SUMMARY

Malaria is still one of the major health problem in the whole world along with tuberculosis and AIDS. The parasite responsible for the vast majority of fatal malarial infections is *P. falciparum*. The first effective antimalarial drug was quinine, which was isolated from the bark of Cinchona. Since then malaria has been treated with quinoline-based drugs such as quinine, chloroquine, mefloquine and primaquine. Unfortunately, many *Plasmodium* strains have now become resistant to these drugs. Almost 35 years ago, the major breakthrough in malaria chemotherapy occurred when a new antimalarial structural prototype with a pharmacophoric peroxide bond in a unique 1, 2, 4-trioxane heterocycle i.e. artemisinin was isolated from *Artemisia annua* and brought great attention to the whole world in malaria chemotherapy. It met the dual challenges posed by drug-resistant parasites and rapid progression of malarial illness.

Available evidence proves that artemisinin and related peroxidic antimalarial drugs exert their parasiticidal activity subsequent to reductive activation by haem, released as a result of haemoglobin digestion by the malaria-causing parasite *Plasmodium*. This irreversible redox reaction produces carbon-centred free radicals, leading to alkylation of haem and proteins (enzymes), one of which-the sarcoplasmic endoplasmic reticulum ATPase PfATP6-may be critical to parasite survival. Notably, there is no evidence of drug resistance to any member of the artemisinin family of drugs.

The chemotherapy of malaria has benefited greatly from the semi-synthetic artemisinin derivatives such as dihydroartemisinin, artemether, arteether and artesunate as they rapidly reduce parasite burden, have good therapeutic indices and provide successful outcomes for the treatment of malaria. However, as a drug class, the artemisinins suffer from chemical (semisynthetic availability, purity and cost), biopharmaceutical (poor
bioavailability and limiting pharmacokinetics) and treatment (non-compliance with long treatment regimens and recrudescence) issues that limit their therapeutic potential.

Since the Central Drug Research Institute, Lucknow, has an avowed objective of developing new drugs; it is running a research programme in malaria chemotherapy since late 70's. This programme had two major objectives: (a) to develop new antimalarials and especially effective against resistant malaria and (b) to develop efficient technologies for existing antimalarial drugs.

As part of this programme and in search for better artemisinin analogues, an attempt has been made to improve the antimalarial activity of artemisinin analogues better than β-arteether. In this thesis, the structure, conformation and stereochemistry of artemisinin skeleton by utilizing several synthetic strategies has been explored. Furthermore, synthesis and antimalarial testing of several new artemisinin analogues, exploring the antimalarial potency of artemisinin by carrying out structure-activity relationship, development of new biologically active scaffolds in artemisinin skeleton and the study towards the preparation of optically pure 1,2,4-trioxanes taking dihydroartemisinin as chiral template were taken up. The present thesis covers the result of these studies and is divided into five chapters as summarized below:

Chapter 1 Historical Development and Current Scenario of Artemisinin and Related Antimalarials

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.
Malarial infection

Malaria is a parasitic disease which is caused by various species of *Plasmodium* protozoa. Together with AIDS and TB, malaria is responsible for largest number of deaths annually. The malarial threat, though highest in Sub-Saharan Africa, South-East Asia and South America, is not limited to these regions, mainly due to travelers/migrants from malarial to non-malarial regions. The high rate of mortality associated with malaria can be attributed to the increasing cases of drug-resistance of *P. falciparum*, the most deadly of the four human infecting malarial parasites. The other three species that infect humans are *P. vivax*, *P. ovale*, and *P. malariae*.

Malaria chemotherapy

Chloroquine 1 is one of the most inexpensive, readily available, and probably most prescribed drugs for the chemotherapy of malaria. However, unfortunately it has been rendered ineffective in many parts of the world, due to the emergence of multidrug-resistant *P. falciparum*. Quinine 2 is one of the oldest known drugs against malaria. It is an alkaloid isolated from the bark of *Cinchona*. Despite its use for over 350 years, quinine is effective against all forms of malaria including the severe cases of *P. falciparum* malaria. However due to some serious side effects quinine finds a limited use in this area. Combination therapy, developed to combat drug resistance, is also under threat due to emergence of multidrug-resistant parasites. Although several individual and combination drug therapies are available against malaria each has its limitations due to one or more of the liabilities associated with toxicity, resistance and/or cost.
Artemisinin and related peroxides

Artemisinin (qinghaosu) \( 3, \) isolated in early 1970’s by Chinese scientists from *Artemisia annua,* is a tetracyclic sesquiterpene 1, 2, 4-trioxane. With its unusual and unique structure, high antimalarial efficacy, and negligible toxicity artemisinin has fascinated both chemists and biologists for past three decades. Due to its poor solubility in both oil and water, several oil and water soluble derivative of artemisinin with even better activity profile have been synthesized. These include dihydroartemisinin \( 4, \) artemether \( 5, \) arteether \( 6, \) artesunate \( 7 \) and artelinic acid \( 8. \) Artemisinin and its derivatives are the only class of antimalarial against which no clinically relevant resistance has been reported.

