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Recent Advances in Antimalarial Drug Discovery – Challenges and Opportunities

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Abstract

Malaria drug discovery is a challenging and difficult task due to the unavailability of the vaccine and lack of newer drugs. The most potent artemisinin and its derivatives, widely used in combination therapies for curing malaria worldwide are also now falling to resistance in some parts of the world. Thus, to combat malaria, new drugs possessing high therapeutic value, minimal toxicity, rapid efficacy and low cost are urgently needed. In this chapter, we will provide an integrated overview on the challenges and opportunities in malaria drug discovery with more emphasis on synthesis of peroxidic antimalarials.

Keywords: Natural Products, Quinine, Chloroquine, Artemisinin, Trioxane, Tetraoxane, Malaria, Antimalarials

1. Introduction

1.1. History

The chronicle of malaria predating humanity is as ancient as mankind.[1] Malaria continues to be a persistent menace wreaking havoc especially in tropical and subtropical regions despite tremendous efforts toward its control and eradication. The unavailability of the vaccine and the emergence of resistance in the parasite against nearly all existing antimalarial drugs have attracted attention of researchers to modify the existing antimalarial drugs with improved efficacy over older therapies and identify new compounds as appropriate clinical candidate. Mortality from malaria is increasing at an alarming rate despite various renewed efforts and

eradication campaigns[2] because the parasites (*Plasmodium* strains) responsible for the majority of fatal infections have become resistant to the existing drugs. Malaria is also the cause of poverty and a major hindrance to economic development, especially in sub-Saharan countries.[3] Mostly, malaria is spread due to local transmission through female anopheles mosquitoes. Occasionally, it can also be transmitted by exposure to infected blood products (transfusion malaria) and also through congenital transmission. The major species of *Plasmodium* strains that infect humans are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Among these, *P. falciparum* causes the most severe form of infection, which could be fatal.

The original picture of the parasitic existence and passage of malaria through historic times remains blurred. It is uncertain whether the human population settlements preceded the arrival of malaria within them.[3] The versions may vary from tentative to widely accepted or even controversial based on the general scientific evidence. However, the effect of malaria wreaking havoc to the human species is prominent, clear, and unmistakable. There was no specific treatment for malaria until the 17th century.[4] The discovery of quinine from the bark of *Cinchona calisaya* began effective treatment of malaria. Further, the synthesis of chloroquine by Hans Andersag in 1934 introduced a cheap antimalarial drug and a substitute for quinine. [5] Until the widespread resistance in 1960, quinoline-related antimalarial drugs played an important role in the treatment of malaria. Fortunately, in 1972, the Chinese discovered artemisinin from sweet wormwood plant *Artemisia annua*. [6] Artemisinin along with its derivatives artemether, arteether (artemotil), and artesunate are the main treatment for malaria that is resistant to conventional therapies.

Recent advances in the molecular genetics and biochemical technologies available for the investigation of malaria parasites within the last half century have enabled us to gain a unique perspective on the human health and health services in relation to malaria.[7]

1.2. Life cycle of malaria parasite

The life cycle of malaria parasites is very complex. It is completed inside two hosts, including the humans (asexual) and the mosquitoes (sexual) (Figure 1).[8, 9] Malaria infection begins when an infected female anopheles mosquito feeding on human blood bites and injects sporozoites into the bloodstream. The parasites then quickly reach liver to form merozoites by asexual multiplication. Subsequently, merozoites exit liver with the rupture of hepatic tissues and enter the bloodstream where they invade and disintegrate red blood cells. Some merozoites transform into gametocytes, which are then circulated in the bloodstream. When the second mosquito bites an infected human, it gets infected and intakes gametocytes. The sexual transformation of gametocytes into ookinetes and ookinetes into oocyst takes place inside the midgut of mosquito. Finally, sporozoites are developed from oocysts, which eventually burst, releasing sporozoites into the salivary gland. Continued infection in humans and mosquitoes alternatively propagates and spreads malaria.

A comparative study with human and rodent parasites revealed the activities of current antimalarial drugs on the life cycle stages of plasmodium.[10] 8-Aminoquinolones are known to be active for liver stage. The most currently available antimalarial drugs primarily target the human blood cell stage. In addition to the asexual blood stage, some drugs (viz., pyronar-

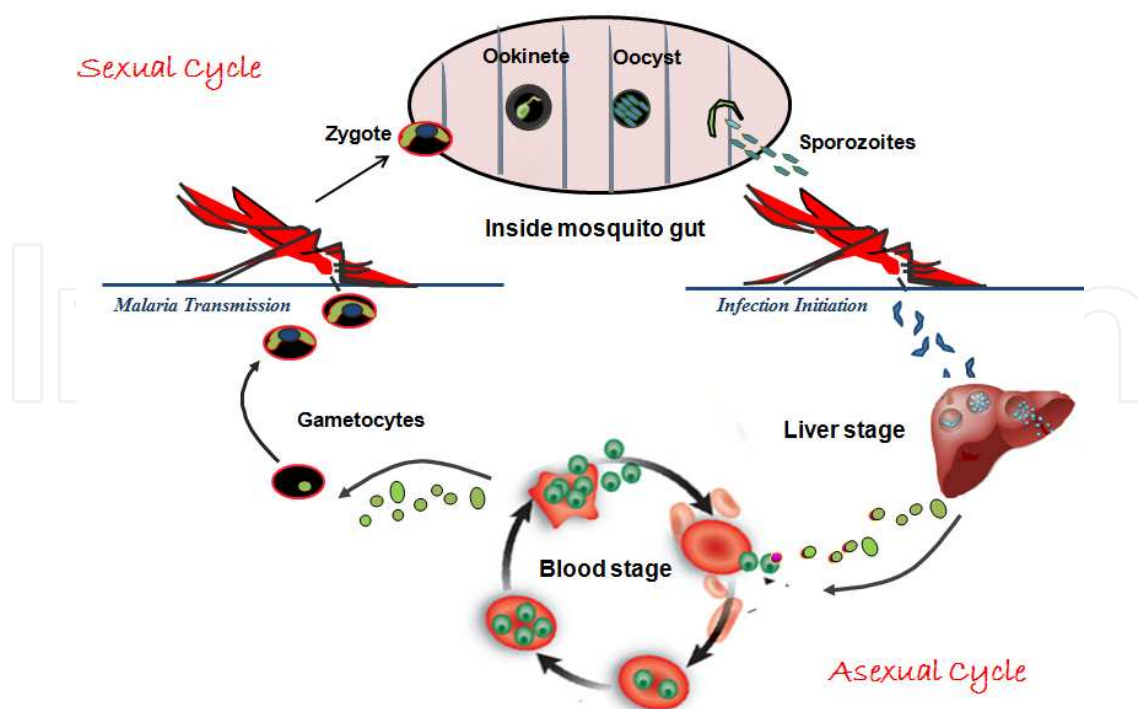


Figure 1. Life cycle of malaria parasite.[9]

idine and atovaquone) can also target both liver and sexual stage. Further, new stable synthetic endoperoxides can inhibit gamete formation and gametocyte maturation.[10] Furthermore, it is important to profile the currently available drugs for specific stage in parasite's life cycle to combat malaria by eradication and circumventing resistance.

