REVIEW

Recent Progress in the Development of New Antimalarial Drugs with Novel Targets

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Abstract: Malaria is a major global health problem that causes significant mortality and morbidity annually. The therapeutic options are scarce and massively challenged by the emergence of resistant parasite strains, which causes a major obstacle to malaria control. To prevent a potential public health emergency, there is an urgent need for new antimalarial drugs, with single-dose cures, broad therapeutic potential, and novel mechanism of action. Antimalarial drug development can follow several approaches ranging from modifications of existing agents to the design of novel agents that act against novel targets. Modern advancement in the biology of the parasite and the availability of the different genomic techniques provide a wide range of novel targets in the development of new therapy. Several promising targets for drug intervention have been revealed in recent years. Therefore, this review focuses on the progress made on the latest scientific and technological advances in the discovery and development of novel antimalarial agents. Among the most interesting antimalarial target proteins currently studied are proteases, protein kinases, *Plasmodium* sugar transporter inhibitor, aquaporin-3 inhibitor, choline transport inhibitor, dihydroorotate dehydrogenase inhibitor, isoprenoid biosynthesis inhibitor, farnesyltransferase inhibitor and enzymes are involved in lipid metabolism and DNA replication. This review summarizes the novel molecular targets and their inhibitors for antimalarial drug development approaches.

Keywords: drug resistance, novel targets, antimalarial agents, mode of action, *Plasmodium*

Background

Malaria is a devastating parasitic infectious disease, especially in Sub-Saharan African, parts of Asia, and South America. Even though several efforts undertaken, nowadays, it is one of the major causes of morbidity and mortality, mainly in pregnant women and children. According to the World Health Organization (WHO) report in 2018, there were 228 million malaria cases and 405 000 deaths in the world. Almost half the world's population is at risk of malaria disease, most cases (93%), and deaths (94%) reported in Africa. About 125 million pregnancies are at risk of malaria every year, and 272,000 Children aged less than five years die due to malaria.[1] Malaria is also the cause of poverty and a major hindrance to economic development, mainly in Africa.[2] The five identified *Plasmodium* species responsible for inflicting malaria in humans are *P. vivax, P. knowlesi, P. ovale, P. malariae*, and *P. falciparum*. Of these, *P. falciparum* is the most virulent and prevalent *Plasmodium* species.[3]

In the absence of an effective vaccine, the therapeutic use of antimalarial agents remains the only method for management and prophylaxis of malarial disease. Several studies showed that the efficacies of most antimalarial agents compromised

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by the emergency of drug-resistant *Plasmodium* species.[4] Resistance reported for nearly all available antimalarial agents that reinforced the urgent need to develop new antimalarial agents against existing validated targets, as well as to search for novel targets.[5] The development of a novel antimalarial agent that acts toward both transmissible gametocytes stages and the intraerythrocytic proliferative asexual, in particular, those of resistant parasite species, is urgently required.[6] Several enzymes, ion channels, transporters, interacting molecules in red blood cell (RBC) invasion, and molecules responsible for oxidative stress in the parasite, lipid metabolism, and degradation of hemoglobin are promising novel targets to develop new antimalarial drugs against rapidly mutating malarial parasites.[7]

The potential of new antimalarial agent judged by several requirements: novel modes of action with no cross-resistance to the current antimalarial agent, single-dose cures, effective against both the asexual blood stages and the gametocytes responsible for transmission. Besides, the new antimalarial agent should have the efficacy to prevent infection (chemoprotective agents), and clear *P. vivax* hypnozoites from the liver (anti-relapse agents).[8]

Traditional drug discovery follows many approaches to identifying a new ant-malarial agent to combat malaria disease. These approaches are optimization of current drug regimens and formulations, modifying the existing antimalarial agents, screening of natural products, isolating resistance-reversal agents, utilizing combination chemotherapeutic approaches, and exploiting drugs indicated for other uses.[8,9]

Besides the traditional drug discovery methods for the identification of new ant-malarial agent, the understanding of *Plasmodium* cell biology and genome has proved a powerful tool for uncovering mechanisms of resistance, and have great potential to design a novel drugs with both high antimalarial activity and transmission-blocking potential to combat malaria once and for all.[10] Genetic screening in *P. falciparum* identified 2680 genes that are important for asexual blood-stage growth resulting identification of crucial cellular processes, which is vital to develop new a drug.[10,11] The new drug should: (i) address drug-resistance issues, (ii) have a rapid onset of action, (iii) be safe, especially in children and pregnant women, and (iv) cure malaria in a single dose.[12] The challenge is to find a drug that addresses all of these features. The objective of this review is to have a view on novel targets in the treatment of malaria parasites,

which are under investigation by several companies so that readers will have information on previously done works.

Antimalarial Drugs

Currently, most antimalarial agents are targeting the asexual phase of malaria infection that causes symptomatic illness. The pre-erythrocytic (hepatic) stage remains unattractive because clinical symptoms not generated. Antimalarial drugs exhibit considerable stage selectivity of action (see Figure 1).Malarial treatment based on natural products, semi-synthetic, and synthetic compounds developed since the 1940s.[13] The existing antimalarial agents grouped into three main classes: quinoline derivative, antifolate, and artemisinin derivatives. There is no single drug has been identified or manufactured that can wipe out all strains of the *Plasmodium* species. Thus, to fight malarial infection efficiently, the combination of drugs frequently administered at the same time. Quinolines are the most widely used antimalarial agents for the management of malaria. Quinine is an alkaloid isolated from the bark of Cinchona trees, was the first antimalarial agent used for the management of disease in the 17th century. Quinine became the standard therapy for malaria from the mid-19th century to the 1940s.[14] The emergence of resistant strains of *P. falciparum*, in addition to toxicity, has limited the therapeutic use of Quinine. However, Quinine still used against severe malaria, most often combined with a second agent to decrease treatment duration and to minimize the side effects.[15,16]

