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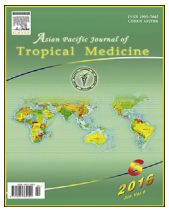

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Insights into the pyrimidine biosynthetic pathway of human malaria parasite *Plasmodium falciparum* as chemotherapeutic target

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ABSTRACT

Malaria is a major cause of morbidity and mortality in humans. Artemisinins remain as the first-line treatment for *Plasmodium falciparum* (*P. falciparum*) malaria although drug resistance has already emerged and spread in Southeast Asia. Thus, to fight this disease, there is an urgent need to develop new antimalarial drugs for malaria chemotherapy. Unlike human host cells, *P. falciparum* cannot salvage preformed pyrimidine bases or nucleosides from the extracellular environment and relies solely on nucleotides synthesized through the *de novo* biosynthetic pathway. This review presents significant progress on understanding the *de novo* pyrimidine pathway and the functional enzymes in the human parasite *P. falciparum*. Current knowledge in genomics and metabolomics are described, particularly focusing on the parasite purine and pyrimidine nucleotide metabolism. These include gene annotation, characterization and molecular mechanism of the enzymes that are different from the human host pathway. Recent elucidation of the three-dimensional crystal structures and the catalytic reactions of three enzymes: dihydroorotate dehydrogenase, orotate phosphoribosyltransferase, and orotidine 5′-monophosphate decarboxylase, as well as their inhibitors are reviewed in the context of their therapeutic potential against malaria.

1. Introduction

Malaria remains as one of the most deadly diseases in tropical and subtropical endemic countries, with almost half of the world's populations at risk of infection, estimated at 515 million clinical cases and 1.3 million deaths annually [1–3]. Of the five *Plasmodium* species that infect humans, including. *Plasmodium vivax* (*P. vivax*), *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*, *Plasmodium falciparum* (*P. falciparum*) is the causative agent of the most lethal and severe form of malaria [1,4,5]. *P. vivax*, responsible for 25%–40% of the estimated annual cases of malaria worldwide, is seldom fatal but relapses often occur even after a primary infection has cleared [6]. Over the past 50 years, the parasites' resistance to both chloroquine and sulphadoxine-pyrimethamine has rapidly emerged and is now widespread in the endemic countries [7].

Artemisinin and its derivatives, considered the most rapid acting and efficacious drug, are the first-line drugs for treatment of *P. falciparum* malaria [8]. However by 2009, resistance to the drug treatment has been reported (Figure 1) [9]. Thus, it is deemed necessary to develop novel antimalarial drugs for malaria chemotherapy [10,11]. Applying lessons learned from malaria research in the post-genomic era, together with increased understanding in genomics, transcriptomics and proteomics [12–16], this review highlights the candidate drug targets for antimalarial drug discovery [11,17–20].

2. Genomics and metabolomics of malaria parasite

Most of the biochemical knowledge on *P. falciparum* has focused on the intraerythrocytic life cycle of the parasite owing to over 60 years of research [21–24], as well the established cultivation method for these stages since 1976 by Trager and Jensen [25]. With the availability of complete genome sequences from various *Plasmodium* species such as, rodent

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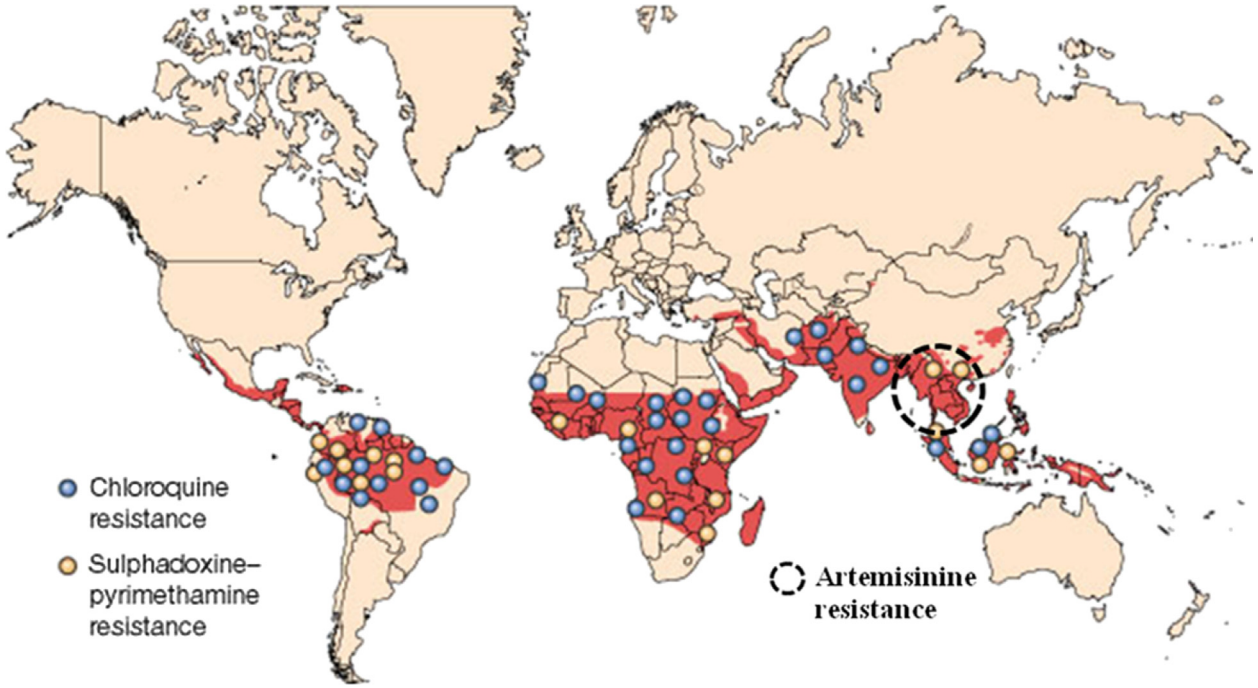