1.3. Status quo

WHO has recommended artemisinin combination therapy (ACT) for the treatment of malaria. [11] Since 2006, artemisinin-based combination therapies remain as the first-line treatment for *P. falciparum* malaria replacing chloroquine and sulfadoxine/pyrimethamine. Combined with other drugs, its derivatives, such as artesunate and artemether, can clear symptoms of malaria in three days. However, a rise in demand has led to a shortage of artemisinin. Artemisinin-based drugs are also more expensive than conventional treatments, in part because large doses are required. Further, with recent reports on the emergence of resistance to artemisinin,[12] it can be foreseen that in the near future, new armamentarium will be required to fight against malaria. Thus, to overcome this problem, there is an urgent need to identify new chemotypes or reexamining old molecules to transform them into an appropriate clinical candidate.

2. Drug resistance

The greatest challenge to malaria control and eradication is the emergence of malaria parasites that are resistant to antimalarial drugs.[13] The reemergence of malaria from the areas where

it was eradicated and spread of malaria to new areas is a major threat. The World Health Organization defined antimalarial drug resistance as the “ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject.”[14] It was modified later to specify that the drug in question must “gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action.”[15] Antimalarial drug resistance occurs through spontaneous mutations that reduces the sensitivity to a given class of drug(s).[16] Only a single point mutation is sufficient to confer resistance to some drugs, while multiple mutations appear to be required for others.

Sl. no	Drug class	Drug	Resistance	Mechanism of action
1.		Chloroquine	Since 1945	
2.	4-Aminoquinoline	Amodiaquine	Yes	Inhibition of hemozoin formation
3.		Piperaquine	Since 1980s	
4.		8-Aminoquinoline	Primaquine	
5.		Quinine	Yes	
6.	Aryl-amino alcohol	Mefloquine	Since 1985	Unknown
7.		Lumefantrine	No	
8.		Pyrimethamine	Since 1967	
9.	Antifolates	Trimethoprim	Yes	Inhibition of DHFR
10.		Proguanil	Since 2000	
11.		Sulfonamides	Yes	
12.	Napthoquinone	Atovaquone	Since 2000	Inhibition cytochrome
13.	Antibiotic	Doxycycline	No	Inhibition of protein synthesis and apicoplast
14.		Clindamycin		
15.		Artesunate	Yes	
16.	Artemisinin	Artemether	Since 2001	Free radical mechanism Heme alkylation
17.		Dihydroartemisinin		

Table 1. Status of resistance in antimalarial drugs.

The malaria parasite has developed some level of resistance against nearly all previous generation antimalarial drugs (Table 1). Recent research has confirmed evidence of artemisinin resistance.[10] Although it is under investigation, immediate actions are needed to restrict resistance to artemisinin from spreading to new areas. It is high time that we should fight this

overwhelming menace with improved tools to aim at controlling the mosquito vector and develop new armaments; otherwise, the future looks bleak and grim.

3. Mechanism of action

The mechanism of action of antimalarial drugs is based on the extensive studies of selected drugs. Most drugs available for the treatment were discovered based on the serendipitous identification of active compounds (natural, synthetic, and semisynthetic).[17] The progress in the understanding of the biochemistry of malarial parasite has shed light on the mechanism of action of new as well as older drugs.

It is believed that artemisinin and related drugs are transported to the food vacuole of the parasite, where they generate free radicals upon interaction with Fe(II)-heme. These free radical's interaction with heme generates oxidative stress and kills the parasite.[18] The mechanism of action of quinoline and related drugs is also well established.[19] It is shown that the drugs enter the RBC and inhabit the digestive vacuole of parasite by simple diffusion. The subsequent inhibition of hemozoin biocrystallization leads to the aggregation and accumulation of cytotoxic heme in food vacuoles resulting in parasite's death. The commercially available quinolone antimalarials target the gyrase and inhibit DNA replication. It results in the delayed death of treated parasites by formation of abnormal apicoplasts.[20]

Based on the mechanism of action, different groups of antimalarials can be classified as follows:

- Artemisinin: binds heme iron and generates oxygen radicals
- Antifolate: inhibits DNA synthesis
- Atovaquone: collapses mitochondrial membrane potential
- Quinoline: inhibits heme crystallization
- Antibacterial: ribosome and DNA gyrase inhibition

4. Toxicity of the antimalarial drugs

The most important determinant of drug use and its effectiveness is the patient compliance. The toxicity of the drug must be balanced with the efficacy of the drug and the risk from malaria, i.e., the drug should cause less harm than the disease itself. The doses given to the patients should be taken into account in determining the treatment of malaria. The assessment of the tolerability of many antimalarial drugs is ongoing, but evaluating adverse drug reactions, events, side effects, and drug-related toxicity is difficult due to the unavailability of good techniques to measure the side effects.[21]

The most promising naturally occurring sesquiterpene lactone drug and its derivatives (artemether, arteether, and sodium artesunate) did not show any serious side effects. However,

insufficient clinical trials to detect the toxicity stopped us from declaring artemisinin 100% safe. However, they have excellent safety profile and remarkable efficacy. The current knowledge obtained from the laboratory and clinical study is that the long-term availability of artemisinins may cause toxicity (rarely produce neurotoxicity and allergic reactions).[22] The short-term peak concentrations followed by rapid elimination of artemisinins after oral intake is relatively safe compared to administration by intramuscular injection. Evidently, the majority of animal experiments showed considerable toxicities in contrast to human studies.

