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In Silico Investigation of 2-Anilino 4-Amino Substituted Quinazolines as Potential Inhibitors of Plasmodium falciparum Dihydroorotate Dehydrogenase

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Abstract. Malaria continues to present significant global health challenges due to emerging resistance against conventional antimalarial drugs, necessitating novel therapeutic agents targeting *Plasmodium falciparum*. Dihydroorotate dehydrogenase (DHODH) is a crucial target in antimalarial drug development due to its essential role in pyrimidine biosynthesis within *Plasmodium* species. This study aimed to evaluate several candidate compounds from previous study - namely ligand 56, 65, 89 and 90 - as potential DHODH inhibitors using *in silico* methods, including molecular docking and molecular dynamics (MD) simulations. Molecular docking was performed using AutoDock Vina against the DHODH receptor structure (PDB ID: 4CQ8). The results indicated that ligand 89 exhibited the highest binding affinity (-9.575 kcal/mol), followed by ligands 90, 56, and 65, all demonstrating superior affinity compared to the control compound chloroquine (-7.462 kcal/mol). Interaction analyses revealed the formation of hydrogen bonds with key residues HIS185, GLY181, and ARG265, along with significant pi-sulfur interactions involving residue CYS184, thereby stabilizing the ligand interactions within the DHODH active site. Pharmacokinetic evaluations conducted using SwissADME revealed that all candidate ligands met Lipinski's rule and demonstrated high gastrointestinal absorption, despite their generally low solubility. MD simulations conducted over 100 ns at 300 K showed that all ligand-DHODH complexes, maintained stability, with Root Mean Square Deviation (RMSD) values ranging between 1.0 and 3.5 Å throughout the simulation. Overall, the findings suggest that ligand 89 and other evaluated ligands hold significant potential for further development as DHODH inhibitors in the pursuit of novel antimalarial drug candidates.

Introduction

Malaria is still an important public health issue worldwide, and notably in tropical regions such as Indonesia, which is due to the large contribution of *Plasmodium falciparum* to the country's high mortality and morbidity rates. The rising resistance of *P. falciparum* to conventional antimalarials, namely chloroquine and artemisinin, is the chief threat to the efficacy of therapy, and hence, urgently necessitates the discovery of new therapeutic modalities with novel mechanisms of actions and targeted approach (Kumar et al., 2018). Recent epidemiological studies consistently highlight that drug-resistant parasites significantly undermine malaria eradication efforts, particularly in endemic regions (Babai et al., 2022; Sharma et al., 2025).

A promising strategy in addressing this challenge involves targeting essential enzymes involved in parasite survival and proliferation. One such critical enzyme is

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dihydroorotate dehydrogenase (DHODH), which is integral to the *de novo* pyrimidine biosynthesis pathway within *Plasmodium* species (Hoelz et al., 2018). Unlike human cells, *P. falciparum* lacks an alternative pyrimidine salvage pathway and is entirely dependent upon the de novo synthesis for its DNA and RNA replication processes. Consequently, DHODH serves as a strategic biochemical target, offering opportunity to develop selective inhibitors that disrupt parasite growth while minimizing adverse effects on human host cells due to structural variations between the parasite and host DHODH enzymes (Singh et al., 2017).

However, the identification of potent DHODH inhibitors is still an arduous and resource-intensive process. To overcome the limitations of experimental screening, computational methods, such as molecular docking and molecular dynamics simulation, have become valuable aids. These *in silico* methods offer cost-effective early-stage evaluation of the interactions of ligands with proteins and the stability of complexes and hence help eliminate unfit candidates before experimental validation (Alzain et al., 2022).

