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New Targets in Malaria Parasite Chemotherapy: A Review

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Abstract

Malaria is a global health problem that causes significant mortality and morbidity annually and a serious problem to drug therapy and discovery as current anti-malarial therapeutics become increasingly ineffective. The need for new therapy for malaria is mandatory because of the emergence of resistance to most of the anti-malarial drugs. There are different approaches and targets proposed by researchers and scientist based on experimental data. Modern advancement in the biology of the parasite and different genomic techniques provide wide ranges of novel targets in the development of new therapy. Therefore, this review will discuss new targets in the chemotherapy of malaria parasite.

Keywords: Malaria parasite Chemotherapy; Emergence of resistance; New targets

Introduction

Malaria is a major human health problem causing high mortality and morbidity, mainly in Sub-Saharan Africa and in some parts of Asia and South America [1]. Malaria is caused by five Plasmodium species, namely, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium Malariae and Plasmodium knowlesi. Of these, P. falciparum is the causative agent of severe malaria in humans resulting in high mortality [2,3]. Every year more than one million deaths are documented due to P. Falciparum and high mortality is reported in infected children [4]. Malaria parasite has a complex life cycle [3] and transmitted to human by the bite of an infected female mosquito of the genus anopheles which harbours the parasite [5]. Once they entered the bloodstream, initially reside in hepatocytes and start asexual multiplication resulting in tens of thousands of merozoites which takes over 5 to 16 days [5]. After bursting from hepatocytes, each merozoite invades erththrocyte and again starts multiplication. Some of the merzoites after hepatocytes burst differentiate into male and female gametocytes. Subsequently, these gametocytes are sooner or later taken up by a mosquito during a blood meal and the life cycle of parasite continues [6]. Asexual erythrocytic stage of this parasite is responsible for the clinical symptoms of malaria [2].

The use of drugs for prophylaxis and treatment are vital to save lives [7]. Therefore, several classes of drugs are used to treat malaria, where most are targeting the erythrocytic stage of parasite, however, some target gametocytes as well [6]. According to World Health Organization (WHO), resistance to all these drugs has been widely reported, even in the case of artemisinin combined therapies (ACT) particularly in South-East Asia [6]. Therefore, researchers are in continuous desire for new targets and therapeutics and have identified several new and potential drug targets to combat anti-malarial drug resistance [8].

The purpose of this review is to have a view on new targets in the chemotherapy of malaria parasite which are under investigation by different organizations and individuals so that readers will have information on previously done works.

Therapeutic and Preventive Anti-Malarial Drugs

Anti-malarial drugs are used for the treatment and prevention of malaria infection. Quinolones, antifolate drugs, artemisinins, atoquavone, and antibiotics such as tetracycline are the most extensively used anti-malarial drugs [1].

Quinolines are historically among the most important anti-malarial drugs. In the 20th century members of this group gives hope for malaria eradication. These drugs include amodiaquine, piperaquine, primaquine, quinine and mefloquine [1,9]. The drugs from this group mostly target blood stages of the parasite's lifecycle but some drugs of quinolines are also believed to target the hepatic stage. These drugs act by forming a complex with haem in the parasite food vacuole. So, haem polymerization is inhibited. As a result, the haem which is released during haemoglobin breakdown builds up to poisonous levels, thereby killing the parasite with its own toxic waste [10].

Chloroquine is a synthetic 4-aminoquinoline formulated as the phosphate salt for oral use and the drug of choice in the treatment of non-falciparum and sensitive falciparum malaria [11]. It rapidly terminates fever (within 24–48 hours) and clears parasitemia (within 48–72 hours) caused by sensitive parasites [11]. Chloroquine mode of action is proposed to be similar to quinine. Chloroquine is the preferred chemoprophylactic agent in malarious regions without resistant falciparum malaria. Eradication of P. vivax and P. ovale requires a course of primaquine to clear hepatic stages [9].