Chloroquine, considered being a safe drug even at higher doses, also causes mild side effects such as reversible effect on optical accommodation, which can potentially affect eyesight. It also binds irreversibly to melanin. Hence, the patients with rheumatoid arthritis treated with the long-term use of high dose chloroquine suffer from accumulation of chloroquine in retinal melanin. Some reports also suggest that chloroquine administered to patients with light intolerant disease can aggravate psoriasis.[22] Proguanil is also assumed to be safe at a dose of 200 mg a day. However, for doses higher than 200 mg, there are reports of reversible alopecia and aphthous ulceration, nausea, and gastric irritation.[23] These side effects are common with other antimalarial agents as well. The combination of chloroquine with proguanil has good tolerability. However, gastrointestinal upset and mouth ulcers are still observed. Sulfadoxine/pyrimethamine is also well tolerated, but it is no longer used because it causes Stevens–Johnson syndrome and toxic epidermal necrolysis. Mefloquine is another valuable drug for the treatment of malaria. Despite good tolerability to most patients, dose-related serious neuro-psychiatric toxicity can occur. Cardiovascular or CNS toxicity is rare for quinine but hypoglycemia may occur. Further, due to its potential for cardiotoxicity, halofantrine is unsuitable for widespread use. Mepacrine, sulfonamides, dapson, and amodiaquine are also withdrawn from the use because of the high frequency of adverse side effects.[24]

5. Malaria vaccine

Malaria vaccine development is a challenging and difficult task because of the antigenic complexity and the complex life cycle of malaria parasite. Research on the development of malaria vaccine is of prime importance because such a discovery can prevent millions of deaths worldwide. The currently available tools are insufficient for malaria eradication. Malaria vaccine could be a transformative tool to help in reduced transmission and future eradication. Extensive research has been carried out in the last two decades, and several vaccines have reached clinical trials, but there is none in the clinical practice due to insufficient immunogenicity. Although parasite vaccines are in development, there is no FDA-approved vaccine for organisms more complex than viruses and bacteria.[25]

5.1. Scientific challenges

The significant hurdle in the development of malaria vaccine is insufficient knowledge about the malaria parasite. Understanding the structure and antigenic variation of parasite popula-

tion requires lengthy, tedious, and difficult lab and field studies. Antigenic variation and parasite polymorphism also create a major scientific barrier. Unfortunately, in nature, there are not many good examples of immunity to malaria, and many vaccine development programs are based only on naturally acquired immunity. Since the mechanism of immune protection is still unknown, it is difficult to comprehend why certain people are protected while others are not. Inadequate animal models and lack of clarity in the definition of desired outcomes create confusion in choosing the best approach to develop a malaria vaccine. Even in particular animal model systems with defined outcomes, there is always uncertainty in translating the success of protection in the model systems with success in humans.[26]

The malaria vaccine development includes recombinant proteins, gene-based (DNA or viral vector) vaccines, attenuated whole organisms, peptides, and prime-boost strategy, which involves a combination of different antigen delivery systems encoding the same epitopes or antigen using various adjuvants. Reports dating back to 1960s[26] demonstrated species-specific and strain cross-reactive protection on immunization with radiation-attenuated sporozoites in primate and experimental rodent models. Studies showed optimistic levels of protective immunity. However, the volunteers immunized against multiple strains of *P. falciparum* malaria were not protected against *P. vivax*. The target antigens were identified from the sera cells of experimental hosts immunized with attenuated sporozoite vaccine and protected volunteers. Circumsporozoite protein, the first cloned and sequenced malaria parasite in *P. knowlesi* and *P. falciparum*, is also the first antigen identified by serological screening. It plays an important role in protection. When the sporozoite was irradiated in the rodent models, antibody and cells showed different roles in malaria species and different strains. Although multifaceted cellular responses are observed, the basic mechanism of immunity is believed to target the intracellular hepatic exoerythrocytic forms by the production of interferon. The antibody eliminates most of the infectious sporozoite inoculum, when the vaccines prove a multipronged approach. The cellular responses target the rest of the intracellular exoerythrocytic forms by direct cytotoxicity or inhibitory cytokines.

The understanding of the research related to vaccine development is greatly benefitted by the lessons learned from discontinued and inactive projects. Recent findings allow us to be optimistic about the possibility of an effective malaria vaccine. Several malaria vaccine candidates have entered field trials. It is now possible to impact the host-parasite relationship using different platforms through vaccine-induced immune responses to multiple antigenic targets. The field has grown rapidly over the last two decades from the first clinical trials to the successful conduct of large-scale field studies, and substantial progress has been made in evaluating many antigens. Despite the daunting task, researchers have produced surprising progress in several areas. The malaria vaccination program has progressed to an assessment and clinical evaluation of RTS,S/AS01E in phase 3 trial.[27] The first malaria vaccine may be considered for licensure in the coming years. Further, there is a possibility of developing more efficacious second-generation vaccines. Researchers are now better equipped to establish clear product profiles. The lessons learned in terms of safety, immunogenicity, efficacy, and trial methodology from malaria vaccine research is summarized in Table 2.

Parameters	Remarks
Safety	· It is often lower in semi-immune populations living in endemic areas than in naïve populations
	· Reactogenicity in young children has not been worse than in adult populations
Immunogenicity	· DNA alone is poorly immunogenic
	· Little clinically significant interference is observed between vaccine antigens and the malarial antigen
Efficacy	· Only RTS,S-based vaccines proved to be effective to reduce morbidity in endemic areas
	· Highly polymorphic blood-stage antigens have tended to lead to allele-specific efficacy, but poor efficacy against the population of circulating strains
Methodology	· In vitro studies and animal studies does not correlate well
	· For testing of new malaria vaccines, ethical and methodological issues may arise
	· There is a need to make formal trial design for phase trials and sample size calculations. Epidemiological studies are required to assess the effectiveness of mosquito antigen vaccines in sexual stage

Table 2. Lessons from two decades of malaria vaccine development research.

6. Antimalarial drugs

Malaria, existing in over 100 countries, is one of the deadliest infectious diseases and major health problem worldwide. Antimalarial drugs are designed to cure malaria, many of which are in market.[28] From the 17th century onward quinine had been the drug of choice for the treatment of malaria. Later on, medication therapies heavily relied on chloroquine, primaquine, mefloquine, etc.⁴ These drugs especially chloroquine have saved more lives than any other drugs in history. Recently, artemisinin and its derivatives have emerged as a new generation of antimalarials (Figure 2).

There is a critical need to develop newer synthetic and more effective drugs that could address the issues associated with the existing and traditional drug therapies. The availability of artemisinin also causes supply constraints because artemisinin and its derivatives constitute an active ingredient of many combination therapy dugs. For example, Coartem contains a fixed combination of artemether and lumefantrine. In 2012, Ranbaxy also launched a new synthetic peroxide antimalarial drug Synriam™ in the market in line with the recommendations of the WHO. It is a fixed dose combination of arterolane maleate and piperazine phosphate. The chemical structures are shown below.