Figure 1. Geographic distribution of antimalarial drug resistant *P. falciparum*.
The resistance to two most widely used drugs, chloroquine and sulphadoxine-pyrimethamine, were represented in blue and yellow circles, respectively. The artemisinin resistance, spreading over the mainland of Southeast Asia from Vietnam to Myanmar in 2014, was shown in black dash area.
Data were adapted from the WHO [7,9].

malaria parasites: *Plasmodium yoelii* [26], *Plasmodium berghei* (*P. berghei*), and *Plasmodium chabaudi* [27]; human malaria parasites: *P. falciparum* [12], *Plasmodium knowlesi* [5], and *P. vivax* [6], substantial genome information is now available for comparative analyses. The genomes of the five human *Plasmodium* species are relatively uniform, ranging from 23 to 27 Mb across 14 chromosomes, and comprising approximately 5500 genes, in which 51% of these have only one intron. The G + C contents of *P. falciparum* and *P. vivax* genomes are 19.4% and 42.3%, respectively. There are two extrachromosomal genomes, the mitochondrion (a linear 6 kb-DNA) and apicoplast (a circular 35 kb-DNA). From genomic, transcriptomic and proteomic data for functional reconstruction, the parasite's metabolic pathways are now mapped in a public database [28,29]. Analysis of metabolic pathways provides strong conceptual frameworks that allow identification of new drug targets resulting in acceleration of preclinical candidates into the drug pipelines.

Several functional key metabolic pathways responsible for survival of the parasite are identified: anaerobic glycolysis, a short version of tricarboxylic acid cycle (Krebs' cycle), a simple mitochondrial electron transport system (mtETS), pentose phosphate pathway, apicoplast fatty acid synthesis, phospholipid synthesis, shikimate pathway, glyoxalate pathway, heme biosynthesis, coenzymes A and Q biosynthesis, amino acid metabolism, hemoglobin catabolism, vitamins B1 and B6 synthesis, coenzyme folate biosynthesis, purine and pyrimidine nucleotide synthetic pathways [7,12,28,29]. Pathways, most relevant to the intraerythrocytic stages of the life cycle, have unique properties. Some pathways are operated in two or three different cellular components, *e.g.*, heme biosynthesis compartmentalizes in cytosol, mitochondrion and apicoplast [30]. A number do not exist in the human host cells, *e.g.*, folate biosynthesis [31], which is a target of several antimalarial drugs (pyrimethamine, cycloguanil, sulphadoxine); hemoglobin catabolism for required amino acid precursors as well as release of potentially toxic heme. Other pathways are different from human metabolism, for instance, the malarial type II fatty acid synthesis that is similar to the bacterial pathway; and the purine and pyrimidine nucleotide synthetic pathways [28,29].

3. Human and parasite purine and pyrimidine nucleotide metabolisms

Nucleotide metabolism, one of the largest metabolic pathways in human cells, provides the building blocks for DNA and RNA synthesis. The nucleotides are also key players in a wide range of cellular functions, ranging from energy transduction, signaling, syntheses of many biomolecules in carbohydrate and lipid metabolisms [32]. Purine and pyrimidine nucleotides can be provided from *de novo* biosynthetic pathways or supplied via salvage pathways where nucleobases and nucleosides/deoxynucleosides are recycled from nutrients or from degraded DNA and RNA. In humans, both *de novo* and salvage pathways are functioning at significant levels for the purine and pyrimidine nucleotide requirements, although the salvage pathways were reported to be more active than the *de novo* pathways [33,34]. This is true also for bacteria, plant and the free-living nematode *Caenorhabditis elegans* [35–37].

