

# Selection of Targets for Drug Development Against Protozoan Parasites

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**Abstract:** Sequencing of parasite genomes opened the possibility to identify potential protein targets for drug development. Several protein targets have been found in the genome of *Plasmodium falciparum*, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*. Bioinformatics analysis is an important tool for the identification of protein targets for drug development against parasitic diseases. In this review we comment about three protein targets, identified in parasite genomes, and discuss the main features that may guide future efforts for virtual screening initiatives.

**Key Words:** Virtual screening, parasites, molecular targets, drug design.

## INTRODUCTION

There has been a substantial progress in drug discovery and development in recent years. All this progress has enabled drugs to be developed to cure and treat a wide spectrum of deadly diseases. Diseases caused by infection of protozoan parasites, such as trypanosomes and leishmania, however, have been neglected by research and development programs, mostly by pharmaceutical industries [1-6].

In order to reach a turning point in this scenario, efforts have been made in the sequencing of parasite genomes. The analysis of these genomes made possible to pursue the identification of a potential portion of a genome that could be targeted by a drug, which is of extreme importance in the early stages of drug development. Among important protozoan parasitic species, four had their genomes sequenced, *Plasmodium falciparum* [7-9], *Trypanosoma cruzi* [10], *Trypanosoma brucei* [11], and *Leishmania major* [12]. The availability of these genome sequences allows for comparative analyses to be carried out in a direct mode, towards a better understanding of biological, genetic and evolutionary aspects. In addition, such analyses makes possible the identification of genes related to pathogenesis, as well as genes involved in crucial metabolic pathways that may lead to drug discovery and development.

Here we discuss three important protein targets for drug development against protozoan parasites, protein kinases, similar to human cyclin-dependent kinase, chorismate synthase, the last enzyme in the shikimate pathway, and purine nucleoside phosphorylase, a key enzyme in the purine salvage pathway. All three enzymes have been identified in parasite genomes, and for at least two of them there is struc-

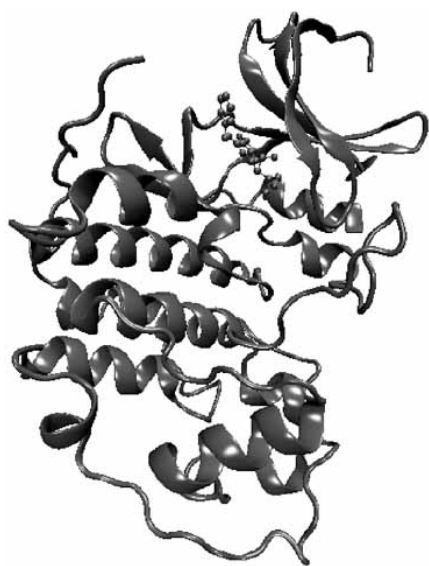
tural information. This structural information opened the possibility to engage virtual screening initiatives focused on these protein targets. In addition, experimental evidence strongly indicates the essential role of these enzymes in the parasite metabolism. The studies focused on structural information and target validation will be described in the present review.

## PROTEIN KINASES

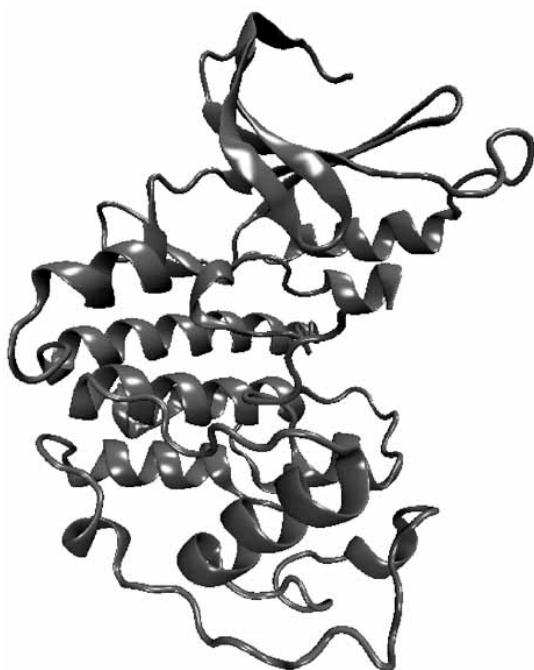
Analysis of parasite genomes identified several protein kinases as potential targets for drug development. PfPK5 and PfPK6 (protein kinase) of *P. falciparum*, and Tgcrk2/TPK2 (cdc2-related kinase and protein kinase 2, respectively) of *Toxoplasma gondii* share a high degree of homology and are most closely related to vertebrate CDK 1 and 2 which are involved in control of the G1/S phase and G2/M phases of the cell cycle. PfPK5, the best characterized of the apicomplexan crks, has been implicated in regulation of DNA synthesis [1, 13] during parasite asexual development, but to date, expression of these apicomplexan crks has not been studied during the sexual cycle [2, 14].

