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REVIEW

Antimalarial Drug Resistance and Novel Targets for Antimalarial Drug Discovery

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Abstract: Malaria is among the most devastating and widespread tropical parasitic diseases in which most prevalent in developing countries. Antimalarial drug resistance is the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of medicine given in doses equal to or higher than those usually recommended. Among the factors which facilitate the emergence of resistance to existing antimalarial drugs: the parasite mutation rate, the overall parasite load, the strength of drug selected, the treatment compliance, poor adherence to malaria treatment guideline, improper dosing, poor pharmacokinetic properties, fake drugs lead to inadequate drug exposure on parasites, and poor-quality antimalarial may aid and abet resistance. Malaria vaccines can be categorized into three categories: pre-erythrocytic, blood-stage, and transmission-blocking vaccines. Molecular markers of antimalarial drug resistance are used to screen for the emergence of resistance and assess its spread. It provides information about the parasite genetics associated with resistance, either single nucleotide polymorphisms or gene copy number variations which are associated with decreased susceptibility of parasites to antimalarial drugs. Glucose transporter PfHT1, kinases (Plasmodium kinome), food vacuole, apicoplast, cysteine proteases, and aminopeptidases are the novel targets for the development of new antimalarial drugs. Therefore, this review summarizes the antimalarial drug resistance and novel targets of antimalarial drugs.

Keywords: antimalarial, drug resistance, novel targets, vaccines

Introduction

Malaria is an infectious, hematologic disease causing death and illness in children and adults, especially in tropical countries[1] Malaria control requires an integrated approach, including prevention, primarily vector control, and prompt treatment with effective antimalarial drugs.[2] Malaria is among the most devastating and widespread tropical parasitic diseases in which most prevalent in developing countries.[3] Malaria is caused by the Plasmodium parasite, which is transmitted by the bite of a mosquito vector. Five species are known to infect humans: *P. falciparum, Plasmodium vivax, Plasmodium ovalae, Plasmodium malariae*, and *Plasmodium knowelsi*. The parasite *P. falciparum* causes the most dangerous, with the highest rates of complications and mortality.[3] Antimalarial drug resistance results in a global resurgence of malaria making a major threat to malaria control. Widespread and indiscriminate use of antimalarial drugs contributes to malaria parasites to evolve mechanisms of resistance.[4,5]

The malaria life cycle is very complex which requires two organisms as host, mosquito, and human being.[6] The most common symptoms of malaria (chills, high

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fever, sweating, malaise, headache, and muscle aches) manifest usually one to four weeks after infection with the parasite; in relapsing Plasmodium parasites it ranges from five to eight years, but these signs and symptoms may also have seen in other diseases.[7] Currently, available malaria diagnostic tools for identification of plasmodium species in human blood samples include microscopy (light or fluorescence)-gold standard method, immunochromatographic lateral flow assays (also called rapid diagnostic tests, RDTs), serology, nucleic acid amplification techniques (NATs) that include polymerase chain reaction (PCR) and isothermal amplification and others.[8,9]

According to the 2018 malaria report WHO estimated that approximately, 219 million cases of malaria from 90 countries, an increase of 2 million cases over 2016. Infants and young children in malaria-endemic countries of Africa typically experience several clinical episodes of malaria before they develop partial immunity. This protects against severe disease and death from malaria.[10,11] Malaria continues to burden the overstretched health services in the sub-Saharan region and is a serious public health problem. The same report indicated that 3.1 billion US$ was invested for malaria control and elimination program, of which US$ 2.2 billion benefited the WHO African Region, followed by the WHO Southeast Asia Region US$ 0.3 billion. The WHO African region with 200 million cases (92%) in 2017, followed by the WHO Southeast Asia region (5%), especially Sub-Saharan Africa suffers by far the greatest malaria burden worldwide and is currently undergoing a profound demographic change. Almost 93% of all deaths due to malaria in 2017 were from Africa. Globally 266, 000 (61%) malaria deaths were estimated to be in children less than 5 years age.[12] In most areas of Africa, *P. vivax* infection is essentially absent because of the inherited lack of the Duffy antigen receptor for chemokine on the surface of red blood cells that are involved in the parasite invasion of erythrocytes.[13] However, in Brazilian Amazon, Madagascar, and Central Sudan implicated that individuals with negative Duffy antigen receptor were infected with *p. vivax. P.falciparum* species are dominant in Africa and the highest-burden of *P. vivax* infection is in Southeast Asia and South America[14] In Ethiopia, major malaria transmission seasons are from September to December and June to August.[15] According to the 2018 federal ministry of health (FMOH) of Ethiopia report many densely populated highland areas are malaria-free including the capital city of Addis Ababa. Health management information system (HMIS) of Ethiopia report between June 2016 and July 2017, 1,530,739 confirmed malaria illnesses (69.24% *P. falciparum*, 30.76% *P. vivax*) malaria illnesses from these 356 deaths were reported.[16]

Drugs Used for the Treatment of Malaria

Currently available antimalarial drugs are broadly categorized into three types. Aryl amino alcohol compounds including quinine, quinidine, halofantrine, lumefantrine, chloroquine, amodiaquine, mefloquine, cycloquine, etc. Antifolate compounds: proguanil, pyrimethamine, trimethoprim, etc. Artemisinin compounds like artemisinin, dihydroartemisinin, artesunate, artemether, arteether, etc.[17,18]

