Drug Targets for *Plasmodium falciparum*: A Post-Genomic Review/Survey

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Abstract: Over 300 million cases of malaria each year cause significant morbidity and mortality. Growing drug-resistance among the Plasmodia that cause malaria motivates the development of additional anti-malarial drugs. This review summarizes the current state of knowledge about potential drug targets for malaria. The recently sequenced malaria genome data clarifies parasite metabolic pathways, and more metabolic targets have been identified.

Keywords: Malaria, drug targets, plasmodium, metabolism, genomics.

With significant human suffering caused by malarial disease and growing resistance to current chemotherapies [1], the search for new methods of combating disease is an urgent one. The search for an effective vaccine is ongoing. However, as hopeful as the quest for a vaccine appears [2], there is still a need for new antimalarial drugs. Resistance to chloroquine, mefloquine, and sulfadoxine/pyrimethamine is widespread, and regional resistance exists for many other antimalarials. With malaria infection remaining a wide-spread problem, drug resistance is expected to increase.

One of the steps in antimalarial drug development is the identification of drug targets in plasmodium. There have been several excellent reviews of malaria drug targets [3-5], supported both by direct experimental evidence and our understanding of malaria biology. In humans, malaria is caused by four species of plasmodia, and the most virulent disease is caused by *Plasmodium falciparum*. The plasmodia are eukaryotic organisms that have complex lifecycle stages. The lifecycle in the human host stages are those that are of importance in the development of human vaccines and antimalarials. For antimalarial development the intraerythrocytic stages are of particular interest as they are the only stages that cause clinical symptoms. The liver stages are targeted by primaquine, which cures recurring malarias. When an infected anopheles mosquito bites a human host, sporozoites are introduced into the bloodstream, and invade hepatocytes in the liver. The sporozoite matures into a schizont in the liver, lyses the hepatocyte, and releases thousands of merozoites into the bloodstream. The merozoites invade erythrocytes, and develop into gametocytes (sexual reproduction) or trophozoites (asexual reproduction). Gametocytes are ingested by a feeding anopheles mosquito. Trophozoites start as ring stage trophozoites and then mature into schizonts within the erythrocyte, which complete the asexual cycle by developing into merozoites.

The genome sequence of *Plasmodium falciparum* 3D7 [6-8] has contributed to our understanding of malaria biology. The sequence and its analysis are publicly available at PlasmoDB: http://plasmodb.org [9, 10]. The genome data has been used to guide direct experiments which biochemically characterize the parasite and its metabolic pathways. In addition, analysis of the genome has in many cases identified potential components of proposed metabolic pathways. An expert view of malaria metabolism is available at the Malaria Parasite Metabolic Pathways website: http://sites.huji.ac.il/malaria/. The genome sequence has enabled attempts to describe the entire metabolism of the organism, and searching for metabolic drug targets based on pathway structure [11].

Once a protein is identified as a possible drug target, verification of the target requires additional information. A good drug target is expressed during the relevant lifecycle stages of the parasite, and its activity in these stages must be essential to the parasite's survival or replication. Furthermore, proteins with essential functions can fail as drug targets if the host requires the function of a similar protein and selective inhibition is not feasible or if the bioavailability, pharmacodynamics or pharmacokinetics of the drug is not suitable. In this review, we catalog the various protein drug targets of *P. falciparum* and discuss the evidence that supports their status as proposed targets.

To better understand the criteria for successful drug targets, we examine what is known about the mechanisms of existing antimalarials. The three main classes of antimalarials in use are the quinolines**,** artemisinin-type compounds, and antifolates. In addition, the hydroxynapthoquinones have yielded one antimalarial drug, atovaquone. Also, fosmidomycin is a relatively new experimental drug that targets isoprenoid biosynthesis.

CURRENT ANTIMALARIALS AND THEIR MECHANISMS OF ACTION

The bark of the cinchona tree was used to treat malarial fever centuries ago, and the active agent, quinine, was isolated in 1820 [12]. Chloroquine, a 4-aminoquinoline derivative of quinine, was widely used due to its safety, efficacy, and low cost before the development of widespread resistance. Chloroquine accumulates in the food vacuole of the parasite and is thought to act through interference with hemoglobin digestion, although its exact molecular mechanism is still a matter of debate. One proposed

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hypothesis for how the parasite is killed by chloroquine that it binds to ferriprotoporphyrin IX, a toxic product of hemoglobin digestion, and causes its build-up in the parasite [13]. This is not thought to be the mechanism of quinine or mefloquine, which are lipophilic and thought to act by an alternative mechanism, perhaps through disruption of phospholipid metabolism [14] or through inhibition of endocytosis [15]. Primaquine is an 8-aminoquinolone, which eradicates the late hepatic stages of *P. vivax* and *P. ovale* (hypnozoites and schizonts). Primaquine also kills gametocytes, preventing transmission of malaria [16]. Artemisinin (also known as qinghaosu) is an extract of sweet wormwood (*Artemisia annua*), a Chinese medicinal plant, which was used for centuries to treat malarial fevers. Artemisinin has good activity against all asexual stages of *P. falciparum* [17]. The artemisinins as a class are widely used throughout Southeast Asia and Africa to treat multi-drug-resistant malaria. The prevailing theory of how the artemisinins work is that ferrous iron in the heme stores of the parasite cleave the peroxide ring of the drug which generates free radicals and increases oxidative stress [18]. This theory is challenged by recent work which suggests artemisinin acts through its inhibition of PfATP6, a sarco/endoplasmic reticulum CA^{2+}-ATPase (SERCA) in *P. falciparum* [19].

The dye methylene blue was noted to have anti-malarial activity in the late 19th century by Paul Erlich [20]. Methylene blue acts by interfering with hemoglobin metabolism as well as by selectively inhibiting plasmodial glutathione reductase, but has only been used sporadically as an anti-malarial, its major use is for septic shock and methemoglobinemia [21].

The antifolates target two enzymes in the folate biosynthesis pathway: dihydropteroate synthase (DHPS; EC 2.5.1.15), which is inhibited by the sulfone/sulfonamide drugs, and dihydrofolate reductase (DHFR; EC 1.5.1.3), the target of drugs such as pyrimethamine and proguanil [22]. In *P. falciparum* both enzymes are expressed throughout the asexual life cycle of the parasite [23]. The antifolates act synergistically, as the inhibition of DHFR and DHPS together has a greater than additive effect.

Atovaquone is a hydroxynapthoquinone, a ubiquinone analog that binds to the ubiquinol-cytochrome C reductase (EC 1.10.2.2) active site of cytochrome bc_1 and blocks mitochondrial respiration [24]. Initial clinical studies showed a high failure rate and rapid development of resistance [25]. However, proguanil acts synergistically with atovaquone and the combination (Malarone) is effective both for treatment and prophylaxis of malaria. Therefore, even drugs that have a low antimalarial effect by themselves have potential for clinical usefulness in combination with other therapies, and such combination therapies slow the development of resistance.

Fosmidomycin is a natural antibiotic that inhibits 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR; EC 1.1.1.267), which is part of the non-melavonate isoprenoid biosynthesis pathway, derived from plants and not found in humans [26]. Inhibition of this enzyme blocks parasite synthesis of isopentenyl diphosphate [27]. Before trials in humans, this drug was found to have activity against the parasite both *in vitro* and in the mouse [27]. Although fosmidomycin has proven efficacy in clinical trials, patients experience recrudescence [28]. However, clinical studies of combination therapies are promising [29]. Studies have been undertaken to develop more effective inhibitors of DXR and compounds have been identified that are more effective against malaria in the mouse [30, 31].

MECHANISMS OF RESISTANCE

The mechanisms of drug resistance have been reviewed [32]. Understanding the causes of resistance can further elucidate mechanisms of drug action and suggest targets for drugs to overcome resistance.

Two genes have been identified that are involved in chloroquine resistance. The *P. falciparum* chloroquine-resistance transporter (Pfcrt) is localized to the digestive vacuole membrane, and five to eight mutations at ten different positions confer resistance [33]. Pfcrt is thought to reduce chloroquine levels in the digestive vacuole [34]. The Pfcrt K76T polymorphism is necessary and sufficient for chloroquine resistance [34]. *P. falciparum* multidrug-resistance 1 (Pfmdr1) is a member of the ABC family of transporters, and mutations in this gene alone do not confer resistance, but both point mutations and gene amplification of Pfmdr1 increase chloroquine resistance in the presence of resistance-conferring alleles of Pfcrt [35]. Pfmdr1 copy number was found to be the most important factor for resistance to mefloquine [36]. Chloroquine resistance is increased by increased levels of glutathione in the parasite [37].

Point mutations in the target enzymes DHPS and DHFR are the major cause of resistance to antifolates. The highest levels of clinical resistance to combination therapies such as pyrimethamine/sulfadoxine, are associated with an allele with four mutations in DHFR (which map to the active site) and two mutations in DHPS [38, 39].

Resistance to atovaquone arises from point mutations in cytochrome bc_1 in the region of ubiquinol oxidation. Mutations in cytochrome bc_1 occur rapidly in the presence of atovaquone because disruption of the electron transport chain increases oxidative stress within mitochondria. This oxidative stress increases the mutation rate of mitochondrial genes, including the cytochrome bc_1 gene [32, 40].

PROPOSED DRUG TARGETS

We discuss drug targets by broad biological functions and emphasize the evidence for each drug target. In order to make new drugs available as quickly as possible, resources should be focused in a coordinated fashion on the most promising drug targets, in some cases even sacrificing the parallel goal of understanding parasite biology. Evidence for the drug targets is summarized in Table **1**.

ENERGY METABOLISM

Within the erythrocyte, the parasite grows and replicates rapidly. Glucose metabolism in *P. falciparum* infected erythrocytes increases more than 50-fold over non-infected erythrocytes, and the parasite depends mainly on glycolysis for energy production [41]. A functional electron transport chain exists in the parasite [42]. Inhibition of the energy

Table 1. Summary of Drug Targets

	EC number	Gene ID	selective inhibition	inhibitors	effect in culture	effect in animal models	effect in humans	Ref.
Energy Metabolism								
aldolase	4.1.2.13	PF14_0425	yes	antisense oligonucleotides	decreases growth			[47]
glucose-6-phosphate isomerase	5.3.1.9	PF14_0341	yes	antiserum				[45]
hexose transporter		PFB210c	yes	O-3-hexose derivatives	kills parasite	antimalarial activity in mice		[43,44]
lactate dehydrogenase	1.1.1.27	PF13_0141	yes	azole-based inhibitors	kills parasite			[51 52]
Mitochondrial electron transport								
NADH dehydrogenase	1.6.5.3	PFI0735c		rotenone	decreases growth			[53]
succinate dehydrogenase	1.3.5.1	PF10_0334 PFL0630w		fumarate, 5-hydroxy-2-methyl-1,4-napthoquinone, antisense oligonucleotides	decreases growth			[54]
ubiquinol-cytochrome c reductase	1.10.2.2	PF14_0248 PF14_0373	yes	atovaquone	yes	antimalarial activity in mice	effective in combination with proguanil	[24]
Cofactor and Prosthetic Group Synthesis								
Folate metabolism								
dihydrofolate reductase	1.5.1.3	PFD0830w	yes	pyrimethamine	yes	antimalarial activity in mice	treats	[22]
dihydropteroate synthase	2.5.1.15	PF08_0095	yes	sulfonamides	yes	antimalarial activity in mice	treats	[22]
de novo heme synthesis								
delta-aminolevulinate synthase	2.3.1.37	PFL2210w	yes	ethanolamine	decreases growth			[61]
Protein Modification								
peptide deformylase	3.5.1.88	PFI0380c		peptide thiol inhibitors	decreases growth			[76]
protein farnesyltransferase		PF11_0483		peptidomimetics	decreases growth	antimalarial activity in mice		[74,75]
GPI Biosynthesis				mannosamine	inhibits trophozoite growth			
N-acetyl glucosaminylphosphatidylinositol deacetylase	3.5.1.89	PFF1190c	yes	suicide inhibitors				[67]
isoprenoid synthesis								
1-deoxy-D-xylulose-5-phosphate reductoisomerase	1.1.1.267	PF14_0641	no human homolog	fosmidomycin, 4-(hydroxyamino)-4-oxobutylphosphonic acid, 4-[hydroxy(methyl)amino]-4-oxobutyl phosphonic acid	decreases growth	antimalarial activity in mice	effective, but recrudescence occurs	[27]

(Table 1) contd…..

