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ORIGINAL RESEARCH

In Silico Screening of *Streptomyces* sp. Metabolites Targeting *P. falciparum* DHODH and DPCK for Antimalarial Discovery

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Background: Malaria is a major global health issue, which significantly affects developing countries, including Indonesia. *Plasmodium falciparum*, the leading cause of malaria mortality, is increasingly resistant to standard treatments. The antimalarial properties of compounds derived from *Streptomyces* sp. have been demonstrated in several in vivo and in vitro studies, although their exact mechanism of action remains unclear. This study explores *Streptomyces* sp. metabolites as potential antimalarials targeting *Pf*DHODH and *Pf*DPCK, essential enzymes for *P. falciparum* survival.

Methods: A comprehensive in silico screening was employed, including phylogenetic analysis of *Pf*DHODH and *Pf*DPCK, network protein analysis, identification and preparation of *Streptomyces* sp. bioactive compounds, structural preparation of target enzymes, molecular docking and visualization, antimalarial efficacy prediction, assessment of drug-likeness and toxicity, and molecular dynamics simulations.

Results: Phylogenetic analysis confirmed that *Pf*DHODH and *Pf*DPCK are distinct from human proteins, reducing off-target risks. Network analysis identified nine proteins linked to *Pf*DHODH and one for *Pf*DPCK. From 27 *Streptomyces* sp. bioactive compounds, caboxamycin, bisanhydroaklavinone, napyradiomycin A1, and gardenomycin A showed the strongest binding to target enzymes. These compounds occupied the active sites of *Pf*DHODH and *Pf*DPCK, mirroring control ligands (FMN, Opera1). PASS analysis indicated strong antiprotozoal potential for bisanhydroaklavinone and gardenomycin A. Toxicity analysis revealed bisanhydroaklavinone's mutagenicity and carcinogenicity risks, while napyradiomycin A1 showed moderate hepatotoxicity. Nonetheless, molecular dynamics confirmed stable interactions, highlighting their promise as antimalarial candidates. However, their toxicity profiles warrant further investigation to ensure safe therapeutic application.

Conclusion: *Pf*DHODH and *Pf*DPCK were identified as selective targets in *P. falciparum*. Four *Streptomyces*-derived compounds showed strong binding, stability, and promising antimalarial potential with acceptable predicted toxicity, warranting further evaluation. However, only caboxamycin and gardenomycin show potential for antimalarial drug development, with acceptable predicted toxicity profiles, making them suitable candidates for in vitro and in vivo testing prior to clinical evaluation.

Keywords: antimalarial, in silico, *Pf*DHODH, *Pf*DPCK, *Streptomyces* sp

Introduction

Malaria is a life-threatening infectious disease and globally remains a challenging epidemiological health problem as a major cause of morbidity and mortality, especially in developing countries.[1] There are 249 million cases of malaria with an estimated 608,000 deaths from malaria in 2022 reported in 85 malaria-endemic countries.[2] Malaria is caused by the protozoan parasite *Plasmodium* sp., which is transmitted through the bite of female Anopheles mosquitoes.[3] In general,

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there are five species of *Plasmodium* sp. that infect humans, which include *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi*. The *P. falciparum* species is reported to be the main cause of most malaria deaths, accounting for more than 90% of malaria mortality rate.[4]

Improving malaria control remains an important program to achieve the target of reducing malaria infection rates. The emergence of antimalarial drug resistance poses an unprecedented threat to global malaria control efforts.[5] Resistance to first-generation antimalarials, such as chloroquine and sulfadoxine–pyrimethamine (SP), has been widely reported in malaria-endemic countries, including Indonesia.[6,7] Artemisinin-based combination therapy (ACT), particularly dihydroartemisinin–piperaquine (DHP), has been the first-line treatment in Indonesia since 2011.[8] However, alarming evidence suggests a decline in artemisinin efficacy even in regions where formal resistance has not yet been documented. This emerging pattern underscores the urgent need for novel antimalarial agents with innovative mechanisms of action.[9] Current resistance mechanisms primarily involve mutations in key parasite proteins that reduce drug target binding affinity or enhance drug efflux. The rapid evolution of *P. falciparum* resistance highlights the importance of targeting essential metabolic enzymes that are biochemically distinct from human homologs, structurally conserved across parasite strains, and indispensable for parasite survival.[10,11] Enzyme inhibition strategies are particularly attractive in antimalarial drug development, as they can disrupt critical biochemical pathways while minimizing off-target effects in human cells.[12]

P. falciparum Dihydroorotate Dehydrogenase (*Pf*DHODH) and *P. falciparum* Dephospho-Coenzyme A Kinase (*Pf*DPCK) represent two particularly attractive but underexplored enzyme targets for antimalarial drug development.[13,14] *Pf*DHODH serves as the rate-limiting enzyme in the pyrimidine biosynthesis pathway, catalyzing the conversion of dihydroorotate to orotate in a flavin mononucleotide-dependent reaction.[13,15] Unlike human cells, which can salvage pyrimidines through alternative pathways, *P. falciparum* parasites are entirely dependent on de novo pyrimidine biosynthesis for DNA replication and RNA synthesis. *Pf*DHODH inhibition results in pyrimidine nucleotide depletion, effectively blocking parasite cell division and growth.[15] Importantly, *Pf*DHODH exhibits significant structural divergence from human DHODH, particularly in the active site architecture, making it an ideal target for selective inhibition. *Pf*DPCK catalyzes the final step in Coenzyme A biosynthesis, converting dephospho-CoA to CoA within the parasite's apicoplast.[14,16] This enzyme is functionally essential because CoA serves as a critical cofactor in numerous metabolic processes, including fatty acid synthesis, the tricarboxylic acid cycle, and amino acid metabolism. Unlike *Pf*DHODH, which has received considerable attention in antimalarial drug discovery, *Pf*DPCK remains significantly underexplored despite its essential role and potential druggability.[16] The lack of effective *Pf*DPCK inhibitors represents a major gap in current antimalarial drug development pipelines.

Natural product-based drug discovery has experienced renewed interest, particularly compounds derived from actinomycetes, especially *Streptomyces* species.[17,18] *Streptomyces* sp. represents the largest genus of Actinomycetes and has been proven as prolific producers of bioactive compounds with antimalarial properties against *P. falciparum* infection.[19] Multiple in vitro and in vivo studies have demonstrated the antimalarial potential of *Streptomyces*-derived metabolites.[20–22] However, critical knowledge gaps exist in the systematic evaluation of *Streptomyces*-derived compounds against specific *P. falciparum* enzyme targets. Most previous studies have focused on phenotypic screening approaches without identifying precise molecular targets or mechanisms of action. Furthermore, no comprehensive study has evaluated the potential for simultaneous inhibition of both *Pf*DHODH and *Pf*DPCK by *Streptomyces*-derived compounds, representing a missed opportunity for dual-target therapeutic strategies that could potentially overcome single-target resistance mechanisms.

In silico screening using molecular docking analysis methods and molecular dynamics (MD) simulations are essential in the process of new drug discovery.[23,24] In silico with molecular docking and molecular dynamic simulation for antimalarial drug discovery has been conducted in several studies and shows promising results so that it can be the basis for further research such as in vitro, in vivo, and clinical trials.[6,25] The stages of the drug candidate search process based on in silico modeling start with unmet medical needs. This is followed by the selection of biological target proteins that can be modulated by specific drugs or compounds. Understanding these target proteins is crucial for elucidating the mechanism of action of the potential treatment. A critical gap in current in silico antimalarial drug discovery is the frequent omission of phylogenetic validation of target proteins. While molecular docking and dynamics simulations are widely employed, few studies incorporate evolutionary analysis to assess target conservation across *Plasmodium* strains

and divergence from human homologs, despite its importance for ensuring broad-spectrum efficacy and minimising off-target effects.[26] Phylogenetic analysis not only identifies conserved functional domains, but also predicts whether inhibitors effective against one strain like *Pf*3D7, will retain activity against genetically distinct isolates.[27] For instance, *Pf*DPCK exhibits around 80% sequence identity across *Plasmodium* species but only 24% with human bifunctional coenzyme A synthase (hsCOASY).[28] Integrating phylogenetics with molecular docking and dynamics addresses a key methodological limitation in natural product studies by simultaneously evaluating target druggability and evolutionary stability. Therefore, based on the above considerations using candidate bioactive components from secondary metabolite compounds of *Streptomyces* sp. this study aims to identify potential drug candidates targeting *Pf*DHODH and *Pf*DPCK enzymes inhibitors of *P. falciparum* as antimalarial drugs based on the in silico screening combining phylogenetic analysis, molecular docking, and molecular dynamics simulations. This comprehensive methodology will provide robust validation of therapeutic targets while identifying promising lead compounds for further antimalarial drug development with implications for broader *P. falciparum* population control.

Materials and Methods

Phylogenetic Analyses of *Pf*DHODH and *Pf*DPCK

Protein sequences of *PfDHODH* and *Pf*DPCK were obtained from the Protein Data Bank (https://www.rcsb.org/), PlasmoDB (https://plasmodb.org/plasmo/), NCBI (http://www.ncbi.nlm.nih.gov/), and UniProt (https://www.uniprot.org/). BLASTp analysis was conducted on NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using *Pf*DHODH and *Pf*DPCK protein sequences (PDB ID 3I65 and PlasmoDB ID PF3D7_1443700, respectively) as query sequences and non-redundant protein sequences (nr). Protein sequences from representative taxa were used as subject sequences. The selected protein sequences have an E value of 1×10^{-10} or less. SeaView software version 4.5.4 was used for sequence alignment and phylogenetic tree reconstruction. Sequence alignment was performed using the MUSCLE program. The phylogenetic tree was reconstructed using the maximum-likelihood (ML) algorithm by selecting the PhyML program. The substitution model chosen was LG, with 1000 bootstrap replications.

Network Protein Analysis

The STRING database (version 12.0; https://string-db.org) was used to identify and analyze the direct protein target of the compound. The organism filter was set to *P. falciparum*, and all available interaction sources, including text mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence, were enabled. The resulting protein–protein interaction (PPI) network, comprising both direct and indirect interactors, was exported and displayed in Cytoscape (version 3.10.3). Cytoscape is an open-source Java-based platform for visualizing molecular interaction networks and associated biological pathways. Network visualization facilitated the identification of functional protein clusters and potential interaction partners relevant to the compound's mechanism of action.

Data Mining and Ligand Preparation of *Streptomyces* sp. Bioactive Compounds

A total of 27 compounds with reported antimalarial potential were selected based on previous studies. Bioactive compounds from *Streptomyces* species were identified through a systematic literature search in PubMed, Scopus, and Web of Science, covering publications from 2019 to 2025. The search terms included "Streptomyces AND antimalarial", "secondary metabolites AND Plasmodium falciparum", and "bioactive compounds AND malaria". Only studies reporting in vitro or in vivo antimalarial activity with IC_{50} or EC_{50} values were included. From these, 27 compounds were shortlisted based on both their antimalarial activity and the availability of their structural data in public chemical libraries (Table 1).

