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In-Silico Evaluation of Phenylisocytosine and Its Analogs as Potent Inhibitors of Plasmodium Falciparum Transketolase: A Strategic Approach in Anti-Malarial Drug Discovery

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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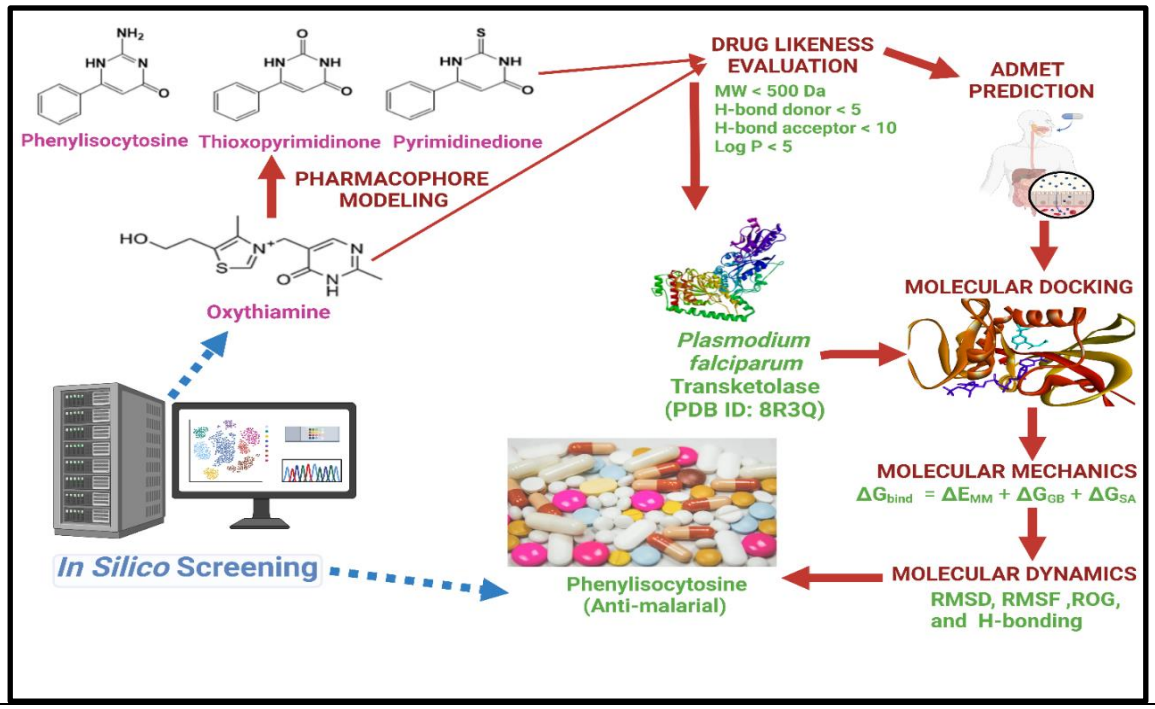
ABSTRACT

Background: The rise of drug-resistant *Plasmodium falciparum* strains, particularly those resistant to artemisinin-based combination therapies (ACTs), underscores the urgent need for alternative antimalarial agents targeting novel biochemical pathways.
Aim: This study investigates the potential of pyrimidine-based compounds (phenylisocytosine, thioxopyrimidinone, and pyrimidinedione) as potential inhibitors of transketolase, a critical enzyme in the pentose phosphate pathway essential for parasite nucleotide synthesis and redox homeostasis.
Methodology: Selected for their structural similarity to oxythiamine—a potent but nephrotoxic and carcinogenic transketolase inhibitor—these compounds were modified to improve safety profiles while retaining inhibitory efficacy. Using a combination of ligand-based and structure-based drug design approaches, comprehensive *in silico* assessments were conducted. Pharmacokinetic and toxicological profiling were evaluated using Lipinski's Rule of Five and ADMET profiling. Binding affinities were determined through molecular docking, while binding free energies were calculated using molecular mechanics. Binding stability was further investigated through molecular dynamics simulations.
Results: Pharmacokinetic evaluations, including drug-likeness and ADMET profiling, indicated favorable drug-like properties and low toxicity across all compounds. Molecular docking studies identified phenylisocytosine as having the highest binding affinity with *Plasmodium falciparum* transketolase (-6.3 kcal/mol in AutoDock Vina and -8.5 kcal/mol in iGEMDock), outperforming both thioxopyrimidinone and pyrimidinedione. Molecular mechanics calculations confirmed phenylisocytosine's superior binding free energy (-26.05 kcal/mol), with the reference drug oxythiamine exhibiting the weakest interaction (-16.85 kcal/mol). Molecular dynamics simulations over 50 nanoseconds further validated phenylisocytosine as the most stable ligand in complex with *Plasmodium falciparum* transketolase, with an RMSD of 0.30 nm, RMSF of 0.12 nm, ROG of 3.01 nm, and H-bond length of 1.01 nm. Although thioxopyrimidinone and oxythiamine showed moderate stability, phenylisocytosine consistently excelled across all parameters.
Conclusion: These findings position phenylisocytosine as a promising candidate for further experimental validation, to evaluate its efficacy, safety, and therapeutic potential as a novel antimalarial drug.

GRAPHICAL ABSTRACT



Keywords: Antimalarial resistance; plasmodium falciparum transketolase; oxythiamine; Phenylisocytosine; ligand-based drug design; structure-based drug design.

ABBREVIATION

2D : Two-Dimensional
3D : Three-Dimensional
ACTs : Artemisinin-Based Combination Therapies
ADMET : Absorption, Distribution, Metabolism, Excretion, and Toxicity
CASTp : Computed Atlas of Surface Topography of Proteins
CGENFF : CHARMM General Force Field
CYP : Cytochrome P450 (e.g., CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4)
hERG : Human Ether-à-go-go Related Gene
LBDD : Ligand-Based Drug Design
MD : Molecular Dynamics
MM-GBSA : Molecular Mechanics Generalized Born Surface Area
NPT : Constant Number of Particles, Pressure, and Temperature (ensemble)
NVT : Constant Number of Particles, Volume, and Temperature (ensemble)
PDB : Protein Data Bank
PPP : Pentose Phosphate Pathway
RMSD : Root Mean Square Deviation
RMSF : Root Mean Square Fluctuation
RO5 : Rule of Five
ROG : Radius of Gyration
SPC : Simple Point Charge (water Model Used in Simulations)
SBDD : Structure-Based Drug Design

1. INTRODUCTION

Malaria remains a major global health challenge, particularly in tropical and subtropical regions, where *Plasmodium falciparum* has developed resistance to existing antimalarial therapies. The widespread emergence of resistance to artemisinin-based combination therapies (ACTs), which have been the standard for malaria treatment over the past decades, underscores the urgent need for new therapeutic approaches to combat drug-resistant strains [1,2]. Despite significant advances in reducing malaria mortality, the World Health Organization (2023) still reports a substantial number of malaria-related deaths each year, particularly in areas where drug resistance is prevalent [3]. Historically, antimalarial drugs such as quinine and chloroquine played crucial roles in malaria control, but resistance to these therapies, and now ACTs, has driven the need to identify alternative drug targets within *Plasmodium falciparum* to develop effective treatments [4,5].

One promising strategy in the search for alternative antimalarial therapies involves targeting the pentose phosphate pathway (PPP), a critical metabolic pathway in *Plasmodium falciparum* responsible for nucleotide synthesis and maintaining redox balance, which are essential for parasite survival. Within the non-oxidative branch of the PPP, *Plasmodium falciparum* transketolase has emerged as a viable therapeutic target, as its inhibition disrupts key metabolic processes, potentially hindering parasite development and survival [6,7]. Recent advancements in computational drug discovery have revolutionized the early stages of antimalarial drug design by enabling accurate predictions of ligand-receptor interactions, binding affinities, and pharmacokinetic properties [8]. Structure-based drug design (SBDD) and ligand-based drug design (LBDD) are now widely employed for screening potential inhibitors and optimizing lead compounds for further development [9,10]. Using these computational techniques, molecular docking, molecular mechanics, and molecular dynamics simulations allow for a comprehensive evaluation of a compound's binding efficiency, stability, and dynamic behavior within the target protein [11].