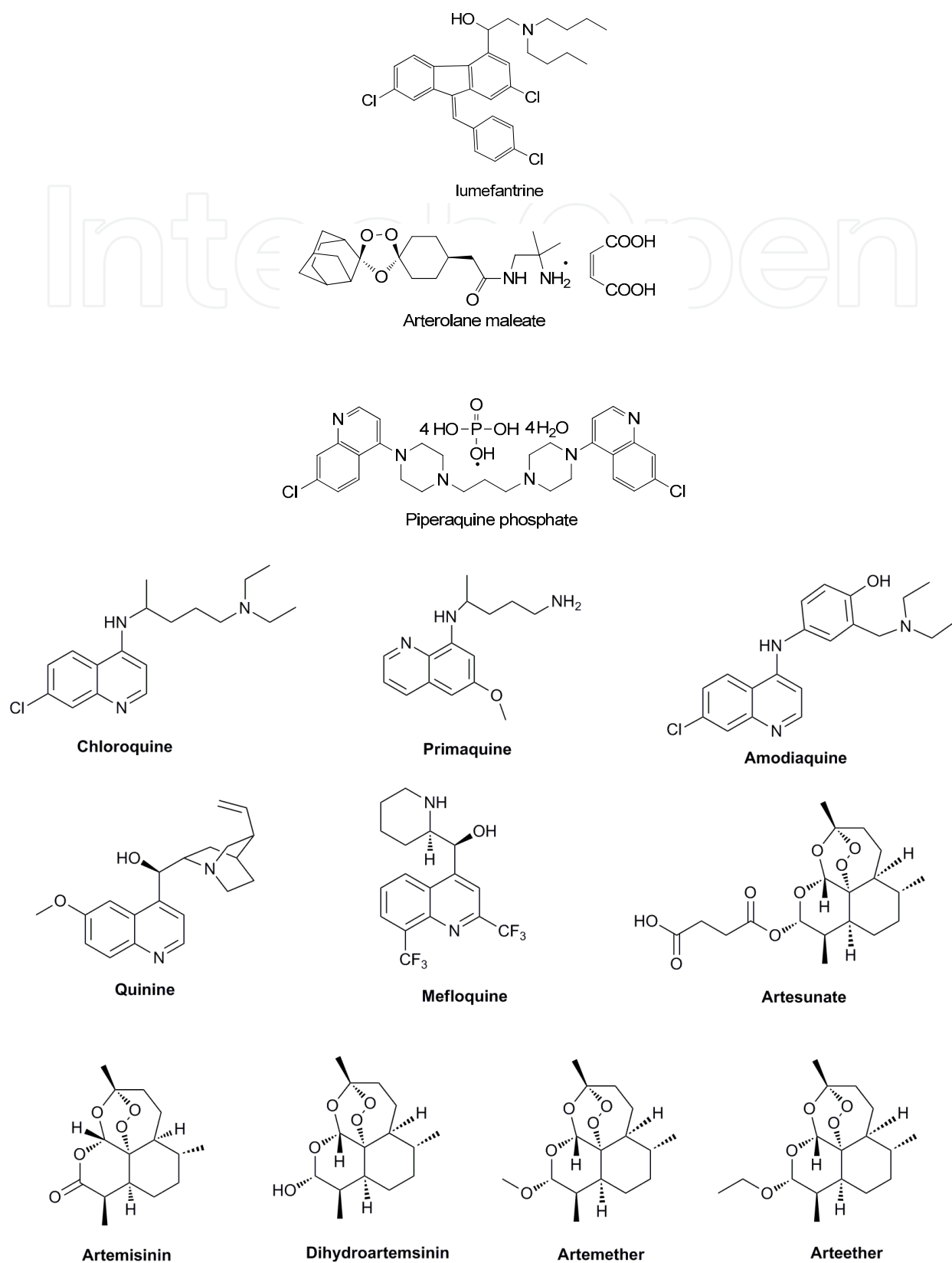


Figure 2. Antimalarial drugs.

Research groups across the world are united in the efforts to discover new chemicals for the treatment of malaria. Attempts to modify the established drugs are also ongoing. Long-term hopes are resting on the modification of the synthetic artemisinin-based drugs containing endoperoxide rings. The following sections will mainly focus on the development of peroxidic antimalarial agents.

6.1. Natural products

Natural products continue to make an immense contribution to malaria chemotherapy. The discovery of quinine and artemisinin proves that nature is a rich source of lead compounds that can provide cure and medicine for malaria. Nature has been extremely generous when it comes to search of new molecular scaffolds for good malarial activity. These scaffolds later serve as template for the development of structurally diverse analogues with more potent activity.[29] For example, quinine a bitter-tasting alkaloid, is one of the earliest natural compounds that helped man in the fight against malaria. It was isolated from the *Cinchona* bark. Later, it also served as a template for the synthesis of more potent and structurally simpler analogues such as chloroquine, primaquine, mepacrine, and mefloquine (Figure 2). Artemisinin extracted from *Artemisia annua* is another example whose diverse pharmacological potential has attracted the researchers worldwide. Artemisinin also gave rise to the development of dihydroxyartemisinin, artemether, arteether and artesunate. Thus, natural products such as quinine and artemisinin have demonstrated the enormous potential of nature in providing lead compounds, which can be further manipulated structurally for the development of more effective antimalarial agents. Many more natural products possessing various chemical structures, such as alkaloids, steroids, chalcones, terpenes, flavonoids, peptides, quinones, xanthenes, coumarines, naphthopyrones, polyketides, phenols, lignans, chromenes, etc., have been tested as antimalarial drugs.[30, 33]

6.2. Semisynthetic drugs

The success of the most potent antimalarial drugs, quinine and artemisinin, has brought some optimism. Due to the widespread emergence of drug-resistant chloroquine, primaquine, mepacrine, and mefloquine (Figure 2) were developed. Despite the remarkable antimalarial activity, artemisinin suffers from limited availability, low solubility, high cost, metabolic stability, short half-life, poor bioavailability, and chemical stability. Thus, there is a need for new compounds more active than the parent artemisinin. To circumvent some of these problems, semisynthetic analogs were prepared. The reduction of artemisinin yields dihydroartemisinin, and the lactol group can be further converted to its ether (artemether, arteether, and artelinic acid) and ester (sodium artesunate) derivatives.[34]

6.3. Synthetic drugs

Artemisinin, a sesquiterpene endoperoxide, has established the role of peroxide ring for potential antimalarial activity. However, the naturally isolated artemisinin is available in short supply and expensive to synthesize. As a consequence, extensive research directed towards the discovery of peroxidic antimalarials inspired researchers to explore structurally simple