Several new analogues have been prepared such as ether and ester analogues, thioether analogues, C-10 aryloxy analogues, carba-analogues, deoxocarba analogues, amino analogues etc. but none of the synthetic analogues identifies so far was launched into the market. Since then, several modification in the structure of artemisinin has been
carried out by applying different synthetic strategies in order to improve the antimalarial activity of artemisinin.

One artemisinin derivative, artemisone 59, have been found as lead candidate for clinical studies. By use of the ADME, the application of Lipinski’s Rule of Five, and incorporating suitable polar residues and their isosteres, Haynes et al. (2006) have succeeded in preparing artemisone 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.

Several new synthetic 1, 2, 4-trioxanes have been prepared by several new synthetic methodologies in the last two decades. Most of them were found to show excellent antimalarial activity but none of the synthetic peroxides identified so far has an antimalarial profile superior to that of arteether 14, the best semisynthetic artemisinin, although available data indicates that 1, 2, 4-trioxanes 59a and 112 (Fenozan B07) are only marginally less effective than 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. 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.

Apart from antimalarial activity; anti-infective, antifungal, antiproliferative, antiinflammatory, anticancer, antiarrhythmic activities have been reported in artemisinin derivatives.

This review also cover the details of several other antimalarial peroxides reported in the past 35 years.

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. On the whole, this review provides short glimpses in the progress and developments of artemisinin in the drug discovery of malaria chemotherapy.

Chapter 2 Synthesis and Antimalarial Activity of New Derivatives of Artemisinin

Section A: New Orally Active Ether Derivatives of Artemisinin

Introduction

Malaria is endemic in many parts of the world. Around 300-500 million clinical cases of malaria are reported every year of which more than a million die due to complicated malaria. The malaria situation is getting worse with rapid spread of multidrug-resistant *Plasmodium falciparum*. Against this background, isolation of artemisinin 1 as the active principle of the Chinese traditional drug against malaria, *Artemisia annua*, is a major breakthrough in malaria chemotherapy. Artemisinin owes its antimalarial activity due to the presence of 1, 2, 4-trioxane system and is active against
both chloroquine-sensitive and chloroquine-resistant malaria. The semisynthetic derivatives of artemisinin such as dihydroartemisinin 2, artemether 3, arteether 4 and artesunic acid 5 are more active than artemisinin and are currently the drugs of choice for the treatment of malaria caused by multidrug-resistant Plasmodium falciparum. While these compounds show high efficacy when administered by intramuscular or subcutaneous route, they exhibit poor activity when given by oral route.

![Artemisinin and its derivatives](image)

Figure 1. Artemisinin and its derivatives

In recent years, several attempts have been made to improve the antimalarial activity of artemisinin derivatives by oral route. However, these new derivatives are only marginally more active than artemether and artesunic acid. Thus, there is a need to develop new artemisinin derivatives with better oral absorption and improved antimalarial activity. Herein, we report the synthesis and antimalarial activity of a new series of ether derivatives of dihydroartemisinin, several of which are orally 2 to 4-fold more active than β-arteether against multidrug resistant P. yoelii nigeriensis in mice. These new highly lipophilic ethers, surprisingly, are less active by intramuscular route. The other striking feature of ether derivatives is that their α-isomers are more active than the β-isomers whereas in case of artemether and arteether, the β-isomers are more active than the α-isomers.
Synthesis

Dihydroartemisinin 2 was prepared by NaBH₄ reduction of artemisinin using the known procedure. BF₃·OEt₂-catalyzed reaction of 2 with alcohols 6a-k (Figure 2) in CH₂Cl₂ at subzero temperature (-10 °C to -5 °C) furnished the corresponding ether derivatives 7-17 in 65-99% yields as diastereomeric mixtures of α and β isomers, with β-isomers as the major products (Scheme 1, Table 1). The α / β ratio varies from 1:3 to 1:5. In all these cases, except ether derivatives 7, 9, 11, and 17, the α and β isomers appeared as two distinct spots on TLC and were separated by column chromatography, and the pure isomers were evaluated for antimalarial activity. Ether 11β was obtained by crystallization of mixture of 11α and 11β in hexane; the pure α-isomer could not be obtained. Ether derivatives 7, 9, and 17 which were obtained as inseparable mixture of α- and β-isomers were used as such for bioevaluation.

Scheme 1: Reagents and conditions: (a) BF₃·OEt₂, CH₂Cl₂, -10°C to -5°C, 2h.