The study aims to evaluate phenylisocytosine and its analogs—thioxopyrimidinone and pyrimidinedione—as potential inhibitors of *P. falciparum* transketolase through rigorous *in silico* assessments. By integrating pharmacokinetic profiling using Lipinski's Rule of Five and ADMET analysis with molecular docking and dynamics simulations, this research seeks to

identify candidates that not only exhibit optimal binding efficiency and stability but also possess favorable safety profiles. Targeting the non-oxidative branch of the PPP offers a strategic advantage in overcoming resistance to current therapies, positioning these compounds as promising candidates for further experimental validation [12]. The findings from this study could contribute significantly to the development of novel antimalarial drugs aimed at combating drug-resistant malaria strains.

2. METHODOLOGY

2.1 Preparation of Target Protein and Determination of Active Site

The essential information on *plasmodium falciparum* transketolase was obtained via the Protein Data Bank (PDB) (https://www.rcsb.org). The homo-domain three-dimensional structure of transketolase from *plasmodium falciparum* as a receptor was utilized as the receptor for this study, with particular focus on its domain D (PDB ID: 8R3Q) with a resolution of 1.88 Å (https://doi.org/10.2210/pdb8r3q/pdb). Biovia Discovery Studio 2021 (http://www.accelrys.com) was employed to optimize the protein structure, ensuring no unintended interactions affected the virtual screening process. The active site of the target protein was predicted using the CASTp 3.0 web server, a widely recognized tool for accurately identifying potential drug interaction sites, which facilitated precise, site-specific docking [13].

2.2 Preparation of Ligands

Oxythiamine, used as a reference drug, was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).
Pharmacophore modeling of oxythiamine was conducted using the ZINC20 database (https://zinc20.docking.org/), yielding phenylisocytosine, thioxopyrimidinone, and pyrimidinedione. These structures were optimized in 3D using ACD/Chemsketch version 2018 (http://www.acdlabs.com/) and saved in PDB format. All compounds were converted to PDBQT format using Open Babel [14], to enable interaction studies with the target protein, facilitating a detailed examination of binding interactions.

2.3 Molecular Docking Validation

To increase the accuracy of virtual screening, consensus scoring was implemented using AutoDock Vina, iGEMDock, and Molecular Operating Environment, each utilizing distinct algorithms [15-17]. These tools assessed the binding affinity and interactions of the co-crystallized ligand, thiamine pyrophosphate, within the D-domain active site of *Plasmodium falciparum* transketolase. AutoDock Vina and iGEMDock produced comparable binding scores and structural conformations, making them suitable for the primary docking phase. This consensus scoring minimized false positives and negatives, ensuring reliable predictions of potential drug efficacy.

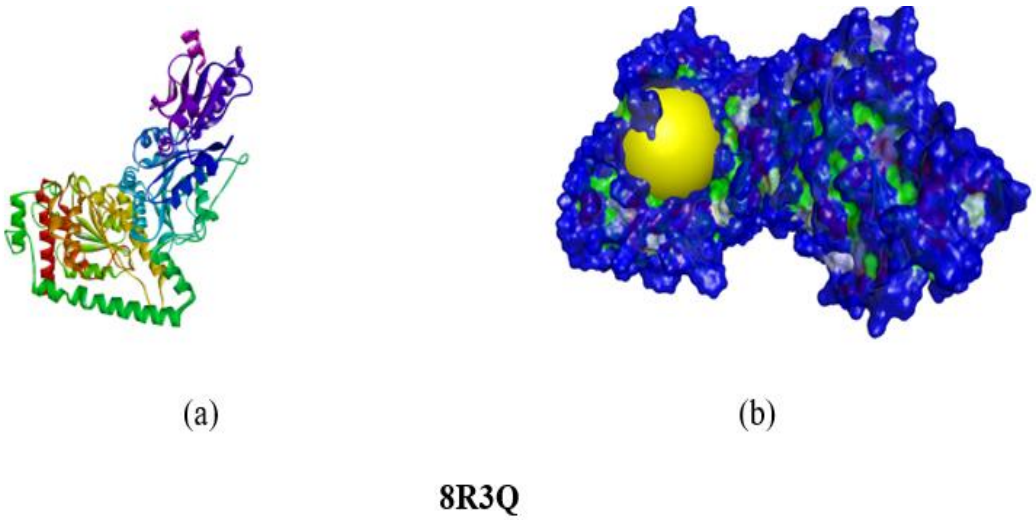


Fig. 1. Visualization of *plasmodium falciparum* transketolase receptor (PDB ID: 8R3Q), (a) cartoon model (b) surface model with the active site colored in yellow

2.4 Molecular Docking

The study employed *in-silico* molecular docking techniques using AutoDock Vina and iGEMDock to evaluate the binding interactions of oxythiamine, phenylisocytosine and two analogues with the *plasmodium falciparum* transketolase enzyme (PDB ID: 8R3Q).

2.4.1 AutoDock vina

AutoDock Vina was employed for structure-based virtual screening to predict ligand binding affinity to *Plasmodium falciparum* transketolase. Docking was performed with an exhaustiveness level of 8 to thoroughly explore conformational space [18]. The grid box, defining the docking site, was positioned around the active pocket of the 8R3Q receptor, with dimensions of (44 × 44 × 44) for the X, Y, and Z axes, and a spacing of (1Å) centered on specific active pocket residues (Trp46, Ser47, Tyr48, Met50, Arg62, Asp63, His109, Thr111, Val114, Glu115, Tyr153, Asp160, Asn190, Ile194, Cys253, His266, Lys306, Asn310, Val427). The protein structure was optimized by adding polar hydrogen atoms and applying Gasteiger charges, then converted to PDBQT format [19,20]. For each ligand, eight docking conformations were generated and scored using the London dG function, with the lowest-energy pose selected for further analysis [21,22].

2.4.2 iGEMDock

Molecular docking validation was conducted using iGEMDock version 2.1, which employed the “prepare binding site” feature to define docking parameters. A grid with an 8.0 Å radius centered on the active site was created. Precision parameters were set to ensure accuracy, including a population size of 800, 10 number of solutions, and 80 [18,23]. This setup enabled an exhaustive exploration of binding orientations and affinities, providing robust docking validation.

2.5 Physicochemical, Pharmacokinetic and Toxicological Profiling

2.5.1 Drug-likeness properties

Lipinski’s Rule of Five (RO5) [24], was used to evaluate the drug-likeness characteristics of the compounds. This evaluation was performed using pkCSM and ADMETLab 3.0. [25,26]. The RO5 assesses essential molecular properties significant for oral bioavailability, including molecular weight, octanol-water partition coefficient (logP), and hydrogen bond acceptors and donors [27]. This criterion, a refinement of drug-likeness, aids in predicting whether a compound possesses the pharmacological or biological activity suitable for oral administration in humans.

2.5.2 ADMET prediction

The evaluation of small molecules in medicinal chemistry and pharmacokinetics necessitates critical Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) studies [28]. pkCSM and ADMETLab 3.0 were used to investigate the ADMET properties of possible therapeutic candidates. Key parameters such as caco2 permeability, human intestinal absorption, p-glycoprotein inhibition, cytochrome P450 enzymes inhibition, half-life, total clearance, acute oral toxicity, ames toxicity, carcinogenicity, hepatoxicity, hematoxicity, nephrotoxicity, and human ether-a-go-go related gene (hERG) inhibition, were assessed [29-31]. This was done by entering the Simplified Molecular Input Line Entry System (SMILES) of the ligands from PubChem into the websever pkCSM and ADMETLab 3.0 respectively.

2.6 Molecular Mechanics (MM-GBSA)

MM-GBSA analysis was performed using the Prime MM-GBSA tool in Maestro version 12.5 to calculate relative binding free energies of each ligand. This method decomposes energy contributions (electrostatic interactions, van der Waals forces, hydrogen bonds, and solvation energies) to determine binding free energy [23,32]. The binding free energy (ΔG_bind) was calculated as:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{GB} + \Delta G_{SA}$$

where ΔE_{MM} equals E_{ele} (electrostatic energy) + E_{vdw} (van der waals energy) + E_{Hbond} (hydrogen bond energy) + Eint (torsional angle energy) and represents molecular mechanical energy, ΔGGB is the polar solvation energy, and ΔGSA is the nonpolar solvation energy. The results from this analysis offered insights into the binding stability of each ligand [33,34].

