Chapter 1

Role of Peroxides in Malaria Chemotherapy: A Voyage from Artemisinin to Synthetic Peroxides
1.1 Introduction

Since ancient times, humankind has had to struggle against the persistent onslaught of pathogenic microorganisms. Malaria is still one of the world's most deadly diseases that threatens nearly 40% of the world's population putting 3.2 billion people at risk in 107 countries and infects approximately 300 to 500 million people annually worldwide mainly in tropical and subtropical areas.\(^1\) It is estimated that there are between 1 million to 3 million deaths every year due to malaria. In Africa alone, more than 1 million people die because of malaria and most of them are children under 5 years of age.\(^2\) The economic toll of malaria is tremendous. It has been estimated that the African continent has forgone almost $100 billion in lost GDP over the last 35 years due to malaria alone.\(^3\) 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.\(^4\)

This review chapter includes a short description of the malaria disease, its cause, a short address to the history of antimalarial drug development and a focus on the development of peroxides that can be used for malaria chemotherapy together with a brief description about their mode of action.

1.2 Life Cycle of Malarial Parasite

Malaria is caused by a protozoal parasite of genus *Plasmodium*, and out of 100s of species so far known only four is found to be infective to human beings which include *P. falciparum* (causes tropical malaria), *P. vivax*, *P. ovale* (both causes tertian malaria), and *P. malariae* (causes quartan malaria). *P. falciparum* and *P. vivax* account for 95% of all malaria infections. Nearly all severe and fatal cases are caused by *P. falciparum* which is the most widely spread geographically, out of the four known species, and the most pernicious one, causing the majority of the malaria related morbidity and mortality, while *P. vivax* and *P. ovale* causes true relapsing malaria. Malaria is found chiefly in tropical regions that includes sub-Saharan Africa, Southeast Asia, Pacific Islands, India, and Central & South America. *P. falciparum* is found throughout tropical Africa, Asia, and Latin America. It is the predominant species in most areas. *P. vivax* is more common in India and South America, but is also found worldwide in tropical and some temperate zones. *P. ovale* is mainly confined to tropical West Africa, while the occurrence of *P. malariae* is worldwide, although its distribution is patchy.\(^5\)
The infectious stages of the malaria parasite reside in the salivary glands of female *Anopheles* mosquitoes that bite humans for a blood meal. During blood extraction, the mosquito injects its saliva into the wound, thereby transferring approximately 15-20 so-called sporozoites into the blood stream. In a matter of minutes, these sporozoites are able to conceal themselves from the host’s immune system by entering into the liver cells. Each sporozoite develops into a tissue schizont, containing 10000-30000 merozoites. The schizont ruptures after one to two weeks and releases the merozoites into the blood stream, starting the erythrocytic phase of the parasite’s life cycle. In the cases of *P. vivax* and *P. ovale*, some sporozoites turn into hypnozoites, a form that can remain dormant in the liver cells, causing relapses months or even years after the initial infection. *P. falciparum* and *P. malariae* lack this liver persistent phase, but *P. malariae* can persist in the blood for many years if inadequately treated. Merozoites released into the bloodstream hide again from the host’s immune system by invading erythrocytes. In the erythrocyte, the parasite develops from a ring stage via a trophozoite stage into a blood schizont. After a time characteristic for each specific *Plasmodium* species, the erythrocyte ruptures and releases 16-32 new merozoites into the blood stream which in turn again invade erythrocytes, thereby starting a new erythrocytic cycle. This asexual life cycle, from invasion of the erythrocytes until the schizont ruptures, spans 48 h for *P. falciparum, P. vivax*, and *P. ovale*, and 72 h for *P. malariae*. After a number of asexual life cycles, some merozoites develop into sexual forms, the gametocytes, which are transferred to a mosquito during another blood meal. These gametocytes undergo sexual reproduction within the mosquito mid-gut producing thousands of infective sporozoites, which migrate to the salivary gland where they are ready for a new infection. With the rupture of the erythrocyte, the parasite’s waste and cell debris is released into the blood stream, causing some of the clinical symptoms of malaria. The main symptom is fever, but rarely in the classical tertian (every 48 h) or quartan (every 72 h) patterns. Further symptoms include chill, headache, abdominal and back pain, nausea, and sometimes vomiting. Thus, the early stages of malaria often resemble the onset of an influenza infection. *P. vivax, P. ovale*, and *P. malariae* show distinct selectivity towards the age of the infected erythrocytes. For that reason, the degree of total parasitaemia is limited. In contrast, *P. falciparum* infects erythrocytes of all ages, leading to high parasitaemia. Although the symptoms of *P. vivax, P. ovale*, and *P. malariae* infections can be severe in non-immune persons, these parasites seldom cause fatal disease. Nevertheless, chronic infection with *P. malariae* can result in an (eventually fatal) nephrotic syndrome. Malaria caused by these three parasites is often called benign malaria. In contrast, *P. falciparum* malaria (also known as tropical malaria) can progress within a few
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days from uncomplicated to severe malaria with a fatal outcome in 10–40% of all cases of severe malaria, depending on the time lag between the onset of the symptoms and effective treatment, as well as on the hospital facilities for the management of complications. Observed complications can include coma (cerebral malaria), respiratory distress, renal failure, hypoglycemia, circulatory collapse, acidosis, and coagulation failure.

1.3 Classification of Antimalarial Drugs

Traditionally, antimalarial agents are classified by the stages of the malaria life cycle that are targeted by the drug.

Blood schizonticides: They act on the asexual intraerythrocytic stages of the parasites; there by terminate clinical attacks of malaria. The drugs belonging to this class include quinine 1, chloroquine 2, mefloquine 3, halofantrine 4, pyrimethamine 5, sulfadoxine 6, sulfones and tetracycline derivatives.
Tissue schizonticides: These kill hepatic schizonts, and thus prevent the invasion of erythrocytes, acting in a causally prophylactic manner. Primaquine and pyrimethamine (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.

Hypnozoiticides: They kill the persistent intrahepatic stages of \textit{P. vivax} and \textit{P. ovale}, thus preventing relapses from these dormant stages. Primaquine 7 is the only prototype drug available for this stage.

Gametocytocides: They destroy the intraerythrocytic sexual forms of the parasites and prevent transmission from human to mosquito. As there are no dormant liver stages in \textit{P. falciparum} malaria (tropical malaria), blood schizonticidal drugs are sufficient to cure the infection. In cases of \textit{P. vivax} and \textit{P. ovale}, a combination of blood schizonticides and tissue schizonticides is required. Chloroquine and quinine have gametocytocidal activity against \textit{P. vivax} and \textit{P. malariae}, but not against \textit{P. falciparum}. However, primaquine has gametocytocidal activity against all human malarial parasite species including that against \textit{P. falciparum}.

