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Structure-Based Virtual Screening and Molecular Dynamics Validation of Tryptoline as a Parasite-Selective PfPNP Inhibitor for Antimalarial Development

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Abstract

Artemisinin resistance in *Plasmodium falciparum* has prompted the search for new antimalarial targets. Purine nucleoside phosphorylase (PNP) from *P. falciparum* offers real promise as a target because the parasite needs purine salvage to survive and lacks adenosine kinase. This study examined whether PfPNP could be selectively targeted through structural comparison and computational drug discovery protocol. BLAST searches with an e-value threshold of 0.001 found no sequence similarity between PfPNP and human PNP. When we compared their 3D structures, we found only 9% sequence identity with an RMSD of 3.08 Å and TM-score of 0.7, indicating they diverge substantially. A key difference emerged in their structure: PfPNP forms a six-unit complex while human PNP forms a three-unit complex, reflecting how differently they've evolved. Screening 326 compounds identified tryptoline as the best lead, showing superior binding efficiency (GLE: -0.638, GLE-SA: -1.500, GLE-IN: -2.326) compared to other candidates. Molecular docking revealed that tryptoline binds through multiple contact points, including aromatic interactions with Tyr160 and electrostatic interactions with Asp206. Molecular dynamics simulation over 100 ns confirmed that tryptoline stays firmly bound, with critical interactions persisting throughout the trajectory. These results support PfPNP as a selective and viable drug target. Cell-based studies and binding assays are needed to validate tryptoline's potential as a new antimalarial.

Introduction

Malaria, a vector-borne infectious disease caused by the *Plasmodium* parasites, is transmitted by the bites of infected female *Anopheles* mosquitoes. The 2023 World Health Organization malaria report recorded 263 million cases and 597,000 deaths, with more than 95% of deaths caused by *Plasmodium falciparum* among five other species that infect humans (World Health Organization, 2025). The history of antimalarial chemotherapy is intimately tied to natural products. Quinine, isolated from cinchona bark in 1820, became the first systematic chemotherapeutic agent and

revolutionized treatment (Renslo, 2013). Decades later, chloroquine was synthesized and introduced as a treatment in the 1940s, but resistance emerged rapidly by the 1950s (Zhou & Yue, 2022). The discovery of artemisinin from *Artemisia annua* in traditional Chinese medicine provided a new avenue for drug development. Artemisinin-based combination therapies (ACTs) emerged as the current first-line treatment following their development in the 1970s and 2000s (Renslo, 2013), yet artemisinin resistance has now risen in Southeast Asia and parts of Africa due to mutations in the *kelch13* (K13) gene (Stokes et al., 2021). The emergence of triple ACT regimens combining amodiaquine with artemether-lumefantrine has been introduced to enhance efficacy and delay resistance, but nearly all antimalarials carry potential adverse effects that raise concerns about developing new therapeutic strategies (Pandey et al., 2023). Resistance is not uniform across all pathways; instead, the parasite often modifies drug targets, alters transporters, and bypasses pathways targeted by existing drugs (Barber et al., 2021). The central theme of modern antimalarial discovery has therefore become identifying new protein targets that are essential for parasite survival, less prone to resistance, and critically parasite-specific to reduce host toxicity (Belen Cassera et al., 2011). The parasites depend entirely on purine salvage because they cannot make purines de novo (Downie et al., 2008). This becomes even more important during the asexual blood stage, where the parasite grows inside erythrocytes. Human red blood cells also lack machinery for de novo purine synthesis, but they contain several salvage enzymes. Among them, purine nucleoside phosphorylase (PNP) and adenosine deaminase (ADA) play major roles (Downie et al., 2008). In *P. falciparum*, the salvage pathway begins with ADA converting adenosine to inosine, followed by PNP converting inosine to hypoxanthine (Chaikuad & Brady, 2009). Hypoxanthine then enters the reaction catalyzed by hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT), forming the essential building blocks for DNA and RNA (Downie et al., 2008). Since hypoxanthine is the major precursor for all purines in the parasite, this pathway directly supports parasite survival and multiplication (Quashie et al., 2010).