The pharmacokinetic profiling program, represented by SwissADME, optimizes the compound selection process via the prediction of essential ADMET (absorption, distribution, metabolism, excretion, toxicity) attributes (Daina et al., 2017). By implementing the program within virtual screening systems, it allows the ranking of lead candidates and optimizes the process based on oral bioavailability, permeability, and likely safety, among others. Among new chemical structures, 2-anilino-4-amino quinazoline derivatives have proved to hold considerable promise. Ashton et al. recorded several quinazoline derivatives with rapid efficacy in the circulation and considerable selectivity towards the catalytic site of dihydroorotate dehydrogenase (DHODH) (Ashton et al., 2021). Their work flagged one particular subset of the derivatives namely 56, 65, 89, and 90, with clearly favourable properties in terms of binding energy and conformation.

The four ligands are studied under an exhaustive *in silico* approach that involves molecular docking, molecular dynamics simulation, and ADMET profiling. Though existing work has primarily focused on the results of docking studies, little consideration has been given to the identification of pharmacokinetic possibilities and dynamical features of the molecules in an overall integrated setting. Filling this void is essential to advance the candidates towards experimental proof and further clinical assessment.

The goal of the current work is the overall evaluation of the binding affinity, interaction stability, and pharmacokinetic properties of the four quinazoline derivatives disclosed in the work of Ashton et al. as promising inhibitors of *P. falciparum* DHODH (Ashton et al., 2021). The results are to guide their likely efficacy as future second-generation antimalarial agents that may address the growing threat of resistance to malaria drugs.

Experimental

Molecular Docking

Molecular docking was carried out to evaluate interactions between candidate compounds and dihydroorotate dehydrogenase (DHODH) receptor using AutoDock Vina software. The candidate compounds selected were those reported with high activity from previous research (Ashton et al., 2021), chosen due to their demonstrated promising antimalarial properties. The three-dimensional structure of DHODH was obtained from the Protein Data Bank (PDB) under the ID 4CQ8 (Rowland, 2014). To prepare the receptor for docking analysis, all water molecules and bound ligands within the crystal structure were removed using PyMOL software. Subsequently, the processed structure was saved in the PDBQT format as dhodh.pdbqt. Ligand structures underwent optimization processes employing Avogadro software, ensuring proper geometry optimization and energy minimization, before saving in PDBQT format as ligand.pdbqt. The docking simulations were performed by defining a specific grid box on the active site of DHODH, guided by the position of the native ligand from the original crystal structure. The grid box center coordinates were set precisely at x = 3.45, y = -0.787, and z = 37.44, with dimensions of 24 Å × 24 Å × 26 Å, thereby encompassing the entire active site pocket of the enzyme. Exhaustiveness parameter was increased to 16, a configuration selected to enhance the accuracy of identifying the optimal ligand conformations within the active site (Trott & Olson, 2010). Docking outcomes were recorded in a results.pdbqt file and assessed by comparing binding affinity scores and analyzing amino acid residue interactions within the active site using Discovery Studio software. Visualization clearly demonstrated the positioning and interactions of candidate ligands relative to key residues in DHODH.

ADMET Analysis

In silico analysis of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiles was conducted to assess the viability of the candidate compounds as potential drug molecules. These compounds were selected based on prior identification of superior activity and utilized consistently through docking phases