	EC number	Gene ID	selective inhibition	inhibitors	effect in culture	effect in animal models	effect in humans	Ref.
polyamine metabolism				agmatine (inhibits HSS in plants)	decreases parasitemia			
ornithine decarboxylase	4.1.1.17	PF10_0322		bis(benzyl)polyamine analogs	arrests schizogeny	cures malaria in mice		[69]
S-adenosylmethionine decarboxylase	4.1.1.50	PF10_0322		MDL 73811	decreases growth			[70]
Lipid Metabolism								
phosphatidylcholine synthesis				TE3		TE3 cures malaria in rhesus monkeys		[81,84]
choline carrier		PFL0620c	yes	bisquaternary ammonium compounds				[83]
phosphoethanolamine methyltransferase	2.1.1.17	MAL13P1.214		miltefosine	decreases growth			[85]
Type II fatty acid synthesis								
3-oxoacyl-ACP synthase I,II, III	2.3.1.41	PFF1275c		thiolactomycin, cerulenin and related compounds	decreases growth			[89,90]
3-hydroxyacyl-ACP dehydratase		PF13_0128		NAS-91, NAS-21	decreases growth			[91]
acetyl-CoA-carboxylase	6.4.1.2	PF14_0664		fenoxaprop, tralkoxydim, diclofop	decreases growth			[88]
enoyl-ACP-reductase	1.3.1.9	PFF0730c		triclosan	decreases growth			[92,93]
Lipases								
phospholipase B	3.1.1.5	PF14_0017 PF14_0737 PF14_0738		sulfhydryl agents	growth arrest, decreased invasion			[94]
phospholipase C	3.1.4.3	PF10_0132		scyphostatin	decreases growth, morphological changes			
sphingomyelinase	3.1.4.12	PFL1870c		scyphostatin	decreases growth			[95,96]
surface phospholipase		PFF1420w		gene disruption in *P. berghei*		decreased hepatic infectivity in mice		[97]
DNA Replication and Transcription								
purine salvage								
adenosine deaminase	3.5.4.4	PF10_0289		L-nucleoside analogs				[105]
GMP synthetase	6.3.5.2	PF10_0123		psicofuranine	interrupts growth			[101]
hypoxanthine guanine phosphoribosyltransferase	2.4.2.8	PF10_0121	yes	antisense oligonucleotides	kills parasite			[108,109]

(Table 1) contd…..

	EC number	Gene ID	selective inhibition	inhibitors	effect in culture	effect in animal models	effect in humans	Ref.
IMP dehydrogenase	1.1.1.205	PFI1020c		bredinin	arrests trophozoite growth			[110]
purine nucleoside phosphorylase	2.4.2.1	PFE0660c	yes	immucillins	kills parasite			[106]
de novo pyrimidine synthesis								
carbamoyl phosphate synthetase	6.3.5.5	PF13_0044	yes	ribozymes	reduces viability in culture			[112]
carbonic anhydrase	4.2.1.1	not identified		acetazolamide, sulfanilamide	decreases growth			[111]
dihydroorotase	3.5.2.3	PF14_0697		6-L-thiodihydroorotate	decreases growth			[113]
dihydroorotate oxidase	1.3.3.1	PFF0160c	yes	5-fluoroorotate, RNAI	decreases growth			[114,115]
orotidine-5'-phosphate decarboxylase	4.1.1.23	PF10_0225		pyrazofurin	decreases growth			[116]
thymidylate synthase	2.1.1.45	PFD0830w		folate based inhibitors	decreases growth			[117]
additional enzymes								
adenosylhomocysteinase	3.3.1.1	PFE1050w		3-deaza-(+/-) aristeromycin	decreases growth			[126,127]
histone deacetylase		PFI1260c PF14_0690		apicidin	decreases growth	reduces *P. berghei* parasitemia in mice		[119,120]
ribonucleotide reductase	1.17.4.1	PF10_0154 PF14_0053	yes	antisense oligonucleotides	decreases growth			[118]
telomerase		PF13_0080		berberine, pyrimidine nucleoside/nucleotide analogs				[121,122]
topoisomerase I	5.99.1.2	PFE0520c		camptothecin	kills parasite			[123]
topoisomerase II	5.99.1.3	PF14_0316		antisense oligonucleotides	decreases growth			[124,125]
Hemoglobin Digestion								
dipeptidyl aminopeptidase 1	3.4.14.1	PF11_0174		gene disruption	lethal			[147]
falcipain-2		PF11_0161 PF11_0165		gene disruption	abnormal morphology of food vacuoles, but normal development			[137]
histo-aspartic protease		PFC0495w		pepstatin A, PMSF				[143]
leucine aminopeptidase	3.4.11.1	PF14_0439		bestatin, nitrobestatin, but inhibitors also inhibit M1-family aminopeptidase	decreases growth			[144]

(Table 1) contd…..

	EC number	Gene ID	selective inhibition	inhibitors	effect in culture	effect in animal models	effect in humans	Ref.
M1-family aminopeptidase		MAL13P1.56		quinolone based inhibitors	decreases growth			[146]
plasmepsin I and II	3.4.23.38 3.4.23.39	PF14_0076 PF14_0077		hydroxyethylamine transition state scaffold based	decreases growth			[138,139]
Antioxidant Defense								
γ-glutamylcysteine synthetase	6.3.2.2	PFI0925w		D,L-buthionine-S,R-sulphoxime	kills parasite			[164,165]
glutathione reductase	1.8.1.7	PF14_0192		5-substituted tetrazoles	decreases growth			[161]
glyoxalase I	4.4.1.5	PFF0230c PF11_0145		S-p-bromobenzylglutathione diethyl ester	induces toxicity			[166]
superoxide dismutase	1.15.1.1	PFF1130c PF08_0071		chalcones and piperazine derivatives (AB93)	decreases growth			[153]
thioredoxin reductase	1.8.1.9	PFI1170c		gene disruption	lethal			[156]
Miscellaneous								
SERA4		PFB0345c		gene disruption	lethal			[172]
SERA5		PFB0340c		gene disruption	lethal			[172]
SERA6		PFB0335c		gene disruption	lethal			[172]
subtilisin 1, 2		PFE0370c PF11_0381		gene disruption	lethal			[167,170]

generating mechanisms of the parasite should at the very least slow its growth and perhaps kill the parasite. The challenge to inhibiting the parasite's energy metabolism pathways is doing so without adverse effects on the patient as these pathways are conserved in humans.

One method of attacking such pathways is to target the point of entry substrates into the parasite. Glucose entry into the parasite occurs through a parasite specific hexose transporter. O-3-hexose derivatives selectively inhibit this transporter, resulting in intracellular pH changes, death of *P. falciparum in vitro* [43] and reduction of *P. berghei* multiplication in mouse [44]. Other methods of selectively inhibiting conserved pathways focus on the areas where the most difference occurs. Malarial glucose-6-phosphate isomerase (EC 5.3.1.9) is inhibited by mouse antiserum, with no effect on the human enzyme [45]. Antisense oligonucleotides targeting *P. falciparum* aldolase (EC 4.1.2.13) mRNA near the translation initiation site (different from the human version) reduce aldolase mRNA and activity and parasite growth *in vitro* [46]. Aldolase also plays a role in host cell invasion by acting with C-terminal domains of the thrombospondin-related anonymous protein family of adhesins, and may function in parasite motility and its metabolic regulation [47]. Triose-phosphate isomerase (EC 5.3.1.1) is a homodimer and individual subunits are not enzymatically active [48]. In *P. falciparum* the homodimer interface contains a methionine to cysteine substitution relative to the human enzyme, and modification of the cysteine in this interface results in decreased activity [49]. This suggests that selective inhibition of this enzyme can be achieved with parasite specific blockade of dimerization. Parasite lactate dehydrogenase (LDH; EC 1.1.1.27) has structural and activity differences from human LDHs [50] and azole-based selective inhibitors of parasite LDH kill the parasite *in vitro* [51, 52].

It is unclear if its main purpose of the malarial electron transport chain is energy production, pyrimidine biosynthesis (through oxidation of dihydroorotate), or to serve as an electron sink. In addition to atovaquone, other inhibitors of the parasite's mitochondrial electron transport chain display antimalarial effects. Rotenone blocks mitochondrial NADH dehydrogenase (EC 1.6.5.3) and has