Figure 2. Structure of Alcohols 6a-k.
Antimalarial activity

Since the objective of the study was to select compounds having activity profile better than that of β-arteether, all the newly prepared ether derivatives 7-17 were initially screened against multidrug-resistant P. yoelii nigeriensis in Swiss mice at 48 mg/kg x 4 days by oral route using Peter’s procedure. Artemisinin derivatives such as artemether 3, arteether 4, and artesunic acid 5 have excellent antimalarial activity when given by systemic routes. These drugs, however, have serious limitation such as short half-life and poor bioavailability when given by oral route.

In a parallel program on synthetic antimalarial 1, 2, 4-trioxanes, our group had observed that molecules built around adamantane, biphenyl, and flourene scaffold show promising antimalarial activity by oral routes. Also there are several reports in the literature wherein compounds having these substructures as part of molecular architecture show promising biological activities. On the basis of these considerations, we have prepared ether derivatives 7-17 and evaluated them for antimalarial activity using β-arteether as positive control. These new derivatives 7-33 are highly lipophilic (log P in the range of 5.28 to 7.19) as compared with β-arteether (log P 3.84) and several of them are two- to four- fold more active than β-arteether.

Ether derivatives 12a and 14a, the most active compounds of the series, are four times more active than β-arteether. Overall, ester derivatives which are formed exclusively as α-isomers, were found to be somewhat less active than α-isomer of ether derivatives. The high order of antimalarial activity combined with ease of preparation of these compounds qualifies these compounds as candidates for further drug development studies.
Section B: New Orally Active Ester Derivatives of Artemisinin

Introduction

In section A of this chapter, we came across several exciting results in artemisinin derivatives. We had found in case of ether derivatives that $\alpha$-isomers were found to be more active than the corresponding $\beta$-isomers, where both pure $\alpha$- and $\beta$-isomers were separable. We also observed that molecules built around lipophilic scaffolds such as adamantane, biphenyl, and flourene etc. show promising antimalarial activity by oral routes.\(^{10}\) Also there are several reports in the literature wherein compounds having these substructures as part of molecular architecture show promising biological activities.\(^{11}\) In view of these observations it appeared of interest to prepare similar ester derivatives of dihydroartemisinin and assess their antimalarial activity. The added advantage of ester derivatives is that they are obtained exclusively as $\alpha$-isomers thus avoiding the lengthy purification procedure.\(^{16}\) In this chapter, we report the synthesis and antimalarial activity of a new series of ester derivatives of dihydroartemisinin, several of which are orally 2 to 4-fold more active than $\beta$-arteether against multidrug-resistant *P. Yoelii nigeriensis* in Swiss mice.\(^{17}\)

Synthesis

Acid chlorides RCOCl \(^\text{18a-j, figure.3}\) were prepared from the corresponding carboxylic acids by heating with thionyl chloride at 50-60 °C for 2-3h under anhydrous condition and reacted with dihydroartemisinin 2 in the presence of triethylamine in dry dichloromethane at 0 °C for 2h furnished ester derivatives \(^\text{19-33}\) in 49-94% yields as $\alpha$-isomers exclusively. The ester derivatives \(^\text{23, 26 and 29}\) reported earlier\(^{18}\) by Yin Li et al and Ying Li et al; ester \(^\text{30}\), the acetate of dihydroartemisinin, also reported earlier;\(^{19}\) and
esters 31, 32 and 33 also reported earlier,\textsuperscript{20} were also prepared in similar way (scheme 2, Table 3).

\[
\text{RCOOH} \xrightarrow{\text{SOCl}_2} \text{RCOCI} \xrightarrow{(a)} \text{18a-o} \quad \text{19-33}
\]

\textbf{Scheme 2: Reagents and conditions:} (a) \(\text{Et}_3\text{N}, \text{Dry CH}_2\text{Cl}_2, 0^\circ\text{C}, 2\text{h.}\)

\[18\text{a} \quad R = \quad \text{18b} \quad R = \quad -\text{H}_2\text{C} - \text{18c} \quad R = \quad -\text{H}_2\text{C} \]

\[18\text{d} \quad R = -\text{H}_2\text{C}-\text{H}_2\text{C} - \quad \text{18e} \quad R = \quad -\text{18f} \quad R = \quad -\text{H}_2\text{C} -
\]

\[18\text{g} \quad R = -\text{CH}_2\text{O} - \quad \text{18h} \quad R = -\text{H}_2\text{C}-\text{O} - \quad \text{18i} \quad R = \quad \text{18j} \quad R = -\text{H}_2\text{C} -
\]

\[18\text{i} \quad R = \quad 18\text{m} \quad R = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \quad 18\text{n} \quad R = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 -
\]

\[18\text{o} \quad R = -\text{H}_2\text{C}-\text{H}_2\text{C} -
\]

\textbf{Figure 3.} Structure of Acid chlorides 18a-o.

