides. Resistance to the commonly used drug chloroquine \( 2 \) is most prevalent, while resistance to most other antimalarials such as alkaloids (e.g., quinine \( 1 \)), sulfonamides (e.g., sulfadoxine \( 7 \)), and diaminopyrimidines (e.g., pyrimethamine \( 6 \)) have also been extensively reported.\(^{16}\) Resistance of *P. falciparum* to Chloroquine \( 2 \) was first reported in the late 1950s in SE Asia. At first resistance was slow to evolve but spread rapidly with in SE Asia in the late 1960s & early 1970s. Resistance was first reported in East Africa in 1979.

The spread of Chloroquine resistance has necessitated the use of alternative drugs, such as sulphonamides \( (7) \) -pyrimethamine \( (6) \) combinations, quinine \( (1) \) / tetracycline, mefloquine \( (3) \) and halofantrine. Despite tremendous efforts, an effective vaccines has not been found yet. Continued problems with parasite resistance to and patient side effects from the quinolines, diaminopyrimidines, and sulfonamides have generated urgent interest in discovery and design of nontraditional antimalarial drugs.

### 1.6 Artemisinin: A major breakthrough in malaria chemotherapy

In 1967, the Chinese government launched a program to discover new antimalarial drugs, and indigenous plants used in traditional medicine were systematically examined.
The first written record of the antipyretic activity of tea-brewed leaves of *Artemisia annua* was described in "The Handbook of Prescriptions for Emergency Treatments" written by Ge Hong (281-340 A.D.). Li Shizen's "Compendium of Materia Medica" (published in 1596) cited the prescription from Ge Hong's book. In 1971, Chinese researchers isolated, by extraction at low temperature from *A. annua* (Sweet wormwood), a stable easily crystallizable compound that they named *Qinghaosu* and later on named artemisinin (8) (Chemical Abstracts, Registry No. 63968-64-9).\(^{17}\)

In the past 36 years, out of all antimalarial drugs discovered during this period, artemisinin was the only natural product whose medicinal properties were known for more than 2000 years. The only one synthetic antimalarial drug, mefloquine, has been discovered during this period.

Since its discovery, artemisinin has distinguished itself as a rapidly acting plasmodial agent against the blood phase of *P. falciparum*, and is potent against both chloroquine-sensitive and chloroquine-resistant strains of the parasite *in vitro*,\(^ {18}\) *in vivo* animal studies,\(^ {19}\) and, most importantly, in humans. Organic chemists have since developed various syntheses of this structurally intriguing natural product.\(^ {20}\)

### 1.7 Pharmacophore for antimalarial activity

Investigations into the antimalarial activity of artemisinin revealed that the central endoperoxide is essential for potent activity.\(^ {22}\) Deoxyartemisinin 9, the peroxide-reduced...
ether form of artemisinin, is a metabolite isolated from urine of patients treated with artemisinin.\textsuperscript{8,23} It is about 300-1000 fold less active than artemisinin against chloroquine-sensitive \textit{P. falciparum} (D6 Strain) \textit{in vitro}.\textsuperscript{24}

Chemists have synthesized a variety of deoxyartemisinin analogs such as Deoxydihydroartemisinin 10, deoxyarteether 11 (approximately 300 and 150 times less activity \textit{in vitro} respectively) etc. that revealed a parallel necessity of the endoperoxide for antimalarial potency.\textsuperscript{25}

1.8 Genetic engineering of artemisinic acid in yeast

\textit{Ro et al.}\textsuperscript{21 (2006)} describe the genetic engineering of \textit{Saccharomyces cerevisiae} to potentially provide a more facile, cheaper route to artemisinin from the precursor artemisinic acid, which has implications for the development of more affordable treatments for malaria (Figure 2). The small quantities of artemisinin, obtained by extraction from the leaves of the wormwood tree (\textit{Artemisia annua}), is quite laborious and costly and its total synthesis render artemisinin-based therapies unaffordable for most malaria sufferers. Artemisinic acid is the final product of a five-step transformation that starts with farnesyl pyrophosphate (FPP). In the FPP biosynthetic pathway, the majority of FPP is converted to sterols. By genetically engineering \textit{S. cerevisiae}, the concentration of FPP available for artemisinic acid biosynthesis was maximized by both increasing FPP production and blocking its use in sterol synthesis.
Figure 2. Schematic representation of the engineered artemisinic acid biosynthetic pathway in *S. cerevisiae* strain EP224 expressing *CYP71AV1* and *CPR*.

The transformation of FPP into artemisinic acid requires only two other enzymes. Amorphadiene synthase (ADS) first catalyses the conversion of FPP to amorpha-4, 11-
diene, which is usually the first committed step in artemisinin biosynthesis. Next, a sequence of three oxidation reactions produces artemisinic acid. A cytochrome P450 (CYP450) has been shown to catalyse the first transformation i.e. the hydroxylation of amorphadiene. Genetic screening of the *A. annua* sequence and BLAST analyses identified a novel CYP450 gene, *CYP71AV1*. Gas chromatography-mass spectrometric analysis of cell cultures and *in vitro* enzyme assays with artemisinic alcohol and artemisinic aldehydes (the two intermediates in this pathway) revealed that *CYP71AV1* catalyses all three reactions. Yields of 100 mg artemisinic acid per litre of culture was reported, with >95% of artemisinic acid being recovered. These high yields led to speculate that the artemisinic acid is transported out of the cells but remains bound to the cell surface.

Taking advantage of this characteristic, the crude product was purified in a single inexpensive step using gel chromatography to furnish pure artemisinic acid (76 mg). The chemistry for the conversion of artemisinic acid to artemisinin is well established and efficient, and so, if the microbial production of artemisinic acid could be optimized, the yields could be high enough to significantly reduce the cost of artemisinin combination therapies.

1.9 Semisynthetic & simplified artemisinin analogs as antimalarials

Although artemisinin 8 has been used clinically in China for the treatment of multidrug-resistant *Plasmodium falciparum* malaria, the therapeutic value of 8 is limited to a great extent by its low solubility in both oil and water. Consequently, in the search for more effective and soluble drugs, Chinese researchers prepared a number of derivatives of the parent drug. Reduction of artemisinin produces dihydroartemisinin 12, which has in turn led to the preparation of a series of semisynthetic first-generation
analogues that include artemether 13 and arteether 14. Both of them showed better oil solubility and improved activity than 8 and are currently in clinical use.  

For treatment of advanced cases of *P. falciparum* malaria, a water-soluble derivative of artemisinin is desired. A water-soluble derivative can be injected intravenously (iv), and thus, the drug can be delivered more quickly than by intramuscular (i.m) injection. The sodium salt of artesunic acid 15 is such a water-soluble derivative, capable of rapidly diminishing parasitaemia and restoring consciousness of comatose cerebral malaria patients. Because of the high recrudescence rate, however, 15 is normally administered in combination therapy, most often with mefloquine. Out of all the first-generation derivatives, 15 is currently the drug of choice. Sodium artelinate 16a, the sodium salt of artelinic acid 16, was designed to overcome the hydrolytic instability experienced with artesunate. In comparison to artemether and arteether, 16 is not only more stable in aqueous solution but also has a much longer half-life (1.5-3 h) than oil-soluble analogues. Artemisinin derivatives are fast acting blood schizontocides and are undoubtedly safe drugs for emergency treatment of severe multidrug-resistant malaria but they should not be used for prophylaxis of malaria or treatment of mild attacks.
1.9.1. Ether and Ester derivatives of artemisinin

Since the discovery of artemisinin, Chinese scientist and ester derivatives of \(8\) in search for a new preparation with good solubility and bioavailability which has become th laboratories round the world.\(^{33,34}\)