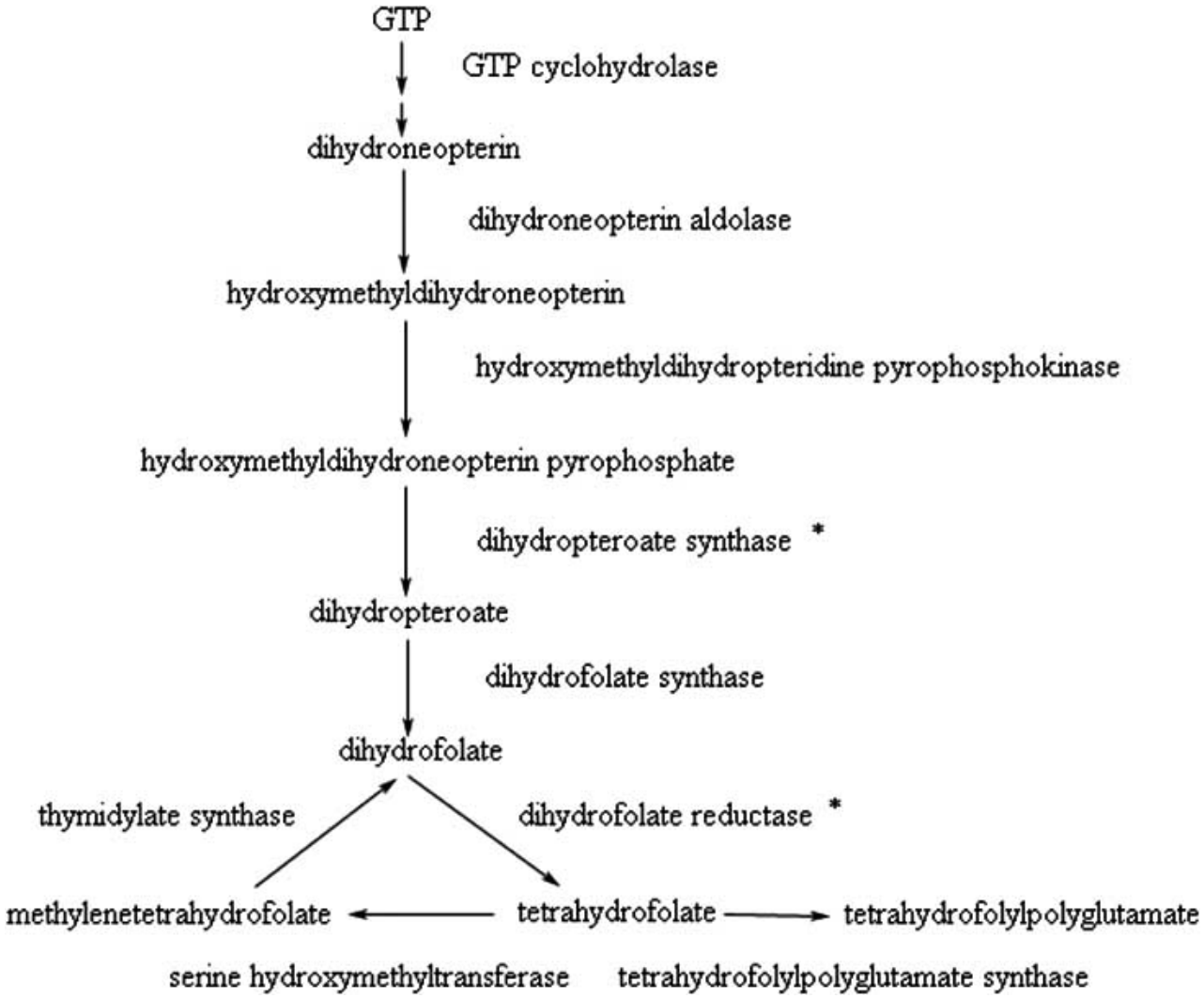


Fig. (1). The folate biosynthesis pathway. Targets of the antifolates are starred. Adapted from the Malaria Parasite Metabolic Pathways.

antimalarial effects *in vitro*. While differential inhibition of parasite NADH dehydrogenase has not been demonstrated, the enzyme has unique properties that may be exploited for parasite specific inhibition [53]. Succinate dehydrogenase inhibitors oxaloacetate, 5-hydroxy-2-methyl-1,4-napthoquinone and antisense oligonucleotides slow *in vitro* growth of *P. falciparum*. Parasite succinate dehydrogenase is not inhibited by an inhibitor of its human homolog indicating that the two homologs have significant structural differences [54].

LDH appears to be the most promising in the energy metabolism group of targets because selective inhibitors of LDH kill the parasite *in vitro*, and can be used as lead compounds in further drug development. The next step in bringing LDH inhibitors closer to use in humans would be screening existing compounds for human safety and bioavailability and further development of the most promising lead compounds if necessary.

COENZYME AND PROSTHETIC GROUP METABOLISM

The parasite obtains cofactors and prosthetic groups by uptake from its environment as well as biosynthesis. Many metabolic functions cannot proceed when necessary cofactors and prosthetic groups are unavailable. In order to increase accessibility of nutrients in its environment, the parasite induces changes in the permeability of the plasma membrane and parasitophorous vacuole of the erythrocyte. This "new permeation pathway" is the subject of active investigation, and has been recently reviewed [55]. Inhibition of this pathway can potentially block essential nutrients from reaching the parasite.

The folate synthesis pathway is a cofactor biosynthesis pathway that is a focus of antimicrobial drug development (Fig. **1**). Because humans lack the ability to synthesize folate de novo and depend on folate from their diet, inhibition of the folate pathway is not expected to have serious side effects. The folate biosynthesis enzymes have all been identified except for dihydroneopterin aldolase (DHNA; EC 4.1.2.25), which is expected to be difficult to find in the genome due to poor conservation among DHNA genes in other organisms [56]. Both malaria parasites and humans have a GTP cyclohydrolase (used for biopterin synthesis in humans) [57]. While the *P. falciparum* enzyme has near identity to the human enzyme around its active site, its N-terminus shows great divergence which may mediate parasite specific functions that can be selectively inhibited [56].

Although erythrocytic parasites have access to large amounts of free heme released from digested host cell hemoglobin, the parasite synthesizes heme *de novo* [58]. Inhibition of heme synthesis leads to parasite death [59]. Careful characterization of each enzyme in heme synthesis must be carried out before proposing it as a drug target, as the parasite is capable of importing at least one host enzyme, δ-aminolevulinate dehydratase (ALAD) for heme synthesis [59], expressing its own ALAD at low levels [60]. However, malarial δ-aminolevulinate synthase (ALAS) was shown to be the only ALAS present in the erythrocytic parasite [61]. Ethanolamine, a selective inhibitor of parasite ALAS slows *P. falciparum* growth in culture [61]. The interplay between human and parasite heme synthesis pathways makes deciphering the role of the parasite enzyme more difficult, but on the positive side, the process of host protein import may yield drug targets once characterized.

P. falciparum depends on external sources of pantothenate, a precursor of coenzymeA (CoA) and the new permeation pathways make the erythrocyte membranes permeable to pantothenate. Once within the parasitophorous vacuole, pantothenate enters the parasite through an H+-coupled transporter, which is different from the Na+-coupled transporters for pantothenate in humans [62]. Thus this H+-coupled transporter has been proposed as a drug target since there is a good chance that selective inhibition of the transporter would choke off the parasite's source of pantothenate, without much effect on the host.

The process of drug development is not very far advanced for proposed drug target of cofactor and prosthetic group synthesis pathways. While successful drugs target folate synthesis enzymes, inhibitors of the other enzymes in the pathway have not yet been identified. Inhibition of ALAS adversely affects the parasite, but parasite heme synthesis in the human lifecycle stages and the role of human enzymes is not well understood. The parasite pantothenate transporter presents a logically attractive target, but it has not yet been proven to be the only mechanism of pantothenate entry into the parasite. Finding inhibitors of other folate synthesis enzymes would be the most useful as these compounds would have activity against other microbes.

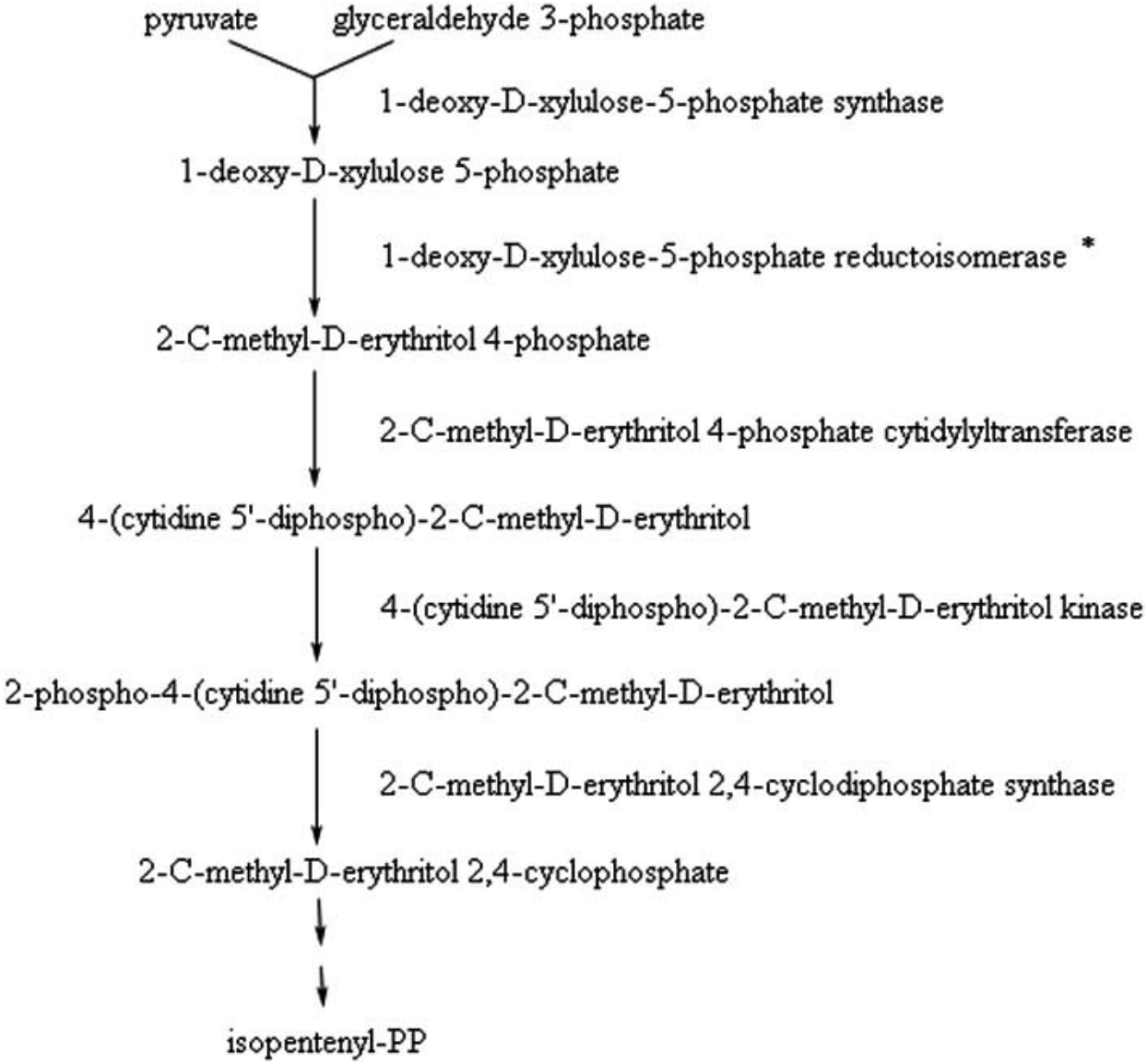


Fig. (2). Isoprenoid Synthesis Pathway. The target of fosmidomycin is starred**.** Adapted from the Malaria Parasite Metabolic Pathways.