*P. falciparum* protein kinase 5 (PfPK5) is the best characterized member of the *P. falciparum* Cyclin-Dependent Kinase (CDK) family [15, 16]. In a sequence comparison of PfPK5 with members of the human CDK family, it shares the greatest degree of sequence identity (about 60%) with human CDK1 and CDK5 [17]. Structural information is available for human CDK2, which has been solved in complex with several inhibitors, in complex with cyclin, and p9 [16-21]. Fig. (1) shows the structure of CDK2 in complex with roscovitine, a potent kinase inhibitor (PDB access code: 2A4L), more recently the structure of PfPK5 was solved at 1.9 Å resolution (PDB access code: 1OB3) [15], and the structure adopts the characteristic bilobate kinase fold [22] (Fig. (2)). The ATP-binding pocket is in the cleft between

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**Fig. (1).** Structure of CDK2 in complex with roscovitine.



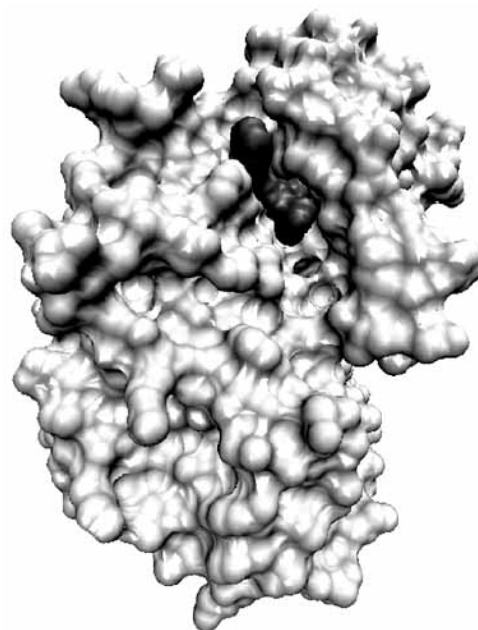
**Fig. (2).** Structure of PfPK5.

the lobes. In the structure of CDK2 in complex with roscovitine we can clearly see complementarity of shape between the inhibitor and the binding pocket, as shown in Fig. (3). Analysis of the intermolecular hydrogen bonds between CDK2 and several different inhibitors indicate a common pattern of contacts, which involve Leu 83 and Glu81.

Analysis of the structures of PfPK5 and CDK2 indicates that these kinases are quite similar to each other, and the superposition of C $\alpha$  generates RMSD of 1.3 Å. In the struc-

tures of CDK2 and PfPK5, the smaller N-terminal lobe consists of five antiparallel  $\beta$ -strands ( $\beta$ 1–5) and a single prominent  $\alpha$  helix containing the sequence PSTTIRE, which corresponds to the PSTAIRE cyclin binding motif of CDKs 1, 2, and 3. This lobe is connected through a hinge region to the larger predominantly  $\alpha$ -helical C-terminal lobe. All features of the monomeric CDK2 fold are well conserved [23–24]. The short CDK2 C-terminal tail (residues Val289–Leu298) that wraps toward the hinge region is absent from PfPK5 [15]. As observed in CDK2, PfPK5 is inactive as monomer. The C helix (residues 44–57), which is displaced from the body of the protein so that the side chain of Glu50 (the E of the PSTTIRE sequence) points towards the solvent and does not interact with Lys32. By analogy with other kinases, it is expected that these two residues will form a close interaction in the active state, which in turn allows Lys32 to correctly orientate the ATP  $\alpha$  and  $\beta$  phosphate groups for phosphotransfer. A second member of the CDK family from a species phylogenetically remote from humans (*P. falciparum*) adopts this distinctive disposition of the C helix provides evidence that the same inappropriate C helix orientation is employed in the wider CDK family to inhibit CDK activity [15]. This fact can be exploited to design new inhibitors against parasitic protein kinases.

Another kinase identified in the *P. falciparum* genome is PfPK6, which was recently isolated in *Plasmodium falciparum*. Analysis of this sequence shows similarity to human cyclin-dependent kinase (CDK) [8]. The sites of regulatory phosphorylation found in other CDKs (Thr14, Tyr15 and Thr160 in CDK2) are conserved in PfPK6 [8].