Most of the antimalarial drugs target the asexual erythrocytic stages of the parasite (blood schizonticidal drugs). Two types either fast-acting (Chloroquine, quinine, and mefloquine) or slow-acting (Pyrimethamine, sulphonamides, and sulphone). Tissue schizonticidal drugs target the hypnozoites (dormant stage of the parasite) in the liver whereas gametocytocidal drugs destroy sexual erythrocytic forms of the parasite in the bloodstream preventing transmission of malaria to the mosquito. Sporontocides prevent or inhibit the formation of malarial oocysts and sporozoites in the infected mosquito.[19]

Quinolines (affects polymerization of hemozoin), antifolates (block dihydrofolate reductase and dihydropteroate synthetase enzymes of the parasite) and artemisinin (have various mechanisms), administered alone or in combination to treat malaria[18] Artemisinin combination therapy is the cornerstone of malaria control in sub-Saharan Africa such as artemether/lumefantrine and artesunate/amodiaquine. Because of the notorious capacities of *Plasmodium falciparum* to develop drug resistance, many antimalarial programs have recently included dihydroartemisinin/piperaquine (DHA/PPQ) as a second-line antimalarial drug.[20]

Resistance to *Plasmodium vivax* and *Plasmodium falciparum*

Before dealing with resistance to malaria it is better to know the terminologies of recurrence, recrudescence, relapse, and resistance (4R's). Recurrence is the recurrence of asexual parasitemia following treatment (in *P. vivax* and *P. ovale* infections only) or a new infection. Recrudescence is the recurrence of asexual parasitemia

after the treatment of the infection with the same infection that caused the original illness. Relapse is the recurrence of asexual parasitemia in *P. vivax* and *P. ovale* malaria deriving from persisting liver stages from persisting hypnozoites. Resistance is the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial medicine in the dose normally recommended.[21] *Plasmodium vivax* is continued to put a substantial burden on the malaria-endemic world with the morbidity and mortality due to its propensity to cause recurrent infections.[22] *Plasmodium vivax* forms dormant liver stages (hypnozoites), which causes relapses of infection weeks to months after the initial attack. Recurrent infections can occur as often as every three weeks, with relapses the main cause of vivax illness. Even though chloroquine is the first-line treatment for *P. vivax* malaria in most endemic countries resistance is the main problem facing chloroquine in different parts of the world. In Africa and South America chloroquine resistance to *plasmodium falciparum* first appeared in 1978 and 1996 respectively.[23,24]

Chloroquine-resistant *Plasmodium vivax* was first reported in 1989 from Papua New Guinea. High-grade chloroquine-resistant *Plasmodium vivax* is prevalent in areas such as Indonesia and Oceania (regarded as epicenters of chloroquine resistance).[25] Both the acute illness and relapses from hypnozoites can be effectively prevented by the administration of a combination of chloroquine with primaquine (radical cure). Primaquine has activity against both blood and liver stages, including against chloroquine-resistant strains. Severe *P. vivax* infections can cause cerebral malaria with generalized convulsions and status epilepticus, severe anemia, hepatic dysfunction and jaundice, acute lung injury, pulmonary edema, splenic rupture, acute renal failure, and severe thrombocytopenia with or without bleeding from different parts of the body.[26–28]

Primaquine has activity against both asexual and sexual blood stages of the parasite as well as against the liver stage schizonts and hypnozoites.[29] Primaquine can result in significant hemolysis in people with glucose-6-phosphate dehydrogenase deficiency (G6PDd). G6PD deficiency is the most common heritable enzymopathy in the world, with a prevalence range of 2% to 40%.[30] The WHO for radical cure of vivax malaria currently recommends the use of a daily dose of 0.25 mg/kg/day (3.5 mg/kg total dose) primaquine taken with food once daily, which can be either co-administered with chloroquine or artemisinin combination therapy depending on chloroquine sensitivity in the area for radical cure of vivax malaria. Current guidelines recommend a 14-day course of primaquine administered either once or twice daily to reduce the risk of hemolysis and improve tolerability from gastrointestinal disturbance.[2]

In Ethiopia first report of *P. falciparum* and *P. vivax* chloroquine treatment failure in Debre Zeit, was in 1995. The invasion of human red blood cells by the extracellular merozoite form of *Plasmodium falciparum* is a process central to the pathogenesis of this devastating pathogen. In the present time control of multidrug-resistant *P. falciparum* malaria has become a very difficult task because endogenous allelic exchanges occurred in *P. falciparum* have increased the therapeutic failures and significantly increased the levels of resistance worldwide. As evolution is an unending process how the formation of drug-resistant mutant alleles stops is a very concerning question.[31] Usually higher mean parasitemia index is seen in infected individuals with *P. falciparum* but *P. vivax* infection generally exhibits low parasitemia index due to its preference to invade reticulocytes rather than erythrocytes.[32]

Mechanism of Antimalarial Drug Resistance

According to the World Health Organization (WHO), antimalarial drug resistance is defined as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of medicine given in doses equal to or higher than those usually recommended but within the tolerance of the subject, provided drug exposure at the site of action is adequate. Resistance to antimalarial arises because of the selection of parasites with genetic mutations or gene amplifications that confer reduced susceptibility.[2]