Antifolates are also used as anti-malarial drugs. These are compounds that inhibit the synthesis of folate cofactors that are required for nucleotide synthesis and amino acid metabolism. The most commonly used antifolates drugs are pyrimethamine (2, 4-diaminopyrimidine), chloroguanide (proguanil, Paludrine), and the sulfa-drugs sulfadoxine, sulfalene and dapsone. Antifolates prevent the nuclear division of Plasmodium at the schizont stage within the hepatocytes and erythrocytes by acting on dihydrofolate reductase (DHFR) or dihydropteroate synthase (DHPS) [1]. Atovaquone inhibits electron transport in plasmodial mitochondria and depolarizes the membranes of plasmodial mitochondria. A fixed combination with chloroguanil has been developed under the trade name MALARONE. They represent a novel class of expensive antimalarial drugs [9,12].

Artemisinins have a broad spectrum of activity against all parasite phases within erythrocytes, in particular younger ring forms and suppress gametocyte transmission. Artemisinin and its analogs are rapidly acting blood schizonticides against all human malaria parasites. However, it have no effect on hepatic stages [9]. The antimalarial activity of artemisinins may result from the production of toxicity of free radicals, produced due to cleavage of the artemisinin endoperoxide bridge in the parasite food vacuole or from inhibition of a parasite calcium ATPase [13].

Existing Targets and Their Limitations

Existing anti-malarial drugs were designed based on the major metabolic differences of malaria parasite with its host. Major pathways of P. falciparum, such as nucleic acid metabolism, heme detoxification, oxidative stress and fatty acid biosynthesis are known targets for anti-malarial drug design. Even though most of them were used for more than decades, however, their use is now limited due to emergence of resistance [8].

According to literatures, there is no existing anti-malarial drug which was developed in a fully rational manner, with a focused attempt to inhibit a known drug target. Instead, in all cases anti-malarial potency has been identified in animal or in vitro models studies. Therefore, the target of action for most available agents within the malaria parasite remains uncertain [6]. In addition, the mechanisms of emergence of resistance are poorly understood for most of the drugs. Genetic, molecular and pharmacological approaches have shown that different targets of older drugs are resistant due to mutations on their key enzymes or transporters [2].

The most potent anti-malarial drug, artemisinins and their derivatives are also not in exception list of drug resistance. Artemisinins are potent inhibitors of phosphatidylinositol-3-kinase (PfPI3K). In clinical resistant strains, increased PfPI3K was associated with the C580Y mutation in P. falciparium Kelch13 (PfKelch13) which is a primary marker of artemisinins resistance [14]. Thus, drug resistance resulting from mutations is a major concern and the identification of new targets is mandatory to design new drugs against resistant malarial parasites [8,15].

The Need of New Target for Anti-malarial Drugs

Malaria elimination need an integrated strategy, including new and old drugs, vaccines, vector control and public health measures [16]. Considering the high mortality, morbidity, the emergence and spread of resistance to existing drugs, there is no question that new drugs are required [8]. To achieve this goal, anti-malarial drug research should focus on validated targets in order to generate new drug candidates.

On the other hand, there is a need to identify new targets for the future by studying the basic metabolic and biochemical processes of the malaria parasite. The need for new metabolic targets stem from two main reasons [17,18]. Firstly, apart from artemisinin-type compounds and atovaquone there is an overall lack of chemical diversity in the anti-malarial drugs in use, which has led to considerable cross-resistance between drugs. Secondly, because of the confusing array of putative chemotherapeutic targets, so many have not been validated. If validated properly it will lead to generate some effective and safe compounds [17].

New Targets against Malaria Parasite Chemotherapy

Identification of novel drug targets and design of new chemical compounds acting on new targets is nowadays widely used approach all over the world to combat issue raised by emergence of resistance to existing drugs [19]. Therefore, investigating inhibitors specific for the new target proteins of malaria parasite has been exploited for drug target identification and currently studies are underway [20].