**Fig. (3).** Molecular surface of the complex CDK2-roscovitine.

Since there is no crystallographic structure for PfPK6, a homology model has been constructed using as the template the structure of active CDK2 extracted from the structure of

the phosphorylated CDK2/cyclin A complex. This homology model shows a typical bilobal structure, with the smaller N-terminal lobe consisting predominantly of  $\beta$ -sheet structure and the larger C-terminal lobe consisting primarily of  $\alpha$ -helices. The N-terminal lobe of PfPK6, as observed for CDKs, consists of a sheet of five antiparallel  $\beta$ -strands. The C-terminal lobe contains a pseudo-4-helical bundle, a small  $\beta$ -sheet and two additional helices [25].

PfPK6 contained a 286-residue catalytic domain similar to that in eukaryotic protein kinases. The identity of PfPK6 ORF with that of human CDK2 and human p38 MAPK was 38% and 33%, respectively. The presence of DIKPEN and GTLWYRAPE motifs in subdomains VI and VIII, respectively, are consistent with the consensus sequence (DLKPEN and GT/SXXY/FXAPE) for serine/threonine kinases. PfPK6 contained 14 of 15 conserved residues found in the catalytic domains of eukaryotic kinases including the GXGXXG (glycine loop) ATP-binding subdomain I motif and the invariant lysine in the catalytic subdomain II [26]. Of these 15 signature residues, only the phenylalanine in the DFG motif in subdomain VII is replaced by a leucine, a conservative change. Several conserved residues whose phosphorylation regulates vertebrate CDK activity, such as Thr14, Tyr15, Thr161 and Ser277, are also conserved in PfPK6 (Thr19, Tyr20, Thr173 and Ser283).

Several lines of evidence suggest that PfPK6 is a cyclin-independent kinase: (a) the recombinant protein shows significant autophosphorylation and histone phosphorylation activity in the absence of cyclin and (b) unlike PfPK5, the PfPK6 activity is not stimulated by incubation with cyclins. By analogy with other well-studied eukaryotic CDKs, it would be predicted that full activation of PfPK6 requires phosphorylation of Thr173, the residue homologous to Thr160 of human CDK2 [8]. The mechanism of PfPK6 cyclin-independent kinase activity is unclear, but presumably the major changes to vertebrate CDKs that are normally induced by cyclin binding must be constitutively present in PfPK6. Interestingly, PfPK6 contains a relatively large insertion (Asp80-Cys94) in the L6 loop, which makes this loop about 10 amino acids longer than that of other CDKs. It is possible that this larger than usual L6 loop may contribute to the constitutive activity of PfPK6, although other protein kinases that are active as monomers do not have an equivalent insertion. [24-26].

PfPK6 exhibits other unusual properties, such as its preference for  $Mn^{2+}$  as the divalent cation, a property usually associated with kinases. PfPK6 also resembles the MAPKs in that its sensitivity to roscovitine is in the micromolar range, rather than the nanomolar range reported for CDK1, CDK2 or CDK5 [26]. The increase in the concentration of roscovitine causes the progressive inhibition of autophosphorylation of PfPK6 in a kinase assay *in vitro* with  $IC_{50}$  value of 30  $\mu M$  [26]. A number of critical domains known to be important for the binding of CDK-activating proteins are also conserved in PfPK6 [26]. Clearly, the determination of the crystallographic structure of PfPK6 will be important to elucidate the mechanism of its cyclin-independent activity and its relationship to MAPKs [8].