Resistance appears to be caused by a change in the structure, function, or quantity of a protein. The change in the protein is mediated by genetic changes such as single nucleotide polymorphisms (SNP) or gene amplification. Because of antimalarial drug resistance is becoming the most difficult hurdle for the success of antimalarial therapy, so scientists are in continuous move researching to overcome the problem. Resistant parasite strains will always emerge, requiring the continual generation of new molecules. The novel drugs with a new mechanism of action are entering into clinical trials.[17]

Several factors facilitate the emergence of resistance to existing antimalarial drugs. To mention some factors, the parasite mutation rate, the overall parasite load, the strength of drug selected, the treatment compliance, and poor adherence to malaria treatment guidelines.[33] Improper dosing, poor pharmacokinetic properties, fake drugs lead to inadequate drug exposure on parasites.[34] Poor-quality antimalarial (falsified antimalarial without active pharmaceutical ingredient (APIs)) may aid and abet resistance by increasing the risk of hyperparasitaemia, recrudescence, and hypergametocyopaenia, wrong APIs such as the use of halofantrine instead of artemisinin which without chemical analysis will be invisible to investigators but not to parasites.[35,36] Frequently targeted biological pathways by antimalarial drugs in parasites of plasmodium are heme detoxification (in digestive vacuole) biosynthesis folate and pyrimidine and electron transport (in mitochondrion). Studies done during treatment with aryl amino alcohols quinine, lumefantrine (LMF), and mefloquine (MFQ) from South East Asia showed that copy-number changes in pfmdr1, as well as PfCRT and PfMDR1 sequence variants, can affect the parasite's susceptibility.[32] Unlike other diseases (eg Tuberculosis, AIDS), malaria drug resistance mechanisms are unique, as the parasite is capable of inducing resistance in the exact cellular target of the drug, drug resistance phenotype is mostly induced due to enhanced and non-specific efflux of drugs through induction of multidrug resistance (MDR) transporters. In malaria, MDR transporters are not the primary mechanism of resistance (Table 1).[37]

The antimalarial activity of Artemisinin is due to its unique trioxane structure with an endoperoxide bond. Usually, semi-synthetic derivatives are used clinically (artemether, artesunate, and dihydroartemisinin) because due to the low solubility of artemisinin.[38] Artemisinin combination therapy (ACT), currently recommended are artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, artesunate + sulphadoxine-pyrimethamine, and dihydroartemisinin + piperaquine, the current gold standard for malaria treatment but resistance is emerging in different areas. Resistance to Artemisinin and its derivatives are emerging and troubling phenomena in malaria treatment.[39] In the Greater Mekong Subregion of Asia, the Artemisinin-based drug resistance is emerging.[40] As resistance to each new malaria drug arises, it becomes necessary to combine two or more component drugs to slow the spread of resistance to reduce the chance of resistance combinations containing an artemisinin derivative that is currently in use.[41] Within the malaria parasite-host hemoglobin is degraded by a series of protease enzymes to release peptides and amino acids required for development and to create space within its digestive vacuole in which buildup of hematin occurs which is potentially toxic to the parasite.

Table 1 Summary of Some Antimalarial Drugs, Mechanism of Action, Site of Action, and Mechanism of Resistance

Antimalarial Drug	Mechanism of Action	Site of Action	Mechanism of Resistance
Antifolates ((pyrimethamine (PYR) and cycloguanil (CYC))	Inhibition of dihydrofolate reductase (DHFR)	Cytosol	Mutations in dihydrofolate reductase (DHFR)
Antifolates (sulfadoxine (SDX))	Inhibition dihydropteroate synthetase (DHPS)	Cytosol	Dihydropteroate synthetase (DHPS)
Naphthoquinones (Atovaquone (ATQ))	Inhibits mitochondrial electron transport	Mitochondria	A single point mutation in the cytochrome b subunit (CYTb) of the bc1 complex
Antibiotics (Clindamycin (CLD) and Doxycycline (DOX))	Inhibit protein translation inside the apicoplast	Inside the apicoplast	A point mutation in the apicoplast encoded 23S rRNA (CLD)
Artemisinin (ART)	Alkylation of proteins and lipids	ER, vesicular structures	Mutation in K13
4- aminoquinolines (CQ, AQ, PPQ, Mannich base pyronaridine (PND))	They bind reactive heme and interfere with its detoxification through incorporation into chemically inert hemozoin.	Digestive vacuole	Point mutations in the transporters PfCRT and PfMDR1, increased expression of the hemoglobinases plasmepsin 2 and 3 (PM2/PM3, in the digestive vacuole), and might in some instances involve mutant PfCRT

Artemisinin and its derivatives have a fast onset of action but are eliminated soon (half-life 0.5–1.4 h) from humans for this reason it is essential to combine with slow clearing drugs to kill residual parasites.[42] Artemisinin and its derivatives can be combined with other antimalarial drugs at least for two reasons, first to prolong the half-life of Artemisinin and its derivatives, second, to prevent resistance.[43] Recent reports in Equatorial Guinea showed that P. falciparum isolate was resistant to artemisinin.[44]