2.7 Molecular Dynamic (MD) Simulation

MD simulations were performed to evaluate the structural binding stability, conformational

dynamics, and interaction modes of the protein-ligand complexes [35]. These simulations provide a powerful approach for examining atomic-level changes within the complexes under dynamic conditions [18]. The simulations were carried out using GROMACS software version 2022, which is widely recognized for its accuracy in simulating proteins, lipids, and nucleic acids. To initiate the simulations, the topologies of the protein and ligands were generated using CHARMM36 force fields and refined following protocols provided by GROMACS and CgenFF [29]. The protein-ligand complex was placed within a dodecahedron box filled with counterions and simple point charge (SPC) water molecules, using the 'genion' tool to create a neutralized, solvated environment suitable for simulation [30].

To ensure system stability, an iterative energy minimization was conducted until the maximum force was reduced to less than 100 kJ/mol/nm, utilizing optimization algorithms like the steepest descent and conjugate gradient methods [32]. Once a stable conformation was established at 5000 steps with the steepest descent approach, the system underwent equilibration using the Verl*et al*gorithm, from 0 to 300 K, over a period of 100 picoseconds (ps) with a time step of 2 femtoseconds (fs) for the NVT ensemble (constant Number of particles, Volume, and Temperature). This was followed by further equilibration using the Berendsen algorithm with a 2fs time step for 100 ps under the NPT ensemble (constant Number of particles, Pressure, and Temperature). The MD simulations were then conducted for a 50-nanosecond (ns) production run of the protein-ligand complexes [29,30]. Post-simulation analysis was performed using Xmgrace, focusing on key parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (ROG), and Intermolecular Hydrogen Bonds [26,29-30]. These analyses provided valuable insights into the stability and dynamic behavior of the protein-ligand complexes, allowing for a comprehensive understanding of their interaction mechanisms and stability under physiological conditions.

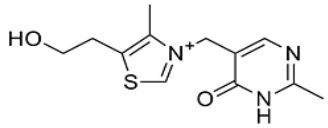
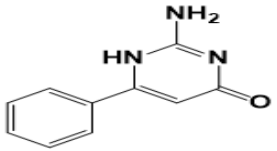
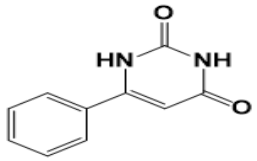
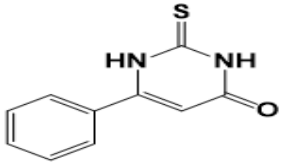
3. RESULTS AND DISCUSSION

3.1 Molecular Docking

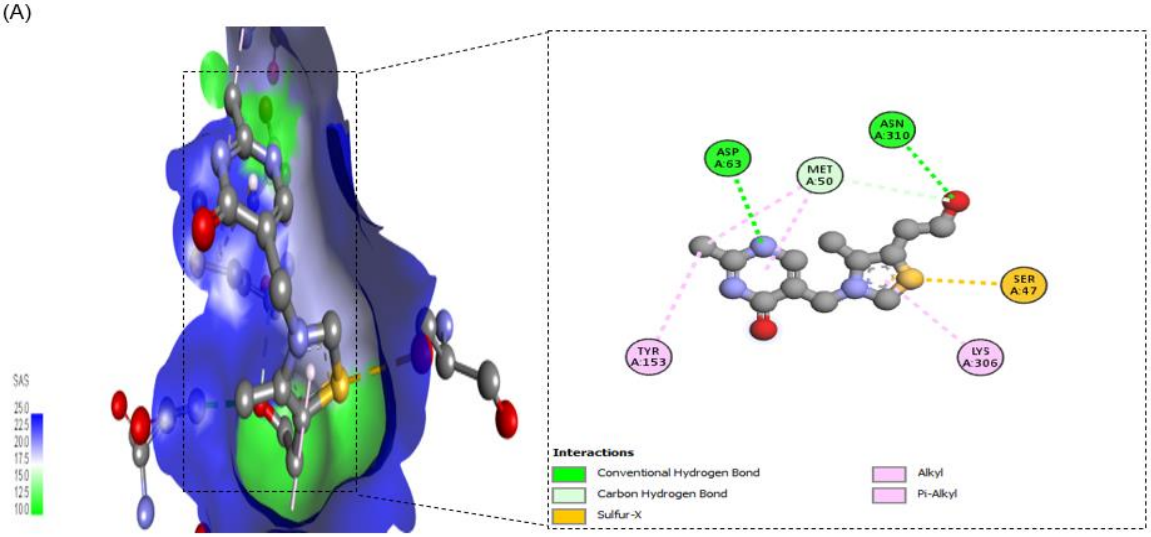
Molecular docking analyses offered critical insights into the binding interactions of phenylisocytosine, thioxopyrimidinone, and pyrimidinedione with *Plasmodium falciparum* transketolase, in comparison to oxythiamine. Strong binding affinities are associated with effective inhibition, as stable interactions within the enzyme's active site are crucial for halting catalytic activity. Oxythiamine demonstrated weaker binding affinities, with scores of -5.2 kcal/mol (AutoDock Vina) and -7.1 kcal/mol (iGEMDock). While oxythiamine formed hydrogen bonds with residues such as Met50, Asp63, and Asn310, additional interactions involving Ser47, Tyr153, and Lys306 contributed to its binding. However, the lower binding energies suggest that oxythiamine may be a less effective inhibitor relative to other ligands, with weaker binding potentially reducing inhibitory potency.

Phenylisocytosine showed the highest binding affinity, scoring -6.3 kcal/mol in AutoDock Vina and -8.5 kcal/mol in iGEMDock. This strong affinity was due to multiple hydrogen bonds with active site residues, including Trp46, Ser47, and Asn310, as well as interactions with Tyr48 and Lys306, which stabilized the phenylisocytosine-transketolase complex. Such strong binding interactions support phenylisocytosine’s potential as an effective inhibitor of transketolase. Thioxopyrimidinone demonstrated a high binding affinity, scoring -6.2 kcal/mol (AutoDock Vina) and -8.5 kcal/mol (iGEMDock), with hydrogen bonds to Asp160, Asn190, Cys253, and His266, and additional hydrophobic interactions with Ile194. The extensive hydrogen bonding and hydrophobic contacts suggest stable binding, supporting thioxopyrimidinone’s efficacy as an antimalarial agent. Pyrimidinedione also exhibited favorable binding, with scores of -6.2 kcal/mol (AutoDock Vina) and -8.3 kcal/mol (iGEMDock), forming hydrogen bonds with residues Arg62, Glu115, Val114, and Ala427, as well as hydrophobic interactions with His109 and Thr111. This binding pattern contributes to stable ligand anchoring in the active site, supporting pyrimidinedione’s inhibitory potential. The comparative analysis of docking results (Table 3, Figs. 2 and 3) highlights the superior binding affinities and interaction profiles of phenylisocytosine, thioxopyrimidinone, and pyrimidinedione over oxythiamine. Stronger binding interactions indicate their potential as effective inhibitors of *Plasmodium falciparum* transketolase, aligning with previous studies that emphasize stable binding as a determinant of inhibitor efficacy [36].