In 1925 the German researchers discovered pamaquine the first synthetic antimalarial agent by modifying methylene blue. Because of limited efficacy and toxicity pamaquine, cannot use for the treatment of malaria. But pamaquine provides lead compounds to develop better antimalarial agents. Mepacrine (quinacrine) is another derivative of methylene blue used to treat malaria during World War II.[17]

Chloroquine developed during World War II to treat malaria. Chloroquine is a drug of choice to treat malarial due to its efficacy, safety, and low cost. But its irrational use soon led to the emergence of chloroquine-resistant *P. falciparum* species.[18] Primaquine therapeutically used to treat relapsing *P. vivax* caused by hypnozoites. Primaquine has a potent gametocytocidal effect against *P. falciparum*. Primaquine causes hemolytic anemia in glucose-6-phosphate dehydrogenase (G6PD) deficient patient.
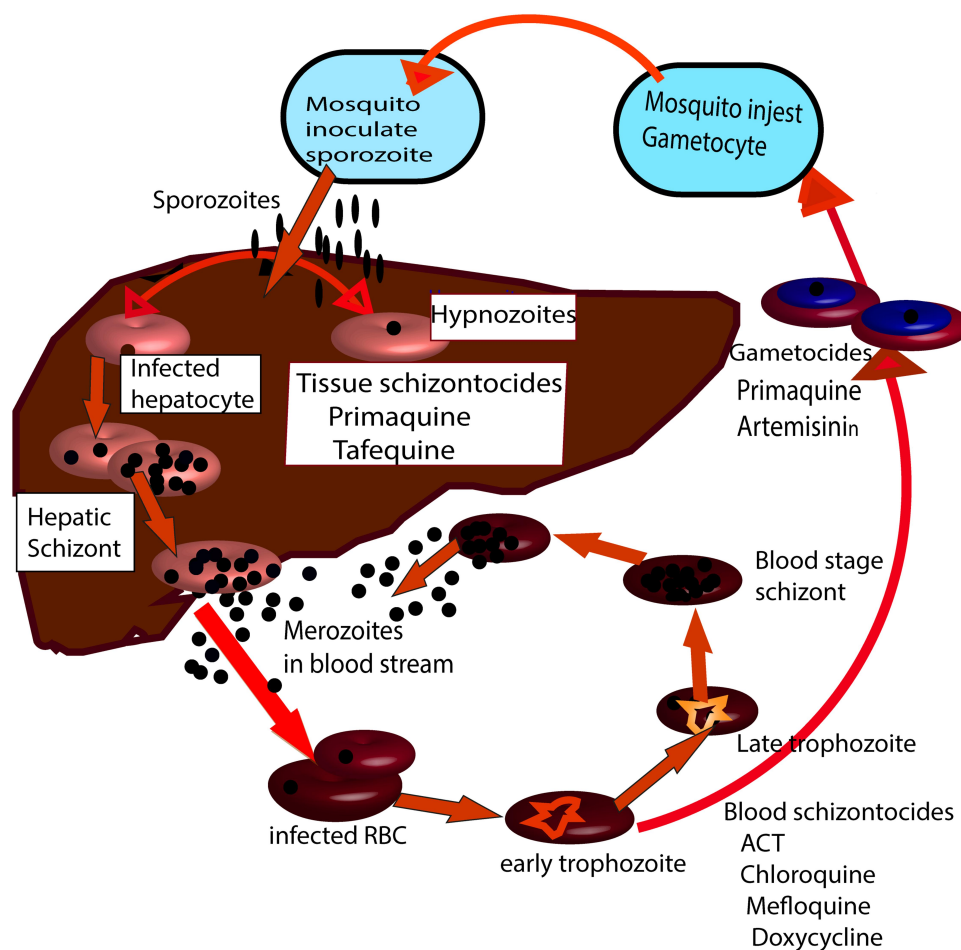
Figure 1 The life cycle of *Plasmodium* parasite in man. Stages and forms of the parasite at which different types of antimalarial drug acts.

This hemolytic effect intensified the need to obtain new drugs with anti-*P. vivax* activity.[19]

New quinoline derivatives were synthesized, giving new drugs such as piperaquine, amodiaquine, and others. After the emergence of chloroquine resistance, amodiaquine, a phenyl-substituted analog of chloroquine, displayed excellent efficacy against chloroquine-resistant strains of P. *falciparum*.[20] Pyronadrine is a Mannich base antimalarial agent developed in 1970 in China. It is effective against drug-resistant *P. falciparum* strains, *P. vivax, P. malariae*, and *P. ovale*. Pyronadrine is now available as ACT with artesunate, which displays excellent efficacy against all malaria parasites.[21] Mefloquine developed in the mid-1980s and currently recommended for chemoprophylaxis of malaria caused by all species including chloroquine resistance strains. However, its use associated with several side effects and drug resistance.[22] Drugs from Quinolines derived mostly act on blood stages of the parasite, but some antimalarial agents act at the hepatic stage. These drugs inhibit by forming a complex with haem in the parasite food vacuole. So, haem polymerization blocked. As a result, the haem that released during hemoglobin breakdown builds up to toxic levels, thereby killing the parasite with its toxic waste.[23]

Antifolates are antimalarial agents that inhibit folic acid synthesis, which is crucial for nucleotide and amino acid synthesis. Antifolates block the nuclear division of the *Plasmodium* species at the schizont stage within the erythrocytes and hepatocytes. Sulfadoxine have similar structures to p-aminobenzoic acid (PABA), a component of folic acid. They inhibit dihydrofolic acid synthesis by inhibiting dihydropteroate synthase, a crucial enzyme for the biosynthesis of nucleic acids.[24]