First generation drugs such as artesunate \(15\) and arteli develop a new class of second generation semisynthetic ant desirable properties. Much effort has been directed at obtaining bioavailability by modification of artelinic acid \(16.35\) C withdrawing functional groups in their aromatic ring showed antimalarial activity. Compounds with a small substituent at showed weaker activity than compounds with a larger s lipophilicity and steric effects of the molecule are also factors.\(^3\)

\[
\begin{align*}
17 & \quad R = \text{CH(CH}_3\text{C=CH} \\
18 & \quad R = \text{CH(CH}_3\text{C=CH} \\
19 & \quad R = \text{(4-OCH}_2\text{C=CHC}_6\text{H}_4})
\end{align*}
\]

Venogopalan et al.\(^{36}\) (1995) have prepared 56 ether de treating hydroxyalkyl, substituted phenol, hydroxyl aralkyl, hy heteroalkyl etc in the presence of BF\(_3\).Et\(_2\)O. Compound 17, 18 in \textit{P. berghei} K-173 infected mice and \textit{P. yoelii nigeriensis} (NS p.o route. C-10 acetal artemisinin dimmer \(20\) was found to be
Haynes et al.\textsuperscript{37} (2002) reported several new C-10 ester and ether derivatives of the dihydroartemisinin 9. β-Artesunate 21, for the first time, has been prepared and its structure has been confirmed by X-ray crystallography. New ester 22 and ether 23 derivatives bearing potential intercalating groups have been synthesised by means of the Schmidt, Mitsunobu and DCC coupling procedures, by acylation in the presence of DMAP, or by hydroxy activation with BF\textsubscript{3} as catalyst. No attempt was made to characterize smaller amount of α-isomer formed in these reactions. Two important observations in his study were:

1. When the hydroxy group of DHA acts as a nucleophile towards activated carboxy groups in acylating agents or the DCC intermediate, α-esters are obtained exclusively.

2. When the hydroxy group is activated for displacement by nucleophiles, as in the Schmidt or Mitsunobu procedures, β-esters and β-ethers are obtained either exclusively or predominantly.

An exception is represented by the Mitsunobu procedure involving DHA and 1- and 2-naphthols, in which mixtures of epimers 24 are obtained; however, exclusive formation of β-aryl ethers takes place when the Schmidt procedure is used, with activation of the intermediate trichloracetimidate by SnCl\textsubscript{2} which is superior method to the patented procedures for the preparation of β-aryl ethers from nonbasic aryl alcohols without detectable rearrangement to C-aryl compounds. However, the Mitsunobu procedure is better when basic aromatic alcohols are used as nucleophiles. The formation of α-products in which the hydroxy group of DHA acts as a nucleophile is of biological significance in relation to enzyme-mediated Phase II glucuronidation of DHA, in which only the α-DHA glucuronide is formed.\textsuperscript{37}
Artemisinin-linked steroid, 10β-[(Cholest-59-en-39-yl) oxy] dihydroartemisinin 25, prepared by reacting cholesterol with DHQ in presence of BF₃-Diethyl ether, have shown antimalarial as well as antiparasitic activities.³⁷

Several modified analogues of artelinic acid 26a-c have been prepared, carboxylic acid 26a showed superior antimalarial activity than artelinic acid in vivo against P. berghei via oral route.³⁸

Other C-10 substituted highly active ether analogues are the bis-trioxane analogues 27a and 27b. Their in vivo activity is comparable to that of 14.³⁶

Delhas et al.³⁹ in 2000 reported a series of dihydroartemisinin derivative 28a-b containing a ferrocene nucleus. Both compound were tested in vitro against P. falciparum and were found to be as active as 8.
26a: X = Cl, R = H  
26b: X = Br, R = H  
26c: X = NO₂, R = Me

27a: β-isomer  
27b: α-isomer

28a R = COCH₂CH₂-Ferrocenyl-alpha (98%)  
28b R = (CH₂)₃NHCH₂-Ferrocenyl

**1.9.2. Water soluble ether derivatives of artemisinin**

29a R = (R) CH₂CH(Me)COOMe  
29b R = (S) CH₂CH(Me)COOMe  
29c R = (R) CH(Me)CH₂COOMe  
29d R = (S) CH(Me)CH₂COOMe

30a R = (R) CH₂CH(Me)COOH  
30b R = (S) CH₂CH(Me)COOH  
30c R = (R) CH(Me)CH₂COOH  
30d R = (S) CH(Me)CH₂COOH

In further attempt to prepare compounds better than artelinic acid, Lin *et al.*²⁸ prepared a series of hydrolytically stable and water soluble derivatives of 9 with optically active side chain and screened for their antimalarial activity. Esters 29a-d were found to be more active than 8, 13 and 14 while corresponding carboxylic acid were 10-100 folds less active. Moreover, S-isomers were found to be more active suggesting the involvement of enzymatic bioactivation as a mode of action.

Taking important clues from the inferences made in the above studies, Lin *et al.*³⁵⁻³⁸ in their attempt to synthesize compounds superior to artelinic acid 16, prepared a number of α-alkyl benzylic ether derivatives of dihydroartemisinin and tested for their *in vitro* antimalarial activity. Compounds 31 was found to be the most active compound
showing activity nearly 10-, 20-, 40-folds better than artemether 13, arteether 14 and artelinic acid 16 respectively.

Singh et al.\textsuperscript{40} also reported the synthesis of hydrolytically stable derivatives of artemisinin 32a-d and 33. Among them compounds 32a-d and 33 were found to have activity comparable to \(\beta\)-arteether 14. While hemisuccinates 34a-d showed activity comparable to artesunic acid whereas 35a-c showed less activity.

Lin et al.\textsuperscript{41} have synthesized several water soluble and hydrolytically stable derivatives of 12. Among them artelinic acid 16 shows better \textit{in vivo} activity and high hydrolytic stability.

1.9.3. C-10 aryloxy derivatives of artemisinin

\begin{align*}
\text{R} & = \text{H, Me, CF\textsubscript{3}, OMe, t-Bu} \\
36 & \\
37 & \\
38 & \text{R} = \text{C\textsubscript{2}H\textsubscript{5}, Cyclohexyl, C\textsubscript{6}H\textsubscript{5}, H\textsubscript{2}COOH, CH\textsubscript{2}CH\textsubscript{2}COOH, CH\textsubscript{3}CH\textsubscript{2}OH, CH(CH\textsubscript{3})\textsubscript{2}, CH\textsubscript{2}CH=CHC\textsubscript{6}H\textsubscript{5}, (4-COOH\textsubscript{C\textsubscript{6}}H\textsubscript{4}), (2-COOCH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}), (4-COOCH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}), CH\textsubscript{2}(2-Furyl), CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{2}-maleate}
\end{align*}
In 1999, P. M. O’Neill reported a series of O-phenoxy derivatives 36 and 37 and their \textit{in vitro} antimalarial activity against chloroquine-sensitive HB3 strains of \textit{P. falciparum}, Compound 36 (\(R = \text{t-Bu}\)) showed IC\(_{50}\) of 3.72 nM.\(^{42}\)

1.9.4. Thioethers of artemisinin

Venogopalan \textit{et al.}\(^{36}\) (1995) have prepared several thioethers 38 of dihydroartemisinin by treating 8 and thiols in the presence of BF\(_3\).Et\(_2\)O. These thioethers were found active in \textit{P. berghei} (K-173)-infected mice and \textit{P. yoelii nigeriensis} (NS)-infected mice via s.c and p.o route and had better activity than 14.

1.9.5. Carba-analogues and deoxocarba-analogues of artemisinin

Several derivatives of artemisinin prepared by replacement of oxygen at C-10 with carbon e.g. 39,\(^{43}\) 40,\(^{44}\) (fig. 2) have shown better activity profile than the parent oxygen containing compounds and the improved activity has been attributed to their improved hydrolytic stability.