1.4 Drug Resistance in Malaria Chemotherapy
Considerable success in gaining control over malaria was achieved in the 1950s and 60s through landscaping measures, vector control with the insecticide DDT, and the widespread administration of chloroquine, the most important and cheapest antimalarial agent so far discovered. In the late 1960s, the final victory over malaria was believed to be within reach,
however, the parasites could not be eradicated because they developed resistance against the most widely used and affordable drugs of that time. Since then the cases of malaria infections were on the rise and has now reached up in record numbers. With due course of time the parasite developed resistance against most of the conventionally used drugs due to their indiscriminate use, incomplete dose regimen, lack of proper antimalarial campaigns. One of the major factors for rise in malaria cases was the development of resistant varieties of vectors against commonly used insecticides. There are numerous reports of resistance of malaria parasite against chloroquine and sulfadoxine-pyrimethamine. This growing emergence of drug-resistance against chloroquine, the cheapest drug so far discovered then led to the use of several other relatively costlier drugs both as single (mono therapy) and in combinations (combination therapy) which include primaquine, mefloquine, halofantrine and lumifantrine but the reports of development of resistance against them as well in several areas enforced to develop new fast acting drugs which are different both in terms of pharmacophore and mode of action.

1.5 Artemisinin: A Lead in Malaria Chemotherapy

Extracts of the herb known as sweet wormwood have been used in China for the treatment of fever for as long as 2000 years. Its earliest mention occurs in the *Recipes for 52 Kinds of Diseases* found in the Mawangdui Han dynasty tomb dating from 168 B.C. In that work, the herb is recommended for use in hemorrhoids. This plant is mentioned further in the *Zhou Hou Bei Ji Fang* (Handbook of Prescriptions for Emergency Treatments) written in 340 A.D. Li Shizhen, the famous herbalist, wrote in his *Ben Cao Gang Mu* (Compendium of Materia Medica) of 1596, that chills and fever of malaria can be combated by *qing hao* (*Artemisia annua* L., sweet wormwood) preparations. It was actually in 1971 that Chinese chemists were able to isolate the substance responsible for its reputed medicinal action from the leafy portions of the plant. This compound, which they called *qinghaosu* (QHS, artemisinin), is a sesquiterpene lactone that bears a peroxide grouping and, unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. Artemisinin is very effective and safe against chloroquine (CQ) sensitive and chloroquine (CQ) resistant strains of *P. falciparum* but has certain limitations like poor oil and water solubility, and high rate of recrudescence. The limited availability of artemisinin and that too from natural source was another lagging factor that led to the development of various synthetic methodologies for the synthesis of artemisinin but none of
them was commercially viable. Hence a lot of efforts have been put only to develop semi synthetic derivatives of artemisinin.

![Artemisinin](image)

### 1.6 Identification of the Pharmacophore

Structure activity relationship studies of artemisinin and its deoxy derivative have revealed that it is actually the endoperoxide linkage of artemisinin in the form of 1,2,4-trioxane is responsible for its activity. Deoxyartemisinin a major metabolite was isolated in the urine of the patients treated with artemisinin which was later obtained by total synthesis. Deoxyartemisinin was found 300 times less active in comparison to artemisinin when tested in *P. falciparum* (D-6 Sierra Leone Clone). The role of peroxide linkage in antimalarial activity was also confirmed by certain other deoxy derivatives, deoxy dihydroartemisinin, deoxyarteether and deoxydeoxygenartemisinin.

![Chemical structures](image)

### 1.7 Semisynthetic Derivatives of Artemisinin as Potent Antimalarials

#### 1.7.1 First generation artemisinin derivatives, their scope and limitations

Artemisinin has been used in China for the treatment of malaria, but poor oil and water solubility as well as poor absorption via gastrointestinal tract were its limiting factor. A lot of attention has been put so far to synthesize better analogs of artemisinin that can have enhanced bioavailability. To overcome this problem Chinese workers made several derivatives of artemisinin and assessed them for their antimalarial efficacy. They reduced the lactone moiety of parent molecule to hemiacetal to synthesize dihydroartemisinin. This compound was although having better oil and water solubility, but suffered the problem of neurotoxicity and
relative instability under acidic conditions. In order to reduce its toxicity and increase its stability it was converted into its corresponding ethyl and methyl ether derivatives arteether 15 and artemether 16 respectively, the first generation analogues of artemisinin. Both of these compounds were found several times more active both in vitro and in vivo against multi-drug resistant malaria in comparison to artemisinin and are at present the drugs of choice for treatment of complicated malaria. Arteether is chiefly used in India and in Netherlands (Artemotil, Emal) but the more prevalent substance is artemether (Paluther, Artenam, Artemos). Currently, the application of artemether 16 with lumefantrine (8) (Coartem or Riamet) is the only artemisinin-based combination therapy available manufactured under Good Manufacturing Practice (GMP) standards. In addition, a formulation for small children (Pediatric Coartem) is in clinical development. Although expensive and for most malaria patients unaffordable, this combination is generally thought to be effective and well tolerated. Another modification of dihydroartemisinin which was developed by Chinese workers was artesunic acid 17 which is another first generation artemisinin derivative, in which the hemiacetal OH group is acetylated with succinic acid. Artesunate 18, its sodium salt is an unstable drug as the succinic ester linkage gets rapidly cleaved, releasing dihydroartemisinin as the active agent. Because of the free carboxylate, artesunate is a water-soluble drug that can be administered via iv route. This is of particular importance for the treatment of severe malaria tropica in which the condition of the patients prohibits any other route of administration. A study on 80 children with
complicated malaria conducted in India showed the superiority of artesunate over quinine.\textsuperscript{28} In a recent study conducted in various regions of Asia, intravenous artesunate was significantly superior to the standard iv regime with quinine in the treatment of adult severe malaria.\textsuperscript{29} Currently available artesunate preparations for parenteral application originate from China or Vietnam and are unable to meet western quality standards. Phase II and III studies were to commence in 2006 in a joint project by the University of Tubingen in Germany (P. G. Kremsner), an industrial partner, and the Walter Reed Army Institute of Research, with the aim of bringing an intravenous artesunate preparation to the market by 2009, to be manufactured according to western drug regulations.\textsuperscript{30} In addition to iv application, artesunate can also be administered via the im, rectal, or oral routes. In a recent study of severe malaria in children, rectally administered artesunate\textsuperscript{18} was at least as effective as im applied artemether\textsuperscript{16} and thus may be useful in settings in which parenteral therapy cannot be given.\textsuperscript{31} Artesunate is the main artemisinin combination partner in artemisinin-based combination therapy (ACT), which is now used as the standard therapy in many countries. Combinations with numerous antimalarials are used, most of which are questionable because of unmatched pharmacokinetic profiles or widespread resistance against the non-artemisinin component of the combination. In particular, the combination of artesunate\textsuperscript{18} with mefloquine\textsuperscript{3} is widely used in Asia.\textsuperscript{32-34}

