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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 600 000 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.

throughput proteomics has revealed 43% of the identified genes being expressed in the sporozoite stage, 35% being expressed during merozoite stage, 42% being expressed in trophozoite and schizont stage and 47% of the identified genes being expressed in gametocyte stage[34]. The quest to identify novel drug targets in the malaria parasite has driven generations of biologists to probe different classes of *Plasmodium* enzymes. Due to the exhaustive nature of this review, we shall limit our discussion to *P. falciparum* targets belonging to the following three classes of enzymes: (i) oxido-reductases, (ii) hydrolases and (iii) protein kinases.

3.1. Crucial oxidoreductases of P. falciparum as the drug targets

Oxidoreductases are enzymes which catalyze the transfer of electrons from the donor molecule (also called reductant) to an acceptor molecule (also known as the oxidant) through the involvement of cofactors such as NADP or NAD^+. These enzymes are essential since they control the redox biochemistry of the cell. In *P. falciparum*, several oxidoreductases have been thoroughly characterized and has been summarized (Table 3). The list of characterized drug targets in the human malaria parasite *P. falciparum* belong to the oxidoreductase group of enzyme nomenclature. Data have been collected through exhaustive literature mining. The dihydroorotate oxidase (DHODH) of the parasite appears to be the most critically studied oxidoreductases for drug development[54]. It is a flavin-dependent mitochondrial enzyme, participating in the *de novo* pyrimidine biosynthesis of the malaria parasite and is an attractive drug target due to the absence of pyrimidine salvage in malaria parasites. The enzyme has been shown to be essential in parasite growth as RNA interfere induced inhibition of DHODH results in growth inhibition[35]. Several inhibitors to *P. falciparum* dihydroorotate dehydrogenase has been reported such as coenzyme Q analogs, orotate analogs and a series of brequinar analogs have been reported to inhibit this crucial parasite enzyme[36-38].

Table 3

Drug targets in the malaria parasite belonging to oxidoreductase group of enzyme classification.

Drug target in *Plasmodium* species	References
DHODH	[35-39]
Succinate dehydrogenase	[40]
Dihydrofolate reductase	[10,41,42]
NADH dehydrogenase	[43]
Glutathione reductase	[44,45]
Thioredoxin reductase	[46,47]
Cytochrome c reductase	[48]
Ribonucleoside-diphosphate reductase	[49-51]
HMBPP reductase	[52,53]

Succinate dehydrogenase is another essential enzyme which participates in the Krebs cycle. The enzyme from *P. falciparum* has been thoroughly characterized and it has been shown to be inhibited by coenzyme Q analog, 5-hydroxy-2-methyl-1,4-naphthoquinone, with an IC_{50} as low as 5 μmol/L[40]. DHFR is an enzyme which reduces dihydrofolic acid to tetrahydrofolic acid, through the involvement of electron donor nicotinamide adenine dinucleotide phosphate. Cycloguanil and proguanil has been shown to inhibit *P. falciparum* DHFR *in vitro*[42]. Interestingly, the anticancer drug methotrexate also inhibits *P. falciparum* dihydrofolate reductase and the drug has been shown to inhibit parasite growth *in vitro*[41]. Similarly, 6-[2'-(3'-methyl)-1',4'-naphthoquinolyl] hexanoic acid, a 5-substituted tetrazoles is shown to inhibit *P. falciparum* glutathione reductase, an essential antioxidant enzyme of the cell[44].