Artemisinin 8 and all other first generation drugs are believed to act as prodrugs for DHA 12. Metabolic studies indicate that 14 is rapidly converted into DHA 12.\(^{45}\) In order to produce derivatives that would not be a prodrug for toxic DHA 12, 10\(\beta\)-alkylartemisinin derivatives were prepared by Ziffer \textit{et al.}\(^{45b}\) (1995). The compound 10\(\beta\)-n-butyl/deoxoartemisinin 41a has \textit{in vivo} activity approximately equal to that of 8.\(^{46}\) 10\(\beta\)-
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Allyldeoxoartemisinin 41b was converted into several promising derivatives, of which 10β-n-propyldeoxoartemisinin 41c was the most promising. The in vivo results with 10β-n-propylartemisinin 41c indicate that its activity against W-2 and D-6 clones of *P. falciparum* and toxicity are comparable to those of 14.47

\[
\begin{align*}
41 & \quad 41a \quad R = \text{n-Bu} \\
41b & \quad R = \text{CH}_2\text{CH}=\text{CH}_2 \\
41c & \quad R = \text{CH}_2\text{CH}_2\text{CH}_3 \\
41d & \quad R = \text{CH}_2\text{CH}_3 \\
41e & \quad R = \text{CH}_2\text{CH}_2\text{OH}
\end{align*}
\]

In another series of 10β-alkyl series, the most promising analogues are alcohols 42a and 42b. They are five to seven times more active in vitro than 8.48

In a series of aromatic C-10 substituted analogues, some show high activity in vitro.49 The C-10 naphthyl substituted derivative 43 exhibited antimalarial activity similar to that of 13 in vivo.50

Jung *et al.* (1991) reported the synthesis of 12-(3'-hydroxy-n-propyl) deoxoartemisinin 44a, b from artemisinic acid. Compound 44a showed 5 times more activity than 8 in vitro against chloroquine-resistant *P. falciparum*.

McChesney *et al.* (1993),52 further reported the synthesis of another series of deoxo-artemisinin analogues 45a-d from artemisinic acid. However, antimalarial activity of these compounds has not been mentioned.

Further, Jung *et al.* (1994) synthesized a series of water soluble 12-deoxoartemisinin analogues from artemisinic acid. Compounds 46b and 46c were found to be as active as 8 in vitro against chloroquine-resistant malaria.
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Vroman et al.\textsuperscript{54} (1997) devised a novel methodology for synthesis of 11-alkyl-12-deoxoartemisinin. Analogues 47a and 47b were reported to be highly potent antimalarials.

Posner et al.\textsuperscript{55} (1999) reported 12-deoxoartemisinin analogues 48a-d which showed high \textit{in vitro} antimalarial activity.

Jung et al.\textsuperscript{56} (2000) in their attempt to generate water soluble, hydrolytically stable novel analogues of deoxoartemisinin synthesized compounds 49a-e. Compounds 49b and 49c were found to be as active as 8 \textit{in vitro}.

\begin{align*}
47a & R = n-C_4H_9 \\
47b & R = CH_2CH_2C_6H_5
\end{align*}

\begin{align*}
48a & R = O \\
48b & R = S \\
48c & R = N \\
48d & R = O
\end{align*}

\begin{align*}
49a & R = CHO \\
49b & R = CH_2NHPh \\
49c & R = CH_2N(CHMe_2)_2 \\
49d & R = CH_2OH \\
49e & R = CH_2O-\text{-D-Glc}
\end{align*}

\begin{align*}
50a & R = 2-\text{Fluorophenyl} \\
50b & R = 3-\text{Fluorophenyl} \\
50c & R = 4-\text{Fluorophenyl} \\
50d & R = 2-\text{trifluorophenyl} \\
50e & R = 2-\text{trifluorophenyl}
\end{align*}

\begin{align*}
51a & R = 2-\text{Fluorophenyl} \\
51b & R = 3-\text{Fluorophenyl} \\
51c & R = 4-\text{Fluorophenyl} \\
51d & R = 3-\text{trifluorophenyl} \\
51e & R = 4-\text{trifluorophenyl}
\end{align*}

P. M. O'Neill et al.\textsuperscript{57} (1999) reported a new series of carba ether 50a-e and ester 51a-e analogues of artemether and tested them \textit{in vitro} against chloroquine-sensitive HB-
3 and resistant K-1 strains of *P. falciparum*. All compounds except 51e were found to be more potent than artemisinin. Compound 50a was fifteen times more potent than 8 and five times more potent than 12.

Posner *et al.*\(^{58}\) (2004) have prepared artemisinin derived trioxane dimers 52 and 53. In mice both 52 and 53 were more effective than clinically used 12 via both oral (p.o.) and intravenous (i.v.) administration (Table 1).

![Chemical structures of compounds 52a, 52b, 53a, and 53b](image)

**Table 1. In vivo antimalarial activity in mice.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>(ED_{50}) (mg/kg/day x 4)</th>
</tr>
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<tbody>
<tr>
<td>52a</td>
<td>i.v.</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>2.0</td>
</tr>
<tr>
<td>52b</td>
<td>i.v.</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>4.5</td>
</tr>
<tr>
<td>12</td>
<td>i.v.</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Posner *et al.*\(^{59}\) (2006) have prepared C-10 carbaartemisinin phthalate dimer 53a and benzyl alcohol dimer 53b as potential anticancer agents. Both have shown high order of antimalarial activity *in vitro*. Bis-benzyl alcohol dimer 53b is approximately 1.5 times more orally efficacious in rodents than the antimalarial drug 15 and is about 37 times more efficacious than 15 via subcutaneous administration. Phthalate dimer 53a is very
highly growth inhibitory but not cytotoxic toward several human cancer cell lines; both
dimers 53a and 53b very efficiently and selectively kill human cervical cancer cells in
vitro in a dose-dependent manner with no cytotoxic effects on normal cervical cells.

Jung et al. (1990) reported C-10 substituted analogues i.e, deoxyartemisinin 54, which lacks the carbonyl group at C-10. It is therefore presumed to be more stable in the body. It is slightly more active than 14 in vitro, but not as active as 12 in vivo.

O’Neill et al. (1999) reported C-10 carba-analogue 55a-e of artemisinin. Out of these, compound 55b (TDR 40292), in collaboration with WHO, was screened and compared with 13. This compound cannot form DHA as a metabolite and contains a side chain that can be formulated as a water-soluble salt. In addition, antimalarial assessment both in vitro and in vivo demonstrates that this compound is superior to 13 and 15.

Haynes et al. (2004) had patented several C-10 α-amino analogues 55f-g of dihydroartemisinin. Several of these analogues have shown exceptional antimalarial activity both in vivo and in vitro.

Bonnet-Delphon and co-workers (2004) attempted to increase the metabolic and chemical stability of arteether and DHA by the incorporation of a C-10 CF₃ group, thereby, making CF₃ analogue of arteether 45 times more stable than 14 itself under "simulated stomach acid conditions."
1.9.6 Azaartemisinin analogues

Avery and coworkers reported one azaartemisinin analogue 56 to be 50% more potent than artemisinin.\textsuperscript{63} The results of the 11-azaartemisinin series correspond to the finding of Avery and coworkers that the antimalarial activities of lactams are as high or higher than that of artemisinin 8 both \textit{in vitro} and \textit{in vivo} tests.\textsuperscript{64} The most promising compound 56 is approximately as active as arteether 14 \textit{in vivo}.\textsuperscript{63b} Further analogues have been prepared with electronegative substituents on the alkyl chain of the lactam substituent in order to improve the antimalarial activity.\textsuperscript{65}

\[ \text{Diagram of 56} \]

1.9.7 Synthetic 1, 2, 4-trioxanes structurally related to artemisinin

A large number of derivatives of artemisinin like 1, 2, 4-trioxanes, including ethers, carboxylate esters, phosphate esters, carbamates and sulfonates have been prepared by Posner and coworkers. Some of the compounds found active \textit{in vitro} were also tested \textit{in vivo} in mice model. Based on their antimalarial potency in mice, two trioxanes 57 and 58 were selected for biological evaluation in Aotus monkeys infected with multidrug-resistant (MDR) \textit{P. falciparum} (Table 2). As can be seen from the activity data in Table 3, both 57 and 58 are as effective as arteether against MDR \textit{P. falciparum} in Aotus monkeys.\textsuperscript{66}
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![Chemical structures](image)

