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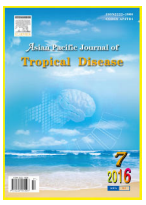

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Emerging drug resistance in *Plasmodium falciparum*: A review of well-characterized drug targets for novel antimalarial chemotherapy

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ABSTRACT

Malaria is a life-threatening, highly infectious parasitic disease caused by the intracellular, protozoan parasites of *Plasmodium* species. The most severe form of the disease is caused by *Plasmodium falciparum*, an Apicomplexa parasite with a remarkable ability to acquire resistance to antimalarial medications. Drug resistant malaria is a serious public health concern in the tropical and sub-tropical nations of the world, rendering conventional antimalarial medication ineffective in several regions. Since an effective malaria vaccine is not yet available to the masses, novel antimalarial drugs need to be developed to control the disease on the face of ever increasing reports of drug resistance. The review acquaints the readers to the current situation of chemotherapy and drug resistance in malaria across the globe, the existing antimalarial medications in use, their mechanism of action and how the malaria parasite evolves resistance to them. The review also focuses on the identification, characterization and validation of a plethora of novel drug targets in *Plasmodium falciparum* from the oxidoreductase, hydrolase and protein kinase groups of enzymes. The work highlights the importance of these drug targets in *Plasmodium* biology and shines a spotlight on novel inhibitors which have the potential to be developed as future antimalarial drugs. The review ends with a detailed discussion on the prospects and challenges in antimalarial drug discovery.

1. Introduction

Malaria is a life-threatening parasitic disease native to the tropical and sub-tropical countries of South-East Asia, the African subcontinent and South America. It is one of the oldest diseases known and recorded by humans and the references to malaria outbreaks have been dated back to the ancient texts of Indian, Chinese, Greek and Mesopotamian civilizations[1]. Despite being an ancient disease, it exists even today and spreads rapidly in the under-developed and developing nations of the world[2]. The disease infects more than 207 million people globally and kills approximately 600000 individuals a year[3]. Among the 2.5 million reported infections in South-East Asia each year, 41% of deaths are reported from India[4,5]. The causative agents are the Apicomplexa parasites of *Plasmodium* species, with *Plasmodium falciparum* (*P. falciparum*) being responsible for the highest recorded morbidity and mortality[6-8]. The disease is prevalent in only tropical and sub-tropical countries of the world. This is due to the fact that the malaria parasite requires two different hosts (the human and the mosquito) and tropical climate coupled with unhygienic environment of the poor nations favoring mosquito breeding. The foremost concern in malaria chemotherapy and treatment is the ever increasing incidences of drug resistance in the malaria parasite[9]. The general idea is that drug resistance results due to a series of spontaneous mutations that confer the parasite with a reduced sensitivity to that particular drug. Also drug resistance appears to develop faster in regions where a large population of parasites are exposed to a specific drug pressure. Natural selection takes care that sensitive parasites are destroyed, while resistant ones would survive and spread[10]. The current scenario is most alarming in several regions of Thailand, Cambodia and Myanmar, where multidrug resistant malaria outbreaks have compelled the medical authority to shift to the last line drug (artemisinin derivatives)[9]. Advances in science and technology has enabled scientists to probe deep into the malaria parasite for the discovery of novel drug targets. In the face of ever increasing instances of drug resistance, the need for new antimalarial molecules to combat malaria in the endemic regions is the pressing need of the hour.

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2. Drug resistance shortening the life-span of antimalarials

Drug resistance in malaria has grown to be a surmounting challenge in the control and eradication of the disease. A reported resistance to a certain antimalarial compound in a malaria endemic region implies that the drug shall no longer be useful for malaria chemotherapy in that area with a fair possibility of spreading of the resistant parasite to other adjoining locations. Currently, there are scientific reports describing resistance to most of the popular antimalarials except the latest artemisinin derivatives which are considered as the last line of defence against the parasite (Table 1). The distribution of drug resistant parasite and resistant malaria infections was reported across the globe. South America and the African subcontinent were the most deadly endemic areas with resistance reported to more than just chloroquine, sulphadoxine-pyrimethamine combinations and mefloquine (Table 1). Quinine was the most primitive antimalarial drug used by man. It was initially extracted from the bark of the cinchona tree[11]. Resistance to quinine was first reported in the 1910 and resulted in synthetic derivatives of quinine such as chloroquine and mefloquine dominating the antimalarial drug market[12]. Table 2 shows the popular antimalarial medications and their year of introduction for malaria chemotherapy, year of publication of first reported resistance and the underlying target whose mutation confers resistance to the drug. Chloroquine resistance against *P. falciparum* was first reported in Thai-Cambodian border of Southeast Asia and Colombia of South America in late 1957 (Table 2). Ever since chloroquine resistance has become a serious concern and has spread to other endemic parts of the world. Soon, chloroquine resistance spreads to most of African subcontinent by 1978 and Asia and Oceania by 1989[21]. In India, the first instance of chloroquine resistance was reported in 1973 in Assam (Karbi-Anglong and Nowgong District)[22].