against the DHODH receptor. ADMET predictions were performed using the SwissADME online server (http://www.swissadme.ch), renowned for robust pharmacokinetic and physicochemical evaluations (Daina et al., 2014, 2017; Daina & Zoete, 2016). Each compound structure was inputted into SwissADME in Simplified Molecular-Input Line-Entry System (SMILES) format, facilitating accurate computational predictions. Critical pharmacokinetic parameters evaluated included gastrointestinal (GI) absorption, Blood-Brain Barrier (BBB) permeability, and the potential to function as substrates or inhibitors of P-glycoprotein (P-gp). Additional assessments encompassed adherence to Lipinski's Rule of Five, bioavailability scores, and solubility predictions. Collectively, these pharmacokinetic parameters provided substantial insights into the feasibility of each compound for oral bioavailability and potential efficacy as therapeutic agents. The resulting data from SwissADME analyses served as preliminary screening tools in the drug development pipeline, informing subsequent *in vitro* and *in vivo* experimental validations.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were implemented to analyze the stability of ligand-DHODH complexes under physiologically relevant conditions. Initial complex structures for MD simulations were derived from the best molecular docking outcomes, employing AutoDock Vina to ensure accuracy and consistency. The subsequent preparation and simulation steps utilized the AMBER25 molecular dynamics package, known for its robust capabilities in simulating biomolecular systems (Case et al., 2023). Topological and coordinate parameters were systematically generated using the ff19SB force field for proteins and the General Amber Force Field (GAFF) for ligands (Tian et al., 2020). Each ligand-DHODH complex was solvated explicitly within a TIP3P water box extending 10 Å from the outermost atoms of the protein-ligand complex, ensuring adequate hydration coverage. Charge neutrality of the systems was achieved by incorporating Na+ or Cl− ions as required. Energy minimization was initially executed to remove unrealistic atomic interactions, applying steepest descent and conjugate gradient algorithms to reach optimized configurations. Systems subsequently underwent controlled heating from 0 K to 300 K over 100 ps within an NVT (constant Number, Volume, Temperature) ensemble, with backbone protein atoms restrained using a harmonic constant of 10 kcal/mol·Å^2 to avoid unnatural structural deviations during initial equilibration. After thermal equilibration, an additional 500 ps equilibration phase was executed within an NPT (constant Number, Pressure, Temperature) ensemble at 300 K and 1 atm, free of positional restraints, to stabilize the density and pressure of the systems fully.

Production MD simulations were conducted for 100 ns at 300 K and 1 atm employing Langevin dynamics for temperature control with a collision frequency set at 2 ps^{-1}, and pressure regulation via the Berendsen barostat. The simulation integration step was fixed at 2 fs, with hydrogen-involving bonds constrained using the SHAKE algorithm to maintain simulation stability and computational efficiency. Coordinate data snapshots were recorded every 10 ps throughout simulations for detailed structural analyses.

Stability and dynamic interactions of ligand-DHODH complexes were analyzed primarily through Root Mean Square Deviation (RMSD) computations using the cpptraj module from AMBER (Roe & Cheatham, 2013). RMSD values provided quantitative measures of conformational stability and fluctuations experienced by ligand-DHODH complexes during simulation trajectories. Additionally, Visual Molecular Dynamics (VMD) software was employed for thorough visual inspection of ligand orientations and critical interactions with active-site residues throughout the simulation, further validating the dynamic stability and binding characteristics of each complex.

Result and Discussion

Molecular Docking

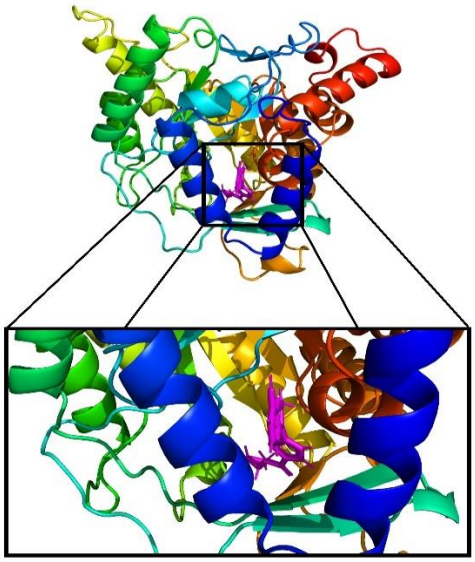

Figure 1. The Structure of *Plasmodium falciparum* DHODH (PDB ID: 4CQ8) with the native ligand (magenta) located within the enzyme's active site.

Binding affinity and interaction profile docking analysis between candidate ligands against the *Plasmodium*

falciparum dihydroorotate dehydrogenase (DHODH) enzyme (PDB ID: 4CQ8) revealed the molecular recognition features of the enzyme's active site. As shown in **Figure 1**, the interaction site of DHODH with its native ligand demonstrates key hydrogen bonding and hydrophobic interactions involving catalytic residues within the binding pocket, which serve as a structural reference for the evaluation of the docked ligands.