Pyrimethamine and Proguanil are schizonticidal antimalarial agents that act at the asexual forms of the *Plasmodium* species. These drugs inhibit the dihydrofolate reductase (DHFR) enzyme, inhibiting the reduction of dihydrofolate to tetrahydrofolate, which is vital for the

biosynthesis of amino acids and nucleic acids. Proguanil is a prodrug that metabolized to cycloguanil. Proguanil is the first antifolate drug developed for malaria treatment. The reason is it destroys the parasites before they invade erythrocytes during their passage into the bloodstream. Besides, proguanil is a safe drug. Pyrimethamine mostly used with other faster-acting drugs. However, its use decreased due to resistance.[24,25]

Atovaquone is the first approved antimalarial agent that targets the *Plasmodium* mitochondria. Atovaquone inhibits electron transport by blocking cytochrome b parts of the cytochrome bc1complex, by acting as a ubiquinone analog. When combined with proguanil, atovaquone is safe and effective for pregnant women and children. Atovaquone is effective against the sexual stages of the parasite in the host and the mosquito. So, it inhibits malaria transmission from mosquito to human. A fixed combination with proguanil developed under the trade name Malarone.[24,26]

In 1972 Artemisinin was extracted from *Artemisia annua*. Artemisinin and its derivatives including, artemether, dihydroartemisinin, arteether, and artesunate have broad-spectrum activity. Artemisinin inhibits all parasite phases within erythrocytes, particularly at the early phase of their development. It also suppresses gametocyte transmission from humans to mosquitoes.[27] Artemisinin and its derivatives are effective against chloroquine and mefloquine-resistant strains. They are safe, potent, and rapid-acting blood schizonticides against all *Plasmodium* species. However, Artemisinin does not clear the latent liver stages of the parasite. These drugs have short a half-life and poor bioavailability which leads to drug resistance, making them ineffective as a monotherapy. Therefore, Artemisinin derivatives recommended for treatment in combination with other antimalarial agents.[28]

The antimalarial effect of artemisinins may result from the production of free radicals, produced due to cleavage of the artemisinin endoperoxide bridge in the parasite food vacuole, which inhibits the parasite calcium ATPase and proteasome.[29,30] Artemether has a high recrudescence rate when used as a monotherapy. It has rapid oral absorption. The bioavailability doubled when administered in the presence of food. Once in the systemic circulation, artemether hydrolyzed in the gut and liver to dihydroartemisinin.

Artesunate is a semisynthetic derivative due to its rapid antimalarial action, lack of significant resistance and greater water solubility. It is recommended as a first-line drug for severe malaria.[31]

Tetracyclines and macrolides are slow-acting antimalarial drugs, used as an adjunct to quinine in treating *P. falciparum*. Doxycycline is also used for chemoprophylaxis in regions with high prevalent drug resistance.[32] The current strategy used to fight antimalarial drug resistance is the therapeutic use of drug combinations. This strategy used in the past through the utilization of fixed combinations. WHO recommended artemisinin-based combination therapy (ACT) regime as a first-line to manage uncomplicated *P. falciparum*. The reason is the combination of the drug decreases the drug resistance and side-effects.[33]

The ACT contains a potent artemisinin component, which rapidly clears the parasites, plus a longer-acting drug, which eliminates remaining parasites and decreases artemisinin resistance. The ACTs recommended by WHO are artesunate/amodiaquine,artemether/lumefantrine, artesunate/mefloquine, artesunate/pyronaridine, dihydroartemisinin/piperaquine, artesunate/sulfadoxine/pyrimethamine, Arterolane/piperaquine and Artesinin/piperaquine/primaquine. Chloroquine plus primaquine remains the first-line for radical cure of *P. vivax*. Quinine + tetracycline/doxycycline has a high cure rate but has severe side effects and contraindicated for children and pregnant women.[34]

Travelers from non-endemic to endemic areas recommended, mefloquine, atovaquone/proguanil, or doxycycline in chemoprophylactic regimens.[35] Intermittent preventive treatment advocated in high-risk populations, including sulfadoxine/pyrimethamine during pregnancy and amodiaquine/sulfadoxine-pyrimethamine as seasonal chemoprophylaxis.[36] Halofantrine is unsuitable for therapeutic use because of its cardiotoxicity. Dapsone, Mepacrine, amodiaquine, and sulfonamides withdrawn from therapeutic use due to their side effects.[36,37] Some of the well known antimalarial agents with their side effects presented in Table 1.

Novel Antimalarial Targets

The current available antimalarial agents identified based on the major metabolic pathway differences of the *Plasmodium* species with its host. The Major metabolic pathways of the parasite, including heme detoxification, fatty acid synthesis, nucleic acid synthesis, fatty acid synthesis, and oxidative stress are some of the novel sites for drug design.[38,39] Although most antimalarial agents used for several years, currently their use limited due to drug resistance. Based on the literature, there is no antimalarial drug identified to inhibit a known drug target.[7,40]