3.2. Crucial hydrolases of P. falciparum as drug targets

Hydrolases are crucial enzymes of the cell which aids in the breakdown of macromolecules such as proteins into smaller fragments or monomers. Several hydrolases of the malaria parasite have been cloned, characterized and probed for inhibitor development (Table 4). The list of characterized drug targets in the human malaria parasite *P. falciparum* belong to the hydrolase group of enzyme nomenclature. Data have been collected through exhaustive literature mining. Protein arginine and lysine methylations are important post-translational modifications within cells which control several aspects of cell biology such as division, apoptosis and transcriptional regulation[63-65]. During methylation event, methyl group from cofactor S-adenosylmethionine is transferred to target arginine or lysine residues of proteins. The byproduct of methylation reactions, S-adenosylhomocysteine (AdoHcy) is recycled by the cellfor reuse. AdoHcy is hydrolyzed to adenosine and L-homocysteine (Hcy) by a crucial enzyme called S-adenosyl-l-homocysteine hydrolase[66]. The crystal structure of AdoHcy hydrolase from *P. falciparum* has been elucidated and is invaluable for inhibitor development against the malaria parasite[55]. Synthetic compounds such as (6'R)-6'-C-methylneplanocin A has been shown to inhibit *Plasmodium* AdoHcy hydrolase[56]. Aminopeptidases are crucial enzymes which breaks down polypeptides to amino acids in cells. *Plasmodium* aminopeptidases have been extensively studied and targeted for antimalarial development. Compounds such as bestatin and nitrobestatin have been shown to inhibit leucine aminopeptidase in *P. falciparum* and *Plasmodium berghei* (*P. berghei*)[59]. The asexual intra-erythrocytic stages of parasite growth largely depend on the parasite's ability to breakdown of host cell hemoglobin for its nutrition. The proteolytic cleavage of hemoglobin into short oligopeptide fragments occur in an acidic organelle known as the food vacuole[67]. *P. falciparum* dipeptidyl aminopeptidase I is such an enzyme which participates in hemoglobin digestion[60]. Aminopeptidases and proteases of the malaria parasite have been a popular class of drug targets due to the fact that hemoglobin digestion and host cell egress are two essential protease-dependent processes key to malaria pathogenesis[68,69]. Plasmepsins are aspartic acid proteases which extensively participate in host hemoglobin degradation in the food vacuole and are the popular choice of drug target for inhibitor development[70]. In *P. falciparum* RNA interfere, mediated blocking of falcipains has demonstrated morphological anomalies, growth inhibition and hemoglobin accumulation within the parasite, demonstrating the essentiality of falcipains in malaria life-cycle[71]. Plasmepsins in *P. falciparum* have been extensively targeted for antimalarial development[61,62].

Table 4

Drug targets in the malaria parasite belonging to hydrolase group of enzyme classification.

Drug target in *Plasmodium* species	References
S-adenosyl-l-homocysteine hydrolase	[55,56,57,58]
Leucine aminopeptidase	[59]
Dipeptidyl aminopeptidase 1	[60]
Plasmepsins (aspartic acid proteases)	[61,62]

3.3. Crucial protein kinases of P. falciparum as drug targets

Protein kinases are defined as enzymes that chemically modify other proteins by adding a phosphate moiety to them, thus modifying the function of the target protein. The phosphorylation of target proteins usually results in a change in its function, cellular localization of rate of enzyme activity. The importance of protein kinases in driving the cellular signaling system can be well understood from the fact that the human genome contains 518 protein kinases, which make up an approximate 2% of the genome[72].

Similarly, the genome sequence of *P. falciparum* has revealed the presence of 65 eukaryotic protein kinases which approximately amounts to 1.6% of the transcriptionally active genes[73,74]. Up to 30% of the *Plasmodium* proteins exist in their phosphorylated state during any instance along the asexual blood stages of the parasite. Protein kinases have always been considered as an important group of drug-targets for many reasons[75]. The primary reason is that the chemical kinetics of a phosphorylation reaction and the overall structural features of most polyketide synthase are basically conserved which means that an array of small molecules can bind to their catalytic site, displacing the natural substrate ATP[76]. Secondly, the reversible protein phosphorylation mechanism is a recurring theme in the regulation of cellular homeostasis, growth, proliferation and differentiation in all forms of life ranging from archaea to human[77]. Several kinase inhibitors have been developed against a myriad of diseases against diabetes, neuropathic diseases, cancer, inflammation and autoimmune disorders[78]. Apart from the clinically approved drugs, a plethora of kinase inhibitors are currently in the late phases of clinical trials or a variety of disease models[79]. Significant differences in the structure and signaling mechanisms of the human and *Plasmodium* protein kinases suggest that selective inhibition of the parasite protein kinases are possible[80]. Many recombinant protein kinases from the *Plasmodium* genome have been characterized in terms of their kinase activity and inhibition (Table 5). The list of characterized drug targets in the human malaria parasite *P. falciparum* belong to the kinase group of enzyme nomenclature. Data have been collected through exhaustive literature mining.