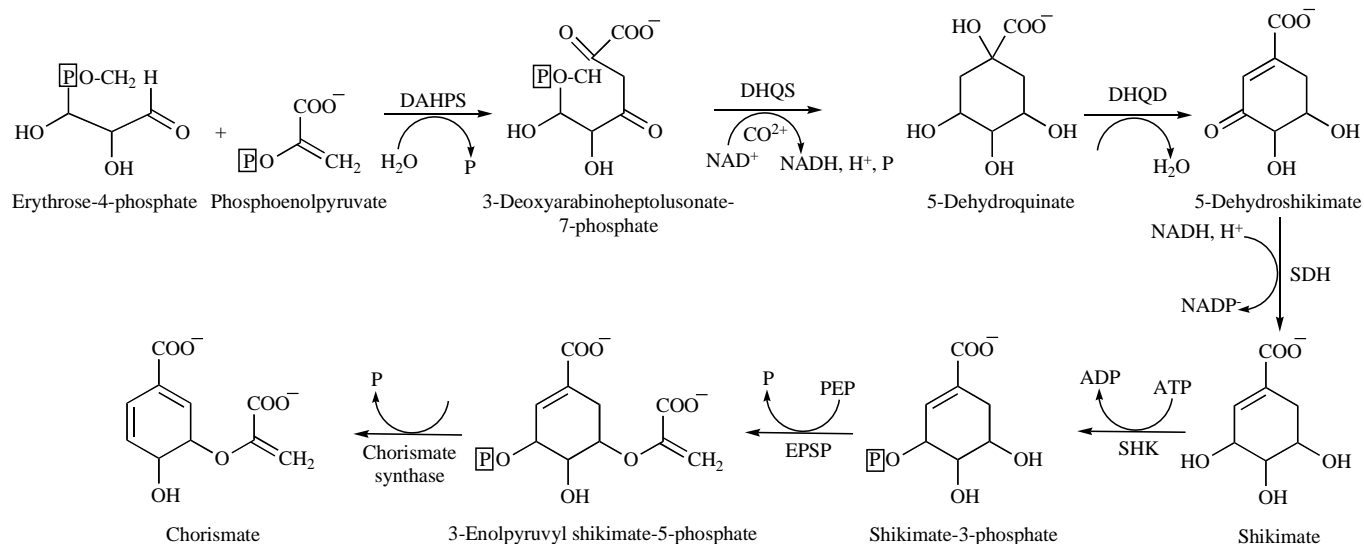
In humans the cell-cycle progression is tightly regulated by cyclins, CDK inhibitors (CKI), and phosphatases. Data-

base mining using cyclin sequences as queries led to the identification of an ORF called Pfcyc-1, which displayed maximal homology to cyclin H from a variety of organisms [27]. Pfcyc-1 presents approximately 17% homology with mammalian cyclin H. It came as a surprise that bacterially expressed Pfcyc-1 was able to efficiently activate PfPK5 *in vitro*. This result suggested that PfPK5 may have a relaxed specificity in its cyclin requirements, a hypothesis that was confirmed by the demonstration that PfPK5 can be activated by other cyclins and cyclin-like proteins such as p25, a CDK5 activator protein structurally related to cyclins despite a lack of homology at the sequence level. Pfcyc-1 also activates Pfmrk, which was somewhat less surprising, since Pfcyc-1 displays maximal homology to cyclin H (the cyclin partner of CDK7) and Pfmrk to CDK7 [26].

Sequence-based searches of the *P. falciparum* genome have failed so far to identify any putative *P. falciparum* proteins with significant sequence homology to any CDK inhibitor homologues. Results previously reported suggest that *P. falciparum* CDK complexes may be subject to regulation by CDK inhibitors and by analogy with the roles of CDK inhibitors in higher eukaryotic cells the identification of bonafide CDK inhibitors in *P. falciparum* would suggest the existence of checkpoint pathways to regulate CDK activity. Hence, it cannot be excluded that the *Plasmodium* CDKs are regulated *in vivo* by CKI functional homologues [8].

## SHIKIMATE PATHWAY

In plants and microorganisms, all the key aromatic compounds involved on the primary metabolism are produced by Shikimate Pathway. It is a seven step biosynthetic route which generates chorismic acid (the major branch point in the synthesis of aromatic amino acids, ubiquinone, and secondary metabolites) from phosphoenol pyruvate and erythrose-4-phosphate. Fig. (4) shows all steps of this biosynthetic route. All pathway intermediates can also be considered branch point compounds that may serve as substrates for other metabolic pathways. Therefore, these enzymes are potential targets for the development of nontoxic antiparasite agents and herbicides. The completion of the genome sequences of several pathogenic organisms is having an enormous impact on our understanding of the pathogenicity of these organisms. The sequencing of genomes allows identification of metabolic pathways present in pathogenic microorganisms, which can be target for the development of new drugs. A possible approach to selective antiparasite chemotherapy has been to exploit the inhibition of unique protein targets, vital to the pathogen and absent in mammals [28]. The shikimate pathway is an attractive example of this kind of targets, since it is present in bacteria, fungi and apicomplexan parasites but absent in mammals [29]. In this pathway the glycolytic intermediate, phosphoenol pyruvate, and the pentose phosphate pathway intermediate, D-erythrose 4-phosphate, are converted to chorismate through seven metabolic steps [30]. The essentiality of shikimate pathway was observed in some microorganisms such as *P. falciparum* and *M. tuberculosis*. The disruption of *aroK* gene, which codes for the shikimate kinase, showed that this enzyme is essential for *M. tuberculosis* viability [31]. In *P. falciparum* the growth was inhibited by glyphosate, a well-characterized inhibitor of the shikimate pathway [32]. These reports provide strong



**Fig. (4).** Shikimate pathway.

evidence that shikimate pathway is essential for the survival of these pathogens; therefore, the enzymes involved in this pathway are potential targets for drug development.