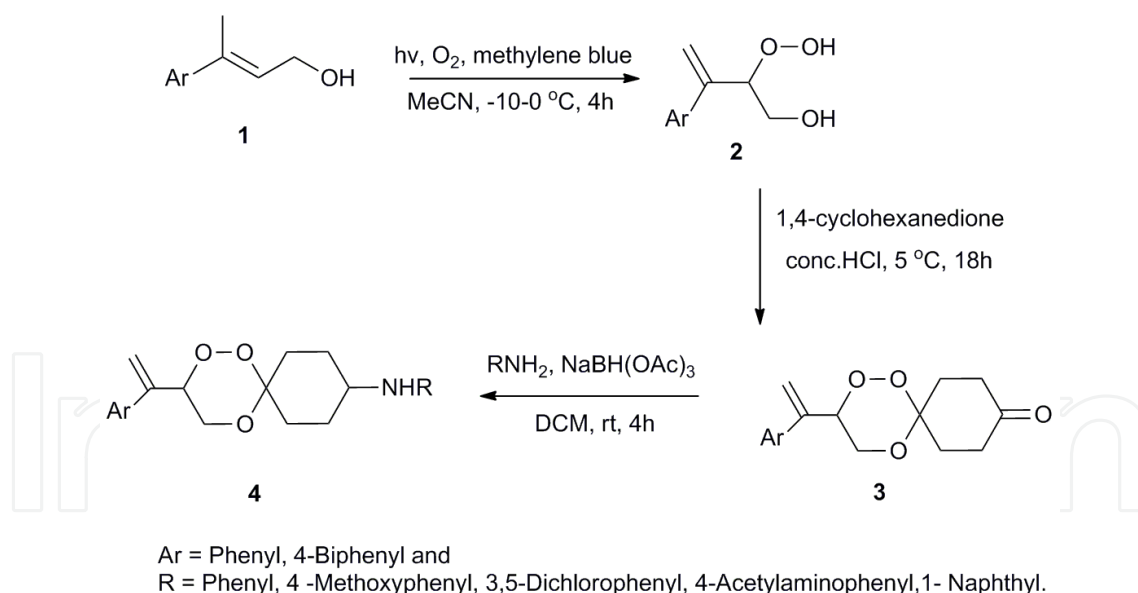
peroxides. Trioxanes, tetraoxanes, and their hybrids were consequently identified as promising candidates for the development of next generation antimalarial drugs.

6.3.1. Various synthetic procedures for the synthesis of trioxanes

Trioxanes can be synthesized from inexpensive starting materials, and their scale-up preparations are feasible. Most methods reported for the synthesis of trioxanes starts with the reaction of singlet oxygen with carbonyls in the presence of Lewis acids. Then acid-catalyzed cyclization of hydroxyperoxides with olefins and reaction of α -peroxy aldehydes with carbonyl compound yields trioxanes in good yields. Many synthetic strategies were developed for the synthesis 1,2,4-trioxanes, which are described below.

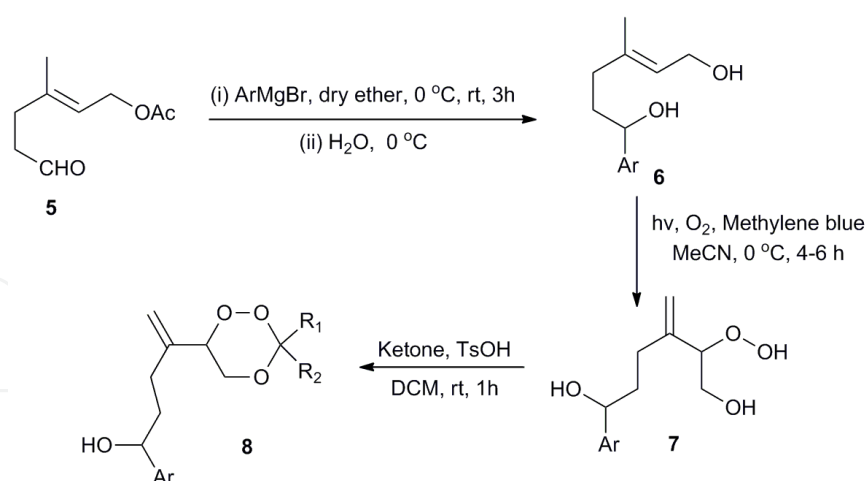
6.3.1.1. Photooxygenation method

Starting from commercially available cyclohexanediones, tricyclic 1,2,4-trioxanes can be synthesized by following simple method. Briefly, photooxygenation of the electron-rich allylic alcohols **1** using singlet oxygen gives β -hydroxyperoxide **2**. Further, β -hydroxyperoxide **2** was condensed with 1,4-cyclohexadiene followed by Lewis acid-mediated cyclization to give keto-trioxane **3**. Amino functionalized trioxanes **4** were also synthesized on reductive amination with various amines in the presence of sodium triacetoxy borohydride (Scheme 1).[35]



Scheme 1. Photooxygenation method for trioxane synthesis.

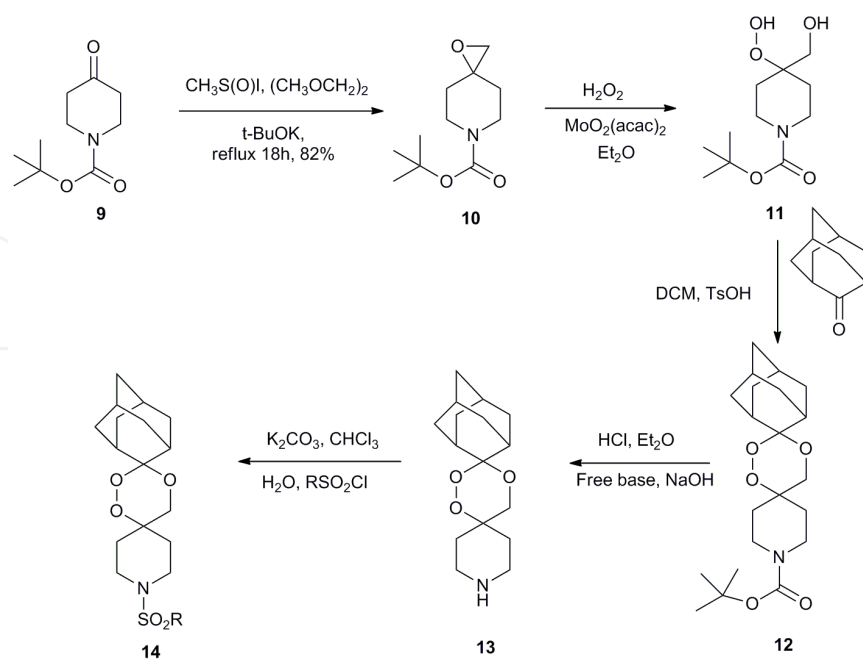
In another synthetic procedure, the geranyl acetate was transformed into aldehyde acetate **5**, which is converted into allylic alcohol **6**. Photooxygenation of **6** followed by subsequent acid catalyzed condensation of β -hydroxyhydroperoxides **7** with various ketones resulted in the formation of new 1,2,4-trioxanes **8** (Scheme 2).[36] The hydroxyl functionalized side chains can be further manipulated for the synthesise of a diverse library of compounds.