Table 1 The Summery of Antimalarial Drugs

Class	Category	Drug (Year of Development)	Therapeutic Use	Side Effect	Ref
Quinoline derivatives	Cinchona alkaloid	Quinine, Quinidine (1820)	Treatment of *P. falciparum* and severe malaria	Hypoglycemia Cichonism (tinnitus, deafness, visual disturbances, headache, dysphoria, nausea, vomiting), hypotension	[96]
	4-aminoquinoline	Chloroquine (1940)	For non-falciparum malaria	Accumulate in retinal and melanin	[97]
		Amodiaquine (1948)	Partner drug for ACT	Hepatitis, myelotoxi city, agranulocytosis	[98]
		Piperaquine (1960)	ACT partner drug with dihydroartemisinin + Piperaquine	Mild headache, dizziness, nausea, abdominal pain vomiting,	[99]
	8-Aminoquinoline	Primaquine (1950)	Radical cure and prophylaxis of *P. vivax* and *P. ovale*; gametocytocidal for *P. falciparum*	Hemolytic anemia in G6PD deficiency	[100]
		Tafenoquine (1978)	Radical cure and prophylaxis of *P. vivax* and *P. ovale*; gametocytocidal for *P. falciparum*	Hemolytic anemia in G6PD deficiency	[100]
	Quinoline-methanol	Mefloquine (1970)	Prophylaxis and partner drug for ACT to treat *P. falciparum*	Depression, psychosis, nightmares	[101]
	Aryl amino alcohol	Lumefantrine (1976)	Combination with artemether as ACT	Headache, anorexia, dizziness, and asthenia with artemether	[102]
		Halofantrine (1980)	TX of Pf malaria	Cardiac toxicity	[102]
	Benzonaphthyridine derivative	Pyronaridine (1970)	Combination with artesunate as ACT	Abdominal pain, vomiting, headache, dizziness, loss of appetite, palpitation and transient ECG changes, but no serious	[103]
Artemisinin	Sesquiterpene lactone endoperoxides	Artemether	ACT: combine with lumefantrine	Have excellent safety profile long-term use may cause nausea, vomiting, abdominal pain, itching and drug fever	[104]
		Artesunate	ACT; treatment of severe malaria		
		Dihydroartemisinin	Chloroquine resistant malaria, Complicated p. falciparum Cerebral malaria		
	Napthoquinone	Atovaquone (1991)	Combined with proguanil to treat *P. falciparum*	Diarrhoea, vomiting, headache, rashes, fever,	[105]
Antifolate	Diaminopyrimidine	Pyrimethamine (1950)	Used only in combination with sulfonamide or dapsone for treatment of falciparum malaria	Nausea and rashes megaloblastic anaemia and granulocytopenia	[106]

(Continued)

Table 1 (Continued).

Class	Category	Drug (Year of Development)	Therapeutic Use	Side Effect	Ref
	Sulfonamides	Sulfadoxine (1960)	Use is restricted to single-dose treatment of uncomplicated CQ-resistant falciparum	Exfoliative dermatitis, Stevens-Johnson syndrome,	
	Biguanide	Proguanil (Chloroguanide) (1945)	Treatment of chloroquine-resistant parasites; Combine with artesunate as ACT	Alopecia, aphthous ulceration, nausea, and gastric irritation	
Antimicrobial	Tetracycline	Doxycycline and tetracycline	Combined to quinine to treat *P. falciparum* when resistance to quinine	Skin and gastrointestinal toxicity	[107]
	Lincisamide	Clindamycin	Clindamycin plus quinine for treating uncomplicated falciparum malaria		[108]
	Macrolide	Azithromycin			

Instead, most antimalarial drugs identified in vivo animal or in vitro model studies. Therefore, the mode of action for most antimalarial agents remains uncertain. Besides, the mechanism of resistance not well understood for most antimalarial agents.[39]

Malaria control needs coordinated strategies, like vector control, effective and safe antimalarial agents, and effective vaccines. Considering the high mortality and morbidity due to malaria, the emergency, and the spread of drug resistance, the ineffectiveness of available antimalarial drugs against non-erythrocyte and sexual stages, It is crucial to identify novel antimalarial agent by understanding the basic metabolic pathway of the parasite. To achieve this objective, drug research should direct on validated novel targets to isolate a new lead compound.[39,41]

The need for identifying novel metabolic targets comes from several reasons. Firstly, except atovaquone and artemisinin derived drugs, most antimalarial agents have no chemical diversity, which may lead to cross-resistance. Secondly, because of the confusing array of putative chemotherapeutic targets, so many have not been validated. If validated it may generate some effective and safe compounds. Identification of novel drug targets and the design of new compounds acting on new targets is nowadays widely used approach all over the world to combat issues raised by the emergence of resistance to existing drugs.[40,41] Therefore, investigating inhibitors specific to the new target proteins of the malaria parasite has been exploited for drug target identification. Since the unveiling of the *P. falciparum* genome, several novel targets for drug intervention have emerged. These potential antimalarial drug targets the crucial metabolite biosynthesis, membrane transport, and signaling system, and the hemoglobin degradation processes.[40,42]

Malaria Parasite Proteases

Plasmodium proteases are a ubiquitous catalytic and regulatory enzyme that has a key role in the survival of the protozoan parasite and the diseases they cause. It catalyzes the hydrolysis of the peptide bond.[43] The effect of Proteases in the pathogenesis malaria disease includes cell/tissue penetration, immune evasion, activation of inflammation, invasion of erythrocyte, hemoglobin and other proteins breakdown, autophagy, and development the parasite.[44]

Malarial proteases (glutamate aspartate, cysteine, metallo, serine, and threonine) are promising therapeutic targets because disruption of the malaria proteases gene inhibits the degradation of hemoglobin and the development of the parasite in the erythrocyte stages.[45]

The rupture of erythrocyte and the subsequent invasion by merozoites require malarial protease enzyme. A Synthetic peptide (GlcA-Val-Leu-Gly-Lys-NHC$_2$H$_5$) inhibits *P. falciparum* schizont cysteine protease Pf 68. It inhibits erythrocyte invasion and parasite development. This suggests that a protease plays a key role in
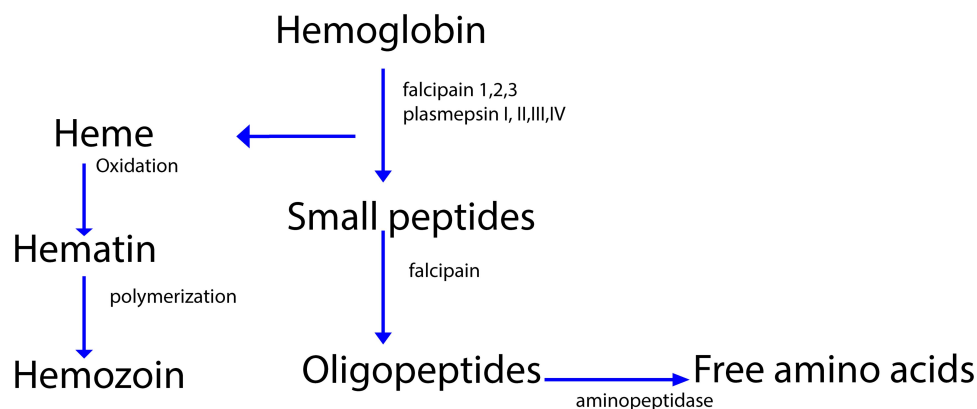
Figure 2 Degradation of hemoglobin by protease.