Molecular Mechanism of Artemisinin Resistance

Artemisinin possesses a long-acting effect against drug-resistant malaria parasites, and also able to reduce the parasite burden in asymptomatic individuals who serve as reservoirs for malaria transmission.[45] Artemisinin and its derivatives (artesunate, artemether, and arteether) are potent and fast-acting drugs that cause a rapid decline in parasitemia during the first days of treatment. Meshnick using mass spectroscopy observed that Artemisinin can alkylate heme resulting in decomposition of the endoperoxide bridge to produce carbon-centered free radicals which are crucial for selectively toxic to malaria parasites.[46] Specific protein or enzyme is used as a molecular target for Artemisinin. Invitro studies show that hemoproteins such as catalase, cytochrome c, and hemoglobin but not free globin, is alkylated by Artemisinin.[47]

Another molecular target is PfATP6; Artemisinin inhibits of a parasite Ca^{2+} transporting ATPases (SERCA – Sarco/endoplasmic reticulum membrane calcium ATPase). SERCA reduces cytosolic free calcium concentrations by actively concentrating Ca^{2+} into membrane-bound stores, an activity critical to cellular survival.[48] In the parasite membrane, Artemisinin accumulates within neutral lipids and causes parasite membrane damage.[49] Artemisinin has shown resistance in P. falciparum cultures[50] and *P. voelii* mouse models[51] and in vitro resistance in field isolates.[52]

P. falciparum Kelch 13 (PfKelch13), the marker for artemisinin resistance in P. falciparum malaria, is not an enzyme or a pump but rather is predicted to be a substrate adapter for a cullin E3 ligase, with a putative substrate of *P. falciparum* phosphatidylinositol 3-kinase (PfPI3K) and a redox sensor.[53] Kelch-like protein K13 is a molecular marker for Artemisinin resistance, but no detectable impact in Africa (except one report with P. falciparum K13-variant infection from western Africa). The reason behind this low impact is the greater degree of acquired immunity there, resulting from repeated exposure to *P. falciparum*, which builds host immunity to help control drug-resistant infections.[44] Mutant K13 results in lowered ART interactions with P. falciparum phosphatidylinositol-3-kinase (PfPI3K).[54] In vitro and in vivo studies in many areas of Southeast Asia show that mutations in the K13 propeller gene (PF3D7_1343700 or PF13_0238) are linked to artemisinin resistance.[55]

Molecular Markers of Antimalarial Drug Resistance

Molecular markers of antimalarial drug resistance are used to screen for the emergence of resistance and assess its spread. It provides information about the parasite genetics associated with resistance, either single nucleotide polymorphisms or gene copy number variations which are associated with decreased susceptibility of parasites to antimalarial drugs. Detection of molecular markers provides a feasible means of tracking the emergence and/or spread of antimalarial drug resistance.[56] *P. falciparum* chloroquine resistance transporter gene (PfCRT), chloroquine accumulates within the DV (digestive vacuole) of the parasites where there is mutant PfCRT, accumulation of chloroquine in parasites is very less as compared to parasites expressing wild type PfCRT,[57] as a result, chloroquine-resistant parasites can export chloroquine via active transport,[58] implying that mutant and wild type PfCRT have different drug transporting properties. P. falciparum multidrug resistance transporter 1 (PfMDR1) locates in the digestive vacuole of the parasite and function as a general importer sequestering toxic metabolites and drugs into the digestive vacuole (DV). Pfmdr1 indirectly influences drug flux by affecting intracellular ions and PH.[59] Studies show that wild type PfMDR1 transports quinine and chloroquine but not halofantrine while mutant PfMDR1 transports halofantrine but not quinine or chloroquine.[60]

The multidrug resistance-associated protein (PfMRP) is a member of the ATP-binding cassette (ABC) proteins family and ABC transporter C subfamily. Genetic disruption PfMRP leads to increased parasite susceptibility to several antimalarial drugs like chloroquine, quinine, artemisinin, piperaquine, and primaquine and accumulates more glutathione (GSH), chloroquine, and quinine.[61] Cytochrome bc1 complex catalyzes the transfer of electrons from ubiquinol to cytochrome c thereby maintaining

the membrane potential of mitochondria used to produce ATP by an ATP synthase. Mutations in the Cytochrome bc1 complex leads to atovaquone resistance this is because atovaquone binds to the ubiquinol binding site, thereby disrupting the electron transfer chain.[62,63]

Imidazolopiperazine (IPZ) class of drug compounds (saturated cyclic amines) has activity in both liver and blood-stage parasites as an antimalarial drug for use in prophylaxis, treatment, and prevention of malaria disease transmission. Whole-genome sequencing done by Prof Paul and his co-workers of these drug-resistant *Plasmodium falciparum* clones, genes associated with drug resistance are identified, an acetyl-CoA transporter (PFACT) and a UDP-galactose transporter (PFUGT). These mechanisms responsible for resistance are members of a family of membrane transporter proteins (major facilitator superfamily or MFS).MFS is the largest and most ubiquitous secondary transporter family responsible for the translocation of small molecules including metabolites, nucleosides, oligosaccharides, amino acids, oxyanions, and drugs.[64,65]

Imidazolopiperazine is promising drug candidates with the potential to aid in malaria elimination include KAF156 and KAF179 (currently in Phase II clinical trials). They possess low Nanomolar potency against *P. falciparum* liver stages, asexual blood stages, and sexual stage gametocytes.[66] P. falciparum V-type H^+ pyrophosphatase (PFVP2) is located in the DV membrane and increased transcription of pfvp2 has been observed in-vitro when P. falciparum are exposed to chloroquine (10-fold up-regulation) and lumefantrine (2-fold up-regulation). The up-regulation of pfvp2 implies that it could be involved in maintaining the H^+ balance in the parasite DV and to compensate for H^+ loss caused by the removal of protonated CQ.[67,68]