The pathway itself plays an important role in the activation of the nucleoside-based prodrug forms or analogs that can be used in therapy or in itself serve as a drug target [38]. In rapidly growing cells, including tumor and cancer cells, total cellular purine and pyrimidine nucleotide pools are reportedly imbalance suggesting therapeutic importance [39–41].

The mature erythrocyte, which provides the host environment for *P. falciparum* growth and multiplication during the intraerythrocytic stages, has a relatively limited ability to salvage

purine and has no capacity for *de novo* purine synthesis. Likewise, the erythrocyte has no ability for *de novo* synthesis of pyrimidines as inferred from the absence or very low levels for enzymes involved in pyrimidine synthesis. In addition, there is little activity of the salvage pathway for utilization of the pyrimidine bases and nucleosides, uracil, uridine and thymidine, eventhough both purine and pyrimidine nucleosides can be taken up by the host erythrocyte [42].

In the intraerythrocytic stages of *P. falciparum*, only one of the 10 enzymes for *de novo* synthesis of purine nucleotide, namely adenylosuccinate lyase which is required to synthesize inosine monophosphate (IMP) from the 5-phosphoribosyl-1-pyrophosphate (PRPP) precursor has been identified [12]. This enzyme also catalyzes the adenosine monophosphate (AMP) synthesis from IMP in the purine salvage pathway. The parasite relies on the salvage of purines or their precursors from the human cell and plasma, particularly for hypoxanthine which is the most abundant purine source in human blood and regarded as the key precursor for other purines [43]. Thus, the great majority of salvaged purine is funneled through hypoxanthine to IMP by a broad substrate specific enzyme, hypoxanthine-guanine-xanthine-phosphoribosyl-transferase (HGXPRT). However, salvage of purine can also occur via the sequential conversion of adenosine to inosine and hypoxanthine by adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP), respectively. Adenosine is known to be rapidly imported into the parasite by at least two effective transporters [43–45]. Moreover, ADA is able to use methylthioadenosine from the polyamine biosynthetic pathway to produce methylthioinosine, which is then catalyzed to hypoxanthine by PNP [43–45]. Thus, the parasite ADA functions at the intersection of polyamine metabolism and purine salvage pathway.

In order to obtain adenylate nucleotides, IMP is first catalyzed by adenylosuccinate synthetase to adenylosuccinate (AMPS), which is then converted to (AMP) by adenylosuccinate lyase. To get guanylate nucleotides, IMP dehydrogenase catalyzes the conversion of IMP to xanthosine monophosphate (XMP), which is then converted to guanosine monophosphate (GMP) via a predominant route for GMP production by GMP synthetase. HGXPRT is also able to catalyze, albeit with limited activity, the conversion of xanthine base to XMP and guanine base to GMP [45,46]. The current understanding on purine salvage pathway and interconversion of salvaged purine derivatives to provide ATP, dATP, GTP and dGTP for the synthesis of DNA and RNA nucleic acids, and the linkage of polyamine and purine pathways are summarized in Figure 2. Details of the parasite transporters that allow reutilization of preformed purine nucleosides and bases, the enzymes involved in the purine salvage pathway, including known three-dimensional crystal structures of these enzymes in *P. falciparum* have been recently reviewed [43,44].

4. Malaria parasite *de novo* pyrimidine biosynthetic pathway

The *de novo* pyrimidine biosynthetic pathway represents one of the oldest and most conserved metabolic pathway, and the six sequential enzymatic steps starting from bicarbonate (HCO_3^-), glutamine (Gln), and ATP, producing uridine 5′-monophosphate (UMP) (Figure 3), have remained intact throughout evolution, although the primary structures of the enzymes responsible for the synthesis deviate significantly among prokaryotes, parasitic protozoa, fungi, animals, and mammals including humans [47–51]. As emphasized above, unlike human cells, *P. falciparum* parasites have little ability to salvage preformed pyrimidine

