New Targets, New Hope: Novel Drug Targets for Curbing Malaria

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Abstract: Malaria continues to plague the tropical and subtropical regions causing high morbidity and mortality. Every year, millions die due to lack of affordable and effective anti-malarial drugs. Malaria poses significant threat to half of the world's population and our arsenal to combat this disease is nearly empty. Pharmaceutical companies shy away from investing in research and development for anti-malarial drugs and have shunned it as non-profitable venture. In wake of emergence and spread of drug resistant malaria to newer territories, there is imperative need to develop new drugs for curbing malaria. This underscores the need of exploring new drug targets and reevaluation of existing drug targets. Availability of genome sequence of both parasite and human host has greatly facilitated the search for novel drug targets. This endeavor is complemented well by advances in functional genomics, structure - based drug design and high throughput screening methods and raises much optimism about winning this battle against malaria. This review discusses potential drug targets in the malarial parasite for designing intervention strategies and suitable chemotherapeutic agents.

Keywords: *Plasmodium*, drug targets, resistance, apicoplast, mitochondria, proteases, membrane biosynthesis and transport.

INTRODUCTION

The uproar over malaria pandemic has been hushed until recently. Malaria is a pestilence of the deprived, easy to neglect. Unfortunately, in this era of unparalleled growth and resources, the world is still burdened with vicious cycle of malaria which takes the life of a child every thirty seconds. Malaria entails mammoth socio-economic burden on mankind, and along with six other diseases (diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia), accounts for 85% of global infectious disease burden [1, 2]. Medical impact of malaria has actually been quite underrated [3] and the massive economic impact of malaria has never been sufficiently considered [4]. The figures are on the increase due to rapidly changing transmission patterns, migration of populations and slow up-scaling of existing control tools. Despite being a preventable and curable disease, malaria has resurged in many parts of the tropics. Not only malaria is a palpable human cataclysm but it also results in huge financial burden as well. Malaria affects six out of eight targets of United Nations Millennium Development Goal. Malaria in human is caused by 4 species of Plasmodium: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale.* Out of these, *P. falciparum* is the most dreaded parasite causing the vast majority of deaths from malaria.

Sequencing of genome of *P. falciparum* in 2002 led to unraveling of new secrets hidden in code providing deep insight of its functions and gave much needed impetus to the drug discovery efforts [5-10]. *P. falciparum* genome numerates to 23 MB and encodes approximately 5300 genes. The development of the more potent anti-malaria drugs and vaccines followed after functional characterization. The information generated from the genome sequence has paid off very well in enlightening the scientific community about the parasite biology and harbingered a new era by paving way for accelerated drug discovery. Comparative genomics techniques have enabled us in differentiating gene expression patterns between species and stages of life cycle [11] which in turn facilitated in exploring the new horizons in anti-malarial drug discovery. Selection and validation of novel molecular targets have become top priority keeping in mind surfeit potential therapeutic drug targets that have emerged from advances in genomics and availability of high throughput technologies which enable us to get an avalanche of data but finally provide only flakes of information. Growing concerns about the emergence of drug resistance in *Plasmodium*, call for exploring novel drugs to lessen the imminent socio-economic impact. Conventional approaches for identifying new therapeutics for malaria rely on optimization of current drug regimens and formulations, development of analogs of existing drugs, exploiting the wealth of natural resources, identification of resistance-reversal agents and drug repositioning [12].

Failure of conventional and time tested widely used anti-malarial drugs chloroquine and sulfadoxine/pyrimethamine in most malaria-endemic regions due to emergence of resistance in malarial parasite has compelled us to overhaul our strategy in order to accelerate the drug discovery process that can be achieved by focusing on validated targets for developing new drug candidates and/or by identifying new potential targets for malaria chemotherapy. But there is a hope around the corner and future holds promise as knowledge of drug targets is seeing an exponential rise. Failure in developing an effective vaccine against malaria necessitates the development of more effective drugs for

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prevention and treatment of malaria. This review is an attempt to describe putative novel molecular targets for the rational design of new anti-malarials.

TARGETING APICOPLAST

Apicoplast is a relic or vestigial organelle of plastid origin found in most parasites of Apicomplexa phylum. Plastid origin of organelle was confirmed with the identification of 35 kb extra-chromosomal DNA in *P. falciparum*. Apicoplast maintains close proximity to the mitochondrion [13]. Its secondary endosymbiotic origin is now confirmed and eubacterial ancestry of apicoplast presents tremendous prospects of developing chemotherapeutic interventions [14-15]. Though its potent druggability has been explored using various interdisciplinary approaches yet we are ignorant about the reasons behind its indispensability for the survival of *Plasmodium* and this has intrigued malariologists [16]. Apicoplast DNA encodes many tRNAs, some ribosomal proteins, genes coding subunits of an RNA polymerase, elongation factor PfTu and a gene involved in the Fe–S pathway [17]. Apicoplast represents a prospective drug target as it contains unique metabolic pathways like fatty acid, isoprenoid and haeme synthesis absent in human that can serve as good source of drug targets.