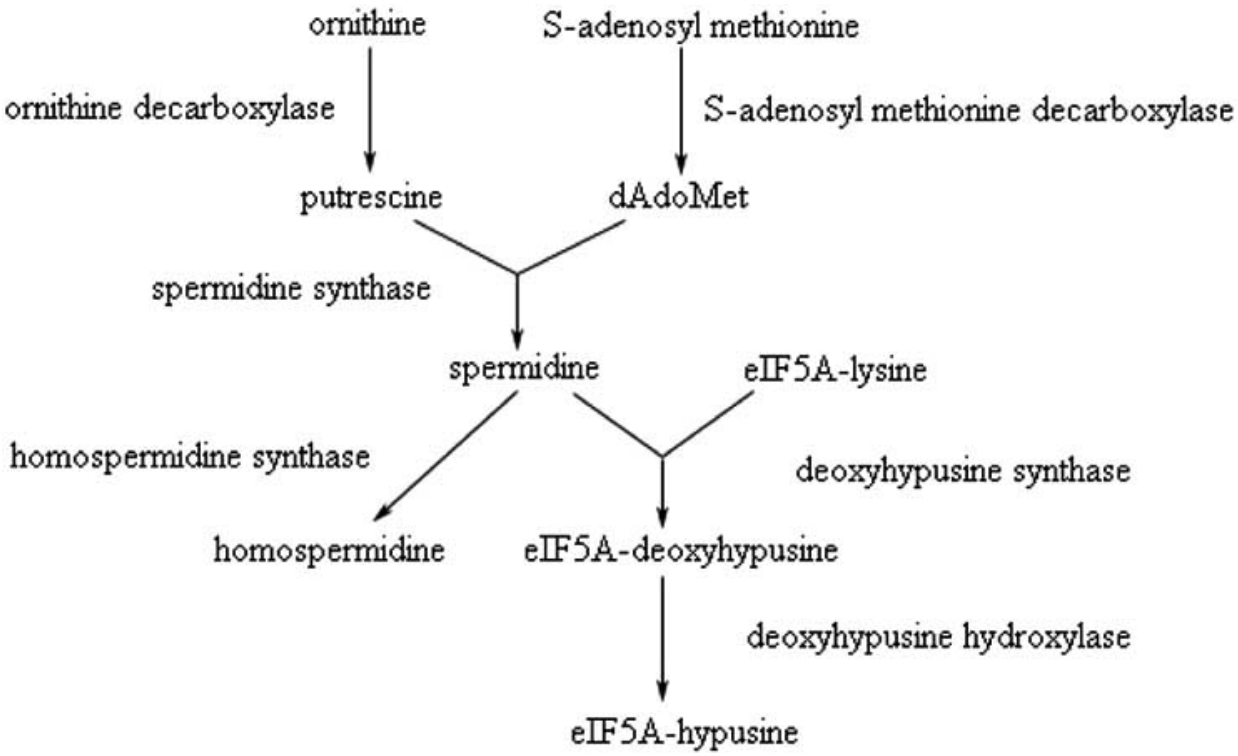


Fig. (3). Spermidine and hypusine synthesis. Adapted from the Malaria Parasite Metabolic Pathways.

PROTEIN MODIFICATION

Post-translational protein modifications are often critical to a protein's function. The covalent attachment of glycosylphosphatidylinositol (GPI) membrane anchors and prenyl groups is necessary for large groups of proteins to function appropriately. Therefore the enzymes involved in these processes may make good drug targets. Other protein modifications may modify specific proteins, but still be crucial to the survival of the cell. Such is the case for spermine dependent synthesis of the modified amino acid hypusine in eukaryotic translation initiation factor (eIF-5A) [63]. Studies in yeast and mammalian cells show that functional eIF-5A is essential to cell proliferation, without it cells arrest at the G1-S boundary of the cell cycle [63]. As eIF-5A requires hypusine for its function, preventing hypusine synthesis should have a detrimental effect on the parasite [63].

Many proteins localized to the parasite's surface contain GPI membrane anchors, and many of these proteins participate in important functions such as host cell invasion [64]. GPIs are glycolipid structures synthesized through multiple enzymatic steps in the endoplasmic reticulum membrane. Free GPIs can act as pathogenic factors, for example by inducing inflammatory cytokines [65]. GPI synthesis in the parasite occurs in maturing trophozoites, and inhibition of GPI synthesis with mannosamine inhibits growth at the trophozoite stage [66]. In the parasite, mannosamine interferes with adding mannose to a phosphoinositol intermediate, which is different from its mechanism of inhibition in other organisms, suggesting parasite specific enzymatic activities [66]. *Plasmodium* specific suicide substrate inhibitors of N-acetylglucosaminylphosphoinositol deacetylase (EC 3.5.1.89), may be useful as lead compounds for drugs that target parasite GPI synthesis [67].

Isoprenoid synthesis in plasmodium has been proposed as a drug target for several reasons, including the fact that it is targeted by fosmidomycin [30]. In the parasite isoprenoid synthesis proceeds through a methylerythritol phosphate (MEP) intermediate as in plants, but not in humans (Fig. **2**). Isoprenoid synthesis produces isopentenyl diphosphate, which is needed for the synthesis of prenyl groups. All enzymes of the MEP pathway of isoprenoid synthesis have been identified in *P. falciparum*, except for 2-C-methyl-D-erythritol-4-phosphate cytidyltransferase, and all the intermediates of the pathway have been identified in the erythrocytic parasite, indicating a complete pathway exists in the parasite [68].

The function of spermidine in the cell is not well understood, but spermidine is a substrate for hypusine synthesis (Fig. **3**). Spermidine biosynthesis in *P. falciparum* is regulated by a bifunctional S-adenosylmethionine decarboxylase (AdoMetDc; EC 4.1.1.50)/ornithine decarboxylase (ODC; EC 4.1.1.17). Inhibitors of ODC arrest schizogony in culture, have moderate *in vivo* antimalarial effects, and cure rodent malaria when given in combination with polyamine analogs [69]. Inhibitors of AdoMetDc slow parasite growth in culture, and this growth inhibition can be partially corrected with supplementation of spermine and spermidine [70]. The bifunctionality of the AdoMetDC/ODC enzyme is unique to plasmodia. The unique hinge region between the two catalytic domains of the protein may represent a unique target for interfering with its enzymatic activities [71]. Once spermidine is synthesized, deoxyhypusuine synthase (DHS; EC 2.5.1.46) transfers an aminobutyl moiety from spermidine to a lysine in eIF-5A [63]. *P. falciparum* eIF-5A has a higher homology to plant

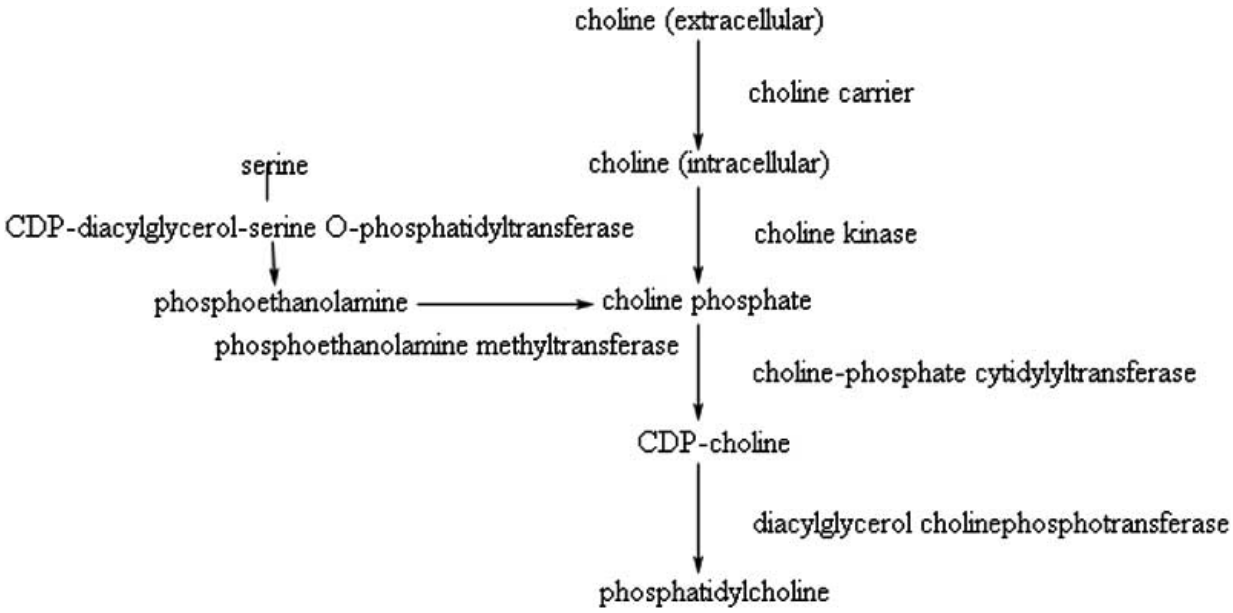


Fig. (4). Phosphatidylcholine synthesis. Adapted from Malaria Parasite Metabolic Pathways.

homologs than to animal or fungal homologs, which indirectly suggests structural differences between human and parasite DHS [72]. Agmatine which inhibits HSS in plants and spermidine metabolism in *P. falciparum*, decreases parasitemia in culture [73].

Protein farnesyltransferase (PFT) transfers farnesyl groups to proteins, and PFT inhibition by peptidomemetics inhibits parasite growth both in culture [74] and in a murine malaria model [75]. Another enzyme which directly modifies proteins is peptide deformylase which removes the N-terminal formyl group from N-terminal formyl-methionines. Proteins synthesized in eukaryotic plastids and mitochondria contain leading formyl-methionines, and PDF has been identified in *P. falciparum* and humans [76]. Inhibitors of PDF slow parasite growth in culture [76]. PDF is currently being investigated as a target for anticancer [77] and antimicrobial [78] drugs.

The drug targets discussed in this section are involved in areas of parasite biology that are not fully understood. Many enzymes in the parasite GPI synthesis pathway remain to be identified and characterized and it is unclear how GPI pathways vary from organism to organism. Therefore it is difficult to infer the pathway in *Plasmodium* from GPI pathways in other organisms [79]. While isoprenoid biosynthesis products are involved in the production of prenyl groups, the pathway may be crucial to additional processes within the parasite. Although eIF-5A-hypusine is required in many organisms, its role in translation is not well understood. One hypothesis is that eIF-5A is involved in translation of specific cell cycle related mRNAs [80]. The most promising drug target involved in protein modification is N-acetylglucosaminylphosphatidylinositol deacetylase, with promising lead compounds identified.

LIPID METABOLISM

The malaria parasite has a high requirement for lipids. With each cell division, the parasite replicates its membranes. The parasitophorous vacuole, in which the erythrocytic parasite is encapsulated, is created from host cell membrane, but its lipid composition is modified by the parasite. In addition to being an integral part of membranes, lipids play roles in growth regulation, differentiation, and apoptosis. The erythrocyte does not synthesize lipids, and thus the parasite synthesizes lipids to meet its metabolic needs as. The lipid composition of an infected erythrocyte changes, with a more than five-fold increase in phospholipids as well as neutral lipids (which include fatty acids) [81]. Thus, disruption of lipid synthesis/absorption may be a potent means of arresting development of the parasite.

Phosphatidylcholine is the most abundant lipid in malaria parasite membranes [82]. The routes of phosphatidylcholine (PC) synthesis in the parasite include de novo synthesis from choline and de novo synthesis of phosphoethanolamine (PE) and its subsequent methylation (Fig. **4**). The pathway through PE is a minor pathway in most human tissues, except the liver, but is expected to contribute significantly to *P. falciparum* PC synthesis. Choline enters the parasitophorous vacuole though the new permeation pathway and enters the parasite through a parasite choline transporter [83]. Orally administered TE3, a bisthioazolium iodide salt and prodrug of T3 (a named drug), cures malaria in rhesus monkeys by inhibiting PC synthesis [81, 84]. These drugs inhibit the parasite choline transporter more than the human choline transporter (which is located in the central nervous system), but the antimalarial activity of the bisquaternary ammonium compounds is thought to be due to interference with another aspect of PC synthesis [83]. Phosphoethanolamine methyltransferase (EC 2.1.1.17) has no human homolog and allows the parasite to synthesize phosphatidylcholine from PE. Inhibition of phosphoethanolamine methyltransferase by miltefosine, a phosphocholine analog, slows growth of *P. falciparum* in culture [85].