Ligands 89 and 90 displayed the highest binding affinities toward the DHODH receptor (-9.575 kcal/mol each), surpassing ligands 56 and 65 (-8.636 kcal/mol and -8.593 kcal/mol), and significantly outperforming chloroquine (-7.462 kcal/mol). These affinity values underscore the potential superiority of these ligands compared to chloroquine, indicating their potential as effective inhibitors of DHODH (**Table 1**). **Table 1** presents a comprehensive summary of the docking results and corresponding affinity values for each ligand, including the control chloroquine. The collective docking results strongly support these ligands as viable candidates for further in-depth analysis via molecular dynamics simulations, owing to their high affinity and stable interaction patterns within the active site of DHODH.

Table 1. Binding Affinities of Candidate Ligands with DHODH Receptor.

No	Ligand	Affinity (kcal/mol)
1	Native	-9.658
2	Chloroquine	-7.462
3	56	-8.636
4	65	-8.593
5	89	-9.575
6	90	-9.571

Visualization of molecular interactions demonstrated hydrogen bonding with active-site residues HIS185, GLY181, and ARG265, in addition to stabilizing pi-sulfur interactions involving CYS184. These were complemented by hydrophobic contacts with LEU172, LEU187, PHE188, and MET536, collectively enhancing ligand binding (**Figure 2**).

