1.1 INTRODUCTION

Malaria is one of the world’s most debilitating diseases and is caused by microscopic parasites of the *Plasmodium* genus. The most recent innovative improvements in health technologies including successes in the discovery and production of new drugs have brought little benefits to the treatment and eradication of malaria. But the disease is still a major threat to the health of the population (Adams *et al*., 2004). Almost half of the global population is under threat of infection with the malaria parasite However, despite the extent and severity of the conditions, global expenditure in malaria research is very low, when compared with the expenditure on conditions such as cancer, HIV/AIDS or asthma (Anderson *et al*., 1996). Another worrying fact is that the occurring resistance to the first line drugs like the quinolines (chloroquine, amodiaquine and mefloquine) and the antifolate combination drugs (sulfadoxine and pyrimethamine) is making the fight against malaria increasingly difficult (Biagini *et al*., 2005). The first reported cases of resistance against the highly effective antimalarial drug artemisinin has occurred in South-East Asia (the Cambodia-Thailand border) and is a further cause of concern (WHO, 2009). Absence of an effective vaccine and availability of artemisinin and its derivatives as the only option in the basket has shifted the circumstances even to worst side (Padmanban *et al*., 2007). All these alarming situations created the urgent need to develop novel drug as well as to explore the new drug targets against malaria (Berwal *et al*., 2008).

1.2 HISTORY

Man has known about malaria and its malicious effect for quite a while. Evidence of a disease causing malaria like symptoms has been found in early Chinese (NeiChing, The Canon of Medicine in 2700 BC), Indian (Sushruta in 500 BC) and Roman scripts (CDC, 2010). Malaria, the oldest disease known to mankind, is defined by the Taber’s Cyclopaedic Medical Dictionary as an acute and sometimes chronic infectious disease due to protozoa of the genus *Plasmodium* infecting red blood cells. Malaria was derived from the Latin word ‘mal-aria’ meaning bad air, as it was thought that the noxious swamp gases around Rome caused the outbreaks of disease. The oldest known malaria DNA was recently identified from a Roman baby graveyard (Abbot A., 2001). The causative agent for human malaria was first identified in 1880 by

Five species of *plasmodium* are identified as zoonotic parasites: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*. These species differ from one another in geographical distribution, appearance, virulence, clinical features, as well as their resistance to antimalarial drugs (Bloland P.B., 2001). Among them *P. falciparum* is known to cause the most severe cases of malaria and death. The parasites of *P vivax* and *ovale* species have the ability to become dormant inside their human host and, at a later date, cause a relapse of malaria. *Plasmodium knowlesi*, was at first thought to infect only non-human primates, but has emerged as a zoonotic malaria parasite (Cox-Singh et al., 2008).

### 1.3 MALARIA TODAY AND GLOBAL BURDEN

An estimated 3.2 billion people were at risk of being infected with malaria and developing disease in 2013, of this, 1.2 billion people are at high risk (>1 case per 1000 population) of malaria (WHO 2015). Over half of all the countries in the world are affected by malaria. According to the latest estimates from WHO, there were 214 million new cases of malaria worldwide in 2015 (range 149–303 million). The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). The worldwide distribution of malaria has been shown in fig 1. In 2015, there were an estimated 438 000 malaria deaths (range 236000–635000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (8%) and the Eastern Mediterranean Region (2%).

Children under five are particularly susceptible to malaria illness, infection and death. According to the WHO malaria report 2015, 453,000 malaria deaths were estimated to occur in children under 5 years, which means 78% of the global total. Over 1,200 children die every day from malaria, which is equivalent to 50 children dying every hour.
1.4 MALARIA BURDEN IN INDIA

Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature like the “Atharva Veda” and “Charaka Samhita”. In the 30’s there was no aspect of life in the country that was not affected by malaria. During the latter parts of nineteenth and early twentieth century, nearly one-fourth of India’s population suffered from malaria, particularly in the states like Punjab and Bengal (Richard Tren, 2002). The economic loss due to malaria was estimated to be at Rs. 10,000 million per year in 1935. At the time of independence in 1947, among a population of 330 million, about 75 million people were estimated to be infected with malaria every year, and the direct mortality due to the disease was estimated at 0.8 million per annum (Shiv Lal et al., 2002). To combat this menace, the Govt. of India launched the National Malaria Control Programme in April 1953. The programme proved highly successful and the number of malaria cases significantly declined to about 2 million by 1958 (NVBDCP, 2013). Encouraged by this, the programme was changed to a more ambitious National Malaria Eradication Programme in 1958. By 1961 the incidence dropped further to a mere 49151 cases,
with no deaths (A.P. Dash et al., 2008). But since then the programme suffered repeated set-backs due to technical, operational and administrative reasons and the cases started rising again (Ashwani Kumar et al., 2007). Early setbacks in malaria eradication coincided with DDT shortages and later it was the result of technical, financial and operational problems. Realising the difficulties in controlling/eradicating malaria, the National Malaria Eradication Programme has been now renamed as National Anti Malaria Programme.