The utility of sodium artesunate, however, is impaired by its poor stability in aqueous solution due to the facile hydrolysis of the ester linkage and short plasma half-life (20-30 min).\textsuperscript{35} Lin \textit{et al} have reported a new series of water-soluble derivatives in which the solubilizing group, carboxylate, is on a moiety that is joined to dihydroartemisinin by ether, rather than an ester, linkage. One of these derivatives, artelinic acid\textsuperscript{19}, is not only considerably more stable than artesunic acid in weakly alkaline solution but is also more active against \textit{P. berghei} in mice. Its sodium salt, sodium artelinate\textsuperscript{20} possesses comparable antimalarial activity both \textit{in vivo} as well as \textit{in vitro} to artemether or arteether. Sodium artelinate was not only found stable in aqueous solution but also has a much longer plasma half-life (1.5-3 h).\textsuperscript{36} Because of its encouraging chemical and biological properties, sodium artelinate was subjected to preclinical testing. In an animal model, intravenous sodium artelinate was shown to be superior to artesunate.\textsuperscript{37} However further development of sodium artelinate has been discontinued in favor of sodium artesunate\textsuperscript{22} because of the higher neurotoxicity of sodium artelinate.\textsuperscript{38,39}
1.7.2 Second generation artemisinin derivatives, a need for better antimalarials

Neurotoxicity\textsuperscript{40} was the major concern with all the first generation artemisinin derivatives owing to their short plasma half-life and biotransformation to neurotoxic dihydroartemisinin \textsuperscript{14}, hence lot of work has been made for the development of second generation artemisinins that can have reduced toxicity and increased bioavailability. Methyl and ethyl residues of the first-generation semisynthetic artemisinins, artemether \textsuperscript{16} and arteether \textsuperscript{15} have been replaced by numerous other residues. Most variations have been carried out at position 10, where the exocyclic oxygen atom is replaced by carbon substituents to remove the metabolically sensitive acetal substructure. Alkyl, aryl, heteroalkyl and heteroaryl residues have been placed at this position. Some substituents have even been used for the formation of dimers that carry two dihydroartemisinin substructures.

1.7.2.1 Artemisinin based monomers

C-10 acetal analogues of artemisinin

Lin et al.\textsuperscript{41} (1987) carried out structure activity relationship various water soluble derivatives of DHA \textsuperscript{21a-c}) by joining various alkyl groups containing free carboxylate group via ether linkage rather than ester linkage as in case of artesunic acid \textsuperscript{19} to insure better stability in aqueous solution.

\textbf{21a: }\text{C=1} \quad \textbf{21b: }\text{C=2} \quad \textbf{21c: }\text{C=3}

\begin{align*}
22a: R &= (R)-\text{CH}_2\text{CH}(_3)\text{COOH} \\
22b: R &= (S)-\text{CH}_2\text{CH}(_3)\text{COOH} \\
22c: R &= (S)-\text{CH}(_3)\text{CH}_2\text{COOH} \\
22d: R &= (R)-\text{CH}(_3)\text{CH}_2\text{COOH} \\
23a: R &= (R)-\text{CH}_2\text{CH}(_3)\text{COOMe} \\
23b: R &= (S)-\text{CH}_2\text{CH}(_3)\text{COOMe} \\
23c: R &= (S)-\text{CH}(_3)\text{CH}_2\text{COOMe} \\
23d: R &= (R)-\text{CH}(_3)\text{CH}_2\text{COOMe} \\
23e: R &= \text{CH}_2
\end{align*}

Lin et al.\textsuperscript{42} (1989) have synthesized various optically active ether derivatives of artemisinin in order to search for new hydrolytically stable and less toxic analogs. He made both water soluble \textbf{22a-d} and oil soluble derivatives \textbf{23a-e}, out of which compound \textbf{23a-d} showed very promising \textit{in vitro} antimalarial activity against \textit{P. falciparum} both in Sierra Leone ($IC_{50} = 0.44$ to 2.15 ng/mL)
and Indochina strains ($IC_{50} = 0.015$ to $0.480$ ng/mL). Compound 23c also showed very good in vivo activity against *P. berghei* in mice.

Lin *et al.* (1992) in search for more and more hydrophilic derivatives of dihydroartemisinin prepared various sugar analogs 24a-d, together with a trimethylsilylated analog 25 which showed much better activity compared to artemisinin.

![](image)

24a: D-glucose  
24b: D-galactose  
24c: 5,6-isopropylidene-D-glucose  
24d: D-cellobiose

Venugopalan *et al.* (1995) prepared several ether derivatives in a search for compounds having better therapeutic index, good solubility and bioavailability. Compound 26, 27 and 28 were found most active derivatives of the series when tested against multidrug resistant *P. yoelii nigeriensis*.

![](image)

26: $R=\text{OCH(CH}_3\text{)C==CH}$  
27: $R=\text{OC(CH}_3\text{)_2C==CH}$  
28: $R=(\text{4-OCH}_3\text{C==CHC}_6\text{H}_3\text{-O})$  
29a: $(R)$ $R^1=\text{CH}_2\text{COOCH}_2\text{CH}_3$  
29b: $(S)$ $R^1=-\text{Me}$  
29c: $(R)$ $R^1=\text{CH}_2\text{COOCH}_2\text{CH}_3$  
29d: $(S)$ $R^1=-\text{CH}_2\text{COOCH}_2\text{CH}_3$

Lin *et al.* (1995) showed that α-alkylbenzylic ethers of dihydroartemisinin 29a-d have much better activity *in vitro* in comparison to artemether, arteether and artesunate against two clones of
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human malaria, *P. falciparum* D-6 (Sierra Leone I clone) and W-2 (Indochina clone). In this study he demonstrated the role of steric factors and lipophilicity in antimalarial activity.

![Chemical structures](image)

30a: R=OH
30b: R=NH₂
30c: R=NC
30d: R=N
30e: R=O

P. M. O'Neill *et al.* (1996), synthesized several mechanism based benzamino 30a-e and alkylamino 31a-b ethers of artemisinin by taking account of this fact, that the food vacuole has a slightly acidic pH, so the introduction of a basic alkyl chain would assist in accumulation of drug inside the parasite.

Lin *et al.* (1997) synthesized several analogs of DHA 32a-e that showed higher efficacy and longer half-life than artelinic acid. Compound 32d was the most active compound of the series.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>R₁</em></th>
<th><em>R₂</em></th>
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<tbody>
<tr>
<td>32a</td>
<td>S(β)</td>
<td>Cl</td>
</tr>
<tr>
<td>32b</td>
<td>R(β)</td>
<td>Cl</td>
</tr>
<tr>
<td>32c</td>
<td>R(α)</td>
<td>Cl</td>
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<tr>
<td>32d</td>
<td>R(β)</td>
<td>F</td>
</tr>
<tr>
<td>32e</td>
<td>S(β)</td>
<td>F</td>
</tr>
</tbody>
</table>

P. M. O’Neill *et al.* (2001) have synthesized C-10 phenoxy derivatives of artemisinin 33a-g and 34a-g in both α and β series respectively. The C-10-phenoxy derivatives were tested *in vitro* against the K-1 chloroquine-resistant strain of *P. falciparum*. The phenoxy derivatives were also tested against the chloroquine sensitive HB3 strain. The most potent β-isomers, the phenyl 33a,
and 4-fluorophenyl 33c were also tested *in vivo* against *P. berghei* and were found more active than arteether.