Interestingly, *P. falciparum* can still grow in erythrocytes that naturally lack human PNP or ADA, showing that the parasite's own enzymes PfADA and PfPNP are sufficient to carry out purine salvage inside the host cell (Chaikuad & Brady, 2009). Earlier studies also showed that PfPNP and PfADA recognize 5'-methylthiopurines in addition to their usual substrates, indicating that the parasite channels 5'-methylthioadenosine (MTA) from the polyamine pathway back

into purine metabolism (Ting et al., 2005). Unlike humans and several related apicomplexan parasites, *P. falciparum* does not have adenosine kinase, leaving PfPNP, PfADA, and PfHGXPRT as the only route for salvage (Riegelhaupt et al., 2010). Because the parasite depends on fewer enzymes than its host, the pathway stands out as a promising target for drug development (Downie et al., 2008). Any disruption in PfPNP can influence both purine supply and the balance of the polyamine pathway (Ting et al., 2005) , making it an attractive target for intervention (Ducati et al., 2018).

Structure-based drug discovery (SBDD) has significantly advanced the drug discovery process by eliminating targets and compounds that fail during the late stage of clinical trials. Access to the three-dimensional (3D) structure of the parasite's enzymes and receptors enables virtual screening against hypothesized candidates or even large libraries. Molecular dynamics, in turn, plays a role in analyzing the stability of predicted complexes (Lionta et al., 2014). Recent work shows steady progress in target-based drug discovery with computational tools (Kataria et al., 2024). Many studies report how structure prediction, virtual screening, and molecular dynamics guide hit identification in short timeframes (Lionta et al., 2014). Research groups have used docking-driven screening to identify selective inhibitors for kinases, proteases, and metabolic enzymes in pathogens and cancer models (Agu et al., 2023; Moreira et al., 2022). Other reports show that MD helps refine binding poses and predict stable complexes before synthesis (Liu et al., 2017), highlighting how in silico methods now support real laboratory outcomes and reduce early-stage failures (Kataria et al., 2024).

The main purpose of this work is to find out whether PfPNP can truly stand out as a safe and specific drug target. We first examined how different it is from human PNP by checking both the sequence and the structure of the two enzymes, ensuring we are not targeting a protein that closely resembles the host. After confirming this distinction, we screened a library of 326 compounds through molecular docking to pick out the ones that bind well. The best complex was then taken for an MD simulation to see how stable it remains under dynamic conditions.

Materials and methods

Data collection

The crystal structure of purine nucleoside phosphorylase (PDB ID: 3PHC) complexed with DADMe-ImmG at 2.00 Å resolution was retrieved from the Protein Data Bank. The protein has a molecular weight of 184.95 kDa with a homo-hexameric assembly. All six chains contain 275 amino acids with no reported mutations (*RCSB*

PDB: Protein Data Bank, 2025). UniProt (ID: Q8I3X4) lists this protein as manually reviewed with an annotation score of 5 (*UniProt*, 2025). Before interaction analysis, the retrieved PNP structure was validated for quality. A total of 326 compounds including approved antimalarial drugs, marine-derived antimalarial compounds, and other malaria-related inhibitors were downloaded as SDF files from PubChem (*PubChem*, 2025).

Sequence similarity search

To ensure target specificity, sequence similarity between parasite PNP and human PNP was examined. The PfPNP FASTA sequence was retrieved from UniProt and analysed using BLAST with an e-value threshold of 0.001 against Homo sapiens (Taxonomy ID: 9606).

Structure similarity

To evaluate any structural resemblance, the tertiary structures of human and parasite PNP were superimposed using the jFATCAT pairwise alignment tool with default parameters (Bittrich et al., 2024).

Protein preparation

The PfPNP structure was prepared using the Protein Preparation Wizard in Maestro (2024-1) (Maestro, 2024). Missing residues, hydrogen atoms, and side chains were added, and orientations of hydroxyl, amino, and thiol groups were corrected. Protonation states were assigned based on predicted pKa values. Non-physical geometries were corrected, and side chains were refined to remove steric clashes. Energy minimisation was performed using the default settings. The co-crystallised ligand was removed before docking.

Active site prediction

The Sitemap tool in Maestro was used to identify potential ligand-binding pockets. The algorithm evaluates cavity depth, volume, hydrophobicity, hydrogen-bonding features, and ionisable regions. SiteScore and DScore values were used to assess druggability. A receptor grid was then generated using the Receptor Grid Generation tool.