The biggest burden of malaria in India is borne by the most backward, poor and remote parts of the country, with >90-95% cases reported from rural areas and <5-10% from urban areas; however, the low malaria incidence in urban areas may be due to almost non-existing surveillance. The state of Orissa, with a population of 36.7 million (3.5%), contributes about 25% of the total annual malaria cases, more than 40% of *P. falciparum* malaria cases and nearly 20–30% of deaths caused by malaria in India, followed by Meghalaya, Mizoram, Maharashtra, Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chhattisgarh, and Jharkhand, which also report significant number of malaria cases and deaths (Ashwani Kumar, 2007, Kounteya Sinha, 2012). The proportion of *P. vivax* and *P. falciparum* varies in different parts of India; *P. falciparum* accounts for 30–90% of the infections in the forested areas inhabited by ethnic tribes and <10% of malaria cases in mostly indogangetic plains and northern hilly states, northwestern India, and southern Tamil Nadu as shown in fig 2.

During 2011, the malaria incidence was around 1.31 million cases, 0.67 million Pf cases and 754 deaths; while during 2012, 1.01 million cases, 0.53 Pf cases and 519 deaths were reported. About 91% of malaria cases and 99% of deaths due to malaria are reported from high disease burden states namely Northeastern (NE) States, Andhra Pradesh, Chhattisgarh, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan and West Bengal (NVBDCP, 2011). However, other States are also vulnerable and have local and focal upsurge. During 2013, 0.88 million cases, 0.46 Pf cases and 440 deaths have been reported (Ashwani Kumar et al., 2007). During 2014 (till October), 0.85 million cases, 0.54 Pf cases and 316 deaths have been reported as shown in fig 3.
Highly endemic areas accounts for 30–90% of the *P. falciparum* infections in the forested areas inhabited by ethnic tribes (shown as blue colored), followed by Meghalaya, Mizoram, Maharashtra, Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chhattisgarh, and Jharkhand (shown as red colored) and <10% of malaria cases in mostly indogangetic plains and northern hilly states, northwestern India, and southern Tamil Nadu (shown as light purple colored).
The survival of the malaria parasite in a certain environment is dependent on the interactions between the parasite, host and vector. For effective malaria transmission to be accomplished, there have to be an abundance of *Anopheles* mosquitoes with a long enough lifespan to support sporogony and enough available hosts. Factors influencing the mosquito population are temperature, altitude, rainfall and the availability of breeding places (Breman, 2001). Genetic and physiologic properties of the human host play a part in the global distribution of malaria. People living in endemic areas can develop immunity to malaria, which protects them against severe illness and death, although the immunity is only effective while the person is continuously exposed to the parasitic pathogens in that region. This type of immunity is called premonition and is lost once a person gets isolated from those malarial antigens by leaving the endemic area (Langhorne *et al*., 2008).

Genetic diseases and polymorphisms have been linked to a decrease in malaria infections. The absence of *P. vivax* infections in western Africa is due to the fact that most of the populations do not have a specific receptor, called the Duffy blood group antigen, on the surface of their erythrocytes. Interaction between this receptor and the Duffy binding protein on the surface of merozoites are necessary for invasion of the erythrocytes. This gives a Duffy negative person complete protection against *P. vivax*.
Introduction To Malaria

(Arévalo and Herrera, 2005). Some inherited erythrocyte disorders can provide protection against malaria. In cases such as ovalcytosis, a mutation in the erythrocytic membrane causes it to become rigid and inaccessible to merozoite invasion. Sickle cell anaemia and glucose-6-phosphate dehydrogenase deficiency are presumed to cause an inability to handle the extra oxidative stress placed upon the erythrocytes, because of the parasitic metabolism. Consequently, the erythrocytes are destroyed before the parasite can complete schizogony (Ayi et al., 2004; Williams, 2006).

1.6 PROGRESS IN DECREASING MALARIA BURDEN SINCE 2000

Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region.