erythrocyte invasion by the parasites. So, protease is a promising target for antimalarial drugs development.[46]

In *P. falciparum* food vacuole several aspartic proteases (plasmepsin I, II, III, IV) and cysteine proteases (falcipain-1, falcipain-2/, falcipain-3) have been isolated which used for degradation of hemoglobin as displayed in Figure 2.

Incubation of cultured *P. falciparum* parasites with the protease inhibitor leupeptin and E-64 causes accumulation of undegraded globin. Leupeptin inhibits both cysteine and some serine proteases, but E-64 specifically inhibit cysteine protease.[47,48] After parasites incubation with aspartic protease inhibitor pepstatin, globin did not accumulate. Several studies showed that cysteine protease inhibitors not only inhibited globin degradation but also inhibited earlier steps in hemoglobin breakdown, like denaturation of the hemoglobin, the release of heme from globin, and production of hemozoin.[49] These results suggest that cysteine protease is required for initial steps in hemoglobin degradation by *P. falciparum*. Both E-64 and pepstatin blocked *P. falciparum* development synergistically. However, only E-64 blocked globin hydrolysis.[48,49] Several cysteine protease inhibitors like fluoromethyl ketones and vinyl sulfones inhibited *P. falciparum* growth and hemoglobin degradation. In the malaria animal model, fluoromethyl ketone inhibits *P. vinckei* protease activity and cured 80% of murine malaria infections. Thus, protease inhibitors are promising candidate antimalarial drugs. Subsequent works identified biologically active falcipain inhibitors, including chalcones, phenothiazines that block parasite metabolism and development.[50]

Serine protease involved in both schizont rupture and erythrocyte reinvasion in the *P. falciparum* life cycle. It can be blocked by several serine protease inhibitors and the best choice because no human enzyme homolog is available. The protease inhibitor LK3 isolated from Streptomyces species degrades serine protease of malaria.[51] Maslinic acid is a natural pentacyclic triterpene that inhibits the maturation of the parasite from the ring stage to the schizont stage, which terminate the release of merozoites and its invasion. A series of highly potent 2-pyrimidine carbonitrile inhibit falcipain-2 and falcipain-3.[52] Inhibition of plasmepsins by statine and allophenylnorstatin-based inhibitors block hemoglobin degradation and kill the parasites. There are several cysteine protease blockers available including, Epoxomicin, lactacystin, MG132, WEHI-842, WEHI-916, and chymostatin.

Phosphatidylinositol 4-kinase (PfPI4K) Inhibitor

Phosphoinositide lipid kinases (PIKs) are ubiquitous enzymes that phosphorylate lipids to regulate proliferation, survival, trafficking, and intracellular signaling.[53] The most widely studied classes of PIKs in the parasite are phosphoinositide 3-kinase (PI3K) and phosphatidylinositol 4-kinase (PI4K). Inhibition of these enzymes identified as a potential target for the development of antimalarial drugs with an ideal activity profile for the prevention, treatment, and elimination of malaria.[54] UCT943, Imidazopyrazines (KAF156), and aminopyridine are a new antimalarial compound class that targets the PI(4)K and inhibits the intracellular development of multiple *Plasmodium* species at each stage of infection in the host. Therefore, targeting (PI3K) and PI(4)K may open up new avenues of target-based drug discovery to identify novel antimalaria drugs. KAF156 is currently in Phase II clinical trial.[55,56] MMV048 is a compound that has good prophylactic

activity against *P.cynomolgi* in vivo and has the promising potential to act as a transmission-blocking drug. MMV048 is currently in Phase IIa clinical trials in Ethiopia.[11]

Targeting Transporters

For rapid growth in infected erythrocyte, the *Plasmodium* species need enough amounts of substrates to fuel their vigorous metabolism. Hence, the parasite prepares the host erythrocyte by inducing specialized transporter, which is significantly different from the host cell transporter for the uptake and removal of metabolites. So, transporters like carrier proteins and channels are potential targets due to their major role as in the transport of metabolites, electrolytes, and nutrients.[57] These are the *plasmodial* surface anion channel (PSAC) and parasitophorous vacuolar membrane (PVM), which provide a sequential diffusive pathway for nutrient entry to the intracellular parasite.[58]

PSAC is the most promising target due to its critical role in different types of nutrient acquisition (hypoxanthine, cysteine, glutamine, glutamate, isoleucine, methionine, proline, tyrosine, pantothenic acid, and choline) into the intracellular parasite. PSAC has no clear homology with known host channel genes.[58,59] Phlorizin, Dantrolene, furosemide, and niflumate are effective anion transporter blockers. Drugs like glibenclamide, meglitinide, and tolbutamide inhibit the influx of choline into parasite-infected erythrocytes.[60,61]

