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Screening Potential Inhibitors of *Plasmodium falciparum* Purine Nucleoside Phosphorylase (PfPNP) Using a Computational Approach

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ABSTRACT

Background: Malaria is a mosquito-borne disease caused by species of *Plasmodium*, with the most severe infections attributed to *Plasmodium falciparum*. This species accounts for 75% of malaria cases, while the remaining 25% are caused by other *Plasmodium* species. Malaria remains a major global cause of mortality and morbidity. Although numerous antimalarial drugs are currently in use, the continuous emergence of drug-resistant parasites necessitates the ongoing search for alternative therapies. One critical target for antimalarial drug development is *Plasmodium falciparum* purine nucleoside phosphorylase (PfPNP), a key enzyme in purine biosynthesis. Inhibiting this enzyme could disrupt purine metabolism, thereby halting parasite growth and leading to purine-deprivation-induced death. This study focused on docking various FDA-approved drugs with PfPNP. **Methods:** The three-dimensional structure of PfPNP was retrieved from the Protein Data Bank. A total of 250 FDA-approved drugs were obtained from ChemSpider and docked using the PatchDock server. Drugs with the highest number of interactions were selected for further analysis. The interactions were visualized using GS Viewer and LIGPLOT+. **Results:** The analysis revealed various interactions, including covalent bonds, hydrogen bonds, and hydrophobic interactions with the active site residues of the target protein. Among the tested drugs, Amoxicillin and Streptomycin exhibited the maximum number of interactions, followed by Ibuprofen, Rifaximin, and Norfloxacin. **Conclusions:** The docking results indicate that these drugs interact significantly with PfPNP and have the potential to induce metabolic disruptions, particularly in purine biosynthesis, leading to the death of *Plasmodium falciparum* through purine deprivation. Further experimental validation is required to confirm their antimalarial efficacy.

INTRODUCTION

Malaria is a parasitic disease caused by a group of protozoans of the genus *Plasmodium*, transmitted by the bite of mosquitoes of the genus *Anopheles*, which breed in a wide variety of surface waters [1, 2]. Parasites require temperatures above 16 ºC to develop in mosquitoes; therefore, in tropical climates, their cycle can be continuous, while in temperate climates, transmission is seasonal [3, 4].

Infection is more frequent in rural areas, suburban areas, and in large non-urbanized human concentrations, where living conditions are deteriorated by poverty, labour exploitation, and social marginalization [5, 6]. In this environment, sanitary conditions are usually very poor, and protection against mosquitoes is minimal [4, 7].

Malaria manifests itself mainly with intermittent fevers, headaches, muscle aches, diarrhoea, and weakness [3, 7]. There are four species of human parasites that cause the disease: *Plasmodium malariae* causes the so-called "quartan fevers, "*Plasmodium vivax* and *Plasmodium ovale* cause "benign tertian fevers," and *Plasmodium falciparum* causes "severe tertian fevers" that can cause death due to severe anaemia, brain damage, or kidney failure [3, 5]. This disease causes between 1.5 and 2.7 million deaths a year [8, 9].

Of the four species of malaria parasites, only *Plasmodium falciparum* usually causes severe forms of the disease and death [5, 6, 9]. Both *P. vivax* and *P. ovale* cause acute attacks that temporarily incapacitate and debilitate the patient but rarely lead to death, and *P.*

malariae is generally milder and can persist as an asymptomatic infection for several decades [3, 4].

Drug resistance is an extreme challenge for controlling malaria nowadays and plays an important role in the spread of malaria to new regions [10, 11]. It has also led to the recurrence of malaria in countries or areas where the disease was previously eradicated. Resistance to antimalarial drug treatment has been shown by two *Plasmodium* species, *P. falciparum* and *P. vivax*. *P. falciparum* has developed resistance to almost all antimalarial drugs [10, 12].

