Exploring Potential Drug Targets in *Plasmodium falciparum* 3D7 Through Computational Analysis

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Abstract

Finding potential drug targets by analyzing the genomic data is an essential step in developing potential therapeutics against malaria parasites. In this work, we focused on the 2,670 essential genes listed in the Database of Essential Genes (DEG) to discover possible therapeutic targets in Plasmodium falciparum 3D7. There are several screening methods such as the BLAST (Basic Local Alignment Search Tool) algorithm to identify the human non-homologous essential genes of the parasite.

Further, the genes obtained were searched in the Panther database and were analyzed for their metabolic pathways in the Kyoto Encyclopedia for Gene and Genome (KEGG) database. Five key genes were identified from the above analysis. A subsequent search of the DrugBank database revealed that no existing (approved or experimental) drug molecules for the enzyme pyridoxal 5'-phosphate synthase subunit (pdx2) are available. Further prediction of the protein-protein interaction network, along with the literature report, indicated that the enzyme pdx2 plays a pivotal role in the biosynthesis of pyridoxal 5'-phosphate (PLP) in the parasite.

Keywords: *Plasmodium falciparum* 3D7, *in silico* methods, drug target identification, database of essential genes, antimalarial, computational methods, Pyridoxal 5'-phosphate synthase subunit.

Introduction

Plasmodium falciparum is a protozoan parasitic that causes the most severe form of malaria in humans. The mode of transmission of this parasite to humans is through the biting activity of infected female *Anopheles* mosquitoes. Once inside the human host, the parasite travels to the liver where it undergoes replication and maturation before entering the bloodstream and infecting red blood cells[17,36]. Despite many advances in the control, malaria remains a major public health challenge, especially in sub-Saharan Africa and Asia where it is endemic.

Malaria remains a significant global health burden with an estimated 229 million cases and 409,000 deaths reported in 2019, predominantly in sub-Saharan Africa (World Health Organization, 2020). Drug treatment is a significant and common measure for the control of malaria. One of the challenges in the chemotherapy of malaria is treatment failure which is caused by various factors of which drug resistance plays a major role. Chloroquine was the gold standard for the treatment of malaria for several years until it became ineffective because of resistance[7]. Accurate diagnosis by several diagnostic aids is an essential criterion for effectively treating the disease. Also, the treatment strategies vary due to the severity of infection, hence integrated therapies are commonly adopted to combat the disease.

However, the development of a therapeutic molecule by experimental methods as well as diagnostic procedures is a challenging task[21,34]. Currently, the *in silico* methods have emerged and have been used to identify novel drug candidates in *Plasmodium falciparum*[3,24,28]. To understand the malarial infection, the genome *Plasmodium falciparum* strain 3D7 has been sequenced and studied thoroughly. The Sanger Institute and The Institute for Genomic Research (TIGR) collaborated to publish the genomic sequence of this parasite.

The genome consists of 14 chromosomes having approximately 23 million base pairs that contain 5,300 protein-coding genes, that code a wide range of proteins used in several metabolic pathways essential for its survival. This comprises of genes associated with drug resistance, host cell invasion and other important metabolisms of the parasite [15]. Using comparative genomics research and genomic analyses, several important characteristics of the *P. falciparum* 3D7 genome have been determined. The erythrocyte membrane protein 1 (EMP1) family, which is involved in immune evasion and host cell invasion, is one of the examples of the many genes involved in antigenic diversity encoded by the genome[18]. Several gene families linked to drug resistance are also present in the genome, including those that encode transporters and enzymes necessary for drug metabolism.

For example, comparative genomics studies revealed that the erythrocyte membrane protein 1 (EMP1) family, encoded by the parasite plays a vital role in the host cell invasion process[18]. Similarly, the genome contains several gene families encoding transporters and enzymes associated with drug resistance metabolism which have been identified.

Furthermore, the comparative genomic analyses have revealed insights into the evolutionary history and genetic diversity of *P. falciparum*. The researchers have identified some conserved gene families that contribute to the parasite's adaptation to its host[1,13]. Identification of novel therapeutic targets in *Plasmodium falciparum* 3D7 will lead

to the creation of effective new antimalarial drugs. The possible drug targets of *Plasmodium falciparum* 3D7 can be predicted by using sophisticated *in silico* methods by using specific databases, algorithms and software tools by analyzing the proteome, genome and metabolic pathways data[4,25]. Several research investigations have revealed potential therapeutic targets in *P. falciparum* 3D7 which are associated with drug resistance, host cell invasion, transporters and enzymes involved in key metabolic pathways[2,11,33]. The present work aimed to analyze all the available essential genes of *Plasmodium falciparum* 3D7 in the DEG database. *In silico* strategies and techniques were employed in the identification of potential drug targets in *P. falciparum* 3D7 to offer valuable insights for researchers.