Malaria Parasite Proteases as Targets for Anti-Malarial Drugs

Proteases constitute a ubiquitous and highly abundant group of catalytic and regulatory molecules having widespread roles in living systems [2]. Studies with protease inhibitors show that there are three processes that require protease activity in erythrocytic parasite of malaria. These are hemoglobin degradation, erythrocyte invasion and erythrocyte rupture [21]. Hemoglobin hydrolysis in the Plasmodium digestive vacuole is thought to be a semi-ordered process mediated by the action of a series of proteases. Plasmepsins (aspartic proteases) and falcipains (cysteine proteases) are involved in the initial steps of the pathway [3,10]. Several researchers have demonstrated peptidyl fluoromethyl ketone, vinyl sulfone and aldehyde inhibitors potency to protect plasmodium-infected mice against lethal malaria, which act as cysteine protease inhibitors [8,22]. Cysteine protease inhibitors such as E64, leupeptin, chymostatin, fluoromethyl ketones, vinyl sulfones, and chalcones appear to be a valuable template for the development of new inhibitors specific to individual plasmodial proteases [17].

The major food vacuole-resident hemoglobin degrading proteases are papain-like cysteine proteases falcipains, aspartic proteases plasmepsins, the metalloprotease falcilysin, dipeptidyl aminopeptidase I, and a M1-family alanyl aminopeptidase. Literature survey indicates that the papain-like cysteine proteases falcipains, particularly falcipain-2 (FP2) and falcipain-3 (FP3), are the major hemoglobin degrading enzymes in P. falciparum. Epoxomicin, E64, lactacystin, MG132, plasmepsin, pepstatin blocks parasite development in P. falciparum erythrocytic stage [23,24].

PfSUB1 is a serine protease involved in both schizont rupture and erythrocyte reinvasion in the P. falciparum life cycle. It can be blocked by serine protease inhibitors and it is the best choice because no human enzyme homolog is available. The protease inhibitor LK3 from Streptomyces species, capable of degrading serine protease of malaria [22]. Maslinic acid (MA), a low toxic natural pentacyclic triterpene has demonstrated ability to hinder the maturation from ring to schizont stage which terminate the release of merozoites and its subsequent invasion [25]. A series of highly potent 2-pyrimi dinecarbonitriles were also reported as inhibitors of falcipain-2 and falcipain-3 [3]. It has also been shown that macromolecular inhibitors such as prodomain,

falstatin, PbICP, Py-ICP are inhibitors of cysteine proteases of malaria parasites [24].

Targeting Apicoplast of the Parasite

The apicoplast is a specialized organelle [26] that maintains certain specific functions such as fatty acid, isoprenoid, heme synthesis and iron-sulfer cluster biogenesis [27] which are also present in bacteria, plants, and apicomplexan parasites but not reported in the human beings [18,28]. Therefore, fatty acid synthesis and isoprenoid precursor synthesis are potential targets for existing antibiotic drugs since these functions are clearly bacterial in nature [27].

Targeting Fatty Acid Pathway

Even though, that apicoplast of fatty acid synthesis is not essential in red blood cell stages or gametocytes, it is valuable as a prophylactic target in mouse models of malaria [28]. Fatty acid within the P. falciparum parasite is synthesized by fatty acid sythase II (FAS II) while animals and humans have the type I fatty acid synthase I (FAS I) [26,27]. Thiolactomycin (a natural antibiotic inhibits fatty acid and mycolic acid synthesis) has exhibited inhibition of β-ketoacyl-ACP synthases of FAS II (FabH, FabB/F) in the culture of P. falciparum [27,29]. Similarly, the antimicrobial Triclosan, a specific inhibitor of the enoyl-ACP-reductases (PfENR or FabI) of FAS II has exhibited inhibition of P. falciparum in in-vitro tests as well as plasmodium-infected mice model [26].