Fatty Acid Biosynthesis

Fatty acids are integral part of membranes and play crucial role in cell signaling [18]. Besides scavenging long chain fatty acids from host [19-21], *Plasmodium* possess *de novo* fatty acid synthesis pathway located in apicoplast. The earlier belief of sole reliance of *Plasmodium* on fatty acids derived from host was uprooted by advent of data mining techniques and occurrence of Type II fatty acid biosynthesis was also validated by biochemical experiments involving incorporation of 14C-labeled acetate into *Plasmodium* fatty acids [22]. FAS II pathway is characterized by presence of distinct enzymes for catalyzing individual reactions for successive steps of fatty acid elongation which is in contrast to FAS I pathway which is brought about by a single multifunctional enzyme [23-25]. Type II fatty acid biosynthesis pathway is *Plasmodium* has been extensively studied and most of its enzymes have been characterized and have received lot of attention for their plausible use in drug development. Vulnerability of *Plasmodium* at multiple points of FAS II pathway has been established by molecular and biochemical data. Though FAS I and FAS II pathways share lot of similarities yet it is possible to selectively inhibit FAS II using Thiolactomycin [26] and triclosan [22]. FAS II pathway presents a good target for development of selective therapeutic drugs for malaria due to differences in fatty acid biosynthesis in malarial parasite and human host. Acetyl CoA carboxylase (ACC)(EC 6.4.1.2) catalyzes the first committed and rate-limiting step in fatty acid synthesis by bringing about the conversion of acetyl CoA to malonyl-CoA utilizing bicarbonate as a source of carbon. As ACC is a crucial metabolic component regulating fatty acid synthesis pathway, it can be regarded as a good target for developing anti-malarial drugs. Inhibition of ACC activity can severely inhibit the growth of organism by affecting fatty acid biosynthesis. Aryloxyphenoxypropionate herbicides specifically block the multi-domain ACC present in plastid of grasses [27]. Apicomplexans like *Toxoplasma* and *Plasmodium* code for similar single-protein type ACC targeted to their apicoplast and both are inhibited by these herbicides [18, 28]. The striking differences in the structure of human and *Plasmodium* ACCs can be exploited for designing chemotherapeutic interventions. Malonyl-CoA: acyl carrier protein transacylase (*fabD*; MCAT, EC2.3.1.39) transfers malonyl moiety to holo-acyl carrier protein ACP forming malonyl-ACP intermediates in fatty acid biosynthesis. MCAT from several species including *P. falciparum* has been cloned and characterized [29] and crystal structures of *E. coli* MCAT [30] *Streptomyces coelicolor* [31] *and Helicobacter pylori* [32] are now available. Though it is considered an important drug target in bacteria owing to its vital role in fatty acid and polyketide synthesis, its potential as a drug target has not been explored in *Plasmodium*. β-ketoacyl-ACP synthase III (KAS III)(EC 2.3.1.180) the first enzyme of elongation pathway uses acetyl-CoA and malonyl-ACP substrates for synthesizing acetoacetyl-ACP. Efforts to inhibit the activity of KAS III with Thiolactomycin(TLM) have met little success but some analogs of TLM showed IC50 value below 10 μM and were found effective against both Chloroquine- sensitive and resistant strains [29]. *β*-Oxoacyl-ACP reductase (EC 1.1.1.100)catalyzes the conversion of acetoacetyl-ACP to *β*-hydroxyacyl-ACP. This enzyme has been characterized in *Plasmodium* [33] but not exploited much as drug target. Next step of elongation cycle is catalyzed by *β*-Hydroxyacyl-ACP dehydratases resulting in formation of enoyl-ACP. Characterization of FabZ of *P. falciparum* has facilitated the identification of inhibitors [34]. Homology modeling and docking studies also aided in identification of NAS91 and NAS21 as important inhibitors of FabZ [34]. Availability of its crystal structure has paved a way for identification of more potent anti-malarials [35]. 3-decynoyl-NAC, a mechanism-based, inhibitor binds covalently to the active site of PfFABZ and inhibits its activity *in vitro*. Enoyl acyl carrier protein reductase has been validated as a promising drug target for developing anti-microbial drugs [32]. Triclosan kills *P. falciparum in vitro* and cure infection in mice infected with *P. berghei* [22]. Many studies focused at testing the activity of analogs of triclosan against PfENR, none showed better results than triclosan [36-38]. Apart from triclosan, substituted pyrazoles and tea catechins also inhibit PfENR [39-40]. Recently, Kumar *et al.* discovered a rhodamine class of inhibitors of PfENR using a combinatorial approach and most potent inhibitor of this class demonstrated an IC50 of 35.6 nM against PfENR and inhibited *Plasmodium* growth in culture in nanomolar concentration [41]. Karioti *et al.* tested linear sesquiterpene lactones derived from *Anthemis auriculata* against *P. falciparum* and found 4-hydroxyanthecotulide and 4-acetoxyanthecotulide inhibited FAS-II enzymes, PfFabI and PfFabG (IC_{50} values 20–75 μg/ml) [42]. KAS I (FabB) and KAS II(FabF), 2 closely related isoforms of ß-ketoacyl-ACP synthase catalyze the elongation of fatty acids and play important roles in elongation of unsaturated fatty acids and controlling fatty acid composition in thermo-sensitive manner [43]. Use of cerulenin as an inhibitor of Fab B and Fab F has been limited as it can affect Fas I pathway also

[44-45]. Thiolctomycin specifically targets FAb B and TLM and its several analogs inhibit growth of *P. falciparum in vitro* [46-47]. It was observed that increasing side chain length and unsaturation in TLM analogs led to a substantial increase in their inhibition potency [45]. It is now known that inhibitors of apicoplast fatty acid synthesis do not cause delayed effects and thus are effective for prophylaxis as well as treatment [22]. Inhibition of regulatory enzymes of fatty acid biosynthesis seems to be worthwhile for curbing malaria.

Isoprenoid Synthesis

Though basic unit of isoprenoids is 5-carbon isoprene, isoprenoids greatly vary in structure and perform different functions in various processes like ETS, photosynthesis, signaling, control of biosynthesis of lipid, meiosis, apoptosis, protein degradation [48]. Two pathways are known for isoprenoid biosynthesis [48]: mevalonate pathway [49] and plastidial methylerythritol 4-phosphate (MEP) pathway/ Rohmer pathway [50-52]. Mevalonate pathway was considered to be the only route for synthesis of isoprenoid before the discovery of mevalonate-independent MEP pathway in chloroplast of plants, eubacteria and apicomplexa. Complete reliance of *Plasmodium* on MEP pathway for isoprenoid synthesis has been established by inhibition of mevalonate pathway by mevastatin [53]. Several enzymes of this pathway have been identified including 1-deoxy-D-xylulose-5-phosphate synthase, 1-deoxy-D-xylulose-5-phosphate reductoisomerase(DOXP reductoisomerase) and 2C-methyl-D-erythritol 2, 4-cyclodiphosphate synthase [48, 54]. Owing to its absence in human hosts, this pathway represents an excellent source of novel drug targets and has come under close scrutiny. It is presumed that specific inhibitors designed against enzymes of this pathway will aid in designing new anti-infective agents, which will be less toxic and cause fewer side effects. Fosmidomycin is an effective inhibitor of DOXP reductoisomerase, an essential enzyme of this pathway as monotherapy as well as in combination with other anti-malarial drugs [55]. It is expected that when used in suitable combination, the regimen will show a marked increase in efficacy in combating malaria.

Haem Biosynthesis

Haem biosynthesis pathway is considered an attractive target as haeme acts as reactive core for many important proteins by acting as prosthetic group. During its life cycle, *Plasmodium* relies on *de novo* synthesis of haem and also accumulates haem by acquiring it from the host R.B.C by degrading it [56]. Malarial parasite shows an essential requirement for haem for sustained growth and protein synthesis [57] which facilitates its survival during exo-erythrocytic cycle. Besides intrinsic parasite pathway present in two endosymbiotically acquired organelles; mitochondria and apicoplast, an extrinsic pathway for haem biosynthesis is also functional in parasite cytosol [58]. Occurrence of haem biosynthesis pathway in mitochondria and apicoplast necessitates the exchange of metabolites across the membranes of these organelles in parasite. Mechanism of such exchange is still not very clear. Haem biosynthetic pathway and its components have been identified [58]. Spatial demarcation for determining the location of various enzymes involved in this pathway is crucial for understanding haem metabolism in parasite. First enzyme of the pathway D-Aminolaevulinic acid (ALA) synthase (PfALAS) that initiates the pathway for synthesis of ALA from glycine and succinyl-CoA, is known to localize to mitochondrion [59]. Plasmodium imports D-aminolaevulinate dehydratase (PfALAD) from host cells and synthesize its own version of ALAD. ALAD is shown to localize to apicoplast [60-64]. Next two enzymes catalyzing subsequent steps of the pathway, porphobilinogen deaminase [65] and uroporphyrinogen III decarboxylase [66] localize to the apicoplast [65]. Careful examination of *P. falciparum* genome reveals the presence of genes encoding all the components of haeme biosynthesis with the exception of Uroporphyrinogen III synthase [8]. The tale of haeme biosynthesis appears to be convoluted as the next 3 enzymes of the pathway coproporphyrinogen oxidase (PfCPO) and protoporphyrinogen oxidase (PfPPO) and ferrochelatase which acts in succession to form haeme, localize to mitochondrion [13]. *De novo* synthesis is imperative for the survival of *Plasmodium* and inhibition of ALA dehydrase by suucinylacetone is reported to cause death of *Plasmodium* [60]. Haeme is the target of many anti-malarials such as artemisinin which forms free radical adducts with haeme detrimental to parasite [67]. Parasite makes use of haeme synthesized *de novo* for meeting its metabolic requirements and stores haeme derived from RBC as inert micro-crystalline form hemozoin. This conversion of haeme to hemozoin by polymerization is unique to *Plasmodium* and we still do not clearly understand the mechanism behind it. Histidine- rich -protein -2 (HRP-2)initiates the process by binding to 50 Fe(III)PPIX molecules per protein as bis-histidyl complexes and b-hematin is formed only after the saturation of hematin binding sites on the protein. Popular drugs like chloroquine interferes with hemozoin formation by interacting with hematin thereby checking its binding to HRP-2.Thus, HRP-2 can be explored as a target for designing new drugs for malaria [68].