Antimalarial Drug Resistance Surveillance

Antimalarial drug resistance surveillance can be performed through in vivo studies such as therapeutic efficacy studies, in vitro/ex vivo studies of cultured malaria parasites, and molecular studies assessing known markers of antimalarial drug resistance. As outcomes have direct clinical relevance in therapeutic efficacy studies it is regarded as the gold standard for informing antimalarial drug resistance and for drug regimen change as well. In malaria-endemic countries, routine monitoring of antimalarial drug efficacy is carried out at sentinel sites by national malaria control programs using a standardized WHO protocol. Treatment response is defined as the absence of parasitemia at follow-up, on day 28 or 42. WHO recommends a switch to another more effective first-line drug if a 10% treatment failure rate is reached.[69,70]

Novel Targets of Antimalarial Drugs

The existing antimalarial drugs were identified based on the major metabolic pathway differences of the parasite with its host. The Key metabolic pathways of the Plasmodium species, including oxidative stress, heme detoxification, fatty acid synthesis, and nucleic acid synthesis are some of the novel targets for antimalarial drug discovery and development.[71,72] Though most of the antimalarial drugs used for many years, presently the use of such drugs is limited as a result of drug resistance. According to previous studies, there are no antimalarial agents recognized to inhibit an identified antimalarial drug targets.[73] In its place, the majority of the antimalarial agents were discovered in both in vitro model and animal models (in vivo). Thus, the exact mechanism of action of most antimalarial drugs is not known. In addition, the mechanism of antimalarial drug resistance was not well known for most antimalarial drugs.[72] In addition to increasing the need to develop new antimalarial drugs, identifying countermeasures either to delay or minimize the development of resistance against new drugs is an important phenomenon.

Glucose Transporter PfHT1

Glucose is a source of energy for Intra erythrocytic malarial parasites in which infected erythrocytes consume higher energy than normal erythrocytes.[74] P. falciparum almost fully depend on glycolysis for energy production, deprived of energy stores; depend on continuous uptake of glucose as a source of energy. The Pyruvate is converted into lactate to yield ATP in the parasite, which necessitates for replicating in the intraerythrocytic site.[75] Initially, via GLUT1 transporter Glucose is transported from the blood into the parasitized erythrocyte, which is abundant in the erythrocyte membrane.[76] The Plasmodium glucose transporter P. Falciparum Hexose transporter (PFHT) is essential for parasite growth and survival,[77] as well as, is the main transporter of glucose.[78] GLUT1 transporter can only transport D-glucose, while P. Falciparum Hexose transporter non selectively transports both D-fructose and D-glucose. Thus, the differences between PFHT and

GLUT1 in their interaction with the substrates, proposed that selective inhibition of P. Falciparum Hexose transporter is a potential novel target for the discovery of new antimalarial agents.[79] In the previous study, Compound 3361 which is a long chain O-3-hexose derivative can hinder the uptake of fructose and glucose by P. Falciparium Hexose transporter nevertheless, it cannot hinders hexose transport by mammalian transporters (GLUT1 and 5). Similarly, Compound 3361 was reported to hinders the glucose uptake by P. vivax of P. Falciparum Hexose transporter.[80]

Targeting the Parasite Protein Kinases

Kinases are involved in phosphorylation, transcriptional control, post-transcriptional control, and protein degradation in the plasmodium parasite life cycle. So, could be the strategic targets for the development of antimalarial drugs. The most studied Cyclin-dependent kinases (CDKs) in *Plasmodium falciparum* are P. falciparum protein kinase 5 (PfPK5), 6, and P. falciparum mitogen related kinase (PfMRK). By in-vitro study two compounds, flavopiridol and lomoucine have shown inhibition of PfPK5, by decreasing DNA synthesis and changing total RNA synthesis and parasite growth.[81]

The P. falciparum kinases play a significant role in the parasite differentiation and growth. Amongst numerous kinases, cyclin-dependent protein kinases (CDKs) are conspicuous targets for the development of drugs, numerous cyclin-dependent protein kinases selective inhibitors were discovered for the management of different diseases such as neurological disorders, infectious diseases, and cancers. Presently, they become a potential novel target in the discovery and development of new antimalarial drugs.[82,83] The PfCDPK4 plays a key role in the formation of infectious sporozoites through the sexual phase of the malarial life cycle. Compound 1294 which is PfCDPK4 inhibitor, revealed an antimalarial effect with a novel mechanism of action through preventing the transmission of parasites from mosquitoes to humans.[84] Likewise, Imidazopyridine derivatives have shown significant PfCDPK1 inhibitory effect with nanomolar antimalarial activity in both in vitro and in vivo models (Table 2).[85]

Table 2 Role of Kinase in the Plasmodium Parasite Life Cycle

Type	Role in the Parasite Life Cycle	Reference
Serine/threonine-protein kinase, casein kinase 2α (PfCK2α)	Crucial for asexual blood-stage parasites	[79]
Calcium-dependent protein kinase 1 (PfCDPK1)	Essential for parasite survival	[79]
Mitogen-activated protein kinase 2 (PfMAP-2)	Essential for completion of the asexual cycle	[80]
cGMP dependent protein kinase (PfPKA)	Essential for parasite growth and survival	[81]
Orphan protein kinase PfPK7	Significant for asexual stage development in humans and oocyst production in mosquitoes	[82]