Material and Methods

Retrieval of essential genes of *Plasmodium falciparum* 3D7: Searching of the essential genes in *Plasmodium falciparum* 3D7 was performed in the Database for Essential Genes (DEG) database. DEG is a comprehensive repository of genes such as protein-coding genes and non-coding RNAs that are vital for the survival of organisms. The latest version of the database (updated September 1, 2020) (available freely at https://tubic.org/deg/public/index.php) was used for searching the genes

Screening criteria for novel drug target identification: After retrieval of all the available essential gene records of *Plasmodium falciparum* 3D7, in the database, an initial filtration process was conducted. This involved screening of genes based on their encoded protein sequence length (ranging from 200-1000 amino acids in length). Additionally, in the dataset genes that are categorized as hypothetical, lacking a specific gene ID, tagged as predicted, or associated with unknown functions was also excluded from further consideration.

Prediction of sequence homology against the human genome: After screening, the resulting protein sequences were searched against the human genome by using the BLAST tool and results were accessed based on their expectation (E) value of matching pairs. To perform the task, the human proteome sequence data was obtained from the UniProt database (https://www.uniprot.org/), accessed on 10.01.2024. Local BLAST searches were conducted with the screened sequences of *P.falciparum* 3D7 by using the BLAST2GO program (http://www.blast2go.com/b2ghome) with default parameter settings[8]. From the BLAST output, only those (essential protein of the parasite) proteins showing no homology with the human genome were selected for further study.

Functional annotation of the sequences: After the screening process, the sequences were analyzed by searching the Panther database (https://www.pantherdb.org/). The Panther (Protein Analysis Through Evolutionary Relationships) database is a comprehensive resource used primarily for protein classification and functional annotation purposes. A wide range of tools and algorithms were designed for the annotation of protein sequences, comprising of classification, phylogenetic analysis and functional annotation [30-31]. In this search, the proteins that do not possess any annotation term, were subsequently removed from the dataset for further study.

Protein-protein interaction (PPI) analysis: The potential targets obtained from the previous steps were subjected to protein-protein interaction (PPI) analysis by using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database available online at https://string-db.org/. The STRING database integrates experimental data, computational predictions and other curated information from different sources for the construction of protein-protein interaction network. The output network properties of PPI enable the researchers to explain protein interactions and functional associations and hence play an important role in studying system-based biological systems and disease mechanisms[29].

KEGG pathway analysis and DrugBank database search: The selected proteins selected from the PPI were subjected to further analysis in KEGG pathways by searching their role in metabolic pathways of *Plasmodium falciparum* 3D7. The KEGG (Kyoto Encyclopedia of Genes and Genomes) database contains metabolic pathways across various organisms including metabolic signaling and their regulatory path. Additionally, the KEGG database contains information about the molecular interactions and relationships among genes, proteins, metabolites and other biomolecules implicated in specific biological processes[19]. Similarly, the DrugBank database provides extensive search options for target compounds, resulting in the experimental and approved drug molecules against the target. In this study, potential drug molecules against the target were also obtained and analyzed using DrugBank (https://go.drugbank.com/targets).

Results

Retrieval and screening process of essential genes from DEG: The database of essential genes contains a total of 2670 sequences for *Plasmodium falciparum* 3D7, which were retrieved for analysis. Screening methods were applied to identify potential drug targets from the selected parasite genes[20,37]. The screening methods followed in the present work are shown in figure 1. Initially, screening criteria were employed to select protein sequences with a length greater than 200 and less than 1000 amino acids. Additionally, genes obtained as predicted, hypothetical, unknown function, or lacking a gene ID in the database were excluded.

The initial screening process resulted in 566 genes out of a total of 2670 genes used in the study. The BLAST P program output of 566 genes against the total reviewed protein sequences of *Homo sapiens* from the Uniprot database yielded 251 non-homologous sequences.