Other regions have achieved impressive reductions in their malaria burden. Since 2000, the malaria mortality rate declined by 72% in the Region of the Americas, by 65% in the Western Pacific Region, by 64% in the Eastern Mediterranean Region, and by 49% in the South-East Asia Region. For the first time, the European Region reported zero indigenous cases of malaria in 2015. Malaria surveillance systems are getting better, but still only 14% of global estimated cases are recorded. Resistance to artemisinin malaria drugs has been detected in five countries, all in South East Asia (WHO World Malaria Report, 2014).

![Estimated malaria cases and deaths 2000-2015](image)

**Figure 4** Data representing the decrease in malaria mortality (2000-2015)
1.7 MALARIA PARASITE AND LIFE CYCLE

Malaria parasite is distributed worldwide, flourishing in the hot and humid conditions of tropical Africa, Asia including South and Central America. The five malaria causing *Plasmodium* species have an overlapping geographical distribution throughout the world, but *P. falciparum* and *P. vivax* cause most of the infections. *Plasmodium falciparum* is the most common species in sub-Saharan Africa while *P. vivax* is the predominant species in India and South-America. *Plasmodium ovale* is mostly found in western Africa, while *P. malariae* is distributed in much the same way as *P. falciparum*, but to a lesser extent. Thus far, *P. knowlesi* cases have been localized to Southeast Asia, especially to Malaysia (Guerra et al., 2006; Cox-Singh et al., 2008). Despite all these, the major public health threat among all is *Plasmodium falciparum* due to which severity may develop and may cause fatality, if not treated early. In India, out of 9 species of Malaria vectors, the major vector for rural malaria is *Anopheles culicifacies*, found all over the country and breeds in clean ground water collections. Other important *Anopheline* species namely *An.minimus* and *An.fluviatilis* breed in running channels, streams with clean water (NVBDCP, 2014-15).

To understand the pathology of malaria, one has to look at the life cycle of the *Plasmodium* parasite (fig 5). A human gets infected with malaria when a *Plasmodium-infected* female *Anopheles* mosquito takes a blood meal and inoculates sporozoites into the skin of the human host. From here the sporozoites enter the bloodstream through capillary endothelial cells. What then followed are three asexual reproductive stages *via* the process of schizogony and a sexual reproductive phase:

- Liver stage or exo-erythrocytic schizogony (in human host)
- Erythrocytic stage or erythrocytic schizogony (in human host)
- Sexual stage or gametogenesis (in *Anopheline* vector)
- Sporogony (in *Anopheline* vector)

The protozoan malaria parasites (*Plasmodium* spp.) are transmitted by infected female mosquitoes when feeding on blood. Parasites soon enter liver cells, and after several days of multiplication, are released into the bloodstream where further cycles of asexual reproduction occur, giving rise to the clinical symptoms of malaria. Some
erythrocytic parasites will differentiate into presexual forms (gametocytes), which when taken up by mosquitoes in further blood meals, mature into gametes and undergo a sexual cycle. With the eventual release of infective sporozoites into the mosquito salivary glands, in this way the life cycle of the parasite is completed (Miller et al., 2002).

1.8 PATHOLOGY

The clinical manifestations of malaria are solely due to activities taking place during the erythrocytic stage (Malaguarnera & Musumeci, 2002). A person infected by any of the malaria species will initially experience flu-like symptoms like headache, slight fever, muscle pain and nausea. What follows are the febrile attacks, known as paroxysms, characteristic of a malaria infection. The periodicity of the paroxysms is due to the synchronized development of the schizonts. All the malarial parasites within a host are approximately at the same developmental stage (i.e., merozoite, trophozoite, schizont) resulting in erythrocytic schizogony to happen in a synchronous manner (24 hours for \textit{P. knowlesi}, 48 hours for \textit{P. falciparum}, \textit{P. vivax} and \textit{P. ovale})

\textit{Figure 5-} Life cycle of \textit{Plasmodium parasite}
and 72 hours for *P. malariae*. This leads to the simultaneous rupture of the infected erythrocytes, the release of merozoites into the host’s circulatory system and the subsequent malarial paroxysms (Clark *et al.*, 2003). The release of pro-inflammatory cytokines, like tumour necrosis factor-alpha (TNF-a), are stimulated as a response to the “dumping” of parasitic waste products and antigens into the host’s bloodstream (Malaguarnera & Musumeci, 2002) and has been linked to the development of the febrile attacks (Karunaweera *et al.*, 1992).