Table 5

Drug targets in the malaria parasite belonging to kinase group of enzymes.

Drug target in *Plasmodium* species	References
CDPK7	[81,82]
CDPK6	[83-86]
CDPK4	[87,88]
PfPK5	[80]
Pfmap1 and Pfmap2	[89]
Phosphatidylinositol 4-phosphate 5-kinase	[90]
Tyrosine kinase-like kinase 3	[91]
PfPK9	[92]
PfPK7	[93]
Phophoenol-pyruvate carboxykinase	[94]
Choline kinase	[95]
Guanylate kinase	[96]

CDPK: Cyclin dependent protein kinase; PfPK: *P. falciparum* protein kinase; Pfmap: *P. falciparum* mitogen activated protein kinases.

Many cyclin dependent kinases (CDKs) have been characterized in *P. falciparum*[74,97]. The kinase activities of two CDKs, *P. falciparum* protein kinase 5 (PfPK5) and *P. falciparum* mitogen activated protein kinase related kinase, have been shown to be regulated by the cyclin binding events. The activities of CDKs from the malaria parasite have been shown to be highly regulated with a high degree of conservation in the catalytic residues. Interestingly, *Plasmodium* CDKs have been shown to exhibit auto-phosphorylation activity *in vitro*[97]. The structure of the *P. falciparum* cyclin dependent protein kinase 4 from *P. falciparum* has been solved and this structure has been exploited for the development of several anti-parasitic compounds (such as pyrazolopyrimidine and imidazopyrazine derivatives) which prevent the transmission of the parasite from human to mosquito[87,88,98].

Interestingly, *Plasmodium* CDKs have also been shown to be inhibited by mammalian cyclin dependent kinase inhibitors *in vitro*, implying a conservation of key structural aspects and functional residues in both the mammalian and *Plasmodium* CDKs[99]. The crystal structure of another parasite CDK PfPK5 coupled with its substrate and inhibitor binding studies have revealed the central role of *P. falciparum* CDKs in the regulation of cellular proliferation[80]. The discovery of several CDPK inhibitors have come in handy in unraveling the cellular functions of CDPKs in the malaria parasite[100]. Knockout of CDPK genes in the murine malaria parasite *P. berghei* has revealed that CDPKs are indispensable for several processes such as parasite ookinete motility, formation of microgametes and the invasion of hepatocytes by the sporozoites[83,101-103]. Ca ions have been shown to regulate the activity of CDPKs by acting as a second messenger and the sub-cellular localization of some of the *Plasmodium* CDPKs have been shown to be governed by the protein's N-terminal acylation[104]. N-terminal acylation along with the stage-specific expression profiles of target genes, contributes to the CDPK signaling specificity in response secondary messenger. A recent study has demonstrated *P. falciparum* CDPK1 to be essential for the parasite to complete the erythrocytic cycle[105]. This study also reports that the treatment of asexual stage parasites with CDPK1 inhibitor prevents merozoite egression.