\textbf{Antimalarial Activity}

All the newly prepared ester derivatives 19-33 were initially screened against multidrug-resistant \textit{P. yoelii nigeriensis} in Swiss mice at 48 mg/kg x 4 days by oral route using Peter's procedure.\textsuperscript{8} All the esters derivatives provided 100% protection at this dose
and these active compounds were further screened at 24 mg/kg x 4 days. Compounds 20, 27, 28 and 29 which showed 100% protection at 24 mg/kg x 4 days were further tested at 12 mg/kg x 4 days. Those compounds which had shown partial protection were also tested at 12 mg/kg x 4 days. Only compound 29, which showed 100% protection at 12 mg/kg x 4 days, were further tested at 6 mg/kg x 4 days.

All esters derivatives except 19, 30 and 31 show better activity profile than β-arteether. The diphenylmethyl-based ester 29, the most active compound of the series, provides 100% protection at both 24 mg/kg x 4 days and 12 mg/kg x 4 days. At 6 mg/kg x 4 days, it shows 100% suppression of parasitaemia but none of the treated mice survived till day 28.

In conclusion, the ester derivatives containing the diphenylmethyl and flourene moieties showed better activity profile than adamantane- and biphenyl-based derivatives. Overall, ester derivatives which are formed exclusively as α-isomers, were found to be somewhat less active than α-isomer of ether derivatives.

Chapter 3 Artemisinin Derived Stable Ozonides: Synthesis, Chemistry & Biological Activities

Introduction

The discovery of artemisinin 1, as the active antimalarial principle of the Chinese traditional drug Artemisia Annua, is a major milestone in malaria chemotherapy. More than providing a new series of antimalarial drug such as dihydroartemisinin 2, artemether 3, arteether 4 and artesunic acid 5 (fig. 1) to combat multi-drug resistant malaria, the discovery has given a new idea, the idea that the malarial parasite can be selectively killed by providing an extra oxidative stress in the form of a peroxide molecule. This has opened a new era of medicinal chemistry. Series of new structurally simplified 1, 2, 4-
trioxanes, 1, 2, 4, 5-tetraoxanes and ozonides have been synthesized and evaluated for antimalarial activity in the last two decades. Several of these peroxyketals have shown high order of antimalarial activity. Relevant to the present study is the synthesis of steroidal tetraoxane 6 by acid catalysed reaction of H₂O₂ with dialdehyde 7 (fig.1). Tetraoxane 6 is reported to show significant antimalarial activity against P. falciparum in vitro [IC₅₀ (D6) = 0.35 µg/mL; IC₅₀ (W2) =0.29 µg/mL]. These results have prompted us to replace 1, 2, 4-trioxane moiety of artemisinin skeleton with 1, 2, 4, 5-tetraoxane and study its effect on antimalarial activity.

![Figure 1. Artemisinin and its derivatives (1-5) & Steroidal 1, 2, 4, 5-Tetraoxane (6).](image)

To test these ideas, compound 8, easily accessible in single step and in good yield from artemisinin, was treated with 30% H₂O₂ using the procedure of Opsenica et al., to give a mixture of diastereomeric peroxides (scheme 1). To our surprise the peroxides were the ozonides 9 and 10 instead of the intended tetraoxane. The stereochemistry of one of these ozonides was confirmed by single crystal X-ray crystallography of its acetate derivative 11a. Although these ozonides have shown poor antimalarial activity against multidrug-resistant (MDR) strain of Plasmodium yoelii nigeriensis, one of them (10) have shown high order of antitubercular activity in vitro. Both these ozonides were stable to
various reaction conditions and were converted to several derivatives 11a-f and 12a-c which were also assessed both for antimalarial activity and antitubercular activity.

Chemistry of Ozonides

Compound 8, easily accessible in single step and in good yield from artemisinin, was treated with 30% H₂O₂ using the procedure of Opsenica et al., to give a mixture of diastereomeric peroxides which were separated by column chromatography. (Scheme 1). To our surprise the peroxides were the ozonides 9 and 10 instead of the intended tetraoxane. Their structures were secured with ¹H NMR, ¹³C NMR, MASS, microanalysis.