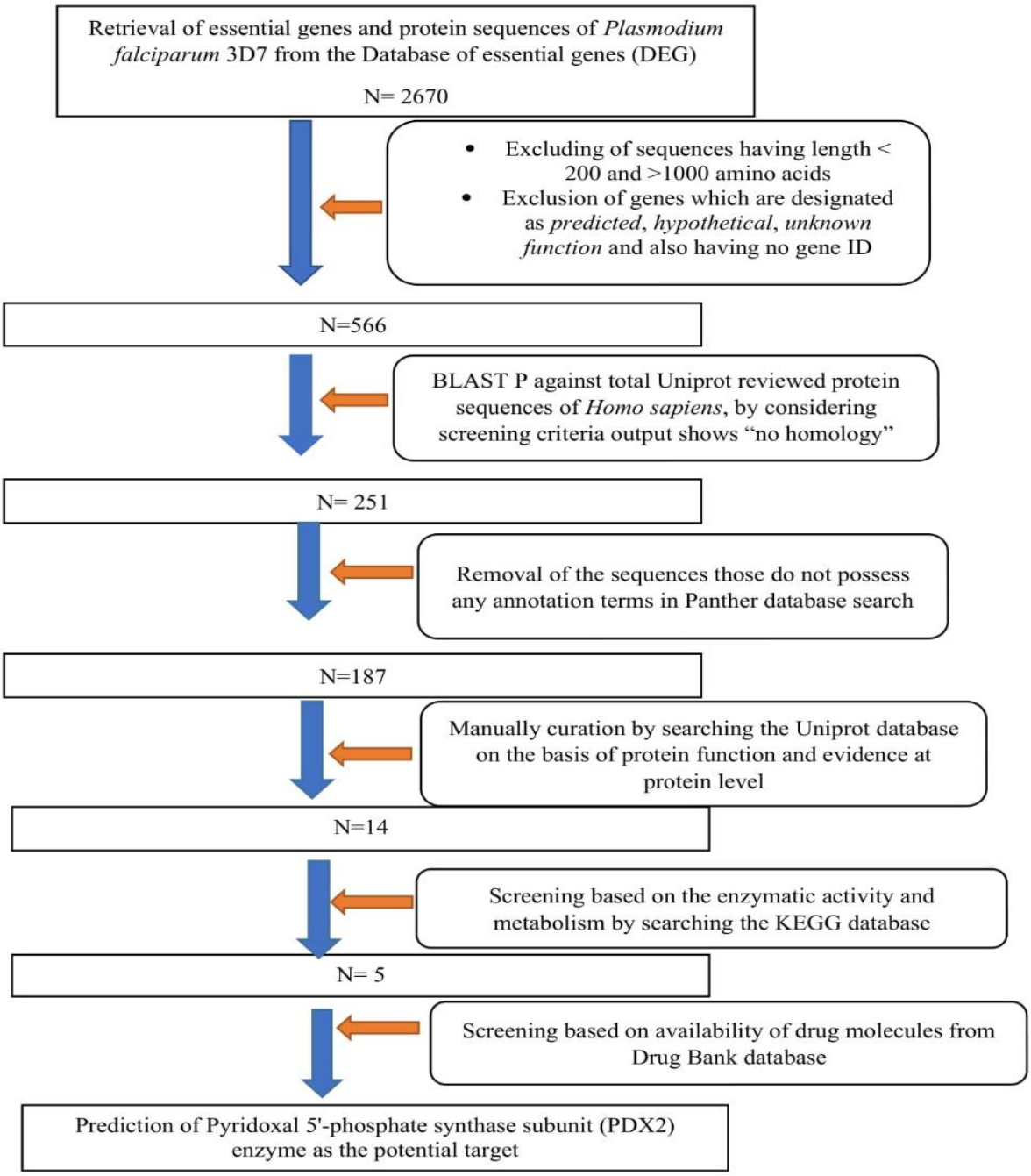

Figure 1: Showing the screening criteria and number of genes (N) obtained after screening

Because of annotation of the 251 sequences by Panther database, a total of 187 annotated sequences were obtained. Further, manual functional analysis was performed through KEGG and UniProt entry analysis to ascertain protein evidence levels, yielding 14 potential drug candidates. Among these, only five gene products have the KEGG functional annotation terms (highlighted in bold in table 1). Subsequently, the amino acid sequences of the five selected proteins were retrieved and target based search was performed in the DrugBank database.