Food Vacuole as a Drug Target

The blend of digestive vesicles provides a large digestive vacuole/food vacuole through the growth of the malaria parasite inside the human erythrocytes. Food vacuole is accountable for the degradation of 60–80% of the host red cell hemoglobin, which has a key role in the attainment of amino acid which is essential for parasite development and growth. Investigation of this degradation pathway can be a promising method for the discovery and development of novel antimalarial agents. This pathway is started by a series of protease enzymes that digest hemoglobin into small peptides. During proteolysis, heme is released from hemoglobin as a toxic byproduct which is detoxified by conversion into hemozoin. In the previous studies, hemozoin comprises about 95% of the free iron synthesized through hemoglobin digestion.[86,87] In addition, two possible mechanisms responsible for the degradation of hemoglobin were reported (degradation by hydrogen peroxide inside the large digestive vacuole and glutathione-dependent degradation within the cytoplasm).[88–90]

Electron Transport Chain (ETC)

The plasmodial mitochondrial electron transport chain is produced from non-proton motive quinone reductases, such as malate quinone oxidoreductase (MQO), (DHODH), (Alternative Complex I), type II NADH dehydrogenase (NDH2, glycerol 3-phosphate dehydrogenase (G3PDH), dihydroorotate dehydrogenase, and succinate dehydrogenase (SDH, Complex II), and proton motive respiratory complexes, such as ATP synthase (Complex V), cytochrome c oxidase (Complex IV), and bc1 complex (Complex III). The electron transport chain needs cytochrome c1 and ubiquinone (coenzyme Q) which serve as

electron carriers between the complexes.[91–93] The pool of electron transport chain and carbon metabolism antimalarial targets that have been under the lamp post in recent years, as well as suggest a promising new avenue for the validation of novel drug targets for the treatment of malaria. The interaction between the pathways vital for the parasite, such as aspartate metabolism, mitochondrial tricarboxylic acid cycle, and pyrimidine biosynthesis, is described to create a road map of novel antimalarial agents.[94]

Apicoplast as Drug Targets

Recently, blocking the P. falciparum ribosome and other parts of the translational machinery accountable for protein synthesis are becoming a promising target for the discovery and development of novel antimalarial agents. The plasmodium species have three genomes: apicoplast, nuclear, and mitochondrial.[95] The apicoplast is a chloroplast like organelle of apicomplexan parasites. The apicoplast resulted from endosymbiosis, leading to an organelle that maintains certain specific functions, probably including fatty acid, heme, and amino acid metabolism.[96] The apicoplast genome of P. falciparum comprises a 35-kb DNA which is small in size.[97] The apicoplast is a non-photosynthetic plastid that is vital for the malarial parasite since it covers a large number of important metabolic biochemical pathways (biosynthesis of fatty acid, isoprenoid precursors, and heme synthesis) for the *Plasmodium falciparum* survival. Human beings do not have these metabolic biochemical pathways which are important for ideal drug targeting.[98,99]

Even though the majority of proteins of this organelle are encoded in the nuclear genome and are subsequently transported to the apicoplast, it also encodes a full set of tRNAs, some ribosomal proteins, three genes for the subunits of an oligomeric RNA polymerase, a gene for the elongation factor PfTu and a gene contributing to the Fe–S pathway.[100] Since the apicoplast possess unique metabolic pathways such as isoprenoid, heme synthesis, and fatty acid, which are not found in the human,[101] it could be a potential drug target for the management of malaria. As reported in the previous study, protein syntheses inhibitors play a key role in the clinical success of potent antibiotics. Azithromycin, Clindamycin, and Doxycycline revealed antimalarial activity since they can inhibit the ribosomes within the apicoplast and Plasmodium species mitochondria, resulting in loss of the normal function of these organelles.[95] For prevention of malaria, azithromycin has shown a noticeable protective effect in Kenyan[102] and Indonesian[103] adults when received daily doses, even though the preventive activity was lower than doxycycline in both trials (protective efficacy in Kenya was 93% for doxycycline vs 83% for azithromycin; in Indonesia 96% vs 72%, respectively). In Kenya, azithromycin preventive activity was fairly deprived when administered weekly (64%). Mass distribution of azithromycin for the control of trachoma was linked with a decrease in malaria parasitemia as compared to controls.[104] Azithromycin + piperaquine was well tolerated in pregnant Papua New Guinean women,[105] even though preventive efficacy data are not obtainable.

Plasmodium Proteases

Plasmodium proteases are a regulatory and ubiquitous catalytic enzyme that play a significant role in the survival of the plasmodium parasite and responsible for the hydrolysis of the peptide bond (Figure 1).[106] The role of plasmodium proteases in the pathogenesis of malaria disease includes activation of inflammation, cell/tissue penetration, invasion of erythrocyte, development of the parasite, immune evasion, autophagy, and hemoglobin and other proteins breakdown.[107] Plasmodium proteases such as aspartate, serine, cysteine, metallo, threonine, and glutamate are auspicious drug targets for the treatment of malaria since the disruption of the plasmodium proteases gene inhibits the degradation of hemoglobin and the growth of the parasite in the erythrocyte stages.[108]

Proteases are generally used for the rupture and subsequent reinvasion of erythrocytes by merozoite-stage parasites and the degradation of hemoglobin by intraerythrocytic trophozoites. For instance, drugs that inhibit Plasmodium cysteine proteases are the potential targets for malarial treatment and shown potential effects.[109] Cysteine proteases have different roles in Plasmodium parasites including hemoglobin hydrolysis (provide amino acids for parasite protein synthesis, maintain the osmotic stability of malaria parasites),[110] erythrocytic rupturing, helps merozoites to be released,[111] erythrocyte invasion also it has a role on non-erythrocytic parasitic stages (Table 3).