Targeting Isoprenoid Pathway

Isoprenoids are a large class of biological compounds that include natural rubbers, cholesterol, ubiquinone, dolichol, farnesol, and many others. This tremendous diversity is derived from a simple five-carbon precursor isopentenyl pyrophosphate (IPP) and its isomeric form dimethylallyl pyrophosphate (DMAPP) [28]. Isoprenoid is one of the important requisite for parasite multiplication in human erythrocytes [31]. Isoprenoid metabolism is critical to P. falciparum [26]. Plasmodium synthesizes isoprenoids using 1-deoxy-Dxylulose-5-phosphate (DOXP) as precursor which is not present in humans. This pathway is a non-mevalonate pathway or methyl erythritol phosphate biosynthetic pathway which is also found in eubacteria and plants but not in humans (mavelonate pathway), expressing a good target for anti-malarias [18,26].

Genes encoding two enzymes in this pathway are DOXP reducto-isomerase and DOXP synthase by P. falciparum and contains apicoplast targeting signals. Isoprenoid metabolism proceeds through DXR is inhibited at the level of the downstream enzyme, methyl erythritol phosphatecytidylyl transferase (IspD) [30]. The antibiotic fosmidomycin and its derivative FR900098 inhibited the activity of recombinant DOXP reductoisomerase as a result inhibited the growth of cultured P. falciparum parasites and cured murine malaria [8,30]. In trials of safety and efficacy in uncomplicated malaria, it exhibited 100% initial cure rate and well tolerated [8,18]. In other study, terpenes such as farnesol, nerolidol, and linalool showed strong inhibitory activity in the biosynthesis of the isoprenic side chain of the benzoquinone ring of ubiquinones in the schizont stage [30].

Targeting Mitochondria of the Parasite

The mitochondrial electron transport chain (ETC) is critical for parasite survival because it is the only way used by Plasmodium to regenerate mitochondrial coenzyme Q [31]. The mitochondrial activity appears to be important when the parasite is outside the host erythrocytes. The heme synthesis may play a role in survival at liver and gametes stages of parasite. Therefore it may be an important drug target for blocking parasite transmission and prophylaxis [29,32].

Compounds such as, 4(1H)-pyridones, acridones, acridinediones and 4(1H)-quinolones are reported to selectively target the parasite mitochondrial electro transport chain (mtETC) and are less expensive, which are under clinical trials nowadays [33,34]. Similarly, myxothiazol, antimycin, and cyanide effect on the intact mitochondria of the parasite were studied by flow cytometric assay. These compounds inhibited mitochondrial electron transfer chain and resulted in collapse of the parasite mitochondrial membrane potential in a dose-dependent manner [35,36].

Similarly, given the structural similarities with other quinolones known to inhibit Plasmodium's cytochrome bc1 complex, decoquinate was found to be potent, selective, and specific inhibitor of P. falciparum mitochondrial bc1 complex [31]. In a study funded by medicine for malaria venture (MMV), compound MK4815 in the lead generation phase displayed activity against P. falciparum dihydroorotate dehydrogenase (PfDHODH), hemozoin formation and P. falciparum falcipain-2 [37].

Targeting Parasite Induced Transporters

In Plasmodium, transporters which also includes those modified by the parasite are currently considered as highly promising targets, due to their involvement, as carrier proteins, channels or pumps, in the movement of nutrients, metabolites and ions into and out of the parasite as well as between sub-cellular compartments within the parasite [26]. New transport pathways are induced by the parasite into the host erythrocyte membrane and these transporters are significantly different from the host cell transport pathway [35]. Two unusual main ion channels have been identified with in the infected host erythrocytes. These are the plasmodial surface anion channel (PSAC), which is induced on the erythrocytes membrane several hours after invasion of the RBC and parasitophorous vacuolar membrane (PVM), which is different from the PSAC. These channels provide a sequential diffusive pathway of nutrient entry to the intracellular parasite [17].