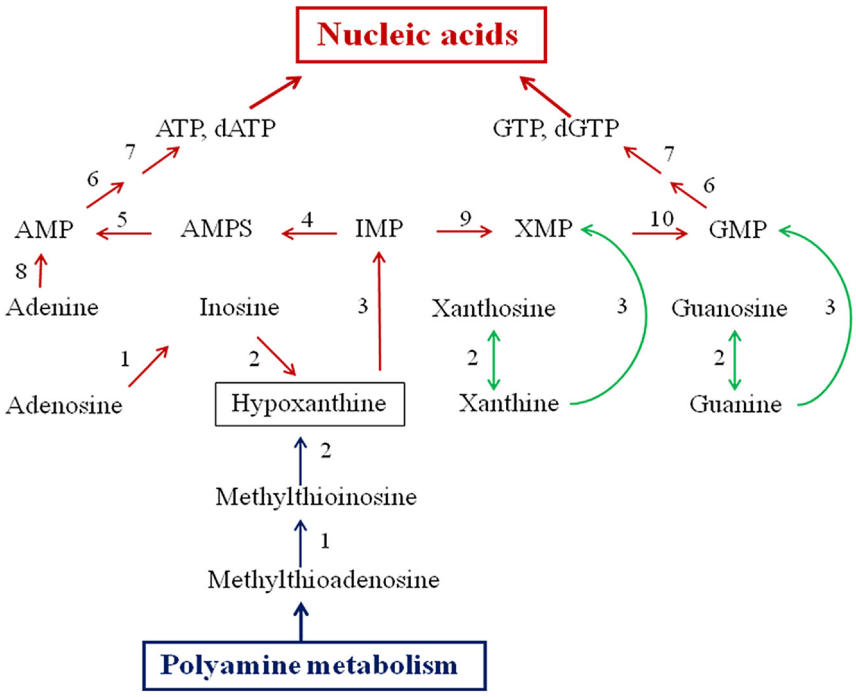

Figure 2. Purine salvage pathway and interconversion of purines in *P. falciparum*.
Red and green arrow indicated predominant and minor pathways, respectively: blue arrow showed polyamine reaction that links purine and polyamine biosynthesis in the parasite. Numbered enzymes were as follows: 1, ADA; 2, PNP; 3, hypoxanthine-guanine-xanthine-phosphoribosyl-transferase; 4, adenylosuccinate synthase; 5, adenylosuccinate lyase; 6, nucleoside monophosphate kinase; 7, nucleoside diphosphate kinase; 8, adenine phosphoribosyltransferase; 9, IMP dehydrogenase; 10, GMP synthetase. Figure adapted from [42].

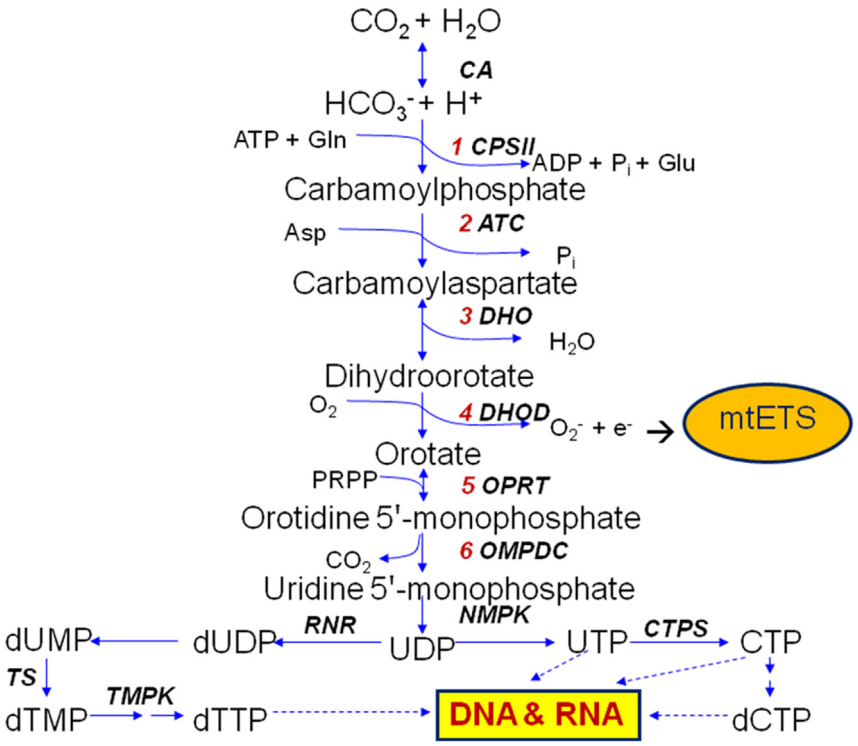

Figure 3. Schematic view of six sequential enzymatic reactions for *de novo* pyrimidine biosynthesis in *P. falciparum*. The enzyme CA was proposed to be associated with the pyrimidine pathway. The mtETS was linked to the enzyme DHOD of the pathway, functioning as electron disposal. NMPK, nucleoside monophosphate kinase.

bases and nucleosides from the host cell and plasma, but rely mostly on nucleotide synthesized through the *de novo* pathway.