A total of 16 interacting drug molecules were identified and are presented in table 2. The drug search for the target Enoyl-Acyl Carrier Reductase enzyme yielded the highest number (nine) of experimental drug molecules including one approved drug. Conversely, no approved drug molecules were identified for the target Pyridoxal 5'-phosphate synthase subunit PDX2. The PDX2 enzyme plays a vital role in the metabolism of vitamin B6 (pyridoxine) in the case of *Plasmodium falciparum* 3D7.

Since vitamin B6 serves as an essential cofactor in numerous enzymatic reactions and it is essential for various cellular processes within the parasite, hence inhibition of PDX2 could potentially interfere to produce an active form of the molecule. In this study, the enzyme PDX2 was proposed as a potential target in *Plasmodium falciparum* 3D7[6,9].

Protein-protein interaction (PPI) and functional annotation of the PDX2: The PPI network study was conducted using the STRING server-generated interacting partners of the PDX2 enzyme, as depicted in figure 2. Within the network, it was observed that the pdx2 gene exhibits strong interactions with its counterpart enzyme, pdx1, which is involved in the biosynthesis of the vitamin B6 pathway in the parasite.

The enzymatic activities of the enzyme pyridoxal 5'-phosphate synthase subunit Pdx1 involve the formation of pyridoxal 5'-phosphate from the substrate ribose 5-phosphate (RBP), glyceraldehyde 3-phosphate (G3P) and ammonia. The ammonia required for this process is generated by the catalytic activity of the enzyme PDX2. [23] The Pyridoxal 5'-phosphate synthase subunit Pdx2 enzyme plays a pivotal role in catalyzing the hydrolysis of glutamine to glutamate and ammonia utilized for the biosynthesis of pyridoxal 5'-phosphate. The resulting ammonia molecule is then directed to the active site of Pdx1. This catalytic mechanism is due to the enzyme pyridoxal kinase (EC: 2.7.1.5) which is associated with the Pdx2 enzyme as depicted in figure 3.

Table 1
Selected 14 targets with enzymatic and KEGG functional information

S.N.	DEG ID	UNIPROT ID	Name of the protein	Enzymatic function	KEGG metabolism	Amino acid length	3D structure availability
1	**DEG20301494**	Q8IIK4	Pyridoxal 5'-phosphate synthase subunit PDX2	lyase	Vitamin B6 metabolism Biosynthesis of co-factors	219	YES
2	**DEG20300100**	P62368	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, apicoplast	synthase	isoprenoid biosynthetic pathway	240	YES
3	**DEG20302622**	Q8IKG4	1-deoxy-D-xylulose 5-phosphate reductoisomerase, apicoplastic	oxidoreductase	Isoprenoid biosynthesis	488	YES
4	DEG20301159	Q8I2S0	Inactive lipoate--protein ligase 2	NIL	Lipoic acid metabolism Biosynthesis of cofactors	384	YES
5	**DEG20300635**	C6KSZ2	Enoyl-acyl carrier reductase	reductase	Fatty acid metabolism and co-factor synthesis	432	YES
6	DEG20300624	C6KSX0	Merozoite surface protein P12	NIL	NIL	347	YES
7	DEG20300556	Q8I3H4	Apicoplast TIC22 protein	NIL	NIL	279	YES
8	DEG20302171	Q8IDM6	Nucleoside transporter 1	transporter	NIL	422	YES
9	DEG20301385	Q8IJ74	Haloacid dehalogenase-like hydrolase	endoribonuclease	NIL	288	YES
10	DEG20300444	Q8I423	Merozoite surface protein P38	NIL	NIL	349	NO
11	DEG20302317	Q8IM30	Pentatricopeptide repeat-containing protein 1, apicoplast	NIL	NIL	608	NO
12	DEG20301196	C0H582	Inactive adenylate kinase	nucleotide kinase	NIL	263	NO
13	**DEG20301912**	Q8I4R4	Chitinase	Glycosidase	Amino sugar and nucleotide sugar metabolism	378	NO
14	DEG20300312	Q8I1X7	Rhoptry surface protein CERLI2	NIL	NIL	579	NO