In malarial parasites, extensive replication of DNA occurs during the liver and blood stages, and there is a great requirement for purine nucleotides. *P. falciparum* lacks the de novo pathway for the biosynthesis of purine nucleotides and highly relies on the salvage pathway to synthesize purines by utilizing the host's supply to meet its requirements [13, 14]. In *Plasmodium*, the purine metabolism pathway has been identified as an essential drug target for antimalarial drugs [14, 15]. The enzyme Purine Nucleoside Phosphorylase (PNP) plays a key role in the salvage pathway, helping to provide purines for DNA and RNA during cell growth by using nucleosides from the host cell. PNP catalyses the reaction of inosine to ribose 1-phosphate and hypoxanthine, which is the main precursor for the salvage pathway [14-16]. The current study was designed to find potential inhibitors of Purine Nucleoside Phosphorylase from *Plasmodium falciparum* (PfPNP) using computational tools.

MATERIALS AND METHODS

Data Retrieval and Visualization

The Purine Nucleoside Phosphorylase from *Plasmodium falciparum* (PfPNP) sequence was retrieved from Protein Database [17]. Subsequently, its 3D structure with a resolution of 2.0 Å was retrieved from RCSB [18]. The PDB ID of the selected protein was 2BSX.

The protein structure was visualized using the DS Visualizer [19], which revealed one protein chain (A), consisting of 1 ligand, NOS 1228 (inosine), and 105 water molecules. The active sites of the protein model were determined for docking studies. The active sites identified were HIS 7, ARG 45, SER 91, CYS 95, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, and TRP 212 in the protein.

For drug-protein interactions, GS Viewer was used [20]. Covalent interactions were indicated by a purple line, hydrogen bonds by a green line, and hydrophobic interactions by a reddish line.

About 250 drugs were retrieved from ChemSpider (www.chemspider.com) and were docked with the protein using the PatchDock server [20]. The top results were analysed and checked in DS Visualizer [19], and the docked amino acids and 2D ligand-protein interactions were observed using the LigPlot+ [21].

RESULTS

In this study, FDA approved drugs were docked with *Plasmodium falciparum* Purine Nucleoside Phosphorylase (PfPNP) to find alternate antimalarial drugs. By screening two hundred and fifty drugs, only five drugs have shown promising result.

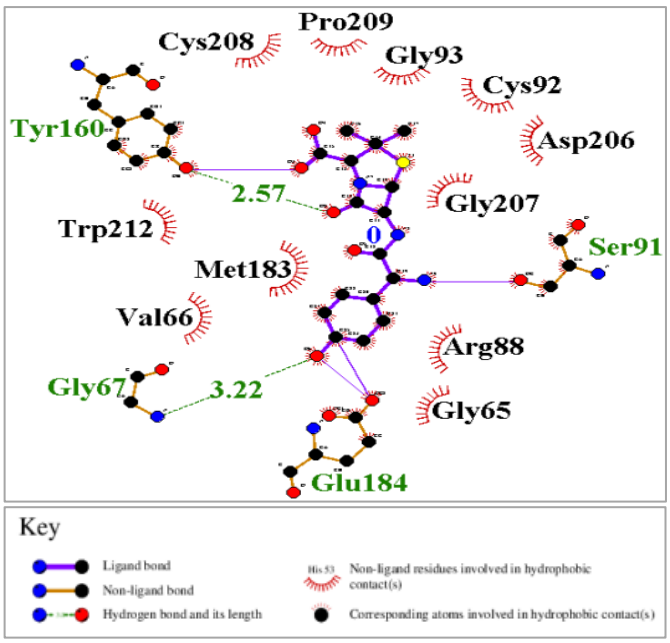
Docking of Amoxicillin with PfPNP

The docking of the drug amoxicillin with PfPNP showed fifteen different interactions, including hydrogen bonds, covalent bonds, and hydrophobic interactions (Table 1). Of these interactions, three residues formed covalent bonds: GLU 184 formed two covalent bonds, and TYR 160, and SER 91, each formed one covalent bond. All these residues are active site residues. Two hydrogen bonds were formed (TYR 160, GLY 67), with TYR 160 being an active site residue. Additionally, 11 hydrophobic interactions were noted, with the active site residues being GLY 93, CYS 92, ASP 206, MET 183, and TRP 212 (Figure 1).