Mitogen activated protein kinases (MAPKs) are important sub-groups of protein kinases which are known to regulate cell cycle proliferation and cellular differentiation in eukaryotes in response to a wide variety of internal or external stimuli[106]. *P. falciparum* contains 2 atypical MAPKs Pfmap1 and Pfmap2. Studies have shown that Pfmap1 and Pfmap2 act in a supplementary fashion *i.e.*, inactivation of Pfmap1 does not hinder detectable parasite growth *in vitro* but the over-expression of Pfmap2 in the Pfmap 1 mutant parasites clearly indicates that Pfmap 1 serves as an important function in the parasites and this function must be taken over or supplemented by the other MAPK (Pfmap2) in the absence of Pfmap1[107]. The function of Pfmap2 appears to be essential for the parasite to complete the erythrocytic asexual cycle[107]. The regulatory mechanisms of *P. falciparum* MAPKs remain to be elucidated although some studies suggest that *Plasmodium* never in mitosis gene A kinase might act as a regulator of Pfmap2[108,109]. Another protein kinase known as glycogen synthase kinase 3 (GSK3) has been well characterized in *P. falciparum*. *P. falciparum* GSK3 has been found to localize within a specific multifunctional organelle common to apicomplexan parasites, the Maurer's clefts[110]. *P. falciparum* GSK3 is believed to be a secretory kinase which is exported to the host erythrocyte[111].

The AGC sub-group of protein kinases (named after the protein kinase A, G, and C families, *i.e*, protein kinase A, protein kinase C and protein kinase G) are serine/threonine kinases of cytoplasmic origin whose activities are regulated by different secondary messengers such as cyclic AMP (cAMP) or lipids. Three kinases of the AGC sub-group have been well characterized in *P. falciparum*. PfPK A is the only cAMP dependent kinase in the parasite. Inhibition of PfPK A blocks parasite growth *in vitro*[112]. Although the mechanism of the effect of PfPK A inhibition on parasite growth is not clear, one hypothesis proposes that PfPK A acts as a cAMP dependent modulator of anion channel conductance of the host erythrocyte[113]. Experiments on *P. falciparum* blood stages indicate that cAMP dependent PfPK A signaling regulates the level of cytosolic calcium thereby keeping a tight control over the parasite cell division *in vitro*[114]. cAMP and Ca-dependent signaling have been shown to regulate the exocytosis of microneme from parasite cytosol in *P. berghei* and hence is an essential step in the invasion of hepatocytes[115]. PfPK G of parasite is found to be selectively inhibited over its human counterpart by trisubstituted pyrrole based compounds[116]. It has also been demonstrated in related apicomplexan parasite, *Toxoplasma gondii*, that a single amino acid substitution in the ATP-binding site, makes the protein kinase G resistant to inhibition by the above compounds[117]. Further studies have revealed the role of *Toxoplasma gondii* protein kinase G in gliding motility, secretion of micronemes, attachment to host erythrocyte and invasion[118]. The above trisubstituted pyrrole based on

compounds failed to block the rounding up of gametocytes *in vitro* in malaria parasites expressing a resistant version of the *PKG* gene. This demonstrates a key role of protein kinase G in gametocyte activation in apicomplexan parasites[119]. PfPK B is the third characterized kinase in this group. Regulation of mammalian protein kinase B is governed by phosphoinositides which interact with the PH domain of protein kinase B[120]. Protein kinase B from *Plasmodium* however lacks a PH domain. It is activated when calcium-bound calmodulin binds to the calmodulin-binding domain at the N-terminal region of the kinase[121,122]. This type of novel calmodulin based signaling pathway, involving activation of protein kinase B was recently discovered in *P. falciparum*. Experimental evidences that PfPK B phosphorylates a glideosome-associated protein which raises an expectation that in the near future, the functions of many more *Plasmodium* kinases would be unraveled.