Delhaes *et al.*49 (2000) reported a new series of dihydroartemisinin derivatives 35a-c containing a ferrocene nucleus. These compounds showed *in vitro* activity comparable to that of artemisinin against *P. falciparum*.

Haynes *et al.*50 (2002) reported various C-10 ether and ester derivatives of DHA. They also first of all reported a convenient synthesis of β-artesunate 36 via base catalyzed esterification. Novel esters derivatives 37a-e were prepared using Mitsunobu and Schmidt reaction procedures. Coupling reaction using DCC or normal acylation conditions were also reported for the synthesis of various esters. Synthesis of various lipophilic ethers 38a-e was reported using either BF₃·Et₂O or TMSOTf as acid catalyst, out of which steroidal ester 39 not only showed good antimalarial activity but also very good antiparasitic activity. Mitsunobu and Schmidt reactions were also utilized for the synthesis of ethers as well.

Singh *et al.*51 have also reported the synthesis of hydrolytically stable derivatives of artemisinin 40a-d and 41 by the incorporation of various alkyl chains. Among these compounds 40a-d and 41 were found to have activity comparable to β-arteether. Hemisuccinates 42a-d showed activity comparable to that of artemisinic acid.
Several workers synthesized various nitrogen containing ethers of DHA that have shown potential antimalarial activity.\(^{48}\)

Liu et al.\(^{52}\) synthesized carbamate derivative \(43\) and assessed its cytotoxicity.

Singh et al.\(^{53}\) (2006) recently reported synthesis and \textit{in vivo} antimalarial assessment of highly lipophilic ether derivatives \(44a-e\) of dihydroartemisinin. He showed that in contrary to arteether where beta isomer is more active \(\alpha\) isomers were far more active than \(\beta\) isomers. They also synthesized various ester derivatives of DHA, \(45a-f\). Several of these derivatives showed better activity profile in comparison to \(\beta\)-arteether.
C-10 carba analogues of artemisinin

Several analogs of artemisinin have been prepared by the replacement of oxygen at C-10 with carbon substituted alkyl or aryl residues. These deoxoartemisinin analogs were designed to be more chemically robust towards acidic hydrolysis due to lack of C-10 acetal
functionality together with the fact that these compounds on oxidative dealkylation would not lead to neurotoxic dihydroartemisinin.

Several approaches have been made in this regard; Jung et al.\(^5\) (1990) first time reported the synthesis and antimalarial activity of 10β-n-butyldeoxoartemisinin 47. It was found to have \textit{in vivo} antimalarial activity comparable to that of artemisinin. Haynes \textit{et al.}\(^5\) (1992) also reported the synthesis of 12α and 12β alkyldideoxoaertemisins using artemisinic acid as starting material. Ziffer \textit{et al.}\(^5\) (1995) have reported synthesis of various 10β-alkyldeoxoartemisinin 48a-c. 10β-allyldideoxoaertemisinin 48a was converted into several promising derivatives, of which 10β-n-propyldeoxoartemisinin 48b was having \textit{in vitro} antimalarial activity approx. equal to that of arteether against W-2 and D-6 clones of \textit{P. falciparum}. Ma \textit{et al.}\(^5\) (2000) utilized 10β-allyldideoxoaertemisinin for the synthesis of various potent carba analogs 49a-c. Several of these compounds showed better activity than artemisinin.

Jung \textit{et al.}\(^5\) (1998) also reported the synthesis and stability of various water soluble carba analogs 54a-c of artemisinin.

Posner and coworkers.\(^6\) (1998) reported various aromatic analogs of 10-deoxoartemisinin. Posner and coworkers \(^6\) (1999) also reported the chemo selective synthesis of 10-deoxoartemisinin analogues 55a-c which showed good \textit{in vitro} antimalarial activity. He also reported the synthesis and antimalarial assessment of several orally active derivatives of artemisinin family in this report.

\textit{P. M. O’Neill \textit{et al.}\(^5\)} (1999) reported the synthesis of carba analogs of first generation 1,2,4-trioxane arteether 56a-e and 57a-e which showed potent antimalarial activity.

\textit{Hindley \textit{et al.}\(^6\)} (2002) reported the synthesis of carba amino derivatives of artemisinin 58a-e out of which compound 58b showed \textit{ED}_{90} less than 10 mg/kg against \textit{P. yoelii} in mice.
Wang et al.\textsuperscript{67} reported the synthesis of 2-hydroxy-naphthyl carba analogs of artemisinin. The C-10 naphthyl substituted derivative 59a and 59b exhibited antimalarial activities similar to that of artemisinin \textit{in vivo}. 

\begin{center}
\includegraphics[width=\textwidth]{diagram.png}
\end{center}
Jung et al.\textsuperscript{68} (2002) reported water soluble, hydrolytically stable (+) deoxoartelinic acid 61 from 60 and assessed its antimalarial activity.

Avery et al.\textsuperscript{69} reported the synthesis and antimalarial activity of novel substituted deoxoartemisinins 62a-e.

Chorki et al.\textsuperscript{70} (2002) for the first time reported synthesis of C-10α trifluoromethyl deoxoartemisinins 63a and 63b.

Haynes et al.\textsuperscript{71} reported stereo selective preparation of 10α and 10β aryl derivatives of artemisinin of prototypes like 64a-e and 65.

Bonnet-Delphon and co-workers\textsuperscript{72} (2004) tried to increase the metabolic and chemical stability of arteether and DHA by the incorporation of C-10 CF\textsubscript{3} group, thereby, making CF\textsubscript{3} analogues of arteether 66a-e 45 times more stable than arteether itself under “simulated stomach acid conditions”.

Liu et al.\textsuperscript{73} reported synthesis and cytotoxicity of various carba analogs 67a-c, 68a-c and 69a-c.
C-10 aza analogues of artemisinin

Lin et al.\textsuperscript{74} first time reported the synthesis and antimalarial activity of C-10 aza analogs of artemisinin 70a-e. These compounds showed very good \textit{in vitro} antimalarial activity but poor \textit{in vivo} antimalarial activity.

Yang et al.\textsuperscript{75} then reported synthesis and antimalarial assessment of aniline substituted aza analogs of artemisinin 71a-j.

Haynes et al.\textsuperscript{76} (2005) carried out detailed structure activity relationship of C-10 aza analogs of artemisinin 72a-e, 73a-f and 74a-e. Out of these compound 73f (artemisone) was chosen for clinical trials on account of its better pharmacokinetic and activity profile.
C-10 thio analogues of artemisinin

Venogopalan et al.44 (1995) have synthesized several C-10 thioether analogs of prototype 75 of artemisinin by treating DHA with various thiols in the presence of BF$_3$.Et$_2$O to furnish $\alpha$ and $\beta$ isomers which were separated. These thioethers were found active both in $P.$ berghei (K-173) infected mice and in $P.$ yoelii nigeriensis (NS) infected mice via subcutaneous and oral route.

