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Research Article
Molecular Docking Studies of Benzamide Derivatives for *Pf*DHODH Inhibitor as Potent Antimalarial Agent

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Abstract

Background and Objective: The most severe form of Malaria is caused by *Plasmodium falciparum*. *Plasmodium falciparum* Dihydroorotate dehydrogenase (*Pf*DHODH) is essential for the growth of this malaria parasite and has been validated as an antimalarial drug target for development of new antimalarial agents. The antimalarial identification using experimental techniques is expensive and requires extensive pains and labor. Several derivatives of triazolopyrimidine, benzamide, naphthamide and urea have been reported to inhibit *Pf*DHODH. Yet, there is a good scope for design and optimization of these molecules owing to either for their toxic nature or poor activity. Therefore, molecular docking techniques can be used to provide new insights into the development of potent chemotherapeutic drug for combating malaria by targeting *Pf*DHODH. **Materials and Methods:** Authors employed biological databases like PubChem, Drug Bank, Protein Data Bank (PDB) and the softwares, namely, Chimera, AutoDock and Python Molecular Viewer. The PDB contains structural information of the experimentally determined macromolecules and AutoDock is an automated docking tool, designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Chimera is a highly extensible program for interactive visualization and analysis of molecular structures while Python Molecular Viewer is a powerful molecular viewer. **Results:** On screening of benzamide derivatives, drug candidate CID 867491 was found to have least docking energy (-4.82 Kcal mol^{-1}), which inhibits *Pf*DHODH and further the interaction between them was validated using python software by formation of hydrogen bond between the CID 867491 and *Pf*DHODH. **Conclusion:** Results obtained from *in silico* study may provide a new insight into the development of potent chemotherapeutic drug for combating malaria by targeting *Pf*DHODH, after further validating the identified target in wet labs.

Key words: Antimalarial, benzamide derivatives, hydrogen bonding, ligand-docking, ligand protein interactions, molecular-docking, *Pf*DHODH

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria is the most dreaded parasitic disease of man and it is still a major health problem in tropical countries[1]. About 216 million cases of malaria were reported in the year, 2016 with an increase of about 5 million cases as compared to year, 2015. *Plasmodium falciparum* is the most prevalent malaria parasite in the WHO African Region, accounting for 99.7% of estimated malaria cases in 2017, as well as in the WHO regions of south-east Asia (62.8%), the eastern Mediterranean (69%) and the Western Pacific (71.9%). Estimated cases of falciparum malaria grew from 211 million in 2015-216 million in 2016 with an increase[1] of 2.4%.

Malaria is caused by hemoprotozoa of the genus *Plasmodium*. These parasites are transmitted to the human by the bites of infected female anopheles mosquitoes. Six malaria species are commonly known to cause human malaria: *P. falciparum*, *P. vivax*, *P. ovale curtisi*, *P. ovale wallikeri*, *P. malariae* and *P. knowlesi*[2]. The majority of malarial deaths are caused by the intracellular protozoan parasite *Plasmodium falciparum*[3]. The commonly used classes of antimalarial compounds include the quinolines (chloroquine, quinine, mefloquine, amodiaquine, primaquine), the antifolates (pyrimethamine, proguanil and sulfadoxine), the artemisinin derivatives (artemisinin, artesunate, artemether, arteether) and hydroxynaphthaquinones (atovaquone)[4]. Drug resistance has been reported to almost every known anti-malarial agent, underscoring the case by which parasite population can adapt and survive[5].

The *P. falciparum* relies exclusively on de novo pyrimidine biosynthesis to supply precursors for DNA and RNA biosynthesis[6]. In contrast, the human host cells contain the enzymatic machinery for both de novo pyrimidine biosynthesis and for salvage of performed pyrimidine bases and nucleosides[7]. Plasmodium purine and pyrimidine metabolic pathways are distinct from those of their human hosts. Thus, targeting purine and pyrimidine metabolic pathways provides a promising route for novel drug development[8]. Dihydroorotate dehydrogenase (DHODH) is a flavin mononucleotide (FMN) dependent mitochondrial enzyme that catalyzes the oxidation of L-dihydroorotate (L-DHO) to produce orotate as part of the fourth and rate-limiting step of the de novo pyrimidine biosynthetic pathway[9].