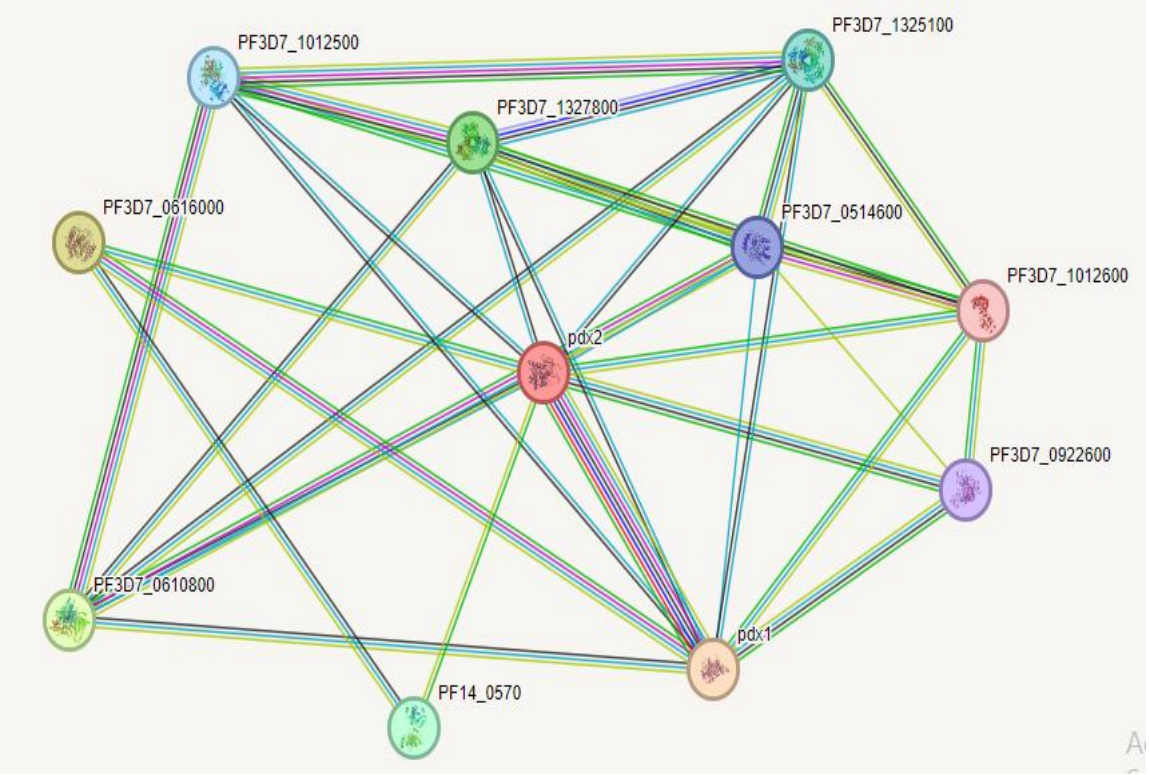

Figure 2: PPI network of pdx2 enzyme

(PF14_0570: NAD(P)H-hydrate epimerase, PF3D7_0514600: Ribose-5-phosphate isomerase, PF3D7_0610800 : Transketolase, PF3D7_0616000: Pyridoxal kinase, PF3D7_0922600: Glutamine synthetase, putative, PF3D7_1012500: Phosphoglucomutase, putative, PF3D7_1012600: GMP synthase, PF3D7_1325100: Phosphoribosylpyrophosphate synthetase, PF3D7_1327800: Ribose-phosphate pyrophosphokinase, putative, pdx1: Pyridoxal 5'-phosphate synthase subunit Pdx1, Pyridoxal 5'-phosphate synthase subunit Pdx2)