Preparation of Target Enzymes Structure

The three-dimensional structure of DHODH (PDB ID: 3I65) was retrieved from the Protein Data Bank (https://www.rcsb.org/). Whereas the 3D structure of DPCK were modelled using the I-TASSER web server (https://zhanggroup.org/I-TASSER/) with the initial protein sequences obtained from UniProt (https://www.uniprot.org/). Newly-formed proteins were subsequently

Table 1 Data Mining of *Streptomyces* sp. Bioactive Compounds From Previous Studies

Compounds	Sources	PubChem CID	References
Adipostatin E	*Streptomyces blancoensis*	171119183	[44,45]
Trichostatin A	*Streptomyces* sp., *Streptomyces* sp. CPCC 203909,	444,732	[46,47]
Chresdihydrochalcone	*Streptomyces chrestomyceticus*	156580637	[48]
Napyradiomycin A1	*Streptomyces ruber, Streptomyces antimycoticus* NT17	6,438,953	[49–51]
Gardenomycin A	*Streptomyces bulli* GJA1	156583029	[52]
Ethyl esther	*Streptomyces* sp. InaCC 1497 and AB8	2733848	[53]
Geosmin	*Streptomyces avermitilis, Streptomyces coelicolor*	29746	[54,55]
2,3-heptanedione	*Streptomyces* SUK 08	60,983	[56]
Butyl propyl ester	*Streptomyces* SUK 08	7184	[56]
1,2,7,7-Tetramethyl-2-norbornanol	*Streptomyces* sp.	11062802	[57,58]
2-Isobutyl-3-methoxypyrazine	*Streptomyces griseus*	32594	[59]
Bisanhydroaklavinone	*Streptomyces griseorubens* strain DSD069	159,297	[60]
Chresphenylacetone	*Streptomyces chrestomyceticus*	156580638	[48]
Indole-3-acetic acid	*Streptomyces fradiae* NKZ-259, *Streptomyces* sp. En-1	802	[61,62]
Cyclo-L-Pro-L-Leu	*Streptomyces s.* strain 22-4	7,074,739	[63]
Dibutyl phthalate	*Streptomyces albidoflavus* 321.2, *Streptomyces coelicolor* M145	3026	[64,65]
Ganicidin W	*Streptomyces* SUK10, *Streptomyces* sp. VITLGK012,	7,074,739	[43,66,67]
Levalbuterol	*Streptomyces hygroscopicus*	123600	[68]
Sedanolide	*Streptomyces hygroscopicus*	5018391	[68]
Usabamycin A	*Streptomyces* sp. NPS853	54,764,239	[69,70]
2-Allyloxyphenol	*Streptomyces sundarbansensis* sp. nov.,	70,772	[71,72]
Caboxamycin	*Streptomyces* sp. NTK 937, *Streptomyces* sp. A-177	135,957,253	[73,74]
Streptokordin	*Streptomyces* sp. KORDI-3238	11,579,160	[74]
Tetracyclines	*Streptomyces rimosus, Streptomyces aureofaciens*	54675776	[75,76]
1-O-acetylstreptenol A	*Streptomyces* sp. MJM3055	170,988,233	[77]
Streptenol A	*Streptomyces* sp.	5384628	[78]
Saliniketal A	*Streptomyces* sp. TP-A0356	16,104,895	[79]

refined using GalaxyRefine Tool (https://galaxy.seoklab.org/), and the quality of the peptide sequences were validated using MolProbity webserver (http://molprobity.biochem.duke.edu/). All proteins were saved in pdb format for molecular docking analysis.

Molecular Docking Simulation and Visualization

Molecular docking simulations were conducted using AutoDock Vina integrated in PyRx software (version 0.8) to evaluate the binding interactions between selected bioactive compounds (ligands) and the target enzymes *Pf*DHODH and *Pf*DPCK. Ligand structures, which had previously been obtained in SDF format from the PubChem database, were

optimized using the Open Babel tool in PyRx to reduce energy. Protein structures of *Pf*DHODH and *Pf*DPCK were retrieved from the Protein Data Bank (PDB) and were preprocessed in Discovery Studio 2019 to remove water molecules, native ligands, and heteroatoms lying within 4 Å of the binding site. Docking grid box parameters for each enzyme (dimensions and center coordinates). Each ligand was docked into the predefined active site using the same grid box dimensions as the respective control ligands (FMN for *Pf*DHODH and Operea1 for *Pf*DPCK), ensuring comparability of binding energies and interaction profiles. Docking results were reported as Gibbs free energy (ΔG, in kcal/mol), where more negative values indicate stronger predicted binding affinity. The docked complexes were analyzed in Discovery Studio 2019 to identify two-dimensional (2D) interaction maps and amino acid residue profiles. Three-dimensional (3D) visualization of binding conformations and pocket geometry was performed using UCSF Chimera.

Antimalarial Probability Prediction

The predicted biological activities of the selected ligands were evaluated using the PASS Online server (http://www.way2drug.com/PASSonline/). The Simplified Molecular Input Line Entry System (SMILES) notation for each compound was systematically retrieved and submitted to a web-based predictive platform to evaluate potential biological activities. The Prediction of Activity Spectra for Substances (PASS) algorithm subsequently generated probabilistic estimates for each activity class, expressed as Pa (probability of the compound being pharmacologically active) and Pi (probability of inactivity). For this study, specifically targeted antiprotozoal activity against *Plasmodium* sp. Compounds had potential action if their predicted probability of activity (Pa) exceeded that of inactivity (Pi), and if Pa was equal to or greater than 0.30. These thresholds were used to identify ligands that were most likely to have antimalarial action.

Drug-Likeness and Toxicity Analysis

Each of *Streptomyces*-derived compounds were screened of its feasibility for drug candidates. Swiss-ADME web server (https://www.swissadme.ch/index.php) were used for pharmacokinetics analysis with regards to the molecular weight, lipophilicity, hydrogen bond donors and acceptors, GI absorption, BBB permeation, P-gp substrate, and topological polar surface area (TPSA). Toxicity screening was also deployed using pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/) by taking into account the AMES toxicity and hepatotoxicity. Selected compounds were further used for subsequent analysis. The AMES toxicity, carcinogenicity, and human hepatotoxicity probability of selected compounds were predicted using ADMETlab 3.0 (https://admetlab3.scbdd.com/). The organ toxicity was investigated through Pro-Tox II web server (https://tox-new.charite.de/protox_II).

Molecular Dynamic Simulation and Analysis

Molecular dynamics (MD) simulations were conducted to evaluate the stability and flexibility of the protein–ligand complexes selected based on the best binding affinity from molecular docking results. All simulations were performed using YASARA (v19.14.12) with the AMBER14 force field, beginning with energy minimization (5,000 steps steepest descent and 5,000 steps conjugate gradient) of protein-ligand complexes solvated in a TIP3P water box (10 Å padding) containing 0.9% NaCl. Systems were equilibrated under NVT (100 ps, 310 K with Berendsen thermostat, $\tau = 0.2$ ps) and NPT (100 ps, 1 atm with Berendsen barostat) ensembles with gradual release of positional restraints (10→1 kcal/mol·Å² on protein heavy atoms), followed by 20 ns production runs (2 fs timestep, periodic boundary conditions). Trajectories were analyzed for backbone RMSD (Cα atoms), residue-specific RMSF, and hydrogen bond occupancy (distance ≤3.5 Å, angle ≥120°) using YASARA's md_analyze module, with validation against apo-protein and native ligand (FMN/Opera1) control simulations. All visualization used UCSF Chimera (v1.16), with frames saved every 10 ps for analysis.

Results

Phylogenetic Profile of *Pf*DHODH and *Pf*DPCK

DHODH and DPCK proteins are present in both *Plasmodium* and humans, as identified through NCBI BLAST searches. Phylogenetic analysis of those protein sequences revealed significant differences in *Plasmodium* and humans (Figure 1). A comparison between *P. falciparum* DHODH (*Pf*DHODH) and human DHODH (*hs*DHODH) reveals a limited identity

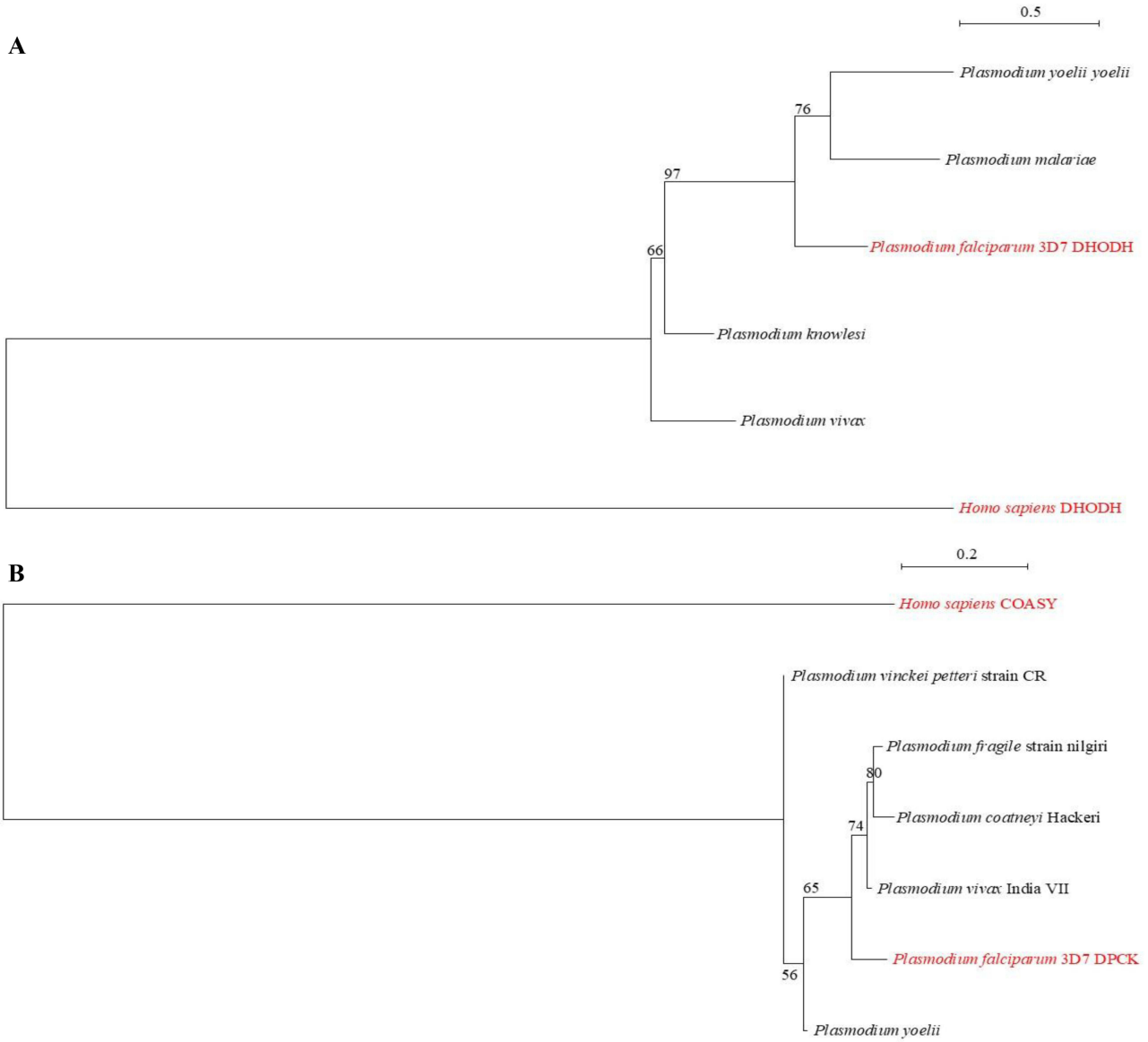

Figure 1 The tree topology is based on phylogenetic analysis of DHODH and DPCK protein sequences from *P. falciparum* and other organisms. The ML tree with LG + Γ4 model is shown. Bootstrap values (>50%) are indicated at branch nodes, representing the percentage of support from 1000 replicates. Phylogenetic analysis of DHODH protein sequences based on 525 aligned positions (**A**) and DPCK protein sequences based on 274 aligned positions (**B**). Scale bars represent 0.5 substitutions (**A**) and 0.2 substitutions (**B**) per amino acid position.

