In Silico Virtual Screening Studies Using Molecular Docking of Isoflavonoid Compounds as Potential Antimalarials on the Plasmodium Falciparum Dihydroorotate Dehydrogenase (PfDHODH) Enzyme

Semuel Sandy*

Research Center for Public Health and Nutrition, National Research and Innovation Agency, Kawasan Kerja Bersama (KKB) BRIN Jayapura, Jl. Isele Kampung Waena, Distrik Heram, Jayapura Selatan, 99358, Papua, Indonesia

Keywords: Isoflavonoids, Malaria, Enzyme, Drug, Docking Simulations.

Abstract: Malaria remains a health problem in Indonesia, leading to an increase in morbidity and mortality. The use of antimalarial drugs has been reported to result in cases of resistance in several Plasmodium spp. Therefore, there is a need for the discovery and development of new drugs to address the broader impact of drug resistance. The aim of this study is to screen flavonoid compounds from plants that have the potential to be developed as antimalarial agents. Virtual screening method is employed using flavonoid compounds obtained from secondary metabolites database server (http://pscdb.appsbio.utalca.cl/viewIndex/index.php). The chemical structures of the flavonoid compounds are then prepared and optimized. The macromolecule used in the docking simulation is the Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH) enzyme, which plays a role in the synthesis of pyrimidine and purine in the parasite's DNA. The protein structure preparation and optimization are performed with 3D protonation using the AMBER10HT force field in the MOE 2015 application. Docking simulations are carried out using the London scoring system (dG) and the results are visualized in two dimensions using the MOE 2015 application. The goal of this study is to identify potential flavonoid compounds from plants that can be further developed as raw materials for antimalarial drugs. The screening results of molecular docking of isoflavonoid compounds against the PfDHODH enzyme yielded seven compounds: Retonone, Retononone, Degueline, 12a-hydroxyrotanenone, Toxicarol, Tephrosin, and Cristacarpin. These isoflavonoid compounds have lower docking scores than the native ligand 2EN603.

1 INTRODUCTION

Globally, an estimated 1.7 billion malaria cases and 10.6 million malaria deaths were averted in the period 2000–2020. Most of the cases (82%) and deaths (95%) averted were in the WHO African Region, followed by the WHO South-East Asia Region (cases 10% and deaths 2%) (WHO, 2021). Malaria is a serious infectious disease caused by the Plasmodium species and is endemic in more than 90 countries. Malaria is caused by protozoan parasites of the genus *Plasmodium*, including *P. falciparum, P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*, and it is exclusively transmitted through the bite of female *Anopheles* mosquitoes. The most deadly form of malaria is caused by *Plasmodium falciparum*. If not treated within 24 hours, *P. falciparum* malaria can progress to severe disease, often resulting in death. Around half of the world's population is at risk of malaria infection, with the highest burden in tropical regions such as Africa, Asia, and Latin America (World Health Organization, 2023).

Malaria remains a common global infectious disease that causes significant morbidity and mortality. Despite the availability of several approved drugs for its treatment, drug resistance has jeopardized many of them, making the development of new drugs for the treatment and prevention of malaria crucial. The completion of the Plasmodium falciparum genome and the growing understanding of parasite biology have triggered the search for new drug targets. However, only a few targets have been chemically validated in vivo. The pyrimidine biosynthesis pathway represents one of the best examples of successful identification of anti-malarial drug targets (Phillips and Rathod, 2010).

Sandy, S.
In Silico Virtual Screening Studies Using Molecular Docking of Isoflavonoid Compounds as Potential Antimalarials on the Plasmodium Falciparum Dihydroorotate Dehydrogenase (PfDHODH) Enzyme.
DOI: 10.5220/0013667000003873

In *Proceedings of the 1st International Conference on Medical Science and Health (ICOMESH 2023)*, pages 131-142
ISBN: 978-989-758-740-5

Purine and pyrimidine bases are essential for the synthesis of RNA and DNA. If a cell cannot synthesize its own RNA or DNA, it will die (Löffler *et al.*, 2005). In human cells, pyrimidine bases can be accessed through salvage pathways or de novo synthesis. If de novo synthesis is inhibited, the cell will rely on salvage pathways, and the cell will not die. However, because Plasmodium species lack the pyrimidine salvage pathway, inhibiting de novo synthesis in the parasite cells leads to their death, making them vulnerable to the inhibition of dihydroorotate dehydrogenase (DHODH) (Vyas *et al.*, 2016). Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH) catalyzes the fourth reaction of de novo pyrimidine biosynthesis in the parasite and is an important target for malaria treatment (Vyas *et al.*, 2016). *PfDHODH is a Class 2 enzyme consisting of 569 amino acids. Inhibition of PfDHODH disrupts the pyrimidine biosynthesis pathway in the parasite, leading to its death, making it a promising target for developing anti-malarial drugs*(Hoelz *et al.*, 2018).

Plants produce a wide variety of organic compounds, which are collectively known as Plant Secondary Compounds (PSC). These compounds, distributed differently among limited taxonomic groups within the plant kingdom, contribute significantly to specific colors, flavors, and odors, as well as provide defense properties. One of the most important applications of plant secondary compounds for humans is their use as traditional medicines and pharmaceuticals. (Wink, 2003).