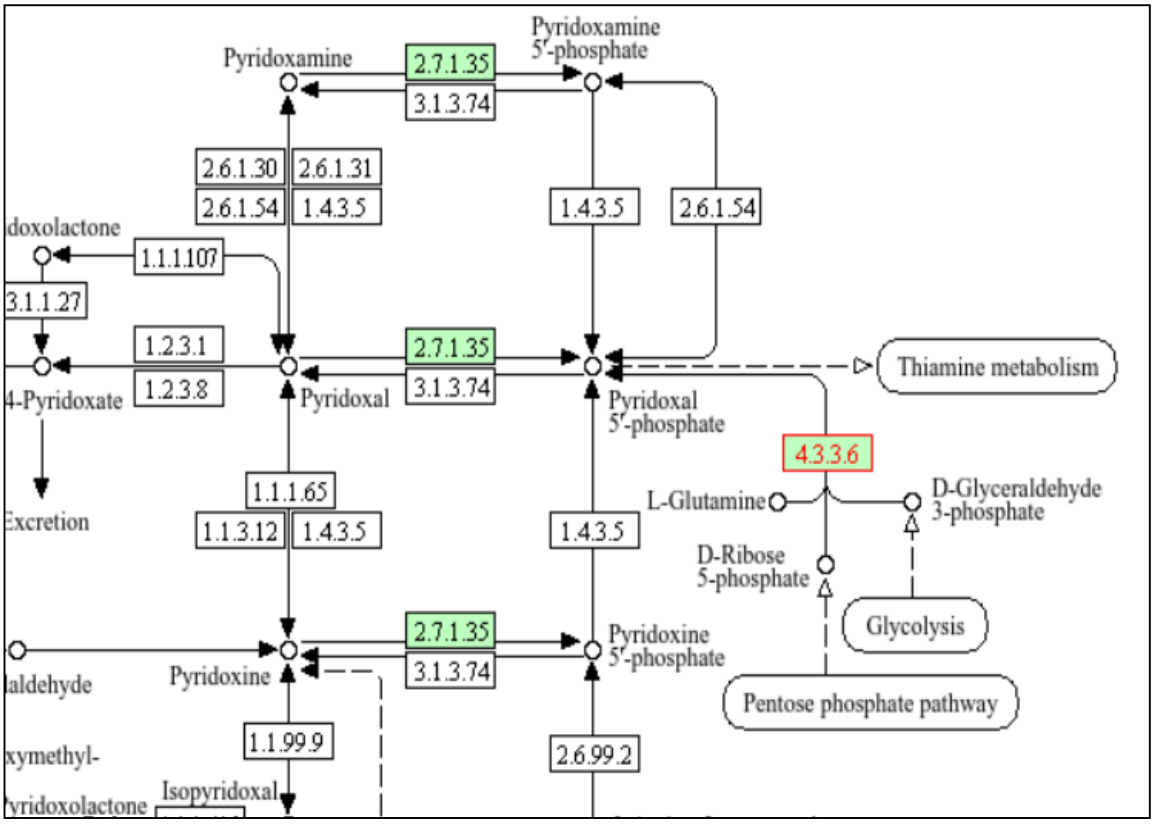

Figure 3: Screenshot showing involvement of major enzymes of Vitamin B6 pathway of *Plasmodium falciparum* 3D7 obtained from KEGG database (PDX2 enzymes highlighted in red)

Table 2
Selected 5 targets with interacting drug molecules obtained from Drug bank search

S.N	Uniprot ID	Name of the protein	Interacting drug molecules from drug bank target search	Drug Group
1	Q8IIK4	Pyridoxal 5'-Phosphate Synthase Subunit Pdx2	Nil	N.A
2	P62368	2-C-Methyl-D-Erythritol 2,4-Cyclodiphosphate Synthase, Apicoplast	Farnesyl Diphosphate	Experimental
3	Q8IKG4	1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase, Apicoplastic	1-Deoxy-D-Xylulose 5-Phosphate	Experimental
			Fosmidomycin	Experimental, Investigational
			[{(5-Chloro-2-Pyridinyl)Amino} Methylene]-1,1-Bisphosphonate	Experimental
4	C6KSZ2	Enoyl-Acyl Carrier Reductase	Indole Naphthyridinone	Experimental
			3-(6-Aminopyridin-3-Yl)-N-Methyl-N-[(1-Methyl-1h-Indol-2-Yl)Methyl]Acrylamide	Experimental
			3-[(Acetyl-Methyl-Amino)-Methyl]-4-Amino-N-Methyl-N-(1-Methyl-1h-Indol-2-Ylmethyl)-Benzamide	Experimental
			1,3,4,9-Tetrahydro-2-(Hydroxybenzoyl)-9-[(4-Hydroxyphenyl)Methyl]-6-Methoxy-2h-Pyrido[3,4-B]Indole	Experimental
			Beta-D-Glucose	Experimental
			2-(Toluene-4-Sulfonyl)-2h-Benzo[D][1,2,3]Diazaborinin-1-Ol	Experimental
			Triclosan	Approved, Investigational
			6-Methyl-2(Propane-1-Sulfonyl)-2h-Thieno[3,2-D][1,2,3]Diazaborinin-1-Ol	Experimental
			4-(2-Thienyl)-1-(4-Methylbenzyl)-1h-Imidazole	Experimental
5	Q8I4R4	Chitinase	Argifin	Experimental
			Argadin	Experimental