*Pf*DHODH is essential for parasite growth and has been validated as an antimalarial drug target for development of new antimalarial agents[10]. Several derivatives of triazolopyrimidine, benzamide, naphthamide and urea have been reported to inhibit *Pf*DHODH[11]. But due to the resistance to these existing drugs against malaria much more efforts are required to develop new anti malarial which overcomes the plasmodium resistance to clinically available drugs. Therefore, considering the high mortality, morbidity, emergence of resistance to existing drugs against malaria, the present study was undertaken for designing and optimization of these putative molecules in order to generate new drug candidates likely to act against malaria.

MATERIALS AND METHODS

Location and time duration of study: The present study was carried out during July 2017-April 2018 at Laboratory of Bioinformatics, School of Biotechnology, IFTM University, Delhi Road (NH-24), Moradabad 244102, Uttar Pradesh, India.

Preparation of protein structure: The 3D coordinates of the crystal structure of *Plasmodium falciparum* dihydroorotate dehydrogenase with a bound inhibitor (PDB id: 1TV5) was retrieved from PDB and taken as the receptor model in flexible docking program. *Plasmodium falciparum* dihydroorotate dehydrogenase was optimized by chimera tool for removal of all heteroatom (A26, FMN, N8F, ORO and Sulphate ion) and water molecules from PDB file of *Pf*DHODH (PDB id: 1TV5) and further polar hydrogen atom were added to protein to make the receptor molecule suitable for docking.

Active site analysis: The active site residues of *Plasmodium falciparum* dihydro-orotate dehydrogenase were obtained from the PDBSUM entry of 1TV5 having binding site residues LEU531, PHE227, GLY535, VAL532, PHE188, MET536, TYR528, HIS185, LEU172, CYS184, ARG265, GLY181, CYS175, PHE171 and ILE263 for inhibitor A26 (2-cyano-3-hydroxy-N-(4-trifluoromethyl-phenyl)-butyramide).

PubChem compound database screening: A total of 25 analogues of benzamide were screened using the criteria (Compounds having similarity value>= 95%) for docking studies (Table 1). Twenty five benzamide derivatives were docked with *Pf*DHODH and validated in two parts: (i) Prediction of docking energy between the docked compounds with *Pf*DHODH and (ii) Hydrogen bond details of the best-ranked docked pose using Python Molecular Viewer.

Molecular docking: Obtained benzamide derivatives against *Plasmodium falciparum* dihydroorotate dehydrogenase structure were docked using molecular docking program AutoDock[12]. Gasteiger charges were added and maximum six