57 $R = P(O)(OPh)_2$

58 $R = CH_2 Ph$

59

Table 2. In vivo activity of 57-58 against Vietnam Smith/RE strain infections of *P. falciparum* in Aotus monkeys by i.m. route.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg/day</th>
<th>No. cured / No. treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>48.0</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>1/2</td>
</tr>
<tr>
<td>58</td>
<td>48.0</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>0/2</td>
</tr>
<tr>
<td>11</td>
<td>48.0</td>
<td>2/2</td>
</tr>
</tbody>
</table>

On the basis of earlier observation by Jefford et al. that replacement of the C₃-methyl by C₃-phenyl improved antimalarial activity, ⁶⁷ various substituted C₃-phenyl analogs of prototype 59 (Table 3), were also prepared by Posner et al. ⁶⁸ Some of these compounds 59a-e have shown promising *in vivo* activity. The activity data of 59a-e in comparison with ⁸ is presented in Table 4. Trioxane alcohol 59a, acetate trioxane 59b and fluorobenzyl ether trioxane 59c are up to twice as potent as ⁸ whereas water soluble carboxylic acid derivative 59e was less active than ⁸. ⁶⁸

Table 3. In vivo antimalarial activity against chloroquine-sensitive *P. berghei*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R$</th>
<th>$ED_{50}$ mg/kg</th>
<th>$ED_{90}$ mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s.c.</td>
<td>p.o.</td>
<td>s.c.</td>
</tr>
<tr>
<td>59a</td>
<td>HOCH₂</td>
<td>3.4</td>
<td>5.5</td>
</tr>
<tr>
<td>59b</td>
<td>MeC(O)OCH₂</td>
<td>2.8</td>
<td>14</td>
</tr>
<tr>
<td>59c</td>
<td>p'-FPhCH₂OCH₂</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>59d</td>
<td>F</td>
<td>6.8</td>
<td>10</td>
</tr>
</tbody>
</table>
In continuation of their work, Posner et al. prepared carboxyphenyl trioxanes 60a and 60b which were more soluble in water at pH 7.4 than 16. These compounds were less effective than their less lipophilic and more easily prepared parent compound 59. Activity data of 60a and 60b against P. berghei in mice, in comparison with 16 has shown in Table 4.

Table 4. Antimalarial efficacy in mice against P. berghei by p.o. route.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED_{50} (mg/kg)</th>
<th>ED_{90} (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60a</td>
<td>17.0</td>
<td>59.0</td>
</tr>
<tr>
<td>60b</td>
<td>15.0</td>
<td>51.0</td>
</tr>
<tr>
<td>16</td>
<td>9.6</td>
<td>29.0</td>
</tr>
</tbody>
</table>

1.10. Molecular Docking and 3D QSAR Studies on Artemisinin

In order to understand the antimalarial mechanism of action and the relationship between the physicochemical properties and the antimalarial activities of artemisinin analogues, molecular docking simulations to probe the interactions of these analogues with hemin were performed, and then performed 3D QSAR studies on the basis of the docking models employing comparative molecular force fields analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). Molecular docking simulations generated probable ‘bioactive’ conformations of artemisinin analogues and
provided a new insight into the mechanism of action. The subsequent partial least squares (PLS) analysis indicates that the calculated binding energies correlate well with the experimental activity values. Good predictive ability of CoMFA and CoMSIA models based on the bioactive conformations have been proved and in turn match well with the docking result, which further testified the reliability of the docking model. Thus, the binding model and activity of new synthesized artemisinin derivatives have been explained through molecular docking and 3-D-QSAR.

With an ever-expanding data set for artemisinin analogues, Avery has recently developed a predictive 3D QSAR for artemisinin analogues, and this is being used to design even more potent peroxide derivatives. Future work in this area will no doubt combine the computer-guided C-10 and C-14 functional group optimization of antimalarial activity coupled with careful incorporation of functional groups that provide both water solubility and resistance to first-pass metabolism.

1.1. Neurotoxicity in ether derivatives

Despite of extensive use of artemisinin during clinical trials early in china, without report of serious human toxicity, animal studies have yielded some cautionary findings. 13 and 14 have short plasma half-lives and produce fatal central nervous system (CNS) toxicity in chronically dosed rats and dogs. Rats dosed with artemether and arteether at 2.5 mg/kg/day showed cardiac abnormalities and neurotoxicity within 2 weeks, and dogs dosed at 20 mg/kg/day with these artemisinin analogs developed progressive neurological defects, leading to death in approximately 1 week. These daily doses are an order of magnitude more than necessary to clear both chloroquine-sensitive and chloroquine-resistant P. Berghei (various strains) in mice in 4 days or less. Given the neurotoxic effects of artemisinin and its analogs in these animal studies, researchers
investigated the effects of these antimalarials on neuronal cells in vitro.\textsuperscript{76} The drugs inhibited both neuronal cell proliferation and formation of neurite outgrowths at concentrations as low as 10 nM,\textsuperscript{77} which is comparable to the effective level of these drugs in vitro against many strains of \textit{P. falciparum}.

As far as potential neurotoxicity is concerned, any analogue with a log \textit{P} higher than that of artemether (3.3-3.5) can cross the blood-brain barrier. By use of the ADME (absorption, distribution, metabolism, and excretion) paradigm for enhancing efficacy through increased absorption, the application of \textit{Lipinski's Rule of Five}\textsuperscript{78} to the design of new semisynthetic analogues have been employed.\textsuperscript{61,79}

1.12. Drawbacks associated with artemisinin

Artemisinin and its derivatives are associated with several serious problems such as high cost, poor solubility in both oil and water, high rate of recrudescence, limited availability from natural sources and poor bioavailability.\textsuperscript{80} Since the discovery of 1, 2, 4-trioxane as the pharmacophore for the antimalarial activity of artemisinin, efforts towards the preparation of simple 1, 2, 4-trioxane have increased tremendously. As a result several new methods for their preparation have been developed in the recent years.

1.13. Synthetic 1, 2, 4-trioxanes & antimalarial peroxides

There have been few reports on the synthesis of 1, 2, 4-trioxanes before the discovery of artemisinin. However, in the post artemisinin era, several new synthetic methodologies have been appeared. In most of these methodologies, singlet oxygen (\textit{^1O}_2) has been the most common source for the introduction of peroxide group, though other sources including H\textsubscript{2}O\textsubscript{2}, ground state oxygen (\textit{^3O}_2) and ozone (O\textsubscript{3}) have also been used.
1.13.1. Synthesis of first 1, 2, 4-trioxanes

Payne and Smith (1957) reported the first synthesis of 1, 2, 4-trioxanes using H$_2$O$_2$ in which cyclohexene epoxide 61 on treatment with 90% H$_2$O$_2$ and tungstic acid (H$_2$WO$_4$) in acetone furnished bicyclic trioxane 62 together with trans diol 63 (Scheme 1). Handling of 90% H$_2$O$_2$ is hazardous and this method is of historical importance in the synthesis of 1, 2, 4-trioxanes.

\[ \text{Scheme 1} \]

1.13.2. Other procedures for the synthesis of 1, 2, 4-trioxanes

In the post artemisinin era, Nojima et al. (1981) firstly reported the synthesis of 1, 2, 4-trioxanes. This was conceptually a new method and involves the treatment of $\alpha$-hydroxy hydroperoxides (such as 64) or their precursors (such as 65 & 66) with epoxides (cyclic or acyclic) in presence of tungstic anhydride (WO$_3$) and CISO$_3$H (as catalyst) to furnish 1, 2, 4-trioxanes 67, 68 and 69 (Scheme 2). The $\alpha$-hydroxy hydroperoxides or their precursors were readily available by treatment of corresponding carbonyl compounds with 30% H$_2$O$_2$ at r.t.