Table 1.
Details of interactions between Ligands/Drugs and Plasmodium falciparum Purine Nucleoside Phosphorylase (PfPNP)

Ligand/Drug	Active Site Residues	Hydrogen Bonds	Covalent Bonds	Hydrophobic Interactions
Amoxicillin	HIS 7, ARG 45, SER 91, CYS 92, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, TRP 212	TYR 160, GLY 67	GLU 184, TYR 160, SER 91	GLY 93, CYS 92, ASP 206, MET 183, TRP 212
Rifaximin	HIS 7, ARG 45, SER 91, CYS 92, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, TRP 212		MET 183, TYR 160, ASP 206, SER 91	TRP 212, GLY 93, GLU 184
Ibuprofen	HIS 7, ARG 45, SER 91, CYS 92, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, TRP 212	MET 183	MET 183	GLU 182, SER 91, GLU 184
Streptomycin	HIS 7, ARG 45, SER 91, CYS 92, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, TRP 212	TRP 212	ASP 206, SER 91	GLU 84, CYS 92, GLY 93
Norfloxacin	HIS 7, ARG 45, SER 91, CYS 92, GLY 93, TYR 160, MET 183, GLU 184, ASP 206, TRP 212	GLU 184, TRP 212	ASP 206, SER 91	CYS 92, MET 183, TYR 160

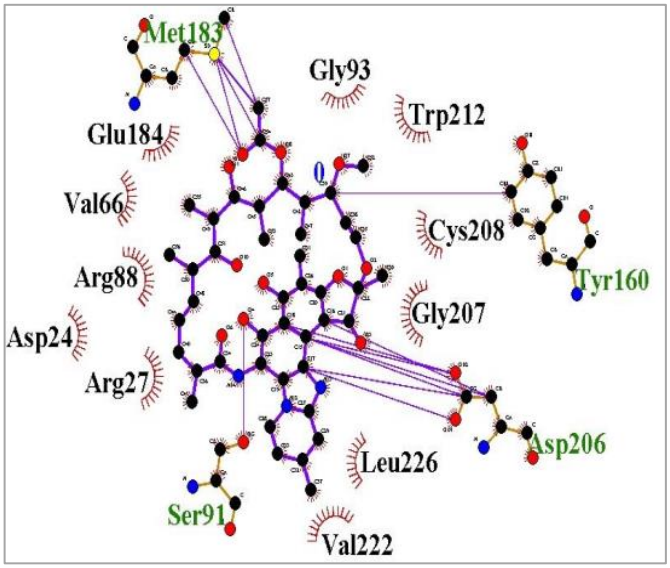
Figure 1
LigPlot+ illustration of Amoxicillin and PfPNP Interactions.



Docking of Rifaximin with PfPNP

Docking of Rifaximin showed numerous interactions including covalent bonds and hydrophobic interactions with the target protein (Table 1). Docking result shown that four active site residues (MET 183, TYR 160, ASP 206 and SER 91) formed covalent bonds. MET 183 and ASP 160 formed 5 and 7 covalent bonds respectively. Eleven hydrophobic interactions were formed in which active site residues were TRP 212, GLY 93 and GLU 184 (Figure 2).

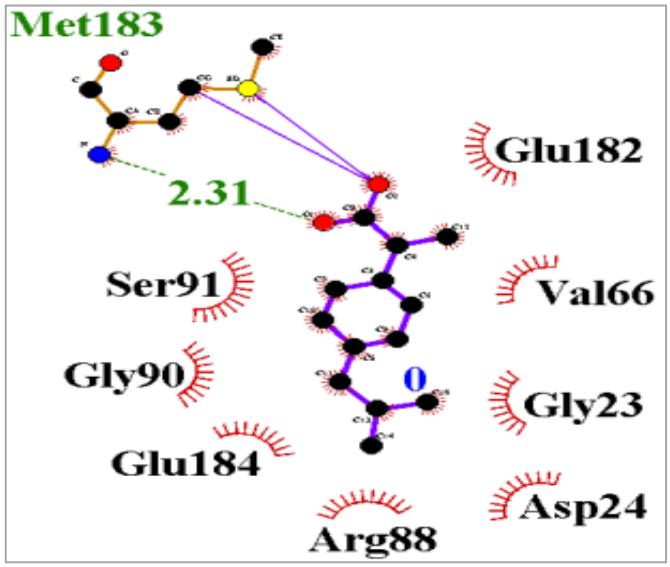
Figure 2
LigPlot+ illustration of Rifaximin and PfPNP interactions