Plasmodium Sugar Transporter Inhibitor

Blood forms of *P. falciparum* almost entirely depend on glycolysis for energy production, without energy stores; rely on constant uptake of glucose. The parasite converts Pyruvate to lactate to produce ATP, which require for replicating in the intraerythrocytic site.[62] Glucose first transported from the blood into the parasitized erythrocyte via a combination of the host cell's glucose transporter called GLUT1, which is abundant in the erythrocyte membrane and the parasite-induced 'New Permeability Pathways'.[63] The glucose transported into the parasite via *P. Falciparium* Hexose transporter (PFHT). PFHT has some of the typical sugar transporter features. GLUT1 is selective for D-glucose, whereas PFHT can transport both D-glucose and D-fructose. Therefore, the differences between GLUT1 and PFHT in terms of their interaction with substrates, proposed that selective inhibition of PFHT is a promising novel target to develop a new antimalarial drug.[64] A long chain O-3-hexose

derivative (Compound 3361) inhibits the uptake of glucose and fructose by PFHT but, it does not inhibit hexose transport by the major mammalian glucose and fructose transporters (GLUT1 and 5). Compound 3361 also inhibits glucose uptake by *P. vivax* of PFHT. In previous studies, Compound 3361 kills' *P. falciparum* in culture and reduces the multiplication of *P. berghei* in the mouse model.[65]

Parasite's Lactate Transporter Inhibitor

Blood form of the malaria parasite relies primarily on anaerobic glycolysis for growth and development.[60] Glucose is uptaken by the parasite-infected erythrocytes up to 100 times faster than uninfected erythrocytes. The parasite metabolizes glucose by glycolysis into lactic acid, which is exported from the parasite by lactate: H+ symport mechanism into the external environment.[66] Both lactate export and glucose uptake are critical for keeping energy needs, intracellular Ph, and osmotic stability of the parasite. The lactate: H+ symport transport system inhibition is a promising novel target to develop a new drug. Several compounds like MMV007839 and MMV000972, kill asexual blood-stage *P. falciparum* parasites via inhibition of the lactate: H+ transporter.[67]

P-Type Na+ATPase Inhibitor (PfATP4) Inhibitor

Like other cell types, erythrocytes maintain a low internal Na+ level. However, the parasite increases the permeability of the erythrocyte cell membrane, facilitates the entrance of Na+ that causes the erythrocyte's cytoplasm Na+ concentration increases to a level of the extracellular medium. Thus, despite the parasite exist in the intracellular site, the parasite finds itself in a high-Na+ medium and must efflux Na+ ions across its plasma membrane to keep a low cytoplasmic Na+ level for survival. In this case, the parasite's influx of Na+ regulated by using a P-type ATPase transporter (PfATP4) that serves as the parasite's primary Na+-efflux pump mechanism as shown in Figure 3.[68] Inhibition of this transporter causes increased Na+ amount inside the parasite, ultimately leading to the death of the malaria parasite. Several compounds including, cipargamin, which is in Phase 2, (+)-SJ733, which is in Phase1 and KAE609, which is in phase 2 target PfATP4 as their Mechanism of action.[67,69]

V-Type H +–ATPase Inhibitor

The *Plasmodium* species control its Na+ level by using a P-type ATPase transporter. It also imports H + by
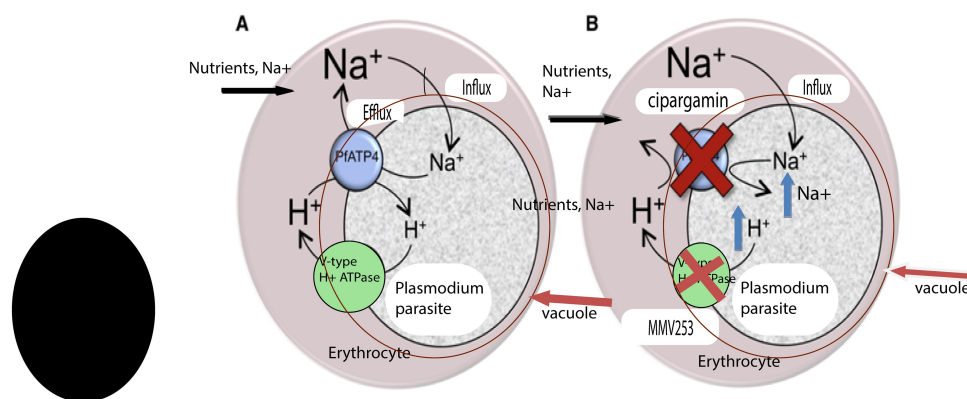
Figure 3 Proposed mechanism of the parasite-induced PfATP4 and V-type H +–ATPase in the death of infected erythrocytes following inhibition by cipargamin.

a similar pathway. To regulate the increasing H + concentration and keep an intracellular pH of 7.3, the malaria parasite uses a complementary V-type ATPase transporter to efflux H +. It is a promising target to develop a novel drug. MMV253 inhibits the V-type H + ATPase as its mechanism of action through the mutant selection and whole-genome sequencing.[70,71]

Aquaporin-3 Inhibitor

Aquaporin-3 (AQP3) is an aquaglyceroporin that facilitates the movement of both water and glycerol in mammalian cells. AQP3 induced in human hepatocytes in response to parasite infection, which has a significant role in the parasite replication. AQP3 provides the pathway for the entry of glycerol into *P. berghei* and contributes to the replication of the parasite during the asexual intraerythrocytic stages.[72] Genetic depletion of AQP3 significantly suppresses in *P. berghei* liver stage of the parasite load. Furthermore, treatment with auphen, an AQP3 inhibitor, reduces both *P. berghei* in hepatocytes and *P. falciparum* parasitemia load in erythrocytes, proposing that the host protein has a crucial role in different life stages of the parasite.[73] The most interesting is the genetic disruption of AQP3 in mice is not lethal, proposing the host protein has the potential therapeutic novel target. This work increases our understanding of host liver processes influenced by *Plasmodium* infection and highlights the potential of targeting these processes for future antimalarial drug development.[71,72]

