

Mechanistic exploration and dynamic binding behaviour of reported antimalarial compounds targeting PfDHODH

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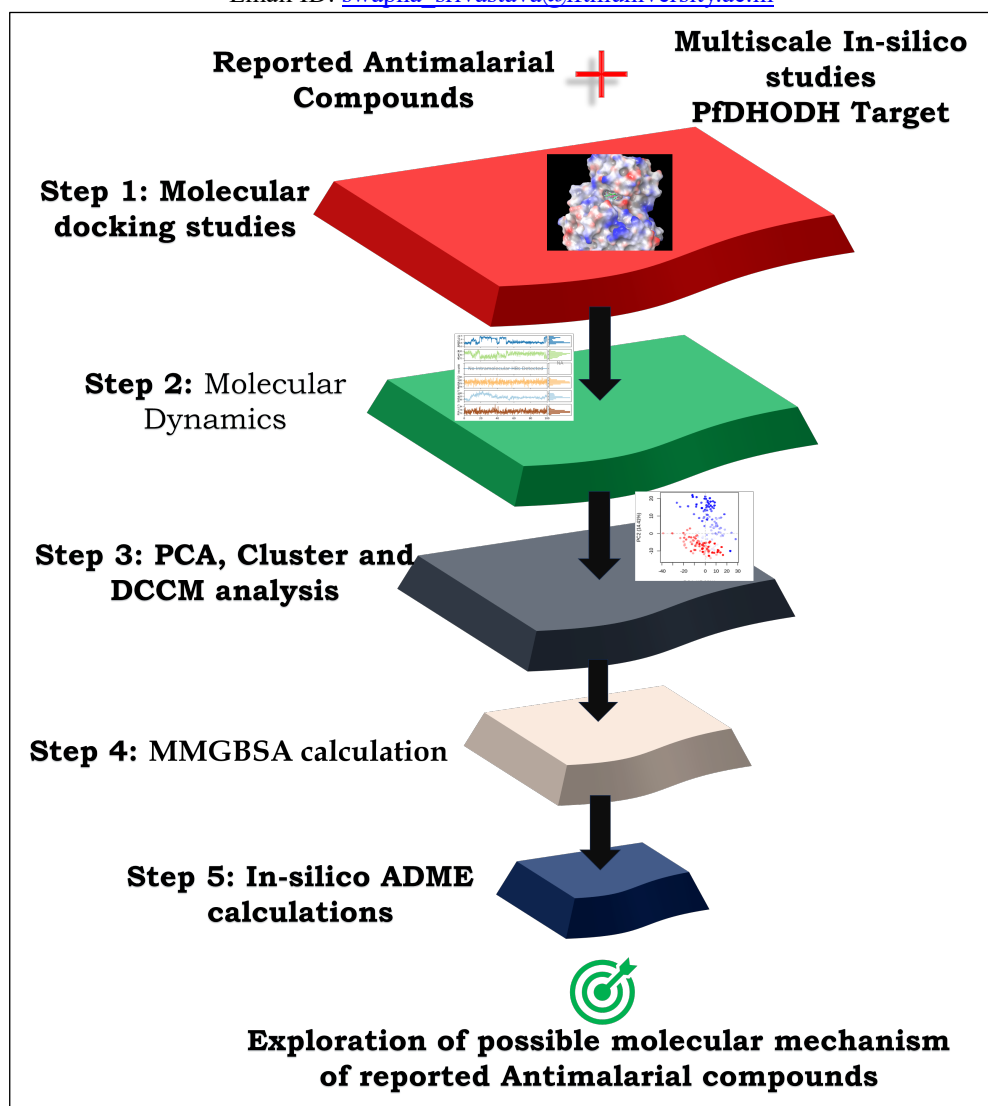
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Graphical Abstract

Abstract:

Malaria continues to be a significant challenge for global health, mainly caused by *Plasmodium falciparum* parasite. Dihydroorotate dehydrogenase (PfDHODH), a key enzyme in pyrimidine biosynthetic pathway and has emerged as an attractive target for antimalarial drug discovery. Despite the several novel antimalarial compounds, the detailed molecular mechanism by which specific inhibitors bind to PfDHODH is still poorly understood. This study aims to study drug-target interactions by molecular docking and simulation studies, further to validate the result PCA, DCCM and MMGBSA analysis were performed.

Molecular docking with PfDHODH showed the strong binding of MMV1557817 with the best docking score (-10.754 kcal/mol). Further stability of complexes was confirmed by MD simulations with low RMSD, RMSF and consistent radius of gyration values. PCA analysis revealed significant conformational stability, while DCCM results showed coordinated residue movements. MMGBSA studies indicated favourable binding energies, especially for MMV1557817 and DSM-421. Moreover, ADME results demonstrated the drug-likeness of molecules and oral absorption, suggesting these compounds as promising candidates for further antimalarial drug development. Among the selected compounds, MMV1557817 displayed the most conserved interaction with PfDHODH during MD simulation. These findings serve as a lead for further drug discovery for antimalarial drugs.

Keywords: Plasmodium falciparum, PfDHODH Inhibitors, Antimalarial Drug Discovery, Molecular Dynamics Simulation, PCA analysis, MMGBSA

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Introduction:

Malaria is among the deadliest infectious diseases globally and infects millions of people each year. Most cases of the disease are caused by parasites from the genus *Plasmodium* (Mugisa et al., 2026). The most dangerous species amongst genus *Plasmodium* is *Plasmodium falciparum*. It is responsible for most cases and deaths from severe malaria infections (S. Sato, 2021). The parasite causes malaria, which is transmitted to humans through the bite of infected female *Anopheles* mosquitoes (da Silva et al., 2023; Venugopal et al., 2020). Once the parasite enters the human body, it reproduces in liver cells followed by infection of red blood cells (Appetecchia et al., 2024). This infection causes fever, chills, fatigue and anaemia. In more severe cases, it can result in organ failure and death (Filipe & Loura, 2022).