DNA Transactions

Parasite apicoplast DNA possesses coding potential and undergoes usual cycle of replication, transcription and translation in a manner akin to those of prokaryotes. Numerous anti-bacterial compounds which block these processes act as slow acting malarials [69].

DNA topoisomerases are enzymatic machines present in all realms of life, from bacteria to higher eukaryotes [70-71]. These enzymes catalyze the breakage of DNA phosphodiester bonds and their reclosure, thus modifying topology of DNA during replication, transcription, recombination and repair processes [70]. Two types of DNA topoisomerases are known: 1. Type I DNA topoisomerases are monomeric ATP-independent enzymes which transiently introduce a cut and then rejoin one DNA strand, thus changing linking number in a step of one. These enzymes relax both positively and negatively supercoiled DNA [72-73] 2. Type II DNA topoisomerases are multimeric enzymes which cut and reseal both strands changing the linking number by step of 2. They not only relax positively supercoiled DNA but also knot/unknot and catenate/

decatenate close circular DNA [74]. Owing to major differences in structure between human host and parasite type I topoisomerase, this enzyme is believed to be an excellent drug target [75-78]. Categories of topoisomerase inhibitors have been the subject of many reviews [77,79]. Camptothecin (CPT), a class I topoisomerase poison produced by the plant *Camptotheca accuminata* is cytotoxic for the erythrocytic forms of *P. falciparum* [80] even at very low concentration. It is reported that DNA gyrase is required for the replication of apicoplast genome of *Plasmodium*. *Plasmodium* genome encodes homologues of gyrA and gyrB [8]. Gyrase specific drug Ciprofloxacin inhibits apicoplast DNA replication without affecting nuclear replication in *Plasmodium*. This clearly establishes DNA replication as a feasible drug target in *Plasmodium* and its use guarantee few side effects as mammalian DNA replication does not require DNA gyrase. But use of ciproflaxin as anti-malarial warrants some caution as some ciproflaxin- resistant bacteria have been reported in past [81,82].

Transcription in apicoplast proceeds with the help of RNA polymerase akin to cyanobacteria and eubacteria [83]. Plastid genome encodes β and β' of RNA polymerase while α unit and sigma factors are encoded by nuclear genome and their products are targeted to apicoplast. This RNA polymerase is sensitive to rifampicin suggesting blockage of apicoplast transcription by the drug [84].

Translational machinery of *Plasmodium* apicoplast is targeted by Fusidic acid, an anti-bacterial derived from *Fusidium coccineum*. This compound binds to elongation factor G(*elongation factor thermo unstable)* and prevents the exit of Ef-G:GDP complex from the ribosome and thus, blocking protein synthesis [85-86]. Fusidic acid shows parasiticidal activity *in vitro* [87] but has not been exploited as anti- malarial drug yet. Antibiotics like lincomycin, clindamycin, erythromycin and azithromycin stall protein synthesis by interacting with the peptidyl transferase domain of 23S subunit of ribosomal RNA in bacteria [88]. These antibiotics can also inhibit the growth of *Plasmodium* by impeding apicoplast translation. Unlike other antibiotics which causes delayed death phenotype in parasite, thiostrepton causes immediate killing by blocking apicoplast translation by binding to GTPase binding domain of large subunit of ribosomal RNA and affects only apicoplast rRNA [89-92]. Many other antibiotics like tetracyclins, doxycycline and chloramphenicol hamper various steps in protein synthesis causing delayed death of parasite *in vitro*. Despite being slow in action, these antibiotics have generated a hope of curbing malaria when used in suitable combinations such as clindamycin and quinine [93] and azithromycin and artesunate [94]. Initially thought as unique to prokaryotes only, peptide deformylase is a metalloenzyme that brings about the deformylation reaction of the amino terminal fMet residue of newly synthesized proteins. Peptide deformylase varies in structure from human counterpart and many inhibitors have been identified that can serve as starting point for development of anti-malarial drugs [95]. Inhibitors targeting Peptide deformylase repress the growth of *P. falciparum* in culture [96-97] implying the possibility of their use for designing new anti-malarial drugs in future.

TARGETING MITOCHONDRIA

Parasites have unique capability of adapting in different environments. Not only are these adaptations seen in unique energy metabolic pathways but also in structural and physiological characteristics of various organelles. For instance, mitochondria of *Plasmodium* display considerable differences during asexual and sexual stages. These adaptations enable *Plasmodium* to survive differences in oxygen tension between human host and Anopheles mosquito. Mitochondrial genome is represented by 6 kb and codes for cytochrome b, cytochrome oxidase I and III, besides fragmented tRNA [98-99]. It attains several nuclear gene coded proteins through import. Computational biology approaches have enlightened us about the organization and function of *Plasmodium* mitochondrion. Mitochondrion, known as power house of cell, plays crucial role in energy generation and electron transport. But these roles of mitochondria in *Plasmodium* appear to be dubious as *Plasmodium* derives energy from glycolysis and even citric acid cycle is also dysfunctional. The citric acid cycle itself appears dysfunctional, although the genes for most of the component enzymes can be identified in the database. These distinctive characteristics of *Plasmodium* mitochondria and related enzymes make potential targets for developing chemotherapeutic agents. The electron transport chain appears to function *via* the FAD-linked tricarboxylic acid cycle enzymes, malate-quinone oxidoreductase and succinate dehydrogenase. Electron transport chain is mediated through FAD-linked tricarboxylic acid cycle enzymes, malate-quinone oxidoreductase and succinate dehydrogenase. ETS has a unique drug target, Coenzyme Q (uniquinone) and 5 enzymes are known to transfer electrons to it reflecting upon its importance. CoQ serves as electron acceptor for dihydroorotate dehydrogenase [13]. Role and localization of NAD(P)H dehydrogenase needs to be understood as it represents a good drug target owing to its absence in human. Atovaquone effectively acts against ubiquinol-cytochrome oxidoreductase (complex III), inhibits electron transport and collapses mitochondrial membrane potential which can prove detrimental to parasite. The major drawback in using it is quick development of resistance in *Plasmodium*. It is found to be effective in combination therapy with antifolate 'proguanil', but this proves to quite expensive [100]. It is interesting to know that *Plasmodium* mitochondrion is also involved in folate, Fe–S cluster and haem biosynthesis. Elucidating the role of mitochondrion in stages other than erytrocytic stage can be a fruitful endeavor and can trace the path for discovery of novel drug targets.