Evidence for a functional pyrimidine *de novo* synthesis in the intraerythrocytic stages comes largely from the following lines of investigation. First, *in vitro* cultures of *P. falciparum* incorporate radioactive HCO_3^- into pyrimidine bases of DNA and RNA. The parasite demonstrates very little incorporation of radioactive orotate, uracil, uridine, thymine and thymidine into its DNA and RNA, whereas the pyrimidines were easily taken up by the *P. falciparum*-infected erythrocytes [21,22]. Second, although most of the enzymes responsible for salvaging preformed pyrimidine bases and nucleosides have been identified, their activities appears to be low, *e.g.*, thymidine kinase, cytosine kinase, uridine kinase, uracil phosphoribosyltransferase. There is one exception – the enzyme uridine phosphorylase (UP), required to add ribose from ribose-1-phosphate to uracil for uridine production. The enzyme was identified in both *P. falciparum* genome (locus: PFE0660c) and the parasite extract [12,52]. The primary structure of the parasite UP suggests that it is one of the moonlighting proteins having both UP and PNP activities on the same polypeptide [53]. Third, all the enzymes required for *de novo* synthesis of UMP, the first pyrimidine nucleotide metabolite acting as the precursor for synthesis of all pyrimidine nucleotides including CTP, dCTP, TTP, and dTTP, were detected in cell extracts from all *Plasmodium* species so far examined (Table 1) [54–56]. Finally, the genes encoding each enzymes in all steps of the *de novo* pathway were detected, whereas most genes for the pyrimidine salvage pathway were conspicuous by their absence with the exception of *pfUP* gene [12,28,29].

The pyrimidine genes (*pyr1* = *pfCPSII*, *pyr2* = *pfATC*, *pyr3* = *pfDHO*, *pyr4* = *pfDHOD*, *pyr5* = *pfOPRT*, and *pyr6* = *pfOMPDC*) encoding the first six enzymes of the pathway were identified in the *P. falciparum* genome [12]. The *pyr1–pyr6* open reading frames (ORFs) are organized on different locations at various chromosomes: *pfCPSII* encoding carbamoylphosphate synthetase II (CPSII) and *pfATC* encoding aspartate transcarbamoylase (ATC) on chromosome 13, *pfDHO* encoding dihydroorotase (DHO) on chromosome 14, *pfDHOD* encoding dihydroorotate dehydrogenase (DHOD) on chromosome 6, *pfOPRT* encoding orotate phosphoribosyltransferase (OPRT) on chromosome 5 and *pfOMPDC* encoding orotidine 5′-monophosphate decarboxylase (OMPDC) on chromosome 10. The location and organization of all six pyrimidine genes are summarized in Table 2. The parasite pyrimidine genes have the following characteristics: 1) single ORF, having no intron, 2) single copy, and 3) the loci are close to the hypothetical proteins on the chromosome. The genes are separated from each other and are not operon-like in its molecular organization. This property differs from its analogous parasitic protozoan *Trypanosoma* and

Table 1
First six enzymes of *de novo* pyrimidine biosynthesis in two malaria parasites, *P. falciparum*, *P. berghei*, *vs* host erythrocytes.[a]

Reaction step	Enzyme	Activity (units per mg protein of crude extract)		
		P. falciparum	*P. berghei*	Erythrocyte
1	CPSII	0.04	0.46	<0.01
2	ATC	11.10	7.24	<0.05
3	DHO	0.22	0.44	<0.01
4	DHOD	0.10	0.42	<0.01
5	OPRT	25.37	1.60	<0.05
6	OMPDC	20.86	1.85	<0.05

[a] Data were taken from [42,86].

Table 2
Organization and character of *de novo* pyrimidine genes and their enzymes in *P. falciparum*.[a]

Gene	Chromosome	Locus	Size (bp)	Enzyme form	Amino acids (residues)
pyr1 (*pfCPSII*)	13	PF13_0044	7329	Mono[b]	2391
pyr2 (*pfATC*)	13	PF13_0240	1435	Mono	339
pyr3 (*pfDHO*)	14	PF14_0697	1076	Mono	358
pyr4 (*pfDHOD*)	6	MAL6P1.36	1709	Mono	569
pyr5 (*pfOPRT*)	5	PFE0630c	846	Complex[c]	281
pyr6 (*pfOMPDC*)	10	PF10_0225	972	Complex	323

[a] Data were taken from [52]. [b] Monofunctional enzyme. [c] Heterotetrameric OPRT-OMPDC enzyme complex.

Leishmania, where *pyr1–pyr6* genes are in an operon-like cluster or synteny constituting of a polycistronic transcript unit on a 25 kb segment of the 800 kb chromosomal DNA [49,50]. The malarial pyrimidine genes are also different from humans in that the fused and single gene *pyr1–pyr3–pyr2* (locus: 2p22–21) encodes the multifunctional CAD protein catalyzing the first three enzymes activities and the gene *pyr5–pyr6* (locus: 3q13) produces the bifunctional UMP synthase activity (Table 3) [47–49]. In addition, the human *pyr4* gene is located separately at locus 16q22. In prokaryotes, *pyr* genes may be localized on different loci as in the bacterial chromosomes, *e.g.*, *Escherichia coli* (*E. coli*) [35]; or may be clustered as an operon similar to *Bacillus subtilis pyr* genes which are transcribed on one large polycistronic message in the 12.5 kb of *Bacillus subtilis* chromosomal DNA [48].