1.8.1 Pathology of *Plasmodium falciparum*

Three properties of the infection by *P. falciparum* parasites that make it more lethal than the other malaria infections are:

- the high level of parasitemia
- their ability to invade all types of erythrocytes
- induction of structural changes to infected erythrocytes

*Plasmodium falciparum* produces ten to hundred times more parasites than other *Plasmodium* species which leads to the destruction of higher quantity of erythrocytes and the release of antigens into the host’s circulatory system; thus a more severe malaria attack (Wiser, 2008). *Plasmodium falciparum* parasites are able to infest all types of erythrocytes, in comparison to *P. vivax* which prefers reticulocytes (Miller *et al.*, 2002). This non-selective invasion of erythrocytes is further supported by an *in vitro* study, which suggested that *falciparum* parasite in patients with severe malaria, is more virulent than parasites from patients with uncomplicated *falciparum* malaria (Chotivanich *et al.*, 2000).

The surface of infected erythrocytes is changed during *P. falciparum* infections. An example is the enhanced permeability of the erythrocytic membrane during the trophic phase which enables the in- and outflux of a wide variety of low molecular weight solutes that helps to satisfy the increased feeding and waste removal needs of the parasites (Kirk *et al.*, 1993). Another modification is the formation of “knoblike” protrusions that is associated with the adherence of infected erythrocytes to endothelial cells. Cytoadherence is mediated by interactions between protein ligands on the surface of the infected erythrocytes e.g. *Plasmodium falciparum* erythrocyte
membrane protein-1 (PfEMP1 or ICAM-1) and various receptors on the vascular endothelial cells. The affected erythrocytes sequestrate in the capillaries and post-capillary venules of the host in organs such as the brain (cerebral malaria), lung, gut, heart and placenta resulting in severe complications. This protects the parasite from being destroyed in the spleen (Craig & Scherf, 2001). Some of the *P. falciparum* infected erythrocytes bind to uninfected erythrocytes to form a rosette like clump; a phenomenon called “rosetting”. These clumps can block micro-vascular flow and contribute to severe malaria (Mercereau-Puijalon *et al*., 2008). Three most common syndromes associated with severe *falciparum* malaria, and most often correlated with death are: cerebral infection, severe anaemia and metabolic acidosis. Other complications of severe malaria are: renal failure, circulatory collapse (shock), hypoglycaemia, impaired consciousness, repeated generalised convulsions, prostration or weakness, abnormal bleeding or coagulation, haemoglobinuria, jaundice and hyperpyrexia.

*P. falciparum* is capable of the most lethal attacks and if left untreated, culminates into severe malaria. Infections by *P. vivax*, *ovale* and *malariae* are rarely lethal, but are a cause of great morbidity. A major fraction of malaria deaths are the consequences of severe complications of malaria and among them cerebral malaria (CM) was considered as the most serious one. CM is a neurological complication of infection with *Plasmodium falciparum*, which has been characterized by coma and asexual forms of the parasite on peripheral blood smears. The chances of death are higher in most of the cases otherwise it leads to long-term neuro-cognitive impairments in surviving patients (Idro *et al*., 2010).

African population is most widely affected from malaria and its serious manifestations. The manifestations of severe malaria includes: cerebral malaria (CM). An analysis which focuses on young African patients with cerebral manifestations which require better understanding and clinical care was performed and revealed that the newer drugs and improved diagnostics, surveillance, and disease management practices and interventions are necessary (Murphy *et al*., 2001). Sequestration is thought to be a specific interaction (cytoadherence) between *Plasmodium* infected RBCs (PfIRBC) and the vascular endothelium, which as a consequence reduces the
microvascular blood flow (Muntendam et al., 2010), which leads to organ and tissue dysfunction such as coma (Newton et al., 2000).

![Figure 6](image.png)

**Figure 6** - Possible mechanism involve in pathogenesis of Cerebral Malaria

Infected erythrocytes have a diverse and varied binding potential, and various host receptors including CD40 and inter cellular adhesion molecule 1 (ICAM-1) support parasite cytoadherence (Gray and Craig, 2003; Berendt et al., 1989). ICAM-1 is supposed to be an important component of binding for infected erythrocytes (IE), as inhibition by particular monoclonal antibodies diminished cytoadherence to almost to background levels despite the presence of other receptors, such as CD40 (Dormeyer et al., 2006). The finding also suggests that the mediating sequestration of brain may be limited if therapies capable of blocking or reversing adhesion of *P. falciparum* parasites in the brain.

The recommended treatment for cerebral malaria is quinine by slow intravenous infusion (Jaffer et al., 1997). However, quinine has several drawbacks, including a short half-life, painful local reactions after intramuscular and intravenous administration and neurotoxicity (WHO, 1998; 2007). Permanent blindness with standard doses of quinine has been well documented. Furthermore, decreasing sensitivity to quinine has been reported in south-eastern Asia and the Amazon region, (WHO, 2007) as well as in parts of Africa (Mutanda LN, 1999).