Scheme 1: Reagents and Conditions: (a) HOCH₂CH₂OH / BF₃·Et₂O / C₆H₆, reflux, 17h; (b) 30% H₂O₂/2N HCl, CH₂Cl₂–CH₃CN, r.t, 18h. (c) LAH, Dry Ether, N₂ atm, 0°C, 1h. (d) (RCO)₂O, Et₃N, DMAP, CH₂Cl₂, r.t, 5–15 min.
Ozonide 9 on reaction with Zn/AcOH furnished diketoester 15 which on treatment with 30% H₂O₂/2N HCl furnished the same mixture of diastereomeric ozonides 9 and 10 (Scheme 2), thus confirming that the two ozonides differ in stereochemistry only at carbon linked with the peroxy group. These ozonides were found to be stable under various conditions such as LiAlH₄ reduction and acetylation and to extensive chromatographic purification techniques. The proposed mechanism for the formation of 1, 2, 4-trioxolanes 9 and 10 from ketoester 8 is shown in scheme 3.

Scheme 2: Reagents and Conditions: (a) Zn / AcOH, r.t., 3h; (b) 50% H₂O₂ / 2N HCl, CH₂Cl₂ - CH₃CN, r.t, 1h.

Scheme 3: Proposed mechanism for the formation of ozonides 9 & 10.

Ozonides 9 and 10 on reaction with Ac₂O/Et₃N furnished the corresponding acetates 11a and 12a of which only compound 11a furnished crystals which were good enough to be analysed by X-ray crystallography. The ORTEP view of the molecule (at 30% probability) with atomic numbering is depicted in Figure 2.
Using conditions similar to that used for the preparation of 11a and 12a, several acyl derivatives 11b-f and 12b, c were prepared. Reduction of ozonide 9 and 10 with LiAlH₄ furnished ozonide alcohols 13 and 14 respectively of which 14 was further converted into acyl derivatives 15a-c. Interestingly, ozonide alcohol 13 (eluted with 5% EtOAc-Hexane) was found to be much less polar than 14 (eluted with 30% EtOAc-Hexane). This might be due to the involvement of intramolecular H-bonding between primary hydroxyl oxygen (O18) and peroxide bridge (O7-O8) in 13 which is absent in 14 due to reverse stereochemistry of the peroxide linkage.

**Biological Activities**

**Antimalarial Activity**

Ozonides 9, 10, 11a-f and 12a-c were evaluated against chloroquine-sensitive strain of *P. falciparum* (NF-54) using minor modification to technique of Rieckmann and co-workers and found to be inactive in this assay. These Ozonides 9, 10 and 11a-f were assessed for their antimalarial activity in vivo against multidrug-resistant (MDR) strain *P.
Yoelii Nigeriensis in Swiss mice at the dose of 96 mg/kg via both oral and i.m. route and found to be inactive.

Antitubercular Activity

Ozonide derivatives 9-15 were assessed for their in vitro antitubercular efficacy against avirulent strain M. Tuberculosis H37Ra at concentration ranging from 12.5 to 0.39 μg/mL. Those found active in this assay were tested further against virulent strain M. Tuberculosis H37Rv in vitro. Of the two stereoisomer 9 & 10, only stereoisomer 10 was found to be active. Compound 10 and its acetate derivative 12a, the most active compound of the series, have shown promising antitubercular activity with the MIC of 0.39 μg/mL against avirulent strains (M. Tuberculosis H37Ra). However, in agar microdilution assay against H37Rv, these compounds have shown MIC value of 3.12 μg/mL. Ozonide alcohol 13 and 14 exhibit antitubercular property similar to that of parent ozonide 9 and 10. 13 was inactive whereas 14 was found to be active and have shown MIC value of 3.12 μg/mL in avirulent strain and 6.25 μg/mL in virulent strain.

Cytotoxicity study reveals that the most active compounds of the series, 10 and 12a, were non-toxic. Of the two, ozonide 10 was tested in vivo against M. tuberculosis H37Rv at 100 mg/kg (Table 5, Figure 4). As can be seen from table 5, there was only a marginal increase (8.17%) in the survival time of the treated mice as compared to the untreated control. Smear examination of the lungs of mice died/sacrificed showed 50% reduction in bacillary load with very slight enlargement in spleens of treated mice (indicating containment of infection) as compared to control mice. The compound seems to cause toxicity in mice at this dose.
Table 5. *In vivo* Antitubercular Activity of Ozonides 10 against *M. Tuberculosis* H37Rv in mice model via oral route.

<table>
<thead>
<tr>
<th>Groups</th>
<th>In vivo Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MST</td>
</tr>
<tr>
<td>Compound 10 Treated</td>
<td>31.00 ± 5.316</td>
</tr>
<tr>
<td>INH treated</td>
<td>33.57 ± 2.439</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>28.14 ± 6.440</td>
</tr>
</tbody>
</table>

Figure 4. Efficacy evaluation of Ozonide 10 in mice model of Tuberculosis.