Ligand preparation

All 326 ligands were prepared using LigPrep. The process involved correcting valency and bond orders, adding missing hydrogens, generating protonation states at pH 7.0 ± 2.0, and creating stereoisomers when applicable. Energy minimisation was performed using the OPLS4 force field.

Molecular docking analysis

Molecular docking was carried out using the Glide engine through XGlide in Maestro (Friesner R. A. et al., 2006). The prepared receptor grid of Pf PNP and all 326 ligands were used as input. Standard Precision (SP) mode was selected, and the maximum number of poses per ligand was set to 1 to identify the best binding conformation.

Molecular dynamics simulation

MD simulation was performed using

Desmond (Schrödinger 2024-1) to study the stability of the top docking complex in a solvated environment. The complex was placed in an orthorhombic TIP3P water box with a 10 Å buffer. Counter ions (Na^+ and Cl^-) were added to neutralise the system. Energy minimisation and relaxation were performed using the OPLS4 force field. The simulation was run for 100 ns under the NPT ensemble at 300 K. Trajectories were recorded every 100 ps and analysed using the Simulation Interaction Diagram to evaluate RMSD, RMSF, and interaction stability.

Results

Sequence and Structural Divergence Between PfPNP and Human PNP

Sequence similarity searches revealed no significant homology between parasite and human PNP. When the BLAST search was conducted against *Homo sapiens* (Taxonomy ID: 9606) with an e-value threshold of 0.001, no hits were identified, establishing that PfPNP shares no detectable sequence similarity with its human counterpart.

Tertiary structure comparison presented a comparable picture of divergence between the two enzymes. The three-dimensional structures of human PNP (7ZSL) and parasite PNP (3PHC) were superimposed using pairwise structure alignment. The structures displayed only 9% identity at the amino acid level, with a root mean square deviation (RMSD) of 3.08 Å and a TM-score of 0.7 (Figure. 1).

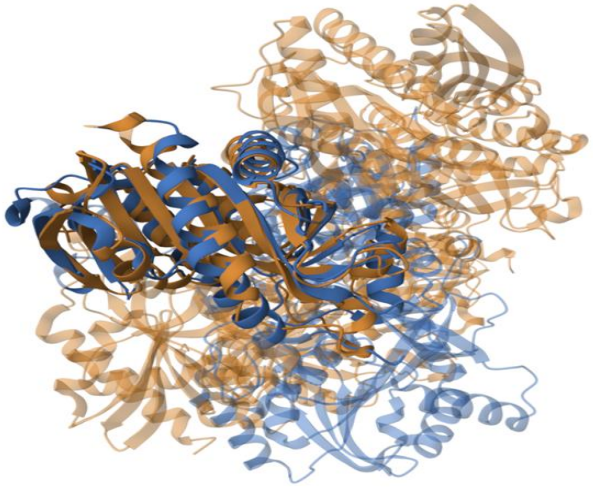

Fig. 1 Superimposed 3PHC and 7ZSL showing the minimal identity of 9%

While the TM-score of 0.7 exceeds the generally accepted threshold for structural similarity, the combination of minimal sequence identity and substantial RMSD reflects considerable divergence in spatial positioning of corresponding atoms. An important structural distinction emerged in the quaternary organization: PfPNP assembles as a homohexamer, whereas

human PNP forms a homotrimer. This fundamental difference in oligomeric state underscores the evolutionary adaptation of the two enzymes to distinct biological contexts.

Molecular Docking Analysis

Screening of 326 compounds against PfPNP identified multiple complexes with favorable binding characteristics. The top 20 complexes were selected for detailed analysis (Table 1). While pentamidine exhibited the highest binding affinity at -9.411 kcal/mol, it lacked optimal ligand efficiency metrics. In contrast, tryptoline (compound ID 107838) demonstrated a docking score of -8.291 kcal/mol with superior efficiency parameters: a GLE value of -0.638, GLE-SA value of -1.500, and GLE-IN value of -2.326. These efficiency metrics, which account for binding affinity relative to ligand size, surface area, and contact interactions, positioned tryptoline as the most favorable candidate for further optimization.

Table 1. Docking scores and ligand efficiency metrics of the top 20 complexes identified from virtual screening.