\[ \text{Scheme 2} \]
Recently Meunier et al.\textsuperscript{83} (2003) have used 30\% H\textsubscript{2}O\textsubscript{2} to prepare polycyclic trioxane 71a. Thus the hetero Diels-Alder dimer 71 of 2-methylene cyclohexanone 70 on treatment with 30\% H\textsubscript{2}O\textsubscript{2} in acidic medium furnished trioxane 71a (Scheme 3).

\begin{center}
\textbf{Scheme 3}
\end{center}

Story and Burgess (1967) first reported ozone-mediated synthesis of a 1, 2, 4-trioxane.\textsuperscript{84} During their studies on ozonolysis of tetramethyl ethylene 72 they isolated a 1, 2, 4-trioxane derivative 73 along with 1, 2, 4, 5-tetraoxane 73a (Scheme 4).

\begin{center}
\textbf{Scheme 4}
\end{center}

Avery et al.\textsuperscript{85} (1990) reported the synthesis of tricyclic 1, 2, 4-trioxanes using ozonolysis of vinyl silanes as the key step. Thus ozonolysis of keto vinyl silane 74 in MeOH, followed by treatment with BF\textsubscript{3}.Et\textsubscript{2}O gave stable endoperoxide aldehyde 75 which on treatment with acetic anhydride and Amberlyst-15 gave tricyclic trioxane 76 (Scheme 5).

\begin{center}
\textbf{Scheme 5}
\end{center}
Bunnelle et al.\textsuperscript{86} (1991) reported another ozone-mediated synthesis of 1, 2, 4-trioxanes (Scheme 6).

\begin{center}
\includegraphics[width=\textwidth]{Scheme6.png}
\end{center}

\textbf{Scheme 6.} Reagents and conditions. (a) (i) n-BuLi, Et₂O-TMEDA, 25°C, 5h; (ii) TMSCI, Et₃N, -60 to 25°C, 4h, 85%; (b) O₃, pentane, NaHCO₃, -78°C, 53%; (c) 1% AcOH in MeOH, 25°C, 6h, 60%; (d) (CF₃SO₂)₂O, 2,6-lutidine, CH₂Cl₂, -78°C, 15 min; (e) MeCN, NaHCO₃, 25°C, 90%.

These authors reasoned that a suitably placed leaving group adjacent to the peroxide linkage of an ozonide can participate in ring expansion, provided the resulting carbocation is stabilized by a trialkyl silyl group. The ozonides 78\texttext{a} and 78\texttext{b} obtained by ozonolysis of 77 were converted to the respective triflates 79\texttext{a} and 79\texttext{b}; of the two triflates only 79\texttext{a} rearranged to 1, 2, 4-trioxane 80 (Scheme 6).

Posner et al.\textsuperscript{87} (1991) reported the synthesis of artemisinin like 1, 2, 4-trioxanes using Et₃SiOOOH as the source of peroxide group. Thus, treatment of enol ether 81 with Et₃SiOOOH at -78°C generated 1, 2,-dioxetane 81\texttext{a} which in situ treatment with t-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) furnished trioxane 82 as the single diastereomer (Scheme 7). Et₃SiOOOH can be easily prepared by reaction of Et₃SiH with ozone in CH₂Cl₂ solution at -78°C.
Dussault and Davies (1996) reported another synthesis of 1, 2, 4-trioxanes, via cyclization of unsaturated hydroperoxyacetals, which themselves were available via ozonolysis of appropriate enol ethers in presence of allylic alcohols. Thus ozonolysis of enol ether 83 in presence of allyl alcohol gave unsaturated hydroperoxyacetal 84 which underwent facile cyclization on treatment first with Hg (OAc)$_2$, followed by KBr, to furnish 1,2,4-trioxane 85 as a 10:1 mixture of diastereomers 85a and 85b (Scheme 8).

![Scheme 8]

Wilson et al. (1973) reported the first ground state molecular oxygen-mediated synthesis of 1, 2, 4-trioxanes by oxidative photocycloaddition of p-benzoquinone 86 to cyclooctatetraene 87. Irradiation of a mixture of 86 and 87 with argon laser in CCl$_4$ in the presence of oxygen gave trioxane 88 together with the normal anaerobic product 89 (Scheme 2.15). Here the triplet diradical 90 formed by reaction of 86 and 87 is trapped by molecular oxygen to give the trioxane 88 (Scheme 9).

![Scheme 9]