Dantrolene derivatives are specific to PSAC, which could be possible to design new and more specific PSAC inhibitors for future anti-malarial drug development. PSAC is the most promising target among other channels because it plays a critical role in various types of nutrient acquisition to the intracellular parasite and there is no clear homology with known host channel genes [8]. Another compound is Phlorizin, which is a naturally occurring in some plants, inhibits the in vitro growth of P. falciparum with a inhibitory concentration (IC50) of 16 µM [33,34]. Other effective anion transporter blockers reported are 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), furosemide, and niflumate [35,38].

Different aromatic derivatives such as cinnamic acid derivatives and aryl amino benzoates are known inhibitor of monocarboxylate transport across mammalian membranes and the translocation of a number of different types of solutes [36]. Glibenclamide and structurally related compounds such as meglitinide and tolbutamide which are used in the treatment of diabetes inhibited the influx of choline into parasite-infected erythrocytes and glibenclamide was reported the most effective one [39].

Plasmodium Sugar Transporters as Anti- Malarial Drug Targets

Intra-erythrocytic stages of P. falciparum are entirely dependent on host glucose for energy [8]. The main source of ATP production in asexual blood stages of malarial parasites is glycolysis, followed by anaerobic fermentation of pyruvate to lactate which provides fast ATP production, which is required for the rapidly replicating intra-erythrocytic parasite [39]. Glucose from blood is delivered to the intera-erythrocytic parasite by both host and plasmodium sugar transporters [39,40]. Glucose is first transported to the host erythrocytes by the facilitative glucose transporter called GLUT1 which is highly abundant in the erythrocyte plasma membrane [40]. P. Falciparium Hexose transporter (PFHT) is a sodium-independent, saturable, facilitative hexose transporter which shares some of the typical sugar transporter features with the major mammalian glucose transporter, GLUT1. GLUT1 is selective for D-glucose whereas PFHT can transport both D-glucose and D-fructose. Therefore, Mechanistic differences that were observed between GLUT1 and P. Falciparium Hexose transporter (PFHT) in terms of their interaction with substrates, suggested that selective inhibition of PFHT may be possible [40]. Compound 3361, an O-3 hexose derivatives, inhibit uptake of glucose and fructose by PFHT but confirmed it has lack of inhibition of hexose transport by the major mammalian glucose and fructose transporters (GLUT1 and 5). Compound 3361 also inhibits glucose uptake by P. vivax ortholoue of PFHT. In previous studies, Compound 3361 kills P. falciparum in culture and reduces multiplication of P. berghei in a mouse model [39].

Targeting the Lipid Metabolic Pathways

Plasmodium infection is followed by a marked increase in the phospholipid content and a significant change in the lipid composition of the infected erythrocyte. So, phospholipids (PL) metabolism is an attractive target for new malaria chemotherapy due to its vital importance to the parasite [41]. Phospholipids metabolism is absent in normal mature human erythrocytes [42] but the erythrocyte phospholipids content increases by as much as 500% after infection, specifically due to the metabolic machinery of the parasite [43]. Growing and dividing malaria parasites need large amounts of phospholipids for membrane structure, signaling and as a source of energy that are synthesized from plasma fatty acids [8].

Hexadecyl-trimethyl-ammonium-bromide, which has a close structural resemblance to a well-known anti-proliferative and anti-leishmanial drug, hexadecy-lphospho-choline (miltefosin) was identified as an inhibitor of P. Falciparium Choline kinase PFCK [41]. Bis-quaternary ammonium salts, structurally analogue to the phospholipids precursor choline, have been shown to be the target of P. falciparum membrane biogenesis by blocking the biosynthesis of Choline kinase (PC) [41]. The lead compound for these compounds is G25, efficiently inhibited the growth of drug-resistant strains of P. falciparum in vitro and abolished Plasmodium infection without recrudescence in rodent and primate models at very low doses [41].