Aminopeptidases

Amino peptidases catalyze the cleavage of amino acids from the amino terminus of peptides and proteins and are distributed widely in prokaryotes and eukaryotes as either integral membrane or cytosolic proteins (Figure 1). They play a role in protein and peptide metabolism, activation/inactivation of biologically active peptides, removal of the N-terminal methionine from newly synthesized proteins, and the trimming of

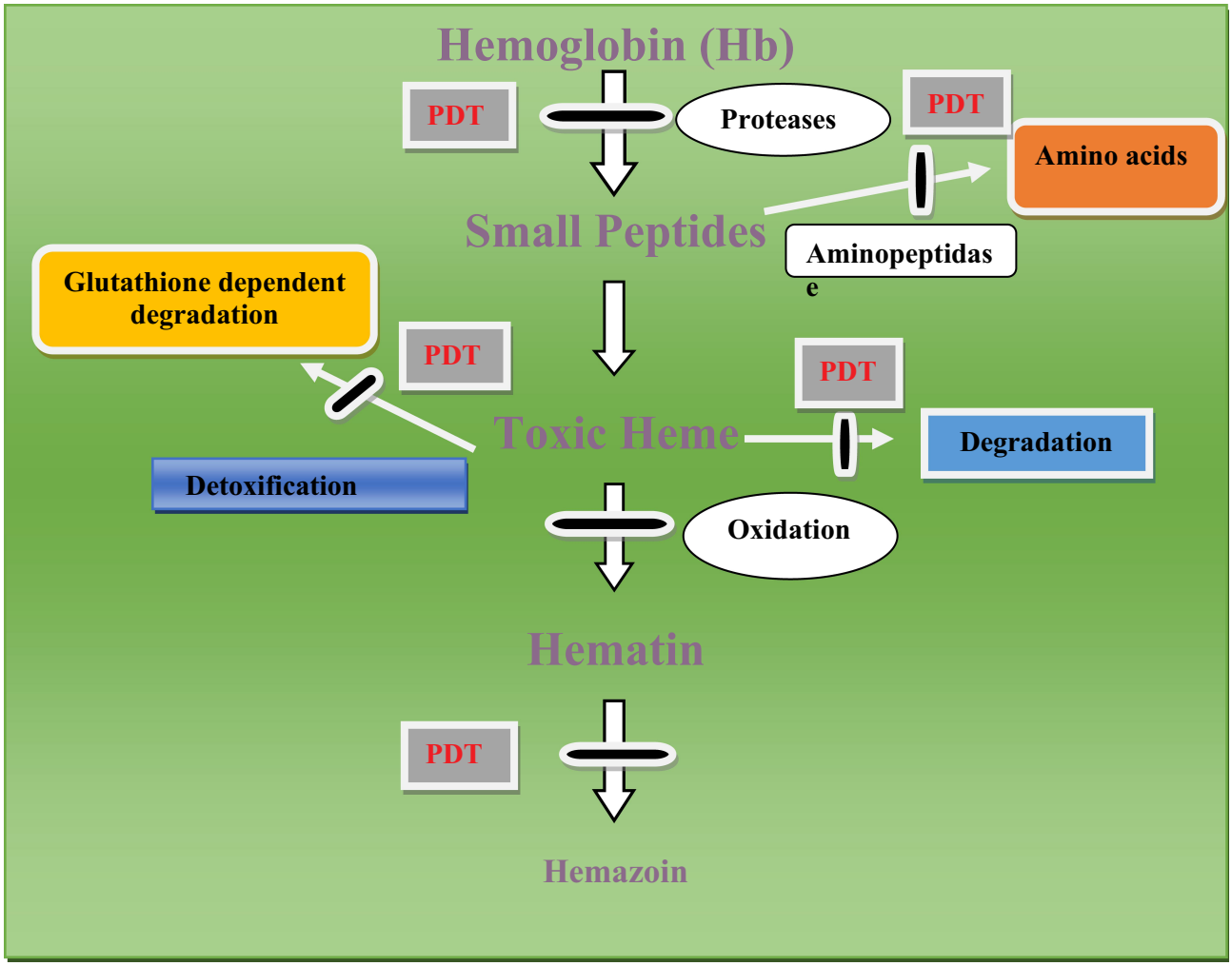

Figure 1 Targets of proteases and amino peptidase, malaria parasite detoxification mechanism.
Abbreviation: PDT, possible drug targets.

antigens for presentation by the major histocompatibility complex-1 system.[112] Bestatin inhibits the growth of *P. falciparum* in vitro and in vivo and is active against the intraerythrocytic stages. Bestatin appears to inhibit both leucine aminopeptidase *(Pf*LAP) and membrane alanine aminopeptidase, (*Pf*A-M1) by chelating the active metal ions in their metal-binding centers. Inhibitors capable of binding compactly within the active site and chelating the tightly bound metal ions of both *Pf*A-M1 and *Pf*LAP. It has two metal-binding sites, a readily exchangeable site, and a tight binding site may prove more potent and show greater anti-malarial activity.[113–115]