TARGETING HISTONE ACETYLATION

Packaging of DNA in nucleus of eukaryotic cell is achieved by formation of highly organized chromatin. Chromatin can be regarded as physiological template for many vital cell processes like DNA replication, repair and transcription. Chromatin based epigenetic mechanism involves a multitude of posttranslational modifications(PTMs) of histones such as acetylation, phosphorylation, methylation, ubiquitination and ADP ribosylation [101]. These PTMs can modify the structure of chromatin thus changing its global environment which in turn affects various biological processes. Specific arrays of

different modifications of N-terminal tails of histone form an epigenetic "histone code" [102]. Among all these PTMs, acetylation is the most widely studied. Histone acetylation is a reversible process catalyzed by action of histone acetyltransferases (HATs) and histone deacetylases (HDACs). *P. falciparum* encodes 2 histone acetyl transferases; PfGCN5, PfMYST and five histone deacetylases viz. PfHDAC1 (homolog of class I HDAC) and PfSir2 (homolog of Class III), PF14_0489, PF14_0690 and PF10_0078 [8,103,104]. Among those, PfHDAC1 are considered a viable drug target.

Although it is still not clear that histone acetylation brings about the regulation of genes in *Plasmodium* yet its importance is underscored by recent revelation of its role in regulating monoallelic expression of *var* gene family which intercedes antigenic switching and virulence of the parasite [105-107]. H3 acetylation mediated by PfGCN5 leads to activation of genes in *Plasmodium* and any interference in histone acetylation results in profound adverse effects on parasite growth. This clearly indicates the role of *Plasmodium* HATs as chemotherapeutic targets. Curcumin inhibits PfGCN5 but does not affect human PCAF [108]. Anacardic acid (AA) derived from plants of family *Anacardiceae* inhibits the PfGCN5 and is effective against both chloroquine-sensitive and resistant *Plasmodium* strains [109]. AA alters gene expression in *Plasmodium* by interfering with HAT activity.

Numerous inhibitors of *Plasmodium* Histone deactelylases selectively showed a high anti-malarial activity against resistant strains of *Plasmodium in vitro*. Drugs inhibiting histone acetylation demonstrate high anti-parasitic activity. It was Darkin-Rattray along with coworkers who first provided experimental proof that apicidin demonstrates parasiticidal activity towards *Plasmodium* by inhibiting histone deacetylase [110]. One group has profiled anti-malarial activity of a group of phenylthiazolyl hydroxamate-based histone deacetylase inhibitors which were previously screened as therapeutic agents for treating pancreatic cancer [111]. It was found that WR301801 which induces hyperacetylation of *P. falciparum* histones like suberoylanilide hydroxamic acid (SAHA), trichostatin A, and 2-aminosuberic acid (2-ASA)analogs, offers several advantages like high selectivity, bioavailability and short half life [111]. WR301801 is now considered one of the most potent HDAC inhibitor exhibiting IC50 of 0.5 to 1.5 nM against several drug-resistant strains of *P. falciparum* [111]. Andrews *et al*, 2008 screened several potent compounds which selectively inhibited the growth of *P. falciparum* (IC50 3-339 nM)[112]. In a recent study, out of 26 compounds designed specifically to bind active site of PfHDAC1, 16 potent compounds showing IC(50) <1000nM and >10 folds more toxicity towards against *P. falciparum* compared to human were identified [113]. Hydoxamate based HDAC inhibitors were evaluated against MDR clinical isolates of *P. vivax* and *P. falciparum* using a modified schizont maturation assay and were shown to inhibit both species at sub-micromolar concentrations [114].

TARGETING NUCLEIC ACID METABOLISM

Nucleotides are building blocks of DNA and RNA. Nucleotides consist of a pentose sugar group linked to a nitrogenous base either a purine (adenine and guanine) or a pyrimidine (cytosine, uracil, and thymine). There are marked differences in nucleic acid metabolism pathways between *Plasmodium* and human host which can be exploited for development of drugs. Plasmodia synthesize purines and pyrimidines by salvage and *de novo* biosynthetic pathways, respectively, while mammalian cells synthesize purines *de novo* and either salvage or synthesize pyrimidine by a *de novo* pathway. There are many enzymes in these pathways that can be targeted.

The Purine Pathway

Purine salvage pathway of *Plasmodium* has been a subject of interest for past many years. Main source of purines in *P. falciparum* is hypoxanthine–guanine–xanthine phosphoribosyltransferase (HGPRT), an extensively studied enzyme which shows 46% homology with host counterpart and substrate specificity [115]. HGPRT catalyses the transfer of a phosphoribosyl group from hypoxanthine to inosinic acid. Prominent significant differences in host and parasite enzyme make it an important drug target. Immucillin-H is a transition state analogue that has been shown to kill the parasite through inhibition of purine nucleoside phosphorylase (PNP) even at very low concentration [116].

The Pyrimidine Pathway

Plasmodium obtains pyrimidine nucleotides through a *de novo* pathway and synthesizes them from bicarbonate and glutamine. Host mammalian cells synthesize pyrimidines by *de novo* and salvage pathways. Salvage pathway is absent in malarial parasite. Carbamoyl phosphate synthase, aspartate carbamoyltransferase, dihydroorotase, dihydroorotate dehydrogenase, orotate phosphoribosyl transferase and orotidine 5-phosphate decarboxylase constitute the *de novo* pathway [117] and their activities have been detected in parasite extracts [118-119].