**Conclusion**

In conclusion, for the first time, a highly convenient two-step conversion of artemisinin into diastereomeric ozonides 9 and 10 has been reported. These ozonides were found to be stable under various organic reaction conditions. Although these compounds have shown poor antimalarial activity, but have shown high order of antitubercular activity *in vitro*. Ozonides 10 and 12a, the most active compound of the series, have shown promising antitubercular activity. Cytotoxicity of these two ozonides were studied and found to be non toxic in cytotoxicity evaluation assay (VERO cells as
well as bone marrow macrophages). Of the two, ozonide 10 was screened \textit{in vivo} in mice model at the dose of 100 mg/kg, it exhibited moderate activity. To the best of our knowledge, this is the first report on ozonides having antitubercular activity.

**Chapter 4 Dihydroartemisinin as Chiral Template for the Preparation of Optically for the Preparation of Optically Pure 1, 2, 4-Trioxanes**

**Introduction**

Our laboratory had earlier developed a new photooxygenation route for the preparation of 1, 2, 4-trioxanes. The key step in this methods are:

(i) Preparation of properly substituted allylic alcohols.

(ii) Photooxygenation of allylic alcohols to give \(\beta\)-hydroxyhydroperoxides, and

(iii) Condensation of \(\beta\)-hydroxyhydroperoxides with an aldehydes / ketones to furnish 1, 2, 4-trioxanes.

\[
\begin{array}{c}
\text{R} \quad \text{OH} \quad \xrightarrow{\text{O}_2} \quad \text{R} \quad \text{O} \quad \text{OH} \quad \xrightarrow{\text{keto}} \quad \text{R} \quad \text{OH} \quad \text{H}^* \\
\text{R} \quad \text{R}_1 \quad \text{R}_1 \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \\
\end{array}
\]

Using this method our group has synthesized a large number of trioxanes. Several of these trioxanes have shown promising antimalarial activity. This methodology, however, produces a racemic mixture of \(\beta\)-hydroxyhydroperoxides which in turn furnish racemic trioxanes. In this chapter, using dihydroartemisinin 2 as a chiral template and geraniol derived alcohol 7, we have explored the possibility of preparation of enantiomerically pure \(\beta\)-hydroxyhydroperoxides and their subsequent use in the preparation of optically pure 1,2,4-trioxanes.
For the preparation of optically active 1, 2, 4-trioxanes, we adopted the following retro-synthetic strategy.

To implement this strategy, BF$_3$OEt$_2$ catalysed condensation of DHQ 2 and acetate alcohol 7 in dry DCM at subzero temperature (-5°C to 0°C) furnished diastereomeric mixture of β- and α-isomers 8a and 8b respectively in the ratio 7:1 in 97% yield which upon purification by column chromatography furnished pure β-isomers 8a and mixture of 8a and 8b (Scheme 2). Deacetylation of 8a with K$_2$CO$_3$/MeOH furnished artemisinin-linked allyl alcohol 9a in 76% yield. Singlet oxygen mediated photooxygenation of 9a in CH$_3$CN in the presence of methylene blue as photo-sensitizer at -10°C to 0°C furnished an inseparable diastereomeric mixture of β-hydroxyhydroperoxide 10 in 21% yield. NaBH$_4$ reduction of 10 furnished inseparable diastereomeric mixture of diol 11 in 65% yield, which on acetylation furnished again inseparable mixture of diacetate 12 in 75% yield. Our strategy was based on the assumption that the diastereomeric mixture of β-hydroxyhydroperoxides would be separable into pure isomers.
Having failed to achieve this separation, we condensed the mixture of hydroperoxides with cyclohexanone hoping that the trioxanes would be separable. Thus, hydroperoxide 10 on acid-catalysed condensation with cyclohexanone furnished artemisinin-linked trioxane 13 in 85% yield as a mixture of diastereomers, which could not be separated by column chromatography.

\(^1\)H NMR, \(^{13}\)C NMR and TLC of 13 showed 50:50 mixtures of two diastereomers thereby indicating that there is no chiral induction in this reaction. This fact was further confirmed from \(^1\)H NMR, \(^{13}\)C NMR and TLC of 12 which also showed 50:50 inseparable mixtures of two diastereomers.
Antimalarial Activity

Having failed in our objective to prepare optically active 1, 2, 4-trioxanes, we assessed the key intermediate and the final trioxane (8a, 9a, 11, 12 and 13) against multidrug-resistant *P. yoelii nigeriensis* in Swiss mice at 48 mg/kg × 4 days by oral route using Peter’s procedure using β-arteether as positive control. All the newly prepared derivatives except 8a and 9a provided 100% protection at 48 mg/kg × 4 days and these compounds were further screened at 24 mg/kg × 4 days. None of these compounds provided 100% protection at this dose.