The identified ORFs of the *pyr1–pyr6* genes of *P. falciparum* are deduced from amino acid sequences of the pyrimidine enzymes. Using multiple sequence alignments and phylogenetic analyses the enzymes *pfCPSII*, *pfDHO* and *pfOPRT* are conserved to bacterial counterparts. The malarial *pfATC*, *pfDHOD* and *pfOMPDC* are mosaic variations, which are homologous to both bacterial and eukaryotic counterparts, including humans. ATC sequence comparison with *Toxoplasma gondii*, a parasitic protozoan, reveals only 30% identity to the *pfATC* gene. The *pfDHO* sequence is close to most bacterial sequences, the yeast *Saccharomyces cerevisiae* and the plant *Arabidopsis thaliana*, indicating that *P. falciparum* may carry the monofunctional *DHO* gene acquired thru horizontal gene transfer from a proteobacterium, *e.g.*, *E. coli*, *Neisseria gonorrhoeae* [52].

In the malaria parasite, there is relatively little information on the sequential enzymatic steps after UMP synthesis before yielding dCTP, dTTP, CTP, TTP which are the building blocks for DNA and RNA synthesis (Figure 3). Genes are present in the parasite genome but few enzymes have been studied to date. *P. falciparum* ribonucleotide reductase (RNR) catalyzes the production of deoxyribonucleotides from ribonucleotides, which is associated with thioredoxin reductase [57–59]. TMP kinase (TMPK), catalyzing the synthesis of dTTP and TTP, is known as type I enzyme by amino acid sequence but has high efficiency in phosphorylation of 3′-azido-3′-dTMP as well as *E. coli* type II TMPK [60]. Notably, the parasite TMPK can also phosphorylate dGMP and dUMP with high specificity, indicating a broad spectrum of substrate specificity [61]. However, CTP synthetase (CTPS) which catalyzes the formation of CTP from UTP, is the only known enzyme for cytosine nucleotide *de novo* synthesis in the parasite [62–65].

In *P. falciparum*, the *de novo* pyrimidine pathway is closely linked to the folate biosynthesis by the thymidylate synthetase (TS) (Figure 3) [66]. The parasite TS is a part of the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS), a validated target for antifolate drugs [67]. The three-dimensional crystal structure of the *P. falciparum* enzyme and substrate channeling domains have been resolved [68]. It is well recognized that the *de novo* folate pathway is operating in the parasite, like in bacteria, whereas the human host is incapable of *de novo* synthesis. In addition, the parasite also has ability to salvage preformed folates from the human host [66,69].

5. Malarial CPSII, ATC and DHO are monofunctional enzymes

Progress towards understanding structures, catalytic mechanisms and regulation of mammalian and human enzymes for *de novo* pyrimidine pathway has been significant in recent years [32,51]. Certain key differences of the enzymes, pathway, and their genomes including the six enzymes of the pathway from precursors HCO_3^-, Gln, and ATP to UMP synthesis warrants a closer look.

By using analytical gel filtration chromatography, the first three enzymes (CPSII, ATC, and DHO) of the rodent parasite *P. berghei* were readily separated into three different molecular

Table 3
Organization and molecular property of *de novo* pyrimidine genes and theirs enzymes in human.

Gene	Chromosome	Locus[a]	Gene character	Enzyme form	Molecular mass[b] (kDa)
pyr1	2			Fused	
pyr2	2	2p22-21	*pyr1-3-2*[c]	Trifunctional CAD	243
pyr3	2				
pyr4	16	16q22	Separated	Monofunctional	43
pyr5	3			Fused	
pyr6	3	3q13	*pyr5-6*	Bifunctional UMPS	52

[a] NCBI database accession number: MIM: 114010, MIM: 274270, MIM: 258900. [b] Data were taken from [51]. [c] Genes *pyr1*, *pyr2*, *pyr3* were fused in the order of *pyr1-3-2*.

masses [70], which is consistent with the presence of three discrete monofunctional proteins. This is similar to that found in another species of protozoa, *Crithidia fasciculata*, and in many prokaryotic systems [35,70]. The characteristic differs from the humans wherein the CPSII, ATC and DHO activities are carried on a 243-kDa multifunctional protein, called CAD [47].