Carbamoyl phosphate synthetase II, the first enzyme of pyrimidine biosynthesis pathway is mono-functional in *Plasmodium* and other members of Apicomplexa and bacteria unlike in eukaryotes, where the enzyme is bi-or trifunctional [120-121]. Thus, the enzyme is quite different from the human counterpart both in structure as well as properties, making the enzyme a lucrative drug target. CPSII has been effectively targeted by utilizing ribozyme based inhibition [122]. Aspartate carbamoyltransferase catalyzes the formation of phosphate and N-carbamoyl-L-aspartate from carbamoyl phosphate and L-aspartate in pyrimidine biosynthesis [123]. In a recent study, homology model of Aspartate carbamoyltransferase was generated and binding efficiencies of PALA based inhibitors were calculated [124]. Orotate phosphoribosyltransferase and orotidine 5'-monophosphate decarboxylase in *Plasmodium* form a complex consisting of 2 subunits retaining their individual kinetic properties which are markedly different from host UMP synthase. Analogs of orotate inhibit pyrimidine synthesis in rodent parasites and cure infection in mice [125-127]. *Plasmodium* dihydroorotate dehydrogenase has been isolated, purified and characterized [125,128]. DHODH is a validated druggable target based on its essential role in pyrimidine synthesis [129] and lack of any functional bypass

in erythrocytic stages of *Plasmodium* for compensating the loss of its activity [130]. It is possible to design specific inhibitors against *Plasmodium* DHODH due to lack of conservation in inhibitor binding sites across species. Phillips *et al*. adapted a 384 well plate and screened a 220,000 compound library for inhibitors of PfDHODH and identified hits which showed selectivity towards *Plasmodium* DHODH and selected compounds based on their IC50 [130]. DSM1, a triazolopyrimidine-based compound showed differential specificity towards *P. falciparum* and also demonstrated potent activity against *P. falciparum* 3D7 in whole cell assays [130]. A number of analogs of DSM1 were synthesized and tested for anti-malarial activity. Structure of *Plasmodium* dihydroorotate dehydrogenase bound with inhibitors has been solved and provided insight on the structural basis for selective binding of various chemical entities [131]. In another study, small molecule library of 208,000 compounds from Genzyme was used for HTS and 5 inhibitors selectively active against *Plasmodium* enzyme were identified and most potent among them showed an IC50 = 40 nM on PfDHODH [132]. Another study established the cellular target and mechanism of action of the enzyme by characterizing DHODH inhibitors against transgenic parasites having DHODH of yeast origin [133].

Thymidylate synthase is a validated drug target but it is extremely hard to design effective specific inhibitors that inhibit only parasite TS owing to its high conservation. N5-N10-methylene tetrahydrofolate analogues are promising candidates for targeting parasite TS but need further investigations [134].

TARGETING FOLATE METABOLISM

The ability to convert folic acid into tetrahydrofolic acid is found in both human and *Plasmodium*. Human meets the requirement of folic acid from dietary resources but *Plasmodium* can derive it from exogenous sources by salvaging preformed folate and also by *de novo* synthesis [135-136]. The exogenous pathway though present and functional in *Plasmodium* is not the primary source of folate and how the parasite maintains a balance between *de novo* and salvage pathway is still unknown and differs among different strains [137]. Antifolates are known to affect all growing stages of *Plasmodium* and also hamper the early development stages in the liver. Folate metabolism is central to the survival of *Plasmodium* and represents an attractive source of drug targets for treatment and prophlaxis of malaria. Use of antifolates against malaria started much before the discovery of the folate pathway in *Plasmodium*. Antifolate agents have been used in treatment of malaria and are instrumental in war against malaria and form important line of defense against chloroquine- resistant *Plasmodium* strains. Despite being in use for such long period, our understanding of folate mechanism is still poor. Antifolates can be classified as: Class I antifolates that inhibit dihydropteorate synthase (DHPS) such as sulfones and sulfonamides and Class II antifolates that target dihydrofolate reductase (DHFR) pyrimethamine and proguanil. They are often administered in combination. Various genes of folate pathway targeted by DHFR [138] and DHPS [139-140] have been cloned and characterized and mutations occurring in these genes are implicated in resistance to antifolate drugs [141]. Major antifolate drugs in use are proguanil, chlorproguanil, sulfadoxine, pyrimethamine and dapsone. Sulfa drugs inhibit *de novo* synthesis of folate and are effective anti-malarials. Despite being most potent inhibitor of DHPS, development of dapsone was abandoned owing to its limited efficacy and high toxicity. To overcome this difficulty, dapsone is being used in combination with chlorproguanil for treating malaria and commercialized by GlaxoSmithKline as Lapdap. Antifolates based on pyrimidines and triazine structures are also being projected as prospective anti-malarials. Another candidate is PS-15, a biguanide precursor of WR 99210 which shows better bioavailability and potency than WR99210 [142] and its analogs are being developed and some have reached preclinical stages. Recent studies carried out in Thailand have identified lead compounds by assessing analogues of pyrimethamine and cycloguanil [143]. Thymidylate synthase is an established target for anti-malarial drugs but owing to high conservation, designing effective inhibitors against this enzyme remains a problem. This problem can be tackled by combining inhibitor of TS with a nucleoside that can be utilized by human host. N5-N10-methylene tetrahydrofolate analogs are considered attractive option in this regard [134]. They also demonstrated that MTX is potent against *P. falciparum* isolates with IC50<50 nM and can be used to treat malaria if proper combination and dosage is formulated and safety issue need to be evaluated and addressed before. It is speculated that MTX can be used as an additive to anti-malarials [144].

TARGETING MEMBRANE BIOSYNTHESIS AND TRANSPORT

Phospholipid Biosynthesis

Once in RBC, malaria parasites synthesize various membranes like food vacuolar membrane, the parasite plasma membrane (PPM) and other subcellular components. As lipids are major constituents of membranes, parasites undergo massive synthesis of lipids. This fact is reinforced by presence of higher amount of lipids in infected RBC as compared to normal RBC. The enzymes involved in synthesis of phospholipids are crucial for replication of parasite and absent in human mature erythrocytes and thus, phospholipid metabolism of *Plasmodium* is considered potential target. Phosphatidylcholine and phosphatidylethanolamine constitutes a major portion of the total phospholipid content in *P. falciparum* and fraction of amount of phosphatidylserine, phosphatidylinositol and cardiolipin present is considerably less [21,145-146]. *Plasmodium* employs various enzymes for the synthesis of phospholipids from precursors produced by parasite metabolism or obtained from human serum [147-148]. Biosynthesis of phosphatidylcholine in *Plasmodium* occurs *via* 2 pathways: the CDP-choline (also called Kennedy pathway) which is the primary route of Phospahtidylcholine and the serine decarboxylase-phosphoethanolamine methyltransferase (SDPM) pathway [149]. Kennedy pathway proceeds through a cascade of events catalyzed by Choline kinase, CDP-choline cytidylyltransferase and CDP-diacylglycerol-choline phosphotransferase. Inhibition of Choline kinase is detrimental to parasite [150-152]. Choubey and coworkers established that hexadecyltrimethylam-

monium bromide (HDTAB) inhibits the purified Choline kinase as well as Choline kinase of *P. falciparum* in culture by interfering with Kennedy pathway and demonstrates high anti-malarial activity [153]. One of the most efficient ways of blocking PtdCho biosynthesis is by using mono- or bis quarternary ammonium salts that mimic choline. Ancelin *et al.* tested such compounds against *P. falciparum* and observed IC_{50} values of 0.7–10 μM [154]. Encouraged with these initial results, new structural analogs were synthesized and assessed for anti-malarial activity and bis-ammonium salts were found to be more potent than mono-ammonium salts [155]. Among these, G25 [1,16-hexadecamethylenebis (*N*-methylpyrrolidinium) dibromide] demonstrated potent anti-proliferative activity when tested against drug- resistant *P. falciparum* strains and it was found to be effective in curing infection in primate and rodent model even at very low doses [151, 156]. In a similar study aimed at testing the effect of N-substitutions for modifying quaternary salts for enhancing the bio-availability, T3 and T4 demonstrated potent anti-malarial activity [157]. As *Plasmodium* cytidylyltrans-ferase, the rate limiting enzyme in Kennedy pathway displays differences with its mammalian counterpart [158], it can be exploited as a potential drug target and needs to be further explored and validated.