Discussion

PLP biosynthetic pathway in *P. falciparum* involves several essential enzymes, having specific roles of action. One such enzyme is Pdx1 (Pyridoxine 5'-phosphate synthase subunit 1) which catalyzes the ribose 5-phosphate and glyceraldehyde 3-phosphate into pyridoxine 5'-phosphate, an intermediate in PLP biosynthesis process. Similarly, the enzyme pdx2 (Pyridoxal 5'-phosphate synthase subunit 2), catalyzes the hydrolysis of glutamine to glutamate and ammonia, essential steps in the synthesis of pyridoxal 5'-phosphate. The resulting ammonia molecule is then utilized by Pdx1. Another enzyme pdxK (Pyridoxal kinase) facilitates the phosphorylation of pyridoxal, converting it into PLP. All three enzymes synergistically catalyze to produce the critical cofactor PLP involved in various essential metabolic processes of the parasite. The PLP acts as a cofactor for several enzymes such as aminotransferases and decarboxylases involved in amino acid metabolism.

Furthermore, PLP is necessary for the functioning of the enzyme aminolevulinic acid synthase (ALAS), which is involved in the heme biosynthesis pathway and is essential to the survival of malaria parasites. PLP also serves as a cofactor for enzymes involved in nucleotide biosynthesis such as serine hydroxymethyltransferase (SHMT) that played crucial roles in the synthesis of DNA and RNA. PLP co-factor-based enzymes, such as glutathione synthetase, catalyze the formation of glutathione from its precursor molecules and contribute to the defense system of the parasite against reactive oxygen species (ROS)[12,14,32].

Due to the essential role of pdx2 in the metabolic processes of the parasite, it can be used as a potential drug target for the development of effective antimalarial compounds. Therefore, inhibition of the Pdx2 enzyme activity can disrupt the PLP biosynthesis process. This may hamper the growth and survival of the parasite.

Barra et al[5,6] conducted a study to understand the impact of pyridoxal 5'-phosphate (PLP) synthesis inhibition on parasite proliferation. Their study highlighted the designing of novel inhibitor/ substrate analogs to target the pathway. Rokkam et al[27] in their research employed both *in vitro* and computational docking methods in combination to investigate the impact of the molecule diarylidene-N-methyl-4-piperidones (DANMPs) molecules on inhibiting the Pdx1enzymatic activity. Their study findings suggested that the DANMPs can be used as novel anti-plasmodial agents inhibiting the PfPLP synthase pathway.

Pina et al[26] investigated the catalytic mechanism of Pdx2 by the use of computational quantum mechanics/molecular mechanics (QM/MM) methods. Their research output concluded that the transition state analog inhibitor molecule of the Pdx2 enzyme of the malaria parasite can be developed to inhibit the ammonia production utilized for pyridoxal 5'-phosphate (PLP) biosynthesis[26].

Moorthy et al[22] synthesized and characterized a series of the molecule 3,5-diarylidenetetrahydro-2H-pyran-4(3H)-ones (DATPs) molecules. By combining both *in vitro* and *in silico* analysis, they identified a specific molecule, 3,5-bis(quinolin-4-ylmethylene) tetrahydro-2H-pyran-4(3H)-one, capable of inhibiting the activity of PfPdx1 enzyme.

Therefore, the *in silico* approach offers a promising approach for creating novel antimalarial medications by developing specific inhibitors against the PLP synthetic pathway of *P. falciparum.* However, the experimental basis of biochemical and functional characterization of these enzymes is crucial in enhancing our understanding regarding the pathogenesis of malaria parasite[16,35].

Conclusion

In silico methods have emerged as a promising approach to identifying potential drug targets through computational analyses of genomic data of pathogens. This research work has focused on the use of databases containing essential genes (DEG), which led to the identification of a set of essential genes specific to *Plasmodium. falciparum* 3D7, out of a total of 2670 essential genes. By employing various *in silico* screening techniques such as BLAST P and Panther, as well as manual methods, the enzyme Pyridoxal 5'-phosphate synthase subunit (pdx2) was predicted as the potential one.

Further exploration of protein-protein interaction networks and metabolic pathway analysis highlighted the role of this enzyme in the biosynthesis of pyridoxal 5'-phosphate, an important cofactor involved in several key metabolic pathways of the parasite. Additionally, from the DrugBank database search, no approved/experimentally approved drug molecules were obtained for this target. Hence, the subsequent immediate approach is to perform molecular docking and molecular dynamics simulation to identify the potential inhibitors against the enzyme.

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