The product of shikimate pathway, the chorismate or chorismic acid, is a dihydroaromatic compound first described by Frank and Margaret Gibson in 1962 [33]. This compound is the branch point in the biosynthesis of several important aromatic molecules. For this reason, it was named chorismate, which means, in Greek, separation, split, or divorce. The chorismate is the common precursor for the biosynthesis of a wide range of primary and secondary metabolites including aromatic amino acids (phenylalanine, tyrosine and tryptophan), folate, naphthoquinones, menaquinones and mycobactins [34]. Chorismate synthase (CS), which is responsible for the synthesis of chorismate, was first described in 1967 by Morell *et al.* [35]. This enzyme catalyses the seventh step in the shikimate pathway (Fig. (4)), the conversion of 5-enolpyruvylshikimate-3-phosphate (EPSP) to chorismate [36]. *Brucella suis*, a gram-negative bacteria, which is responsible for animal brucellosis in a variety of mammalian hosts, had the *aroC* encoded CS gene disrupted in an array for oral vaccine against brucellosis. The *B. suis* mutant is highly attenuated in tissue culture and murine virulence models [37]. Other pathogenic bacteria as *Salmonella enterica* and *Yersinia enterocolitica* with *aroC* knockout [38-39] also grow slowly *in vivo*. The *in vivo* behavior of the bacterial *aroC* mutants (low-level residual virulence) suggests the importance of the CS for these microorganisms and shows that mutants can be candidate vaccines and the protein can be targeted for drug development. The CS enzyme has been described in bacteria, fungi, plants and apicomplexan parasites. However, the requirement of reduced flavin cofactor is an intriguing factor, because the CS does not do net redox change when the substrate EPSP is converted to chorismate. The reaction catalyzed by CS involves a 1,4-antielimination of the 3-phosphate group and of the C-(6*proR*) hydrogen from EPSP, which is unusual and unique in nature [40-41]. The determination of CS structure from *Streptococcus pneumoniae* complexed with EPSP and FMN

[42] could elucidate or confirm the role of the flavin in the chemical reaction catalyzed by CS. Kinetics studies could show that reduced flavin plays a pivotal role in the reaction. In the structure of *S. pneumoniae*, EPSP molecule is stacked above the *si*-face of the isoalloxazine ring in an average distance of 3.3 Å. This close juxtaposition of FMN and EPSP supports earlier suggestions for a direct role of reduced FMN in the elimination reaction, such as a radical mechanism, in which electron transfer from the reduced cofactor to the substrate initiates C-O bond breakage of the phosphate group to yield a substrate-derived neutral radical [41, 43-47]. As there is not net redox change in the reaction, a reverse transfer of the transiently donated electron is required to complete the catalytic cycle [41]. This unusual reaction catalyzed by CS also makes it unique among flavin-dependent enzymes. The herbicide glyphosate, a potent inhibitor of the shikimate pathway enzyme EPSP synthase, has been shown to inhibit the *in vivo* growth of the apicomplexan parasites *Toxoplasma gondii*, *P. falciparum* and *Cryptosporidium parvum*. This effect has provided evidence for the presence of shikimate pathway in apicomplexan parasites. The CS was the first shikimate pathway enzyme identified in apicomplexan parasites by anaerobic assay [32]. The genome projects have identified the ORFs from *aroC* encoded CS gene by sequence homology in apicomplexan parasites. Many introns were found in *aroC* gene from *T. gondii*, but none was identified in *P. falciparum aroC* gene. The lack of introns allows the direct expression of *P. falciparum* CS in bacterial systems. Moreover, the *T. gondii aroC* is in a single copy, which allows gene deletion experiments. Other interesting feature in the *T. gondii aroC* gene is a potential GCN4 binding motif (TGACTC) in the 5' noncoding region. This specific upstream promoter element is present in genes that are regulated by the control-activator protein GCN4. In *S. cerevisiae* the expression of genes encoding shikimate pathway enzymes DAHP synthase (3-deoxy-D-arabino-heptulosonate 7-phosphate synthase) and the pentafunctional arom complexes (which catalyzes the steps second to sixth in the pathway) are regulated by GCN4 protein [48], which indi-

cates a possible regulation of shikimate pathway by CS expression in apicomplexan parasites.