Targeting Parasite Transporters

Plasmodium spends a major part of its life cycle in erythrocytes and soon after entering the erythrocytes, parasites start rapid growth and replication. This imposes a high demand of nutrients which can not be quenched solely by the host cells, thus necessitating acquisition of new permeability pathways (NPPs) to obtain supply of nutrients from extracellular milieu as well as erythrocytes [159]. These NPPs also aid in metabolite removal which if left to accumulate in the cell can prove toxic and also for regulating the volume of cell by amino acid release. These NPPs are essential for parasite survival and thus are considered good palpable drug targets. A fact reinforced by the death of parasite by specific inhibitors of NPP. Furosemide analogues have been assessed for blocking *Plasmodium* NPPs [160].

The requirement for a constant glucose supply suggests the plausible role of Hexose transporter as a drug target [161]. O-3-hexose derivatives block uptake of glucose and fructose by PfHT in *Plasmodium berghei* [162] Understanding the mechanism of transport processes in the parasite will pave a way for their use in developing new chemotherapeutic agents.

TARGETING PARASITE PROTEASES

Beginning of erythrocytic life cycle of *Plasmodium* is marked by invasion of erythrocytes by free merozoites and it completes with release of invasive merozoites on rupture of erythrocytes by mature schizonts. Parasitic proteases carry out the rupture of blood cells and degradation of hemoglobin and hydrolyse a considerable fraction of the host erythrocyte proteins into constituent amino acids which the parasite utilizes for its own protein synthesis [163-164]. Proteases are also crucial for reinvasion as the release of merozoites by erythrocytes rupture is required for the introduction of merozoites into plasma for malarial cycle. *Plasmodium* proteases can be classified into two categories: 1. Proteases involved in invasion and rupture of blood cells 2. Proteases involved in degradation of hemoglobin. Mechanism of merozoite release has been investigated using cysteine protease inhibitors using advanced imaging techniques. These inhibitors obstruct breaking of erythrocyte membrane causing aggregates of merozoites structures. These inhibitors provide a starting point for development of new drugs. The processes involved in host–parasite interactions are still not properly understood. Several proteases in *Plasmodium* such as cysteine (cathepsins B, H, and L) and aspartic (cathepsin D) proteases have been characterized. Many proteases like plasmepsin and falcipains are believed to be good targets for rational drug designing. Fluoromethyl ketones, vinyl sulfones and chalcones target the cysteine proteases [165] while statine, allophenylnorstatine and diphenylurea derivatives target the aspartic proteases [166]. Elucidation of structure and detailed biochemical characterization will aid in evaluating their potential as drug targets. Another group of proteases that have received a lot of attention as potential anti-malarial targets recently is Methionine aminopeptidases (MetAPs) which catalyze the removal of the N-terminal initiator methionine during protein synthesis [167]. Recently one group identified a family of structurally related inhibitors containing a 2-(2-pyridinyl) - pyrimidine core on screening a library of 175000 compounds against one of MetAP [168].

HEAT SHOCK PROTEINS

Molecular chaperones regulate protein folding in cell and are implicated in diverse cellular processes like signal transduction, differentiation and development and protein trafficking [169]. Heat shock proteins (HSPs) are ubiquitous proteins induced in response to cellular stress arising from sudden change in the environment. HSPs act as molecular chaperones and facilitate the refolding of misfolded proteins [170]. HSPs are one of the most abundant proteins expressed in eukaryotic cell under normal conditions [171]. They display high degree of evolutionary conservation among species and are considered crucial for cell survival. They are best known and classified according to their molecular weight. It is perceived that *Plasmodium* heat shock proteins play a cytoprotective role during its transition across 2 varied habitats i.e. from poikilothermic mosquito to homothermic man host and enable the parasite to overcome stress arising due to physiological and temperature fluctuations [172]. Studies have revealed that *Plasmodium* genome encodes 43 Hsp40, 6 Hsp70 and 3 Hsp90 [173]. Existence of several isoforms of PfHsp70 points to the pivotal role they play in translation and export of proteins into the apicoplast in the life cycle of malarial parasite [174-178]. Despite this, it is difficult to design drugs against PfHsp70 owing to their high sequence similarity with the human counterparts. Among various heat shock proteins expressed in *Plasmodium*, heat shock protein 90 has received a lot of attention during past several years. Heat shock protein90 is also implicated in increasing the pathogenesis of parasite during febrile episodes of malaria where temperature of the patient rises considerably [179]. Hsp 90 acts in coordination with many other cochaperones and is involved in regulation of proper conformation of many signaling proteins. Heat shock protein

90 is crucial for intra-erythrocytic development of *P. falciparum* [180-181]. Hsp90 promotes parasite development during febrile episodes of malaria and its inhibition by geldanamycin (GA) stalls parasite development from trophozoite to ring stages during asexual growth of *Plasmodium* in erythrocytes. Thus, the dependence of *Plasmodium* intra-erythrocytic development along with its central role in modulating activity of transcriptional factors makes Hsp90 an attractive drug target. Availability of structure of Hsp90 will pave a way for development of new anti-malarial molecules [182] and it is also anticipated that some of the analogs of GA like 17-AAG and others which have reached clinical trial stages can be exploited in treatment of malaria as well [181].

TARGETING CYCLIN DEPENDENT KINASES

Cyclin dependent kinases (CDKs) regulate the progression of cell cycle, growth and differentiation [183]. The association of the constituent catalytic subunit and regulator cyclin unit results in time windows for activation of CDKs [184]. Progression of cell cycle is mediated by various combinations of CDKs during distinct phases of cell cycle. The conservation of these CDKs across species underscores their crucial role in *Plasmodium*. There are marked deviations in cell cycle of *Plasmodium* and host. In contrast to host where single round of DNA replication per cell cycle results in two identical cells, several rounds of DNA replication and karyokinesis occur in *Plasmodium* before cell division during intra-erythrocytic asexual cycle resulting in single multinucleate syncytium. Hence, regulation of cell cycle in parasite is achieved in way completely different from the host. Several CDK like sequences have been identified in *Plasmodium* such as PfPK5, the MO15-related Pfmrk, the CDK ⁄ mitogen-activated protein kinase (MAPK] hybrid protein PfPK6, and several cyclin-related kinases (Pfcrk-1, Pfcrk-3, Pfcrk-4, and Pfcrk-5) [185]. These CDKs demonstrate notable structural and functional differences from the human host. Significance of these CDKs in regulation of various check points of cell cycle renders them attractive drug targets [186]. Inhibition of these CDKs seems to be a rewarding option for developing anti-malarials.