Table 1. Provides information about oxythiamine and the three ligands, presenting data on each ligand name, binding affinities, number of hydrogen bond interactions, other interactions and 2D structures

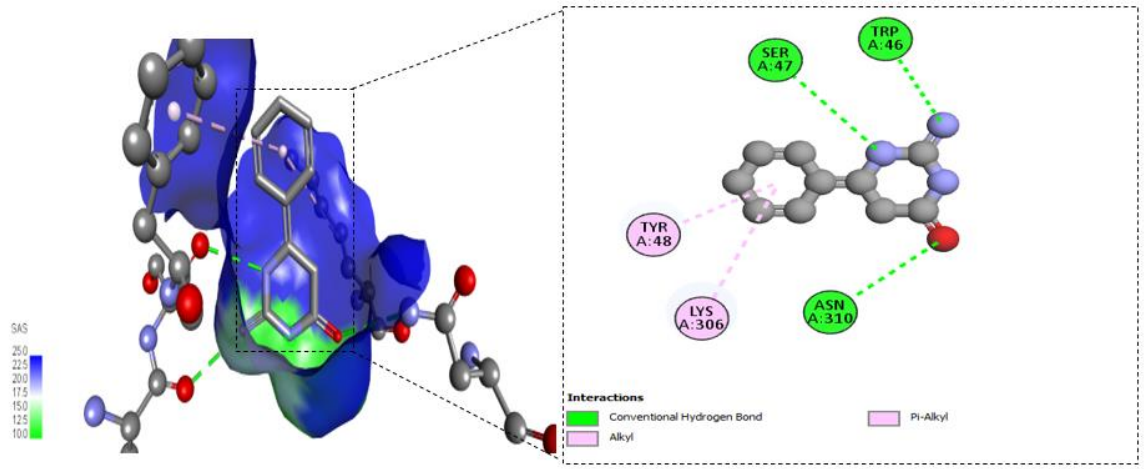
Ligands	Binding Energy (AutoDock Vina) Kcal/mol)	Binding Energy (iGEMDock) Kcal/mol)	H-bond Interaction	Other Interaction	2-D structure
Oxythiamine (Reference Drug)	-5.2	-7.1	Met50, Asp63, Asn310	Ser47, Tyr153, Lys306	
Phenylisocytosine	-6.3	-8.5	Trp46, Ser47, Asn310	Tyr48, Lys306	
Thioxopyrimidinone	-6.2	-8.5	Asp160, Asn190, Cys253, His266	Ile194	
Pyrimidinedione	-6.2	-8.3	Arg62, Glu115, Val114, Ala427	His109, Thr111	

(A)



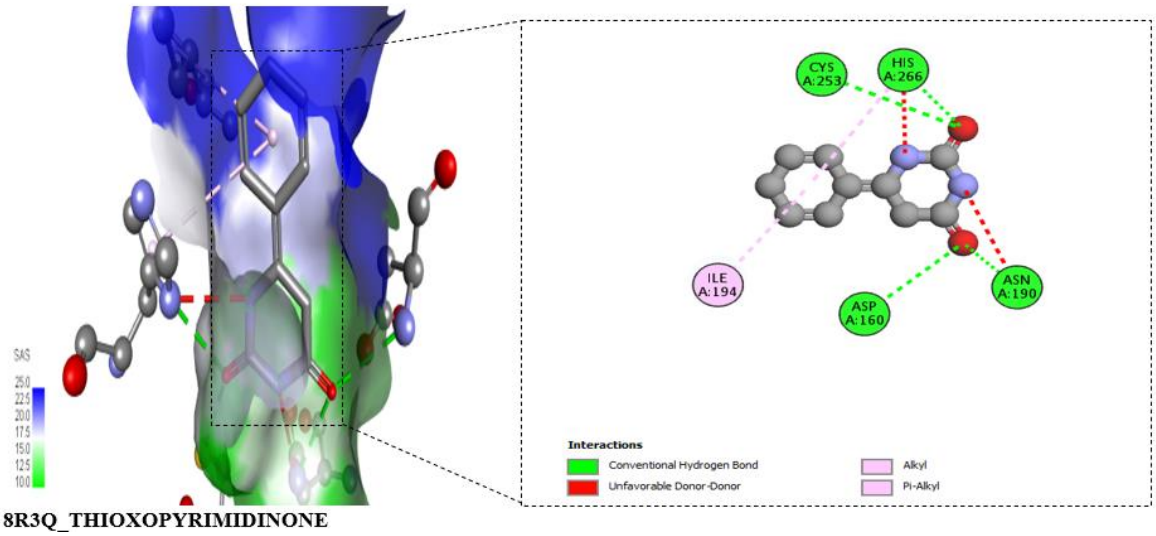
8R3Q_OXYTHIAMINE

(B)



8R3Q_PHENYLISOCYTOSINE

(C)



8R3Q_THIOXOPYRIMIDINONE

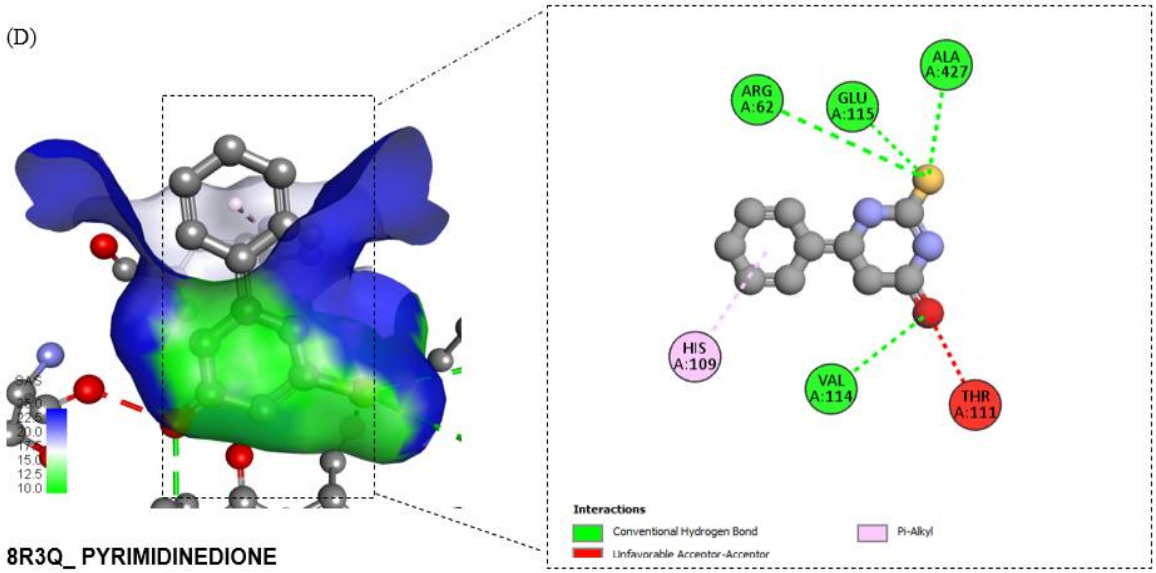


Fig. 2. Molecular interaction between oxythiamine and the three ligands at the active site of *plasmodium falciparum* transketolase (8R3Q) (a) Oxythiamine (reference drug) (b) Phenylisocytosine (c) Thioxopyrimidinone (d) Pyrimidinedione. The structures were rendered using Biovia Discovery Studio 2021

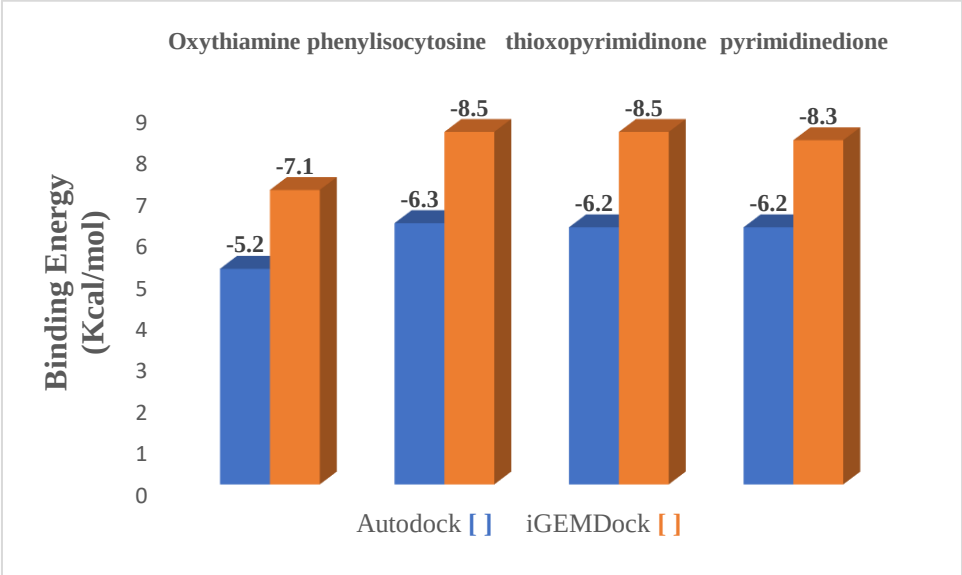


Fig. 3. Binding affinities of oxythiamine and the three ligands in Auto-dock vina and IGEM Dock

3.2 Physicochemical, Pharmacokinetic and Toxicological Profiling

3.2.1 Drug likeness prediction

The drug-likeness of phenylisocytosine, thioxopyrimidinone, and pyrimidinedione was assessed against Lipinski's Rule of Five (RO5) criteria, which are essential for predicting oral bioavailability in potential drug candidates. According to RO5, an ideal drug candidate should have a molecular weight <500 Da, <5 hydrogen bond donors, <10 hydrogen bond acceptors, and a log P <5, balancing hydrophilicity and lipophilicity to support efficient absorption and distribution [24,26]. Table 1 summarizes the properties of each compound, including oxythiamine as a reference drug.