Scheme 2. Synthesis of Geraniol derived 1,2,4-trioxanes.

6.3.1.2. Epoxidation method

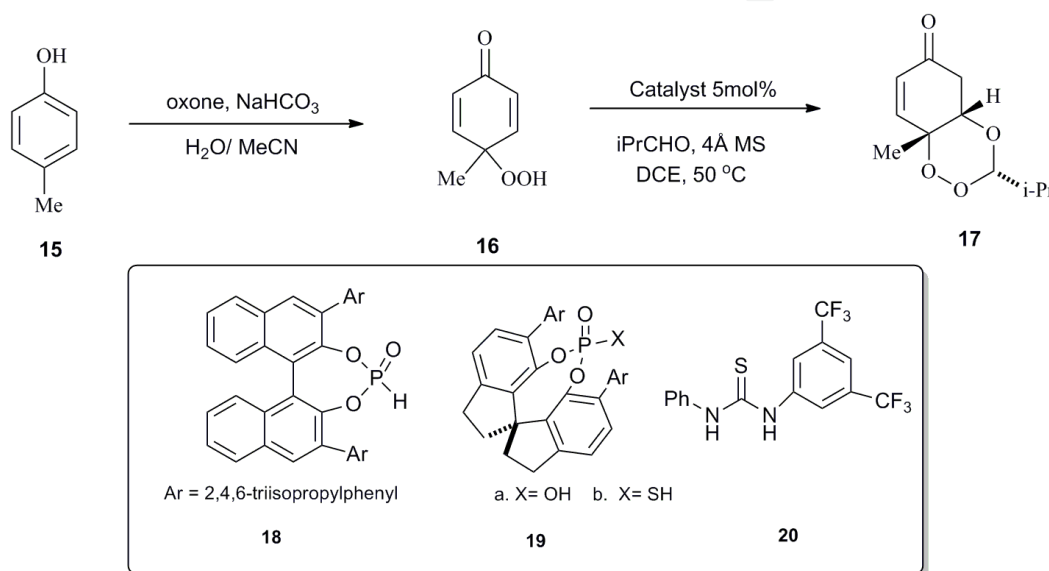
The epoxidation of N-Boc piperidone **9** gives N-Boc spirooxirane **10**. Dispiro N-Boc-protected 1,2,4-trioxane can then be synthesized by MoO₂(acac)₂ catalyzed perhydrolysis of N-Boc spirooxirane **10**, as shown in Scheme 3.[37] Subsequent condensation of the resulting β-hydroperoxy alcohol **11** with 2-adamantanone gives N-Boc 1,2,4-trioxane **12**, which can be converted into the amine 1,2,4-trioxane hydrochloride salt **13**. Further, alkylation may result in a diversified sulfonamide trioxane derivatives **14**.



Scheme 3. Trioxane synthesis using epoxidation method.

6.3.1.3. Catalytic enantioselective synthesis

Trioxanes can also be synthesized by catalytic enantioselective synthesis. Para-cresol **15** is converted into *p*-peroxyquinols **16**. The desymmetrization of *p*-peroxyquinols **16** occurs via an acetalization/oxa-Michael cascade reaction (Scheme 4).[38] The reaction proceeds via a dynamic kinetic resolution of a peroxyhemiacetal intermediate. Various derivatized trioxanes **17** can be easily obtained by this method. The use of chiral Brønsted acid catalyst **18** gave a single diastereomer trioxane **17** in 86% ee, while using bis-(2,4,6-triisopropylphenyl)spirobiindane phosphoric acid **19** gave 96% ee. The use of thiourea **20** as cocatalyst helped to restore the reactivity even at lower catalyst loading.



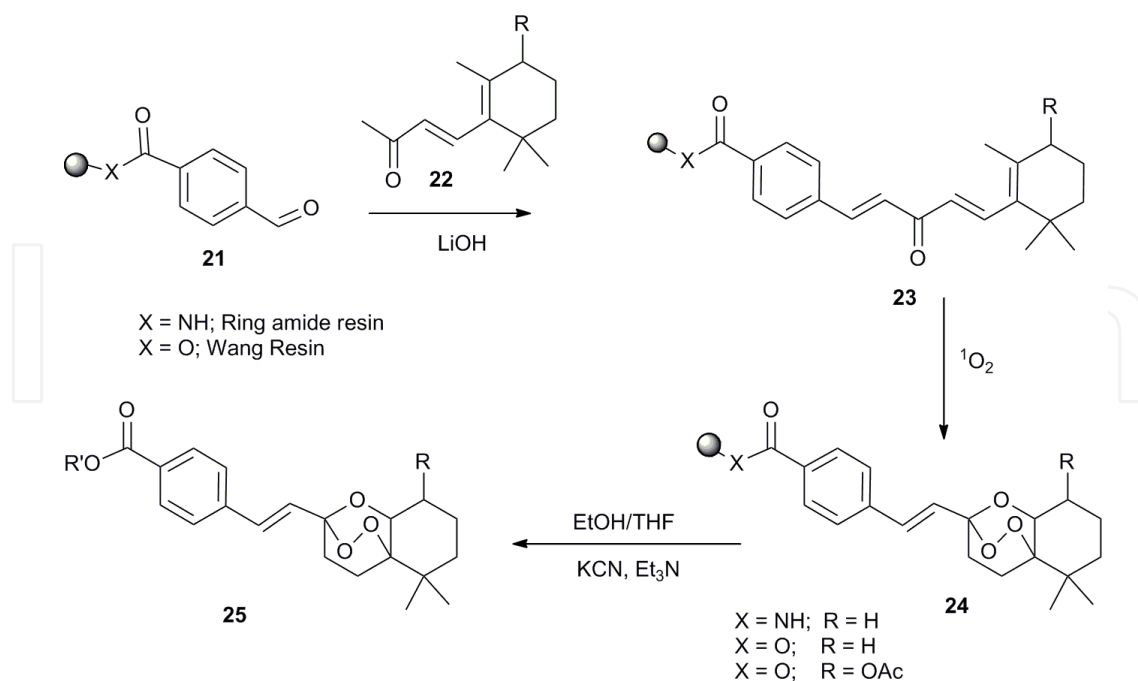
Scheme 4. Enantioselective synthesis of trioxanes.