Compounds that block PfPK5 are the ones that have been reported to inhibit CDKs like purvalanol, hymenialdisine, and indirubin-3-monoxime but not purine derivatives olomoucine and roscovitine [187-188]. Several 1,3-diaryl-2-propenones (chalcone derivatives) which exclusively hamper pfmrk at low concentrations have been described [186]. Purines, quinolinones, oxindole-based compounds, thiophene sulfonamides selectively block the parasite enzyme without affecting human CDKs [189-191]. 8-nitro and 7-chloro-4-azatryptanthrins and their derivatives are reported to considerably inhibit Pfmrk activity [192].

TARGETING REDOX MECHANISM

Plasmodium has to maintain itself in different environments being a digenetic parasite and thus needs to cope with oxidative stress [193-194]. This means it has to develop defense armory for protection against oxidative stress in intra-erythrocytic stage of its life cycle. Defense against Reactive Oxygen Species (ROS) generated either by parasite or human host from the metabolism of hemoglobin is mediated by a number of antioxidant enzymes and mechanisms adopted by parasite. Enzymes that enable the parasite to survive through periods of oxidative insults include superoxide dismutases and thioredoxin-dependent peroxidases. Thioredoxin and glutathiome systems are involved in antioxidant defence mechanisms in malarial parasites. Thioredoxin redox cycle (comprising NADPH, thioredoxin reductase and thioredoxin) is crucial for existence of blood stage of *Plasmodium*. *P. falciparum* Thioredoxin reductase has different peripheral redox centre when compared with host and the difference can very well be exploited for drug design. Inhibitors are being screened and tested for Pf Thioredoxin reductase. 5, 8-Dihydroxy- 1, 4-naphthoquinone and 5-nitro-2-furanacrolein block thioredoxin reductase and kill malarial parasite *in vitro* [195]. Glutathione redox system is functional in malarial parasite and glutathione buffers the system and also acts as cofactor for glutathione S-transferase and glutaredoxin. *Plasmodium* has single Glutathione S -transferase isozyme that brings about the nucleophilic addition of glutathione to a variety of hydrophobic substances into non-toxic compounds. It is considered a promising target owing to its high abundance and considerable structural differences from the host enzyme [196]. It will be interesting to identify inhibitors of *Plasmodium* GST as it is presumed to intensify the effect of drugs that impede haeme polymerization in blood. Apart from cytosolic redox system, mitochondrial antioxidant system is also functional in *Plasmodium*.

TARGETING THE SHIKIMATE PATHWAY

The shikimate pathway responsible for biosynthesis of aromatic compounds is indispensable to bacteria, fungi and plants [197] as well as apicomplexan parasites [198-200]. Shikimate pathway proceeds in a sequential manner in 7 steps which culminates in generation of a dihydroaromatic compound chorismic acid, a major branch point in synthesis of many aromatic amino acids, folate, ubiquinone, vitamin K, enterochelin and many secondary metabolites [201]. This reaction is catalyzed by Chorismate synthase enzyme which brings about the conversion of 5-enolpyruvylshikimate- 3-phosphate (EPSP) to chorismate.

Owing to its absence in metazoans, this pathway is of prime importance and offers many promising targets for the development of antimicrobial drugs [202], herbicides [203] and antiparasitic agents. Shikimate pathway has attracted a lot of attention as a source of drug targets due to its crucial role in *Plasmodium* and absence in mammals [198]. The requirement of this biosynthetic pathway in apicomplexan parasites was initially substantiated by isolating *P. falciparum* mutants requiring para-amino benzoic acid for growth [204]. This has also been confirmed by disruption of expression using RNA interference [205-206]. Even though there is volume of sequence information available after the sequencing of *Plasmodium* genome, except Chorismate synthase, genes for other six enzymes of the Shikimate pathway could not be identified conclusively till date. Chorismate synthase thus represent a lucrative target for drug development and has been the subject of many studies in the recent past. Though only Chorismate synthase has

been identified conclusively in *P. falciparum*, activities of other enzymes have also been observed in crude extracts of *P. falciparum* [198,207]. As the 3-dimensional structure of Pf Chorismate synthase is not yet available on PDB, there have been efforts to elucidate its structure [208-209] using homology modeling and the binding efficiencies of potent inhibitors were calculated in nano-molecular ranges [208-209]

Glyphosate(N-phosphonomethyl glycine), a widely used weedicide and analogs of shikimate like 6-S-fluoroshikimate and 6-R-fluoroshikimate are known to interfere with the growth of the malarial parasite *P. falciparum in vitro* [198-199,210]. A successful endeavor at establishing CS as a viable drug target involved a dsRNA encoding a 900 bp fragment of aroC for inhibition of the growth of *P. falciparum* growth which confirmed the essentiality of Chorismate synthase for the normal growth of malarial parasite [211]. Fluorinated analogs of shikimate are also potent inhibitors of *P. falciparum* [199]. These results definitely show promise for use of shikimate pathway inhibitors for treatment of malaria [212]. Other enzymes are being identified and characterized using bioinformatics tools and comparative genomics.

ERYTHROCYTE G PROTEIN AS A NOVEL TARGET

Major symptoms of malaria are associated with the blood stage infection that initiates with the invasion of mature R.B.C with merozoite stage, a phenomenon often considered a gateway to malaria and a source of targets for developing chemotherapeutics for malaria [213-214]. Very little information is available about erythrocytic mechanism of invasion. It is now known that signaling occurs in erythrocytes during this phase and is mediated by host erythrocyte heterotrimeric G protein [215]. Erythrocyte guanine nucleotide regulatory protein Gs are crucial for entry and intracellular parasite proliferation. Thus, G–protein signaling represents a novel anti-malarial drug target [216]. Efficient blocking of malarial action by G-protein antagonists has been reported [215-216]. Propranolol and other second and third generation beta blockers have been suggested for use in combination with other anti-malarial drugs [216].

TARGETING GLYCOLYSIS PATHWAY

A functional Krebs cycle is absent for some stages in *Plasmodium* life cycle [217-218]. Blood-stage *Plasmodium* depends on a constant supply of glucose and glycolysis for ATP generation as main source of energy. *Plasmodium* exhibits very high energy requirement and utilization of glucose in infected cells is hundred fold when compared to normal erythrocytes [219-220]. A major proportion of glucose is converted to lactose while other intermediates generated are diverted to other routes for synthetic purposes. The steps of glycolysis in *Plasmodium* are same as in other organisms and activities of all enzymes in *Plasmodium* have been reported. Glycolytic pathway has 11 enzymes and hexokinase, phosphofructokinase and pyruvate kinase are considered good drug targets being rate limiting in nature [221]. Lactate dehydrogenase (LDH), a choke point in glycolytic pathway which catalyze the conversion of pyruvate into lactate and simultaneously the conversion of NADH to NAD+, is considered a validated drug target. *Plasmodium* LDH has several unique amino acids in the active site and shows structural and kinetic differences with human counterpart. Unique binding site for NADH cofactor owing to sequence changes at the binding pocket raise the possibility for design of molecules capable of effectively inhibiting parasite LDH [222]. Taking advantage of the structural differences, inhibitor molecules aimed at blocking LDH but steering clear of mammalian LDH can be designed and serve as anti-malarials. Azoles and gossypol demonstrate anti-malarial activity [218,223]. An effective synthetic strategy was adopted for synthesis of oxamic acid and ester libraries for screening of lead molecules for developing anti-malarials [224]. Therapeutic effect of 2-deoxy-D-glucose and 2-fluoro-2-deoxy-D-glucose has been reported in malaria [225]. But these molecules need to be examined further before their induction in drug development programme.