Table 2. Drug-likeness (Rule of 5) evaluation and physiochemical properties of oxythiamine (Reference drug), phenylisocytosine, thioxopyrimidinone, and pyrimidinedione

Ligands	Molecular weight	H-bond donor	H-bond acceptor	Log p	Inference
Compound Identifier	**< 500**	**< 5**	**< 10**	**< 5**	**MEET R05**
Oxythiamine (reference Drug)	266.1	2	5	-0.977	Accepted
2-amino-6-phenylpyrimidin-4(1H)-one (Phenylisocytosine)	189.09	3	4	0.921	Accepted
6-Phenylpyrimidine-2,4(1H,3H)-dione (thioxopyrimidinone)	190.07	2	4	1.083	Accepted
6-Phenyl-2-thioxo-2,3-dihydropyrimidine-4(1H)-one (pyrimidinedione)	206.05	2	3	1.489	Accepted

All tested compounds met RO5 requirements, indicating their potential as orally active agents. Oxythiamine, with a molecular weight of 266.1 Da, 2 hydrogen bond donors, 5 acceptors, and a log P of -0.977, was within RO5 limits, yet its high hydrophilicity (negative log P) could hinder membrane permeability, impacting bioavailability unless aided by active transport [37]. Phenylisocytosine displayed ideal drug-likeness characteristics with a molecular weight of 189.09 Da, 3 hydrogen bond donors, 4 acceptors, and a log P of 0.921, suggesting a favorable hydrophilicity-lipophilicity balance that could facilitate effective membrane permeability and oral bioavailability.

Thioxopyrimidinone and pyrimidinedione, with molecular weights of 190.07 Da and 206.05 Da, respectively, also adhered to RO5, exhibiting 2 hydrogen bond donors and acceptable acceptor values. Their log P values, 1.083 for thioxopyrimidinone and 1.489 for pyrimidinedione, suggest better lipophilicity compared to phenylisocytosine, which could enhance absorption through lipid membranes. However, increased lipophilicity also raises bioaccumulation risks, potentially causing toxicity with prolonged use [38]. Therefore, although favorable, careful monitoring is essential to ensure safe pharmacokinetic profiles.

3.2.2 ADMET evaluation

ADMET properties—Absorption, Distribution, Metabolism, Excretion, and Toxicity—of the ligands were evaluated using pkCSM and ADMETLab 3.0 to assess clinical efficacy and safety [26]. All compounds demonstrated excellent Caco-2 permeability and high human intestinal absorption, supporting their likelihood for effective oral bioavailability. However, oxythiamine displayed poor Caco-2 permeability and limited human intestinal absorption, indicating potential issues with passive absorption that may limit clinical efficacy [39]. Notably, all ligands inhibited P-glycoprotein, which could reduce drug efflux, though oxythiamine's poor permeability remains a challenge. In terms of metabolism, all ligands were non-inhibitors of CYP450 enzymes such as CYP2D6 and CYP3A4, reducing the risk of drug-drug interactions, a significant factor for potential co-therapies. Oxythiamine, however, moderately inhibited CYP1A2, suggesting a risk of interactions when co-administered with drugs metabolized by this enzyme. The absence of CYP inhibition in phenylisocytosine and its analogs indicates a favorable metabolic profile, further supporting their potential safety in combination therapies. Regarding excretion, all compounds demonstrated excellent clearance rates, aside from oxythiamine, which had poor clearance and a prolonged half-life, increasing the risk of toxicity from accumulation [40]. Conversely, the other ligands' effective clearance suggests reduced toxicity risks, enabling safe excretion.

Toxicity profiles highlighted that none of the ligands were associated with hERG inhibition, mitigating cardiotoxicity risks. While oxythiamine showed nephrotoxicity, posing a risk for kidney damage, phenylisocytosine, thioxopyrimidinone, and pyrimidinedione were non-toxic in terms of hepatotoxicity and hematotoxicity, indicating their suitability as safer agents. Additionally, oxythiamine exhibited a higher risk of carcinogenicity compared to the other ligands, suggesting a limited therapeutic window. The moderate AMES toxicity and carcinogenicity of the other ligands warrant monitoring but do not preclude further investigation as potential antimalarial drugs [41,42].

Table 3. ADMET properties of oxythiamine, phenylisocytosine, thioxopyrimidinone, and pyrimidinedione (Reference drug)

Ligands	Absorption and Distribution				Metabolism						Excretion and Toxicity							
Compound Identifier	Caco2 Permeability	aco2 permeability	Human Intestinal absorption	P-glycoprotein Inhibitor	CYP2D6	CYP3A4	CYP1A2	CYP2C19	CYP2C9	Half-life	Total Clearance	Nephrotoxicity	Acute oral toxicity	AMES toxicity	Carcinogenicity	HERG inhibitor	Hepatoxicity	Hematoxicity
Oxythiamine (Reference Drug)	R		R	G	G	G	B	G	G	0.894	B	R	R	G	R	G	G	G
Phenylisocytosine	G		G	G	G	G	G	G	G	1.17	G	G	R	B	B	G	G	G
Thioxopyrimidinone	G		G	G	G	G	G	G	G	1.091	G	G	R	B	B	G	G	G
Pyrimidinedione	G		G	G	G	G	G	G	G	1.074	G	G	R	B	B	G	G	G

G = Excellent B = Medium R = Poor

3.3 Molecular Mechanics-Generalized Born Surface Area (MM-GBSA)

MM-GBSA analysis was conducted to provide insights into the binding energies and stability of the ligand-protein complexes, with the results summarized in Table 4 and Fig. 4. The MM-GBSA approach enabled a breakdown of binding free energy into key components, including van der Waals forces, hydrogen bonding, covalent bonding, and polar solvation. Understanding these energy components is essential for interpreting ligand-protein interactions and stability within the complexes.

Van der Waals interactions emerged as a significant contributor to binding stability across all ligands. Oxythiamine demonstrated the strongest van der Waals interaction (-33.6 kcal/mol), enhancing its binding affinity; however, its overall binding energy was limited to -16.85 kcal/mol due to high polar solvation energy (30.91 kcal/mol). This high solvation energy indicates substantial desolvation costs, which counter the binding stability provided by van der Waals forces, thus weakening oxythiamine's stability as a ligand. In contrast, phenylisocytosine exhibited a balanced combination of interactions, achieving the lowest overall binding energy (-26.05 kcal/mol), making it the most stable ligand. Despite a high desolvation penalty (polar solvation energy of 45.45 kcal/mol), phenylisocytosine's favorable van der Waals interactions (-24.22 kcal/mol) and moderate hydrogen bonding (-3.16 kcal/mol) contributed to a strong binding with the target protein. This combination highlights phenylisocytosine's stability and suggests its potential as an effective inhibitor of *Plasmodium falciparum* transketolase. Thioxopyrimidinone showed comparable binding stability, with an overall binding energy of -25.48 kcal/mol, supported by strong hydrogen bonding (-3.34 kcal/mol) and moderate van der Waals interactions (-20.65 kcal/mol). Its lower polar solvation energy (19.3 kcal/mol) indicated a reduced desolvation cost, enhancing its binding stability. These factors indicate that thioxopyrimidinone forms a balanced and stable interaction with the enzyme. Pyrimidinedione, while showing promising van der Waals interactions (-20.95 kcal/mol), had a slightly weaker binding energy (-24.44 kcal/mol), largely due to its polar solvation energy (-4.95 kcal/mol). Despite forming stable hydrogen bonds (-3.38 kcal/mol), pyrimidinedione's binding stability was slightly lower than that of phenylisocytosine and thioxopyrimidinone, making it a somewhat less stable ligand.