Docking of Ibuprofen with PfPNP

The docking results of Ibuprofen with PfPNP revealed enough interactions which includes hydrogen bonds, covalent bonds and hydrophobic interactions. In these interactions, one active site residues, MET 183, formed covalent as well as a hydrogen bond. Eight residues formed hydrophobic interaction, of which 3 (Figure 3) are active site residues (GLU 182, SER 91 and GLU 184).

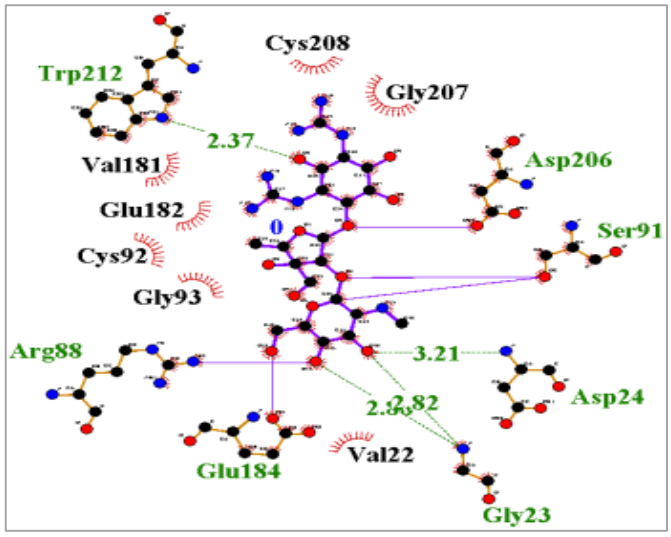
Figure 3
LigPlot+ illustration of Ibuprofen and PfPNP interactions



Docking of Streptomycin with PfPNP

Streptomycin when docked with PfPNP, fifteen different interactions were observed (Table 1). Three residues formed hydrogen bonds (TRP 212, ASP 24 and GLY 23), in which TRP 212 is an active site residue. Four residues (ASP 206, SER 91, ARG 88 and GLU 184) formed covalent bonds, in which ASP 206, SER 91 and GLU 184 are the active site residues. Seven residues formed hydrophobic interaction which active site residues are CYS 92 and GLY 93 (Figure 4).

Figure 4
LigPlot+ illustration of Streptomycin and PfPNP interactions

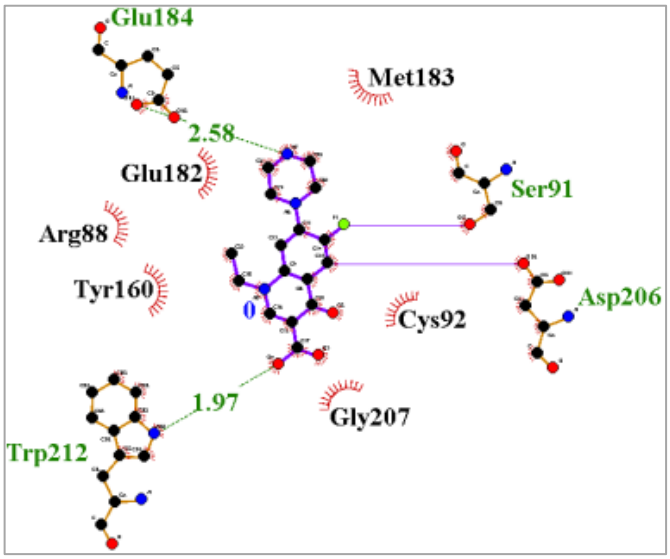


Docking of Norfloxacin with PfPNP

Different kinds of interactions were observed when Norfloxacin was docked with PfPNP (Table 1). In these interactions, two residues (active site) ASP 206 and SER 91 formed covalent bonds, two formed (GLU 184 and TERP 212) hydrogen bonds which are also the active site residues. Six hydrophobic interactions were also noted, in which active site residues are CYS 92, TYR 160 and MET 183 (Figure 5).