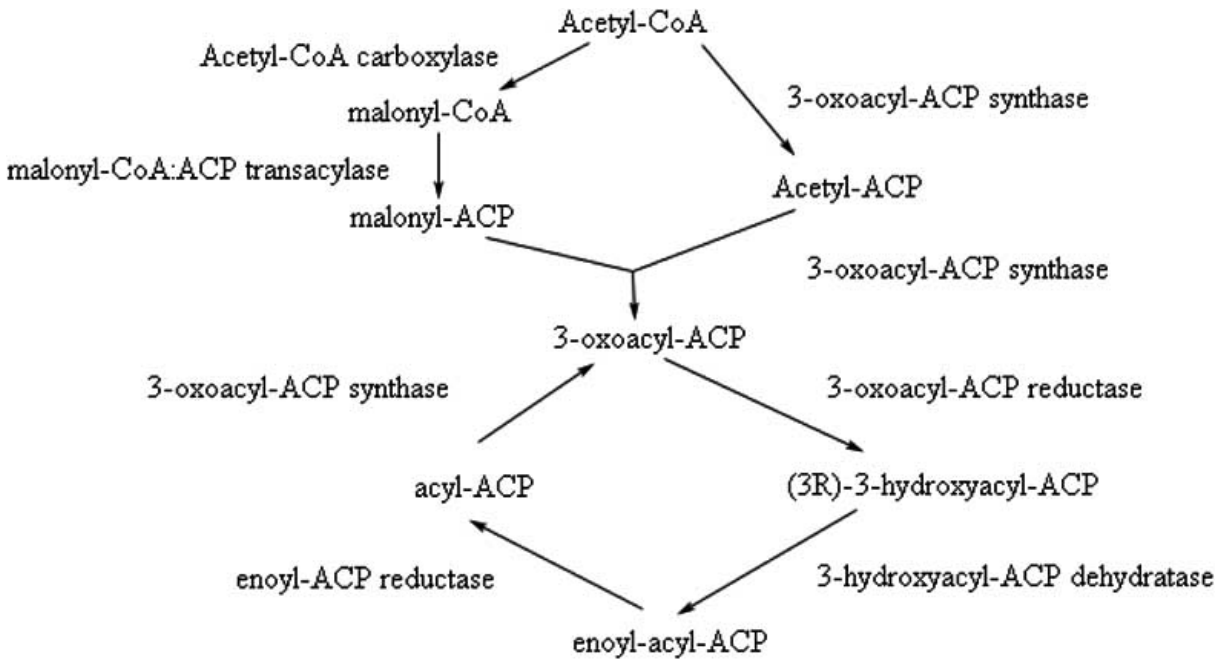


Fig. (5). Type II Fatty Acid Synthesis. Adapted from the Malaria Parasite Metabolic Pathways.

The enzymes of Type I and Type II fatty acid synthesis have major structural differences. Type I synthesis occurs in humans, while Type II occurs in the malaria parasite and in plants [86]. Therefore, the enzymes of Type II fatty acid synthesis may make good drug targets, offset perhaps by parasite import of fatty acids from its environment (Fig. **5**) [87]. Acetyl-CoA carboxylase (EC 6.4.1.2) activity is inhibited by herbicides fenoxaprop, tralkoxydim, and diclofop, which also inhibit *P. falciparum* growth *in vitro* [88]. Thiolactomycin and cerulenin which inhibit 3-oxoacyl-ACP synthase I, II (OAS; EC 2.3.1.41) slow *P. falciparum* growth *in vitro* [89]. Compounds structurally related to thiolactomycin inhibit OAS III and *P. falciparum* growth in culture [90]. Definitive experiments to determine whether inhibition of one specific OAS or some combination exhibits antimalarial activity have not yet been done. 3-hydroxyacyl-ACP dehydratase has been cloned and characterized, and lead compounds with inhibitory activity have been synthesized and shown to inhibit *P. falciparum* growth in culture [91]. Enoyl-ACP-reductase (EC 1.3.1.9) catalyzes the final step of fatty acid elongation and controls the rate of elongation [92]. Its activity is inhibited by triclosan, a broad spectrum antibiotic and antifungal, which shows activity against plasmodium in culture. Triclosan works synergistically with cerulenin [93], strengthening the evidence that these drugs work through the fatty acid synthesis pathway.

Lipases are also good drug targets related to lipid metabolism. Cleavage of lipids is involved in signal transduction and regulation of membrane composition. Lysophospholipase (EC 3.1.1.5) cleaves lyso-phosphatidylcholine and its inhibition by sulfhydryl agents causes growth arrest and decreased erythrocyte invasion in culture [94]. Sphingomyelinase (EC 3.1.4.12) generates ceramide, which modulates proliferation, differentiation, apoptosis [95]. Inhibition of sphingomyelinase by scyphostatin decreases parasite growth in culture and the parasite exhibits morphologic abnormalities in lifecycle stages that correspond with the transcriptional expression pattern of sphingomyelinase [96]. Surface phospholipase (which is most similar to lecithin: cholesterol acyl transferase (EC 2.3.1.43) in humans) has been disrupted in *P. bergei* (*P. falciparum* has a highly similar homolog) resulting in decreased hepatic infectivity and impaired movement across epithelial cell layer [97].

DNA REPLICATION AND TRANSCRIPTION

The parasite undergoes exponential growth during its erythrocytic stages, with high rates of RNA and DNA synthesis. Therefore, the parasite has a high requirement for nucleotides. While humans have salvage and de novo pathways for production of purines and pyrimidines, the parasite only has pathways for purine salvage (Fig. **6**) and *de novo* pyrimidine synthesis (Fig. **7**) [7]. Thus, the parasite should be particularly sensitive to inhibition of nucleotide synthesis based on its replication rate and streamlined metabolism [98-102]. Enzymes that directly regulate and participate in DNA synthesis have been investigated as potential drug targets.

The parasite obtains adenosine and other nucleosides for purine salvage through a nucleoside transporter localized to its plasma membrane [103]. This transporter has significant differences from its human homologs and is not sensitive to inhibitors of its closest human homolog, suggesting that significant structural differences between the parasite and human transporters exist [104]. The parasite transporter allows L-nucleosides to enter the parasite, including an L-nucleoside analog that inhibits *P. falciparum* adenosine deaminase (EC 3.5.4.4) [105]. Through this parasite transporter, toxic L-nucleosides can be selectively administered to *P. falciparum*, side-stepping the problem of differential inhibition of parasite and human target enzymes with differential administration.

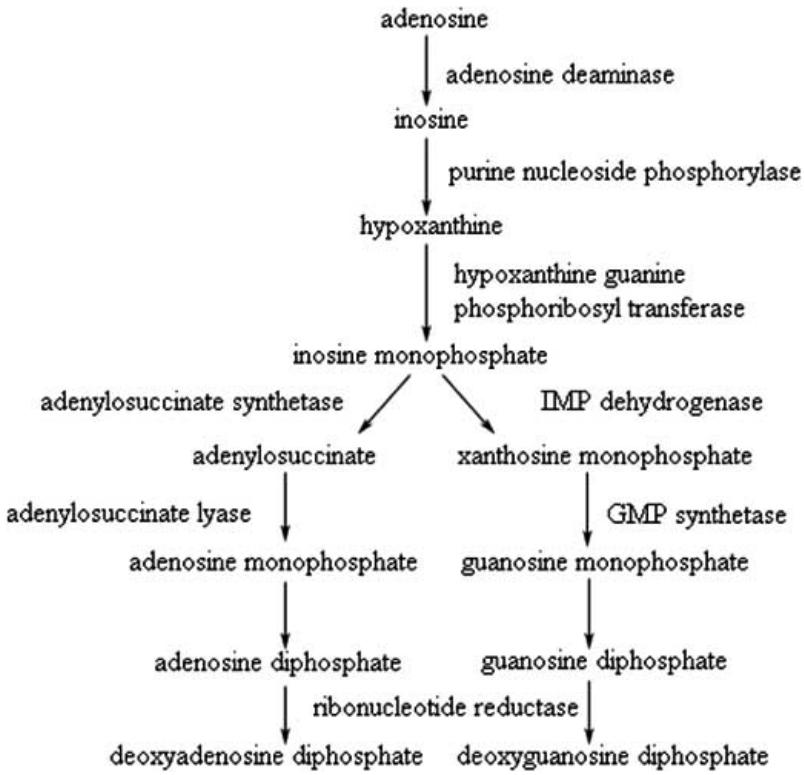


Fig. (6). Purine Salvage Pathway. Adapted from the Malaria Parasite Metabolic Pathways.

Immucillins are transition state analogs of purine nucleoside phosphorylase (PNP; EC 2.4.2.1) which inhibit PNP activity and kill *P. falciparum* in culture [106]. Immucillins can be designed to selectively inhibit parasite PNP [107]. Inhibition of hypoxanthine guanine phosphoribosyl transferase (HGPRT; EC 2.4.2.8) by antisense oligonucleotides kills parasites in culture [108]. Potent inhibitors of HGPRT have been developed [109]. Bredinin inhibition of IMP dehydrogenase (EC 1.1.1.205) arrests trophozoite growth in culture [110]. GMP synthase inhibition by psicofuranine interrupts parasite growth *in vitro* [101]. Thus there are many promising drug targets in the purine salvage pathway, many with lead compounds.

Carbonic anhydrase (CA) produces bicarbonate from carbon dioxide and water, and is integral to the import of carbon dioxide into the parasite (utilized in pyrimidine synthesis). The plasmodial CA enzyme has distinct catalytic activity and its inhibition by acetazolamide and sulfanilamide inhibit parasite growth in culture [111]. Carbamoyl-phosphate synthetase II (EC 6.3.5.5), the rate-limiting enzyme of pyrimidine synthesis, is inhibited by ribozymes which target unique inserts in its mRNA. These ribozymes reduce parasite growth in culture [112]. Inhibition of dihydroorotase (EC 3.5.2.3) by 6-L-thiodihydroorotate slows parasite growth in culture. The growth inhibition can be reversed with orotate supplementation [113]. Inhibition of dihydroorotate oxidase (DHOD; EC 1.3.3.1) by 5-fluoroorotate also slows parasite growth in culture [114]. DHOD can also be inhibited by RNA interference, and the decrease in growth corresponds with the decrease in DHOD mRNA [115]. Pyrazofurin inhibits orotidine 5'-monophosphate decarboxylase (ODCase; EC 4.1.1.23) and causes growth arrest of *P. falciparum* in culture [116]. Thymidylate synthase (EC 2.1.1.45) is potently inhibited by folate-based inhibitors that inhibit growth of both drug-sensitive and resistant strains of *P. falciparum* [117].