Azaartemisinins

Avery et al.77 (1995) gave a synthetic methodology for the synthesis of 11-aza-9-desmethylartemisinins 76a-f and assessed their antimalarial activity. Torok et al.78 (1995) developed semisynthetic method for preparation $N$-alkyl-11-azaartemisinins 77a-f and screened them for antimalarial activity. One of the compounds in them showed much better activity than artemisinin in vivo. Mekonnen et al.79 have also synthesized several analogs of artemisinin of prototype 78. Haynes et al.80 (2007) carried out detailed thermal stability and in vitro efficacy study of various $N$-sulfonyl derivatives of 11-azaartemisinin of prototype 79.
1.7.2.2 Artemisinin based dimers

The first report of artemisinin based dimer and its antimalarial activity comes from Chinese group, who isolated the compound 80 as self dimer of dihydroartemisinin formed during the course of acetal formation reaction under acidic conditions. This compound has been mentioned in literature by various other workers as well.\textsuperscript{81}

Physical properties and antimalarial activity of dimers of dihydroartemisinin 81-83 with intercalating succinyl group have also been reported by several groups.\textsuperscript{82}

Venugopalan \textit{et al.}\textsuperscript{83} (1995) synthesized various ring contracted dimers of artemisinin 84 and 85 and assessed them for antimalarial activity.

Posner \textit{et al.}\textsuperscript{84} (1997) reported the antimalarial and antiproliferative activity of various artemisinin based dimers 86.
Posner et al.\textsuperscript{85} (1999) reported the antimalarial, antiproliferative and antitumor activity of artemisinin derived chemically robust trioxane dimers 87. He in his ongoing research developed varieties of artemisnins derived dimers 87a-d and assessed them for their antimalarial and anticancer activity.

Ekthawatchai et al.\textsuperscript{86} reported the antimalarial activity of various prototype dimers 88 and 89 of artemisinin formed upon nucleophilic addition to artemisitene.
Jung et al.\textsuperscript{87} also synthesized various artemisinin based dimers 90 and 91. Jeyadevan et al.\textsuperscript{88} carried out synthesis and antimalarial assessment of C-10 non acetal dimers of artemisinin 92.

Grellepois et al.\textsuperscript{89} synthesized various artemisinin based dimers having prototype 93 via self cross metathesis reaction using Grubbs catalyst.\textsuperscript{90}

Posner et al. published several papers regarding the synthesis and bio evaluation of artemisinin based dimers 94-105.\textsuperscript{91}
1.7.2.3 Non privileged analogs of artemisinin

Together with these privileged analogs of artemisinin there are various non privileged analogs as well which have been synthesized by various workers. Jung et al.\textsuperscript{92} (2001) reported the synthesis of (+)-deoxoartemisinin and its novel C-11 derivatives. Avery et al.\textsuperscript{93} reported the synthesis and activity of various C-13 analogs of artemisinin. They also synthesized design based C-9β substituted analogs of artemisinin and assessed their structure activity relationship.
1.8 Drawbacks and problems associated with artemisinins

Although artemisinin and its derivatives are still the best known antimalarials, they are often associated with several serious problems such as high cost, limited availability from natural sources, sometimes poor oil and water solubility as in case of artemisinin itself and in some cases high rate of recrudescence, short plasma half life, toxicity and poor bioavailability as well. These factors limit the use of artemisinin as continuous source of drug for the treatment of malaria. The extensive use of artemisinin during clinical trials early in China, without report of serious human toxicity, animal studies have yielded some cautionary findings. Arteether and artemether 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. The drugs inhibited both neuronal cell proliferation and formation of neurite outgrowths at concentrations as low as 10 nM, which is comparable to the effective level of these drugs in vitro against many strains of *P. falciparum*. As far as potential neurotoxicity is concerned, any analogue with a log \( P \) 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 Lipinski’s Rule of Five to the design of new semisynthetic analogues have been employed.

The discovery of 1,2,4 trioxane, a peroxide moiety as the pharmacophore for the antimalarial activity of artemisinin, several efforts have been made in past few decades towards the preparation of simpler synthetic peroxides in order to meet the growing demand of new and cheaper antimalarials.

1.9 Synthetic Peroxides as Potential Antimalarial Agents

1.2 Dioxanes

Yingzhaosu A, a natural product endoperoxide with antimalarial properties was isolated from Chinese herb, Yingzhao, *Artabotrys uncinatus*, but its scarcity in nature and difficult total synthesis had led to the development of its various structurally simpler synthetic analogs.
Roche's group reported variety of analogs of containing its 2,3 dioxabicyclo[3.3.1]nonane core were prepared from the enantiomers of carvone. Endoperoxide , the core structure of , has weak antimalarial activity in vivo. Replacement of the methyl group at position 4 with n-alkyl chains of 9-11 carbon atoms led to a nearly order of magnitude increase in in vivo activity; analogs with shorter or longer chains were less active. As illustrated by , compounds containing polar functional groups such as alcohols, acids esters, or amines at position 4 showed little or no activity, although reduction of the ketone group at position 7 to the more polar alcohol did not affect activity significantly. As shown by , replacement of the undecyl chain in with a styryl group abolished antimalarial activity. However, analogs of , including quinoline , and especially , the 2,4-di(trifluoromethyl)styryl derivative, had very good antimalarial profiles.

Although is an order of magnitude less potent than the semisynthetic artemisinins in vitro, it is only 3-fold less active than artemether in vivo. Other attractive properties of include a chemically more stable 1,2-dioxane (endoperoxide) versus the 1,2,4-trioxane in artemisinin, and a lower rate of recrudescence and a longer plasma half-life than either artemether or arteether. From these data, (arteflene) was selected as the clinical candidate, and it progressed to Phase II clinical trials in semi-immune African patients with mild P. falciparum malaria. In these trials, the drug was given orally as a lipid suspension, but the results were inconsistent and the compound was abandoned.
A short and efficient synthesis of 4,8-dimethyl-4-phenylsulfonylmethyl-2,3
dioxabicyclo[3.3.1]nonanes from the enantiomers of limonene or R-(−)-carveol afforded a new
series 108 of analogs of yingzhaosu 106 with a variety of substituents at C-8.106 Relative to
benzyl ether 108a, activity declined substantially for the more polar carbinol 108c, a trend that
was partially reversed by acetylation to form 108d. In contrast, the less polar olefin, a
dehydration product of 108c, and its fully saturated hydrogenation product, were less active than
108c. Although 108b was marginally less potent than its diastereomer 108a in vitro, it was
significantly more active than 108a and only slightly less active than artemisinin when it was
administered orally.