Compound ID	docking score	glide ligand efficiency	glide ligand efficiency sa	glide ligand efficiency ln	glide gscore	glide emodel	glide energy
4735	-9.411	-0.376	-1.101	-2.231	-9.411	-92.838	-52.769
57519507	-9.122	-0.351	-1.039	-2.142	-9.156	-69.312	-52.344
5281404	-8.426	-0.602	-1.451	-2.315	-8.486	-58.858	-34.036
60490	-8.337	-0.521	-1.313	-2.21	-8.36	-68.367	-46.71
107838	-8.291	-0.638	-1.5	-2.326	-8.303	-63.756	-37.119
57519507	-8.186	-0.315	-0.933	-1.922	-8.22	-68.281	-47.836
4212	-8.053	-0.252	-0.799	-1.803	-8.053	-89.048	-54.897
2746	-7.8	-0.39	-1.059	-1.952	-7.8	-58.626	-46.593
9837769	-7.774	-0.486	-1.224	-2.061	-7.774	-62.748	-45.2
9837769	-7.774	-0.486	-1.224	-2.061	-7.774	-62.748	-45.2

5280953	-7.744	-0.484	-1.22	-2.053	-7.792	-57.081	-35.764
10771337	-7.713	-0.22	-0.721	-1.693	-7.74	-70.474	-50.645
68617	-7.619	-0.381	-1.034	-1.907	-7.635	-52.035	-36.418
68617	-7.563	-0.378	-1.026	-1.893	-7.578	-52.176	-42.665
2955	-7.48	-0.44	-1.131	-1.951	-7.48	-42.103	-34.153
4046	-7.44	-0.286	-0.848	-1.747	-7.444	-22.468	-23.504
60490	-7.426	-0.464	-1.17	-1.968	-7.449	-58.433	-41.26
444	-7.343	-0.459	-1.156	-1.946	-7.589	-64.725	-40.622
136216112	-7.326	-0.366	-0.994	-1.833	-7.626	-47.379	-37.52
8530	-7.304	-0.609	-1.394	-2.096	-7.304	-39.324	-27.616

The PfPNP-tryptoline complex featured two pi-pi stacking interactions between the ligand's aromatic rings and Tyr160, measured at distances of 4.76 and 4.78 Å. Two salt bridges were also present: one between the ligand's protonated amine (N^+H_2) and Asp206 at 3.91 Å, and another between the same amine group and a phosphate ion (PO_4) at 4.99 Å. These aromatic and electrostatic interactions provided multivalent binding contacts within the active site.