Adam et al. (1988) prepared artesinin type 1, 2, 4-trioxanes by molecular oxygen trapping of 1, 4-diradicals obtained from 3, 4-dihydro-4, 4-dimethyl-2H-pyran-2-
one 91 and quinones (p-benzoquinone and phenanthroquinone). Thus irradiation of a mixture of 91 and p-benzoquinone 86 in CCl₄ under oxygen furnished trioxanes 92, 93 and 94 in a ratio of 28:9:18 (Scheme 10).

```
  O'Neil et al.³¹ (2001) reported Co (acac)₂-mediated regioselective Mukaiyama-Lsayama peroxysilylation of 2-alkyl- or 2-aryl-prop-2-ene-1-ols as the key step for the synthesis of 1, 2, 4-trioxanes. Reaction of 2-phenyl-prop-2-en-1-ol 95 with Et₃SiH, Co(acac)₂ and O₂ followed by acid catalyzed condensation of the intermediate triethyl silyl peroxy alcohol 95a with cyclopentanone and cyclohexanone afforded spiro trioxanes 96 and 97 respectively (Scheme 11).
```

```
  Scheme 10
```

```
  Scheme 11
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```
  Scheme 12
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Bloodworth et al.\textsuperscript{92} (1991 & 1993) reported a new synthesis of 1, 2, 4-trioxanes using allylic hydroperoxides, obtained by photooxygenation of appropriate alkenes. The intermediate hemiperoxyacetal 99 obtained \textit{in situ} from allylic hydroperoxide 98 and acetaldehyde was cyclized either with N-bromosuccinimide (NBS), N-iodosuccinimide (NIS) or Hg(OAc)\textsubscript{2} to furnish trioxanes 100, 101 or 102 respectively (Scheme 12).

Jefford et al.\textsuperscript{93} (1983) reported the first \textit{1}O\textsubscript{2}-mediated synthesis of structurally simple 1, 2, 4-trioxanes. Bicyclic endoperoxides and 1, 2-dioxetanes were used as starting materials, which themselves were obtained by photooxygenation of suitable cyclic dienes and enol ethers. Thus endoperoxide 103b obtained from 1, 4-dimethylnaphthalene 103a on treatment with acetaldehyde and acetone in presence of catalytic amberlyst-15 gave cis-fused bicyclic 1, 2, 4-trioxanes 104 and 105 respectively. Similarly endoperoxide 106b gave cis-fused cyclopenteno trioxanes 107 and 108 (Scheme 13).

\begin{center}
\includegraphics[width=\textwidth]{scheme13.png}
\end{center}

\textit{Scheme 13}

Jefford \textit{et al.}\textsuperscript{94} also reported (1989) the synthesis of artemisinin like 1, 2, 4-trioxanes. Photooxygenation of keto enol ether 109 in CH\textsubscript{2}Cl\textsubscript{2} at -78\degree C, followed by treatment with amberlyst-15 provided two epimeric trioxanes 110, 111 (Scheme 14).
Out of several 1, 2, 4-trioxanes screened by Jefford et al. only cts-fused
cyclopenteno trioxanes (Fenozans) showed the most promising results in vivo. 4-
Fluorophenyl substituted trioxane 112 (Fenozan B07, earlier Fenozan 50F) was the best
compound of the Fenozan series. To study the role of chirality on antimalarial activity,
the same compound was resolved into enantiomers 112a and 112b using chiral HPLC.
The in vivo activity data of both the enantiomers and racemic 112 by oral (p.o.) and
subcutaneous (s.c.) routes in mice model against chloroquine-resistant Plasmodium yoelii
has been presented in Table 2.1. As can be seen from the activity profile of each
enantiomer (Table 5), there is no correlation between configuration and activity.
Racemates and enantiomers have commensurate activities.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>( P. \text{yoelii NS mg/kg/day} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( E_{D50} )</td>
</tr>
<tr>
<td>112a</td>
<td>s.c.</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>1.4</td>
</tr>
<tr>
<td>112b</td>
<td>s.c.</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>1.1</td>
</tr>
<tr>
<td>Racemic 112</td>
<td>s.c.</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>5.6</td>
</tr>
<tr>
<td>13</td>
<td>s.c.</td>
<td>5.8</td>
</tr>
</tbody>
</table>
1.14. Other antimalarial peroxides

1.14.1. Saturated 1, 2-dioxanes (endoperoxides)

Yingzhaosu A 113, a sesquiterpene endoperoxide similar to artemisinin with putative antimalarial properties was isolated from the traditional Chinese herb, Yingzhao, *Artabotrys uncinatus*. It contains unique 2, 3-dioxabicyclo [3.3.1] nonane ring system bearing a dihydroxyolefinic side chain. It's a traditional Chinese medicine for the treatment of malaria. But now a days it is no more in use. As reported by the Roche group, a number of structurally simplified analogs of 113 (Fig. 2) were prepared from its difficult 14-step total synthesis from R-(-)-carvone. In this series of endoperoxides, the activity was remarkably insensitive to changes in absolute and relative stereochemistry.

Endoperoxide 114a, the core structure of 113, showed weak antimalarial activity in vivo and replacing methyl group at position 4 with n-alkyl chains of 9–11 carbon atoms 114b led to slight increase in in vivo activity; analogs with shorter or longer chains were less active. As illustrated by 114c and 114d, compounds containing polar functional groups such as alcohols, acids esters, showed little or no activity. As shown by 114e, replacement of the undecyl chain in 114b with a styryl group abolished antimalarial activity. However, analogs of 114e, including quinoline 114f, and especially 114, the 2, 4-di (trifluoromethyl) styryl derivative, had very good antimalarial profiles. Although 114 was many folds less potent than the semisynthetic artemisinins in vitro, it is only 3-fold less active than 13 in vivo.
With having lower rate of recrudescence, longer plasma half-life, more stability, \textbf{114} (arteflene) was selected as the clinical candidate, and it progressed to Phase II clinical trials in semi-immune African patients with mild \textit{P. falciparum} malaria giving drug orally in lipid suspension. The compound was abandoned due to inconsistent results, long synthesis and poor oral bioavailability.\textsuperscript{99}
Posner et al. reported symmetrical bicyclo [3.2.2] nonane 115a-g and bicyclo [2.2.2] octane 116 endoperoxides. In vitro activity against chloroquine-sensitive *P. falciparum* (NF-54) showed that bicyclic endoperoxides 115a and 115b showed antimalarial activity about 1/7th that of artemisinin on a molar basis. Gem-dimethyl bicyclic endoperoxides 115c was found to be almost inactive. *p*-Methoxy-substituted 115b, and *p*-fluoro-substituted 116, only 10-fold less potent than 8, were the best of their respective classes.¹⁰⁰ Kamata et al.¹⁰¹ noted that the *p*-fluoro derivative of 115b was equipotent to the unsubstituted prototype bicyclo [3.2.2] nonane. However, Posner et al.¹⁰⁰c reported that the *p*-fluoro derivative was more potent than its unsubstituted prototype bicyclo [2.2.2] octane. In sum, there was no discernable aromatic substituent SAR for these bicyclic endoperoxides. As illustrated by sulfone 115g, seven heterocyclic analogs 115d-g containing sulfur, oxygen or nitrogen atoms were synthesized and found to be less potent than their carbocyclic analog 115a.¹⁰⁰b

1.14.2. Unsaturated 1, 2-dioxanes (endoperoxides)

Unsaturated 1, 2-dioxanes 117–121 have been prepared from the corresponding cyclohexadienes by 4 + 2 cycloaddition with photogenerated singlet oxygen (Figure 5).

Endoperoxide carbinol 117, the most potent compound in this series, was completely inactive in vivo.¹⁰² All 118, 119, and 120 were completely devoid of antimalarial activity.¹⁰³ Unsaturated bicyclic [2.2.2] endoperoxides 121, synthesized in
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three steps from cyclohexane-1, 4-dione, was the best and only 8-fold less potent than 8.

1.14.3. 1, 2-Dioxane (endoperoxide) ketal

Peroxyplakoric acid 122 is an antimalarial constituent obtained from sponge. Compound 123, methyl ester of 122, was less potent than its parent natural product prototype. Treatment of bis (cyclohexyl)-2, 2'-dione with 30%H₂O₂ furnished modestly more potent peroxy bis-hemiketal 124. Its more polar mono half ester-acid derivative was found to be less potent than the parent compound.

\[ \text{RO} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{CH}_3 \]
\[ \text{122 R = H} \]
\[ \text{123 R = CH}_3 \]

1.14.4. Simple bicyclic 1, 2, 4-trioxanes

The bicyclic trioxanone 125 was prepared from 2-methyl-2-cyclopenten-1-ol in six steps which involves the rearrangement of an ozonide to an unsaturated trioxane followed by cleavage of the terminal double bond with ozone as one of the key step. Bicyclic trioxane 126, 2, 3, 5- trioxabicyclo [2.2.3] nonane, was prepared from 6-tetrahydrooxepanol by treatment of its mesylate with acidic methanol to form the methyl acetal. Successive reactions of this methyl acetal with anhydrous hydrazine, 30% aq. H₂O₂/sodium peroxide and perchloric acid afforded 126. A series of 2,3,5-trioxabicyclo[2.2.2]octane homologs of 126 were similarly prepared from 5-hydroperoxy-2-methoxytetrahydroxyprans. However, these bicyclic trioxanes had only marginal antimalarial activity.

1.14.5. Tricyclic 1, 2, 4-trioxanes