One promising chemical compound as a candidate for antimalarial treatment is the isoflavonoid. Molecular docking screening analysis is conducted to select isoflavonoid compounds that have similar interactions with the inhibitor compound of the PfDHODH enzyme, in this case, the native ligand E2N603. The compound E2N603 has inhibitory abilities at the active site with specific amino acids on the PfDHODH enzyme (Vyas and Ghate, 2011). Newer classes of antimalarial agents target molecular-based enzymes in nucleoside biosynthesis pathways, such as PfDHODH for pyrimidine biosynthesis inhibition, purine nucleoside phosphorylase for purine nucleoside biosynthesis inhibition, adenosine deaminase for nucleoside synthesis inhibition, and dihydrofolate reductase for folate biosynthesis in the parasite (Leartsakulpanich *et al.*, 2002; Belén Cassera *et al.*, 2011; Frame *et al.*, 2015).

The goal of the docking study is to identify new hits from the database of natural compound flavonoids that have the potential to be potent and selective antimalarial agents against the PfDHODH enzyme.

2 MATERIAL AND METHODS

2.1 Protein Preparation for Molecular Docking

Molecular docking simulations for drug discovery were conducted using the crystal structure of Plasmodium falciparum dihydroorotate dehydrogenase (DHODH) co-crystallized with 3-Hydroxy-1-methyl-5-((3 (trifluoromethyl)phenoxy) methyl)-1H-pyrazole-4-carboxylic acid (ID: 6I4B) obtained from X-ray diffraction with a resolution of 1.98Å, R-free = 0.226, and R-work = 0.192. The crystal structure of the DHODH enzyme was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/structure/6I4B). Ligands, heteroatoms, and water molecules were separated from the PfDHODH enzyme structure prior to the molecular docking simulations (Shivanika *et al.*, 2020). The PfDHODH enzyme's structure was optimized and subjected to 3D protonation using the AMBER10HT force field with the assistance of the MOE 2015 application (Abhimanyu, Srivastava and Jain, 2022).

2.2 Ligands Preparation for Molecular Docking

Chemical compounds of isoflavones from various plants were downloaded from the Plant Secondary Compounds database (http://pscdb.appsbio.utalca.cl/viewIndex/index.php) in sdf format. A total of 122 groups of isoflavone chemical compounds were screened using the Lipinski rule of five, resulting in 103 chemical compounds (Lipinski, 2000, 2004; Valdés-Jiménez *et al.*, 2021). These compounds were then prepared by adding hydrogen atoms and force field AMBER10HT charges, followed by energy minimization of the test compounds using the MOE 2015 application.

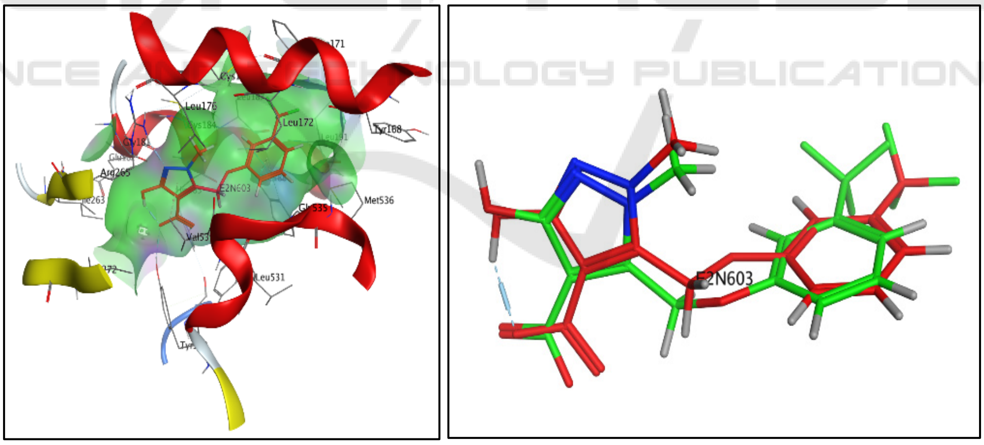
2.3 Molecular Docking Simulation

Molecular docking was performed by identifying the binding site of the PfDHODH enzyme using MOE 2015 software. The binding site was automatically identified and saved as a target for the docking simulations of the native ligand E2N603 as the reference and the docking simulations of the test ligands. The docking process was conducted with flexible ligands and a rigid enzyme PfDHODH receptor. The obtained docking results were scored using the London scoring system (dG) (Rachman and Mutalib, 2008; Sarwar, 2013)

3 RESULT AND DISCUSSION

The results of the binding site analysis for the E2N603 compound can be seen in Figure 1a. The binding site of the PfDHODH enzyme is dominated by a hydrophobic zone, with only a small portion of the hydrogen bonding and polar zones found. Molecular docking validation is performed to ensure the validity of the docking scores. One commonly used validation method is to perform redocking on the active site of the compound with known conformation and orientation, usually obtained from co-crystal structures. A program capable of reproducing poses with a Root Mean Square Deviation (RMSD) value below the pre-selected threshold (usually 1.5 or 2 Å, depending on the ligand size) is considered successful. The selection of poses is then followed by assessment and ranking to determine which scoring function provides the most accurate ranking of poses based on their RMSD values (Hevener *et al.*, 2010) In the redocking simulation using the reference ligand E2N603 on the PfDHODH enzyme, a root mean square deviation (RMSD) value of 0.9 A was obtained, indicating that the docking simulation process conducted is valid.