A number of antimalarial drugs have also been developed in the past decades to control the disease (Ampornnanai et al., 2022; Hidayati et al., 2023). In malaria treatment, drugs including chloroquine, mefloquine and artemisinin derivatives have been broadly used (Sankineni et al., 2023). In terms of treatment, Artemisinin-based combination therapies are presently the most effective for management of *P. falciparum* malaria (Irwin et al., 2020). But the malaria parasite has slowly acquired resistance to many of the drugs now in use. Resistance to chloroquine has already been widely disseminated in many areas (Palmer et al., 2021). Reduced sensitivity to artemisinin-based therapies has also been reported in recent years. The ongoing emergence of drug-resistant strains of parasite has posed a great threat to malaria control program. Hence, it is desperately needed to

explore new drug target and discover efficacious antimalarial agents (Kotepui et al., 2020).

A drug discovery strategy that for malaria is targeting essential enzymes of parasite that do not share homology with humans (Azad, 2023). Therefore, targeting of the parasite-specific enzymes can lead to selective inhibition of the parasites itself without disturbing their hosts (Nguyen et al., 2023; Probst et al., 2025). Among the important enzymes, *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) has drawn much attention (Suryavanshi et al., 2025). This enzyme is involved in the de novo pyrimidine biosynthetic pathway of the parasite. Pyrimidines: An essential molecule for DNA and RNA synthesis. They are essential for generating new cells and copying them (van Niekerk et al., 2023).

The malaria parasite cannot utilize pyrimidines from the host cell, unlike human cells (Calic et al., 2020; Calit et al., 2024). Rather, it uses only its own biosynthesis pathway. This pathway incorporates several significant steps, one of which is catalysed by PfDHODH (Nie et al., 2025). During pyrimidine synthesis, it catalyses the conversion of dihydroorotate to orotate. This step is critical for pyrimidine nucleotide production (Rajaram et al., 2022). Thus, blockade of pyrimidine biosynthesis can effectively stop parasite propagation by inhibiting PfDHODH (Awalt et al., 2024; Shibeshi et al., 2020). Due to this critical role, PfDHODH represents an attractive target for antimalarial drug discovery (Lahree et al., 2023).

Here we report a comprehensive computational study for exploring the mechanistic insights of reported antimalarial compound targeting the PfDHODH

system(Goswami et al., 2022). Docking of molecules was performed to find the orientation and interactions between compounds and active site of enzyme (Evers et al., 2021). The stabilities and flexibilities of the protein-ligand complexes were subsequently evaluated using long-timescale molecular dynamics simulations (Alday et al., 2021; Sun et al., 2024). Secondary analyses including PCA and DCCM were performed to understand the collective motions and conformational dynamics of the protein (Pou et al., 2024).

Estimating binding free energy using certain algorithm such as MM-GBSA is another crucial insight provided in this study (Birkholtz et al., 2022). Helps to assess the robustness of ligand-protein interactions, and shed light on which compounds investigated are relatively more stable with respect to one another (Dodean et al., 2025).

This study provides in-depth mechanistic knowledge on the interaction of anti-malarial reported compounds (DSM-74, TCMD-124418, DSM-421 and MMV1557817) with PfDHODH(Kancharla et al., 2020). The findings advance knowledge about how these molecules stabilize inside the enzyme active site and affect its structure dynamics (Dorjsuren et al., 2021). This information could provide valuable insight for structural-based rational design and optimization of future PfDHODH inhibitors(Luo et al., 2023; Yang et al., 2021). Thus, the outputs of this work could also support existing work in antimalarial drug discovery and development efforts (Komatsuya et al., 2022).

Material and Methods

The current study utilized Glide (Schrödinger Suites 2017-2, LLC, Cambridge, USA) for molecular docking to conduct the preliminary binding analysis (Rawat & Verma, 2020). *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) is a protein acquired through the Protein Data bank (www.rcsb.org) using the PDB ID 7WYF. The composition of PfDHODH has a resolution of 3.30 Å and consists of a single chain (A chain) comprising 431 amino acids in length. The reliability of the protein structure is assessed through computational studies and all residues along with any absent hydrogen bonds are identified using protein preparation panels. It is essential to verify the docking results and perform a comprehensive examination of the behaviour of the ligand within the moored structures of the ultimate therapeutic candidates were forwarded to MDS, focusing on the binding pocket of the target proteins. An investigation into Molecular Dynamics Simulation was conducted utilizing the Desmond V3 software developed by the DE Shaw group (Onyango et al., 2024). The established procedures were carried out on an Intel CTM i7-8700 processor functioning at 3.20 GHz and 3.19 GHz, with 16 GB of RAM on a 1 TB

Ubuntu system. The detailed methodology is explained in Figure 1.

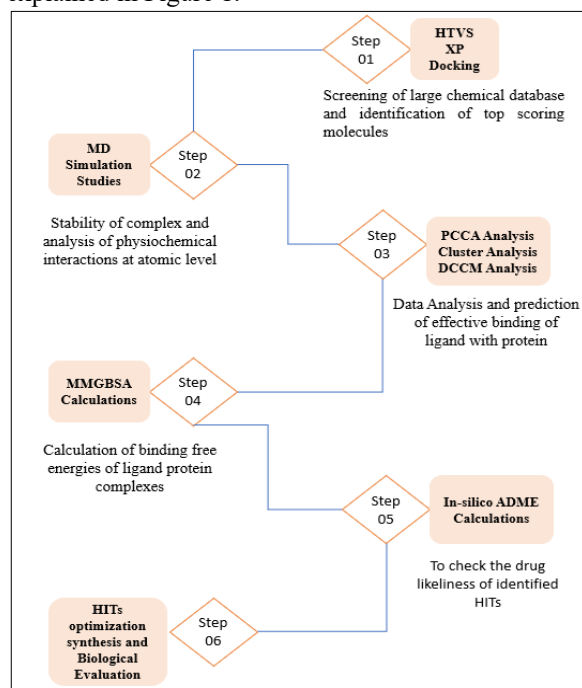


Figure 1: Process flowchart for identifying new PfDHODH inhibitors targeting *Plasmodium falciparum*.