The malarial DHO enzyme has been purified from *P. falciparum* and its gene has been cloned, expressed and characterized in detail by our groups [71]. It is a Zn^{2+} enzyme belonging to the amidohydrolase family, sharing characteristics of both mammalian type I and eubacterial type II DHO by overall amino acid sequence homology, structural properties, kinetics and inhibitor characteristics [71–73].

In mammals, the CPSII domain of the CAD is a rate limiting step of the pathway, which is subject to UTP feedback inhibition and allosteric activation by PRPP [39–41]. Responses to allosteric effectors are modulated by phosphorylation, through signaling cascades of mitogen-activated protein kinase (MAP kinase) and protein kinase A (PKA) when demand of the pyrimidine nucleotides is greatest; and by protein kinase C (PKC) when its demand is least [51]. In the bacterial system, the ATC activity is sensitive to feedback inhibition by CTP, and is activated by ATP using allosteric regulation [35]. At present, the malarial CPSII and ATC enzymes are still poorly characterized and regulation of these pyrimidine enzymes is largely unknown.

6. Malarial DHOD is the mitochondrial enzyme

Numerous studies have focused on DHOD, the fourth enzyme in the pathway, particularly as a target for antimalarial agents [74–78]. The *P. falciparum DHOD* gene has been cloned, expressed and characterized [79]. Immunogold labeling localized DHOD in the inner membrane of mitochondrion [77]. The three-dimensional crystal structure of the parasite enzyme has been elucidated and compared to the human DHOD structure [80,81]. Crystal structures of human and parasite DHOD identifies completely different binding sites for the inhibitor leflunomide. The overall structure is α/β-barrel, similarly to that of other family 2 DHOD of eukaryotic origin (Figure 4A). It contains flavin mononucleotide prosthetic group, ubiquinone binding site and active site for dihydroorotate substrate, consistent with the previous assumptions using kinetic analyses [76,77]. Furthermore, the pyrimidine pathway is linked to the mtETS through the DHOD and ubiquinone coenzyme [77]. The mtETS are valuable targets in malaria chemotherapy [30,82–85].

7. Malarial OPRT and OMPDC are multienzyme complex

We characterized the functional, kinetic, and structural properties of OPRT and OMPDC, the fifth and sixth enzymes of the *de novo* pathway [86–88]. The OPRT and OMPDC enzymes were purified directly from *P. falciparum* culture. The native enzymes are organized in an $\alpha_2\beta_2$ heterotetramer structure having two subunits each of OPRT and OMPDC [86]. We also expressed both genes in *E. coli* [87,88]. Co-expression recombinant *P. falciparum OPRT* and *OMPDC* genes also exhibited the $\alpha_2\beta_2$ complex formation [89,90]. Most recently the parasites' low complexity region was found to be responsible for the protein–protein interaction in the heterotetrameric formation of the malarial OPRT and OMPDC enzymes, $[(OPRT)_2(OMPDC)_2]$, as identified by means of a unique insertion of low complexity amino acid sequence characterized by single amino acid repeat which was not seen in homologous proteins from other organisms (Figure 5) [91]. The steady-state kinetics of initial velocity and product inhibition studies of the monofunctional OPRT and OPRT domain in the heterotetrameric complex follows a random sequential kinetic mechanism [87,89].

Furthermore, three-dimensional crystal structures of the parasite OPRT and OMPDC have been elucidated (Figure 4B and C) [92–95] and compared to the recently characterized human OPRT and OMPDC structures [32]. Direct decarboxylation mechanism for OMPDC catalysis was proposed based on the crystal structures of the OMPDC-OMP substrate and OMPDC-UMP product complexes. In this catalytic mechanism, the decarboxylation of OMP is initiated by charge repulsion between the C6 carboxylate group of OMP and the carboxyl group at the side chain of Asp136, followed by a proton transfer from Lys138 to the C6 position of UMP, and Lys102 hydrogen bonded to Asp136 instead of the carboxyl group of the pyrimidine ring after decarboxylation. There is a strong network of charged residues around the C6 position that has the potential to destabilize the ground state and stabilize the transition state for the decarboxylation of OMP [93]. By far, the inhibitors of OMPDC have been designed and chemically synthesized for therapeutic potential against malaria using the structure-based drug-design approach [94,96,97].