The redocking simulation of the native ligand E2N603 resulted in a docking score of -7.87 kcal/mol. The hydrogen bond interactions between the PfDHODH enzyme complex and the E2N603 ligand involve the amino acid residues ARG265, HIS185, and TYR258. Additionally, ionic interactions are formed with HIS185, HIS185, and Pi-Interactions with PHE188 and VAL532 (Table 2). The 2-dimensional visualization of the interaction between the PfDHODH enzyme complex and the ligand E2N603 can be seen in Figure 2(a) and Figure 2(b). A total of 122 ligands were obtained from the database, and after screening using the Lipinski's rule of five, 103 isoflavonoid compounds were selected (Hebbar *et al.*, 2022). These isoflavonoid compounds were then subjected to molecular docking simulations. The results of the screening and molecular docking simulations can be seen in Table 1. Among the isoflavonoid compounds, Rotenone showed the highest docking score (-8.62 kcal/mol), while Piscerythramine exhibited the lowest docking score (-1.49 kcal/mol).



(a) (b)

Figure 1: a) Pink color: hydrogen bonding zone, green color: hydrophobic zone, blue color: polarity zone. (b) Redocking results of the E2N603 ligand. The red color represents the native ligand E2N603, while the green color represents the redocking results of the native ligand E2N603. The redocking results obtained a rmsd value of 0.9 Å.

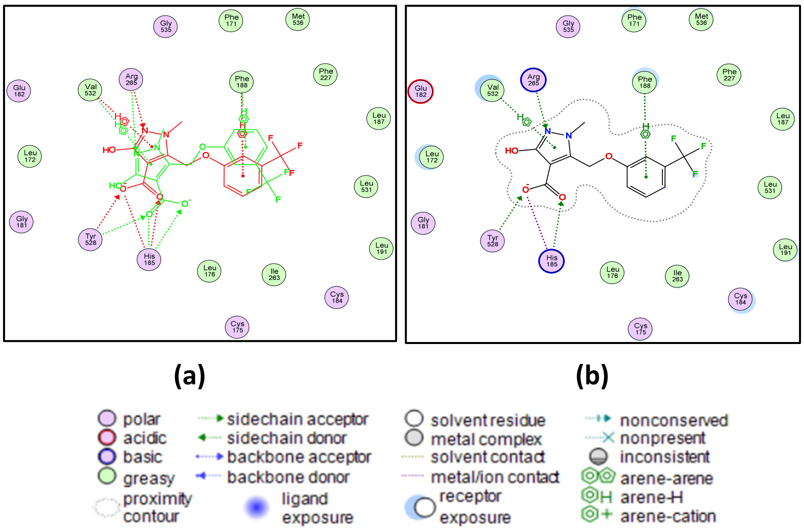


Figure 2: (a) Overlay of native ligand E2N603 interactions before docking and after redocking. (b) E2N603 ligand interactions at the PfDHODH enzyme receptors.

Table 1: The result of docking isoflavonoid compounds at the PfDHODH enzyme receptor

Com-pounds ID	Names	Mole-cular Mass (>500 Dalton)	Formula	N Rota-te Bond	N Hbond Acceptors (<10)	N Hbond Donors (<5)	Molar refract-tivity (<130)	TPSA (<140A)	W-logP	Dock-ing Score (Kcal /mol)
PSCdb 00419	Rotenone	394.42	C23H22O6	3	6	0	106.15	63.22	3.7	-8.62
PSCdb 02048	Rotenonone	406.38	C23H18O7	3	7	0	112.31	88.11	3.95	-8.43
PSCdb 01996	Deguelin	394.42	C23H22O6	2	6	0	106.98	63.22	3.9	-8.28
PSCdb 02015	12a-Hydroxyrotenone	410.42	C23H22O7	3	7	1	107.2	83.45	2.7	-8.12
PSCdb 02059	Toxicarol; alpha-Toxicarol	410.42	C23H22O7	2	7	1	109.01	83.45	3.6	-8.12
PSCdb 02057	Tephrosin	410.42	C23H22O7	2	7	1	108.03	83.45	2.89	-8.09
PSCdb 01831	Cristacarpin	354.4	C21H22O5	3	5	2	97.93	68.15	3.19	-7.91
PSCdb 02032	Mucronulatol	302.32	C17H18O5	3	5	2	82.11	68.15	2.83	-7.80
PSCdb 02025	Lotisoflavan	302.32	C17H18O5	3	5	2	82.11	68.15	2.83	-7.80
PSCdb 02034	Ononin; Formononetin 7-O-glucoside	430.4	C22H22O9	5	9	4	108.56	138.82	0.65	-7.78
PSCdb 02550	7-Hydroxy-2',4',5'-trimethoxyisoflavone	328.32	C18H16O6	4	6	1	89.42	78.13	3.19	-7.75
PSCdb 02019	Irisolidone	314.29	C17H14O6	3	6	2	84.95	89.13	2.89	-7.73
PSCdb 02031	Millettone	378.37	C22H18O6	0	6	0	100.06	63.22	3.61	-7.65
PSCdb 02052	Sophoraisoflavanone A	370.4	C21H22O6	4	6	3	101.78	96.22	3.68	-7.63