Artemisinin derivatives, a relatively new group of antimalarials that produce a very rapid therapeutic response and are effective against multidrugresistant *P. falciparum,
have been used increasingly over the past decade (WHO, 2006). Although resistance to artemisinin derivatives has been reported along the Thai–Cambodian border (WHO, 2007), it has not been detected anywhere else. The neurotoxic effects of artemisinin derivatives have been observed in pre-clinical animal studies at doses about 10 times higher than those used for human treatment (Lugt CB, 2000), but no such toxic effects have been reported in humans.

1.9 CONTROL OF MALARIA

The closely integrated symbiosis of the parasite, the human host and the mosquito vector provide two strategies that can be used in the fight against the disease:

1.9.1 Vector control
1.9.2 Disease control

1.9.1 Vector Control

Vector control is aimed at killing the malaria infected female *Anopheles* mosquitoes (or their larvae) or minimizing the contact between human hosts and the mosquitoes. To this end, the World Health Organisation prescribes the use of insecticide treated nets and the Indoor Residual Spraying (IRS) of targeted households that are at high risk (WHO, 2009). Complimentary to these measures, environment-based interventions such as the drainage of breeding sites or the managing of stream water flow to kill the mosquito’s larvae (Konradsen *et al.*, 2004). However, resistance to the pyrethroid insecticides used in treating the nets is a cause of concern. In 2000, South Africa had a dramatic increase in malaria incidence (64 000 cases and 423 deaths) and it was linked to the appearance of the *Anopheles funestus* mosquito, a species showing metabolic resistance to the pyrethroids. The controversial toxin DDT had to be re-introduced in the IRS strategy, and it took immediate effect with the death toll falling to 67 in 2005 (Tren and Bate, 2004).

1.9.2 Disease Control

Control of the disease is achieved by chemotherapeutic treatment of people with malaria or the prophylactic treatment of people living in or visiting malaria endemic areas. To exercise the most effective antimalarial therapy, factors such as the parasite species, severity of the disease as well as the age, and immune status of the patient need to be considered.
1.9.2.1 Antimalarial Drugs

Preventive and/or curative drug therapies play an important role in the control malaria (Gregson and Plowe, 2005). Although drugs in use target different stages of the malaria life cycle, majority of them act on the asexual, intra-erythrocytic stage: the phase responsible for the clinical symptoms of disease (Maitland et al., 2004). The choice of an antimalarial agent largely depends on the patient’s level of immunity, the drug’s side effect profile, cost and the site where the infection was acquired—an indicator of a particular drug’s resistance probability (White, 2004). The WHO and the National Vector Borne Disease Control Programme of government of India has published guidelines on the standard treatment with malarial chemotherapy (WHO, 2015; NVBDCP, 2015). Various antimalarial drugs, which are generally prescribed for the treatment include, Quinolines, hydroxynapthoquinones, folate antagonists, antibiotics and artemisinin derivatives. Each class of these drugs exhibit unique properties as explained below.

1.9.2.1.1 Quinolines

This class is comprised of: (i) 4-aminoquinolines (chloroquine and amodiaquine), (ii) quinoline methanols (quinine, quinidine and mefloquine) and (iii) phenathrene methanols (halofantrine and lumefantrine). Drugs in this class exert their action on the parasite’s food vacuole (Maitland et al., 2004). Predominantly in the trophozoite and early schizont stages, hemoglobin is ingested with the cytoplasm of the host erythrocyte by a phagocytosis-like mechanism into the food vacuole where it is degraded to generate free heme (Ginsburg et al., 1999). Free heme is potentially toxic to the parasite and is therefore disposed of by conversion into long insoluble polymers of hemozoin (heme polymerization). Heme polymerization and the oxidative and glutathione-dependent heme degradation pathways are inhibited by the quinolines. They bind to heme through a π-π stacking of their planar aromatic structures (Ridley et al., 1997). Moreover, mefloquine and quinine have been shown to block the uptake of hemoglobin from the host cell (Famin and Ginsburg, 2002). The activity of quinolines seems to depend on the weak base effect whereby presence of a basic amino function enables concentration of the drug in the acidic food vacuole in its membrane impermeable protonated form (Ginsburg et al., 1989).
Members of this class are widely used in malaria treatment as exemplified by the use of chloroquine as a first line agent in many African countries for a long time before the emergence of chloroquine resistance (Winstanley et al., 2000). Although generally safe, several adverse effects which include pruritis, retinopathy, shock, cardiac arrhythmias, psoriasis, leukopenia and aplastic anaemia have been reported for chloroquine. Amodiaquine through its active metabolites has been shown to cause hepatitis and agranulocytosis (Winstanley et al., 1990). Parenteral quinine is the drug of choice for severe malaria as compliance with oral quinine is poor due its bitter taste (Newton and White, 1999). The therapeutic index for quinine is narrow and severe side effects such as hypoglycemia, coma, hemolytic uraemic syndrome and cardiovascular disorders may arise. Cinchonism, (characterized by tinnitus, deafness, dizziness, nausea and visual problems) is, however, the commonest side effect.