Posner’s group reported the mechanism-based design of a series of easily prepared symmetrical
bicyclo[3.2.2]nonane 109 and bicyclo[2.2.2]octane 110 endoperoxides. As illustrated by sulfone
109b, seven heterocyclic analogs of 109a containing sulfur, oxygen or nitrogen atoms were
synthesized; however, these were all an order of magnitude less potent than their carbocyclic
analog 109a even though they are reduced by ferrous iron to form reactive carbon centered
radicals and epoxides.107

Varieties of dioxanes have been prepared so far and have been assessed for their antimalarial
activity but none of them have shown potent antimalarial activity.
1,2,4-Trioxanes
This class of compounds have been known in literature since 1957, when Payne and Smith first of all synthesized first synthetic trioxane.\textsuperscript{108} Later on several researchers developed various methodologies for the synthesis of different types of trioxanes only from synthetic point of view.\textsuperscript{109} It was only after the disclosure of the fact that it is actually the endoperoxide linkage of artemisinin in form of 1,2,4-trioxane, which is responsible for its antimalarial activity large emphasis has been made towards the synthesis and bio-evaluation of various types of synthetic trioxanes.

The bicyclic trioxanone\textsuperscript{109e} \textsuperscript{111} was prepared from 2-methyl-2-cyclopenten-1-ol as in six steps. Bicyclic trioxane\textsuperscript{110} \textsuperscript{112} (2,3,5-trioxabicyclo[2.2.3]nonane), easily recognizable as the pharmacophoric core of artemisinin, was prepared from 6-tetrahydrooxepanol as starting material. However, these bicyclic trioxanes had only marginal antimalarial activity.

The epimeric 1,2,4-trioxanes \textsuperscript{113a} and \textsuperscript{113b} were synthesized by the photooxygenation reaction. Compound \textsuperscript{113a} was just an order of magnitude less potent than artemisinin, whereas \textsuperscript{113b} was quite less potent than artemisinin. Jefford \textit{et al.} showed that replacement of the bridgehead C-3 methyl group by C-3 phenyl group in \textsuperscript{113a} improved 6-fold antimalarial potency.\textsuperscript{111} Based on these facts Posner\textsuperscript{112} \textit{et al.} synthesized various substituted C-3 phenyl analogs of prototype \textsuperscript{114}.

Some of these compounds \textsuperscript{114a-e} have shown promising \textit{in vivo} activity. Trioxane alcohol \textsuperscript{114b}, acetate trioxane \textsuperscript{114c} were more potent to artemisinin whereas water soluble carboxylic acid derivative \textsuperscript{114d} was less active than artemisinin.

In continuation of their work, Posner \textit{et al.}\textsuperscript{113} prepared carboxyphenyl trioxanes \textsuperscript{115a} and \textsuperscript{115b} which were more soluble in water at pH 7.4 than artesunate. These compounds were less effective than their less lipophilic and more easily prepared parent compound \textsuperscript{114}.

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 \textsuperscript{116} and \textsuperscript{117} were selected for biological evaluation in Aotus monkeys infected with multidrug-resistant (MDR) \textit{P. falciparum}. The activity data revealed that both \textsuperscript{116} and \textsuperscript{117} are as effective as arteether against MDR \textit{P. falciparum} in Aotus monkeys.\textsuperscript{114}

Spiro ring-fused trioxane \textsuperscript{118} was synthesized starting with (-)-isopulegol. This trioxane was only slightly less potent than artemisinin. The analog in which the spirocyclopentane ring was replaced with geminal methyl substituents was 9-fold less potent than \textsuperscript{118}.\textsuperscript{111,115}
1,4-Endoperoxides, formed from photooxygenation of 1,4-diaryl-1,3-cyclopentadienes, reacted with aldehydes or ketones in reactions catalyzed by Me₃SiOTf to produce a large series of cis-fused cyclopenteno-1,2,4-trioxanes, exemplified by 119a (Fenozan B07). Several such analogs 119b-f were synthesized and assessed for antimalarial activity. Among these cis-fused cyclopenteno-1,2,4-trioxanes, 119a (Fenozan B07) had the most promising activity profile and was chosen for further development. Spiro trioxanes 120 and 121 and their analogs were prepared by photooxygenation of the corresponding allylic alcohols followed by peroxyacetalization reactions with aldehydes or ketones. Griesbeck et al. (2002) reported synthesis of antimalarial 1,2,4-trioxanes via photooxygenation of chiral allylic alcohol 4-methyl-3-penten-2-ol followed by subsequent BF₃ catalyzed peroxyacetalization with aldehydes or ketones afforded four monocyclic and spirobicyclic 1,2,4-trioxanes, of which 120 was the most potent. O’Neill et al. (2001) reported Co(II)-mediated regioselective Mukaiyama hydroperoxysilylation of 2-alkyl- or 2-aryl-prop-2-en-1-ols furnished peroxyxsilyl alcohols which were treated with aldehydes or ketones to provide various spiro trioxanes. Trioxane 121, the best of these, was only an order of magnitude less potent than artemisinin.
Singh (1990) reported a new and convenient $^1$O$_2$-mediated synthesis of 6-arylvinyl-1,2,4-trioxanes. The key steps of this method are the preparation of $\beta$-hydroxyhydroperoxides by photooxygenation of suitably substituted allylic alcohols and then elaboration of these $\beta$-hydroxyhydroperoxides into 1, 2, 4-trioxanes by acid catalyzed condensation with various ketones or aldehydes. This method is safe and has been used for the preparation of trioxanes on multigram scale.

Singh et al. have prepared several 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 in vivo. In the preliminary study on 6-arylvinyl-1,2,4-trioxanes, compounds 122-128 showed promising activity by intra peritoneal (ip) route against chloroquine-sensitive P. berghei in mice but these compounds were poorly active against chloroquine-resistant P. yoelii in mice. Among geranol
derived 6-arylalkylvinyl trioxanes, compound 126 showed 100% survival rate at 96 mg/kg against MDR *P. yoelii* in mice by oral and im routes. Although no *in vitro* data was presented for these trioxanes, the *in vivo* data showed that the order of efficacy was spiroadamantane > spirocyclopentane > spirocyclohexane. Introduction of a methyl group at the carbon atom bearing the α-arylvinyl group abolished activity.

Singh *et al.* in continuation of their SAR have prepared several highly lipophilic synthetic trioxanes 127a-g and amino functionalized trioxanes 128a-d and trioxane quinoline hybrids (trioxaquines) 129a-f. Compound 127a and 127b showed 100% survival at 12 mg/kg and 24 mg/kg dose respectively, by oral route against multi drug resistant *P. falciparum* in swiss mice. Water soluble trioxanes 127e is active by both oral and i.m. route at 72 mg/kg dose and has been selected for clinical trials on account of its better pharmacokinetic profile. Among amino derivatives compound 128d showed 80% survival rate at 24mg/kg dose by oral route against MDR *P. yoelii* in mice. The trioxaquines 129a-e were found to have poor activity.