Compounds ID	**Names**	**Molecular Mass (>500 Dalton)**	**Formula**	**N Rotate Bond**	**N Hbond Acceptors (<10)**	**N Hbond Donors (<5)**	**Molar refractivity (<130)**	**TPSA (<140A)**	**W-logP**	**Docking Score (Kcal /mol)**
PSCdb 02036	Pachyrrhizone	366.32	C20H14O7	1	7	0	92.21	76.36	3.29	-7.54
PSCdb 01826	Betavulgarin	312.27	C17H12O6	2	6	1	82.5	78.13	2.9	-7.54
PSCdb 02022	Licoisoflavone A; 2',4',5,7-Tetrahydroxy-3'-(3,3-dimethylallyl) isoflavone	354.35	C20H18O6	3	6	4	99.73	111.13	3.79	-7.50
PSCdb 02053	(-)-Sparticarpin	300.31	C17H16O5	2	5	1	79.66	57.15	2.69	-7.45
PSCdb 02001	(-)-Glyceollin II	338.35	C20H18O5	0	5	2	91.84	68.15	2.75	-7.42
PSCdb 00770	Moracin A	286.28	C16H14O5	3	5	2	78.68	72.06	3.53	-7.41
PSCdb 02049	(-)-Sativan	286.32	C17H18O4	3	4	1	80.09	47.92	3.13	-7.39
PSCdb 02002	Hildecarpin	330.29	C17H14O7	1	7	2	80.27	86.61	1.41	-7.38
PSCdb 02033	(-)-Nissolin	286.28	C16H14O5	1	5	2	75.19	68.15	2.39	-7.37
PSCdb 02530	Glyceocarpin; 2-Dimethylallyl-(6aS,11aS)-3,6a,9-trihydroxypterocarpan	340.37	C20H20O5	2	5	3	93.46	79.15	2.89	-7.32
PSCdb 02038	(-)-Phaseollin; Phaseolin	322.35	C20H18O4	0	4	1	90.8	47.92	3.75	-7.30
PSCdb 02562	(-)-Sophorol	300.26	C16H12O6	1	6	2	75.61	85.22	2.19	-7.30
PSCdb 02026	Luteone	354.35	C20H18O6	3	6	4	99.73	111.13	3.79	-7.28
PSCdb 01998	Ferreirin; 2,3-Dihydro-5,7-dihydroxy-3-(2-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one	302.28	C16H14O6	2	6	3	78.06	96.22	2.17	-7.24
PSCdb 02050	Sayanedine	298.29	C17H14O5	3	5	1	82.93	68.9	3.18	-7.17
PSCdb 00045	(-)-Vestitone; Vestitone	286.28	C16H14O5	2	5	2	76.04	75.99	2.47	-7.14
PSCdb 02013	4-Hydroxyhomopterocarpin	300.31	C17H16O5	2	5	1	79.66	57.15	2.69	-7.14
PSCdb 02040	Pisatin; (+)-Pisatin	314.29	C17H14O6	1	6	1	78.25	66.38	1.7	-7.13
PSCdb 02532	Glyceollin III	338.35	C20H18O5	1	5	2	91.01	68.15	2.55	-7.13
PSCdb 01824	Afrormosin	298.29	C17H14O5	3	5	1	82.93	68.9	3.18	-7.11

Compounds ID	**Names**	**Molecular Mass (>500 Dalton)**	**Formula**	**N Rotate Bond**	**N Hbond Acceptors (<10)**	**N Hbond Donors (<5)**	**Molar refractivity (<130)**	**TPSA (<140A)**	**W-logP**	**Docking Score (Kcal /mol)**
PSCdb 00685	Albafuran A; 4-((2E)-3,7-Dimethyl-2,6-octadienyl)-5-(6-hydroxy-2-benzofuranyl)-1,3-benzenediol	378.46	$C_{24}H_{26}O_4$	6	4	3	115	73.83	6.45	-7.11
PSCdb 02559	(-)-Vestitol	272.3	$C_{16}H_{16}O_4$	2	4	2	75.62	58.92	2.83	-7.10
PSCdb 02054	Sumatrol	410.42	$C_{23}H_{22}O_7$	3	7	1	108.18	83.45	3.41	-7.09
PSCdb 02557	(-)-Medicocarpin; Medicarpin 3-O-glucoside	432.42	$C_{22}H_{24}O_9$	4	9	4	105.29	127.07	0.16	-7.09
PSCdb 02043	Pratensein	300.26	$C_{16}H_{12}O_6$	2	6	3	80.48	100.13	2.59	-7.09
PSCdb 02448	2'-Hydroxybiochanin A; Dehydroferreirin	300.26	$C_{16}H_{12}O_6$	2	6	3	80.48	100.13	2.59	-7.05
PSCdb 02017	Irilone	298.25	$C_{16}H_{10}O_6$	1	6	2	78.03	89.13	2.6	-7.05
PSCdb 02549	7,2'-Dihydroxy-4'-methoxy-isoflavanol; DMI; (3R,4R)-4'-Methoxyisoflavan-2',4,7-triol	288.3	$C_{16}H_{16}O_5$	2	5	3	76.78	79.15	1.99	-7.05
PSCdb 02046	Psoralidin	336.34	$C_{20}H_{16}O_5$	2	5	2	97.53	83.81	4.61	-7.04
PSCdb 01827	Bowdichione	298.25	$C_{16}H_{10}O_6$	2	6	1	77.53	93.81	1.56	-7.04
PSCdb 02062	Vestitol	272.3	$C_{16}H_{16}O_4$	2	4	2	75.62	58.92	2.83	-7.04
PSCdb 02561	(+)-Sophorol	300.26	$C_{16}H_{12}O_6$	1	6	2	75.61	85.22	2.19	-7.03
PSCdb 00895	Sainfuran	286.28	$C_{16}H_{14}O_5$	3	5	2	78.68	72.06	3.53	-7.02
PSCdb 00116	(-)-Glyceollin I; Glyceollin	338.35	$C_{20}H_{18}O_5$	0	5	2	91.84	68.15	2.75	-7.00
PSCdb 02063	Wedelolactone	314.25	$C_{16}H_{10}O_7$	1	7	3	82.32	113.27	2.82	-7.00
PSCdb 02056	Tectorigenin	300.26	$C_{16}H_{12}O_6$	2	6	3	80.48	100.13	2.59	-6.99
PSCdb 02027	(-)-Maackiain; Inermin	284.26	$C_{16}H_{12}O_5$	0	5	1	72.74	57.15	2.41	-6.99
PSCdb 02560	2',7-Dihydroxy-4',5'-methylenedi	298.25	$C_{16}H_{10}O_6$	1	6	2	78.03	89.13	2.6	-6.98