There are several protein kinases that do not resemble structurally to any of the established eukaryotic protein kinase groups, and hence are classified under orphan kinases. PfPK7 is one such orphan kinase in the malaria parasite that has been extensively studied. The C-terminal lobe of PfPK7 shows the highest homology to mitogen-ativated protein kinase kinases, while its N-terminal domain is homologous to fungal protein kinase A[93]. Malaria parasites in which PfPK7 is deleted shows slower growth rate, produces lesser number of daughter merozoites, as well as demonstrates impairment in oocyst formation, suggesting this kinase as an important drug target[123]. Another well studied orphan kinase from *Plasmodium* is the PfPK9 which demonstrates the ability to auto-phosphorylate itself as well as phosphorylate exogenous substrates *in vitro*[92]. Auto-phosphorylation specifically occurs on three residues. When any of the critical residues are mutated, enzyme activity is abolished, which suggests a potentially complex mechanism of regulation of this kinase *in vivo*. Recombinant PfPK9 displays selective phosphorylation of an E2 ubiquitin-conjugating enzyme from parasite extract, thereby down-regulating its ubiquitin-conjugating activity. These findings suggest the pivotal role played by PfPK9 in proteasome regulation of *Plasmodium*[92].

4. The prospects and challenges in target based antimalarial discovery

Malaria is rightly classified as a neglected tropical disease[124,125]. The term (neglected) arises due to the non-involvement of pharmaceutical companies in antimalarial drug research and development[126,127]. This is exemplified by the fact that out of the 1 393 drugs approved by the Food and Drug Administration for clinical use between 1975–2000, only four drugs (0.3%) were discovered and approved for malaria chemotherapy[126]. The primary reason for this negligence is the poverty of the infected populations and the meager economy of the countries in the endemic regions[128]. To quote Mr. Bill Gates (http://www.gatesfoundation.org/Media-Center/Speeches/2011/10/Bill-Gates-Malaria-Forum), the malaria parasite has been killing children and sapping the strength of whole populations for tens of thousands of years. It is impossible to calculate that the harm malaria has done to the world. The solution to malaria can only come when focused research from academia and public sector is merged with the infrastructure of the pharma industries through active partnerships and collaborations[129,130]. This perceived negligence for tropical anti-parasitic drug discovery has gradually moved towards betterment since 2000, with more than 20 new antimalarial agents discovered and approved for clinical use. This success is primarily attributed to the progress in partnerships between the public research organizations and top notch pharmaceutical companies[131]. With the grant of new funds for anti-parasitic drug development particularly by organizations and non-governmental organizations like the Bill and Melinda Gates Foundation, The Wellcome Trust, the Irish Aid, Medicines for Malaria Venture and the Drugs for Neglected Diseases initiative, more focused research has been in-progress towards the development of novel antimalarial drugs.

Despite the success, there are several challenges in the path towards development of novel drugs for malaria. The drug discovery process in general is an arduous challenge with high risk and attrition rates. For antimalarial discovery, the challenges seem to be even more due to the following reasons. Firstly, pharmaceutical industries are more keen towards spending on the research and development for chronic and life-style related diseases such as cancer, heart-care and diabetes rather than neglected diseases such as malaria for reasons pertaining to profit[128]. Secondly, many of the candidate antimalarials in human trial in the endemic regions require follow-up health checkups to determine any long term side-effect of the drugs. This is not always possible in the under-developed nations due to a serious lack in health-care infrastructure. Thirdly, antimalarials are primarily developed keeping in mind the endemic populations who dwell in high transmission zones. Many of these endemic regions are teeming with malaria patients, with limited medical resources. Oral drug formulations are thus the ideal. However, obtaining drugs with high efficacy as well as good bioavailability is a challenge. Lastly, drug resistance in the endemic regions shorten the life-span of antimalarials. Such widespread resistance is alarming since the available repertoire of antimalarials is limited. To keep this ever-evolving, life-threatening parasite at bay, novel drugs need to be discovered targeting well characterized, crucial enzymes of *P. falciparum* of next generation antimalarial drugs.

Conflict of interest statement

I declare that I have no conflict of interest.

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