Posner et al.\textsuperscript{107} (1992) prepared $^{18}$O labeled trioxanes 127a-c in order to gain a better understanding of mechanism of antimalarial activity of artemisinin and its simple 1, 2, 4-trioxane analogues at the molecular level.

Posner et al.\textsuperscript{108} (1994) reported preparation and antimalarial activity of 128a-c and 58. Compounds 128a was found to be as potent as artemisinin and nearly 100 times more potent than 128b-c \textit{in vitro} against chloroquine sensitive and resistant \textit{P. falciparum}. This study suggests the importance of H$_{4a}$ in the mechanism of action of artemisinin like trioxanes.

\[ O = ^{18}O + ^{16}O \]

127a $R = \text{SiMe}_2$-Bu-t
127b $R = \text{H}$
127c $R = \text{SO}_2$Tol.

128a $R_1 = \text{H}, R_2 = \text{Me}$
128b $R_1 = \text{H}, R_2 = \text{H}$
128c $R_1 = \text{Me}, R_2 = \text{Me}$

Posner et al.\textsuperscript{109} (1995), in continuation of study reported synthesis and antimalarial activities of 11 structurally simplified 1, 2, 4-trioxane 129. Ten compounds out of 11 showed significant \textit{in vitro} antimalarial activity against chloroquine sensitive and resistant \textit{P. falciparum}. Compound 129 where $R_1 = \text{Me}, ZR_2 = \text{OMe}, R_3 = \text{Me}$ and compound 129 where $R_1 = \text{H}, ZR_2 = \text{OMe}, R_3 = \text{Me}$ showed antimalarial activity similar to that of 8.

Posner et al.\textsuperscript{110} in the same year reported C$_4$-benzyl analogue 130a and C$_4$-(trimethylsilyl) methyl analog 130b. 130a showed in vitro antimalarial activity comparable to that of 8 against chloroquine-sensitive \textit{P. falciparum} (NF-54).
Later in 1995 Posner et al.\textsuperscript{111} reported several benzylic ether derivatives 131\textsuperscript{a-d}. \textit{In vitro} antimalarial activity of these analogues against \textit{P. falciparum} showed that compound 131\textsuperscript{b} was more active than 8. In \textit{in vivo} studies against \textit{P. berghei} in mice, compound 131\textsuperscript{b} showed significant activity.

Posner et al.\textsuperscript{112} (1996) reported trioxanes 132\textsuperscript{a-e} and 133\textsuperscript{a-f} which were designed to study structure-activity relationship and to provide possible clues for the mode of action of artemisinin like trioxanes.

Posner et al.\textsuperscript{113} in continuation to work reported a series of 3-aryl-1, 2, 4-trioxanes 134\textsuperscript{a-n}. Both \textit{in vivo} and \textit{in vitro} antimalarial evaluation of these new trioxanes showed that 12β-methoxy-3-aryltrioxanes 134\textsuperscript{g,h,k} and 1 were highly potent; 134\textsuperscript{k} showed potent activity even when administered to rodents orally.
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### 1.14.6. Spiro 1, 2, 4-trioxanes

Spiro trioxanes 135 and 136 and their analogs were prepared by photooxygenation of the corresponding allylic alcohols followed by peroxyacetalization reactions with aldehydes or ketones.

Griesbeck et al.\textsuperscript{114} (2002) reported synthesis of antimalarial 1,2,4-trioxanes via photooxygenation of chiral allylic alcohol 4-methyl-3-penten-2-ol followed by subsequent BF$_3$ catalyzed peroxyacetalization with aldehydes or ketones afforded four monocyclic and spirobicyclic 1,2,4-trioxanes, of which 135 was the most potent.

O’Neill et al.\textsuperscript{115} (2001) reported Co(II)-mediated regioselective Mukaiyama hydroperoxysilylation of 2-alkyl- or 2-aryl-prop-2-en-1-ols furnished peroxyaryl alcohols which were treated with aldehydes or ketones to provide 11 target spiro trioxanes. Trioxane 136, the best of these, was only an order of magnitude less potent than 8.
C Singh (1990) reported a new and convenient $^{1}O_{2}$-mediated synthesis of 1, 2, 4-trioxanes.\(^{116}\) The key steps of this method are (i) preparation of $\beta$-hydroxyhydroperoxides by photooxygenation of suitably substituted allylic alcohols and (ii) elaboration of these $\beta$-hydroxyhydroperoxides into 1, 2, 4-trioxanes (Scheme 15). The two sequences of reactions can be performed in one pot, avoiding workup and purification of unstable hydroperoxide intermediates. The method is safe and has been used for the preparation of trioxanes on gram scale.

\[
\begin{array}{c}
\text{Scheme 15}
\end{array}
\]

Singh et al.\(^{117}\) have prepared several \textit{in vivo} potent spiro 1, 2, 4-trioxanes of different prototypes and were the first to report antimalarial potency of synthetic 1, 2, 4-trioxanes \textit{in vivo}. In the preliminary study on 6-arylvinyl trioxanes, compounds 137-140 showed activity at 30 mg/kg by intra peritoneal (i.p.) route against chloroquine-sensitive \textit{P. berghei} in mice but these compounds were poorly active against chloroquine-resistant \textit{P. yoelii} in mice. Among 6-arylalkylvinyl trioxanes, compound 141 showed 100\% survival rate at 96 mg/kg against MDR \textit{P. yoelii} in mice by p.o and i.m. routes. Although no \textit{in vitro} data was presented for these trioxanes, the \textit{in vivo} data showed that the order of efficacy was spiroadamantane > spirocyclopentane > spirocyclohexane. Introduction of a methyl group at the carbon atom bearing the $\alpha$-arylvinyl group abolished activity. Among amino derivatives of 6-arylvinyl trioxanes, compound 142 showed 100\% survival rate at 96 mg/kg and 60\% survival rate at 48 mg/kg against MDR \textit{P. yoelii} in mice by p.o. route.

Out of geraniol derived hydroxy diene spiro trioxanes 143a and 143b, trioxane 143b ($IC_{50} = 93$ nM), with its bulky spiroadamantane, was 4.7-fold more potent than its
relatively sterically unhindered spirocyclohexyl analog 143a (IC$_{50}$ = 440 nM). 143b was administered by i.m. route at the dose of 96 mg/kg and cured (survival >10 days) all of the malaria-infected mice; unfortunately, no corresponding in vivo data is available for 143a. several 6-cycloalkylvinyl substituted 1, 2, 4-trioxanes such as 144 and 145 have been prepared using regioselective photooxygenation of cycloalkyl substituted respective allylic alcohols and found to be inactive in vivo.

![Chemical structures](image)

Singh et al.\textsuperscript{117} have prepared several highly active synthetic trioxanes 146a-d and amino functionalized trioxanes 147a-b (Table 6). As can be seen from table 6, 146a, 146b, 146c showed 100% survival at 24 mg/kg, 12 mg/kg and 48 mg/kg respectively by oral route. Water soluble trioxanes 146d is active by both oral and i.m. route at 72 mg/kg. Among amino derivatives compound 147a showed 100% survival rate at 96mg/kg and 60% survival rate at 48 mg/kg by oral route against MDR \textit{P. yoelii} in mice by p.o. route (Table 6).
Table 6. In vivo activity against MDR P. yoelii in Swiss mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>Route</th>
<th>% Supression on day 4</th>
<th>Mice alive on day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>146a</td>
<td>24 p.o.</td>
<td>100</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 p.o.</td>
<td>100</td>
<td>9/11</td>
<td></td>
</tr>
<tr>
<td>146b</td>
<td>12 p.o.</td>
<td>100</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 p.o.</td>
<td>100</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96 i.m.</td>
<td>100</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>146c</td>
<td>48 p.o.</td>
<td>100</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 p.o.</td>
<td>100</td>
<td>8/10</td>
<td></td>
</tr>
<tr>
<td>146d</td>
<td>72 p.o.</td>
<td>100</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 p.o.</td>
<td>100</td>
<td>2/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72 i.m.</td>
<td>100</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 i.m.</td>
<td>100</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>147a</td>
<td>96 p.o.</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 p.o.</td>
<td>100</td>
<td>3/5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>48 i.m.</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 i.m.</td>
<td>100</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>48 p.o.</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 p.o.</td>
<td>100</td>
<td>1/5</td>
<td></td>
</tr>
</tbody>
</table>

*percent suppression = [(C-T)/C] x 100; where C=parasitaemia in control group, and T=parasitaemia in treated group.

Meunier et al.\textsuperscript{118} have synthesized several trioxane-quinoline 'hybrids' (trioxaquines), some of which have shown promising activity profile in vitro and in vivo. Ascaridole-derived, trioxaquine 148 was the best compound of the series. It exhibited \(ED_{50}\) values of 5 mg/kg/day and 18 mg/kg/day by i.p. and p.o. routes respectively against \(P. vinckei\) in mice. This compound completely cleared parasitaemia in \(P. vinckei\) infected mice, without recrudescence, at an i.p. dose of 20 mg/kg/day.
1.14.7. Steroid-1, 2, 4-trioxane Hybrid

In trioxane 149, Rong and Wu\textsuperscript{119} melded most of the structural elements of 8 in a cholestane-type steroid-trioxane hybrid structure. Compound 149 has been prepared in five steps from methyl 3-oxocholest-4-en-6β-y1 acetate using photooxygenation reaction as key step. Both 149 and its diastereomer were more effective than 8 \textit{in vivo}.\textsuperscript{120}