Compounds ID	**Names**	**Molecular Mass (>500 Dalton)**	**Formula**	**N Rotate Bond**	**N Hbond Acceptors (<10)**	**N Hbond Donors (<5)**	**Molar refractivity (<130)**	**TPSA (<140A)**	**W-logP**	**Docking Score (Kcal /mol)**
	oxyisoflavone; DMI									
PSCdb 02039	(-)-Phaseollinisoflavan	324.37	C20H20O4	1	4	2	93.25	58.92	3.89	-6.98
PSCdb 00911	Vignafuran	270.28	C16H14O4	3	4	1	76.66	51.83	3.82	-6.97
PSCdb 00182	Dihydrobiochanin A; 2,3-Dihydrobiochanin A	286.28	C16H14O5	2	5	2	76.04	75.99	2.47	-6.96
PSCdb 00050	Biochanin A	284.26	C16H12O5	2	5	2	78.46	79.9	2.88	-6.95
PSCdb 02061	(-)-Variabilin	300.31	C17H16O5	2	5	1	78.68	57.15	1.98	-6.93
PSCdb 02051	Sojagol	336.34	C20H16O5	0	5	1	95.59	72.81	4.5	-6.92
PSCdb 00104	Calycosin	284.26	C16H12O5	2	5	2	78.46	79.9	2.88	-6.92
PSCdb 01829	Cajanol	316.31	C17H16O6	3	6	2	82.53	85.22	2.47	-6.92
PSCdb 01997	5-Deoxykievitone; (+-)-5-Deoxykievitone	340.37	C20H20O5	3	5	3	95.29	86.99	3.67	-6.91
PSCdb 02045	Pseudobaptigenin	282.25	C16H10O5	1	5	1	76.01	68.9	2.89	-6.87
PSCdb 01828	Cajanin	300.26	C16H12O6	2	6	3	80.48	100.13	2.59	-6.86
PSCdb 02551	2,7-Dihydroxy-4'-methoxyisoflavanone	286.28	C16H14O5	2	5	2	75.18	75.99	2.08	-6.86
PSCdb 02531	4-Glyceollidin; 4-Dimethylallylglycinol; 4-Dimethylallyl-(6aS,11aS)-3,6a,9-trihydroxypterocarpan	340.37	C20H20O5	2	5	3	93.46	79.15	2.89	-6.86
PSCdb 02028	Medicarpin; (-)-Medicarpin	270.28	C16H14O4	1	4	1	73.17	47.92	2.69	-6.85
PSCdb 00193	2'-Hydroxyformononetin; 2'-Hydroformononetin	284.26	C16H12O5	2	5	2	78.46	79.9	2.88	-6.83
PSCdb 02064	Wighteone	338.35	C20H18O5	3	5	3	97.71	90.9	4.09	-6.82
PSCdb 00108	Kievitone	356.37	C20H20O6	3	6	4	97.31	107.22	3.38	-6.79
PSCdb 01823	(-)-Acanthocarpan	328.27	C17H12O7	0	7	1	77.82	75.61	1.42	-6.77