percentage of 38.3%. Furthermore, *P. falciparum* DPCK (*Pf*DPCK) exhibits an identity percentage of 25.7% compared to *hs*COASY. These findings highlight the safety of targeting DHODH and DPCK as specific sites for inhibitors. The substantial differences between *P. falciparum* and human DHODH and DPCK proteins provide an opportunity to develop anti-malarial drugs targeting these proteins without interfering with their function in humans. The *Plasmodium* species (subject sequence) was compared to *Pf*DHODH and *Pf*DPCK, the percentage identity was 68–78% and 86–91%, respectively. The similarity of DHODH and DPCK proteins among *Plasmodium* species indicates a biological relationship, suggesting that these enzymes have the potential to serve as specific targets for malaria treatment.

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class 2 DHODH, however in humans, DHODH has an alternative mechanism for pyrimidine synthesis called the salvage pathway, so targeting *Pf*DHODH has no effect on essential human functions.[15] Then also significant variation exists in the structure of the ubiquinone binding site, as revealed by the X-ray structure of DHODH in *P. falciparum* and humans. These variations serve as the structural basis for identifying specific inhibitors in each species.[80] Additionally, the crystal structures of *hs*DHODH and *PfDHODH* show that the human enzyme and *Plasmodium* enzyme have different amino acid sequences.[13] *Pf*DPCK is a highly conserved protein among *Plasmodium* species with 88–93% amino acid identity to *Plasmodium yoelii, Plasmodium vivax, Plasmodium knowlesi, Plasmodium ovale*, and *Plasmodium malariae*. In contrast, *Pf*DPCK exhibits very limited similarity (24%) to its human counterpart (*hs*COASY).[15]

Protein Network Analysis

Protein network analysis for malaria is a method to identify and analyze the interactions between proteins in the malaria parasite and in the host (human body). This analysis can help identify potential drug targets and diseases that are similar to malaria.[81] A number of proteins which may be co-expressed with *Pf*DHODH and *Pf*DPCK are found by STRING dataset, then visualized alongside the direct target using Cytoscape software version 3.10.3. In the *Pf*DHODH analysis results, there are 9 main proteins that are top relative to *Pf*DHODH. While in *Pf*DPCK there is only 1 protein that is relatively the same as *Pf*DPCK, namely Holo-[acyl-carrier-protein] synthase (Figure 2 and Table 2).

Data Mining of *Streptomyces* sp. Bioactive Compounds

Bioactive compounds isolated from *Streptomyces* sp. have long been used as a natural source of antibiotics and are still used clinically. Existence of biosynthetic gene clusters encoding enzymes involved in the production of secondary metabolites is one of the unique characteristics of bioactive compounds of *Streptomyces* sp. It was reported that 80–95% of the total 200 antibiotics were produced by *Streptomyces* sp.[82,83] In previous studies on biological control agents conducted in recent years, the genus *Streptomyces* sp. has been identified as the largest source of bioactive metabolites with antibacterial, antifungal, anticancer, antioxidant properties, and has promising potential for antimalarial therapy against *Plasmodium falciparum* infection. The antimalarial effect has also been proven through in vitro, in vivo, and in silico tests. There is one unique feature.[7] In this study, 27 bioactive compounds derived from secondary metabolites of

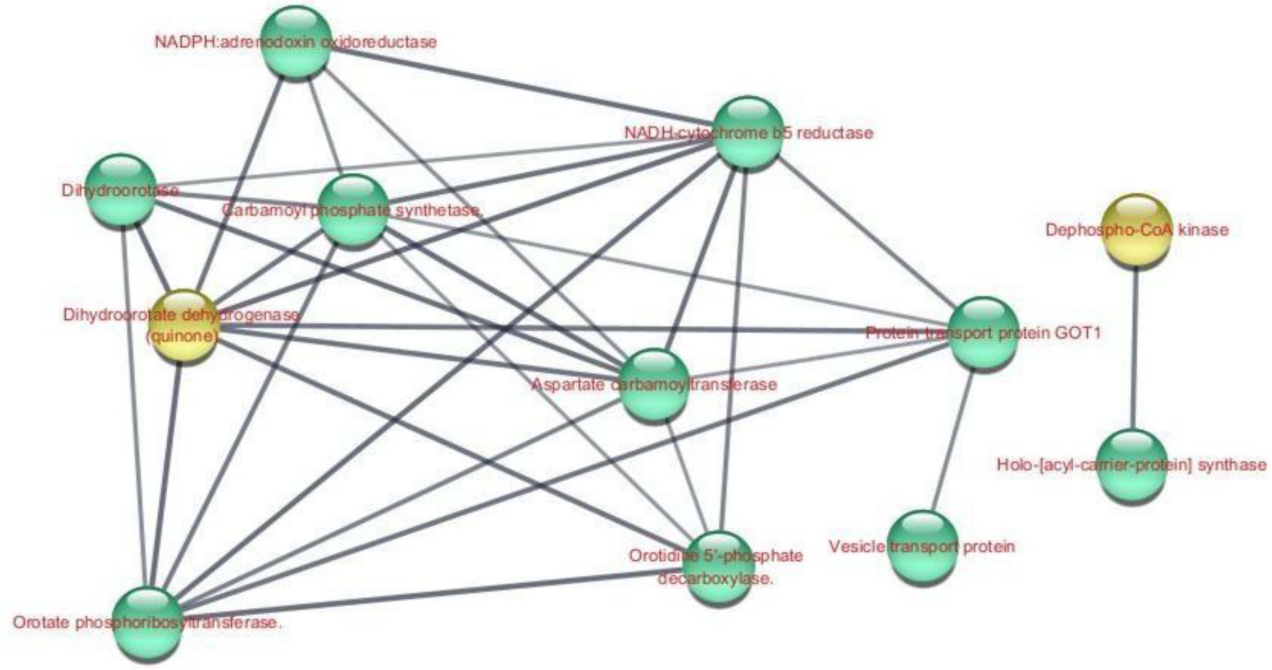

Figure 2 Protein network analysis of *P. falciparum* DHODH and DPCK. : Direct Target. : Indirect Target.

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Table 2 Protein Network Co-Expressed by *P. falciparum* DHODH and DPCK

Protein	Direct Target	Description	References
Dihydroorotase (DHOtase)	*Pf*DHODH	The third enzyme in the de novo pathway, DHOase is a Zn^{2+} amidohydrolase superfamily that catalyzes reversible cyclization of N-carbamoyl-L-aspartate (L-CA) to form L-dihydroorotate (L-DHO).	[29,30]
Carbamoyl phosphate syngthetase (CPS)	*Pf*DHODH	Catalyzes the formation of carbamoylphosphate from C02, ATP, and ammonia or glutamine, for pyrimidine biosynthesis, arginine biosynthesis, or the urea cycle	[31,32]
Orotate phosphoribosyltransferase (OPRTase)	*Pf*DHODH	The fifth enzyme in the indispensible *de novo* pyrimidine-synthesis pathway of the parasite and catalyses the conversion of orotate and 5-phospho-d-ribosyl 1-pyrophosphate (PRPP) into orotidine 5′-monophosphate (OMP)	[33]
NADPH: adrenodoxin oxidoreductase	*Pf*DHODH	NADPH-adrenodoxin reductase, which catalyzes the reduction of adrenodoxin by NADPH	[34,35]
NADH-cytochrome b5 reductase (cytb5r)	*Pf*DHODH	Is a FAD-containing flavoprotein that mainly binds to the endoplasmic reticulum membrane of cells, accepts two electrons from NADH, and transfers one electron to cytochrome b5. reduction of methemoglobin and cytochrome b5 for the soluble isoenzyme, a fatty acid desaturation complex for the membrane-bound microsomal enzyme, and participation in drug metabolism	[36,37]
Aspartate carbamoyltransferase	*Pf*DHODH	Catalyzes the formation of phosphate and *N*-carbamoyl-l-aspartate from carbamoyl phosphate and l-aspartate in pyrimidine biosynthesis	[38]
Orotidine 5'-phosphate decarboxylase (ODCase)	*Pf*DHODH	Conversion of dihydroorotate (L-DHO) to UMP is catalysed by the last three enzymes of the de nova pyrimidine biosynthetic pathway	[39,40]
Protein transport protein GOT1	*Pf*DHODH	Support DHODH-mediated pyrimidine synthesis through alpha-ketoglutarate and aspartate production	[41]
Vesicle transport protein	*Pf*DHODH	Helping to transport any molecules, especially that needed for the cell to growth	[42]
Holo-[acyl-carrier-protein] synthase	*Pf*DPCK	For moving the acyl groups from one enzyme to another to complete the fatty acid synthesis cycle, acyl carrier protein (ACP) is essential to fatty acid biosynthesis. The synthesis of acyl-ACPs, which serve as the donor and carrier for a variety of metabolic processes, requires holo-ACP as a necessary substrate.	[43]

Streptomyces sp. species were collected from a comprehensive critical review of previous studies (Table 1). The structure of each compound was retrieved from the PubChem database with all having CID and canonical SMILES notation. All bioactive compounds were screened for feasibility as drug candidates based on pharmacokinetic analysis (molecular weight, lipophilicity, hydrogen bond donor and acceptor, GI absorption, BBB permeation, P-gp substrate, and polar surface area topology (TPSA)) using the Swiss-ADME web server (https://www.swissadme.ch/index.php) (Table 3). The results showed that all 27 bioactive compounds were feasible to be used as drug candidates and continued with molecular docking analysis.