A Recent Achievement in the Discovery and Development of Antimalarial Agents

Drugs currently in Phase I, II and III trials for blood-stage treatments of malaria includes KAE609 (cipargamin) inhibit Na+-TPase 4 ion channel,[116] KAF156/GNF156/(Cyclic amine resistance unknown mechanism of locus (PfCARL) inhibitor),[117] Albitiazolium/SAR9727/(Inhibit the transport of choline into the parasite),[118] DSM265 (Inhibit dihydroorotate dehydrogenase enzyme),[119] Methylene Blue (Prevents haem polymerisation by inhibiting P. falciparum glutathione reductase),[120] Sevuparin/DF02/(Anti-adhesive polysaccharide derived Blocks merozoite invasion and sequestration),[121] MMV048 (Inhibiting the parasite enzyme phosphoinositol 4-kinase enzyme),[122] MMV390048 (Phosphatidylinositol 4-kinase (PfPI4K) inhibitor),[123] Fosmidomycin + piperaquine (DOXP pathway), Artefenomel (oz439) + Piperaquine (Synthetic endoperoxide),[124] OZ277+ Piperaquine (Inhibit Pf-encoded sarcoplasmic endoplasmic reticulum calcium ATPase), P218 (PfDHFR inhibitor),[125] M5717/DDD498/(Protein-making machinery of the malaria

Table 3 Mechanism of Action of Protease Inhibitors

Name of Drug	Mechanism of Action on Cultured Parasite
Leupeptin	Causes the food vacuole to swell and fill with dark-staining material, blocks the processing of hemoglobin, Inhibits lysis of erythrocyte membranes
Anti-pain	Inhibits lysis of erythrocyte membranes
E-64	Causes the food vacuole to swell and fill with dark-staining material, inhibits lysis of parasitophorous vacuole, Blocks the processing of hemoglobin
Chymostatin (serine protease inhibitor)	Inhibits erythrocyte invasion

parasite, liver- stage P. falciparum),[126] SJ733 (The P-type Na+–ATPase transporter),[116] and Spiroindolone (cipargamin) inhibits PfATP4, a parasite plasma membrane Na+-ATPase that regulates sodium (maintains low-level Na^+ in the cytosol) and osmotic homeostasis. Cipargamin is used in the treatment of falciparum and vivax malaria. Inhibition of PfATP4 increases a Na^+ in the cytosol as Na^+ moves into the cell, down its electrochemical gradient leading to a concomitant increase in cytosolic pH (PfATP4-mediated acid load). Mutation in PfATP4 results in cipargamin.[127] Artefenomel is a new synthetic antimalarial peroxide that clears parasitemia rapidly in both *P falciparum* and *P vivax* malaria. It has a good safety profile and long half-life (for a single dose malaria cure).[128]

Conclusion

In conclusion, present and future therapeutic targets for the discovery and development of novel antimalarial agents were reviewed. The frequently emerging antimalarial drug resistance including combination therapies globally forces the scientists to search and develop antimalarial drugs with novel mechanisms of action. Resistance to two highly dominant species *Plasmodium falciparum* and *Plasmodium vivax* is highly predominant in south East Asia, Africa, and South America. The complex life cycle of malaria parasite provoke obstacle in the discovery of new therapeutic agents, nevertheless, the discovery of novel biochemical pathways in the malaria parasite offers new opportunities for development antimalarial agents. Due to the resistance of antimalarial agents globally, searching for novel cellular targets and developing new therapeutic agents targeting old targets is both imperative aspects in fighting drug-resistant malaria. The future antimalarial drug development will better target medicines with a distinctive mechanism of action.

Abbreviations

ABC, ATP binding cassette; ACT, artemisinin combination therapy; ART, artemisinin; ATQ, atovaquone; CDKs, cyclic dependent kinases; CLD, clindamycin; CQ, chloroquine; CYC, cycloguanil; Cytb1, cytochrome b subunit 1; DHA/PPQ, dihydroartemisinin/piperaquine; DHFR, dihydrofoliate reductase; DHPS, dihydropteroate synthase; DV, digestive Vacuole; G6PDd, glucose-6-phosphate dehydrogenase deficiency; GSH, glutathione; IPZ, imizolopiperazine; LMF, lumefantrine' MFQ, mefloquine; MFS, major facilitator superfamily; NATs, nucleic acid amplification; PAM, pregnancy associated malaria; PCR, polymerase chain reaction; Pfact, *Plasmodium falciparum* acetyl CoA transport; *Pf*A-M1, Pf membrane alanine aminopeptidase; PfCRT, Pf chloroquine-resistant transporter; PfKelch13, Pf kelch like protein 13; *Pf*LAP, Pf leuci ne aminopeptidase; PfMDR1, pf multidrug resistance 1; Pfmrk, Pf mitogen related kinases; pfMRP, Pf multidrug resistance-associated protein; PfPI3K, Pf phosphatidylinositol-3-kinase; Pfpk5, *Plasmodium falciparum* protein kinase 5; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase.

Ethical Approval

Not applicable.

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The authors declare that they have no competing interests.

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