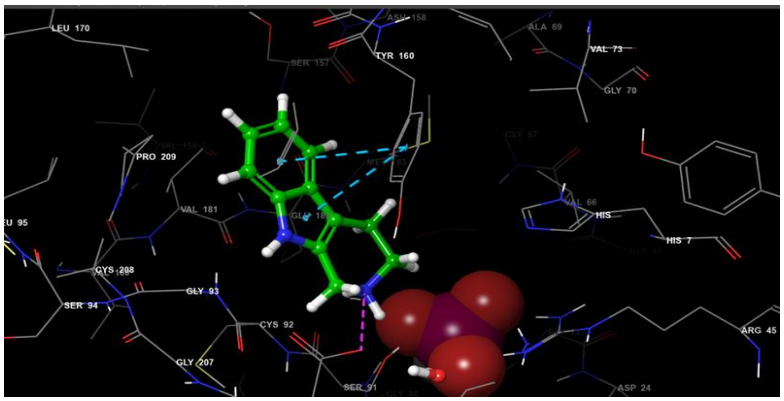

Fig. 2 3D interaction between target protein PfPNP and tryptoline

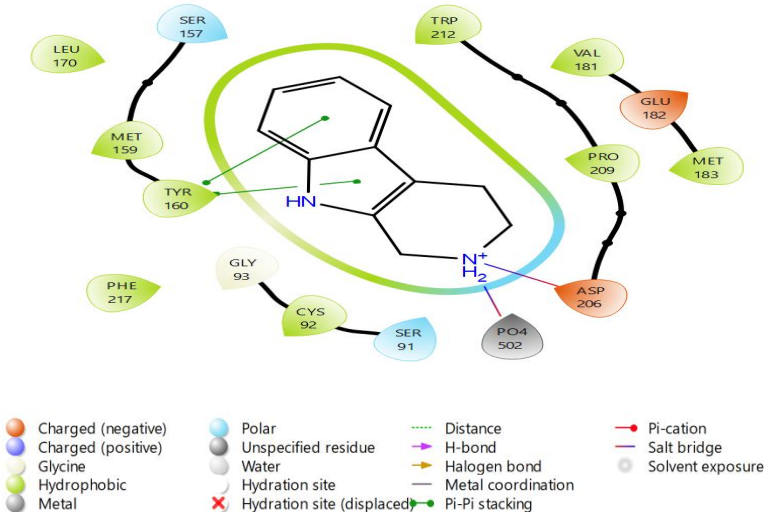

Fig. 3 2D interaction between target protein and ligand

Molecular Dynamics Simulation

The PfPNP-tryptoline complex underwent 100 ns MD simulation to evaluate binding stability under physiological conditions. The protein RMSD reached equilibration at approximately 1 Å following the initial heating phase and remained stable throughout the simulation. The ligand RMSD fluctuated between 0.5 and 2.0 Å, indicating stable positioning within the enzyme active site while retaining conformational flexibility to optimize interactions. The overall complex RMSD remained within the acceptable range of 1–3 Å, confirming system stability throughout the trajectory.

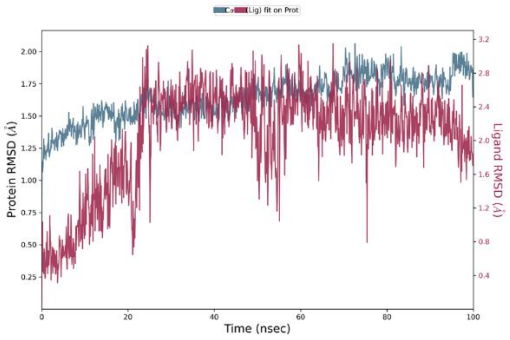

Fig. 4 RMSD plot of 100 ns MD simulation of PfPNP and tryptoline complex

Root mean square fluctuation (RMSF) analysis showed differential regional dynamics within the protein. The terminal regions (N- and C-termini) exhibited greater fluctuation than structured elements, reflecting the expected rigidity of alpha-helices and beta-strands in contrast to loop regions. Residues directly engaging with tryptoline maintained low RMSF values, with active site residues showing minimal deviation relative to more distant regions, indicating stabilization of the binding interface throughout the simulation.

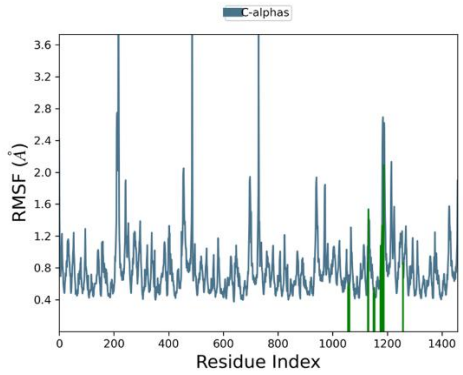

Fig. 5 RMSF plot of 100 ns MD simulation of PfPNP and tryptoline complex

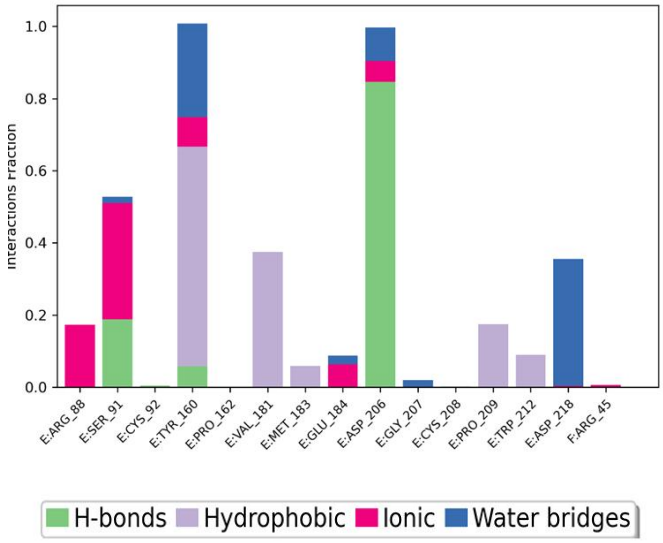

Fig. 6 The histogram of protein-ligand interaction with different residues in the active site of the protein throughout the simulation