Choline Transport Inhibitor

Phospholipids play a key role in the intra-erythrocytic life cycle of *P. falciparum*, both as structural components of the membranes and as regulatory molecules that regulate several enzymatic activities. These molecules are vital for parasite multiplication inside RBC. After the invasion of RBC, the level of phospholipids increases, with phosphatidylcholine as the major lipid of its cell membranes component. The parasites synthesize phosphatidylcholine de novo using choline as a precursor. This de novo pathway is essential for parasite growth and survival. Inhibition of choline transport into the parasite, inhibit phosphatidylcholine biosynthesis resulting in parasite death.[74] Albitiazolium is a drug that has reached Phase II trials, acts primarily by inhibiting the transport of choline into the parasite. Albitiazolium accumulates in the *Plasmodium* up to 1000-fold and inhibits parasite growth without recrudescence. It is active effective in severe conditions. Notably, a single injection is curative at high parasitemia levels.[75,76]

Cholinephosphate cytidylyl transferase is a rate-limiting step in phosphatidylcholine de novo biosynthesis.[77] The bisquaternary ammonium compound G25 and bis-cationic compound T3 inhibits phosphatidylcholine synthesis in the parasite. G25 is 1000-fold less toxic to mammalian cell lines. These agents are crucial lead compounds for antimalarial agent discovery and development.[78,79]

Dihydroorotate Dehydrogenase Inhibitor

A key-step for the spreading of *Plasmodium* species in the human host is the extensive and rapid division of parasite DNA, which depends on the availability of essential metabolites, such as pyrimidines. In the malaria parasite, pyrimidine nucleotide has a vital role in the synthesis of DNA, phospholipids, and glycoproteins. Nucleotide synthesis follows two major pathways: the salvage pathway and the de novo pathway.

Dihydroorotate dehydrogenase (DHODH) is an important enzyme that catalyzes the oxidation of dihydroorotate to orotate, a rate-limiting step in the de novo pyrimidine synthesis. So, DHODH represents a potentially promising target for antimalarial drug development.[80] A human cell access pyrimidines by salvage of already formed pyrimidine or by de novo synthesis. If the de novo biosynthesis pathway inhibited the cell will depend on the salvage pathway, and the cell will not die. However, inhibition of de novo pyrimidine biosynthesis in the parasite causes the death of those cells because the malaria parasite lacks the pyrimidine salvage pathway, which makes the parasite vulnerable to inhibition of DHODH.[81] DSM190 and DSM265 are a selective inhibitor of the parasite DHODH enzyme, the later is currently in phase 2 clinical trials. P218 is a DHODH inhibitor effective against all pyrimethamine-resistant strains currently in phase 1. KAF156 (Ganaplacide) is currently in Phase 2b clinical trials with lumefantrine.[82]

Isoprenoid Biosynthesis Inhibitor

Isoprenoids are necessary for post-translational lipid modification of proteins and asexual replication of *P. falciparum*. Isoprenoid synthesized from five-carbon precursor isopentyl diphosphate (IPP) or its isomer dimethylallyl diphosphate (DMAPP) synthesized by one of the two independent pathways. The mevalonate pathway and the 2C-methyl -D-erythritol 4-phosphate (MEP) pathway. The two pathways are mutually exclusive in most microorganisms. Bacteria and *P. falciparum* rely exclusively on the MEP pathway, but humans do not. So, Enzymes in the MEP pathway explored as potential therapeutic novel targets. The rate-limiting step in the MEP pathways catalyzed by *P. falciparum* 1-deoxy-Dxylulose- 5-phosphate reductoisomerase (pfDxr), making this parasite enzyme a promising target to develop a novel antimalarial agent.[83,84] PfDXR inhibitor inhibited *P. falciparum* growth, without being toxic to human cells. PfDXR is a potential novel target in antimalarial drug development.[83] Fosmidomycin, MMV019313, and MMV008138, inhibits DOXP reductoisomerase, a key enzyme of the DOXP pathway which is absent in human. Because inhibition of protein prenylation in the malaria parasite disrupts asexual parasite growth and which is a potential antimalarial target.[85]

Farnesyltransferase Inhibitor

Prenylated protein has a vital role in several cellular processes including vesicular trafficking, signal transduction, regulation of DNA replication, and cell division. Such post-translational modifications help the binding of intracellular proteins to membranes and promote protein-protein interactions. Farnesyl transferase catalyzes the transfer of the farnesyl group, a 15-carbon isoprenoid lipid unit, from farnesyl pyrophosphate to the C-terminus of proteins containing the CaaX motif. Farnesyltransferase enzyme is a promising novel target to develop an antimalaria drug because its inhibition kills the parasite.[86]

Previously, resistance evolution of the parasite with the farnesyltransferase inhibitor BMS-388,891 a tetrahydroquinoline, showed mutations in the protein at the peptide substrate-binding domain. In selections with BMS-339,941 another tetrahydroquinoline, mutation identified in the farnesyl pyrophosphate binding pocket. In another study, mutations found in the beta subunit of the farnesyltransferase in *P. falciparum* strains resistant to MMV019066. Modeling studies revealed that the mutations distort the critical interaction site of the small molecule with the farnesylation active site, thereby conferring resistance.[87]

P. falciparum Translational Elongation Factor 2 Inhibitor

One of the promising targets for developing a new drug is the blocking *P. falciparum* ribosome, and other parts of the translational machinery responsible for protein synthesis. *Plasmodium* species possess three genomes: nuclear, mitochondrial, and apicoplast (from a relic chloroplast). All genomes require translational machinery to function. Protein syntheses Inhibitors have major clinical success as potent antibiotics. Doxycycline, clindamycin, and azithromycin, have an antimalarial therapeutic use, because they inhibit the ribosomes within the *Plasmodium* specie's mitochondria and apicoplast, leading to loss of effect of these organelles.[88] Interestingly, the *P. falciparum* ribosomes occupy an evolutionary middle ground between prokaryotic and eukaryotic, differentiating it significantly from the human ribosome to give an important promising novel target. *P. falciparum* elongation factor 2 (pfEF2) is a component of a ribosome that catalyzes the GTP-dependent translocation of the ribosome along messenger RNA, and is crucial for protein synthesis in eukaryotes. PfEF2 isolated as a novel target for antimalarial drug development.[87,89]