The rising chloroquine resistance had shifted the first line of drug choice to sulphadoxine-pyrimethamine combination. Sooner resistance to sulphadoxine-pyrimethamine was also reported along the Thai-Cambodian border in 1960s. Resistance to another popular drug, mefloquine was first reported near the borders of Thailand-Cambodia in late 1980s[23]. Table 2 depicts the present status of popular antimalarial drugs, the year of their discovery and the year in which the first report of resistance was published for the drug.

P. falciparum is an extremely complex organism and can easily adapt to changes in microenvironments, different hosts, metabolic changes and drug pressure. Molecular causes of resistance to different antimalarials have been probed by scientific community. For example, chloroquine resistance of *P. falciparum* is attributed to the enhanced capacity of the resistant parasite to expel chloroquine out of the cell (through transporter proteins) at a rate that does not allow chloroquine to accumulate in levels required for the inhibition of heme polymerization[24].

Recent studies have identified genes *pfmdr*-1 and 2 and *pfcrt*, that play crucial role in the resistance to quinolone based drugs such as chloroquine and quinine[25,26]. Similarly, folate inhibitors like sulphadoxine-pyrimethamine inhibit the *Plasmodium* dihydrofolate reductase (DHFR). Mutations in DHFR confers sulphadoxine-pyrimethamine resistance to *P. falciparum*[27]. Similarly, the molecular target of sulphonamide is the dihydropteroate synthase of the parasite. Mutations in dihydropteroate synthase result in resistant strains which are 400–800 fold less sensitive to the drug[28]. Drug resistance has raised the fear, morbidity and mortality associated with malaria and has rendered treatment very difficult. Newer approaches to tackle drug resistance (such as the use of combination therapies) have already been resorted[29,30]. The alternative strategy is to discover novel antimalarial molecules targeting the well-established drug targets in *P. falciparum* using a thorough literature mining. Throughout several decades, scientists have identified a plethora of drug targets in the malaria parasite and many of which have been shown to be essential for parasite survival.

3. Potential drug targets in *P. falciparum*

The sequencing of the *P. falciparum* genome in 2002 is a milestone development in malaria research[31]. Since then, the identification of key parasite genes and metabolic pathways has been a research priority. High throughput transcriptomics and proteomic profiling of different stages of the malaria parasite have helped in identifying proteins playing key roles in specific life cycle stages[32,33]. High

Table 1

Distribution of drug resistance across the globe.

Regions	Resistance reported		
	Chloroquine	Sulphadoxine-pyrimethamine	Mefloquine
Central America (Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, NW Panama)	No	No	No
Caribbean (Haiti and Dominican Republic)	No	No	No
South America (Southeast Panama, Columbia, Peru, Brazil, Venezuela, Ecuador, Bolivia)	Yes	Yes	Yes
Western Africa	Yes	Yes	Yes
Southern Africa	Yes	Yes	No
Eastern Africa	Yes	Yes	No
Indian subcontinent	Yes	No	No
South-East Asia and Oceania	Yes	Yes	Yes
East Asia (China)	Yes	Yes	Yes

Table 2

Present scenario of popular antimalarial medications and resistance against them.

Antimalarial medications	Target pathway/process in parasite	Year of introduction	Year of first reported resistance	Proteins associated with resistance	Function	References
Quinine	Heme polymerization	1632	1910	Pfmdr1, PfCRT, Pfnhe1	Transporters	[13,14]
Chloroquine	Heme polymerization	1945	1957	PfCRT	Transporter	[15,16]
Sulfadoxine-pyrimethamine	Folate metabolism	1967	1969	PfDHPS, PfDHFR	Folic acid biosynthesis	[12,17]
Mefloquine	Hemoglobin digestion	1977	1982	Pfmdr1	Transporter	[18,19]
Artemisinin	Antioxidant system, membrane transport	1994	2010	Pfmdr1, SERCA	Transporter	[20]

Pfmdr1: *P. falciparum* multi-drug resistance transporter 1; PfCRT: *P. falciparum* chloroquine resistance transporter; Pfnhe1: *P. falciparum* sodium/hydrogen exchanger; PfDHPS: *P. falciparum* dihydropteroate synthetase; PfDHFR: *P. falciparum* dihydrofolate reductase; SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase.