Chapter 2

Malaria: At a Glance
2.1 INTRODUCTION

Malaria, a vector (female Anopheles mosquito) borne parasitic disease, is a leading cause of morbidity and mortality in the developing world and control of this is a high priority task. According to World Health Organization (WHO), malaria causes approximately 300 million clinical cases every year. It is estimated that between 1.5 to 2.7 million people die from malaria every year either directly or in association with anemia and up to one million of those deaths are among children in the age group less then 5 years. Malaria manifests clinically when any one of the human malarial parasite namely Plasmodium falciparum, P. vivax, P. ovale, and P. malariae enter the intraerythrocytic cycle. Among these parasites, P. falciparum is the cause of most severe and life threatening malaria in human beings. It is responsible for about 80% of infections and 90% of deaths. The control of malaria vector (female Anopheles mosquito) by spraying chemicals agents like DDT has limited utility in the control of disease. Once the parasite establishes dwelling in the human host, chemotherapy is the only way to tackle and cure the disease.

2.2 LIFE CYCLE OF MALARIA PARASITE

The Lifecycle of the malaria parasite may be divided into two stages (i) the definitive (vertebrate) host - e.g. man and (ii) the intermediate (invertebrate) host - e.g. female anopheline mosquitoes. A mosquito becomes infected when it takes a blood meal from an infected human. Once ingested the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and fuse in the mosquito gut. This produces an ookinete that penetrates the gut lining and developed as an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito’s body to the salivary glands, where they are then ready to infect a new human host. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal. Following an infected mosquito bite, some of the sporozoites rapidly enter liver parenchymal cells, where they undergo exoerythrocytic schigony forming tissue schizonts which mature and release thousands of merozoites in to the blood on rupture of the cell. Some of the merozoites enter erythrocytes where they transform in to trophozoite. These produce blood schizonts which rupture and release merozoites in to circulations and infect other erythrocytes. This is termed erythrocytic cycle.
Fig. 1. The figure describes the different stages of lifecycle of malaria parasite and various targets for antimalarial drugs.

After several erythrocytic cycles some erythrocytic forms develop into sexual gametocytes. It is ingestion of infected blood containing gametocytes by biting a female mosquito which allows the lifecycle to be completed with the sexual phase in the mosquito. In *P. vivax* and *ovale* infections, some of the sporozoites enter to the liver cells and remain in latent tissue stage in the form of hypnozoites which are responsible for the recurrence of malaria caused by these organisms. Only female mosquitoes feed on blood, thus males do not transmit the disease. An outline of lifecycle of malaria parasite is presented in Fig. 1.3,4

2.3 CLINICALLY USED ANTIMALARIAL DRUGS

There are several classes of drugs used to treat malaria. The majority of antimalarial drugs act on the erythrocytic stages of the parasite, and thus, are used for treatment of the acute attack of malaria. These start with the quinoline-methanols *Quinine*, an alkaloids isolated from bark of cinchona tree. Quinine was used as a first antimalarial agent. The structure of quinine was elucidated in 1908 and provided
evidence that the quinoline nucleus could be useful component of an antimalarial. However synthetic route of quinine nucleus is very complex for commercial production. In the 1940s, as a result of large-scale search for analogues of quinine, the 4-aminoquinolines chloroquine (CQ) and amodiaquine was produced. Later Mefloquine, a synthetic analogue of quinine and halofantrine, a phenanthrene methanol structurally related to quinine was developed by the Walter Reed Army Institute of Research, U.S.4

The antimalarial endoperoxide, Artemisinin was isolated from Artemisia Annua (Asteraceae). Because of its low solubility, more hydrosoluble analogues, artemether (R = -OMe) and arteether (R = -OEt) were developed and are active against multi-drug resistance strains of Plasmodium parasite. The drugs available for the treatment of the liver forms of the parasite are the primaquine (8-amionquinoline analogue) and dihydrofolate reductase inhibitors e.g., proguanil. The structures of clinically used antimalarial drugs are given in Fig. 2.3,4

The antimalarial drugs are classified as follows:

1. **4-methanolquinoline**: quinine, quinidine, mefloquine
2. **4-aminoquinolines**: chloroquine (CQ), hydroxychloroquine, amodiaquine
3. **8-aminoquinolines**: primaquine, pamaquine, bulaquine
4. **Phenenthrenemethanol**: halofentrine
5. **9-aminoacridines**: quinacrine
6. **Hydroxynephthoquinone**: atovaquone
7. **Biguanides (antifolates)**: proguanil, chlorguanil
8. **Diaminopyrimidine (antifolates)**: pyrimethamine
9. **Sfalanilamide (antifolates)**: suladoxine, sulfamethoxazole, sulfalane
10. **Sesquiterpenes endoperoxides**: artemether, arteether, artesunate, arteflene, fenozan, yingzhaosu
11. **Antibiotics**: tetracyclin, doxyclin, clindamycin, azithromycin etc.
2.4 MECHANISM OF ACTION OF ANTIMALARIAL DRUGS