Analysis of intermolecular interactions revealed a diverse binding network maintained over the 100 ns trajectory. Hydrogen bonding was observed among Ser91, Cys92, Tyr160, Asp206, and Cys208, with Asp206 maintaining hydrogen bond contacts for approximately 80% of the simulation duration. Hydrophobic interactions involved Tyr160, Val181, Met183, Pro209, and Trp212; Tyr160 sustained these contacts for ~60% of the simulation while Val181 participated for ~40%. Ionic interactions were recorded between the ligand and residues Arg88, Ser91, Tyr160, Glu184, Asp206, Asp218, and Arg45. Water-mediated interactions occurred between Ser91, Tyr160, Glu184, Gly207, and Asp218, comprising more than 30% of the simulation time and involving a single bridging water molecule. Notably, Tyr160 consistently participated in both hydrophobic and pi-pi stacking interactions throughout the simulation, emerging as a critical residue in the binding interface.

Discussion

Malaria drug discovery stands at a critical juncture. With artemisinin-based combination therapies (ACTs) remaining

the backbone of malaria treatment, the emergence of resistance in Southeast Asia and parts of Africa presents an urgent challenge (Tilley et al., 2016). The parasite's dependence on purine salvage for nucleic acid synthesis and energy metabolism has long been recognized as a metabolic vulnerability (Downie et al., 2008), yet most efforts to exploit this weakness have yielded drugs with poor selectivity or pharmacokinetic profiles. PfPNP, in particular, offers a compelling target because *P. falciparum* lacks adenosine kinase and must therefore salvage purines to survive (Madrid et al., 2008). The structural and sequence divergence between human and parasite PNP suggested that a truly selective inhibitor might be possible but this needed rigorous validation (Chung et al., 2024).

What we found in sequence searches was essentially a clean separation: BLAST returned zero hits between PfPNP and human PNP at an e-value threshold of 0.001. This complete absence of homology set a strong foundation, but we knew sequence similarity alone doesn't guarantee structural divergence (Larson et al., 2008). At the three-dimensional level, the picture became more nuanced. The TM-score of 0.7 technically exceeded the similarity threshold, yet the 9% sequence identity and 3.08 Å RMSD clearly demonstrated fundamental divergence. The RMSD in particular representing spatial deviation between corresponding atoms showed that these enzymes are organized quite differently in space, which matters enormously for drug design (Forte et al., 2021). The real kicker was the quaternary structure: PfPNP assembles as a homohexamer while human PNP forms a homotrimer. This difference isn't trivial. It reflects adaptation to entirely different metabolic contexts and severely restricts any possibility of cross-reactivity (Chaikuad & Brady, 2009). Earlier work had documented similar divergence in *P. vivax* PNP, attributing it to the parasite's distinct salvage pathway, and our findings aligned with that conclusion (Ting et al., 2005). The combination of sequence absence, structural divergence, and different oligomeric organization made a compelling case that PfPNP could be hit selectively without worrying extensively about hitting the human enzyme.

Screening 326 compounds through docking revealed an unexpected complexity in interpreting results. Pentamidine exhibited the highest binding affinity at -9.411 kcal/mol, which initially looked promising. But when we examined ligand efficiency metrics GLE, GLE-SA, and GLE-IN the interpretation shifted (Reynolds et al., 2008). These metrics matter because they account for binding quality relative to molecular size, surface area, and the nature of contacts. A

compound with exceptional affinity but requiring a large molecular scaffold often struggles during lead optimization due to poor pharmacokinetic properties (Hopkins et al., 2014). Pentamidine's structure, a diamidine with a rigid backbone and two positively charged amidinium groups illustrates these concerns. The compound's inherent polarity and charge density, while creating strong electrostatic interactions with acidic residues, generates significant liabilities for drug development (Wenzler et al., 2013). High polar surface area and multiple positive charges typically result in poor cell membrane permeability and reduced oral bioavailability, critical properties for a drug intended to reach infected erythrocytes and accumulate within the parasite's cytoplasm (Hastings & Hodel, 2014). Furthermore, pentamidine's extensive hydration shell around these charged groups increases the energetic barrier for crossing cellular membranes and reaching the blood-brain barrier. From a biochemical perspective, pentamidine's binding appears overly dependent on a narrow set of electrostatic interactions with key acidic residues. This binding brittleness raises concerns about resistance even minor mutations in these anchor residues could potentially abolish activity (Barber et al., 2021). The lack of favourable ligand efficiency metrics compounded these concerns, suggesting pentamidine wasn't an ideal lead despite the high affinity number.