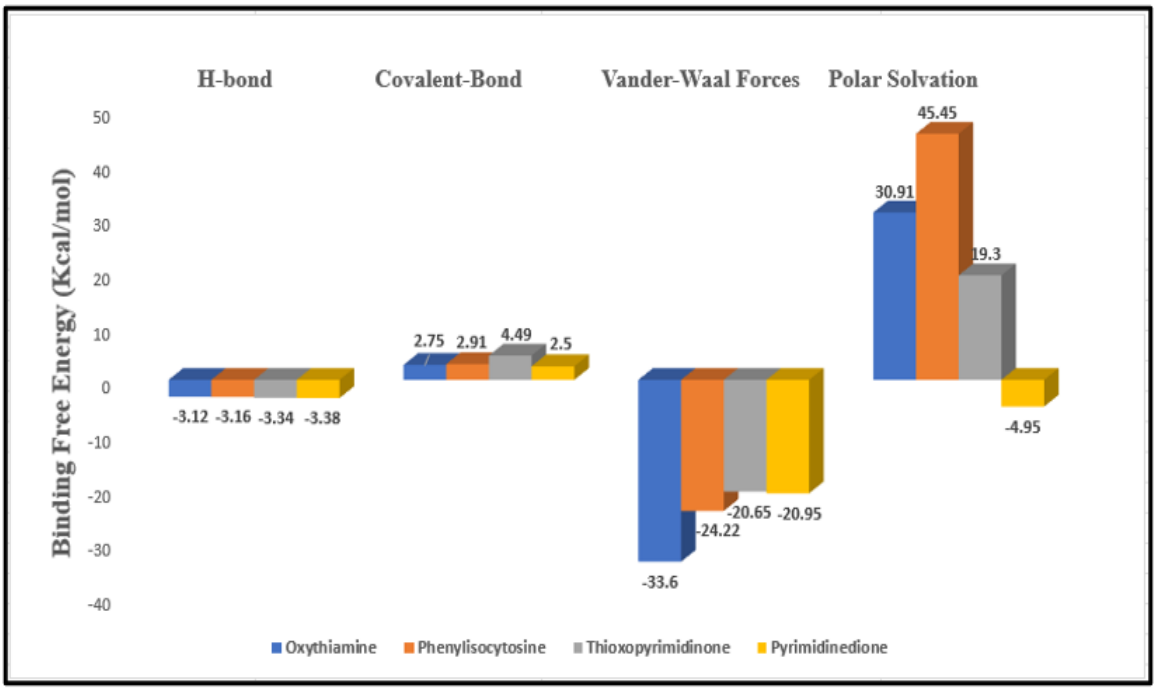


Fig. 4. MMGBSAΔG Bind H-bond, Covalent bond, Vander waal forces, and Polar solvation of Oxythiamine, Phenylisocytosine, Thioxopyrimidinone, and Pyrimidinedione in Maestro 12.5

Table 4. MM-GBSA binding free energies of Oxythiamine, Phenylisocytosine, Thioxopyrimidinone, and Pyrimidinedione

Ligands	MMGBSA ΔG Bind H-bond (Kcal/mol)	MMGBSA ΔG Bind Covalent bond (Kcal/mol)	MMGBSA ΔG Bind Vander Waal Forces (Kcal/mol)	MMGBSA ΔG Bind Polar Solvation (Kcal/mol)	MMGBSA ΔG Overall Binding energy (Kcal/mol)
Oxythiamine	-3.12	2.75	-33.6	30.91	-16.85
Phenylisocytosine	-3.16	2.91	-24.22	45.45	-26.05
Thioxopyrimidinone	-3.34	4.49	-20.65	19.3	-25.48
Pyrimidinedione	-3.38	2.5	-20.95	-4.95	-24.44

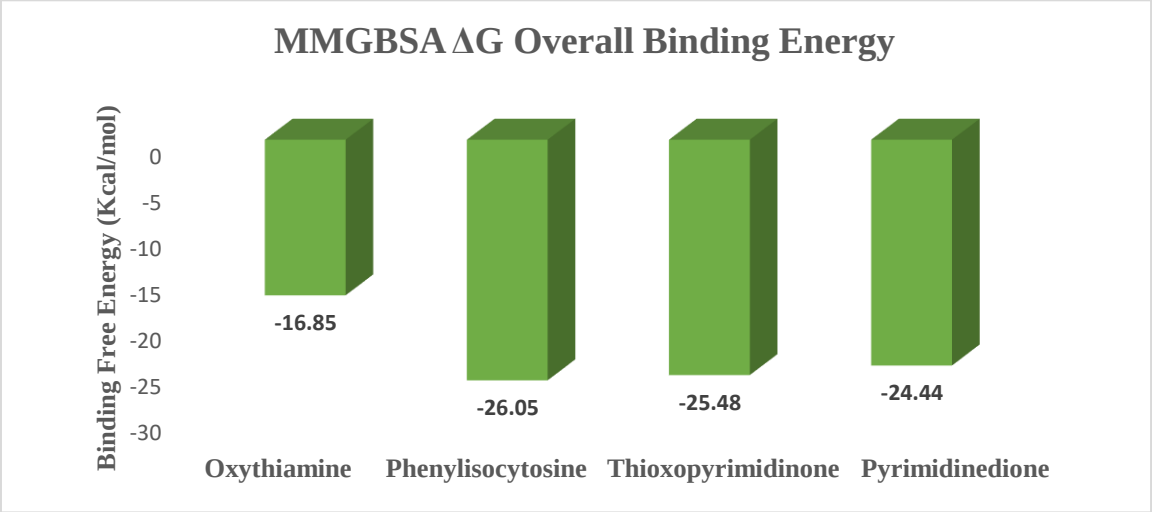


Fig. 5. MMGBSAΔG overall binding energy for Oxythiamine, Phenylisocytosine, Thioxopyrimidinone, and Pyrimidinedione in Maestro 12.5

3.4 Selection of Lead Compounds for MD Simulation

After an initial filtering process through structure-based virtual screening (molecular docking and molecular mechanics) and ligand-based virtual screening, including Lipinski's rule of five and ADMET evaluation, 2-amino-6-phenylpyrimidin-4(1H)-one (Phenylisocytosine) and 6-Phenylpyrimidine-2,4(1H,3H)-dione (Thioxopyrimidinone) were selected for MD simulations alongside the reference drug, Oxythiamine. These two ligands exhibited favourable pharmacokinetic profiles, meeting drug-likeness criteria while demonstrating strong binding affinities in molecular docking studies. In particular, their ability to engage in stable interactions with the target protein and their promising ADMET properties made them viable candidates for further molecular dynamic simulation.

3.5 Molecular Dynamics (MD)

MD simulations assessed the stability, flexibility, and interaction strength of the protein-ligand complexes over time, analyzing key parameters such as RMSD, RMSF, ROG, and H-bond (Table 5). These metrics provide insights into the complexes' behavior under physiological conditions.

3.5.1 RMSD

RMSD values reflect the overall stability of the protein-ligand complex by measuring the deviation in atomic positions over time. Oxythiamine displayed the highest RMSD value (0.474 nm), which suggests that it undergoes significant conformational changes during the simulation. This high value indicates that oxythiamine forms a less stable complex, likely resulting from weaker interactions with the protein, leading to greater flexibility and movement away from the initial binding site. In contrast, phenylisocytosine exhibited the lowest RMSD value (0.303 nm), reflecting a highly stable complex with minimal deviation from its initial conformation. This lower RMSD suggests that phenylisocytosine maintains a strong and consistent interaction with the target protein throughout the simulation, reinforcing its potential as an effective inhibitor. Thioxopyrimidinone showed a slightly higher RMSD value (0.327 nm) than phenylisocytosine, but it still demonstrated good stability. These results highlight that phenylisocytosine forms the most stable complex, followed by Thioxopyrimidinone, while oxythiamine exhibited the least stable interactions.