Figure 5

LigPlot+ illustration of Norfloxacin and PfPNP interactions



DISCUSSION

Malaria is a debilitating protozoan parasitic disease that cause severe mortality particularly in least developing countries. The most severe form of the disease is caused by *Plasmodium falciparum*. The immediate and appropriate treatment of malaria aims to prevent severe forms of the disease, as well as reduce mortality, in addition to eliminating the source of infection for the mosquito, and, consequently, reduce the transmission of the disease. While many antimalarial drugs are available in the market, resistance to most of these drugs is emerging. Thus, finding alternative FDA approved drug is essential. This study aimed to find FDA approved drugs that can inhibit PfPNP. Among the docked drugs, 5 showed promising results – Amoxicillin, Streptomycin, Ibuprofen, Rifaximin and Norfloxacin.

The purine pathway of metabolism is identified as an essential drug target for antimalarial drugs [14], the enzyme PfPNP plays an essential role in this pathway by catalysing different reactions. *Plasmodium falciparum* is a purine auxotroph, and its deficiency may lead to purine-less death of the parasite [22].

Amoxicillin is a broad-spectrum antibiotic active against many gram-negative and gram-positive microorganisms [23]. It is used to treat pneumonia, strep throat, skin infections, and urinary tract infections [24].

The docking revealed different interactions with the target protein. Interactions particularly covalent interaction along with hydrogen and hydrophobic interactions are known to act between ligand and drugs [25]. Amoxicillin diverse interactions with the target protein includes covalent bonds, hydrogen bonds, and hydrophobic interactions (Table 1). These maximum interactions might be sufficient to disrupt the metabolic pathway of purine synthesis, potentially halting the growth of the parasite and other activities necessary for its survival.

Rifaximin is a derivative of Rifamycin, a semi synthetic antibacterial drug and is used for various diseases including traveller diarrhoea [26]. Mechanism of action of Rifaximin involve the binding of the beta subunit of bacterial DNA dependent RNA polymerase which inhibit initiation of chain formation in RNA synthesis [27, 28]. In our study, this drug showed diverse interactions with the active site residues (Table 1, Figure 2) which reflects its potential antimalarial properties.

Ibuprofen is an anti-analgesic and anti-inflammatory drug mostly, used in the treatment of rheumatic disorder, fever and pain [29]. The docking result of Ibuprofen in the current study revealed diverse interactions that could disturb the metabolism of purine in *Plasmodium falciparum* and ultimately result in its death.

Streptomycin is used for the treatment of tuberculosis in combination with other drugs and have mixed type inhibitory properties [30]. Numerous interactions were observed after docking Streptomycin with PfPNP.

Norfloxacin is commonly used for the primary and secondary prophylaxis of spontaneous bacterial peritonitis and spontaneous bacteraemia in cirrhosis [31]. It stops infections caused by gram negative bacilli and is also used to treat urinary tract infection [32]. The docking of Norfloxacin with the target showed interactions including covalent bonds, hydrogen bonds and hydrophobic interactions, which hints on its potential antimalarial properties.

CONCLUSION

This study aimed to find new FDA approved inhibitors for *Plasmodium falciparum* Purine Nucleoside Phosphorylase (PfPNP) using in silico approach. Various FDA approved drugs were selected and docked with PfPNP. Among the docked drugs, Amoxicillin and Streptomycin showed the highest number of interactions with 15 interactions each. The docking results suggest that these drugs interact with the target protein and can induce metabolic changes especially in purine biosynthesis. This can lead to purine deprivation induced death of the parasite. Further studies and experiments are needed to validate their antimalarial activity.

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