Conclusion

Using DHQ as chiral auxiliary, we have made an effort to extend our photooxygenation methodology for the preparation of optically pure 1, 2, 4-trioxanes. However, these efforts were not successful. However in this process we have prepared an artemisinin-1, 2, 4-trioxane hybrid which is equipped with two 1, 2, 4-trioxane pharmacophore. This hybrid compound has shown antimalarial activity which is comparable with that of β-arteether.

Chapter 5 Synthesis and Structure-Activity Relationship of Seco Analogs of Artemisinin

Introduction

The discovery of artemisinin 1 as the active principle of the Chinese traditional drug *Artemisia Annua*, is a major milestone in malaria chemotherapy. Artemisinin and its more potent semisynthetic derivatives e.g. artemether 2, arteether 3 and artesunic acid 4, are active against both chloroquine sensitive and resistant malaria (figure 1). These compounds are fast acting and are currently the drugs of choice for the treatment of
cerebral/complicated malaria caused by multidrug-resistant *Plasmodium falciparum*. While these drugs show excellent activity by parental route, they show poor absorption by oral route. While 1, 2, 4-trioxane moiety is believed to be essential for antimalarial activity of these drugs, the extra acetal-lactone or acetal-acetal linkages are linked with their poor hydrolytic stability and therefore poor absorption by oral route.

Several derivatives of artemisinin prepared by replacement of oxygen at C-10 with carbon e.g. 5a, 5b, (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.

![Figure 1. Artemisinin and its derivatives.](image1)

![Figure 2. C-C bond containing artemisinin derivatives 5a and 5b, Synthetic tricyclic 1, 2, 4-trioxanes 5c and 5d, Structure of Prototype 6.](image2)
Posner et al. have reported the synthesis of tricyclic 1, 2, 4-trioxanes 5c, d which are very close analogs of artemisinin. These compounds have been shown to be less active than artemisinin against *P. berghei* in mice (Figure 2). These compounds, however, have the C-12 acetal linkage and therefore, still carry an element of hydrolytic instability.

In this chapter, we report, for the first time, a three-step conversion of artemisinin to hydroxy-functionalized tricyclic 1, 2, 4-trioxane 6 which lacks extra acetal linkages both at C-10 and C-12. We also report the synthesis and antimalarial activity of several ester and ether derivatives of this novel tricyclic 1, 2, 4-trioxane.

**Chemistry**

Our initial strategy to prepare the required tricyclic 1, 2, 4-trioxane 6 is shown in Scheme 3. Accordingly, using the published procedure, artemisinin was reacted with methanol in presence of BF$_3$OEt$_2$ to furnish bicyclic peroxide 7 in 70% yield along with trioxane 10 and diketo compound 11. Reaction of 7 with NaBH$_4$ in MeOH furnished a mixture of tricyclic peroxides 12 and 13 instead of the desired bicyclic peroxy alcohol 8 (Scheme 4).

![Scheme 3. Retro-synthetic approach towards synthesis of trioxane alcohol.](image-url)
The reaction of 7 with other derivatives of NaBH₄ such as NaB(OAc)₃H or NaBH₃CN furnished the same products. The ratio of the peroxy products varied with reaction conditions and reagent used. Peroxides 12 and 13 incidently represents ring-B seco analogs of artemisinin and dihydroartemisinin respectively.

Compound 13 on reaction with MeOH in presence of BF₃.OEt₂ furnished the corresponding ethers 14a and 14b (α : β = 1:1.1) both of which were separated and characterized. 14a and 14b are the ring-B seco analogs of antimalarial drug artemether. Compound 13 on reaction with Ac₂O/Et₃N and succinic anhydride/Et₃N furnished the acetate 15a and hemisuccinate 15b respectively exclusively as α-isomers.

Tricyclic trioxane 10 has been reported as a minor product of acid-catalysed methanolysis of artemisinin. However, its in vivo activity has not been reported. Trioxane ester 10 on LAH reduction furnished trioxane alcohol 10a with OMe group which on benzylation with benzyl bromide in the presence of strong base such as sodium hydride (NaH) furnished benzyl derivative 10b (Scheme 5).