3.5.2 RMSF

RMSF values provide insight into the flexibility of individual residues within the protein-ligand complex. Phenylisocytosine once again exhibited superior performance with the lowest RMSF value (0.122 nm), indicating minimal fluctuations and strong interactions with specific protein residues. This suggests that the binding region remains stable when interacting with phenylisocytosine, further supporting its potential as a potent inhibitor. Thioxopyrimidinone exhibited an RMSF value of 0.131 nm, indicating

slightly more flexibility than phenylisocytosine but still maintaining a stable interaction. Oxythiamine, with the highest RMSF value (0.138 nm), showed greater residue flexibility, suggesting weaker binding and a less stable complex. These findings reinforce the conclusion that phenylisocytosine forms the most rigid and stable interactions with the protein, while oxythiamine exhibits greater flexibility and weaker binding, which could undermine its inhibitory effectiveness.

3.5.3 ROG

ROG is a measure of the compactness of the protein-ligand complex, with lower values indicating a more tightly packed and stable structure. Oxythiamine exhibited the highest ROG value (3.022 nm), indicating a less compact and more expanded structure. This suggests that the complex involving oxythiamine is not as tightly bound, resulting in weaker interactions and a less stable binding profile. Phenylisocytosine, with an ROG value of 3.013 nm, formed a more compact and stable complex, further confirming its strong interaction with the enzyme's active site. Thioxopyrimidinone demonstrated the lowest ROG value (2.996 nm), indicating that it forms the most compact complex among the tested ligands. This compactness is a positive indicator of stable binding, as the ligand remains tightly seated within the binding pocket, leading to greater stability. The ROG results align with the RMSD and hydrogen bonding data, suggesting that both phenylisocytosine and Thioxopyrimidinone form more stable and tightly packed complexes compared to oxythiamine, which showed the least favorable compactness.

Table 5. Average values of RMSD, RMSF, ROG, and H-bond of all simulated complexes

Compound Identifier	Average RMSD values (nm)	Average RMSF values (nm)	Average H-bond values (nm)	Average ROG values (nm)
Oxythiamine	0.474347	0.138127	0.08173	3.022909
2-amino-6-phenylpyrimidin-4(1H)-one (Phenylisocytosine)	0.303705	0.122734	1.023494	3.013614
6-Phenylpyrimidine-2,4(1H,3H)-dione (Thioxopyrimidinone)	0.327217	0.131814	0.422394	2.99662

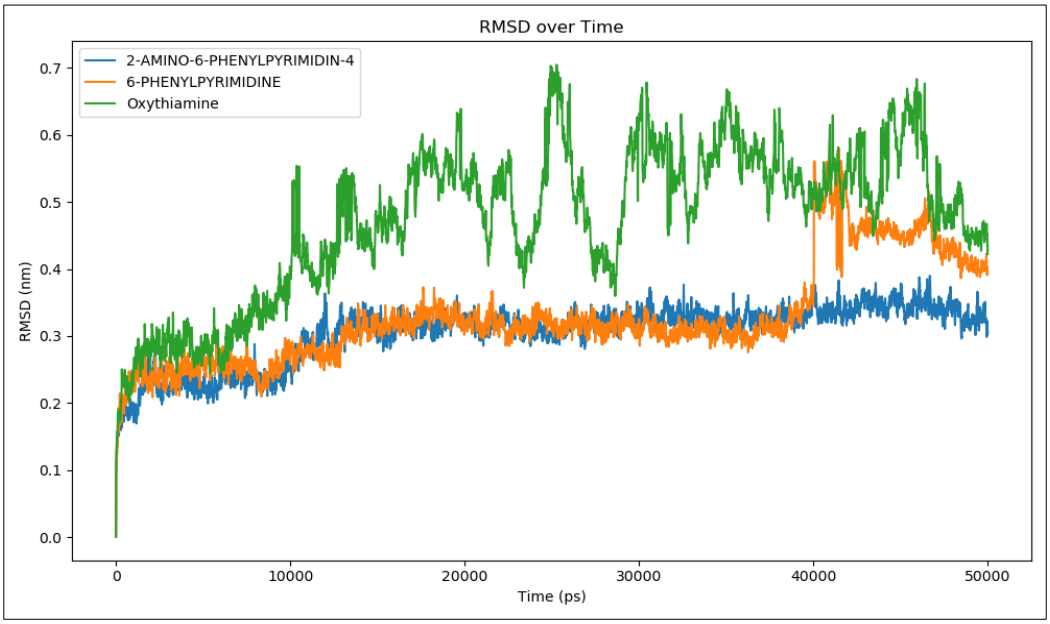


Fig. 6. RMSD Plot Showing the Stability of 2-Amino-6-Phenylpyrimidin-4 (Phenylisocytosine), 6-Phenylpyrimidine (Thioxopyrimidinone), and Oxythiamine Over 50 ns of Molecular Dynamics Simulation

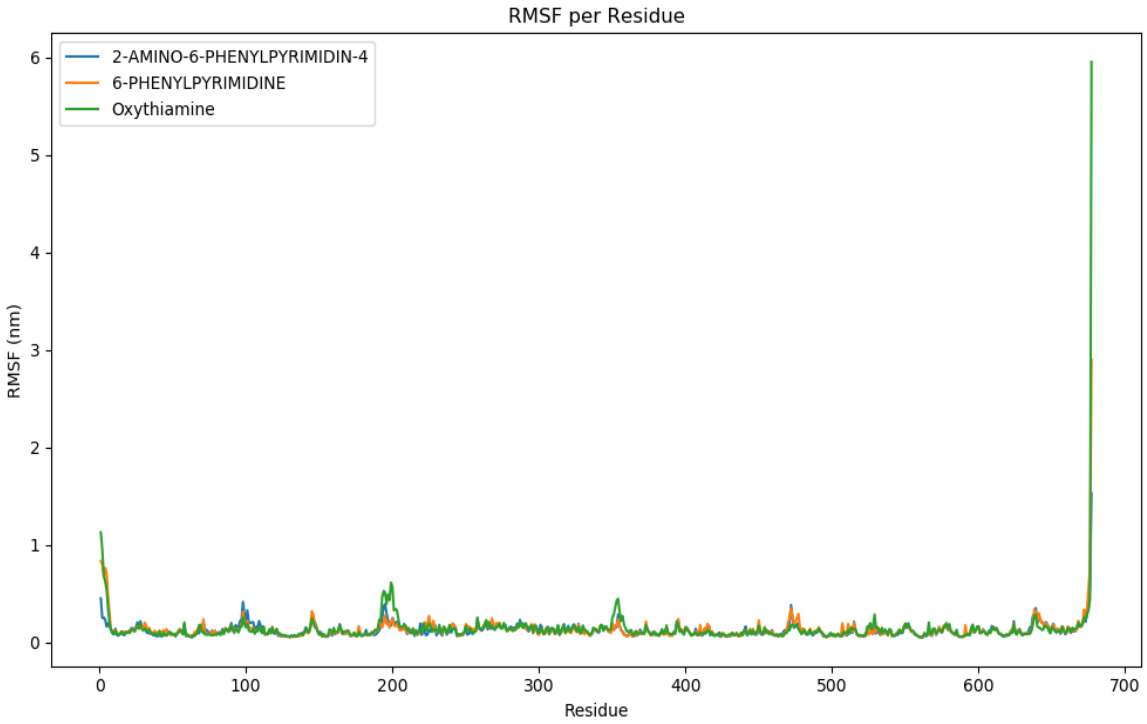


Fig. 7. RMSF Plot Showing the Stability of 2-Amino-6-Phenylpyrimidin-4 (Phenylisocytosine), 6-Phenylpyrimidine (Thioxopyrimidinone), and Oxythiamine Over 50 ns of Molecular Dynamics Simulation