Tryptoline presented a starkly different profile. The docking score of -8.291 came with substantially better efficiency values: GLE of -0.638, GLE-SA of -1.500, and GLE-IN of -2.326. These efficiency metrics were considerably higher than pentamidine's and represented the best among all 326 compounds screened. In drug discovery practice, this is exactly what we look for a smaller, more efficient compound that leaves chemical space for later optimization (Chandraghatgi et al., 2024). Tryptoline belongs to the indole alkaloid family, a well-established class in medicinal chemistry with proven success in natural product-derived pharmaceuticals (Chadha & Silakari, 2017). The indole scaffold offers genuine advantages: it's relatively compact, aromatic enough to support multiple interaction types without excessive polarity, and the indole nitrogen can participate in hydrogen bonding. Unlike pentamidine's rigid structure, tryptoline's architecture allows for chemical modification at multiple sites to enhance aqueous solubility, metabolic stability, or cellular uptake all critical for a compound aimed at the parasite cytoplasm (Fola et al., 2023). The balanced physicochemical properties of indole-based structures have made them preferred starting materials in many drug discovery programs (Liu et al., 2017). The binding mode reinforced why this efficiency values

translated to real advantages at the molecular level. Two pi-pi stacking interactions between tryptoline's aromatic rings and Tyr160 occurred at distances of 4.76 and 4.78 Å, within optimal ranges for aromatic-aromatic contacts (Brylinski, 2018). Two salt bridges one between the protonated amine and Asp206, another between the same amine and a nearby phosphate ion provided electrostatic anchors. This multivalent binding pattern meant multiple interaction types contributed independently to stability (Agarwal et al., 2015). If a single mutation disabled one interaction, others would still hold the compound in place. This redundancy matters for resistance considerations; compounds locked through multiple interaction types are intrinsically more resilient to mutation-driven escape.

The 100 ns molecular dynamics simulation was essential for moving beyond a static docking picture. Docking generates a single snapshot; MD reveals what happens in a thermal environment mimicking physiological conditions (Hopkins et al., 2014). The protein RMSD reached equilibrium at approximately 1 Å following initial heating, indicating the system was well-established. The ligand RMSD fluctuated between 0.5 and 2.0 Å, showing tryptoline remained relatively fixed in the active site while retaining the conformational flexibility necessary for enzyme function. Overall complex RMSD stayed within the 1–3 Å range considered acceptable for viable drug candidates. About 94% of binding poses identified as correct in crystal structures remain stable during equilibrium MD simulations, suggesting these results meaningfully reflected experimental binding modes (Liu et al., 2017). The RMSF analysis reinforced this picture. Terminal regions fluctuating more than structured cores is expected in proteins; alpha helices and beta strands are inherently more rigid than loops. The critical observation was that residues directly contacting tryptoline showed consistently lower fluctuations. This stabilization of the contact interface rather than loose or frustrated binding suggested the interactions were productive (Calic et al., 2020). Residues with high RMSF values at binding interfaces often signal weak or transiently formed contacts; that wasn't occurring here.

The interaction profile over 100 ns painted a picture of robust, persistent binding. Asp206 maintained hydrogen bonds for approximately 80% of the simulation persistent enough to matter physiologically. Tyr160 proved particularly versatile, maintaining both hydrophobic interactions for ~60% of the time and consistently making pi-pi stacking contacts with the aromatic ligand. Val181 contributed hydrophobic interactions for ~40% of the trajectory. Water-bridged interactions

involving Ser91, Tyr160, Glu184, Gly207, and Asp218 occurred for more than 30% of the simulation time (Liu et al., 2017). In a dynamic cellular environment where thermal motion constantly buffets protein-ligand complexes, interactions present only 5–10% of the time may not translate to real biological relevance (Riegelhaupt et al., 2010). The high occupancy percentages we observed suggested tryptoline's binding mode was fundamentally robust. This stability matters for resistance prospects: compounds locked through redundant, persistent interactions are less vulnerable to single mutations and better equipped to overcome resistance (Barber et al., 2021). Tyr160 especially deserves attention functioning simultaneously as a hydrophobic contact point and pi-pi interaction partner, it essentially anchored the compound across multiple modes.