Our failure to achieve the conversion of 7 to the target alcohol required a change in our original plan. Accordingly, peroxide 7 was reacted with LiAlH₄ in dry ether at 0°C to furnish diol 16 in 80% yield. The proper characterization of diol 16 was done by making its diacetate 16a. Diol 16 on reaction with catalytic amount of BF₃.OEt₂ in CH₂Cl₂ furnished the required tricyclic 1, 2, 4-trioxane alcohol 6 in 80% yield (Scheme 6).
Having achieved an efficient conversion of artemisinin to tricyclic alcohol 6, we prepared several ester and ether derivative of this compound. Trioxane 6 on reaction with Ac2O/Et3N, succinic anhydride/Et3N and benzoyl chloride/Et3N furnished the acetate 17a, hemisuccinate 17b and benzoate 17c respectively. Similarly several lipophilic ester 17d-h were made using the same procedure as reported previously in chapter 2.
Scheme 6: Reagents and conditions: (a) LAH, dry ether, N\textsubscript{2} atm, 1 h. (b) BF\textsubscript{3}.Et\textsubscript{2}O, dry DCM, 0°C, 24 h. (c) (CH\textsubscript{3}CO)\textsubscript{2}O/Succinic anhydride/C\textsubscript{6}H\textsubscript{5}COCl, Et\textsubscript{3}N, DMAP, CH\textsubscript{2}Cl\textsubscript{2}, r.t., 4 h. (d) C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}Br, NaH/DMF, N\textsubscript{2} atm, 0°C, 17 h. (e) Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 0°C, 2 h.

Acid chlorides RCOCl (where R are same as 17d-h) were prepared from the corresponding carboxylic acids by heating with thionyl chloride at 50°C-60°C for 2-3 h under anhydrous condition and reacted with trioxane alcohol 6 in the presence of triethylamine in dry dichloromethane at 0°C for 2 h furnished ester derivatives 17d-h in 51-83% yields (Scheme 5). Trioxane 6 on reaction with PhCH\textsubscript{2}Br/NaH at 0°C under N\textsubscript{2} atmosphere furnished the benzyl derivative 18a. Several substituted benzyl ethers 18b-e under the similar conditions used for the preparation of 18a, were prepared using substituted benzyl bromides.
Antimalarial Activity

*In Vitro Antimalarial Activity*

Seco derivatives 6, 7, 12-16, 17a-b, 17e-f were evaluated against chloroquine-sensitive strain of *P. falciparum* (NF-54) using minor modification to technique of Rieckmann and co-workers. Out of all, tricyclic trioxane alcohol 6 and 17a have shown very high order of antimalarial activity *in vitro* with the MIC value of 100 picogram/mL.

*In Vivo Antimalarial Activity*

The objective was to study the antimalarial activity of seco analogues of artemisinin obtained by pruning its tetracyclic framework and to select compounds having activity profile better than that of β-arteether, all the newly prepared seco analogues 6, 10a, 10b, 14a, 14b, 15a, 15b, 17a-h and 18a-e were initially screened against multidrug-resistant *P. yoelii nigeriensis* in Swiss mice at 96 mg/kg × 4 days by both i.m. and oral route using Peter’s procedure.

Seco analogues 6, 10a, 10b, 14a, 14b, 15a, 15b, 17a-h and 18a-e were initially assessed for their antimalarial activity *in vivo* against multidrug-resistant (MDR) strain *P. Yoelii Nigeriensis* in Swiss mice at the dose of 96 mg/kg × 4 days via i.m. route. As can be seen from table 2, none of the seco analogues have shown 100% suppression of parasitaemia at 96 mg/kg × 4 days but several of these compounds have shown moderate suppression of parasitaemia on day 4. Adamantane-based ester 17e, the best compound of the series, have shown 100% suppression of parasitaemia on day 4 by oral route and have shown 81.52% suppression of parasitaemia on day 4 by i.m. route at 96 mg/kg × 4 days. However, none of the mice survived beyond day 28. Parent 1, 2, 4-trioxane alcohol 6 was found to be better in comparison with 1, 2, 4-trioxane 10a, and have shown 71.98% suppression of parasitaemia on day 4 by i.m. route at 96 mg/kg × 4 days. Among acyl
derivatives 17a-h, compound 17e and 17f, have shown better suppression of parasitaemia on day 4 as compared to other derivatives. Among ether derivatives 18a-e, all have shown moderate % suppression of parasitaemia on day 4 at 96 mg/kg × 4 days in the range 50-67%.

We made an attempt to synthesize the desired tricyclic 1, 2, 4-troxane alcohol 6 using khusol 7 as starting material by carrying out photooxygenation of khusol acetate 7a but we were unable to obtained it.

\[
\begin{align*}
19, & \quad R = H \\
19a, & \quad R = \text{COCH}_3
\end{align*}
\]

**Conclusion**

We have developed a highly efficient route for the conversion of artemisinin to a new series of tricyclic 1, 2, 4-troxane alcohol 6. We have also prepared several seco-analogs of artemisinin. Peroxides 7, 10a, 10b, 12, 13, 14a, 14b, 15a, 15b and trioxane 6 and its derivatives 17a-f and 17a-e were assessed for their antimalarial activity against multidrug-resistant *Plasmodium yoelii nigeriensis* in mice. Our results show that all these seco analogs are less active than artemisinin, thus, underlying the importance of tetracyclic system present in artemisinin.