Additional enzymes that are more directly involved in DNA synthesis have also been proposed as drug targets:

- Ribonucleotide reductase converts ribonucleotides to deoxyribonucleotides and inhibition of ribonucleotide reductase with antisense oligonucleotides reduces parasite growth *in vitro* [118].
- Histone modifications regulate chromatin structure, affecting both replication and transcription of DNA. Apicidin, a tetrapeptide isolated from Fusarium spp., inhibits histone deacetylase activity and has antiplasmodial activity *in vitro* [119]. Apicidin is also effective against *P. berghei* in mice [120].
- Telomerase is an enzyme that replicates the ends of chromosomes, prevents chromosome shortening over generations, and is recognized as a drug target for rapidly replicating cells, such as in cancers. While parasite telomerase can be inhibited by berberine and pyrimidine nucleoside and nucleotide analogs [121, 122], the effect of these inhibitors on the parasite remains to be determined.
- DNA topoisomerases solve the winding problem of DNA replication and prevent DNA tangling. Topoisomerase I interconverts topoisomers of DNA through intermediates with single-strand breaks and its inhibition by camptothecin (also being investigated as a cancer chemotherapy agent) kills the parasite *in vitro* [123]. Topoisomerase II interconverts topoisomers and untangles DNA through

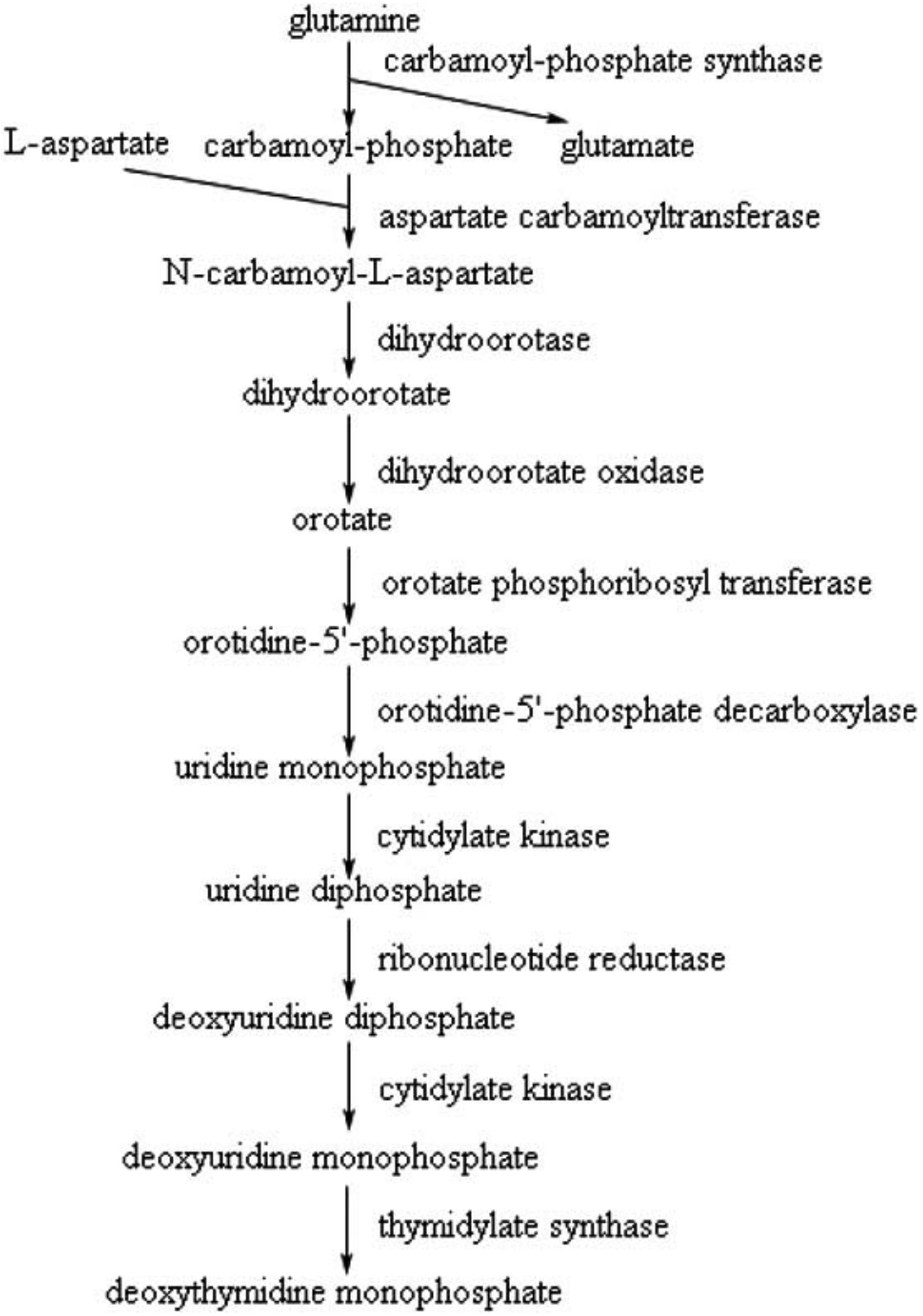


Fig. (7). Pyrimidine *de novo* synthesis pathway. Adapted from the Malaria Parasite Metabolic Pathways.

intermediates with double-strand breaks and inhibition of *P. falciparum* topoisomerase II inhibits growth of the parasite in culture [124, 125].

- Inhibitors of S-adenosyl-L-homocysteine hydrolase (adenosylhomocysteinase) which regulates methylation states in the cell, slow *P. falciparum* growth, and the enzyme has different specificities compared to its human counterpart [126, 127].

HEMOGLOBIN DIGESTION

Erythrocytic malaria parasites pinocytose host cell cytoplasm, which they digest in their acidic food vacuole. The parasite degrades hemoglobin as a source of amino acids for protein synthesis, in the process releasing free heme which is detoxified by conversion to the malaria pigment hemozoin. However, most of the amino acids resulting from hemoglobin digestion are exported from the parasite [128].

Recent models suggest that hemoglobin digestion by the parasite prevents premature host-cell lysis [129]. The parasite has many proteases which are involved in digestion of hemoglobin [130]. Cysteine protease inhibitors block hemoglobin digestion [131] and can kill the parasite in culture [132].

P. falciparum has four members of the falcipain family of cysteine proteases: falcipain-1, 2, 2' (a highly similar copy of falcipain-2) and 3. Falcipain-1 was thought to be critical in erythrocyte invasion and a promising drug target [133], but disruption studies show that this protein is not essential to the erythrocytic parasite [134]. Falcipain-2 and 3 are both expressed in the trophozoite and localized to the food vacuole, most likely participating in hemoglobin digestion [135, 136]. A recent study of falcipain-2 disruption showed decreased cysteine protease activity in the trophozoite and morphologic changes with swollen food vacuoles, supporting the hypothesis that falcipain-2 plays an important role in hemoglobin digestion. The abnormal phenotype was only transient with maturation into parasites that appear normal [137], indicating that falcipain-2 is not essential to parasite development. However, the experiment is inconclusive as the highly similar falcipain-2' gene was left intact. The authors propose that falcipain-2, 2' and 3 have similar activities due to their sequence similarity and expect that simultaneous inhibition of falcipain-2 and falcipain-3 would be needed for good anti-malarial effect.

The plasmepsins are aspartic proteases in *P. falciparum* localized to the food vacuole (there are 6 other aspartic proteases) and are also involved in hemoglobin digestion. Protease inhibitors have been developed that exhibit differential inhibition against plasmepsin I and II compared with cathepsin D, the closest human homolog, and inhibit parasite growth *in vitro* [138, 139]. A highly potent peptidomimetic plasmepsin II inhibitor has been developed [140]. However, recent individual knockout experiments have shown that no one member of the plasmepsins is essential to the parasite. *P. falciparum* growth was reduced with plasmepsin 1 and 4 knockouts combined, and abnormal mitochondrial morphoplogy was observed in the plasmepsin II knockout [141]. The authors suggest that an inhibitor of multiple plasmepsins would have antimalarial activity. Histo-aspartic protease (HAP; formerly named plasmepsin 3) has similarity to plasmepsin 1 and 2 but contains a histidine substitution of its catalytic aspartate [142]. HAP is of interest as a drug target due to its role in hemoglobin digestion and its unique inhibition profile. HAP is inhibited by pepstatin A, which inhibits aspartic proteases, as well as PMSF, a serine protease inhibitor, but not several other serine protease inhibitors that were tested [143].Aminopeptidases are required to digest the products of the proteases to free amino acids. Leucine aminopeptidase has been proposed as a drug target, as the enzyme in rodent malaria *P. chabaudi chabaudi* is strongly inhibited by bestatin and nitrobestatin, and these inhibitors block *P. falciparum* growth *in vitro* [144]. However, these inhibitors also inhibit other aminopeptidases, such as PfA-M1, an M1 family aminopeptidase which cleaves basic, hydrophobic and aromatic amino acids, and has been detected in trophozoites and schizonts [145]. This protein shows homology (44%) to human aminopeptidase-N and leukotriene A4 hydrolase. Specific inhibitors of PfA-M1 have been synthesized, which differentially inhibit PfA-M1 from human aminopeptidase, and these inhibitors inhibit parasite growth *in vitro* [146]. Dipeptidyl aminopeptidase 1 has been experimentally localized to the food vacuole of *P. falciparum*, and genetic disruption of the gene was not possible, implying it has an essential enzyme [147].

ANTIOXIDANT DEFENSE

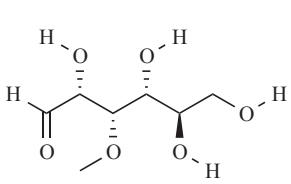
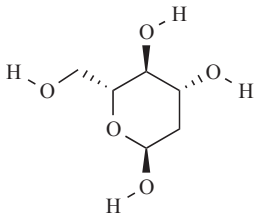
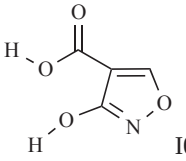
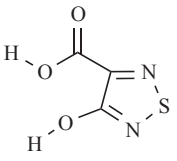
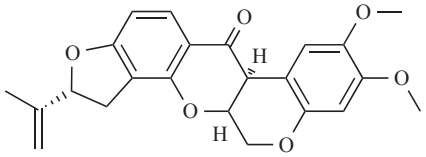
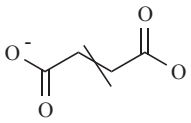
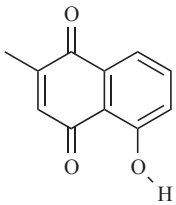
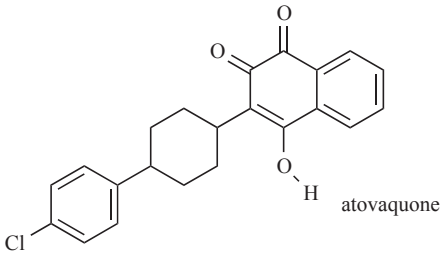
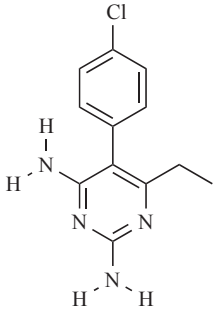
The digestion of hemoglobin by the erythrocytic parasite results in high levels of free heme which is highly toxic due to is oxidative power. Decreased resistance to antioxidant stress in the erythrocyte is thought to be the protective effect of glucose-6-phosphate dehydrogenase deficiency against malaria infection [148, 149]. The major antioxidant systems in the parasite are the superoxide dismutases, and the thioredoxin (Trx) and glutathione (GSH) systems, these and other antioxidants are reviewed by Muller [150].

Superoxide dismutase (SOD) is the first line of defense against superoxide anions, and the parasite SODs, of which there are two, contain iron, in contrast to mammalian SODs that contain Cu/Zn or Mn [151] and were thus proposed as drug targets [152]. A screening of a chemical library of synthetic compounds for inhibition of *P. falciparum* SOD identified compounds that inhibit SOD activity and also inhibit *P. falciparum* growth *in vitro* [153]. In addition peroxiredoxins in *P. falciparum*, 1-Cys peroxiredoxin and 2-Cys peroxiredoxin are capable of reducing hydrogen peroxide, and 1-Cys peroxiredoxin has been identified in some erythrocytic forms of the parasite [154].