2.1. Molecular Docking:

The 3D structure of PfDHODH, also known as dihydroorotate dehydrogenase (PDB ID: 7WYF) was prepared for molecular docking by assigning bond orders, deleting water molecules from hetero groups larger than 5 Å, adding hydrogens, removing original hydrogens and generating zero-order bonds to metals (Rawat & Verma, 2020). Protein preparation involved removing the water molecules from the structure, incorporating hydrogen atoms and rectifying side chains (Pinzi & Rastelli, 2019). To prepare the protein, the Maestro software is utilized, in which the protein is protonated and transformed into pdb format prior to performing docking (Joshi et al., 2024). To find the active site for ligand binding, co-crystallized ligands were re-docked to the receptor by varying the grid box size and the binding site coordinates (Sugita, 2023). For this, the OPLS-2005 force field utilized in the Schrodinger Ligprep version 4.7 module was utilized to create three-dimensional natural ligand molecules while preserving their initial conformation(Shamsuddin et al., 2021). Given that it indicates the site of the protein where the binding affinity score functions are provided and the ligand will dock, grid formation around a protein's three-dimensional structure is an essential stage in the docking process (Alzain et al., 2022). In this study, grids were created to position ligand molecules within

the binding site, utilizing a grid box in three dimensions with dimensions of 10 Å, which includes consist of the active site regions of all desired proteins (Zakaria et al., 2020). This task was completed by the Glide Program v8.2's grid creation tab (Naik et al., 2024).

2.2. Molecular Dynamics Studies:

Desmond V3 developed by the DE Shaw group was utilized to do MD simulations of best scoring. The procedure involves the three basic steps, system builder, energy minimization and MD simulation (Rawat & Verma, 2021). First step deals with the positioning of the selected ligand-protein complex within an SPC (single point charge) water box, which extends 10 Å beyond each atom within the complex. To replicate physiological conditions, a salt concentration of 0.15 M was set for both sodium and chloride ions (Ibrahim et al., 2022). Subsequently, the box simulation was neutralized through the addition of both positive and negative ions, specifically sodium and chlorine. The energy was minimized using the steepest drop for one nanosecond; when the energy hit 100 kJ/mol, the decline was stopped. To maintain system stability for 1 ns each, the NVT and NPT ensembles were run independently utilizing a 2-fs time step at a temperature of 300 K and a pressure of 1 atm, utilizing the Verlet cut-off (Hermawan et al., 2024). For every system that fitted its massive atoms utilize the initial configuration for manufacturing dynamics as the benchmark. point and independently calculate the root-mean square deviation (RMSD). Hydrogen interactions (H-bonds) for complexes containing protein cofactor (FMN) and ligand were evaluated. A hit was recognized when the separation between two polar heavy atoms, each connected to at least one hydrogen atom, dropped below 3.5 Å and the H-donor angle surpassed 120° (Evaristo et al., 2025).

2.3. PCA Analysis:

PCA has been used to analyse MD trajectories and experimental structure distributions. It has also been shown to offer significant insights into the characteristics of conformational alterations among different protein families (Hartuti et al., 2021). In summary, PCA relies on diagonalizing the covariance matrix, C , where elements C_{ij} are derived from the superimposed and aligned Cartesian coordinates, r , of identical $C\alpha$ atoms. We employed the Bio3D R software tool to analyse protein structures to aid in the comparison process (Favuzza et al., 2020). To achieve this, we brought the Desmond trajectory files (.dtr) are imported into VMD as well as then transformed to CHARMM/NAMD trajectory formats. To prepare PCA data, we initially arranged atoms regarding each source trajectory frame containing simplified structural PDB files (Pramisandi et al., 2020). As a result, we generated quantitative coordinates (x, y, z)

for C imported atoms into RStudio and corresponding .dcd files. The main motion is obtained by examining the conformation of the sample by applying a basic component analysis (PCA) to the protein trajectory, commonly referred to as essential dynamics (ED) (D. Sato et al., 2020; Zeng et al., 2021). The particular protein motions (at the chosen coarse-grained level) can be described by a linear combination of the 3m orthogonal modes derived from principal component analysis (PCA). As a result, significant protein movements are defined by a restricted number of PCA modes exhibiting the greatest variances (Belete, 2020a). Looking specifically at the small movements, the PCA method may be effectively utilized by targeting the specific areas of the protein, thereby elucidating the description of the movement in the specific area of the protein (Abdel-Magid, 2020; Duffey et al., 2021).

2.4. Dynamics cross-correlation matrix (DCCM) analysis:

Dynamic Cross-Correlation Matrix (DCCM) analysis represents an advanced computational technique employed in molecular dynamics (MD) simulations to explore the interrelationships in motion among residues or atoms in biomolecular systems like proteins, nucleic acids, or complexes (Gholam et al., 2024; Kleandrova et al., 2025). This method entails computing a matrix of correlation coefficients that measure the relationships between the movements of various atoms or residues during a simulation (Elamin et al., 2023). The DCCM is constructed using the correlation coefficient C_{ij} is between the displacement vectors of atoms or residues i and j over the course of the simulation (Gehlot & Vyas, 2023; Heidarian & Zahedi-Tabrizi, 2020). The equation for calculating C_{ij} is

$$ij = (\Delta r_i \cdot \Delta r_j) \div \sqrt{(\Delta r_i \cdot \Delta r_i) (\Delta r_j \cdot \Delta r_j)}$$

Where, Δr_i represents the deviation in relation to the average position of atom i .

Δr_i denotes the deviation of the location of atom j in relation to its average position.

2.5. MMGB/SA (Molecular Mechanics/Generalized Born Surface Area):

The pulling forces within components of the ligand-protein complex entropy were assessed using the Prime MMGB-SA approach (Satarker et al., 2022). The Prime approach combines the Generalized Born/Surface Area continuum solvent model with the Molecular Mechanics Poisson Boltzmann approach (Qureshi et al., 2020). By measuring the change in binding free energy (ΔG_{Bind}), MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) assesses the thermodynamic stability of the ligand-receptor interaction (Belete, 2020b; Custódio et al., 2021). A negative ΔG_{bind} reflects favourable binding and indicates that when bound, the ligand has

A higher level of attraction for the desired receptor in comparison to its free state. Greater ligand stability associated with the protein's binding site results in a lower score (Owoloye et al., 2022; Singh et al., 2022). $\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T\Delta S$

- The variation in energy during molecular mechanics, done during binding, is described by the term E_{MM} .
- The GB model and SA calculations apply in the determination and assessment of the changes in solvation free energy, G_{solv} .

2.6. In-silico ADME calculation:

The formation of novel pharmacological agents largely depends on factors such as those associated with their "absorption, distribution, metabolism, and excretion (Godinez-Macias & Winzeler, 2024)." These have a direct impact on how a specific dose of an agent should be used. To assess and predict a ligand's characteristics, such as those concerning its absorptivity, distribution, metabolism, and excretion, QikProp was used, which is a tool for virtual bioactivity screening and analysis (Olusola et al., 2024). A ligand's "drug likeness" was based on Lipinski's Rule of 5, which involved important considerations such as its capacity to function as a hydrogen bond acceptor or a hydrogen bond donor, its molecular weight, its capacity to cross the blood-brain barrier, its ability to undergo CYP metabolism, its capacity to be polar, its capacity for oral bioavailability in human beings, and its ability to cross a cell culture of human intestinal cells (Nwonuma et al., 2023).

3. Result and Discussion:

In the current work, a systematic computational analysis was performed to evaluate potential inhibition of parsing reported antimalarial molecules on PfDHODH. First, molecular docking analysis was carried out to study the binding orientation and interaction patterns of compounds into protein active site. Based on the docking results and interaction profiles, four of them were chosen for subsequent investigation. Long-timescale molecular dynamics simulations were performed to gain insights into stability and dynamic behaviour of the protein-ligand complexes. To assess complex stability, various structural and dynamic parameters were examined. Moreover, advanced analyses were performed including PCA, DCCM, NMA and MM-GBSA binding free energy calculations. ADME prediction was used for the assessment of drug-like characteristics and evaluation of their pharmacokinetic properties. In following sections, we discuss the results of this analysis in details.

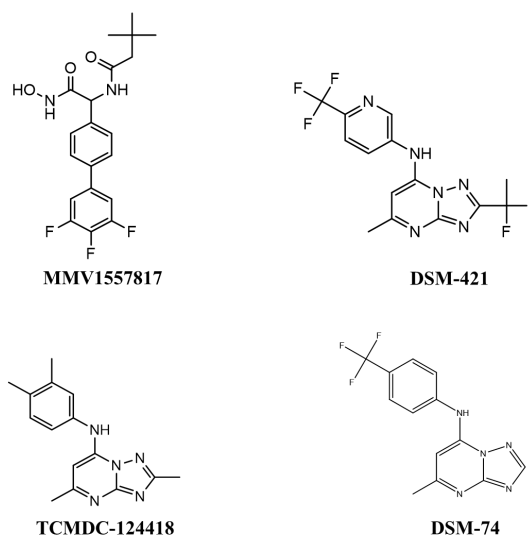


Figure 2: 2D Structures of selected antimalarial molecules for in-silico exploration of possible mechanism DSM-74, TCMDC-124418, DSM-421 and MMV1557817.

3.1. Molecular Docking Study:

To identify the active site of dihydroorotate dehydrogenase (PfDHODH, PDB ID 7WYF), the amino acid residues of PfDHODH that engage with the ligands were examined (Figure 2). The residues participating in the binding site are Ala225, Gly226, Lys229, Asn230, Thr249, Ile263, Ile272, Asn274, Cys276, Ser311, Lys429, Asn458, Ser477, Gly478, Gly507, Tyr528 & Ser529, which are considered the ligands target site and are used to formation of grid box in molecular docking studies. The docking scores of the selected ligands and receptor interactions were compared with reference molecule, FDA approved drugs (shown in Table 1 & 2) and comparative analysis is done to screened the ligands for molecular simulation. In this case, we utilized the OPLS-2005 standard is the force field employed in the Ligprep v4.7 module. The docking results achieved for the highest-scoring molecule are summarized in Table 1, while Figure 4 and 5 illustrate Three-dimensional and two-dimensional dynamic diagrams, respectively. All molecules with good binding scores occupied a binding site that displayed identical features to those shown by a reference molecule, is supported due to an abundance of hydrophobic interactions through amino acid residues (Table 1 and Figure 5 & 6). The leading four compounds exhibited docking scores between -10.754 and -9.430 kcal/mole as illustrated in Table 1, which suggests a favourable interaction of these compounds with the protein. This represents a significant computational method for assessing the precision of docking processes. The results confirmed the

advantageous binding with scores varying from -9.430 to -10.754 kcal/mol.



Figure 3: 3D structure of dihydroorotate dehydrogenase (PfDHODH, PDB ID 7WYF) is associated with flavin mononucleotide (FMN), orotic acid, and ethyl 2-(naphthalen-2-ylamino)-4-oxidanylidene-furan-3-carboxylate.

The top scoring compound MMV1557817 showed hydrogen bonding interaction with Arg-265 and hydrophobic interaction with Phe-227, Cys-184, Ile-272, Leu-189, Val-532, And Met-536 were observed. Other compounds with substantial binding affinities were DSM-74 (-9.430) TCMDC-124418 (-9.683) and DSM-421 (-9.684). Hence, the attachment of these compounds to PfDHODH resulted in alterations in the protein's conformation and influenced the dynamics of PfDHODH-catalysed phosphorylation. In general, the identified compounds demonstrated the essential affinities and connections with the remnants of the active location PfDHODH indicate that these compounds are likely to be incorporated into the later Phases of the pharmaceutical creation and development process.

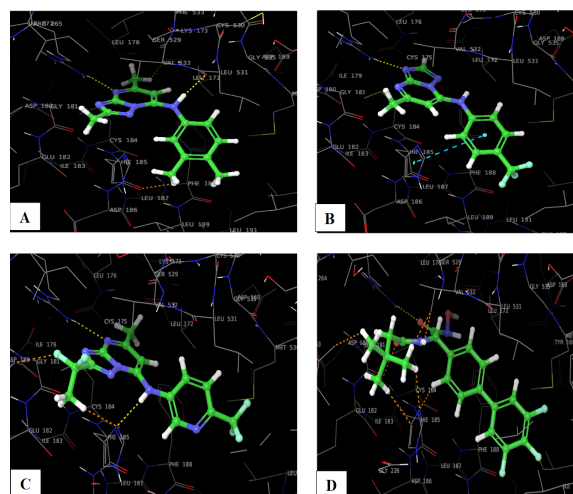


Figure 4: 3D interactive models of designed molecules positioned at the active site of 7WYF. Where A: DSM-74 (5308127); B: TCMDC-124418 (25263414); C: DSM-421 (122552662); D: MMV1557817 (124196387). Various forms of include connections like hydrogen bonds, pi-pi stacking contacts, hydrophobic bonds, and polar interactions among residues, have been demonstrated in Figureure and tabulated in the table1. The yellow line represents the H bonding between drug and active site residues. The turquoise line shows the p-p stacking interactions between two hydrophobic group of drug and receptors.

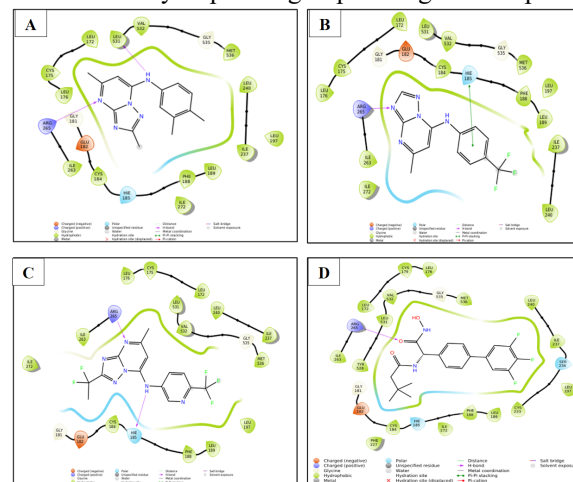


Figure 5: 2D interactive diagrams of designed molecules at the active pocket of 7WYF. Where A: DSM-74 (5308127); B: TCMDC-124418 (25263414); C: DSM-421 (122552662); D: MMV1557817 (124196387). The H bond interactions are shown by pink line, green coloured residues represent the hydrophobic interactions, turquoise-coloured residues show the polar interactions.

Table 1: Results acquired from the docking studies and various interactions between the drug and active site residues are presented

Sr No	Compound	Docking Score	Interaction	Active site residue
1.	A: DSM-74 (5308127)	-9.430	H-Bond	Arg265, Leu531
			Hydrophobic	Ile263, Cys184, Ile272, Phe188, Leu189, Ile237, Leu197, Leu240, Met536, Val532, Leu531, Leu172, Cys175, Leu176, Ile263, Cys184, Ile272, Phe188, Leu189, Ile237, Leu197, Leu240, Met536, Val532, Leu531, Leu172, Cys175, Leu176
			Polar	Hie185
			Glycine	Gly535, Gly535
			Charged (Positive)	Arg265
			Charged (Negative)	Glu182
2.	B: TCMDC-124418 (25263414)	-9.683	H-Bond	Arg265
			Pi-Pi stacking	Hie185
			Hydrophobic	Leu240, Ile237, Leu189, Phe188, Leu197, Met536, Cys184,

				Val532, Leu531, Leu172, Cys175, Leu176, Ile263, Ile272
			Polar	Hie185
			Glycine	Gly535, Gly181
			Charged (Positive)	Arg265
			Charged (Negative)	Glu182
3.	C: DSM-421 (122552662)	-9.684	H-Bond	Arg265, Hie185
			Hydrophobic	Cys184, Phe188, Leu189, Leu197, Ile272, Ile263, Met536, Ile237, Val532, Leu531, Leu240, Leu172, Cys175, Leu176
			Polar	Hie185
			Glycine	Gly535, Gly181
			Charged (Positive)	Arg265
			Charged (Negative)	Glu182
4.	D: MMV155781 7 (124196387)	-10.754	H-Bond	Arg265
			Hydrophobic	Phe227, Cys184, Ile272, Phe188, Leu189, Cys233, Leu197, Ile237, Leu240, Met536, Leu176, Cys175,

				Val532, Lu531, Leu172, Ile263, Tyr528
			Polar	Hic185, Ser236
			Glycine	Gly535, Gly181
			Charged (Positive)	ARG26 5
			Charged (Negative)	GLU18 2

Table 2: Binding Energy of FDA Approved Inhibitors

S.N o.	Drug	Molecular Formula	PubCh em ID	Energy (kcal/ mol)
1.	Mefloquin e	C ₁₇ H ₁₆ F ₆ N O	4046	-7.7
2.	Piperaqui ne	C ₂₉ H ₃₂ Cl ₂ N ₆	12226 2	-7.7
3.	Artemisin in	C ₁₅ H ₂₂ O ₅	68827	-7.3
4.	Amodiaqu ine	C ₂₀ H ₂₂ CIN O	2165	-7.1
5.	Pyrimetha mine	C ₁₂ H ₁₃ CIN 4	4993	-6.7
6.	Quinine Sulphate	C ₄₀ H ₅₀ N ₄ O 8S	16051 948	-6.7
7.	Tafenoqui ne	C ₂₄ H ₂₈ F ₃ N O ₃	11535 8	-6.4
8.	Chloroqui ne Phosphate	C ₁₈ H ₃₂ CIN O ₈ P ₂	64927	-5.9
9.	Primaquin e	C ₁₅ H ₂₁ N ₃ O	4908	-5.8
10.	Proguanil	C ₁₁ H ₁₆ CIN 5	61781 11	-5.5

3.2. MD Simulation:

The docking complex that achieved the highest score, along with the reference, were subjected to molecular dynamics simulation study using the SPC model for a period of 300 ns. The results of these studies lead us to investigate the validation of the protein, which may take place when they are in solution. In the 300 ns simulation run that produced frames of the protein-ligand complex trajectories were analysed, which included the α -carbon RMSD, a ligand RMSD concerning the amino acid, RoG, the Root Mean Square Fluctuation (RMSF), also the assessment of Protein-ligand interactions. This measures the variation from the original (native structure) displacement position of the atom in the protein-ligand

complex over a specified duration. It acts as an essential numerical evaluation for analysing the consistency of the structures of both the protein as well as ligand throughout the duration regarding the activation. For better understanding of stability of docked structures, the comparative RMSD Ca and ligand fit on protein of all five identify HITs are given Figure.6 and values are summarized in Table 3. The average RMSD of the protein Ca atom was determined to be 2.531 Å, 1.910 Å, 2.356 Å, 1.601 Å, DSM-74, TCMDC-124418, DSM-421 and MMV1557817 respectively, whereas 1.9316Å is the mean RMSD for apoprotein Ca (Table 3). The MMV1557817 protein complex showed the best RMSD Ca (Å) atom of 1.601 Å in comparison to reference molecule, which were stable for complete simulation protocol (Figure 6).

The RMSD backbone results indicated that each molecule maintained consistent interactions with the protein across the entire 300 ns simulation protocol. No significant variations were noted, indicating optimal connections amongst ligands and proteins. The reference molecule showed fluctuations between 2.5 and 3.0 Å from 58 to 75 ns, subsequently, it remained unchanged throughout the whole simulation. In comparison, every other screened molecule exhibited stable interactions, varying between 1.0 and 2.5 Å during the simulation protocol. The root mean square deviation (RMSD) (Å) The measurement of the column was determined to be 2.531, 1.902, 2.351, 1.636 Å bound with reference, TCMDC-124418, DSM-74, DSM-421, and MMV1557817 molecules respectively. The findings of this research are illustrated in Figure 6. In order to assess the reliability of ligand-protein interactions were analysed by capturing images from the MD trajectory at intervals of 0, 20, 40, 60, 80, and 300 ns. These images can be found in the ESI file. A thorough analysis of the obtained images led to the conclusion that both complexes exhibited considerable stability throughout the entire 300 ns duration of the MD trajectory.

In the molecular dynamic simulations under the category of protein-ligand complexes, the testing of the reliability of the “complexity” involves the calculation of the “root mean square fluctuation,” which refers to the examination of the “flexibility of protein remnants on the basis of the fluctuation observed in the atoms of the protein that are recorded as part of the simulation process. The details of the RMSF values of the recognized strikes are included in the ESI files. The RMSF (Å) values obtained were 0.953, 0.970, 1.038, and 0.926, associated with reference and DSM-74, TCMDC-124418, DSM-421, and MMV1557817 compounds. Moreover, there were no considerable changes in amino acid residues during the course of the simulation, and this may be explained by the low values observed in Figure 5. The

differences observed ranged between 0.87 and 4.87 Å. The changes were not noted beyond 5 Å units, and this points to how paramount protein-ligand complexes stability can be and how this affects changes in protein structures. Secondly, examining changes in the radii of gyration can be employed as a method to measure changes in root average Squared range among a collection of molecules from a shared centre of mass. The changes in radius of gyration (Rg) values for each image are illustrated in Figure 9 as a temporal variable. The Rg was found to be 3.980, 3.698, 4.478, 4.803 (Å) bound with reference, DSM-74, TCMD-124418, DSM-421 and MMV1557817 molecules. The values with respect to Rg are explained in Table 3, and Figure 8 indicates that there are no significant differences between different complexes with respect to Rg values. Some differences are noted in Rg values, but in all cases, similar protein structures are noted to be similar in nature because most protein structures contain a high percentage of Alpha helices in their structures. Thus, to conclude, from the values with respect to root mean square deviation (RMSD), root mean square fluctuation (RMSF), and Rg that are derived and obtained from the general molecular dynamics (MD) simulations, it can be easily concluded that structural stability with respect to the Complexes consisting of protein and ligand structures are maintained over time with respect to 300 ns derived from MD simulations.

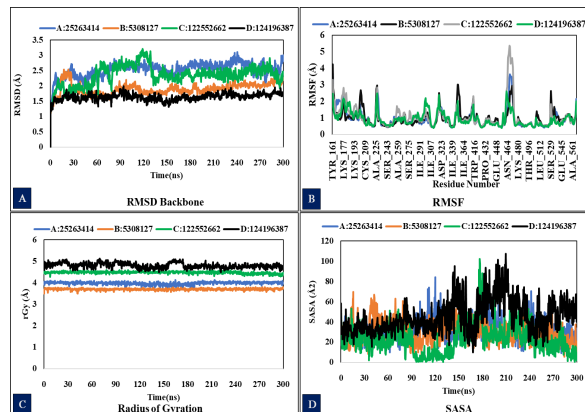


Figure 6: A) Root Mean Square Deviations about the protein backbone throughout a 300 ns molecular dynamics simulation. Various Amino acids constitute represented on the Y-axis, B) while the RMSF values (Å) displayed on the Y-axis demonstrate the degree of fluctuation of each residue about its average location throughout the simulation. The graph shows that lower RMSF values indicate. The assemblies of the ligand and protein resilience process of the whole 100 ns molecular dynamics trajectory. C) The graph shown for the radius of the gyration (Rg) for various complexes of the ligand and the protein was calculated based on a 300-nanosecond simulation carried out by

the molecular dynamic simulation method. D) Solvent Accessible Surface Area of top scoring molecules.

Table 3: Values obtained after the successful 300ns MD run of top scoring molecules.