Construction of Protein Target Enzymes Structure

In this study, enzyme target proteins selected were *P. falciparum* Dihydroorotate Dehydrogenase (*Pf*DHODH) and *P. falciparum* Dephospho-Coenzyme A Kinase (*Pf*DPCK). Target proteins were selected based on their important function for the survival of *P. falciparum* parasites so that their inhibition or inactivation can result in parasite death. In addition, Target proteins have native ligands that are neither peptides nor proteins.[6] Two target proteins have different control ligands and are used to compare the binding affinity values formed between the target protein and the compound as well as the target protein and the compound in molecular docking analysis.[12] The control ligands of each enzyme were used as a threshold to consider the strength of the interaction, specifically FMN (*Pf*DHODH) and Opera1 (*Pf*DPCK). Three-dimensional structure of DHODH (PDB ID: 3I65) was taken from Protein Data Bank (https://www.rcsb.org/) in SDF format. The 3D structure of DPCK was modeled using the I-TASSER web server (https://zhanggroup.org/I-TASSER/) with the initial protein sequence obtained from UniProt (https://www.uniprot.org/). The reconstruction of *Pf*DHODH and *Pf*DPCK was done by residue separation and protein target optimization through the removal of residual water, ligands

Table 3 Drug Candidates Analysis of Bioactive Compounds From *Streptomyces* sp.

Compound	Pharmacokinetic Analysis								
	Molecular Weight (≤500 g/mol)	Lipophilicity mLogP (≤5)	nOHNH (≤5)	nON (≤10)	Violation	GI absorption	BBB permeation	P-gp substrate	TPSA (<150)
Adipostatin E	334.5	4.44	2	2	1	Low	No	No	40.46 Å²
Trichostatin A	302.37	1.9	2	3	0	High	Yes	No	69.64 Å²
Chresdihydrochalcone	390.38	2.48	3	8	0	High	No	Yes	122.52 Å²
Napyradiomycin A1	481.41	3.85	2	5	0	High	No	Yes	83.83 Å²
Gardenomycin A	358.34	0.4	4	7	0	High	No	Yes	124.29 Å²
Ethyl esther	208.25	1.98	1	3	0	High	Yes	No	46.53 Å²
Geosmin	182.30	2.88	0	1	0	High	Yes	No	17.07 Å²
2,3-heptanedione	128.17	1.52	0	2	0	High	Yes	No	26.30 Å²
Butyl propyl ester	194.23	2.23	1	3	0	High	Yes	No	46.53 Å²
1,2,7,7-Tetramethyl-2-norbornanol	168.28	2.88	0	1	0	High	Yes	No	17.07 Å²
2-Isobutyl-3-methoxypyrazine	166.22	0.66	0	3	0	High	Yes	No	35.01 Å²
Bisanhydroaklavinone	376.36	3.03	2	6	0	High	No	No	100.90 Å²
Chresphenylacetone	210.23	1.89	2	4	0	High	Yes	No	66.76 Å²
Indole-3-acetic acid	60.05	−0.49	1	2	0	High	No	No	37.30 Å²
Cyclo-L-Pro-L-Leu	210.27	0.64	1	2	0	High	No	No	49.41 Å²
Dibutyl phthalate	278.34	3.43	0	4	0	High	Yes	No	52.60 Å²
Ganicidin W	210.27	2.28	1	2	0	High	No	No	49.41 Å²
Levalbuterol	239.31	0.95	4	4	0	High	No	No	72.72 Å²
Sedanolide	194.27	2.65	0	2	0	High	Yes	No	26.30 Å²
Usabamycin A	272.34	2.99	1	2	0	High	Yes	Yes	41.57 Å²

(*Continued*)

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Table 3 (Continued).

Compound	Pharmacokinetic Analysis								
	Molecular Weight (≤500 g/mol)	**Lipophilicity mLogP (≤5)**	**nOHNH (≤5)**	**nON (≤10)**	**Violation**	**GI absorption**	**BBB permeation**	**P-gp substrate**	**TPSA (<150)**
2-Allyloxyphenol	150.17	1.98	0	2	0	High	Yes	No	26.30 Å²
Caboxamycin	255.23	2.09	1	4	0	High	No	No	76.37 Å²
Streptokordin	151.16	0.91	2	2	0	High	Yes	No	49.33 Å²
Tetracyclines	444.4	−2.08	6	9	1	Low	No	Yes	181.62 Å²
1-O-acetylstreptenol A	228.28	1.22	1	4	0	High	Yes	No	63.60 Å²
Streptenol A	207.23	1.13	1	3	0	High	Yes	No	49.77 Å²
Saliniketal A	395.5	1.61	3	5	0	High	No	Yes	102.01 Å²

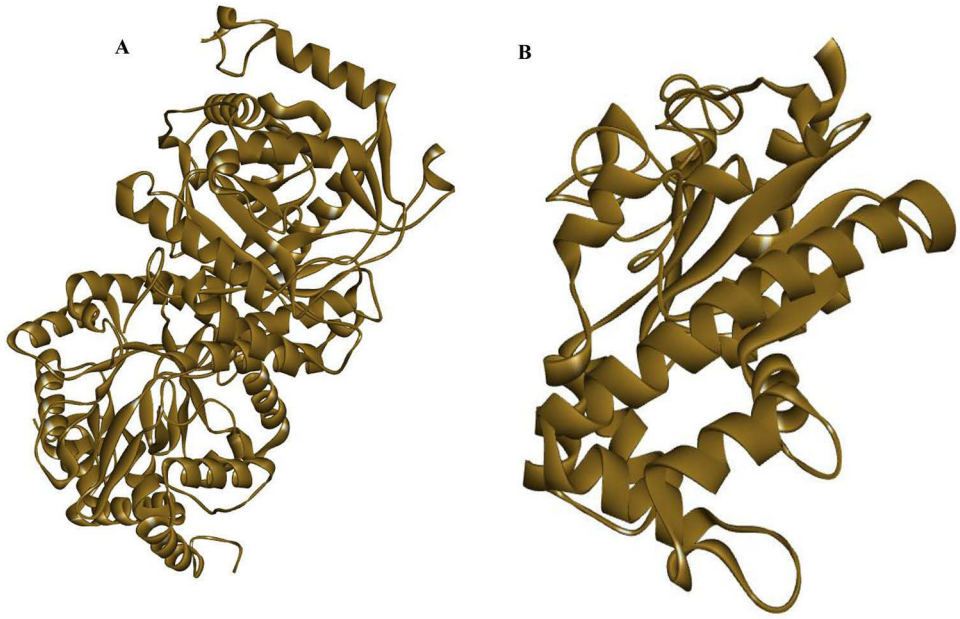

Figure 3 The 3D structure of (**A**) DHODH (PDB ID: 3165) and (**B**) DPCK.

from the crystallization process, and cofactors with the Biovia Discovery Studio program. In order for residues that are still in the protein not to affect the bond between the protein and the ligand, it needs to be separated first so that the docking results can be optimal and results of this process are saved in pdb format (Figure 3).

Molecular Docking Simulation and Visualization

Prediction analysis of bioactive compounds from *Streptomyces* sp. in inhibiting parasite development and growth was carried out by predicting the chemical bond energy of bioactive compounds with *Pf*DHODH and *Pf*DPCK target protein receptors studied through a molecular docking screening approach using PyRx 0.8 with the appropriate specific grid box dimensions (interaction space between ligand and receptor) based on the original ligand protein target (Table 4). The grid box scale will determine positions of the binding site and active site of the protein, which has previously removed water molecules to achieve its actual state in the form of amino acids. Binding images of target proteins and bioactive compounds were visualized in the form of two-dimensional images with Discovery Studio 2019 and three-dimensional images with UCSF Chimera. The results of data obtained are the binding affinity values from the results of molecular docking simulations against *Pf*DHODH and *Pf*DPCK which are compared with the docking results of native ligands, namely FMN and Opera1 as controls.

The results of molecular docking simulations conducted on 27 bioactive compounds from *Streptomyces* sp. showed that four compounds consisting of Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A (Figure 4), have low binding affinity values to *Pf*DHODH and *Pf*DPCK compared to the original ligand or control (Table 5). Binding affinity is the ability of the bioactive compound ligand to bind to the target protein in the interaction between the receptor/target protein so that the binding affinity value becomes the most important parameter in molecular

Table 4 Grid Box Scale of Molecular Docking

Target Protein		Grid Box Scale of Center (Å)		
		X	Y	Z
*Pf*DHODH	*P. falciparum* Dihydroorotate dehydrogenase	22.7849	−33.3761	16.5286
*Pf*DPCK	*P. falciparum* Dephospho-Coenzyme A Kinase	22.6815	20.9107	28.192

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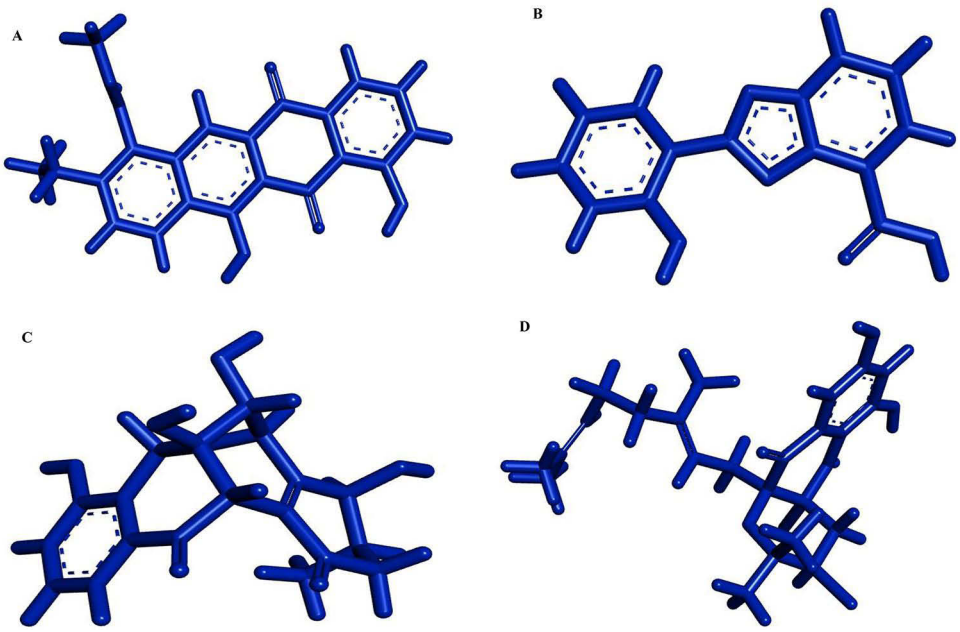

Figure 4 The 3D structure ligan of (**A**) Bisanhydroaklavinone, (**B**) Carboxamycin, (**C**) Gardenomycin (**A** and **D**) Napyradiomycin A1.

docking. The highest binding affinity values of the four compounds Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A indicate that the four bioactive compounds tested have high potential biological activity as strong inhibitors to bind to *Pf*DHODH and *Pf*DPCK. All four compounds exceeded the threshold for strong protein–ligand interactions (<-5.00 kcal/mol), the negative binding affinity value will be directly proportional to the strength of the bond energy that occurs.[84,85] In general, the category of active ligands that form strong bonds to target proteins have binding affinity values lower than −5.00 kcal/mol and indicating potential as effective dual-target inhibitors.