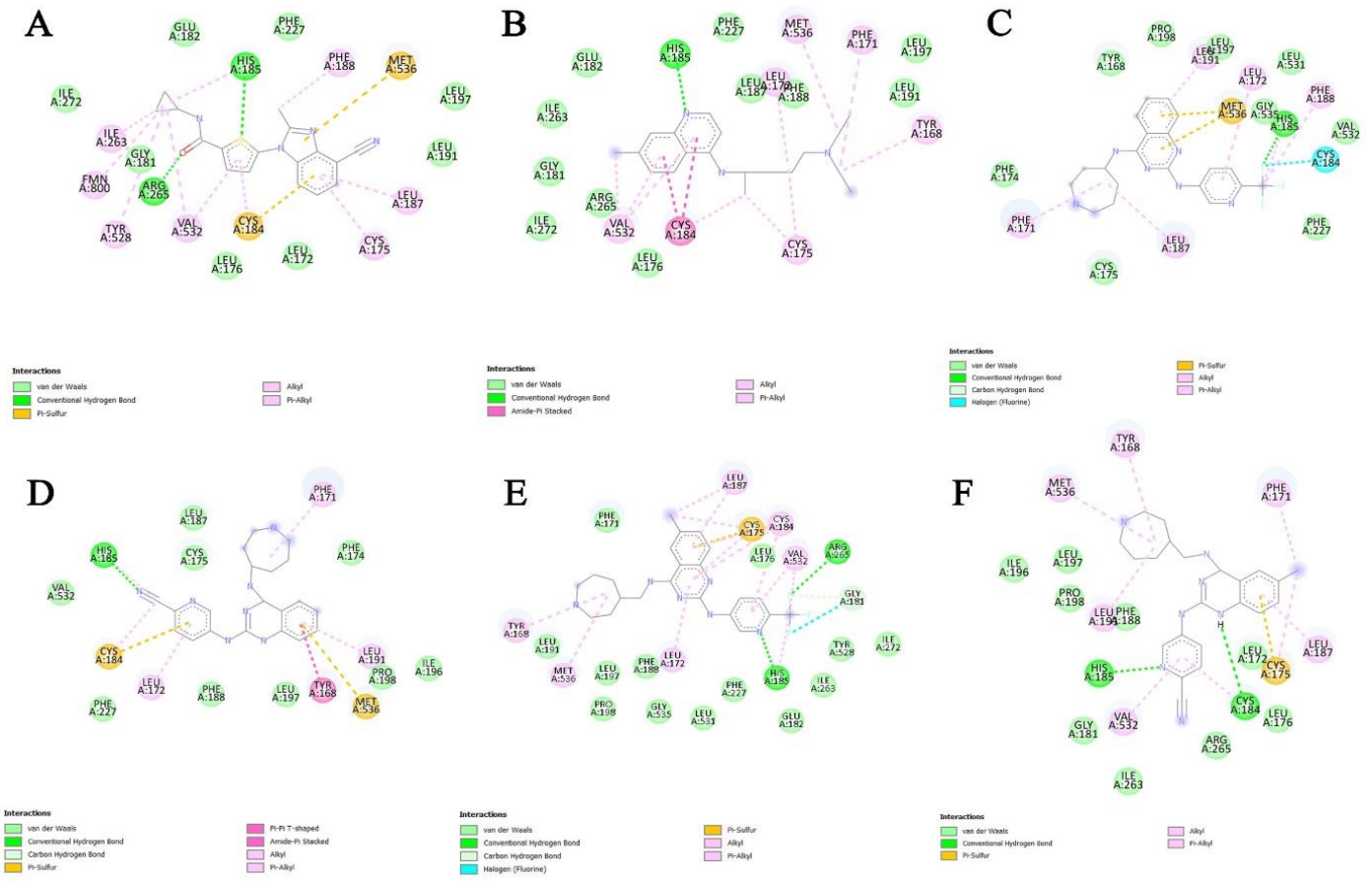

Figure 2. 2D Ligand–Protein Interaction Diagrams of DHODH Complexes with Candidate Inhibitors. Subfigures A–F illustrate the ligand positions and interactions for native, chloroquine, 56, 65, 89, and 90, respectively.

The molecular docking analysis showed that ligand 89 had the strongest binding affinity for PfDHODH (-9.575 kcal/mol), followed by ligand 90. Both values exceeded the affinity observed for chloroquine (-7.462 kcal/mol), suggesting these quinazoline derivatives may inhibit DHODH more effectively. Greater negative binding energies generally indicate more stable ligand–enzyme complexes (Alsedfy et al., 2024).

Detailed structural interaction analysis identified essential hydrogen bonds between ligands and key stabilizing DHODH active site residues, including HIS185, GLY181, and ARG265 (Alzain et al., 2022). The presence of pi-sulfur interactions involving residue CYS184 further contributed significantly to ligand stabilization within the enzyme's binding pocket. Additionally, extensive hydrophobic interactions, including pi-alkyl and alkyl bonds with LEU172, LEU187, PHE188, and MET536, enhanced ligand binding stability, aligning with earlier studies highlighting these residues' contributions to binding affinity (Manhas et al., 2017; Owoloye et al., 2020; Rawat & Verma, 2021; Xu et al., 2013).

ADMET Properties

Drug-Likeness and Absorption Characteristics Pharmacokinetic prediction using SwissADME indicated that all candidate ligands that all candidate ligands successfully complied with Lipinski's criteria, suggesting favorable potential for oral bioavailability. All ligands exhibited high gastrointestinal absorption profiles, indicating their potential effectiveness when administered orally. However, the solubility assessments generally indicated low solubility, a common characteristic that may necessitate further formulation strategies to enhance bioavailability. Specifically, chloroquine displayed poor solubility alongside BBB permeability, whereas the candidate ligands notably demonstrated low BBB permeability, a beneficial property in reducing potential central nervous system side effects.

Furthermore, ligands 56, 65, 89, and 90 were identified as potential substrates of P-glycoprotein, a factor that may influence their intracellular concentrations and necessitate consideration in further drug development phases. Detailed pharmacokinetic predictions are presented in **Table 2**, offering an extensive comparison of all evaluated compounds, underscoring their suitability for further drug development processes with considerations of optimization to overcome the solubility constraints.