Compounds ID	Names	Molecular Mass (>500 Dalton)	Formula	N Rotate Bond	N Hbond Acceptors (<10)	N Hbond Donors (<5)	Molar refracttivity (<130)	TPSA (<140A)	W-logP	Docking Score (Kcal /mol)
PSCdb 02522	Glycitein; 7,4'-Dihydroxy-6-methoxyisoflavone	284.26	$C_{16}H_{12}O_5$	2	5	2	78.46	79.9	2.88	-6.75
PSCdb 02044	Prunetin	284.26	$C_{16}H_{12}O_5$	2	5	2	78.46	79.9	2.88	-6.74
PSCdb 02444	Isoformononetin; 4'-Hydroxy-7-methoxyisoflavone	268.26	$C_{16}H_{12}O_4$	2	4	1	76.43	59.67	3.17	-6.71
PSCdb 00055	Formononetin	268.26	$C_{16}H_{12}O_4$	2	4	1	76.44	59.67	3.17	-6.70
PSCdb 00401	7-O-Methylluteone	368.38	$C_{21}H_{20}O_6$	4	6	3	104.2	100.13	4.09	-6.69
PSCdb 01994	(+-)-Dalbergioidin	288.25	$C_{15}H_{12}O_6$	1	6	4	73.59	107.22	1.87	-6.69
PSCdb 01832	Cyclokievitone	354.35	$C_{20}H_{18}O_6$	1	6	3	95.69	96.22	3.24	-6.68
PSCdb 02058	Texasin; 6,7-Dihydroxy-3-(4-methoxyphenyl)-4-benzopyrone	284.26	$C_{16}H_{12}O_5$	2	5	2	78.46	79.9	2.88	-6.66
PSCdb 00173	2'-Hydroxydaidzein; 3-(2,4-Dihydroxyphenyl)-7-hydroxychromen-4-one	270.24	$C_{15}H_{10}O_5$	1	5	3	73.99	90.9	2.58	-6.59
PSCdb 02035	Orobol	286.24	$C_{15}H_{10}O_6$	1	6	4	76.01	111.13	2.28	-6.58
PSCdb 02447	2'-Hydroxygenistein; 2',4',5,7-Tetrahydroxyisoflavone	286.24	$C_{15}H_{10}O_6$	1	6	4	76.01	111.13	2.28	-6.57
PSCdb 02564	(+)-6a-Hydroxymaackiain	300.26	$C_{16}H_{12}O_6$	0	6	2	73.78	77.38	1.4	-6.57
PSCdb 00206	2'-Hydroxydihydrodaidzein; 2'-Hydroxy-2,3-dihydrodaidzein	272.25	$C_{15}H_{12}O_5$	1	5	3	71.57	86.99	2.16	-6.56
PSCdb 02000	Glabridin	324.37	$C_{20}H_{20}O_4$	1	4	2	93.25	58.92	3.89	-6.53
PSCdb 00262	Phaseollidin; (-)-Phaseollidin	324.37	$C_{20}H_{20}O_4$	2	4	2	92.42	58.92	3.89	-6.51
PSCdb 02488	2-Hydroxy-2,3-	288.25	$C_{15}H_{12}O_6$	1	6	4	72.73	107.22	1.48	-6.50

Compounds ID	Names	Molecular Mass (>500 Dalton)	Formula	N Rotate Bond	N Hbond Acceptors (<10)	N Hbond Donors (<5)	Molar refract-tivity (<130)	TPSA (<140A)	W-logP	Docking Score (Kcal /mol)
	dihydrogenistein									
PSCdb 00228	3,9-Dihydroxypterocarpan; (6aR,11aR)-3,9-Dihydroxypterocarpan	256.25	$C_{15}H_{12}O_4$	0	4	2	68.7	58.92	2.38	-6.47
PSCdb 02563	(+)-Maackiain	284.26	$C_{16}H_{12}O_5$	0	5	1	72.74	57.15	2.41	-6.46
PSCdb 02566	2,6,7,4'-Tetrahydroxyisoflavanone	288.25	$C_{15}H_{12}O_6$	1	6	4	72.73	107.22	1.48	-6.44
PSCdb 02514	Equol	242.27	$C_{15}H_{14}O_3$	1	3	2	69.13	49.69	2.82	-6.40
PSCdb 00387	Genistein; 5,7,4'-Trihydroxyisoflavone	270.24	$C_{15}H_{10}O_5$	1	5	3	73.99	90.9	2.58	-6.40
PSCdb 02003	Hispaglabridin A	392.49	$C_{25}H_{28}O_4$	3	4	2	116.97	58.92	5.4	-6.38
PSCdb 02536	2,7,4'-Trihydroxyisoflavanone	272.25	$C_{15}H_{12}O_5$	1	5	3	70.71	86.99	1.78	-6.38
PSCdb 02519	3',4',7-Trihydroxyisoflavone	270.24	$C_{15}H_{10}O_5$	1	5	3	73.99	90.9	2.58	-6.30
PSCdb 00119	(+)-Medicarpin; 3-Hydroxy-9-methoxypterocarpan	270.28	$C_{16}H_{14}O_4$	1	4	1	73.17	47.92	2.69	-6.29
PSCdb 01833	Daidzein	254.24	$C_{15}H_{10}O_4$	1	4	2	71.97	70.67	2.87	-6.28
PSCdb 00075	3,6,9-Trihydroxypterocarpan; (6aS,11aS)-3,6a,9-Trihydroxypterocarpan; (-)-Glycinol	272.25	$C_{15}H_{12}O_5$	0	5	3	69.74	79.15	1.38	-6.25
PSCdb 00772	Mulberrofuran A	392.49	$C_{25}H_{28}O_4$	7	4	2	119.47	62.83	6.75	-6.08
PSCdb 01830	Coumestrol	268.22	$C_{15}H_8O_5$	0	5	2	73.81	83.81	3.1	-5.99
PSCdb 01825	Anhydroglycinol; 3,9-Dihydroxypterocarpen	254.24	$C_{15}H_{10}O_4$	0	4	2	70.23	62.83	3.25	-5.93
PSCdb 02042	Pomiferin	420.45	$C_{25}H_{24}O_6$	3	6	3	121.83	100.13	5.16	-5.73
PSCdb 02023	Lonchocarpenin	448.51	$C_{27}H_{28}O_6$	5	6	1	130.77	78.13	5.77	-5.12
PSCdb 02041	Piscerythramine	451.51	$C_{26}H_{29}NO_6$	6	6	4	132.33	126.15	5.19	-1.49