Molecules	RM SD Ca (Å)	RMS D Backbone (Å)	RMS F (Å)	Radius of Gyration (Å)	SA SA (Å ²)
TCMD C-124418 (25263414)	2.531	2.531	0.953	3.980	32.15
DSM-74 (5308127)	1.910	1.902	0.970	3.698	30.36
DSM-421 (122552662)	2.356	2.351	1.038	4.478	22.36
MMV1557817 (124196387)	1.601	1.636	0.926	4.803	45.42

3.3. PCA Analysis:

Principal Component Analysis is an efficient method that can reduce dimensionality and can provide significant insights into the essential group actions and changes in conformation of biomolecular systems using MD simulations effectively. Extensive Molecular Dynamics simulations provide large amounts of data from simulations conducted. To ensure complete and precise analysis of a medium-sized protein structure is done effectively and correctly, data sampled at a rate of a minimum of 10,000 atoms every 10-15 seconds is a necessity. Principal Component Analysis is used to reduce dimensionality in molecular dynamics simulations and make visualization and capture of basic protein dynamics simpler for experiments conducted using molecular dynamics simulations. PCA can be delineated into five fundamental phases viz data centering, calculating the covariance matrix, finding eigenvectors as well as eigenvalues, preparing the eigenvectors according to the eigenvalues obtained in the second step, and finally transforming data to this newly formed space. It was evident from the PCA analysis done for MMV1557817 that the initially formed principal components PC1, PC2, as well as PC3, explained the variance at 24.23%, 9.85%, as well

as 5.85%, respectively (see Figure 7), as compared with the other principal components whose contribution was less than 9%. Thus, these initial principal components were responsible for the variance in the data of the initial distribution, which gave a very understandable view of variations in the atomic position throughout the conformation set. Consequently, our study focused on the structural changes reflected in the subdomain defined by PC1 and PC2 that provide insight within the paths of the modelled systems across this multidimensional space. The resulting image depicted the three-dimensional variations of the C α atoms in a reduced-dimensional format, enhancing comprehension of the conformational changes. Mapping the paths taken following the PC1 and PC2 axes revealed a clear separation among the blue and red rations, indicating that the simulation systems dynamically changed in their initial state, represented together on this principal motion trajectory.

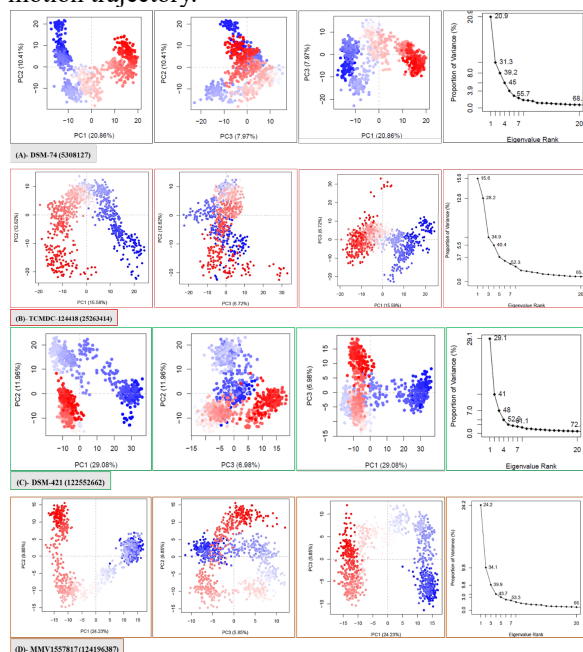


Figure 7: Each frame of the simulation is shaded from blue to red to emphasize progress, and the PCA of the chemicals uncovered numerous evolving structures over time. The first period of the simulation is visualized as blue, the next section as white and the concluding stages as red. You can observe the extent of structural changes with the help of this color gradient. The structural changes for the leading component (PCA-1) are highlighted on the left, so that one can see the chief structural movements during the simulation. Where; A. DSM-74 (5308127); B. TCMDC-124418 (25263414); C. DSM-421 (122552662); D. MMV1557817 (124196387)

3.4. Dynamic Cross-Correlation Matrix (DCCM) Analysis:

DCCM is used for better understanding of the connections among proteins and corresponding ligands. It hence acts as a significant resource for exploring the mechanisms of flexibility and adhesion, especially the identification of novel HITs by means of an algorithmic analysis. The purpose is to assess the influence of the HIT MMV1557817 with respect to conformational behavior for PfDHODH. The trajectory of the complex between 7WYF and MMV1557817, derived from a 300 ns particle dynamics simulation, was assessed by means of the dynamic cross-correlation matrix. The correlation spectrum illustrated in Figure 8 ranges from -1.0 to 1.0 and is depicted through a colour gradient, where -1.0 is represented by a deep purple shade and a deep blue shade of 1.0. Colour intensity reflects the degree of correlation among residue pairs, where darker tones indicate stronger correlations. Correlation coefficients in this range reflect the level of coordinated movement among residues: positive values signify concurrent dynamics, whereas negative values suggest anti-correlated or opposing behaviours. The correlation coefficients, which varied from -1 to 1, represented the presence of positive as well as negative correlations in residual movement signifies conflicting motion, whereas positive correlations suggest synchronised motions of the residue. The analysis of DCCM spectrum suggested that presence of both correlated and non-correlated motions indicates modulation of conformational landscape by protein which potentially alter its pharmacological activity and potency.

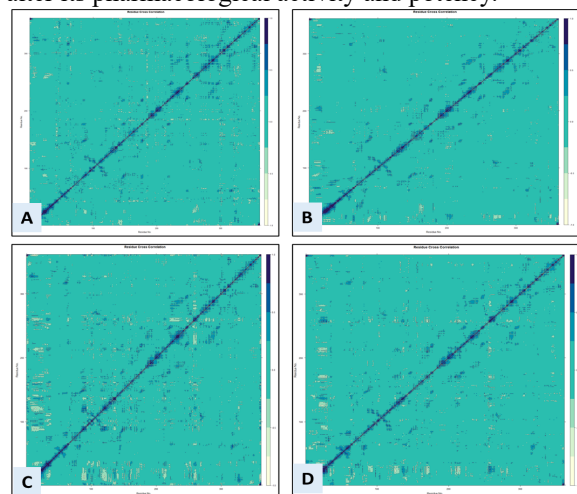


Figure 8: The DCCM study of A. DSM-74 (5308127); B. TCMDC-124418 (25263414); C. DSM-421 (122552662); D. MMV1557817 (124196387), trajectories were employed as input for computing the 2D Dynamical cross-correlation map using the DCCM function. It reveals the movement of ligands and protein together and the x and y axes identify certain protein residues. If residues show a positive correlation, it means they move together in harmony,

but with a negative correlation opposing movements are observed. These two types of correlation motions are each assigned a color from blue through white to red. If both kinds of crosses are correlated in any way, they will act in the same or opposite directions. The allosteric changes and conformational stability are used to show simulations of the protein and its interaction in the ligand binding site.