6.3.1.4. Solid phase synthesis

The solid support synthesis of 1,2,4-trioxanes also needs light mediated oxygenation on polystyrene polymer support. Wang and Rink amide resins can be used as linkers. The reaction of resin-bound *p*-carboxybenzaldehydes **21** with excess of ionone derivatives **22** gave immobilized dienones **23** in the presence of LiOH in DME (Scheme 5).[39] Resin-bound trioxane **24** was obtained upon irradiation of compound **23** with UV light (354 nm) in toluene yielded. After cleavage from the solid support, the formation of **25** was confirmed by ¹³C NMR. Peaks at 82.4 and 94.4 ppm corresponded to the peroxy-bearing carbon and peroxyketal carbon of the trioxane ring system.

6.3.2. Various synthetic procedures for the synthesis of tetraoxanes

The chemical modification of artemisinin retaining the crucial endoperoxide ring has resulted in yet another simplified structure known as 1,2,4,5-tetraoxane. Tetraoxanes show significantly higher stability and exhibit even higher activity than natural peroxidic drugs for curing malaria



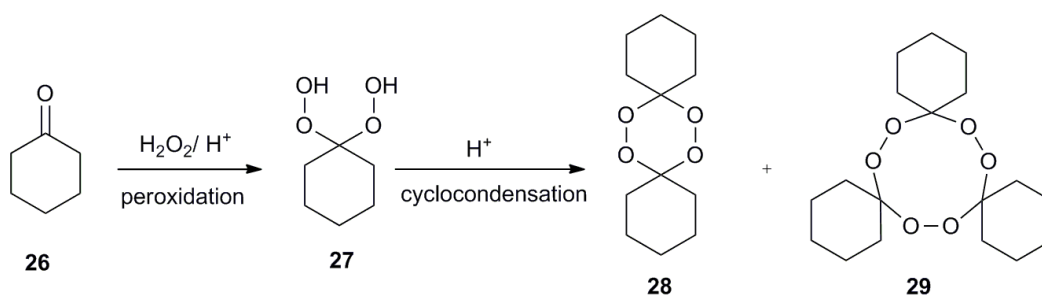
Scheme 5. Solid phase trioxane synthesis.

infections. In 1899, Baeyer and Villiger reported the synthesis of the first dimeric acetone peroxide upon treatment of acetone and Caro's acid in ether. Since then, the field has moved ahead significantly and newer synthetic routes and efficient methodologies were developed. The synthesis can be carried out by several methods as described below.

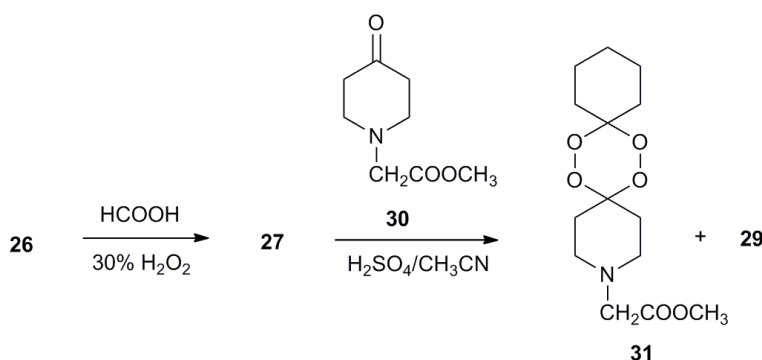
6.3.2.1. Peroxidation method

The most commonly and widely used method for tetraoxane synthesis is known as peroxidation method. In this method, acid-catalyzed cyclocondensation of ketones or aldehydes gives the *gem*-dihydroperoxide as an important active intermediate. Generally, the acid-catalyzed addition of hydrogen peroxide to carbonyl compound **26** produce *gem*-dihydroperoxide **27**, which on subsequent cyclocondensation in the presence of strong acid such as sulfuric acid, perchloric acid, or methanesulfuric acid yield more stable symmetrical tetraoxane **28** along with side product hexaoxane **29**, as shown in Scheme 6. It is also known that the trimeric cyclic peroxide by-product hexaoxonane is formed in the presence of excess hydrogen peroxide. Dimethyl sulfide and potassium iodide can be used for the removal of hydroperoxide-related impurities. Hexaoxonanes could be removed by washing the reaction mixture with cold methanol. [40]

In our lab, we also attempted the synthesis of a new series of tetraoxane by incorporating nitrogen within the cyclohexyl ring. [41] Methyl 2-(4-oxopiperidin-1-yl)acetate **30** on reaction with *gem*-dihydroperoxide **27** may give very small amount of tetraoxane **31** and trimer **29**, as shown in Scheme 7. We characterized hexaoxonane **29** as a main side product by spectroscopy and x-ray crystallography.



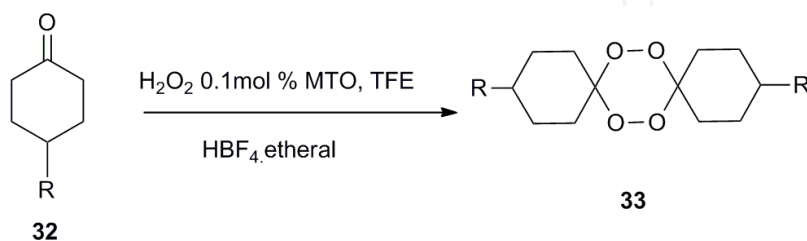
Scheme 6. Acid catalyzed synthesis of tetraoxanes and hexaoxonanes.