Table 5 Results of The Chemical Interaction Analysis of Ligand and Protein Target Complex

Protein Target	Ligand-Protein Complex	Binding Affinity (kcal/mol)	Amino Acid Residues
*Pf*DHODH	FMN*	−7.6	TYR199, ASP200, THR201, SER202, ASN203, ILE218, LEU238, LYS239, LEU240, GLY241, PHE242, LEU302, HIS306
	Caboxamycin	−8.4	ASP200, SER202, ASN203, ASP204, ILE218, LEU238, LYS239, LEU240, GLY241, PHE242, LEU302, LYS305, HIS306, LYS543
	Bisanhydroaklavinone	−7.9	TYR199, ASP200, THR201, ASN203, ILE218, LEU238, LYS239, GLY241, LEU302, LEU303, LYS305, HIS306
	Napyradiomycin A1	−7.8	TYR199, ASP200, THR201, SER202, ASN203, ILE218, LYS239, LYS301, LEU302, SER304, LYS305, HIS306
*Pf*DPCK	Opera1*	−7.6	ILE81, ALA82, LYS85, ASP103, THR106, TYR110, ARG141, VAL144, PHE145, VAL151, ILE154, ASN155, THR158, HIS159, ILE162, PRO187, LEU188, ARG218, ILE230, ASN233, GLN234
	Napyradiomycin A1	−8.9	ILE81, LYS85, ALA102, THR106, TYR110, ARG141, VAL144, PHE145, VAL151, ILE154, ASN155, THR158, HIS159, ILE162, ALA186, PRO187, LEU188, LEU189, THR192, LEU194, ILE230, ASN233, GLN234
	Gardenomycin A	−8.1	ALA102, ASP103, THR106, VAL141, VAL144, PHE145, THR158, HIS159, ILE162, PRO187, LEU188, ASN233, GLN234
	Bisanhydroaklavinone	−7.9	ILE81, ALA82, LYS85, ASP103, TYR110, LEU140, ARG141, VAL144, PHE145, ILE154, ASN155, PRO187, LEU188, ARG218, ILE230, ASN233, GLN234

Note: *control ligand.

The dual-target approach employed in this study represents a significant advancement over conventional single-target antimalarial strategies, as simultaneous inhibition of both pyrimidine biosynthesis (*Pf*DHODH) and salvage pathways (*Pf*DPCK) could potentially overcome resistance mechanisms that often emerge with monotherapies. Notably, these *Streptomyces*-derived bioactive compounds demonstrated consistently stronger binding affinities than reference inhibitors for both targets, with Caboxamycin showing the most promising dual-target activity. Binding site analysis revealed conserved interaction patterns across both targets such as key hydrogen bonds with catalytic residues, favorable hydrophobic contacts within active sites, and minimal steric clashes, then indicating good complementarity. Importantly, the molecular scaffolds of these *Streptomyces* metabolites differ substantially from existing antimalarial compounds, suggesting novel mechanisms of action that could circumvent current resistance pathways. The dual-target binding capability of these four compounds represents a novel finding in secondary metabolite compounds in *Streptomyces* sp. for antimalarial drug discovery, as previous studies have primarily focused on single-target approaches or have not demonstrated simultaneous high-affinity binding to both *Pf*DHODH and *Pf*DPCK by natural product compounds. This dual-target selectivity, combined with the unique structural features of *Streptomyces*-derived metabolites, positions these compounds as promising leads for next-generation antimalarial therapeutics that could address the urgent need for resistance-breaking drug candidates.

To predict the potential interactions of compounds with *Pf*DHODH (PDB ID: 3I65) targets, molecular docking was performed. The binding energy and amino acid residues of *Pf*DHODH that interacted with each compound, along with the hydrogen bonds formed, are presented in Table 5. Three candidate compounds were selected based on their stronger binding affinities compared to FMN (−7,6 kcal/mol) and against other compounds. The potential compounds include Caboxamycin, Bisanhydroaklavinone, and Napyradiomycin A1. The docking analysis results indicate that Caboxamycin exhibits a significant binding affinity to *Pf*DHODH, achieving a docking score of −8.4 kcal/mol. This score serves as a quantitative measure of the compound's ability to interact with the target protein. Notably, Caboxamycin demonstrates robust interactions, characterized by strong hydrogen bonds with SER202 and hydrophobic interactions with LEU302, LYS239, and ILE218. These interactions are critical determinants of molecular affinity, positioning Caboxamycin as a promising candidate for further investigation. On the other hand, the binding affinity of Bisanhydroaklavinone to *Pf*DHODH was assessed, yielding a docking score of −7.9 kcal/mol. Molecular modeling revealed that Bisanhydroaklavinone forms hydrogen bonds with four key amino acid residues: ASN203, LEU238, LYS239, and HIS306. Furthermore, hydrophobic interactions were identified between Bisanhydroaklavinone and residues such as TYR199, LEU302, and LYS305. The interaction of Napyradiomycin A1 with DHODH resulted in a docking score of −7.8 kcal/mol, suggesting potential engagement between the two molecules. Our analysis found that Napyradiomycin A1 establishes a hydrogen bond with LEU302. Additionally, several hydrophobic interactions were noted between Napyradiomycin A1 and DHODH, specifically involving residues TYR199, LYS239, LEU302, LYS305, and HIS306. These findings provide a valuable foundation for elucidating the mechanism of action of Napyradiomycin A1 in malaria treatment.

Molecular docking simulations demonstrated significant interactions between bioactive compounds and *P. falciparum* Dephospho-CoA Kinase (*Pf*DPCK). Napyradiomycin A1 exhibited the strongest binding affinity (−8.9 kcal/mol), engaging with 23 amino acid residues including key hydrophobic residues (PHE145, VAL144) and polar interaction sites (THR158, HIS159). Gardenomycin A similarly showed high binding affinity (−8.1 kcal/mol), forming stable interactions with active site residues ASP103 and GLN234. In contrast, Bisanhydroaklavinone (−7.9 kcal/mol) established stable contacts primarily with LYS85 and ASN155. Comparative analysis revealed Napyradiomycin A1 interacts with a more diverse range of amino acid residues than Bisanhydroaklavinone, suggesting greater conformational flexibility in its enzyme binding. These findings position Napyradiomycin A1 as the most promising candidate for further investigation of its *Pf*DPCK interactions, particularly for developing novel mechanisms of action against drug-resistant malaria strains. All three compounds demonstrated potential to inhibit *Pf*DPCK's catalytic function, which plays an essential role in coenzyme A biosynthesis in malaria parasites. This study presents the first in silico exploration of *Pf*DPCK as a therapeutic target for malaria. Notably, *Pf*DPCK shows only 24% sequence similarity with its human ortholog (HSCOASY), suggesting minimal off-target effects in human biochemistry. Furthermore, *Pf*DPCK exhibits high

sequence conservation across *Plasmodium* species, indicating its potential as a broad-spectrum target against multiple malaria parasites.

The molecular docking data (Table 5) show that the bond interactions formed between the four bioactive compounds with the two target proteins, *Pf*DHODH and *Pf*DPCK, are able to work on the side of the target protein so that it is considered to be able to interact better than the control ligand. This is based on the number of bonds formed and the number of bond similarities compared to the original ligand. In addition, the four compounds were also found to have many bond similarities with the control ligand so that, based on the active side that is bound, bonds are formed in the same area as FMN and Opera1, which indicates that the four compounds can inhibit *Pf*DHODH and *Pf*DPCK with the same mechanism as FMN and Opera1. To illustrate the binding mode of the test compound ligand alongside the control compound ligand at the receptor's active site, molecular docking was performed (Figure 5). The visualization results indicate that the bonds of the four bioactive compounds and the control ligand reside within the same pocket, specifically at the receptor's active site. This implies a considerable potential for these compounds to function as inhibitors of *Pf*DHODH and *Pf*DPCK, akin to the control ligands FMN and Opera1. According to Nursamsiar et al,[86] if the bioactive compound has many residues on the active side, a stable and stronger bond will be formed. The similarity of residue bonds between bioactive compounds and control ligands to target proteins on the active side means that the tested bioactive compounds have similar activity to control ligands and have the potential to continue biological responses or as antagonistic compounds.[87]

Antimalarial Probability Prediction

The four bioactive compounds tested, Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A, were analyzed to determine the probability of antimalarial properties based on the ability of the compound to perform its main activity as antiparasitic and antiprotozoan (*Plasmodium*) as well as other biological activities (Figure 6) using PASS online (https://www.way2drug.com/passonline/). These activities are evidenced by Pa values (probability "to be active") that are greater than Pi values (probability "to be inactive").[88] Through online PASS analysis, it shows that two compounds, Bisanhydroaklavinone and Gardenomycin A, have high Pa values for antiprotozoal (*Plasmodium*) activity, both of which are 0.734, while the caboxamycin compound has a Pa value of 0.273. Meanwhile, the antiparasitic activity is owned by three compounds in a row from the highest, namely Napyradiomycin A1 of 0.552, Bisanhydroaklavinone, and Gardenomycin A with the same value of 0.143. According to,[89] if the Pa value is >0.7, then a compound is estimated to have high potential activity as an antiprotozoal or antiparasitic because it has a high similarity with compounds that have previously been proven as antiprotozoal (*Plasmodium*) or antiparasitic, while a Pa value >0.3 and <0.7 (medium confidence) indicates that the compound still has low similarity with compounds that have been proven as antiprotozoal (*Plasmodium*) or antiparasitic.

Hepatoxicity and Toxicity Analysis

Analyzing the pharmacokinetic profile of a test compound, it is important to evaluate its toxicity, especially the hepatotoxicity effect, which is a crucial aspect in drug development. The eligibility of a drug compound candidate for hepatotoxicity and toxicity assessment is generally used to understand the mechanisms underlying its biological effects along with potential implications in further drug development. Based on molecular docking simulations, this study proposes four substances as potential drug candidates for malaria treatment, interacting with key proteins involved in anti malarial development: *Pf*DHODH and *Pf*DPCK. Among the compounds tested, Bisanhydroaklavinone and Napyradiomycin A1 interacted with all three essential proteins, while Gardenomycin A interacted with *Pf*DPCK. Caboxamycin, on the other hand, showed interactions primarily with *Pf*DHODH. To further assess the clinical potential of these candidates, their toxicity profiles were evaluated using ADMETlab 3.0, with a focus on three key toxicity aspects: AMES toxicity, carcinogenicity, and human hepatotoxicity (Figure 7A). Bisanhydroaklavinone exhibited a high likelihood of inducing genetic mutations (AMES toxicity 0.965) and a significant probability of carcinogenicity (0.825), suggesting the need for further testing to assess its safety. However, its risk for human hepatotoxicity is relatively low, indicating a potentially safer profile in terms of liver toxicity. Napyradiomycin A1 showed a moderate risk for AMES toxicity (0.556) and carcinogenicity (0.601), but it has a higher probability of hepatotoxicity (0.686), suggesting that this