The results of molecular docking simulations of isoflavonoid compounds were then screened based on docking score values above -7.87 kcal/mol, which is the score of the E2N603 ligand inhibitor. This screening resulted in 7 isoflavonoid compounds with the lowest docking scores. The interactions of these isoflavonoid compounds from the docking simulation with the PfDHODH enzyme complex included hydrogen bond donors involving residues CYS184 and LEU531, as well as Pi-H interactions with the amino acid VAL532, while no ionic interactions were found in the complex. The complete interactions of the PfDHODH complex with the isoflavonoid compounds can be seen in Table 2. The results of the molecular docking simulations for the isoflavonoid compounds did not show the same amino acid interactions as the native ligand E2N603. The screening results of the molecular docking simulations identified seven isoflavonoid compounds with lower docking scores than the native ligand. The differences in hydrogen bond, ionic, and Pi-H interactions formed in the PfDHODH complex with the test compounds can be considered for further in vitro experimental testing to determine whether these compounds are capable of inhibiting the activity of the PfDHODH enzyme.

Table 2: Molecular interactions of isoflavonoid compounds docked on the PfDHODH enzyme receptor

Compounds ID	**Names**	**Docking Score (Kcal/mol)**	**Reseptor**	**Interactions distance (A)**				**Energy interactions (kcal/mol)**			
				Hbond acceptor	**Hbond domor**	**Ionic**	**Pi-H**	**Hbond acceptor**	**Hbond domor**	**Ionic**	**Pi-H**
Native ligand	E2N603	-7.87	ARG 265	3.14	-	-	-	-4.4	-	-	-
			HIS 185	2.79	-	-	-	-4.7	-	-	-
			TYR 258	3.11	-	-	-	-3.4	-	-	-
			HIS 185	-	-	2.79	-	-	-	-6.1	-
			HIS 185	-	-	3.39	-	-	-	-2.3	-
			PHE 188	-	-	-	4.39	-	-	-	-0.5
			PHE 188	-	-	-	3.68	-	-	-	-0.6
			VAL 532	-	-	-	3.65	-	-	-	-0.7
PSCdb 00419	Rotenone	-8.62	CYS 184	-	3.48	-	-	-	-0.7	-	-
			LEU 531	-	3.48	-	-	-	-0.7	-	-
PSCdb 02048	Rotenonone	-8.43	CYS 184	-	3.61	-	-	-	-0.5	-	-
			VAL 532	-	-	-	3.97	-	-	-	-0.6
			VAL 532	-	-	-	4.21	-	-	-	-0.5
PSCdb 01996	Deguelin	-8.28	CYS 184	-	3.42	-	-	-	-0.8	-	-
			LEU 531	-	3.25	-	-	-	-0.7	-	-
			VAL 532	-	-	-	4.18	-	-	-	-0.5

Compounds ID	Names	Docking Score (Kcal/mol)	Reseptor	Interactions distance (A)				Energy interactions (kcal/mol)			
				Hbond acceptor	Hbond donor	Ionic	Pi-H	Hbond acceptor	Hbond donor	Ionic	Pi-H
PSCdb 02015	12a-Hydroxyrotenone	-8.12	CYS 184	-	3.29	-	-	-	-0.8	-	-
PSCdb 02059	Toxicarol; alpha-Toxicarol	-8.12	CYS 184	-	3.43	-	-	-	-0.8	-	-
			LEU 531	-	3.29	-	-	-	-0.6	-	-
			VAL 532	-	-		4.18				-0.6
PSCdb 02057	Tephrosin	-8.09	CYS 184	-	3.19	-	-	-	-0.8	-	-
PSCdb 01831	Cristacarpin	-7.91	LEU 531	-	2.69	-	-	-	-1.1	-	-

4 CONCLUSION

The screening results of molecular docking of isoflavonoid compounds against the PfDHODH enzyme yielded seven compounds: Retonone, Retononone, Degueline, 12a-hydroxyrotanenone, Toxicarol, Tephrosin, and Cristacarpin. These isoflavonoid compounds have lower docking scores than the native ligand 2EN603. Further in vitro experimental research can be conducted on these compounds to test their inhibitory activity against the PfDHODH enzyme.

REFERENCES

Abhimanyu, Srivastava, P. and Jain, C.K. (2022) 'In-Silico Investigation of Plant-Derived Natural Allosteric Compounds Towards Enhanced Drug-Protein Interaction of MOA Protein Complex in Depression Based on Molecular Docking and Molecular Dynamic Simulation Approaches', *Current Trends in Biotechnology and Pharmacy*, 16(4), pp. 529–539. Available at: https://doi.org/10.5530/ctbp.2022.4.86.