Meunier *et al.* 123 have also synthesized several trioxane quinoline hybrids (trioxaquines), some of which have shown promising activity profile *in vitro* and *in vivo*. Ascaridole-derived, trioxaquine 130 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.
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Rong and Wu\textsuperscript{124} melded most of the structural elements of artemisinin in a cholestane-type steroid trioxane hybrid structure. Compound 131 has been prepared in five steps from methyl 3-oxocholest-4-en-6b-yl acetate using photooxygenation reaction as key step. Both 131 and its diasteromer were more effective than artemisinin \textit{in vivo}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{128a-128c.png}
\caption{128a, 128b, 128c}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{129a-129f.png}
\caption{129a: n=2, 129b: n=3, 129c: n=4, 129d: n=2, 129e: n=3, 129f: n=4}
\end{figure}

1,2,4,5-Tetraoxanes

Symmetrical meso dispiro 1, 2, 4, 5-tetraoxane 132a, readily obtained by reaction of 2-methylcyclohexanone with acidified hydrogen peroxide, was found to be only 6-fold less active than artemisinin. Tetraoxane 132a is synergistic with chloroquine, quinine, mefloquine, and artemisinin against \textit{P. falciparum}.\textsuperscript{125}

Sixteen dispiro tetraoxane analogs of 132a with various alkyl substitutions 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 there was any correlation between antimalarial activity and neurotoxicity. Dispiro tetraoxanes 132b and 132c bearing unsaturated and polar
functional groups were prepared to improve antimalarial activity of prototype tetraoxane 132a by oral route. But both 132b and 132c were found to be inactive. However, the more lipophilic ethyl ester of 132c (IC_{50} 6.4 nM) and methyl ether of 132b (IC_{50} 15 nM) showed significant \textit{in vitro} antimalarial potency. These tetraoxanes possessed poor activity \textit{in vivo}.

Mixed tetraoxanes possessing spirocycloalkane and spirocholic acid-derived steroid substructures were prepared 133 and found to be 6-fold more potent than artemisinin. Mixed tetraoxanes with a spirocyclohexane were more potent than the corresponding spirocyclopentane and spirocyclooctane analogs.\textsuperscript{126} Several diester and diamide cholic acid-derived tetraoxanes were synthesized, best one of these, cis diamide tetraoxane 134, was only 4-fold less potent than artemisinin.\textsuperscript{127} Cholestane-type steroid tetraoxane hybrid 135 was found to be less active than artemisinin.\textsuperscript{128} Solaja and his coworkers have developed several bile acid derived highly potent tetraoxanes in past few years.\textsuperscript{129}

\begin{center}
\includegraphics[scale=0.5]{tetraoxanes.png}
\end{center}

1,2,4-Trioxepanes

There are only few methods\textsuperscript{130} reported in literature for the synthesis of 1,2,4-trioxepanes, the next higher homolog of 1,2,4-trioxanes and only two reports of their antimalarial activity. O’Neill\textsuperscript{131} and his coworkers first of all reported \textit{in vitro} activity of 1,2,4-trioxepanes having prototype 136 and 137. Singh\textsuperscript{132} \textit{et al.} have also reported \textit{in vivo} assessment of new class of aryl vinyl 1,2,4-trioxepanes 138a-c.
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**1,2,4,5-Tetraoxepanes, 1,2,4,5-Tetraoxocanes & 1,2,5,6-Tetraoxonanes**

Tricyclic 1,2,4,5-tetraoxepane 139 and 1,2,5,6-tetraoxonane 140 were 35 to 40-fold less potent than artemisinin, but 140 had notably better *in vivo* activity (ip). Both 139 and 140 however, were completely inactive when they were administered orally. 1,2,4,5-tetraoxocanes 141a and 141b exhibited excellent *in vitro* potency, however, both were less effective than artemisinin *in vivo*. 134

**1.2.4-Trioxolanes**

The 1,2,4-trioxolane system is well known to organic chemists as secondary ozonide, a highly reactive intermediate of the ozonolysis reaction.

De Almeida Barbosa *et al.* 135 firstly reported antimalarial activity of a new series of tricyclic trioxolanes (8,9,10,11-tetraoxatricyclo [5.2.1.12.6] undecan-4-ones) which were synthesized from various 8-oxabicyclo [3.2.1] oct-6-en-3-ones by ozonolysis. Trioxolane 142 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. 142, the best of these, had an IC\textsubscript{50} of 7,300 nM which was three orders of magnitude less potent than artemisinin.

Research efforts made by Vennerstrom *et al.* 136 led to the discovery of various novel antimalarial 1,2,4-trioxolanes 143. Out of which trioxolane 143c displayed *in vitro* IC\textsubscript{50} values of 0.39 ng/mL and 0.42 ng/mL versus chloroquine-resistant *P. falciparum* K1 and chloroquine sensitive NF-54.
strains, and was found to be more active than chloroquine, and mefloquine in vivo after a single 3 mg/kg dose administration against *P. berghei* infected mice. Compound 143c OZ-277 displayed high activity against field isolates from Gabon (median IC₅₀ 0.47 nm; range: 0.13–2.23 nm). Its stage specificity is similar to that of artemisins. The activity against *P. vivax* is in the same range as the activity against *P. falciparum*. This compound had been taken upto clinical trials but the clinical development of OZ-277 has been discontinued because areas under the curve (AUC) in malaria patients were less than 50% of those recorded in healthy volunteers (W. Gutteridge, personal communication).

Amine Peroxides

Amine peroxides 144 were synthesized from the corresponding secondary amines by treatment with formaldehyde and t-butyl hydroperoxide. Morpholine peroxide 144b, the most potent member of this class, was only 20-fold less potent than artemisinin, but with the exception of the weakly active 144c, each was inactive in vivo. 4-Aminoquinoline peroxide 144d was only weakly potent and at dose of 640 mg/kg was toxic (Vennerstrom, unpublished results).

Miscellaneous Peroxides

Together with above reported various class peroxides, different other structurally modified peroxides have also been synthesized and assessed for their antimalarial activity. Perketal derivatives of unsaturated fatty acid hydroperoxide such as 145 was found inactive although terpene derived perketal 146 is considerably more potent than 145, it is still some two orders of magnitude less potent than artemisinin. Compound 147 was one of the most potent
molecule with an \( IC_{50} \) value of 86 nM. More importantly, 147 is the first acyclic peroxide with demonstrable \textit{in vivo} efficacy.\textsuperscript{143}

\begin{align*}
\text{145} & \quad \text{146} & \quad \text{147}
\end{align*}

Various other acyclic peroxides have been synthesized and assessed for their antimalarial efficacy but none of them was found to have activity comparable to that of artemisinin.