Bis-thiazolium compounds, T3 and T4 exerted a highly rapid cytotoxic effect against malaria parasites in vitro at very low doses and recently researchers detected a down regulation of the choline/ethanolamine-phosphotransferase protein by using a proteomic approach and validated it as a potent anti-malarial drug. Choline/ethanolamine-phosphotransferase is a key enzyme involved in the final step of biosynthesis of phosphatidylcholine (PC) [8].

Targeting the Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase (Serca)

The SERCA belongs to the family of P-type ATPases that are responsible for active transport of cations across bio-membranes [13,44]. Thapsigargin is a sesquiterpene lactone and the most widely used SERCA inhibitor that inhibits SERCA in the nanomolar concentration range [45]. It is also highly selective, since it does not appreciably inhibit other related Ca^{2+}-ATPases such as the PMCA (plasma membrane $Ca2^{+}$-ATPase) or the SPCA (secretory pathway $Ca2^{+}$-ATPase) at these very low concentrations [38]. Another compound extracted from traditional Chinese medicinal herb is Alisol B, steroid in nature with a ketone group at the C-3 position and a branched acyl hydroxylated epoxide side chain at the C-17 position [45]. Alisol B was likely to bind best to the trans-membrane domain at the same site occupied by thapsigargin [46].

Targeting Eukaryotic Protein Kinases of Malaria Parasites

Protein kinases are valid drug targets in numerous diseases such as cancer and this also created an interest in plasmodial protein kinase. Based on a sequence homology with the mammalian Cyclin-dependent kinases (CDKs), several CDKs have been identified in the malaria parasite; however, only P. falciparum protein kinase 5 (PfPK5), 6 and P. falciparum mitogen related kinase (Pfmrk) have been well characterized in P. falciparum [47]. Compounds such as Flavopiridol and olomoucine have exhibited inhibition of PfPK5, decrease in DNA synthesis and changes in total RNA synthesis and parasite growth in in vitro study. Therefore, PfPK5 has been suggested to regulate S phase of the plasmodial cell cycle. This indicates that modification of these compounds may produce potent anti-malarial drug [7].

Targeting Proteasome of Malaria Parasites

Malaria parasites appear to have a typical 26S proteasome which is composed of two multi-subunit assemblies: a core protease complex, called the core particle (CP) or 20S proteasome; and a regulatory element, known as the 19S regulatory particle (RP) [48]. The proteasome is part of the ubiquitin-proteasome system (UPS), which manages proteostasis in the cell [23,48]. Via an UPS-specific enzymatic cascade, proteins become labeled with a small ubiquitin (Ub) tag. The type of ubiquitination then determines whether a protein is designated for further roles in cellular processes like DNA repair, trafficking or signal transduction, or whether it will be degraded by the proteasome. Previously, it has been presumed as an anti-cancer drug target. In plasmodium parasite studies it illustrated that it has an essential role in the liver, blood and transmission stages which suggests that proteasome is a multi-stage target in malaria therapy [48].

Several kinds of proteasome inhibitors have been identified. Natural products such as lactacystin [23,48], epoxomicin, fellutamide B and designed compounds (MG-132, bortezomib) have been intensively characterized. Most of these carry a chemically reactive "warhead" at the C-terminus to deactivate the proteasome intermittently or even irreversibly [23].

Anti-Malarial Compounds on Pipeline

In present findings there are other new molecules which have remarkable activity in the fight against malaria. New molecules are required to shorten the duration of treatment and increase compliance;

and to prevent the transmission of the parasite back to the insect vector. From its phase II clinical information, ferroquine is a third-generation addition to the chloroquine family which has the standard 4-aminoquinoline scaffold with its basic centers that enable its accumulation in the acidic environment of the parasitic digestive vacuole and an iron atom sandwiched between two aromatic rings (a ferrocene core) that generate toxic free-radicals, providing an additional way for the drug to kill the parasite [49,50].