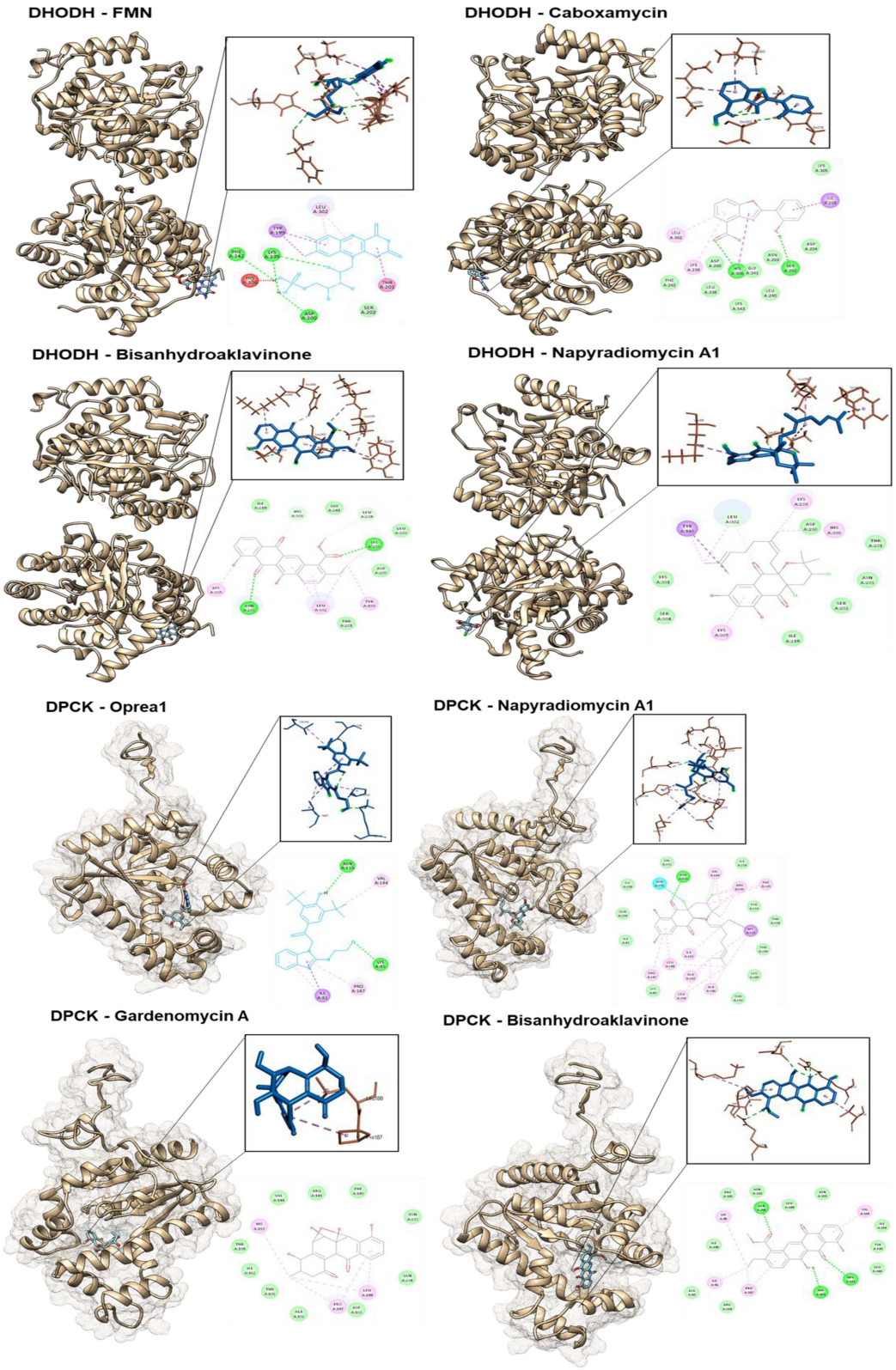

Figure 5 Molecular docking analysis of *Streptomyces* sp. bioactive compounds with target protein receptor. Binding mode visualization showing 3D protein-ligand complex with control ligand positioned in the active site, and 2D interaction diagram depicting hydrogen bonds (dashed lines), hydrophobic contacts, and key amino acid residues involved in ligand recognition. The ligand is displayed in stick representation with conventional atom coloring.

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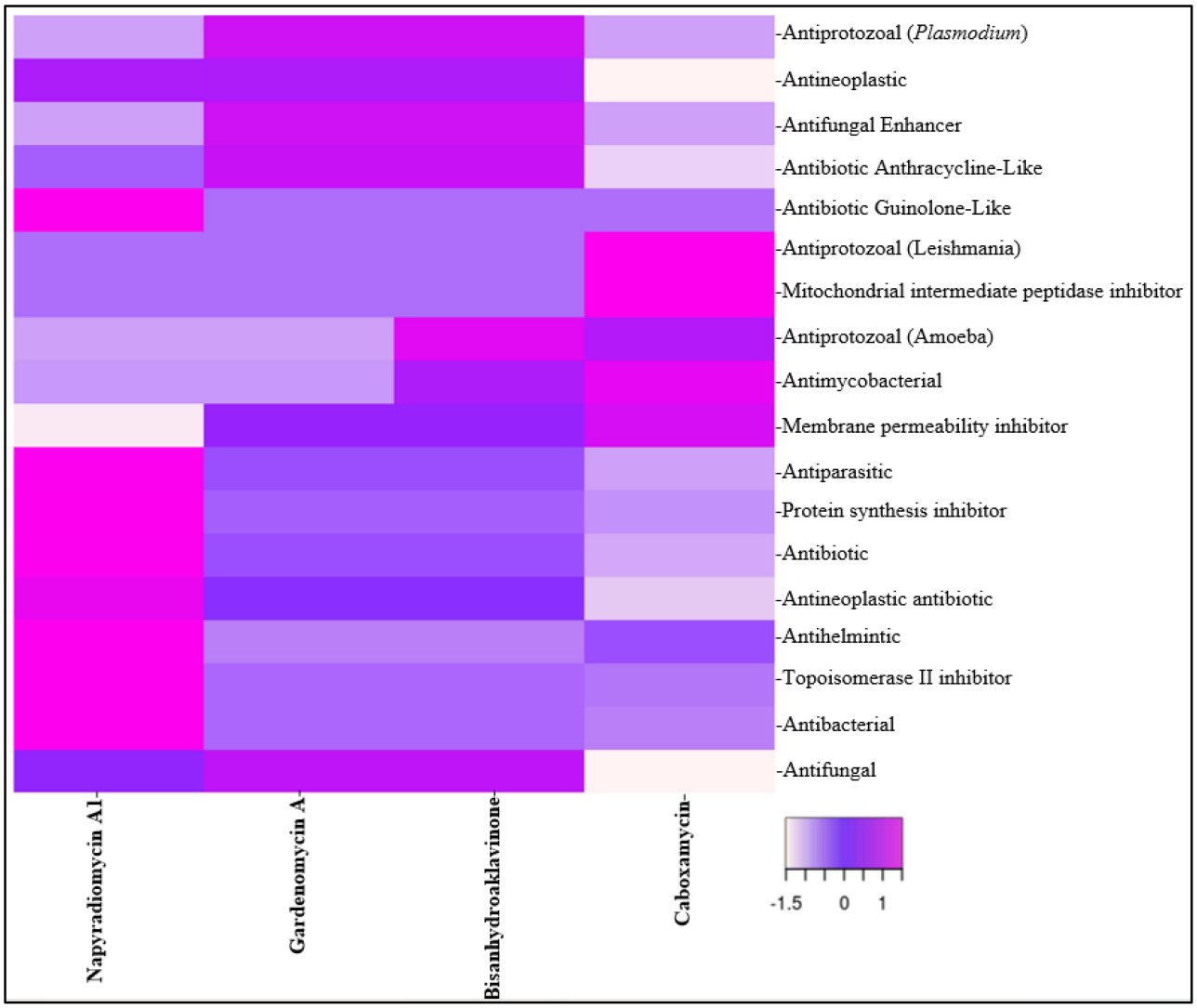

Figure 6 Biologycal Activities Prediction of Four Bioactive Compounds *Streptomyces* sp. Collected from PASS Online. The compounds were analyzed using the Prediction of Activity Spectra for Substances (PASS) algorithm, with Pa > 0.7 indicating high probability of activity and Pi < 0.3 suggesting low probability of side effects.

compound could potentially cause liver damage and should be closely monitored for hepatotoxic effects. Both Caboxamycin and Gardenomycin A exhibited moderate toxicity risks across all three toxicity aspects, signaling a medium concern for potential toxicity. These compounds require thorough evaluation of their overall safety profile.

Organ toxicity was further assessed using Pro-Tox II (Figure 7). Napyradiomycin A1 demonstrated an active hepatotoxicity property with a relatively moderate probability of 0.81, indicating a potential risk to liver health that requires close monitoring. Regarding cardiovascular and neurotoxicity, all compounds appear to be safe, as none exhibit significant toxicity probabilities. This suggests that these substances do not pose major risks to cardiovascular or neurotoxic effects under the current conditions. However, Napyradiomycin A1 and Gardenomycin A show moderate to high probabilities of nephrotoxicity, which requires caution, particularly for individuals at higher risk of kidney damage. A serious concern arises regarding respiratory toxicity, as all compounds exhibit relatively high probabilities (0.75 or higher). Based on this data, it is not recommended to administer these substances via routes that could affect respiratory health. Therefore, careful consideration should be given to the route of administration. Additionally, each of these compounds has a different LD50 value, highlighting the need for individualized approaches when considering the safety of these substances in any therapeutic setting (Figure 7).

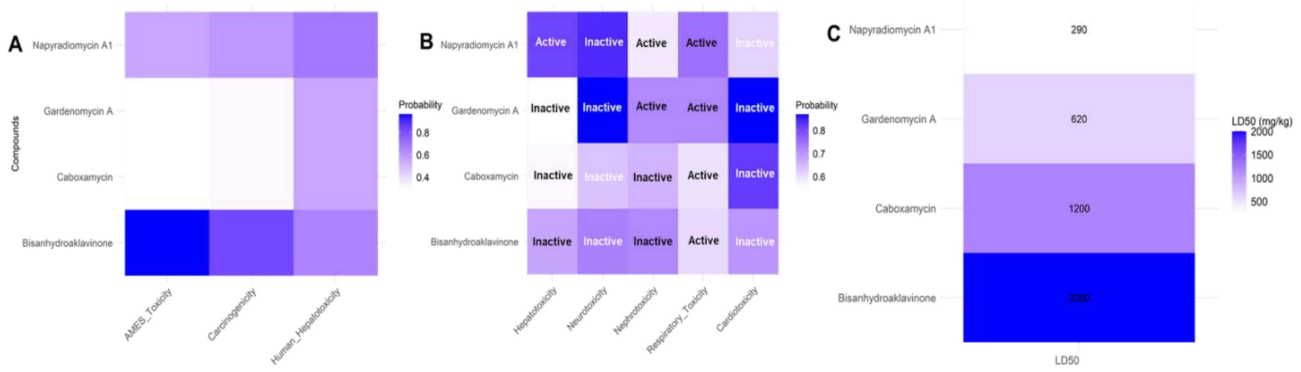

Figure 7 Predicted toxicity profiles of four antimalarial candidate compounds. Comprehensive toxicity assessment showing (**A**) ADMET properties including absorption, distribution, metabolism, excretion, and toxicity parameters, (**B**) organ-specific toxicity predictions for liver, kidney, and cardiac systems, and (**C**) mutagenicity and carcinogenicity risk assessment. Compounds meeting acceptable toxicity thresholds are highlighted for further development consideration.