3.5. Free Energy Calculations (MMGB/SA):

Such is the importance of the MMGBSA in the field of pharmaceutical development, particularly in the field of molecular interaction understanding and optimization. One of the main applications of the MMGBSA is in rationalizing drug development. Thus, the major contributors to understanding such an objective are quantitatively determined through interaction analysis, thereby aiding in the identification of the main contributors, intensification of Their bond of affinity, as well as rational judgment in the course of drug development. For the present research, every trajectory obtained in the molecular dynamic simulations is used for the objective of MMGBSA analysis. As shown in Table 4, the free energy values associated with specific interaction pairs are provided. Also, the dG binding energy values are provided in the same table for every individual complex. For example, the binding free energy denoted as ΔG_{bind} is a measure of the energy change that is implemented when a ligand is in interaction with a protein.

Every complex that was examined showed negative ΔG_{bind} values, suggesting that the identified HIT chemicals interact with the PfDHODH protein in a thermodynamically favourable way. Notably, TCMDC-124418 and DSM-74 displayed the highest binding affinities, with intrinsic energy that is free of -87.8055 and -69.7805 kcal/mol, respectively, which suggests a stronger affinity for the Plasmodium receptor. Moreover, various elements of binding energy namely $DG_{\text{Bind_Coulomb}}$, $DG_{\text{Bind_Covalent}}$, $DG_{\text{Bind_Hbond}}$, $DG_{\text{Bind_Lipo}}$ and $DG_{\text{Bind_Packing}}$ (stack interaction) were found to be negative and further supported the concept of favourable interactions among all recognized HITs. These results indicate that the target protein prefers interactions with compounds exhibiting lower overall binding free energies, underscoring these molecules as potential candidates for further research.

Table 4: Binding free energy assessment of various molecules determined by utilizing the final 100 frames produced during the MD simulation process

S. No.	Molecules	dG	dG _{Bind}	dG _{Bind}	dG _B	dG _{Bind}	dG _{Bind}

		Bi nd kcal/ mole	_Cou lomb kcal/ mole	_Cov alent kcal/ mole	in d_ H bond kcal/ mole	Lip o kcal/ mole	_Pac king kcal/ mole
	TC MD C- 124 418 (25 263 414)	- 69 .7 80 5	- 10.08 19	0.981 00	- 0. 57 15 2	- 25.7 667	- 1.84 69
	DS M- 74 (53 081 27)	- 72 .9 63 4	- 12.05 46	1.693 3	- 0. 05 36	- 32.0 203	- 1.46 63
	DS M- 421 (12 255 266 2)	- 85 .9 99 1	- 14.63 51	2.397 7	- 0. 28 76	- 33.1 814	- 1.09 61
	M MV 155 781 7 (12 419 638 7)	- 87 .8 05 5	- 25.98 73	4.447 9	- 0. 70 92	- 37.9 930	- 0.96 66

3.6. In-silico ADME calculation:

Evaluations of ADME (Consumption, Transmission, metabolism, and Excretion) are essential in developing pharmaceuticals process, as numerous drug candidates do not succeed in clinical trials because of insufficient ADME characteristics. In this document, the attributes were evaluated by means of in silico assessment of ADME on the highest-ranked molecule and were compared with standard compounds. The findings gathered were presented in Table 5. Crucial

components for orally effective medications include Lipinski's Rule of Five, logP_{o/w} (the oil/water split ratio), and cytochrome P450 metabolic process. The findings indicate that both the reference compound and the compound with the highest score satisfy Lipinski's criteria. Furthermore, the anticipated the barrier between the brain (plogBB) metrics imply that these compounds cannot penetrate the barrier, suggesting a minimal risk of Toxicity of the CNS. The ADME information revealed a distinction among the apex candidate and the reference compound, highlighting the importance of successful drug compound must stay stable post-absorption and arrive at its target location to achieve the expected therapeutic effect.

Table 5: Calculated in-silico ADME properties of identified HITS against 7WYF

Molecular Name	Molecular Weight	HBD	HBA	LogP _{o/w}	LogCB	LogBB	Molar Absorption	% Human Oral Absorption	PSA	RO5 Violation	RO3 Violation
DSM-74 (5308127)	267.3	4	1	3.22	1.90	0.29	4	100	54.88	0	0
TMDC-12	293.2	4	1	2.9	1.41	-0.10	1	100	54.8	0	0

4418 (25263414)	51			92	.805	88				03	
DSM-421 (12255262)	358.23	5	1	3.892	1.63027	0.303				65.382	00
MMV-157817 (124196387)	394.3	5	2	2.552	1.48151	-1.024				80.854	92.9300

4. Summary and Conclusion:

In this study, a systematic computational exploration was performed to evaluate the binding of select reported antimalarial molecules with *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH). Molecular docking studies showed that the selected compounds fitted into the active site of the enzyme with favourable binding modes. Results of the docking pointed important interactions with key active site residues of PfDHODH.

To further assess the stability of these interactions, 300 ns molecular dynamics simulations were carried out. The RMSDs analysis indicated that the protein-ligand complexes were structurally stable in all instances during the entire simulation period. The stability of the complexes was further supported by structural parameters like RMSD, RMSF and radius of gyration.

Dynamic analyses such as PCA, DCCM, and NMA were performed to better describe the dynamical behaviour of the enzyme-ligand complex. The MM-GBSA method confirmed the stability of the interactions, shown for the interaction energies. Of these compounds, MMV1557817 exhibited relatively consistent binding behaviour.

At last, this study offers unique mechanistic information on the interaction and dynamic stability of reported antimalarial compounds targeting PfDHODH. Together these findings add to the understanding of PfDHODH inhibition, which may assist future structure-based optimization of antimalarial drug candidates.

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