Belén Cassera, M., Zhang, Y., Hazleton, K.Z. and Schramm, V.L. (2011) 'Purine and Pyrimidine Pathways as Targets in Plasmodium falciparum', *Top Med Chem*, 11(16), pp. 2103–2115.

Frame, I.J., Deniskin, R., Arora, A. and Akabas, M.H. (2015) 'Purine import into malaria parasites as a target for antimalarial drug development', *Annals of the New York Academy of Sciences*, 1342(1), pp. 19–28. Available at: https://doi.org/10.1111/nyas.12568.

Hebbar, S., Nandan, S.K., Shetty, C.R., Nayak, P., Dhas, N. and Naha, A. (2022) 'In-silico screening and molecular docking studies of active constituents of Withania somnifera to investigate its kinase inhibitory activities', *Journal of Pharmaceutical Negative Results*, 13(4), pp. 1342–1349. Available at: https://doi.org/10.47750/pnr.2022.13.04.187.

Hevener, K.E., Zhao, W., Ball, D.M., Babaoglu, K., Qi, J., Stephen, W. and Lee, R.E. (2010) 'Validation of Molecular Docking Programs for Virtual Screening against Dihydropteroate Synthase', *J Chem Inf Model*, 49(2), pp. 444–460.

Hoelz, L.V., Calil, F.A., Nonato, M.C., Pinheiro, L.C. and Boechat, N. (2018) 'Plasmodium falciparum dihydroorotate dehydrogenase: A drug target against malaria', *Future Medicinal Chemistry*. Future Medicine Ltd., pp. 1853–1874. Available at: https://doi.org/10.4155/fmc-2017-0250.

Leartsakulpanich, U., Imwong, M., Pukrittayakamee, S. and Yuthavong, Y. (2002) 'Molecular characterization of dihydrofolate reductase in relation to antifolate resistance in Plasmodium vivax', *Molecular & Biochemical Parasitology 119*, 119, pp. 63–73.

Lipinski, C.A. (2000) 'Drug-like properties and the causes of poor solubility and poor permeability', *Journal of Pharmacological and Toxicological Methods*, 44, pp. 235–249.

Lipinski, C.A. (2004) 'Lead- and drug-like compounds: The rule-of-five revolution', *Drug Discovery Today: Technologies*, pp. 337–341. Available at: https://doi.org/10.1016/j.ddtec.2004.11.007.

Löffler, M., Fairbanks, L.D., Zameitat, E., Marinaki, A.M. and Simmonds, H.A. (2005) 'Pyrimidine pathways in health and disease', *Trends in Molecular Medicine*, 11(9), pp. 430–437. Available at: https://doi.org/10.1016/j.molmed.2005.07.003.

Phillips, M.A. and Rathod, P.K. (2010) 'Plasmodium dihydroorotate dehydrogenase: a promising target for novel anti-malarial chemotherapy', *Infect Disord Drug Targets*, 10(3), pp. 226–239. Available at: http://www.mmv.org/.

Rachman, A. and Mutalib, A. (2008) 'Molecular Docking Fevicordin on Human Estrogen α Receptor Using MOE Software', in *Proceeding of The International Seminar on Chemistry 2008*, pp. 325–331. Available at: www.intel.com.

Sarwar, M.W. (2013) 'Insilico Characterization and Homology Modeling of Arabitol Dehydrogenase (ArDH) from Candida albican', *Bioinformation*, 9(19), pp. 952–957. Available at: https://www.researchgate.net/publication/259224502

Shivanika, C., S, D.K., Ragunathan, V., Sumitha, A. and P, B.D. (2020) 'Molecular docking, validation, dynamics simulations , and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main- protease', *Journal of Biomolecular Structure and Dynamics*, pp. 1–27. Available at: https://doi.org/10.1080/07391102.2020.1815584.

Valdés-Jiménez, A., Peña-Varas, C., Borrego-Muñoz, P., Arrue, L., Alegría-Arcos, M., Nour-Eldin, H., Dreyer, I., Nuñez-Vivanco, G. and Ramírez, D. (2021) 'Psc-db: A structured and searchable 3d-database for plant secondary compounds', *Molecules*, 26, p. 1124. Available at: https://doi.org/10.3390/molecules26041124.

Vyas, V.K. and Ghate, M. (2011) 'Recent Developments in the Medicinal Chemistry and Therapeutic Potential of Dihydroorotate Dehydrogenase (DHODH) Inhibitors', *Reviews in Medicinal Chemistry*, 11, pp. 1039–1055.

Vyas, V.K., Qureshi, G., Ghate, M., Patel, H. and Dalai, S. (2016) *Identification of novel PfDHODH inhibitors as antimalarial agents via pharmacophore-based virtual screening followed by molecular docking and in vivo antimalarial activity*, *SAR and QSAR in Environmental Research*. Taylor and Francis Ltd. Available at: https://doi.org/10.1080/1062936X.2016.1189959.

WHO (2021) *World malaria report 2021*. 1st edn. Geneva: World Health Organization.

Wink, M. (2003) 'Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective', *Phytochemistry*, 64(1), pp. 3–19. Available at: https://doi.org/10.1016/S0031-9422(03)00300-5.

World Health Organization (2023) *Malaria Fact Sheet*, *Malaria*. Available at: https://www.who.int/en/news-room/fact-sheets/detail/malaria.