In plants the shikimate pathway enzymes, like other nuclearencoded plastid enzymes, are post-translationally targeted to the plastid by an amino-terminal leader sequence. The apicomplexan parasites have a plastid-like organelle called apicoplast [49]. Although the apicoplast proteins from apicomplexan parasites also have leader sequences, there is no evidence that shikimate pathway enzymes are localized in the apicoplast. Neither the *T. gondii* CS gene nor *P. falciparum* CS gene have an obvious amino-terminal leader sequence similar to that found on CSs from higher plants, which strongly suggests that CS apicomplexan enzymes are in the cytosol, and not in the apicoplast [50]. The shikimate pathway in fungi also occurs in cytosol, and phylogenetic analysis has demonstrated that CSs from apicomplexan parasites are closer to fungal CSs than plants CSs, which is consistent with the idea of a nonapicoplast CS. Moreover, it also indicates a common ancestor to fungal and apicomplexan enzymes [51]. Studies with CS from *P. falciparum* have characterized the enzyme biochemically and immunologically. Confocal immunofluorescence and cellular fractions followed by Western blot analysis have shown that CS is located in the cytosol of this organism in different stages of infection, which is in agreement with the phylogenetic analysis. However, despite the close relationship with fungal proteins, the enzymatic assay has demonstrated that *P. falciparum* CS is monofunctional, as in plants and bacteria [52]. Therefore, the CS from *P. falciparum* has a combination of properties from plant and fungal enzymes and it seems to possess properties distinct from previously described CSs. It was found that both *T. gondii* and *P. falciparum* enzymes differ from other known CS in possessing a number of unique insertions. Although the CSs in apicomplexan parasites are larger in length, they share all of the amino acids to be highly conserved in CSs from other species [32]. The implication of a cytosolic location for the development of new drugs for apicomplexan parasites therapy is clear, as fewer membranes will have to be crossed by the drug in order to reach the enzyme. The inhibition of *P. falciparum* growth with a dsRNA encoding a 900 bp fragment of *aroC* has shown that CS is required for the normal growth of this pathogen [53], which suggests that CS is a viable target for chemotherapy. Moreover, two fluorinated analogs of shikimate: (6*R*)-6-fluoroshikimate and (6*S*)-6-fluoroshikimate have been shown to be inhibitors of the *P. falciparum* growth *in vitro* [54]. The possible function of apicomplexan CS in

shikimate pathway regulation makes this enzyme a promising target for drug development. Increasing our understanding of the apicomplexan shikimate pathway enzymes may expedite the discovery and design of new antiparasitic agents. Based on the structural knowledge of the enzymes of the shikimate pathway and their mechanisms of catalysis, it is possible to design potential inhibitors in a rational way against pathogenic bacteria, fungi and apicomplexan parasites.

## PURINE NUCLEOSIDE PHOSPHORYLASE

Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) is a well-studied enzyme of the purine salvage pathway. This protein has been submitted to intense structural studies, which generated detailed information about its active site and also the structural basis for its inhibition. A recent review summarizes the main results of these studies [55]. PNP is responsible for the inter-conversion between (deoxy) nucleosides and bases, which in turn may be converted to uric acid for excretion or reused in nucleic acid biosynthesis [56]. Fig. (5) shows the reaction catalyzed by PNP. This reversible cleavage occurs in the presence of inorganic phosphate ( $P_i$ ), of *N*-ribosidic bonds of purine nucleosides and deoxynucleosides, except adenosine, to generate ribose 1-phosphate and the corresponding purine base [57].

Several enzymes of the purine salvage pathway were identified in *P. falciparum* genome [57], including PNP, which suggests that the parasite must salvage purine bases from the mammalian host to survive [55]. This hypothesis needs further experimental evidence. Nevertheless, previously published studies indicated that *P. falciparum* growth is reduced by culturing in the presence of xanthine oxidase, which depletes both the extracellular medium and the erythrocyte of hypoxanthine, the major purine precursor for purine salvage [55, 59]. The sole pathway of hypoxanthine production in *P. falciparum* and in human erythrocytes is through the phosphorolysis of inosine to hypoxanthine, a reaction catalyzed by PNP [55, 60]. Therefore, it is tempting to speculate that inhibitors for *P. falciparum* PNP (PfPNP) might have the potential to prevent hypoxanthine salvage and interrupt the parasite life cycle, suggesting that PNP might be a protein target for drug development against protozoan parasites, at least for *P. falciparum*.