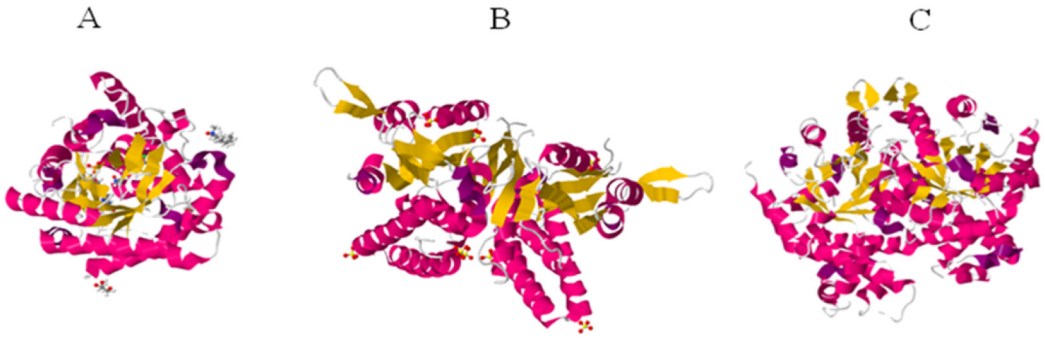

Figure 4. Three-dimensional structure of three *P. falciparum* enzymes: DHOD (A), OPRT (B), and OMPDC (C).
All structures were α/β-barrel, with α-helix shown in violet and β-strand shown in gold. The DHOD, as monomeric form, was in complex with DSM265 triazolpyrimidine inhibitor. OPRT and OMPDC were in dimeric forms as apoenzyme. The DHOD, OPRT, and OMPDC were taken from Protein Data Bank with PDB ID: 5DEL, 4FYM, and 2ZA2, respectively. The models were built using the Java program.

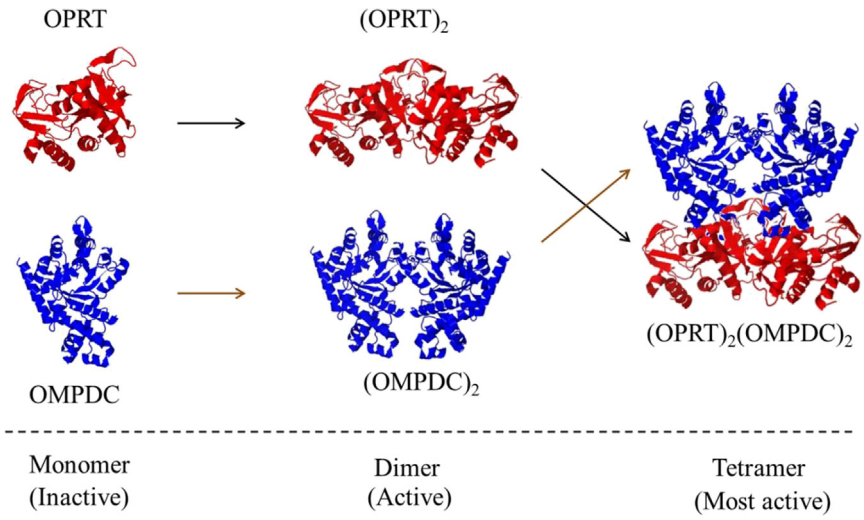

Figure 5. Sequential steps for dimer and tetramer formation of *P. falciparum* OPRT and OMPDC. The inactive monomer OPRT and OMPDC form its homodimer $(OPRT)_2$ and $(OMPDC)_2$. Both homodimers are then associated into heterotetrameric $[(OPRT)_2(OMPDC)_2]$ complex. The OPRT and OMPDC models are shown in red and blue, respectively.

8. Carbonic anhydrase (CA) is linked to the *de novo* pyrimidine pathway in the parasite

Functional and kinetic properties of CA were performed from *P. falciparum* [98]. The parasite CA catalyzes the interconversion of HCO_3^- and CO_2 possessing catalytic properties distinct from that of the human host CA isozymes I and II. Low amino acid sequence identity in the primary structures and phylogenetic analyses is being tapped for malaria chemotherapy, which is now in preclinical phase for drug development [99–101]. The CA supplies HCO_3^- as substrate for the CPSIIof the *de novo* pyrimidine synthetic pathway, linking the parasite CA to the pyrimidine pathway (Figure 3) [102–104].

9. Concluding remarks and future prospects

Until very recently, artemisinin-resistant parasites have spread over the mainland of Southeast Asian from Vietnam to Myanmar (Figure 1) [105]. The emergence and spread of these parasites entails novel measures for malaria treatment and control. Hopefully, one inhibitor, namely DSM265 (a triazolopyrimidine compound (Figure 4A)), targeting the DHOD enzyme of the pyrimidine pathway would prove promising as it progresses to preclinical and clinical phase I trials for drug development [106,107]. Moreover, as structure-based design of antimalarial drug development continues to tapped X-ray crystal structures of the enzyme, *in silico* screening and surface plasmon resonance analysis, especially for the parasite OMPDC [94], the possibility of modulating potential toxicity through the biochemical pathway might have therapeutic potential against human malaria [108].

Conflict of interest statement

We declare that we have no conflict of interest.

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