1.14.8. 1, 2, 4, 5-tetraoxanes

Symmetrical meso dispiro 1, 2, 4, 5-tetraoxane 150a, readily obtained by reaction of 2-methylcyclohexanone with acidified hydrogen peroxide, was found to be only 6-fold less active than 1. Tetraoxane 150a is synergistic with chloroquine, quinine, mefloquine, and artemisinin against \textit{P. falciparum}.\textsuperscript{120}

Sixteen alkyl substituted dispiro tetraoxane analogs of 150a were synthesized and found to be inactive or weakly active because of steric effects preventing or hindering
peroxide bond access to parasite heme. For these tetraoxanes, there was no apparent relationship between tetraoxane structure and \textit{in vitro} neurotoxicity, nor was there any correlation between antimalarial activity and neurotoxicity. Dispiro tetraoxanes 150b and 150c bearing unsaturated and polar functional groups were prepared to improve antimalarial activity of prototype tetraoxane 150a by oral route.\textsuperscript{120} But both 150b and 150c were found to be inactive. However, the more lipophilic ethyl ester of 150c (IC\textsubscript{50}-6.4 nM) and methyl ether of 150b (IC\textsubscript{50}-15 nM) showed significant \textit{in vitro} antimalarial potency. These tetraoxanes possessed less activity \textit{in vivo}.

Mixed tetraoxanes possessing spirocycloalkane and spirocholic acid-derived steroid substructures were prepared 151 and found to be 6-fold more potent than 8. Mixed tetraoxanes with a spirocyclohexane were more potent than the corresponding spirocyclopentane and spirocyclooctane analogs.\textsuperscript{121}

Several diester and diamide cholic acid-derived tetraoxanes were synthesized, best one of these, cis diamide tetraoxane 152, was only 4-fold less potent than 8.\textsuperscript{122} Cholestane-type steroid–tetraoxane hybrid 153 was found to be less active than 8.\textsuperscript{123}

1.14.9. 1, 2, 4-tetraoxepanes, 1, 2, 4, 5-tetraoxocanes & 1, 2, 5, 6-tetraoxonanes.

Tricyclic 1, 2, 4, 5-tetraoxepane 154 and 1, 2, 5, 6-tetraoxonane 155 were 35- to 40-fold less potent than 8, but 155 had notably better \textit{in vivo} activity (i.p.). Both 154 and 155 however, were completely inactive when they were administered orally.\textsuperscript{124} 1, 2, 4, 5-tetraoxocanes 156a and 156b exhibited excellent \textit{in vitro} potency, however, both were less effective than 8 \textit{in vivo}.\textsuperscript{125}
1.14.10. 1, 2, 4-trioxolanes (Ozonides)

De Almeida Barbosa et al.\textsuperscript{26} firstly reported antimalarial activity of a new series of tricyclic trioxolanes (8, 9, 10, 11-tetraoxatricyclo [5.2.1.1\textsuperscript{2}·6\textsuperscript{1}] undecan-4-ones) which were synthesized from various 8-oxabicyclo [3.2.1] oct-6-en-3-ones by ozonolysis. Trioxolane 157, was prepared in five steps from 3-(2-furyl) propan-1-ol in a sequence of hydroxy group protection, cycloaddition, deprotection, methoxylation, and ozonolysis. With their low potencies ranging from 7,300 to 90,000 nM, these tricyclic trioxolanes were found to be inactive. 157, the best of these, had an IC\textsubscript{50} of 7,300 nM which was three order of magnitude less potent than 8.\textsuperscript{26}

\begin{center}
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{157_158.png}
\end{figure}
\end{center}

Research efforts made by Vennerstrom et al.\textsuperscript{27} led to the discovery of a novel antimalarial 1, 2, 4-trioxolane 158, of which 158c displayed \textit{in vitro} IC\textsubscript{50} values of 0.39 ng/mL and 0.42 ng/mL versus chloroquine-resistant \textit{P. falciparum} K1 and chloroquine-sensitive NF-54 strains, and was found to be more active than 13, 15, chloroquine, and mefloquine \textit{in vivo} after a single 3 mg/kg dose administration against \textit{P. berghei} infected mice. Now it is under clinical trials.

1.15. Other Biological activities in artemisinin

Apart from antimalarial activity, several other biological activities such as anti-infective,\textsuperscript{28} antifungal,\textsuperscript{29} antiproliferative,\textsuperscript{30} antiinflammatory,\textsuperscript{30} anticancer,\textsuperscript{30, 31}
antiarrhythmic\textsuperscript{132} activities have been reported in artemisinin derivatives. Recently, Posner et al. have shown anticancer activity of artemisinin-derived trioxanes.\textsuperscript{131}

\section*{1.16. Discussion}

One artemisinin derivative, artemisone \textit{59}, have been found as lead candidate for clinical studies.\textsuperscript{133} By use of the ADME, the application of \textit{Lipinski's Rule of Five,}\textsuperscript{78} and incorporating suitable polar residues and their isosteres, Haynes \textit{et al.}\textsuperscript{133} \textit{(2006)} have succeeded in preparing artemisone \textit{59} that have considerably reduced neurotoxicity and have much improved properties over the first-generation analogues and represents the success of the ADME approach to drug design.

None of the synthetic peroxides identified so far has an antimalarial profile superior to that of arteether \textit{14}, the best semisynthetic artemisinin, although available data indicates that 1,2,4-trioxanes \textit{59a} and \textit{112} (Fenozan B07) are only marginally less effective than \textit{14}. Within a given peroxide chemical family, the more lipophilic members are more potent and possess better oral antimalarial activity in animal models than their more polar counterparts. This poses a challenge to identify peroxide structures with suitable "drug-like" physicochemical properties.\textsuperscript{78} Synthesis complexity, source of peroxide oxygen atoms (hydrogen peroxide, singlet oxygen, ozone), reduced stability of unsaturated versus saturated peroxide heterocycles, and stereochemistry, are other
important chemical parameters that must be considered in synthetic peroxide design and development. Another necessary objective is to identify synthetic peroxides with good biopharmaceutical (ADME) properties.\textsuperscript{79}

1.17. Conclusion

The efforts to eradicate malaria have failed due to emergence of parasites resistant to conventional drugs, imposing a lot of pressure on public health systems to introduce new treatments. Using the effective drug against the particular parasite strain in the affected area can solve this problem. In some areas combination therapy may be helpful where resistance against a single drug has been reported. Artemisinin and its derivatives are effective antimalarials against which no resistance is reported in clinical cases. Further some of the synthetic 1, 2, 4-trioxanes have shown better activity and likely to be drugs of the future for the chemotherapy of malaria. For malaria researchers, sequencing of genome of \textit{P. falciparum} has provided an unprecedented opportunity. The analysis of the genome sequence should provide valuable information to identify promising new leads for vaccine development. A number of new potential target pathways have already been identified and efforts to develop lead compounds for these putative targets hopefully will allow treatment of malaria infections in a uniform sustained way.

To conclude, several new lead compounds have been discovered in the past 35 years, which offers exciting opportunities for developing novel, efficacious and probably more safer antimalarial drugs.

1.18. Summary

Many semisynthetic artemisinins and synthetic peroxides have been prepared; a fair number are quite potent in vitro, but most suffer from low oral activity. Some
compounds are found to be good prototypes, and for others, additional chemistry and SAR will be required. To identify orally active synthetic antimalarial peroxides with good pharmacokinetic profiles, it will, at least, necessitate p.o. activity data from relatively simple in vivo experiments, and where possible, additional ADME data.\textsuperscript{134} To identify orally active peroxide structures with suitable developability characteristics, several efforts have been made by scientist all over the world. Neurotoxicity, a potential liability of the artemisinins,\textsuperscript{77} and possibly all synthetic antimalarial peroxides,\textsuperscript{135} is another issue that must be taken into consideration in the journey of drug discovery and development. Despite all of these challenges, substantial progress had been made in the identification of a new generation artemisinin analogues and synthetic antimalarial peroxides. As new peroxide-compatible synthetic methods are identified or developed, and as the mode of action of artemisinin is now more fully understood, the synthesis of more potent antimalarial peroxides with superior pharmaceutical properties should be realized.

1.19 References

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Historical development & current scenario of Artemisinin

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