Mefloquine, mostly used in prophylaxis due to its long terminal half-life (14-21 days) has been linked with neuropsychotic side effects. Halofantrine by prolonging the QT interval causes ventricular arrhythmias. By targeting the dormant liver forms (hypnozoites) of \textit{P. vivax} and \textit{P. ovale} infections, the 8-amino quinoline, primaquine, prevents relapses of these infections. Primaquine is, however, contraindicated in glucose 6-phosphate dehydrogenase deficient patients in whom it causes hemolysis.

\subsection*{1.9.2.1.2 Hydroxynapthoquinones}

The prototype of this class is atovaquone which blocks mitochondrial electron transport through inhibition of the cytochrome \textit{bc1} complex (Fry and Pudney, 1992). When used alone though, resistance emerges rapidly due to a point mutation of cytochrome \textit{b} gene localized in the mitochondrial genome (Srivastava et al., 1999). As a result, atovaquone is used as a fixed-dose combination with proguanil which is thought to enhance the atovaquone-induced collapse of the mitochondrial membrane potential (Srivastava et al., 1999). In prophylaxis, atovaquone-proguanil activity against liver stages is advantageous as the parasites are killed before an infection of erythrocytes can be established (Berman et al., 2001).

\subsection*{1.9.2.1.3 Folate antagonists}

Inhibition of enzymes of the folate pathway results in decreased pyrimidine synthesis and consequently reduced DNA, serine and methionine formation (Olliaro, 2001).
Available antifolates include synergistic mixtures such as pyrimethamine-sulfadoxine (Fansidar®), chlorproguanil-dapsone (LapDap®), sulfalene-pyrimethamine (metakelphin®) and proguanil-atovaquone (Malarone®). These combinations are carefully selected for matching pharmacokinetics to maximize the synergy.

Malaria parasites are particularly susceptible to inhibition of DHFR because unlike mammalian cells, transcriptional inhibition (mediated by the protein binding to its own message) is not relieved by the accumulation of substrate that occurs in the presence of inhibitor (Zhang and Rathod, 2002).

1.9.2.1.4 Antibiotics

Several antibacterial agents also exhibit antimalarial activity. This activity stems from the fact that malarial parasites, like other Apicomplexan parasites, possess a plastid-like organelle, the apicoplast (Kohler et al., 1997) which fulfills some metabolic functions such as the synthesis of isoprenoids, fatty acids and probably heme (Wilson, 2002). The apicoplast contains a residual genome that encodes tRNA’s, rRNA’s, RNA polymerases and ribosomal proteins all of which ensure self-replication of this organelle (Wiesner et al., 2003). Antibiotics act through inhibition of the prokaryote-like RNA and protein synthesis in the apicoplast (Ralph et al., 2001). The tetracyclines may also block mitochondrial protein synthesis in the parasite (Ralph et al., 2001). Rifampicin, tetracyclines, lincosamides and macrolides are being increasingly used in combination with other antimalarials to augment their activity. However, due to their slow effect, antibiotics are mostly used for prophylaxis in which case doxycycline is the most popular agent.

1.9.2.1.5 Artemisinins

This class consists of a unique family of sesquiterpene lactone endoperoxides. The parent compound, artemisinin, was first extracted from the Chinese herb Artemisia annua (qinghao). Several semisynthetic derivatives which include artemether, arteether, artelinate and artesunate are in use. They are metabolized to dihydroartemisinin which is the main active agent in the body (Ridley, 2002). Members of this group act on all phases of the asexual intra-erythrocytic schizogonic cycle and also possess gametocytocidal activity (Ridley, 2002; Newton and White, 1999). Although the mechanism of action is not known with certainty, the prevailing
hypothesis is that the essential pharmacophore, an endoperoxide bridge, undergoes reductive cleavage by ferroheme ferrousprotoporphyrin IX (Fe(II)PPIX) to generate carbon-centered free radicals that alkylate protein and damage parasites’ microorganelles and membranes (Meshnick et al, 1996).