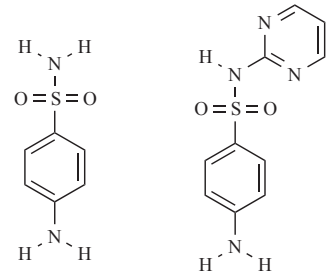
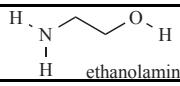
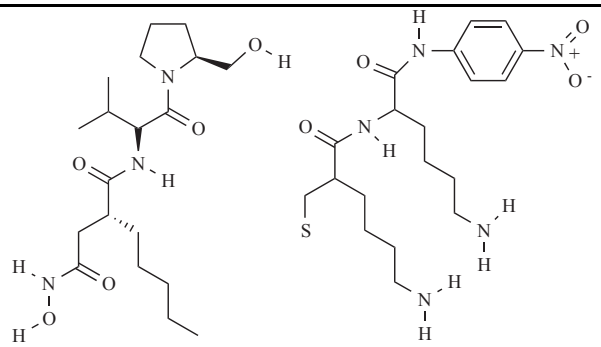
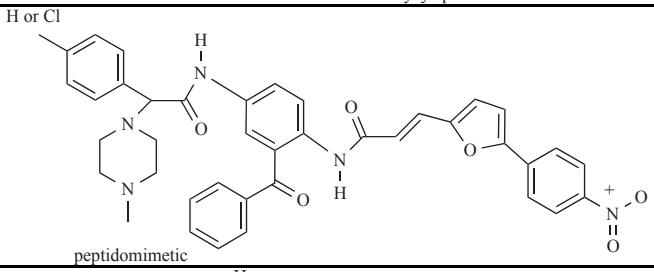
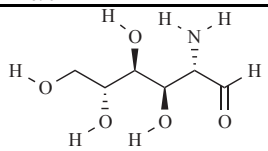
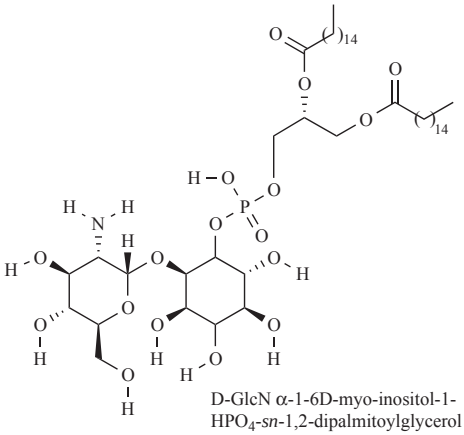
Trx is a protein with neighboring cysteines that can be either in an oxidized or reduced form. Thioredoxin reductase (TrxR) reduces the oxidized form of Trx, and the parasite enzyme is similar to the human enzyme, but with significant differences at the active site, suggesting differential inhibition is possible [155]. Gene disruption of thioredoxin reductase in *P. falciparum* was unsuccessful, suggesting that the enzyme is essential to the parasite [156]. *P. falciparum* also contains glutaredoxin (Grx), which belongs to the thioredoxin superfamily and is reduced by glutathione [157]. Plasmaredoxin (Plrx) is a member of the thioredoxin superfamily, is unique to malaria parasites, and expressed in erythrocytic stages. Plrx can be reduced by glutathione and other dithiols [158]. Reduction of ribonucleotide reductase requires reduced Trx or Grx or Plrx.

GSH is a tripeptide thiol redox buffer. It was noticed that chloroquine killing concentration is inversely related to parasite glutathione levels [159], suggesting that decreasing the parasite's GSH levels is deleterious, and that chloroquine acts through decreasing glutathione levels [37]. Glutathione reductase (GR) is the enzyme that reduces oxidized GSH, thus, methylene blue which has been shown to inhibit parasite glutathione reductase (GR) can potentially reverse chloroquine resistance [160]. Inhibitors of glutathione reductase show *in vitro* antimalarial activity [161]. GR prefers Trx to GSH as a substrate [162]. The crystal structure of *P. falciparum* GR was recently solved [163], constraining structure based design of parasite specific GR inhibitors. γ-glutamylcysteine synthetase (γ –GSH, EC 6.3.2.2) participates in the synthesis of GSH which is important to maintaining parasite GSH levels. Inhibition of γ-GCS by

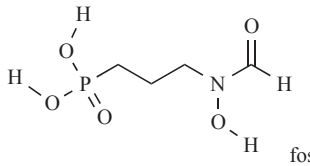
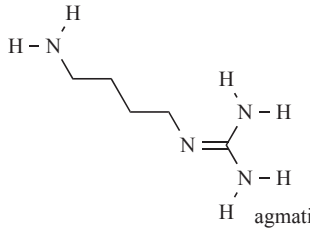
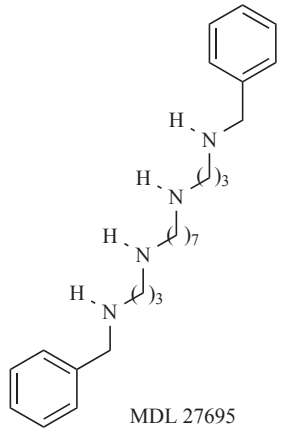
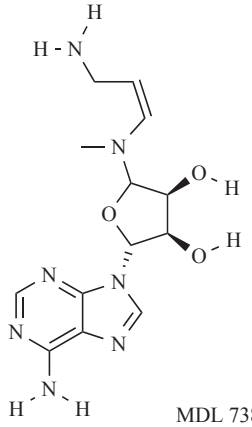
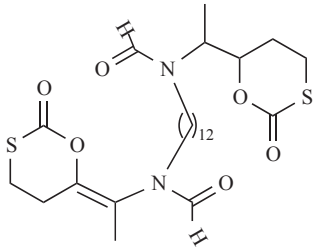
Table 2. Enzymes and their Inhibitors

	Inhibitors and structures
Energy Metabolism	
hexose transporter	 3-O-methylglucose  deoxyglucose
lactate dehydrogenase	 I0A1  TDA1
mitochondrial electron transport	
NADH dehydrogenase	 rotenone
succinate dehydrogenase	 fumarate  5-hydroxy-2-methyl-1,4-napthoquinone
ubiquinol-cytochrome c reductase	 atovaquone
Cofactor and Prosthetic Group Synthesis	
folate metabolism	
dihydrofolate reductase	 pyrimethamine

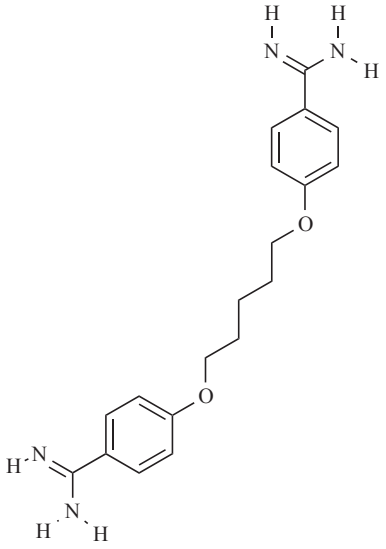
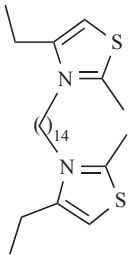
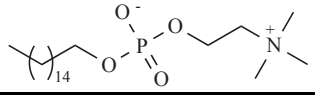
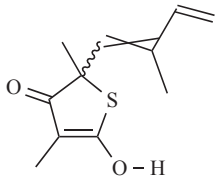
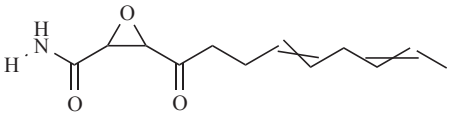
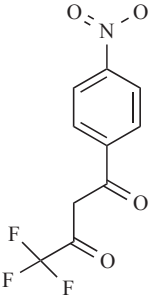
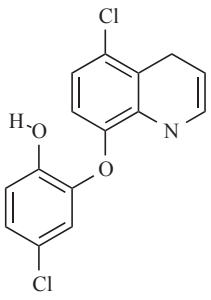
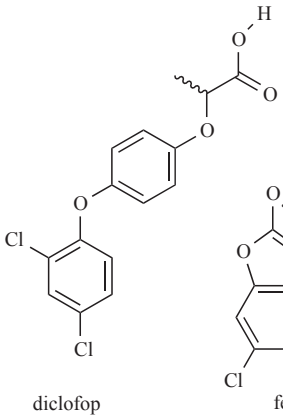
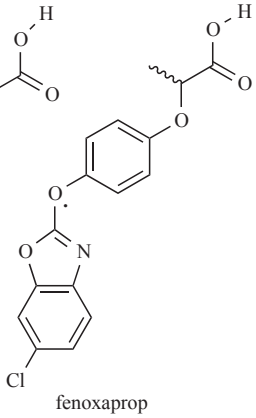
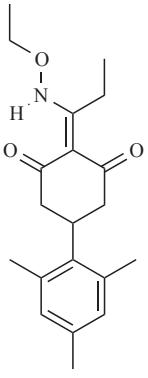
(Table 2) contd…..

	Inhibitors and structures
dihydropteroate synthase	 sulfanilamide sulfadiazine
***de novo* heme synthesis**	
delta-aminolevulinate synthase	 ethanolamine
Protein Modification	
peptide deformylase	 actinonin N-[(α-mercaptomethyl) caproyl]-L-lysyl-p-nitroanilide
protein farnesyltransferase	 peptidomimetic
GPI Biosynthesis	 mannosamine
N-acetyl glucosaminyl-phosphatidylinositol deacetylase	 D-GlcN α-1-6D-myo-inositol-1-HPO_4-*sn*-1,2-dipalmitoylglycerol

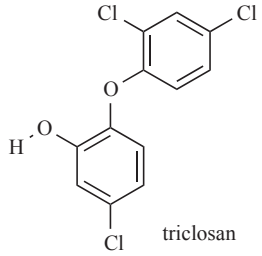
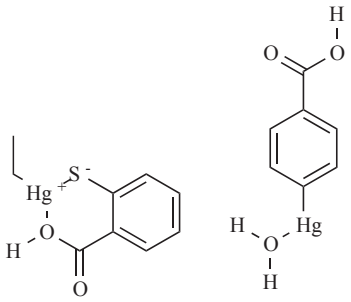
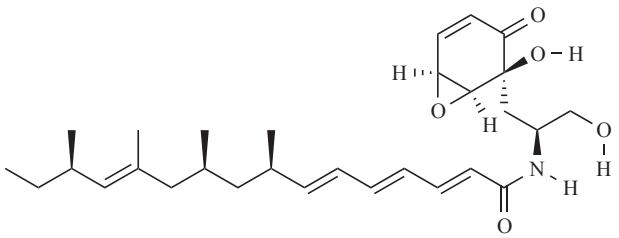
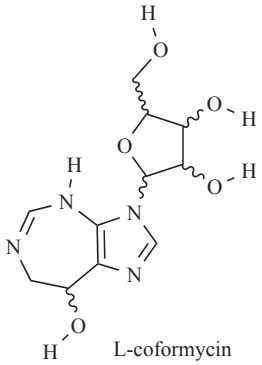
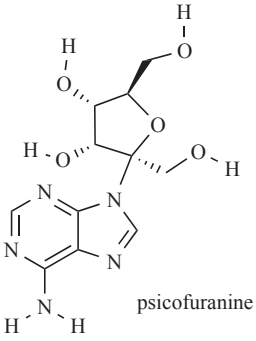
(Table 2) contd…..