In silico ADMET analysis conducted via SwissADME further substantiated the therapeutic potential of these candidate ligands. All evaluated ligands conformed to Lipinski's Rule of Five, indicating favorable physicochemical profiles and potential oral bioavailability (Lipinski, 2004). Ligand lipophilicity (log P) values ranged moderately between 1.67 and 4.5, suggesting suitable membrane permeability while retaining adequate biological solubility (Chen, 2009; de Souza et al., 2025). Despite high gastrointestinal absorption predictions for all ligands, low solubility emerged as a potential challenge, urging formulation optimizations to enhance bioavailability as observed in the case of chloroquine (Neumaier et al., 2021).

Table 2. Summary of In Silico Pharmacokinetic Analysis Results of Candidate Compounds.

No	Ligand	MW (g/mol)	Log P	Acceptor	Donor	Molar Refractivity	Lipinski	GI	BBB	P-gly	Sol
1.	Native	322.38	2.9	4	3	89.12	Yes	High	No	No	Moderately soluble
2.	Chloroquine	319.87	4.15	2	1	97.41	Yes	High	Yes	No	Poorly soluble
3.	56	402.42	3.55	7	3	109.82	Yes	High	No	Yes	Poorly soluble
4.	65	361.44	1.67	5	4	116.13	Yes	High	No	Yes	Poorly soluble
5.	89	450.89	4.5	7	3	119.64	Yes	High	No	Yes	Poorly soluble
6.	90	409.92	2.28	5	4	125.95	Yes	High	No	Yes	Poorly soluble

Importantly, low blood-brain barrier (BBB) permeability predictions for candidate ligands represent a crucial advantage, significantly minimizing potential central nervous system side effects (Huang et al., 2024). Additionally, the prediction that these ligands act as potential substrates for P-glycoprotein (P-gp) influence

intracellular concentration and distribution. However, this characteristic may also reduce systemic toxicity risks, emphasizing the importance of comprehensive pharmacokinetic considerations in subsequent developmental stages (Tia et al., 2025).

Molecular Dynamics Simulastion

Structural Stability of Ligand-Receptor Complexes Molecular dynamics simulations over 100 ns at a physiologically relevant temperature of 300 K, confirmed the structural stability of the ligand-DHODH complexes. Throughout the simulation duration, all ligand-DHODH complexes displayed fluctuations in RMSD within a narrow and acceptable range of 1.0–3.5 Å. This observed range indicates that all complexes maintained structural stability under simulated physiological conditions, with minimal deviations from their initial conformations post-equilibration phase (**Figure 3**).

Ligand 65 demonstrated the lowest and most stable RMSD profile among the studied complexes, signifying strong and consistent interactions with DHODH throughout the simulation. Ligand 89, despite having the highest binding affinity during docking, exhibited slightly greater RMSD fluctuations. However, these fluctuations remained within the acceptable stability range, confirming the persistent ligand-enzyme interactions within the active site during simulation.

Molecular dynamics simulations over 100 ns demonstrated that all ligands remained stably bound within the PfDHODH active site under simulated physiological condition, with Root Mean Square Deviation (RMSD) values below 3.5 Å. Ligand chloroquine and compounds 56, 65 display good stability in their binding, with lower and more stable RMSD values, while ligand 89 showed minor fluctuations without dissociation, confirming the durability of the predicted binding modes under near-physiological conditions.

The overall predictive dock-ADMET-MD results show that the following ligands, 89, 90, 56, and 65, are the best candidates to inhibit PfDHODH. These computational results agree with the previous work regarding the quinazoline scaffolds and fast antiplasmodial activity (Ashton et al., 2021).

Thus, this comprehensive in silico evaluation robustly supports the continued development of ligands 89, 90, 56, and 65. Subsequent research stages should include rigorous experimental validation through enzymatic inhibition assays and in vitro antimalarial activity tests, which will critically verify computational predictions and further elucidate these compounds' pharmacological potential. By confirming these initial computational findings experimentally, the candidate ligands can be advanced as viable therapeutic options to overcome current antimalarial resistance challenges.