![Figure 7: Structures of Antimalarial Drugs](image)

As a class, artemisinins are fast acting and potent but due to their short half-lives, they are currently used in combination with longer half-life drugs. This strategy forms the basis of artemisinin combined therapies (ACTs) and is hoped to improve individual compliance and prevent or retard the development of drug resistance. Several combinations are already in the market and include artemether-lumefantrine (Coartem®), dihydroartemisinin-piperaquine (Artekin®) and the triple combination of chlorproguanildapsone-artesunate undergoing phase II trials in Malawi (Maitland et al., 2004).
Although relatively lacking in adverse effects, embryonic toxicity has been reported for this class of antimalarials in China although a later study allayed fears of artemisinin related birth defects (McGready et al., 2001).

1.10 ANTIMALARIAL DRUG RESISTANCE

The effectiveness of drugs in malaria control has depreciated considerably over the last few years due the development of drug resistant strains of the parasite. This has been attributed to widespread misuse of common antimalarials together with their extensive deployment particularly chloroquine (CQ) in the tropical regions of the world (White, 2004).

Globally therefore, malaria control programmes such as the WHO funded Roll Back Malaria campaign, are under the threat of failing unless measures are put in place to curtail resistance progression (Yeung et al., 2004). Resistance to antimalarials is mainly due to point mutations and changes in steady-state transcript levels (Arav-Boger and Shapiro, 2005). The mutations in, or changes in, the copy number of genes encoding the drug’s parasite target e.g. membrane transporters or enzymes affect the intraparasitic drug accumulation (White, 2004).

CQ resistance has mainly been attributed to the accumulation of multiple point mutations in the gene coding for *Plasmodium falciparum* chloroquine resistant transporter (*PfCRT*), a putative transporter protein located in the parasite’s digestive vacuole membrane. This 424 amino acid protein with ten predicted transmembrane domains (Fidock et al., 2000) is thought to be involved in drug fluxes and/or pH regulation in the digestive vacuole (Wellems and Plowe, 2001). Two mutations, K76T and A220S, have been shown to be critical to the evolution of resistance (Fidock et al., 2000; Wellems and Plowe, 2001), a fact that lead to propose that either these two are the pharmacologically relevant mutations or that the other mutations serve to compensate for impaired protein function after acquisition of the critical mutations (Hastings et al., 2002). Sidhu et al. 2002, by swapping the (*PfCRT*) gene in chloroquine (CQ) sensitive strain with the one from CQ-resistant strain, also demonstrated the role of K76T mutation in CQ resistance and further proved that up-regulation of *PfCRT* is not obligatory for resistance to occur. Although the actual resistance mechanism remains unclear, several hypotheses have been posited and
include drug expulsion through an acquired efflux system (Sanchez et al., 2003), leaking of the drug out of the digestive vacuole (Bray et al., 2005) and variation of vacuolar pH due to altered chloride conductance across the vacuolar membrane (Zhang et al., 2002). These fit in with the presumed role of PfCRT. Reversal of chloroquine resistance has been observed with several agents among them verapamil, a calcium channel blocker and chlopheniramine, a histamine H₁ receptor antagonist. These agents do not however restore full chloroquine sensitivity (Bray et al., 1996). Verapamil has been proposed to act via its hydrophobic binding to the mutated PfCRT protein. By so doing, it replaces the lost positive charge and, therefore, repels the access of 4-aminoquinoline cations to PfCRT (Henry et al., 2006; Warhurst, 2003).

Apart from PfCRT, the multidrug resistance gene Pfmdr1 has also been implicated in chloroquine resistance. This gene encodes the ATP-dependent P-glycoprotein homolog 1(Pgh-1), a transmembrane protein also found in the parasite digestive vacuole (Cowman et al., 1991; Wilson et al., 1989). A widely reported defect in Pgh-1 associated with chloroquine resistance is the N86Y mutation (Dorsey et al., 2001). A further four Pgh-1 mutations (Y184F, S1034C, N1042D and D1246Y) have been shown to play a role in other quinoline antimalarials resistance in addition to modulating the parasite’s sensitivity to chloroquine and artemesinin (Reed et al., 2000).