1.10 Mechanism of Artemisinin and Related Peroxides

Efforts to elucidate the antimalarial action of artemisinin started in the 1970s, and last three decades large no of papers have been published by various workers regarding the mode of action of artemisinin and related peroxides.\textsuperscript{144-151}

\begin{align*}
\text{Artemisinin} & \quad \text{Path 1} & \quad \text{Path 2} & \quad \text{C-Centered Secodary Free Radical} & \quad \text{Alkylation of Biomolecules}
\end{align*}

\textbf{Mechanism of formation of carbon centered free radicals}

Despite the growing importance of artemisinins, their exact mechanism of action is still unresolved and remains a matter of intense debate. It has been proposed that Fe\textsuperscript{2+} mediated cleavage of the endoperoxide leads to the formation of different C-centered radicals which may
be primary or secondary in nature. Which, if not possibly both, of these radicals is the active species is unclear. For a long time it was thought that the formation of C-centered radicals takes place in the digestive vacuole and that ferrousprotoporphyrin IX is the activating species. The reactive C-centered radicals are thought to subsequently react more or less indiscriminately with different protein targets as well as with ferriprotoporphyrin IX itself, thus preventing heme detoxification and inhibiting a multitude of enzymes. O’Neill and Posner formulated the mechanism of artemisinins as “iron-triggered cluster bombs”.

![Diagram of the iron-triggered cluster bomb](image)

The “iron-triggered cluster bomb”

![Diagram of radical mediated inhibition of Ca^{2+} ATPase (SERCA) called as PfATP6](image)

Radical mediated inhibition of Ca^{2+} ATPase (SERCA) called as PfATP6

Although very attractive, the development of resistance against a drug that acts nonspecifically against multiple targets is unlikely, so this concept has been questioned owing to some
contradictory findings: Artemisinins act against all developmental parasite stages, including those which do not produce hemozoin. Several experiments detected labeled artemisinin derivatives localized not within but only outside the digestive vacuole, and there are some highly active artemisinin derivatives that are more or less insensitive to Fe$^{2+}$ mediated cleavage.\footnote{157}

Recently, Krishna and co-workers put forward another theory which says that the endoperoxide cleavage should take place in the cytoplasm catalyzed by a cytoplasmic Fe$^{2+}$ source. The resulting reactive species then very specifically inhibits an ATP-dependent Ca$^{2+}$ pump located on the endoplasmic reticulum. The pump, called PfATP6, is a homologue of a mammalian sarcoplasmic/endoplasmic reticulum Ca$^{2+}$ ATPase (SERCA).\footnote{158}

\subsection*{1.11 Discussion}

In the last few decades none of the peroxide based compounds other than clinically used arteether, artemether and artesunate, both semisynthetic and synthetic has come up to the task of becoming drug of future although a lot of effort in this regard has been made. Artelinate \textsuperscript{20} was one such compound which was thought to replace artesunate \textsuperscript{17} as it was more stable and water soluble and has better activity profile; however, further development of artelinate has been discontinued in favor of artesunate because of the higher neurotoxicity of artelinate. Artemisone \textsuperscript{73f}, have been found as lead candidate for clinical studies. This compound was designed on the basis of ADME parameters by the application of \textit{Lipinski’s Rule of Five}, and incorporating suitable polar residues and their isosteres. It was found to 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. Artemisone had been in clinical studies, but at present, further development of this promising drug is allegedly uncertain.

None of the synthetic peroxides identified so far has an antimalarial profile superior to that of arteether \textsuperscript{16}, the best semisynthetic artemisinin, although available data indicates that 1,2,4-trioxanes \textsuperscript{114a} and \textsuperscript{119a} (Fenozan B07) are only marginally less effective than \textsuperscript{16}. Recently a compound \textsuperscript{127e}, from Singh’s group has been chosen for Phase I clinical studies.

In case of synthetic peroxides other than 1,2,4-trioxanes, compound \textsuperscript{143c} (OZ-277) was taken up to clinical trials but withdrawn because of poor performance in malaria affected patients.

Of course, the very instability of the peroxide bond that endows these compounds with their unique antimalarial specificities also precludes a number of synthetic transformations and reaction conditions that could normally be considered for nonperoxidic compounds. However,
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like the semisynthetic artemisinins, 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. Synthesis complexity, source of peroxide oxygen atoms (hydrogen peroxide, singlet or triplet oxygen, ozone), reduced stability of unsaturated versus saturated peroxide heterocycles, and stereochemistry, are other chemical parameters that must be considered in synthetic peroxide design and development.

1.12 Conclusion

The growing emergence of resistant varieties of malaria parasites against conventional therapies have enforced medicinal chemists to search for new antimalarial drugs. The real problem in developing an antimalarial drug is of course its cost and toxicity. The major problem in research and development for antimalarial drugs is that, this disease is considered to be as a disease of poor and developing countries where patients can’t afford to buy expensive drugs, consequently pharmaceutical industries have not paid lot of emphasis in this regard as they do not see it as a profitable business. The partnership between research institutions, academia, private industries and international agencies can prove as mile stone in the development of new antimalarial drugs. Growing emergence of resistance against artemisinin derivatives in some areas have made researchers to think about combination therapies. Various artemisinin based combination therapies are now being used in areas where parasite has become resistant against conventional monotherapy.

Although no alternatives to artemisinin based therapies is currently available for the treatment of complicated malaria, efforts are now being made to develop synthetic peroxides as possible alternative to artemisinin based therapies in order to search for a cost effective remedy. Most of the peroxide based compounds other than conventionally used artemisinin derivatives that have come up to clinical stages have been withdrawn because of their toxicity. Various synthetic peroxides like 107a (arteflene), 143c (OZ-277), 119a (Fenozan B07) come up to clinical trials, but failed due to toxicity. Recently compound 127e (CDRI-97/78) which have shown very good preclinical results have been taken up for Phase I clinical testing.

The analysis of the genome sequence can provide some valuable information regarding the development of new leads for vaccine development, but such efforts are still at laboratory stages. A number of new potential target pathways have already been identified so far and efforts are
now being made to develop lead compounds for these putative targets which will allow treating
the malaria infections in a uniform and sustained way.

In conclusion, in order to combat the growing resistance of malarial parasite against various
conventionally used artemisinin based drugs, efforts are now being made to develop new
synthetic peroxides as potential antimalarial agents on account of their relative cheapness and
versatility of structural modification, enabling them to tailored to fit a drug profile characterized
by potency superior to that of natural product artemisinin, by enhanced bioavailability and
minimal toxicity.

1.12 Summary
In the last few decades lot of effort has been put to develop new antimalarial drugs that have
better activity profile and reduced neurotoxicity. Knowledge of mechanism of artemisinin based
therapies has allowed to researchers to develop compounds that have reduced neurotoxicity.
Compounds based upon ADME parameters have been developed to insure optimum activity and
reduced toxicity. Various scientific tools are now being used to design and develop new
structurally modified synthetic peroxides in order to search for new leads for malaria
chemotherapy. As the mode of action of artemisinin based compounds and various other
antimalarial synthetic peroxides is fully known scientists are now trying to develop mechanism
based compounds in order to combat the malaria, but the search is still going on.

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