One of the strategies to develop new medicines is to improve existing drugs. For instance, artemisinin endoperoxides and their derivatives is the mainstay of current therapy. These endo peroxides are logically good starting points for drug discovery. But most of them are from natural products that might take long time and results in lag in demand and supply. So making synthetic molecules as effective as artesunate and affordable at its best price are required [50]. Based on this, OZ277 was developed through a partnership between the MMV and Ranbaxy and subsequently called Rbx11160 or arterolane. A limited Phase III programme has led to the submission and eventual approval of arterolane - piperaquine (under the trade name Synriam) in India in 2013, followed by approval in seven African Nations in 2014 [50]. Arterolane is a rapidly acting blood schizonticide against all blood stages of P. falciparum without effect on liver stages and acts by inhibition of PfATP6, a sarcoplasmic endoplasmic reticulum calcium ATPase encoded by P. falciparum [51].

The original team that developed OZ277 continued with the discovery and development of other new synthetic endoperoxides called OZ439 (also known as artefenomel), which progressed to Phase IIb combination studies, whereby being tested in combination with piperaquine. In vitro data on the early ring stages confirmed that OZ439 is fully active on artemisinin-resistant strains. There are no clinical data on the use of OZ277 or OZ439 in pregnancy, but both OZ277 and OZ439 show a safety margin almost a hundred times higher than artemisinin derivatives in preclinical embryo–fetal developmental safety studies [51]. The global portfolio of antimalarials contains two other interesting endoperoxides: artemisone (also known as BAY-44-9585) and the tetraxoane TDD E209, the next generation compound following RKA182, which was discovered by researchers at the University of Liverpool, UK [50,52]. Artemisone provides a single-dose cure in Aotus monkeys infected with P. falciparum at 10 mg/kg body weight when combined with mefloquine (5 mg/kg) [52].

DSM265 is another potent inhibitor of the Plasmodium enzymes, P.falciparum dihydroorotate dehydrogenase (PfDHODH) and P. vivax dihydroorotate dehydrogenase (PvDHODH) with excellent selectivity versus human dihydroorotate dehydrogenase (DHODH) and efficacious against both blood and liver stages of P. falciparum and active against drug- resistant parasite isolates [50,53]. DSM430 and DSM450 also inhibit P.Falciparum dihydroorotate dehydrogenase (PfDHODH), but their activity against P. falciparum parasites and their species selectivity profiles are substantially different [53]. Another most advanced compound is the spiroindolone KAE609 (also known as cipargamin) developed in a collaboration between the Novartis Institute for Tropical Diseases in Singapore, its Genome Foundation in San Diego, USA, and the Swiss Tropical and Public Health Institute [50,54]. Spiroindolones inhibit PfATP4, a parasite plasma membrane Na^{+-}ATPase that regulates sodium and osmotic homeostasis and has potent activity against both the asexual and sexual stages of the malaria parasite with in vitro model [55]. KAE609 rapidly progressed through to Phase I and has now completed the first studies in human patients

in Thailand and it kills the parasite even faster than artesunate and also maintains a plasma concentration above the MIC for several days [50]. Several other chemical series have emerged that also target PfATP4, including a pyrazoleamide, PA21A092 from Drexel University, USA and a dihydroisoquinolone, SJ733 from collaboration between St Jude Children's Research Hospital and Rutgers University, USA6 [50] which reported to affect the parasites' ATP4 enzyme are in preclinical development [55]. Another candidate that has been developed from a phenotypic hit is MMV390048; where its target was identified to be lipid phosphatidylinositol 4-kinase (PfPI4K). MMV390048 has successfully completed the preclinical programme and began Phase I human studies in Cape Town in the first half of 2014 [50].

Conclusion

Understanding the biological and the biochemical processes of malaria parasites, many approaches to anti-malarial drug discovery are available. There are also different approaches taken into account to identify novel anti-malaria targets which range from minor modifications of existing agents to the design of novel agents that act against new targets.

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