Scheme 7. Synthesis of piperidinetetraoxane

6.3.2.2. One pot synthesis

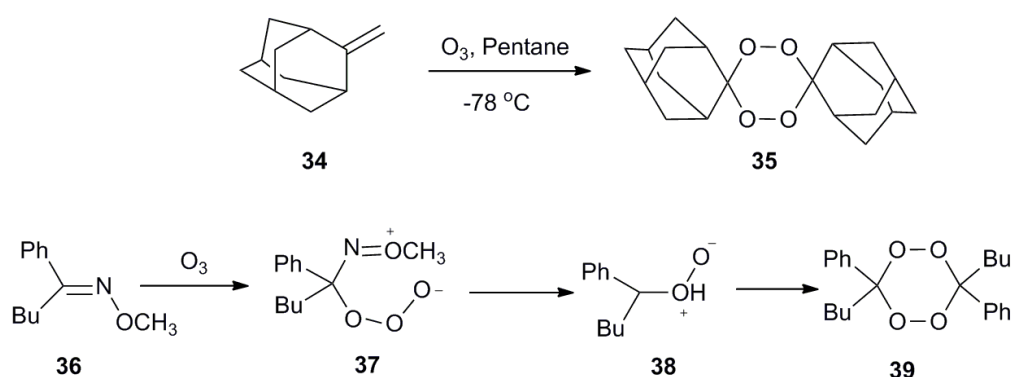
Iskara et al.[42] developed the first one-pot synthesis of tetraoxane. Simple carbonyl compounds **32** in the presence of $30\% \text{H}_2\text{O}_2$, $0.1\% \text{MTO}$, and fluororous alcohols (TFE and HFIP) selectively gives tetraoxanes **33** (Scheme 8). Fluorous solvents TFE and HFIP activate both H_2O_2 and MTO for oxidation reactions. The one-pot synthesis of mixed tetraoxanes begins with the oxidation of the most reactive carbonyl compound, and then less oxidizable carbonyl compound is added in the presence of acid. In this reaction, no trimeric product is formed.



Scheme 8. One pot tetraoxane synthesis.

6.3.2.3. Ozonolysis method

The most prolific strategy for the synthesis of tetraoxanes is the ozonolysis of suitable olefins and oximes. This method has dual advantage over others: (1) the absence of hexaoxonane (a usual by-product), which is very common in acid catalyzed reactions, and (2) it is useful for the synthesis of aromatic tetraoxanes, which could not be obtained by other methods. In the 1970s, Keul et al. reported the synthesis of dimeric adamantane peroxide **35** by ozonization of methyleneadamantane **34** in pentane at -78°C . The ozonolysis of valerophenone oxime *o*-methyl ether **36** produces carbonyl oxide **38** via an intermediate ozonoid **37** to give the crystalline dimeric valerophenone peroxides **39** in the absence of carbonyl compounds or protic solvents.[43]



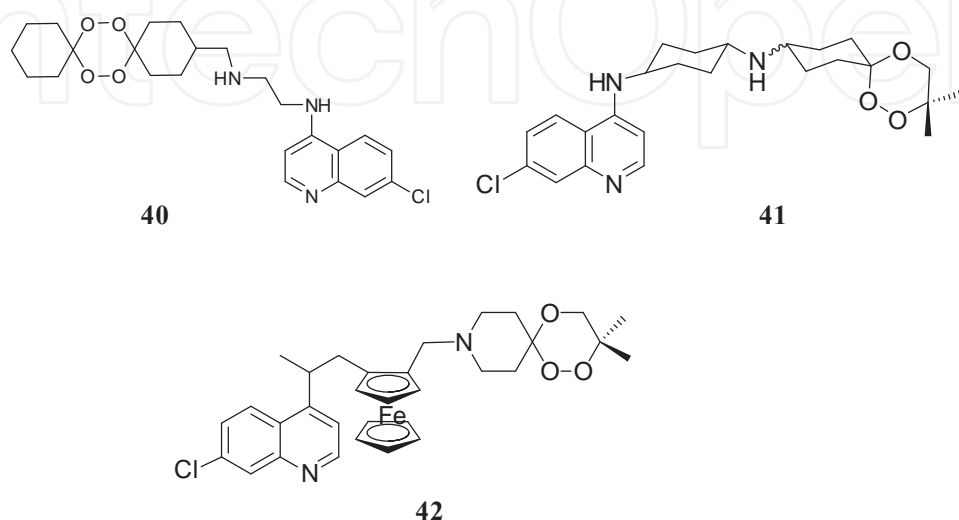
Scheme 9. Tetraoxane synthesis by ozonolysis method.

7. Prodrug and combination therapies

The search of newer drugs and the enhancement of antimalarial activity of the existing ones have led to the development of prodrug and combination therapy approaches. It presents a good platform for the usage of readily available drugs in combination with other effective drugs. The potential of drug hybrids, prodrugs, and combination therapy as new approaches are immense.[44]

Tetraoxaquine **40** contain two covalently linked pharmacophores, i.e., a tetraoxane (a radical donor) and an aminoquinoline (interferes with hemo polymerization).[45] Moreover, trioxaquine **41** contains covalently attached trioxane to a 4-aminoquinoline moiety.[46] The chimeric drug penetrates (enabled by aminoquinoline) into infected erythrocyte and targets the free heme. The hemoglobin digestion of the schizonts within infected red blood cells liberates free heme, which is alkylated by the peroxidic part. Trioxaferroquine **42** consists of a trioxane, a substituted quinoline, and an iron (II) species within a single structure.[47]

These new chimeric molecules containing two covalently attached moieties can be expected to possess synergistic therapeutic value, reduce resistance, and toxicity. These strategies offer a rational drug design approach for the development of next generation drug candidates. Notwithstanding few selected examples, which are discussed in this section, it explains the concept and potential applications.



8. Conclusion and future prospect

The development of new drugs for malaria presents a challenging situation. Lack of alternatives and increasing ineffectiveness of the existing drugs are the main reasons for increased mortality. Traditional medicines have provided few drugs, but to combat malaria, new drugs are urgently needed. These new drugs must ideally possess minimal toxicity, rapid efficacy, and low cost. However, there is consensus among scientific community that drug combinations may create optimal control of malaria because the combination therapies are believed to be additive in potency, provide synergistic activity, and is more advantageous than monotherapies. Unfortunately, these requirements are not met by any combination at the current window of time. Besides all the challenges, failures, and setbacks, the global importance of fighting malaria is recognized. Dedicated efforts and academic engagement to discover, develop, and deliver new, effective, and affordable antimalarials have thus increased dramatically. Natural products, semisynthetic drugs, and synthetic compounds offer vast opportunity for the drug development process. Further, assessment and clinical evaluation of RTS,S/AS01E for malaria vaccination offers hope that we may soon expect some good news. Malaria drug discovery is undoubtedly challenging, but scientists are optimistic as they also have got various opportunities too. The *status quo* seems balanced. However, we believe that we have to provoke the *status quo* to gain the upper hand in the battle against this tropical scourge.

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