MICROTUBULE AS DRUG TARGET

Microtubules are hollow filaments formed by polymerization of alpha and beta tubulin units and show high variation in length and are present in all eukaryotes [226-227]. Microtubules perform an array of important functions in cytokinesis, being principal component of mitotic spindle; determine cell shape and polarity by providing an architectural framework and aid in motility and vesicular transport [228-229]. Microtubules can serve as excellent drug targets owing to their involvement in variety of functions crucial for the survival and growth of organism. Attempts at targeting microtubules for development of anti-parasitic drugs have been successful and have received a lot of attention from researchers across the globe [230]. Thus, microtubules represent excellent targets for blocking various stages of *Plasmodium* and subsequently for rational design and development of anti-malarials [231-232].

Azadirachtin, a limonoid extracted from seeds of Neem tree, inhibits sexual development of malarial parasite, a crucial step in transmission of the parasite to human host [233]. It is yet unclear that what step of exflagellation of microgametogenesis is affected by azadirachtin. It is believed that azadirachtin blocks some microtubule based action [234]. Recently, the scope of inhibiting growth and survival of *Plasmodium* by targeting microtubules has been reviewed [235-236]. Dolastatins and dolastatin based compounds were found to inhibit *P. falciparum* in culture [237]. Other compounds that deserve special mention as microtubule inhibitors for malarial parasite are cochicine, taxol, taxotere, tubulozole, dinitroaniline, some trifluoro-methyl derivatives [238-242]. Despite their high potential therapeutic index, only few microtubule inhibitors have hit the shelves. This can be possibility attributed to off-target effects due to lack of selectivity owing to high conservation among different organisms [238]. Still, there is a ray of hope of effective use of microtubule inhibitors in curbing malaria as a dinitroaniline/ phosphorothioamidate-binding site of *P. falciparum* tubulin is not conserved in human and is projected as plausible drug target [188, 238].

HELICASES AS DRUG AND THERAPEUTIC TARGET

Spread of multi-drug resistant *Plasmodium* strains has warranted the search of novel drugs and consequently new safe and effective drugs. Reliable flow of genetic information from one generation to other relies upon many molecular processes which requires unwinding of double helical structure to generate a single stranded template. Invariable presence and conservation of helicases in all living forms underscores their significance in various aspects of nucleic acid metabolism like replication, recombination and repair, transcription, ribosome biogenesis and RNA processing, translation, and decay [243-248]. Often called 'motor proteins', helicases are associated with cellular macromolecular machinery in which they work [249-250]. Helicases belong to a class of enzymes which catalyze the transient opening in nucleic acid duplexes viz. DNA–DNA, RNA–DNA or RNA–RNA in an NTP-dependent manner by disrupting hydrogen bonds that holds two complementary strands together [246,249, 251]. Helicases demonstrate a directional bias with few reported exceptions [252-253]. It is now known that these helicases are characterized by the presence of 7-9 highly conserved motifs or signature sequences known as Q, I, Ia, Ib, II, III, IV, V and VI [254-257]. This observation led to further classification of these enzymes in further categories viz SF1, SF2 and SF3 super-families based on similarity and occurrence of characteristic signature motifs [249,256,258-260]. The core region containing these signature motifs is flanked by divergent carboxy terminal and amino acid sequences that show considerable variation both in length and sequence [261-263] which confer the ability to perform diverse functions like substrate specificity [264]. Owing to presence of various functional domains and enzymatic action and their crucial role in nucleic acid metabolism, helicases are propounded as attractive drug targets [246]. Some helicase genes have been reported in *P. falciparum* and *P. cytomolgi* [265]. As many as 22 helicases have been identified in *P. falciparum* genome using traditional biochemical approaches as well as bioinformatics analysis [261, 266-267]. Any compound that can interrupt any of three key steps of helicase reaction i.e. nucleic acid binding, ATP binding and hydrolysis can be considered as potential inhibitor [249]. Cisplastin, 4′6′-diamidino-2-phenylindole, daunorubicin and nogalamycin were reported to inhibit helicase activity in *P. cytomolgi* [268]. In a recent study, daunorubicin and nogalamycin were found to effectively inhibit helicase activity and hence, growth of *P. falciparum* in culture [268-269]. This raises a possibility of identification of an effective lead compound that can inhibit helicase activity of malarial parasite without affecting host in near future.

As *Plasmodium* genome is highly rich in AT compared to human, it is assumed that several DNA targeting enzymes that specifically target *Plasmodium* DNA and introduce deleterious changes will be effective in curbing the infection. But this approach has met with limited success due to problem of cytotoxicity. Encouraged with preliminary studies regarding use of Zinc finger nuclease (ZFN) with engineered DNA recognition motifs in various cases like HIV and Hepatitis [270-271], it is hypothesized that such ZFN combined with Cell-penetrating peptides(CPP) can be effective against *Plasmodium* [272]. This approach offers several advantages as it can exploit difference in sequences among structurally and functionally conserved enzymes of human and *Plasmodium* protein and ZFN can be designed against target sites conserved across different *Plasmodium* species. But this optimistic approach needs to be tested and validated experimentally for realizing its true potential.

CONCLUSION

In the present time, drugs like chloroquine and SP which were considered the mainstay in treatment of malaria have been rendered ineffective in wake of emergence of multi-drug resistant *Plasmodium* species and the situation gets worsened by unavailability of affordable alternatives. Evolution of drug resistance towards multitude of existing conventional anti-malarial drugs has necessitated exploration of new drugs to treat malaria. The spread of resistance to current anti-malarial drugs has spurred a new era in research efforts for development of new medicines with novel mechanism of action for control of malaria. Availability of genome sequence of *P. falciparum* has ushered a new era of knowledge and led to identification of numerous potential target pathways. Such information in conjunction with the genome information of host will provide a platform for gaining a better understanding of biochemical properties and functions of metabolic enzymes of *Plasmodium* and will aid in prioritization of targets for therapeutic intervention and fostering the development of inhibitors specifically targeting malarial parasite. It is anticipated that this emerging knowledge will provide information for development of new lead molecules for the putative targets. Enzymes that are distinct from their human homologues and can be examined against available small molecule libraries seem to be promising drug targets. The growing body of structural results provides insights about the reaction mechanism and offer tantalizing opportunity for rational drug design. With the increase in funding in drug discovery and research efforts, it is anticipated that an information base of targets and drugs aimed at curbing malaria can be generated in the foreseeable future.

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