Drugs in use today target different stages of the malaria life cycle. The majority of them act on the erythrocytic phases of development of the malaria parasite (Fig. 1). These drugs exert their antimalarial action through different mechanisms. 5-8
2.4.1 Quinoline analogues. It is suggested that quinoline-containing drugs act primarily on heme disposal, a process by which malaria parasites detoxify toxic heme. The *Plasmodium* parasites digest the host hemoglobin as a source of amino acids in their food vacuoles which generates globin and toxic heme (ferriprotoporphyrin IX or FPIX). The free heme can lyse the membranes, lead to the generation of reactive oxygen intermediates and inhibit many other processes of *Plasmodium* and thus is quite toxic to parasite. The free heme is detoxified in the food vacuole via a biocrystallization process in which the heme is sequestered into large insoluble crystals called hemozoin (malarial pigment, Fig. 3). The chloroquine and similar analogues exert its toxic effect by interfering with the conversion of free heme to hemozoin which result in the accumulation of toxic heme in parasite food vacuole and lead to the parasite cell death.\(^5\)

![Fig. 3. The conversion of free heme to hemozoin](image)

2.4.2 Sesquiterpene endoperoxides. The specific mechanism of action of artemisinin is not well understood and there is ongoing research directed at elucidating it. It is proposed that artemisinin transported to the food vacuole of the parasite, interact with \(\text{Fe}^{++}\)-heme and converted into a free radical. These free radicals then thought to modify and inhibit heme, lipids and at least four proteins, resulting in parasite death. Also the activated artemisinin specifically and irreversibly binds and inhibits Pf-ATP6, and inhibits parasite growth (Fig. 4).\(^6\)
2.4.3 Antifolates. The antifolates act by inhibition of enzymes of the folate pathway results in decreased pyrimidine synthesis, hence, reduced nucleic acids, serine, and methionine formation. Their activity is exerted at all growing stages of the asexual erythrocytic cycle and on young gametocytes. Antifolates are classified into two classes:

(i) Type-1 antifolates (sulfonamides and sulfones) mimic p-aminobenzoic acid (PABA) and prevent the formation of dihydropteroate from hydroxymethyldihydropterin catalyzed by dihydropteroate synthase (DHPS) which is a bifunctional enzyme in plasmodia coupled with 2-amino-4-hydroxy-6-hydroxymethyl-dihydropteridine pyrophosphokinase [PPPK].

(ii) Type-2 antifolates (pyrimethamine, biguanides and triazine metabolites, quinazolines) inhibit dihydrofolate reductase (DHFR, also a bifunctional enzyme in plasmodia coupled with thymidylate synthase [TS]), thus preventing the NADPH-dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by DHFR. THF is a necessary cofactor for the biosynthesis of thymidylate, purine nucleotides, and certain amino acids.

2.4.4 Atovaquone. Atovaquone is used for both the treatment and prevention of malaria in a fixed combination with proguanil. The atovaquone acts on the mitochondrial electron...
2.5 DEVELOPMENT OF CHLOROQUINE-RESISTANT STRAINS OF PLASMODIUM PARASITE

The chloroquine (CQ) is the first line drug for the treatment of malaria. The indiscriminant use of chloroquine led to the development of CQ-resistance strains of *plasmodium* parasites. The emergence and spread of resistant strains has further complicated the effectiveness of the treatment and control of malaria. The resistance to CQ began from 2 epicentres – Columbia (South America) and Thailand (South East Asia) in early part of 1960s. Since then, resistance has been spreading world wide and reached the Indian state of Assam in 1973. Biochemical studies indicate that chloroquine-resistant isolates of *P. falciparum* accumulate less amount of drug than their sensitive counterparts. The food vacuole of the parasite is acidic (pH 5.5) and based on the pH gradient between this compartment and the extracellular environment, chloroquine should accumulate 60,000 fold based on the simple Hasselbasch consideration. Therefore resistance could result from any process that reduces extent of drug accumulation. This could involve enhanced efflux, reduced uptake or a combination of both. It was shown that the chloroquine resistance results from mutation in a new vacuolar transporter *PfCRT*, a putative transporter or channel that is probably involved in chloroquine flux and equilibrium across the digestive vacuole membranes. However, while opinion remains divided on the mechanistic explanation for this reduction, its reversal by molecules such as verapamil, desipramine, and chlorpromazine suggests that an enhanced CQ efflux by a multidrug-resistant protein may be involved. In this endeavor, a detailed knowledge of the mechanism of action of chloroquine could provide the basis for rational design of novel antimalarials.