	Inhibitors and structures
isoprenoid synthesis	
1-deoxy-D-xylulose-5-phosphate reductoisomerase	 fosmidomycin
polyamine metabolism	 agmatine
ornithine decarboxylase	 MDL 27695
S-adenosylmethionine decarboxylase	 MDL 73811
Lipid Metabolism	
phosphatidylcholine synthesis	 TE3

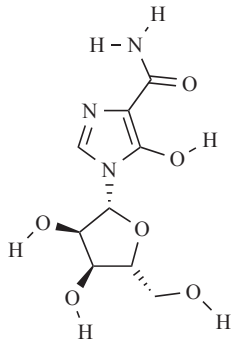
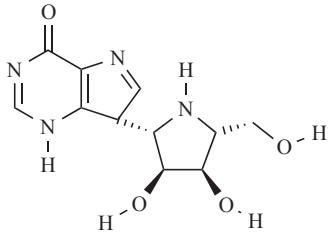
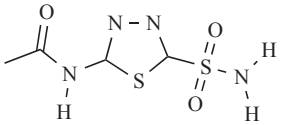
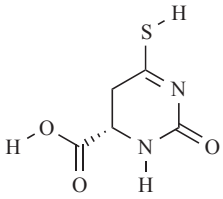
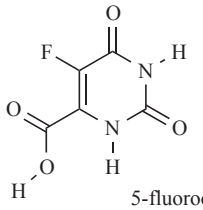
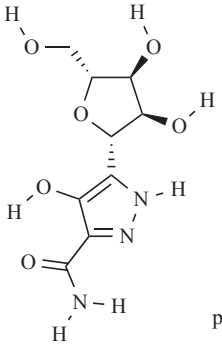
(Table 2) contd…..

	Inhibitors and structures
choline carrier	 pentamidine  T-16
phosphoethanolamine methyltransferase	 miltefosine
Type II fatty acid synthesis	
3-oxoacyl-ACP synthase I,II, III	 thiolactomycin  cerulenin
3-hydroxyacyl-ACP dehydratase	 NAS-21  NAS-91
acetyl-CoA-carboxylase	 diclofop  fenoxaprop  tralkoxydim

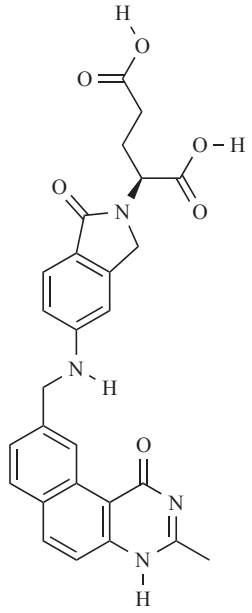
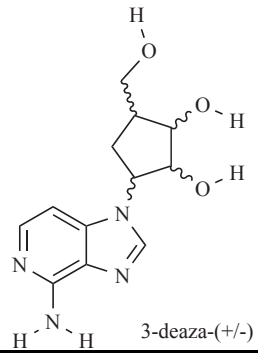
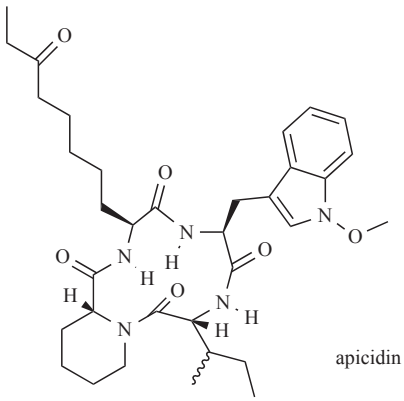
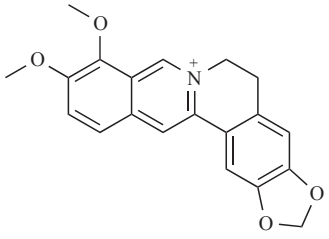
(Table 2) contd…..

	Inhibitors and structures
enoyl-ACP-reductase	 triclosan
Lipases	
phospholipase B	 thimerosal p-hydroxymercuribenzoate
phospholipase C and sphingomyelinase	 scyphostatin
DNA replication and transcription	
purine salvage	
adenosine deaminase	 L-coformycin
GMP synthetase	 psicofuranine

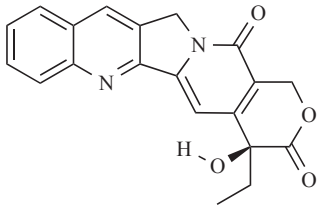
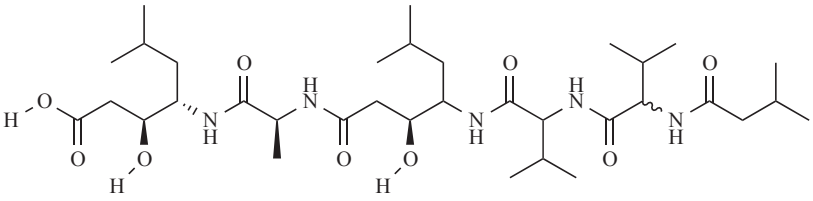
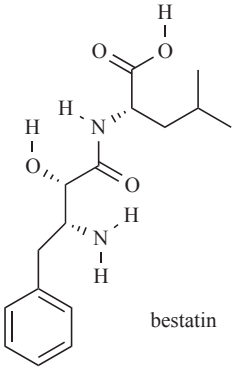
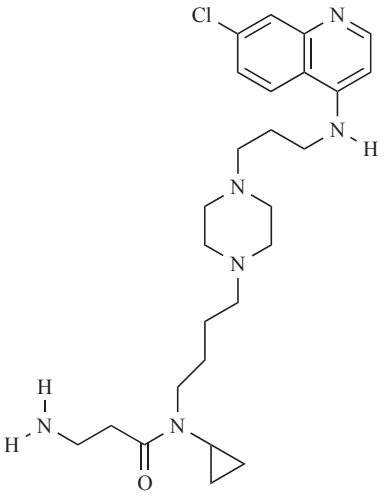
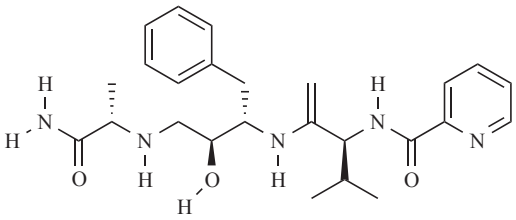
(Table 2) contd…..

	Inhibitors and structures
IMP dehydrogenase	 bredinin
purine nucleoside phosphorylase	 immucillin-H
***de novo* pyrimidine synthesis**	
carbonic anhydrase	 sulfanilamide (see above) acetazolamide
dihydroorotase	 6-thiodihydroorotate
dihydroorotate oxidase	 5-fluoroorotate
orotidine-5'-phosphate decarboxylase	 pyrazofurin

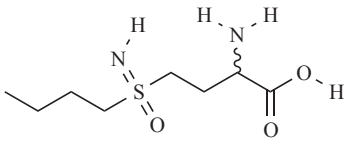
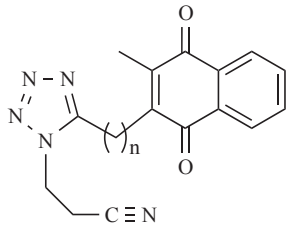
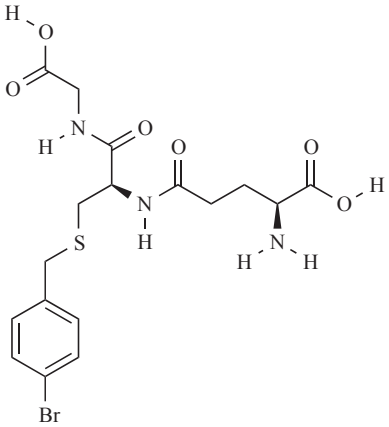
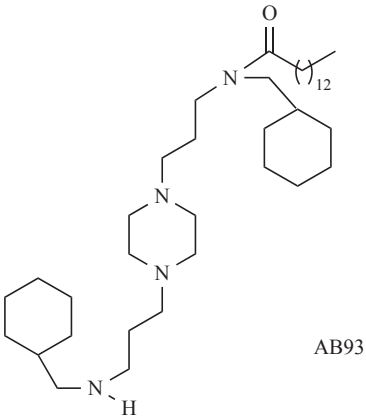
(Table 2) contd…..

	Inhibitors and structures
thymidylate synthase	 1843u89
additional enzymes	
adenosylhomocysteinase	 3-deaza-(+/-) aristeromycin
histone deacetylase	 apicidin
telomerase	 berberine

(Table 2) contd…..

	Inhibitors and structures
topoisomerase I	 camptothecin
Hemoglobin Digestion	
histo-aspartic protease	 pepstatin A PMSF
leucine aminopeptidase	 bestatin
M1-family aminopeptidase	 starting point for analogues
plasmepsin I and II	 hydroxyethylamine transition state scaffold based

(Table 2) contd…..

	Inhibitors and structures
Antioxidant Defense	
γ-glutamylcysteine synthetase	 D,L-buthionine-S,R-sulphoxime
glutathione reductase	 cyanoethylprotected tetrazole
glyoxalase I	 S-P-bromobenzylgluatathione diethyl ester
superoxide dismutase	 AB93

Structures Taken and Adapted from PubChem (http://pubchem.ncbi.nlm.nih.gov/).

D,L-buthionine-S,R-sulphoximine inhibits parasite development *in vitro* [164], and partially reverses chloroquine resistance of *P. berghei in vivo* [165]. Glyoxylase I uses reduced GSH as a coenzyme for detoxifying toxic compounds in the parasite, and the *in vitro* antimalarial activity of S-p-bromobenzylglutathione diethyl ester, is thought to be due to inhibition of this enzyme [166].

The antioxidant systems are important to the parasite, and the nuances of their interactions are not completely understood. However, progress has been made as inhibitors

of enzymes in the three major redox systems have been identified and reduce parasite growth or even kill the parasite *in vitro*. The next step is to develop differential inhibition methods which may be possible due to occasional differences between parasite and human enzymes.

MISCELLANEOUS DRUG TARGETS

In some cases, enzymes become drug targets before the processes in which they function are understood, which do not preclude them from leading to the development of an effective drug.

• *P. falciparum* contains three subtilisin-like serine proteases, which have been previously reviewed [167]. The precise role of these proteases in the parasite has not been determined, but the expression of Pfsub-1 and Pfsub-2 in asexual erythrocytic stages [168, 169], and the inability of disruption these two genes in plasmodium strongly suggests these proteins have an essential function [167, 170].

• The *P. falciparum* serine repeat antigens (SERAs) contain a papain-like protease domain, but have uncharacterized functions. There are nine SERAs in *P. falciparum*, and proteolytic activity has been demonstrated for SERA5 [171]. The most promising as drug targets are SERA 4, 5, 6 which have been demonstrated in blood stage parasites by antibody studies, and are essential to blood-stage parasite growth as evidenced by targeted genetic disruption [172].

CONCLUSION

We review drug targets in the current literature and outline the reasoning and biological evidence supporting further research and development of particular drug targets and their inhibitors (Table **1**). The goal of publicly funded research is to develop a drug (by developing lead compounds for inhibitors, testing inhibitors in animal models) to the point where a pharmaceutical company will begin industrial development and testing. We have presented evidence for target proteins in several general areas of parasite biology. The list of targets has blossomed because of the efforts of scientists, who have taken advantage of the information provided by the sequenced genome. The current set of drug targets is heavily weighted toward metabolic pathways, but this may change with increased understanding of parasite signaling mechanisms and the parasite's interactions with host cells. The next decade will require careful evaluation of these targets (and others that emerge) in the context of the biology of the parasite, and its interactions with the host.

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