Inhibition of protein synthesis exemplified in the discovery of the sordarin, a natural product which selectively blocks fungal protein synthesis by inhibiting the yeast

eukaryotic elongation factor 2. Similarly, M5717 (previously DDD107498), a selective inhibitor of the 80S ribosome interacting PfEF2, is currently in phase 1 study, validating the potential of the PfEF2as an effective target for antimalarial drugs.[88,90]

Anti-Adhesive Polysaccharide

The main features of severe malaria are the sequestration of parasite-infected erythrocytes, inflammation, and Microvascular obstruction. The falciparum parasite uses heparan sulfate during attachment to the endothelium and other blood cells causing obstructions of blood flow. Inhibition of these abnormal cells and pathogen interactions with drugs restore hampered blood flow and affect the parasite growth.[91]

Several studies showed that sevuparin is an antiadhesive polysaccharide agent manufactured from heparin with eliminated antithrombin effect. Sevuparin inhibits invasion of merozoites into erythrocytes, binding of infected erythrocytes to uninfected and infected erythrocytes and binding to vascular endothelial cells. Besides, sevuparin binds to the N-terminal extracellular, heparan sulfate binding

Table 2 Overview of Some of the Ongoing Clinical Trial Performed in Malaria Drug Development

Name of Drug	Company	Phase	Mechanism of Action	Ref
KAE609 (cipargamin)	Novartis	2a	Na+-TPase 4 ion channel inhibitor	[69]
M5717 (DDD498)	Merck, KGaA, Darmstadt	I	Protein-making machinery of the malaria parasite, liver-stage *P. falciparum*	[90]
Albitiazolium (SAR9727)	CNRS/University of Montpellier/Sanofi	2	Inhibiting the transport of choline into the parasite	[75]
SJ733	St Jude/Eisai (Rutgers)	I	The P-type Na+–ATPase transporter	[69]
KAF156(Ganaplacide)/ lumefantrine	Novartis	2b	Cyclic amine resistance unknown mechanism of locus (PfCARL) inhibitor	[55]
DSM265	Takeda (Univ.of Texas Southwestern	2a	Inhibit dihydroorotate dehydrogenase enzyme	[82]
Methylene Blue	University of Heidelberg	2	Prevents haem polymerisation by inhibiting *P. falciparum* glutathione reductase	[17]
Sevuparin (DF02)	Dilaforette-Karolinska Institute	2	Anti-adhesive polysaccharide derived Blocks merozoite invasion and sequestration	[92]
P218	Medicines for Malaria Venture	I	PfDHFR inhibitor	[82]
MMV048	Univ. of Cape Town	2a	Inhibiting the parasite enzyme phosphoinositol 4-kinase enzyme	[11]
MMV390048	University of Cape Town	2a	Phosphatidylinositol 4-kinase (PfPI4K) inhibitor	[9]
Artefenomel (oz439) + Piperaquine	Medicines for Malaria Venture	3b	Synthetic endoperoxide	[94]
AQ 13	Tulane University and University of Bamako	2	Not known	[95]
OZ277+ Piperaquine		2–3	Inhibit Pf-encoded sarcoplasmic endoplasmic reticulum calcium ATPase	
Fosmidomycin + piperaquine	Medicines for Malaria Venture and Jomaa Pharma GmbH	2a 2b	DOXP pathway	[115]

Notes: Phase 2a studies focus on monotherapy, whereas Phase 2b studies investigate combination therapy.

structure of *P. falciparum* erythrocyte membrane protein 1, the Duffy-binding like domain 1α (DBL1α), known as a vital contributor to sequestration of infected erythrocytes.[92,93] Some of the clinical trials that are undergoing in different phases summarized in Table 2.

Besides, new drug discovery effective vaccine development is crucial to control malaria. Several vaccines developed in the past targeting the different parasitic life cycle stages. Transmission blocker like PpPf S25, targets the parasite stages in the mosquito vector. Vaccines targeting blood-stage forms, such as the apical membrane antigen-1 (AMA-1) and merozoite surface protein 1 (MSP1) showed disappointing results due to the complex metabolic pathways of erythrocytes and merozoites. Vaccines targeting the pre-erythrocytic sporozoite stage remain the most promising approach. RTS, S/AS02$_A$ vaccine induces an anti-circumsporozoite antibody that has completed Phase III efficacy trials.[94,95]

Conclusion

It is very concerning that the recent emergence of resistance of malaria parasites to available drugs continues to grow, increasingly limiting our ability to control this serious disease. However, it is reassuring that many new approaches to antimalarial drug discovery are now under evaluation, as reviewed here. Recent increases in the pace of progress in this area suggest that, if support for antimalarial drug discovery is adequate, new approaches should lead to the development of new antimalarials that can act through novel mechanisms of action soon. Over the past few years, high-throughput screens have identified several novel chemotypes that developed into highly promising antimalarial candidates. The pace of research progress is high; meaning updates to reviews such as this will always be needed. Apart from efficacy, toxic side effects, pharmacokinetic compatibility, and the potential to develop resistance would all be the major parameters in the eventual development of a successful drug. An understanding of the mechanisms underlying antimalarial drug resistance should also help us to circumvent the emergence of resistance to new generations of antimalarials soon.

Abbreviations

AQP3, Aquaporin-3; pfEF2, *P. falciparum* elongation factor 2; WHO, World Health Organization; DHODH, Dihydroorotate dehydrogenase.

Disclosure

The author declares that they have no competing interests for this work.

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