Molecular Dynamic Simulation and Analysis

Molecular dynamics is used to evaluate the stability of protein-ligand complexes formed over a certain time span. The simulation will predict how each atom in a protein or other molecular system moves over time and is based on a physics model that regulates interactions between atoms.[90,91] Molecular dynamic simulation in this study was carried out to analyze the interaction between protein-compound complexes tested based on molecular docking results that showed the level of binding affinity, which are Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A. Molecular dynamics simulations were carried out on the bonding of test compounds with protein targets *Pf*DHODH and *Pf*DPCK (Figure 8). The results of the molecular dynamics simulation were then analyzed for RMSD (Root Mean Square Deviation) and RMSF (Root Mean Square Fluctuation) analysis using YASARA software (version 19.14.12) with the AMBER14 force field for 20 ns.

Analysis of the RMSD results will show a measure that is often used in 3D molecular geometry in comparing changes or movements in molecular conformation so that it can describe how far the state of the protein-ligand complex changes each time until the end of the simulation time ends and the final result is obtained in the form of stability of the structure of the protein-ligand complex, while the RMSF will analyze the size of the deviation between particle positions with several reference positions counting each amino acid residue that makes up proteins aimed at seeing the extent of fluctuations in movement of each amino acid residue during simulation.[92,93]

The results of molecular dynamic simulations in this study will analyze the structural stability and conformational fluctuations of Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A interacting with *Pf*DHODH and *Pf*DPCK during the 20 ns simulation (Figure 8). The interaction of ligands (test compounds) of Caboxamycin, Bisanhydroaklavinone, and Napyradiomycin A1 with *Pf*DHODH are considered stable from the beginning to the end of the simulation because the RMSD values are less than 3 Å with slight fluctuations for Napyradiomycin A1 compounds in RMSD ligand conformation. In general, the stability of protein structures in molecular dynamic simulations has minimal fluctuations with RMSD and RMSF values below 3 Å.[94] Meanwhile, the inhibitor compound (ligand control) experienced significant fluctuations of RMSD backbone values within 5.5–7.5 ns which increased up to 10 Å indicating the instability of the protein backbone structures. When the RMSD value is more than 3 Å, a conformational change occurs so that the complex structure formed becomes unstable.[95] The results of RMSD interactions between proteins and *Pf*DPCK show a stable trend based on the results of RMSD ligand conformation in Bisanhydroklavinone, Napyradiomycin A1, and Gardenomycin A compounds with a value of less than 3 Å for 20 ns, but the backbone RMSD value experiences unstable fluctuations in Napyradiomycin A1, and Gardenomycin starting at 3 ns until the end of the simulation. According to[96] the stability of the interaction of ligands with proteins of a complex in molecular dynamic simulations is declared stable if the RMSD value is low with minimal fluctuations. The tendency of the stability of the RMSD value is supported by hydrogen bonds and RMSF values. The number of hydrogen bonds formed in both protein-*Pf*DHODH/*Pf*DPCK complexes with protein-Caboxamycin, Bisanhydroaklavinone,

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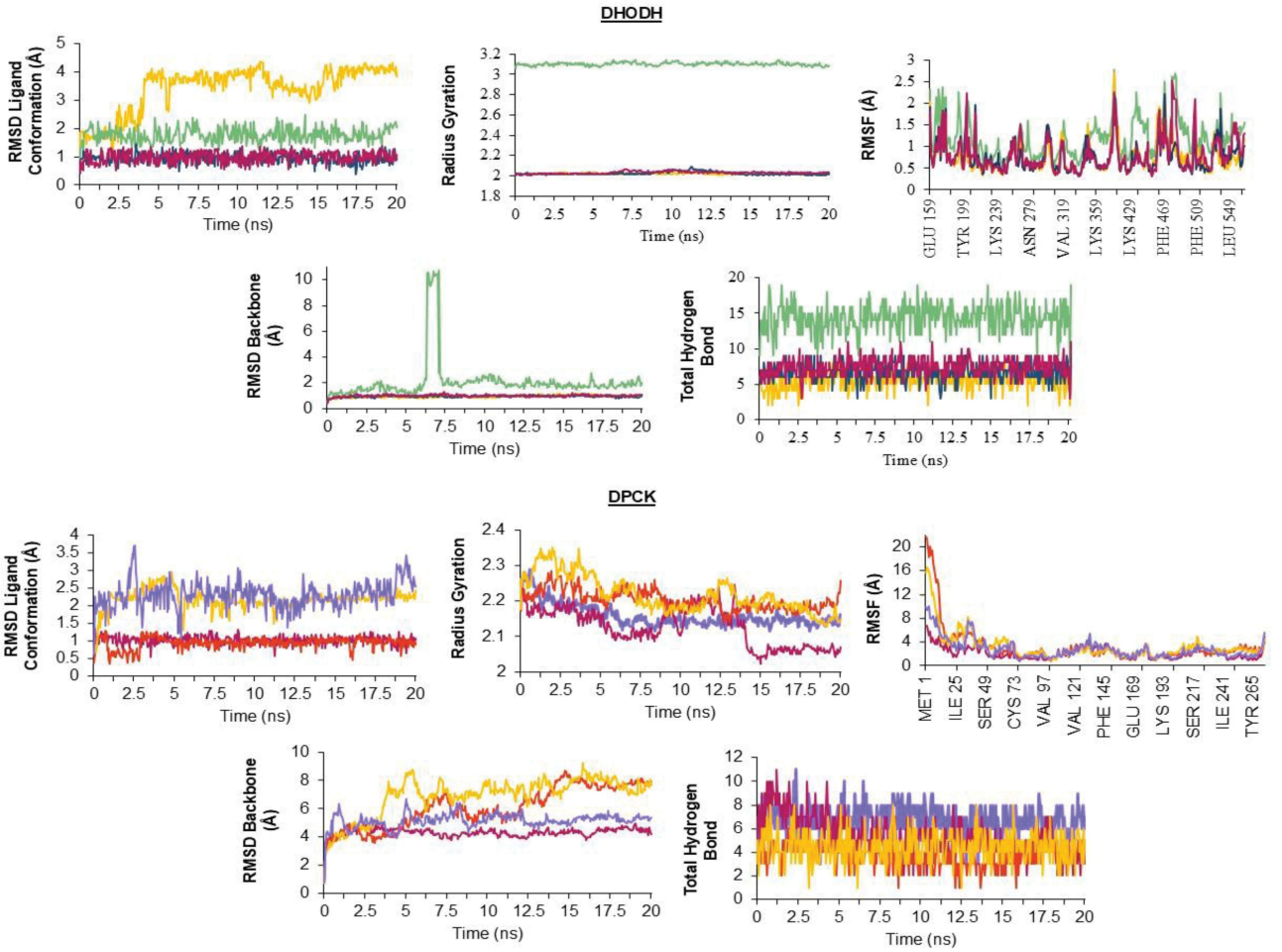

Figure 8 Molecular dynamics simulation analysis of protein-ligand complexes. Time-dependent analysis showing Root Mean Square Deviation (RMSD) of ligand position relative to the initial conformation, RMSD of protein backbone atoms indicating structural stability, radius of gyration reflecting protein compactness over simulation time, total number of intermolecular hydrogen bonds between ligand and protein, and Root Mean Square Fluctuation (RMSF) of individual amino acid residues highlighting flexible regions. : FMN. : Napyradomycin A1. : Caboxamycin. : Opera1. : Bisanhydroclavinone. : Gardenomycin.

Napyradiomycin A1, and Gardenomycin A complexes is not much different in all complexes. The RMSF value displays the stability of the amino structure of the acidic residues, with the overall RMSF value in all complexes below 3 Å for 20 ns. The radius of gyration also shows that the protein remains in a compact and stable state (Figure 8).

Discussion

DHODH proteins are present in the biological systems of malaria parasites and humans. Both of them contain class 2 DHODH, however in humans, DHODH has an alternative mechanism for pyrimidine synthesis called the salvage pathway, so targeting *Pf*DHODH has no effect on essential human functions.[15] Significant variation also exists in the structure of the ubiquinone binding site, as revealed by the X-ray structure of DHODH in *P. falciparum* and humans. These variations serve as the structural basis for identifying specific inhibitors in each species.[80] Additionally, the crystal structures of *Hs*DHODH and *Pf*DHODH show that the human enzyme and *Plasmodium* enzyme have different amino acid sequences.[15] Phylogenetic analysis based on sequence alignment using BLASTp revealed a distant evolutionary relationship between *Pf*DHODH and *Hs*DHODH, as indicated by a sequence identity of 35%.[97] *Pf*DPCK is a highly conserved protein among *Plasmodium* species with 88–93% amino acid identity to *Plasmodium yoelii, Plasmodium vivax, Plasmodium knowlesi, Plasmodium ovale*, and *Plasmodium malariae*. In contrast, *Pf*DPCK exhibits very limited similarity (24%) to its human counterpart (*Hs*COASY). *Pf*DPCK exhibits significant evolutionary, sequence-structural, and functional domain divergence compared to its human homolog. Phylogenetic analysis of 43 DPCK sequences from

various species revealed that the *Plasmodium* DPCK forms a distinct clade separate from that of humans. This indicates that *Pf*DPCK is not only divergent at the sequence level but has also undergone evolutionary divergence accompanied by functional specialization or unique adaptations to the parasite's metabolic pathways. *Pf*DPCK is a monofunctional enzyme containing only the kinase domain responsible for catalyzing the phosphorylation of dephospho-CoA. In contrast, the human homolog is part of the bifunctional coenzyme A synthase (*Hs*COASY), which also contains a phosphopantetheine adenylyltransferase (PPAT) domain.[14] *Hs*COASY harbors two functional domains (DPCK and PPAT) within a single polypeptide, whereas in *Plasmodium*, these two enzymes are encoded by separate genes. This separation provides an opportunity to develop selective inhibitors that do not affect the human enzyme.[98]

Protein network interaction, or protein–protein interaction (PPI), will visually represent the functional interactions between proteins based on the identification of functional modules and conservative interaction patterns, thereby enabling the discovery of new protein functions based on network connectivity to support target proteins in drug discovery for the analysis of complex infectious diseases.[99] Additionally, this analysis of protein networks and pathways is also part of systems biology, which integrates functional data to understand molecular interactions in the context of disease and therapy. This study revealed that DHODH and DPCK in *P. falciparum* can be predicted to interact with several proteins that can control cell metabolism. This interaction can lead to a metabolism change that favors the *P. falciparum* growth in the host cell. Particularly, with inducing nutrient transport into cells to fulfill their glucose, and nucleic base.