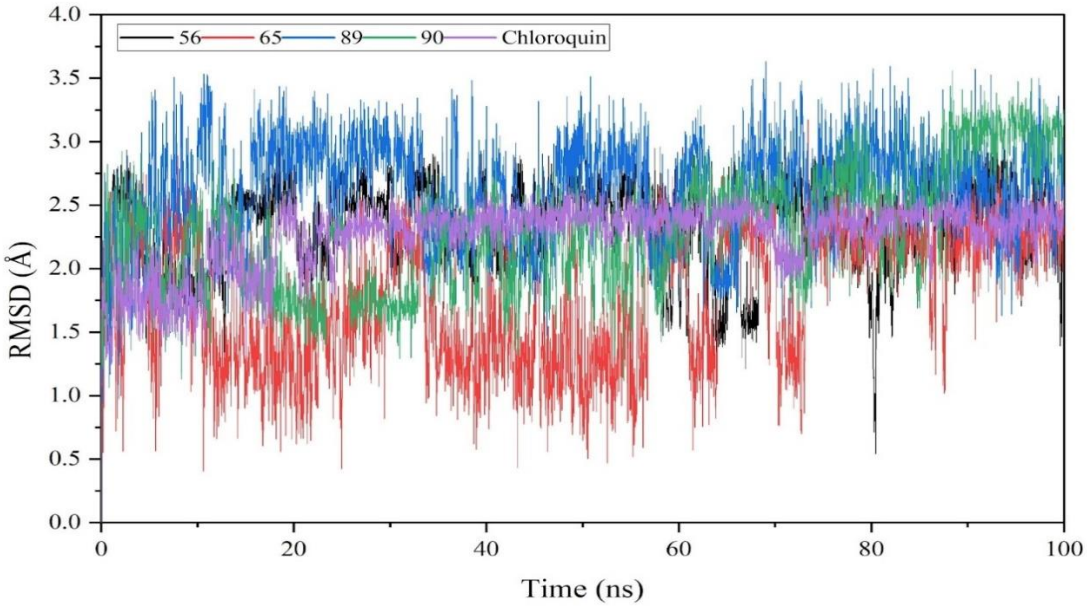

Figure 3. RMSD plots of DHODH–ligand complexes (ligands 56, 65, 89, 90, and chloroquine) during 100 ns MD simulations.

Conclusion

In this study, an integrated computational strategy was employed to assess four quinazoline derivatives namely compounds 56, 65, 89 and 90 as inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase. Molecular docking identified ligand 89 as the strongest binder with a calculated affinity of 9.575, followed by ligands 65 and 56, all of which showed stronger binding than the chloroquine benchmark. Detailed interaction analyses confirmed that these compounds engage key active-site residues (HIS185, GLY181, ARG265 and CYS184) through stable hydrogen bonds, hydrophobic contacts and pi-interactions, thus supporting their potential to block enzyme activity. Pharmacokinetic analysis indicated that all candidate ligands conformed to Lipinski's Rule of Five, exhibited high gastrointestinal absorption potential, albeit with limited solubility, and showed capability as substrates for P-glycoprotein (P-gp). These pharmacokinetic properties underscore their potential for oral administration, though formulation optimization may be necessary to address solubility issues effectively. Molecular dynamics simulations over a duration of 100 ns at 300 K further confirmed the stability of ligand-DHODH complexes, maintaining consistent interactions within the enzyme's active site, as evidenced by RMSD values within the acceptable stability range of 1.0–3.5 Å. Together, these findings provide a compelling *in silico* rationale for advancing these quinazoline derivatives as selective PfDHODH inhibitors. The next phase should encompass enzymatic assays and *in vitro* antiplasmodial testing to confirm their inhibitory potency and to guide further optimization toward new antimalarial therapies capable of overcoming existing drug resistance.

Conflict of Interest

The authors declare that there is no conflict of interest.

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