Resistance to the antifolate antimalarials is conferred by single point mutations of the gene encoding for the respective enzyme, resulting in substitutions in the amino acid side chains. High-level pyrimethamine resistance results from the accumulation of mutations in DHFR gene (Ridley, 2002). Highest level of clinical resistance result from parasites with four mutations in DHFR and two in DHPS, which may suggest the maximum number of mutations that can be tolerated in competition with less affected strains (Arav and Shapiro, 2005). Dihydrofolate reductase –thymidylate synthase crystal structure provides evidence that the critical mutations mediating clinical drug resistance map to the dihydrofolate reductase active site (Yuvaniyama et al., 2003).
Resistance to atovaquone is due to mutations affecting five amino acids clustered in a highly conserved fifteen amino acid sequence of cytochrome b from *P. yoelii* (Arav-Boger and Shapiro, 2005). Analysis of *P. falciparum* isolated from patients who failed atovaquone monotherapy confirmed predilection for mutations at residue Y268 (Korsinczky *et al*., 2000).

**1.11 ORIGIN OF THE CURRENT STUDY**

Today the more efficient strategies in the quest to develop an effective and cheaper antimalarial drug are still in need, particularly for severe complications such as cerebral malaria. The re-design of existing drugs candidates, for example the concept of hybrid-drugs in which the moieties from already available antimalarials are merged with the new pharmacophoric features, may add some benefit. The “drug cocktails” or “hybrid drugs” is a tactic used by many researchers nowadays, to get some better therapeutic hits. A hybrid molecule can be defined as a “chemical entity with two or more structural domains, having different biological functions and dual activity” and thus describing a single molecule that acts as two distinct pharmacophores. Moreover, the simultaneous treatment of multiple drug targets (polypharmacology) has also been used by most of the clinicians in their practice to achieve an optimal patient outcome.

Various researchers have reported that heterocyclic compounds based on quinoline and pyrazole are the well known for their antimalarial potential (Manohar *et al*., 2010; Kaushik *et al*., 2010; Batra *et al*., 2009; Cochin *et al*., 2008; Riggione *et al*., 2002; Chauhan *et al*., 2010. Likewise, Some scientist have also been reported 2-(3H)-furanones (butenolides), bearing quinoline moiety, as newer antimalarial agents (Alam *et al*., 2011; Akhter *et al*., 2014). In addition to this several natural compounds containing furan ring such as Sesquiterpene lactones based elemanolide, heliangolide, vernolide and vermodalin (Chukwujekwu *et al*., 2009), gomphostenin (Sathe *et al*., 2010), andrographolide (Misra *et al*., 1992; Najib Nik *et al*., 1999), domesticulide (Nisakorn Saewan *et al*., 2005), sergeolide (Frandeur *et al*., 1985), neurolenin B, hirsutinolide and bulaquine (Marrero *et al*., 2006) have been found as effective antiplasmodial agents. The mucobromic acid and mucochloric acid having 2(5H)-furanone as sub-structural part have been reported for antimalarial activity (Pillay *et al*., 2007).
Furthermore, strategies such as exploring the main target receptor involved in the pathology (for example ICAM-1 for cerebral malaria) and parasite-specific targets (for example Parasite lactate dehydrogenase) may add some benefits (Biagini et al. 2005). Various enzymes which have been essential for life of parasite *Plasmodium* and having significant different properties than human host, may serve as the potential drug targets and this kind of strategy may help in designing of better therapeutic agents with more specific action and lesser side effects (Makler et al., 1998, Winter et. al., 2003). Moreover, the techniques like structure based drug designing derived from three-dimensional structural information of the drug target proteins, may help us to identify new chemical entities that bind to the parasite in more specific manner and inhibit its growth. Taken as a whole, the above observations, such as the importance of parasite specific drug target and furanone, quinoline, azole based hybrids as promising antimalarial drug candidates, prompted us to design libraries of hybrid ligands based on furanone-Quinoline and furanone-pyrazole moieties. Further the role of computational tools in drug discovery and designing (Khokra et al., 2013), driven our interest to explore these strategies in our aim to search for new antimalarial compounds.
REFERENCES


• Ayi K, Turrini F, Arese, P. Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against
Introduction To Malaria


Introduction To Malaria


- Clark IA, Cowden WB. The pathophysiology of falciparum malaria. Pharmacology & Therapeutics. 2003; 99:221-260.


• Makler MT, Piper RC, Milhous WK. Lactate Dehydrogenase and the Diagnosis of Malaria. Parasitology Today. 1998; 14(9):376-77.


• Sinha K. India to raise malaria toll figure 40-fold. Times News Network. 2012.


Introduction To Malaria


• Winter VJ, Cameron A, Tranter R, Sessions RB, Brady RL. Crystal structure of Plasmodium berghei lactate dehydrogenase indicates the unique structural differences of these enzymes are shared across the Plasmodium genus. Molecular & Biochemical Parasitology. 2003; 131:1–10.