Streptomyces sp. is a bacterium that is naturally active in producing secondary metabolites with various biological activities. It is estimated that to date, more than 10,000 bioactive compounds have been derived from the *Streptomyces* genus, exhibiting high functional and structural diversity, particularly as antiparasitic agents, especially against *Plasmodium*, as demonstrated by previous studies.[100] The metabolite extract from *S. hygroscopicus* subsp. hygroscopicus has shown various targets for antimalarial action, previously demonstrated by its ability to inhibit the ubiquitin-proteasome system (UPS), which results in a decrease in parasitemia levels in mice infected with *P. berghei*.[101] The natural product derived from *Streptomyces* sp., 5′-O-sulfamoyl-2-chloroadenosine (also known as dealanylascamycin or DACM), demonstrates significant inhibition of *P. falciparum* growth, exhibiting efficacy that is comparable to the leading antimalarial treatment, dihydroartemisinin. When *P. falciparum* is exposed to DACM, it results in the inhibition of protein translation, which is mediated by the phosphorylation of eIF2α. This finding indicated that DACM targets several aminoacyl-tRNA synthetases (aaRSs), including the cytoplasmic aspartyl tRNA synthetase (AspRS).[102] Another compound derived from *Streptomyces hygroscopicus* subsp. hygroscopicus has been identified through LC/MS as 6,7-dinitro-2-[1, 2, 4]triazole-4-yl-benzo[de]isoquinoline-1,3-dione which is classified as one of the isoquinoline derivatives. This compound has the potential to be developed as an antimalarial drug candidate with mechanism of action involves the compound's interaction with several critical proteins based on their binding affinities, which include: adenylosuccinate synthetase, falcipain 2, glucose-6-phosphate isomerase, glycerol kinase, enoyl-acyl carrier protein reductase, and dihydrofolate reductase-thymidylate synthase.[6]

The selection of Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A from the initial pool of 27 candidates can be justified according to the following criteria. Their binding affinity values were lower (more negative) than those of the control ligands FMN and Opera1, indicating stronger binding affinity toward the target enzymes and which demonstrated exceptional binding affinities to both *Pf*DHODH and *Pf*DPCK targets. The binding affinity values obtained demonstrate the superior potential of our compounds compared to control ligands. Caboxamycin exhibited the strongest interaction with *Pf*DHODH (−8.4 kcal/mol), surpassing the reference ligand FMN (−7.6 kcal/mol), while Napyradiomycin A1 showed exceptional binding to *Pf*DPCK (−8.9 kcal/mol). These values fall well below the −5.00 kcal/mol threshold that characterizes active ligands forming strong bonds to target proteins, indicating high potential for biological activity. Similar strong binding affinities have been reported for effective *Pf*DHODH inhibitors, with successful compounds typically showing binding energies below −7.0 kcal/mol. Binding affinity is one of the aspects that need to be considered in the interaction between ligands and receptors/target proteins. A low binding affinity value indicates that a ligand requires less energy to interact or bind with the receptor. Ligands with the lowest binding affinity values have a greater potential to interact with target proteins. As the binding affinity value decreases, the affinity between the ligand and receptor increases.[24,26]

The structure resulting from the docking process reveals the main interactions between the ligand and the protein, including hydrogen bonds, hydrophobic interactions, and electrostatic forces. Information from these interactions can provide a deep understanding of how the ligand works and form the basis for efforts to improve or refine the structure of the compound.[103] Hydrogen bonds are one type of non-covalent interaction commonly found in biological systems, such as proteins and nucleic acids, and are used as an important indicator in assessing pharmacological interactions between compounds and receptors.[104] Hydrophobic bonds in complexes between proteins and ligands play a crucial role in enhancing protein structural stability, particularly through the process of reorienting hydrophilic amino acid residues in a hydrophobic environment. The presence of hydrophobic interactions also contributes to ligand stability on receptors by reducing contact between nonpolar residues and water molecules.[104,105] Van der Waals interactions are a type of weak, non-specific attractive force that forms when two atoms are in very close proximity to each other. This force arises due to temporary fluctuations in electric charge that create transient dipoles, resulting in intermolecular attraction at short distances. Although weak in nature, these interactions play a crucial role in maintaining the stability of molecular structures and biological complexes.[106]

The PASS Online prediction result for Caboxamycin showed a low PA score in terms of antiprotozoal activity, meanwhile the molecular docking and molecular dynamic results were promising. This may be due to the lack of efficacy data in related tests for Caboxamycin or similar compounds tested on protozoa, particularly *P. falciparum*. PASS Online uses structure–activity relationship (SAR) analysis, which considers chemical characteristics, such as functional groups, stereochemical structure, size, and shape of a compound, to predict its biological and pharmacological effects.[107] Therefore, this can explain the discrepancy between the predicted results of antiprotozoal activity and the results of molecular docking and molecular dynamics.

This study evaluated the toxicity profiles of four proposed antimalarial compounds based on their interactions with key *P. falciparum* proteins. While toxicity predictions varied among the compounds, several insights can be drawn regarding their potential clinical applicability. Among the candidates, Napyradiomycin A1 warrants particular attention. In addition to its dual-target engagement, previous studies[108] have reported its antibacterial activity against Gram-positive bacteria, including *Staphylococcus* and *Bacillus* species, with MIC values ranging from 0.25 to 32 μg/mL. Furthermore, Napyradiomycin A1 demonstrated moderate cytotoxic effects against human cancer cell lines, with IC_{50} values below 20 μM. These findings, together with the current predictions of hepatotoxicity and nephrotoxicity, reinforce the need for close safety monitoring in preclinical studies. Although Napyradiomycin A1 lacks antioxidative activity, its bioactivity spectrum and interaction profile suggest potential for derivatives development, provided toxicity concerns can be addressed. For Bisanhydroaklavinone, Gardenomycin A, and Caboxamycin, no prior toxicity or pharmacological studies were identified. Nevertheless, their favorable binding profiles and manageable predicted toxicity suggest these compounds are worth exploring further as novel antimalarial candidates. Importantly, the high likelihood of respiratory toxicity across all compounds highlights the need for cautious selection of administration routes in future in vivo models. Altogether, while additional experimental validation is essential, the compounds proposed here offer promising scaffolds for antimalarial drug development. Napyradiomycin A1 presents a promising balance of efficacy and toxicity, warranting further investigation. To mitigate its hepatotoxicity and nephrotoxicity, structural modifications such as altering metabolically reactive groups or adding steric hindrance to reduce enzyme interaction should be explored. For Bisanhydroaklavinone, reducing DNA-reactive moieties like quinones may help suppress its mutagenic potential. Developing derivatives or synthetic analogs through rational design, supported by computational toxicology and in vitro screening, may retain antimalarial activity while minimizing off-target effects.

Molecular dynamics (MD) simulations were utilized to assess the stability of Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A in complex with *P. falciparum Pf*DHODH and *Pf*DPCK. RMSD investigation illustrated that caboxamycin, bisanhydroaklavinone, and Napyradiomycin A1 kept up conformational soundness in *Pf*DHODH, with RMSD values remaining underneath 3 Å all through the 20 ns reenactment. In contrast, the control ligand FMN showed critical spine deviation, coming to up to 10 Å between 5.5 and 7.5 ns, showing auxiliary flimsiness.[109] For *Pf*DPCK, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A moreover showed ligand RMSD values beneath 3 Å, recommending steady official. Be that as it may, Napyradiomycin A1 and Gardenomycin A appeared fluctuating spine RMSD values from 3 ns ahead, recommending direct basic strain in spite of protected ligand

situating.[110] The radius of gyration over all complexes remained between 2.0 and 2.4 Å, demonstrating reliable protein compactness. Hydrogen bond investigation uncovered steady interaction profiles over all ligand-protein complexes, whereas RMSF values, overwhelmingly underneath 3 Å, affirmed negligible residue-level change in key official districts. This information recommends vigorous complex arrangement and constrained neighborhood adaptability. Collectively, the reenactment highlights Bisanhydroaklavinone and Napyradiomycin A1 as promising dual-target inhibitors of *Pf*DHODH and *Pf*DPCK, illustrating favorable soundness profiles comparable or superior to reference ligands. These discoveries emphasize the potential of *Streptomyces*-derived metabolites as antimalarial candidates. In any case, the 20 ns reenactment window and nonappearance of organic setting (eg, film environment, solving impacts) restrain generalizability. Assist in vitro approval and expanded MD runs are essential to authenticate these discoveries and back downstream sedate advancement endeavors.

Compounds with strong pharmacological potential may also exhibit nonspecific interactions leading to toxicity. In silico predictions do not always reflect actual biological conditions. Therefore, in vitro or in vivo validation is essential to distinguish clinically relevant toxicity from "false positive" predictive results. Alternative bioprospecting strategies include semi-synthetic modification or derivatization of candidate compounds to reduce toxicity, as well as low-dose combination therapy with synergistic agents to mitigate cumulative toxicity risk. Cross-source bioprospecting such as exploration of metabolites from endophytic fungi, marine microbes, or other Actinobacteria may yield structurally analogous but safer chemical scaffolds.

The dual-target approach involves the simultaneous inhibition of two essential enzymes, *Pf*DHODH and *Pf*DPCK. Previous investigations have primarily focused on a single molecular target, most notably *Pf*DHODH[29] or, less frequently, *Pf*DPCK.[14] To our knowledge, this work constitutes the first in silico study to investigate *Pf*DPCK inhibition by natural products derived from *Streptomyces* sp. Four compounds Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A have not previously been identified as inhibitors of either *Pf*DHODH or *Pf*DPCK. Some of these molecules, such as napyradiomycin A1, have established anticancer or antibacterial properties,-[49,111] yet their potential as dual-target antimalarial agents has not been reported. The novelty of this dual-target mechanism is particularly significant when compared to existing *Streptomyces*-derived compounds. While traditional antimalarials from *Streptomyces* sources have shown limited efficacy against drug-resistant strains due to their single-target nature, our compounds offer a synergistic approach by simultaneously disrupting. Pyrimidine biosynthesis pathway (via *Pf*DHODH inhibition) is essential for DNA synthesis, previously validated as a promising antimalarial target with numerous inhibitors showing potent activity in recent studies and Coenzyme A biosynthesis pathway (via *Pf*DPCK inhibition), critical for metabolic processes, representing a novel and unexplored therapeutic target with significant potential based on our findings.

This investigation is based solely on in silico analyses predicted affinity and stability of the molecules toward *Pf*DHODH and *Pf*DPCK which still require validation through in vitro assays. Such validation includes enzyme inhibition assays to determine IC_{50} values, measurement of the inhibition constant (*Ki*) against *Pf*DHODH and *Pf*DPCK, and cell-based antimalarial assays using *P. falciparum* cultures to assess compound potency and selectivity. These steps are essential to confirm that the interactions observed in silico translate into biologically relevant effects, as demonstrated in previous studies on *Pf*DHODH inhibitor development and *Pf*DPCK.[11,14]

Conclusion

Molecular docking of 27 bioactive compounds from *Streptomyces* sp. highlighted four *Streptomyces*-derived compounds (Caboxamycin, Bisanhydroaklavinone, Napyradiomycin A1, and Gardenomycin A) showed strong binding, stability, and promising antimalarial potential to *Pf*DHODH and *Pf*DPCK as selective targets in *P. falciparum*. with strong inhibitory potential against these targets. Ligand-protein visualization and molecular dynamics analysis confirmed their active-site binding and stability, similar to control ligands. Those compounds were predicted to exhibit superior inhibitory *P. falciparum* activity, however only Caboxamycin, and gardenomycin are potential for antimalarial drug development with acceptable predicted toxicity, allowing them to undergo in vitro and in vivo test prior to clinical use, thus have the potential to become antimalarial drug candidates.

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Disclosure

All authors report no conflicts of interest in this work.

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