One significant advantage lay in the complete absence of sequence homology and substantial structural divergence between PfPNP and human PNP. Many older antimalarials suffered from off-target toxicity precisely because they hit multiple parasite and host targets simultaneously (Belen Cassera et al., 2011). The selectivity demonstrated here meant developers could optimize PfPNP inhibitors without constantly worrying about hitting the human enzyme and causing host toxicity (Edwards & Odom John, 2016). This represents a major advantage over highly conserved targets where selectivity remains perpetually challenging (Barber et al., 2021). The computational evidence from docking and dynamics also informed clinical development strategy. Tryptoline's efficiency metrics suggested it was a genuine starting point for chemical optimization rather than an advanced scaffold requiring little modification. During lead optimization, medicinal chemists could explore additions or modifications to improve aqueous solubility, metabolic stability, or cell penetration essential properties for getting a compound into infected erythrocytes (Vial et al., 2013). The firm, multivalent binding mode and stable MD results suggested there was flexibility to make these modifications without immediately losing activity.

That said, computational predictions require experimental validation. No amount of docking or MD, however careful, substitutes for actual binding assays surface plasmon resonance, isothermal titration calorimetry, cell-based uptake studies (Kataria et al., 2024). Tryptoline would need testing for selectivity against other parasite enzymes beyond just PfPNP to rule out off-target effects within the parasite itself (Heidebrecht et al., 2012). The homohexameric structure of PfPNP versus trimeric human PNP was striking, but future work should determine whether this also

manifests in differences in cellular localization or regulatory mechanisms (Yahiya et al., 2019). The screening of 326 compounds was reasonably thorough but necessarily finite. A larger chemical library or de novo computational design approaches could uncover additional leads (Mohamed Abdul Cader et al., 2024). While tryptoline showed genuine promise, advancing a few of the other top-ranking compounds in parallel would hedge against failure and maximize success probability.

The evidence collectively supported PfPNP as a parasite-selective target with real tractability for drug development. The convergence of zero sequence homology, substantial structural divergence including fundamentally different oligomeric states, identification of tryptoline as a lead with persistent multivalent binding, and robust stability under MD simulation suggested solid footing for moving to experimental work (Chung et al., 2024). The parasite's absolute dependence on purine salvage and absence of adenosine kinase made pathway disruption a viable strategy (Madrid et al., 2008). Our computational work suggested tryptoline-based inhibitors could achieve that disruption selectively. Experimental validation was the natural next step, but the structural and computational foundation had been carefully established.

Conclusion

PfPNP has emerged as a genuinely promising parasite-selective drug target for antimalarial discovery. Its lack of meaningful sequence homology to human PNP, together with the clear structural differences between the *P. falciparum* hexamer and the human trimeric form, supports the idea that selective inhibition can be achieved with minimal risk of host toxicity. In our screening set of 326 molecules, tryptoline showed better ligand efficiency than pentamidine and most other candidates, which made it stand out early in the analysis. Molecular-dynamics simulations further indicated stable binding, mainly through persistent interactions involving residues Tyr160 and Asp206. These observations now need to be backed by experimental work binding-kinetics assays, parasite growth inhibition, and tests on cellular uptake to confirm whether tryptoline truly discriminates between parasite and host PNP. If those studies hold up, there is room for medicinal-chemistry refinement to improve potency and drug-like properties and possibly move towards a clinical lead. Considering that *P. falciparum* relies entirely on purine salvage and lacks adenosine kinase, targeting PfPNP remains a logical route for developing new antimalarials, particularly in the context of increasing artemisinin resistance.

CRediT authorship contribution statement

Sethupathi Virumandi: Writing – original draft, Methodology, Data curation, Formal analysis. **Elumalai Balamurugan**: Investigation, Validation, Supervision. **Aakash Ganesan, Sowmiya Ganesan, Srinidhi Raveenthiran** – Data curation, proof reading and editing.

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