Sequence alignment of human PNP and *P. falciparum* and other apicomplexan indicates that PfPNP shares low degree of sequence identity (about 20%). PfPNP presents

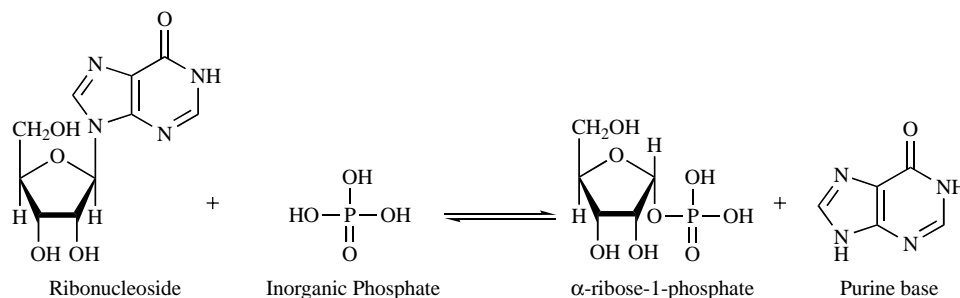
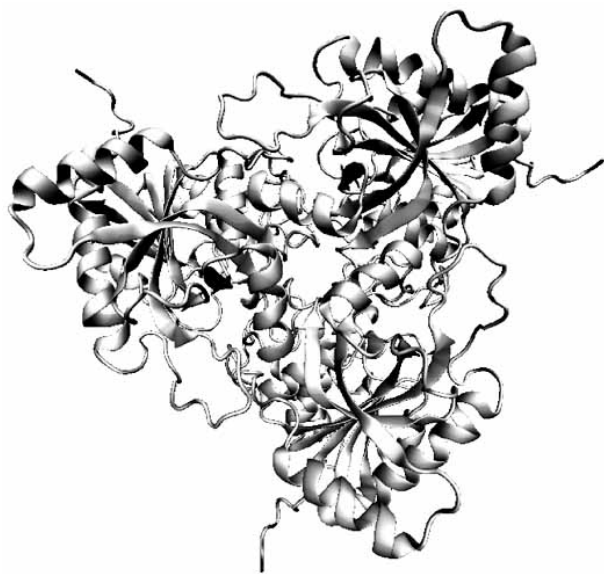


Fig. (5). Reaction catalyzed by PNP.

40% similarity to hexameric PNPs and 20% similarity to trimeric PNPs [61], and its three-dimensional structure resembles that of the *E. coli* PNP [62]. On the other hand, it does not accept adenosine as substrate as *E. coli* PNP does, and its catalytic activity with 2'-deoxynucleosides is low, as compared to human PNP. Therefore, PfPNP is not classified as member of trimeric nor hexameric PNP families [61].

PNPs from *Plasmodium lophurae* and *Toxoplasma gondii* have been briefly characterized, and the inhibitory activity of few compounds were tested [63, 64]. Indeed, some compounds were identified as inhibitors of *T. gondii* PNP, but they exhibited low affinity for the enzyme [65]. One of these inhibitors was Immucillin-H, which was shown to inhibit *T. gondii* PNP, but was unable to kill wild-type *T. gondii* [66].

Structure-based drug design is an interesting approach to rational development of enzyme inhibitors. HsPNP has been solved at 2.3 Å resolution, and its structure is shown in Fig. (6). It is characterized by two groups of internal, mixed  $\beta$ -sheets, joining to form a distorted  $\beta$ -barrel, surrounded by  $\alpha$ -helices [67-70]. A number of structures of human and bovine PNPs in complexes with several ligands have been published in the last few years, allowing to clearly identify residues that form the purine base, ribose, and phosphate (sulfate) binding sites, mapping the interactions that may play a role in binding and catalysis [71, 72-82]. Residues Asn243, Glu201, Phe200, Val217, Ala116, and Thr242 are responsible for the most frequently reported interactions with the base, either through hydrogen bond or van der Waals forces, whereas Ser33, Arg84, His86, and Ser220 are positioned to form hydrogen bonds with the sulfate group. The ribose binding site is mainly composed of aromatic residues, such as Tyr88, Phe200, Phe159, His257, and His86, as well as the non-aromatic but hydrophobic Met219 [67, 68, 78, 80, 81].