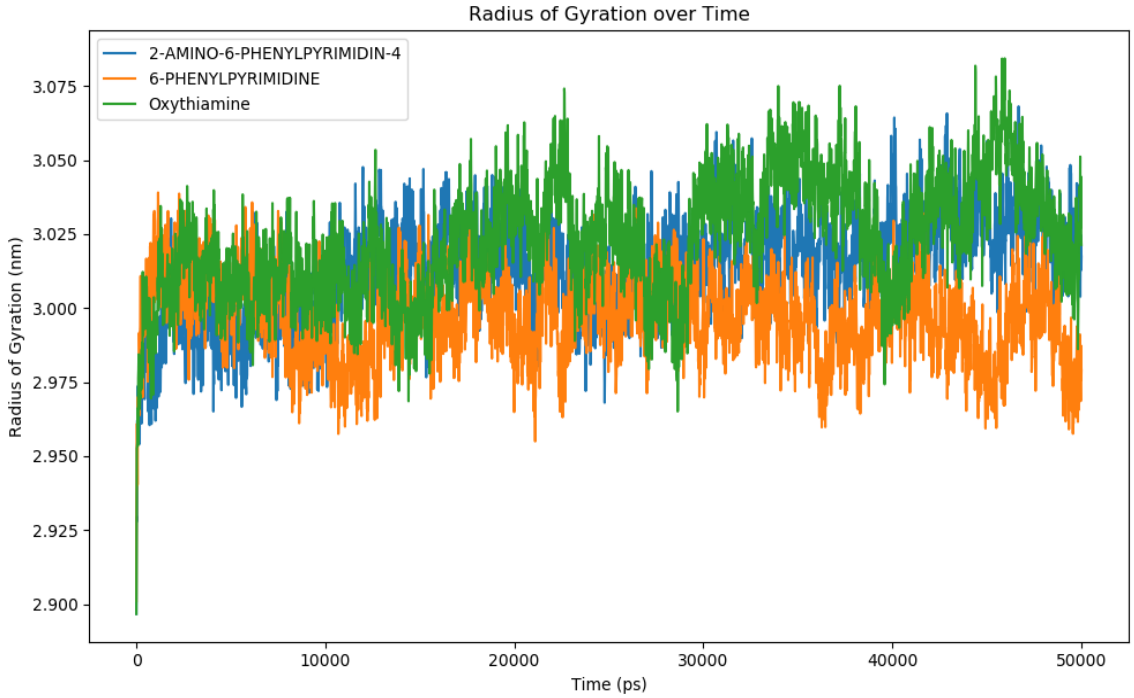


Fig. 8. RoG Plot Showing the Stability of 2-Amino-6-Phenylpyrimidin-4 (Phenylisocytosine), 6-Phenylpyrimidine (Thioxopyrimidinone), and Oxythiamine Over 50 ns of Molecular Dynamics Simulation

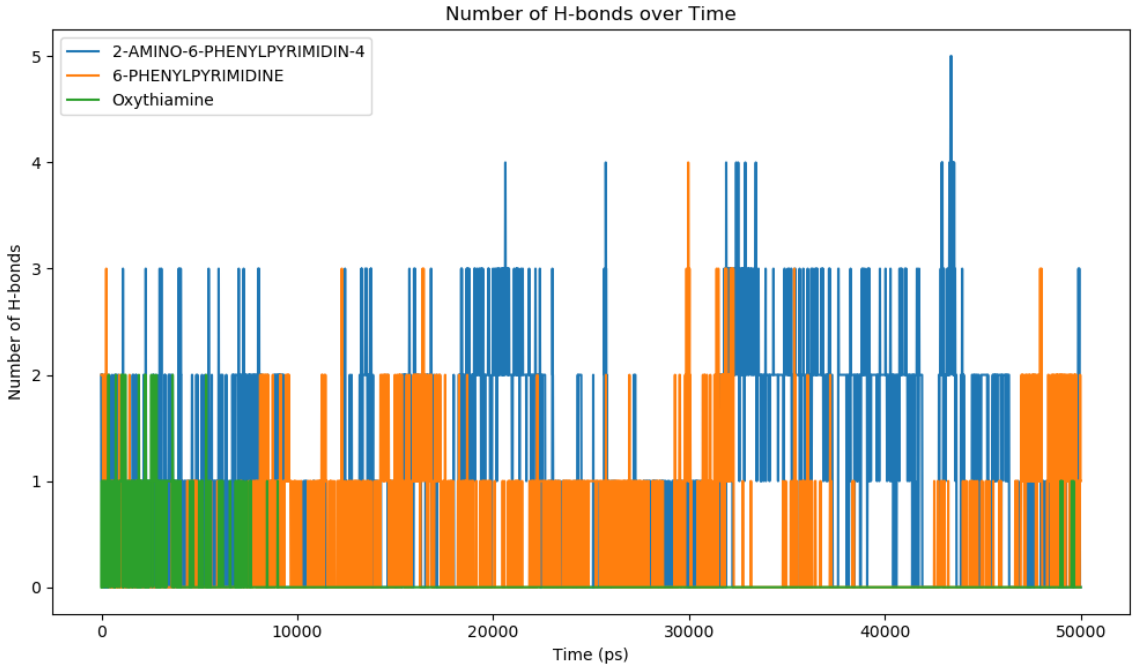


Fig. 9. H-bonding Plot Showing the Stability of 2-Amino-6-Phenylpyrimidin-4 (Phenylisocytosine), 6-Phenylpyrimidine (Thioxopyrimidinone), and Oxythiamine Over 50 ns of Molecular Dynamics Simulation

3.5.4 H-bonding

Hydrogen bonding is crucial for stabilizing protein-ligand interactions, and the number of hydrogen bonds formed during the MD simulation directly correlates with interaction strength. Phenylisocytosine formed an average of 1.023 hydrogen bonds, indicating strong and stable interactions with the target protein. This relatively high number of hydrogen bonds supports the hypothesis that phenylisocytosine establishes robust and stable interactions, contributing to the overall stability of the complex. Thioxopyrimidinone formed 0.422 hydrogen bonds on average, which, although lower than phenylisocytosine, still suggests a relatively stable interaction. In contrast, oxythiamine formed only 0.081 hydrogen bonds, reflecting much weaker interactions and reduced stability. The limited hydrogen bonding capability of oxythiamine suggests that it does not effectively stabilize the protein-ligand complex, likely contributing to its poorer performance in maintaining binding stability.

4. CONCLUSION

This study evaluated phenylisocytosine and its analogs—thioxopyrimidinone and pyrimidinedione—as potential inhibitors of *Plasmodium falciparum* transketolase, a critical enzyme in the pentose phosphate pathway, to combat drug-resistant malaria. Comprehensive *in silico* analyses, including pharmacokinetic and toxicological profiling, molecular docking, molecular mechanics, and molecular dynamics simulations, identified phenylisocytosine as the most promising candidate demonstrating superior drug-likeness, moderate safety profiles, strong binding affinity, and exceptional stability in comparison to both its analogs and the reference drug, oxythiamine, which showed weaker binding and poor pharmacokinetics. While the compound exhibit moderate safety profile, its primary safety concern lies in potential acute oral toxicity, emphasizing the need for further experimental validation. These findings highlight the strategic potential of targeting alternative metabolic pathways in *P. falciparum* and establish a strong foundation for the development of phenylisocytosine as a novel antimalarial agent. Future studies, including synthesis and experimental validations through *in vitro* and *in vivo* evaluations, are essential to confirm its therapeutic efficacy and safety, advancing efforts to overcome drug-resistant malaria and improve global health outcomes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that generative AI technologies were used during the writing and

editing of this manuscript. Specifically, the QuillBot paraphrasing tool was employed to enhance clarity, improve language precision, and streamline complex ideas. QuillBot was used solely for paraphrasing and grammar checking, ensuring the manuscript was clear and concise.

Details of AI usage are as follows:

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After using this tool, the authors carefully reviewed and edited the content to ensure it met the standards of quality and accuracy required for publication. The authors take full responsibility for the content of the manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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