2.6 DRUGS FOR CHLOROQUINE-RESISTANT MALARIA

The quinine remains effective against CQ resistant *P. falciparum*. The combination of mefloquine or atovaquone with proguanil is an effective treatment for CQ-resistant malaria. Amodiaquine is effective against resistant strains and has the same mechanism of action as CQ. It has been observed that CQ resistant *plasmodium* parasites are not cross-resistant to amodiaquine. But there are concerns about its possible toxicity if
taken repeatedly to treat malaria. Halofantrine is also effective against drug-resistant parasites but has significant cardiac side-effects. The artemisinin analogues, e.g. arteether, artesunate have the broadest antimalarial activity, against parasites from the ring (blood) stage to early schizonts (sequestered) stage and cause the fastest decline in parasite counts. These derivatives are quite effective in CQ resistant strains of malaria parasites.\textsuperscript{7,8}

2.7 NEW TARGETS AND LIGANDS FOR MALARIA

The advances in the understanding of molecular and structural biology of malaria parasite lead to the identification of various molecular targets for the design of new molecule against \textit{plasmodium} parasite. These targets are various enzymes vital for the growth as well as for the synthesis essential metabolites of parasite. The list of various biological targets and their inhibitors are given in Table 1.

Apart from this the few quinoline based analogues are also designed against CQ resistance strains of malaria. These studies show that the CQ-resistant strains are not automatically cross-resistant to other quinoline based analogues.\textsuperscript{12} Carlton and co-workers showed that chloroquine resistance results from mutations in a new vacuolar transporter, \textit{PfCRT}.\textsuperscript{9} The CQ accumulates in parasite food vacuole and inhibit the hemozoin formation which leads to the death of parasite. Biochemical studies have clearly indicated that isolates of the CQ-resistant parasites accumulate less drug content than their more sensitive counterparts. Its reversal by molecules such as verapamil, desipramine, and chlorpromazine suggests that an enhanced CQ efflux by a multidrug-resistant protein may be involved.\textsuperscript{10,11} A method to overcome CQ efflux consists of designing quinoline-based drugs that are not recognized by the proteins involved in the drug efflux. In this respect, bulky bisquinolines were synthesized and suggested to be extruded with difficulty by a proteinaceous transporter. These includes \textit{N,N}-bis(7-chloroquinolin-4-yl)alkanediamines,\textsuperscript{13} bisquinolinemethanols,\textsuperscript{14} bis(9-amino-6-chloro-2-methoxyacridines)\textsuperscript{15} and bis-, tris- and tetraquinolines with linear / cyclic amino linkers.\textsuperscript{16} The other aminoquinoline based antimalarials are tebuquine analogues,\textsuperscript{17} 7-substituted 4-aminoquinolines,\textsuperscript{18} amodiaquine analogues\textsuperscript{19} and isoquine analogues.\textsuperscript{20,21} These analogues were found to be effective against CQ-resistant strains of malaria at low concentration. They are effective against both CQ-sensitive and CQ-resistant parasites.
Table 1. New biological targets for malaria and their inhibitors

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<th>Target</th>
<th>Enzyme / process</th>
<th>Inhibitor</th>
<th>Structure</th>
<th>Ref.</th>
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<td>Thioredoxin reductase</td>
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2.8 PRESENT QSAR STUDY

In the present QSAR (Quantitative Structure-Activity Relationship) studies we have selected two different leads associated with antimalarial activity i.e. 4-(3', 5'-disubstitutedanilino)quinoline analogues and substituted tetrahydroquinoline analogues. The 4-(3', 5'-disubstitutedanilino)quinoline analogues are reported to be active against resistant strains of *Plasmodium falciparum* and exert their action in same manner as that of chloroquine and amodiaquine. The substituted tetrahydroquinoline analogues have been reported to act via inhibition of protein farnesyltransferase of *Plasmodium*. The former has been studied using various class of constitutional, structural and topological descriptors obtained from Dragon software (Chapter 3). The study on the substituted tetrahydroquinoline analogues has been carried out using various physicochemical, surface areas, atom and bond counts, topological, H-bond donor/acceptor features, partial charges, and potential energy descriptors from MOE software (Chapter 4).
2.9 REFERENCES