**Fig. (6).** Crystal structural of human PNP.

Recently, a computational analysis of the binary complexes involving PNP and several different ligands was able

to determine an empirical scoring function that can predict ligand-binding affinity [83-90]. This computational approach might guide future efforts to identify a new generation of PNP inhibitors.

## FINAL REMARKS

The knowledge obtained from many years of development of inhibitors against human CDKs may help in the discovery of a new generation of inhibitors against plasmodial CDKs. Several inhibitors of human CDKs have been reported, many have reached the nanomolar IC<sub>50</sub> level, and present good selectivity [2].

Analysis of the crystallographic structures of binary complexes of enzymes and inhibitor provides a snapshot of the structure, which in certain point of view omits relevant features of the intermolecular interaction between the inhibitor and the enzyme, which should be considered in the development of more specific inhibitors. These features may be accessed using molecular dynamics simulations. Recent studies identified important features of the intermolecular interactions between R-roscovitine and related inhibitors and CDK2 and CDK5 [91]. The preference of the both CDKs for R-roscovitine over the S enantiomer, was also identified by the analysis of molecular dynamics simulations. Furthermore, the computer simulations showed that the cause of the stronger affinity for the R enantiomer is the presence of an intermolecular hydrogen bond between R-roscovitine and the CDKs not observed in S-roscovitine complexes. Two amino acid mutations in the ATP-binding pocket of CDK5/R-roscovitine that favor binding-enhanced electrostatic contributions, making the inhibitor more effective for CDK5 than for CDK2, were also identified in the molecular dynamics simulations. This suggests that the roscovitine-like inhibitors can be improved by enhancing their electrostatic interaction with the CDKs [2]. The use of the same molecular dynamics protocols may be able to identify the major structural features important for the inhibition of kinases [92-94].

Structural studies of human CDK2 complexed with inhibitors, using biocrystallography and structural bioinformatics [2], were able to explain the specificity and potency of the human CDK inhibitors, and suggest the use of structure-based design and combinatorial library design may improve the chance of discovering new inhibitors of CDK2 and also plasmodial CDKs.

CS is an important enzyme of the shikimate pathway, having a critical role in metabolism of microorganisms. The determination of various structures of this protein would contribute to an understanding of the details of the mechanism of catalysis and reveal the role played by important active site residues, such as the His10 and His110 in *S. pneumoniae*. Future research could also contribute to the understanding of the unprecedented flavin-dependence in the reaction mechanism, which can be through of site-directed mutagenesis and the utilization of substrate and cofactor analogues. The major challenge today, is to precisely determine the role of NAD(P)H in bifunctional CSs and the residues that are involved in their binding. On the other hand, today, major new opportunities in structure-based drug design are possible due the determination of CS structure com-

plexed with both substrate and cofactor. This knowledge could contribute to the development of new drugs including the use of combinatorial chemistry and virtual screening methods that could render inhibitors more specific to compete with substrate and cofactor in binding to the enzyme [95-104].

Purine nucleoside phosphorylases identified in apicomplexan genomes are targets for treatment of parasite-caused diseases, such as malaria and toxoplasmosis. Analysis of crystallographic structures of human PNP and PfPNP strongly indicates that the differences in the active sites of human and parasite enzymes may be useful to develop specific inhibitors of these enzymes, which could kill parasites with little effect on the human host.

Finally, one of the most defying challenges in the Post-genomic Era is the understanding of protein networks and their complexation with small drugs. Information obtained from the sequencing of parasite genomes, integrated with structural studies of protein targets will pave the way for identification of a new generation of drugs against protozoan parasites.

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#### ABBREVIATIONS

|      |   |   |
|------|---|---|
| ATP  | = | Adenosine triphosphate                      |
| cdc  | = | Cell division cycle                         |
| CDK  | = | Cyclin-dependent kinase                     |
| crk  | = | cdc2-related kinase                         |
| CS   | = | Chorismate synthase                         |
| DAHP | = | 3-deoxy-D-arabino-heptulosonate 7-phosphate |
| DNA  | = | Deoxyribonucleic acid                       |
| EPSP | = | 5-enolpyruvylshikimate- 3-phosphate         |
| FMN  | = | Flavin mononucleotide                       |
| IC50 | = | Inhibition concentration 50%                |
| mrk  | = | MO15-related kinase                         |
| ORF  | = | Open reading frame                          |
| PDB  | = | Protein data bank                           |
| Pf   | = | <i>Plasmodium falciparum</i>                |
| PK   | = | Protein kinase                              |
| PNP  | = | Purine Nucleoside Phosphorylase             |
| Tg   | = | <i>Toxoplasma gondii</i>                    |

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