Table 1: List of benzamide derivatives screened from Pubchem compound database

Compound CID No.	IUPAC name	M.Wt. (g mol^{-1})	Molecular formula	XLog P	HBD	HBA
798434	N-[3-(butanoylamino)phenyl]-2-methyl-3-nitrobenzamide	341.36116	C18H19N3O4	30	2	4
878248	N-[2-(hydroxymethyl)phenyl]-2-methyl-3-nitrobenzamide	286.28266	C15H14N2O4	2.6	2	4
878150	N-(3,5-dimethoxyphenyl)-2-methyl-3-nitrobenzamide	316.30864	C16H16N2O5	2.9	1	5
878176	N-(3,4-dimethoxyphenyl)-2-methyl-3-nitrobenzamide	316.30864	C16H16N2O5	2.9	1	5
870944	2-methyl-3-nitro-N-(4-propan-2-ylphenyl)benzamide	298.33642	C17H18N2O3	4.1	1	3
596245	2-methyl-3-nitro-N-(2,4,5-trichlorophenyl)benzamide	359.59186	C14H9Cl3N2O3	4.8	1	3
796489	N-(2,4-dimethylphenyl)-2-methyl-3-nitrobenzamide	284.30984	C16H16N2O3	3.7	1	3
796617	N-(2-chlorophenyl)-2-methyl-3-nitrobenzamide	290.70174	C14H11ClN2O3	3.6	1	3
803481	N-(3-chlorophenyl)-2-methyl-3-nitrobenzamide	290.70174	C14H11ClN2O3	3.6	1	3
803490	N-(3-methoxyphenyl)-2-methyl-3-nitrobenzamide	286.28266	C15H14N2O4	2.7	1	4
796554	2-methyl-N-(3-methylphenyl)-3-nitrobenzamide	270.28326	C15H14N2O3	3.3	1	3
874186	2-methyl-3-nitro-N-[4-(trifluoromethoxy)phenyl]benza mide	340.25405	C15H11F3N2 O4	4.1	1	7
843100	1,3-benzodioxol-5-amine	312.363	C18H20N2O3	4.5	1	3
878054	N-[4-(dimethylamino)phenyl]-2-methyl-3-nitrobenzamide	299.32448	C16H17N3O3	3.1	1	4
878125	N-(3-chloro-4-methoxyphenyl)-2-methyl-3-nitrobenzamide	320.72772	C15H13ClN2 O4	3.5	1	4
779960	2-methyl-N-(4-methylphenyl)-3-nitrobenzamide	270.28326	C15H14N2O3	3.3	1	3
779633	N-(3-chloro-2-methylphenyl)-2-methyl-3-nitrobenzamide	304.72832	C15H13ClN2 O3	3.9	1	3
779694	2-methyl-N-(2-methylphenyl)-3-nitrobenzamide	270.28326	C15H14N2O3	3.3	1	3
779768	N-(2,3-dimethylphenyl)-2-methyl-3-nitrobenzamide	284.30984	C16H16N2O3	3.7	1	3
779820	N-(2-ethyl-6-methylphenyl)-2-methyl-3-nitrobenzamide	298.33642	C17H18N2O3	4.1	1	3
761421	N-(2-methoxyphenyl)-2-methyl-3-nitrobenzamide	286.28266	C15H14N2O4	2.9	1	4
867491	N-(4-bromo-3-methylphenyl)-2-methyl-3-nitrobenzamide	349.17932	C15H13BrN2O3	4.0	1	3
870712	N-(3-hydroxyphenyl)-2-methyl-3-nitrobenzamide	272.25608	C14H12N2O4	2.6	2	4
743451	N-(3-acetamidophenyl)-2-methyl-3-nitrobenzamide	313.308	C16H15N3O4	2.1	2	4
755803	N-(4-chloro-2-methylphenyl)-2-methyl-3-nitrobenzamide	304.72832	C15H13ClN2O3	3.9	1	3

numbers of active torsions were given to the lead compounds using AutoDock tool, Kollman charges and the salvation term were then added to the protein structure using the same. The spacing parameters of grid points were adjusted to cover the entire active site residues of the *Pf*DHODH and the default value 0.375Å was set between grid points. The Lamarckian genetic algorithm was implemented and docking parameters were set as follows: 30 docking trials, population size of 150, maximum number of energy evaluation ranges of 25,0000, maximum number of generations is 27,000, mutation rate of 0.02, cross-over rate of 0.8, while Other docking parameters were set to the software's default values.

RESULTS AND DISCUSSION

The retrieved crystal structure of *Plasmodium falciparum* dihydroorotate dehydrogenase with a bound inhibitor (PDB id: 1TV5)[13] from PDB was modification using chimera tool and the above modified molecule was docked using Autodock software tool to study its interaction with ligands. The docked molecule was further analyzed through Python Molecular Viewer[14].

Molecular docking: The docking results of 25 screened compounds with *Pf*DHODH are shown in Table 2 After screening the results based on docking energy, it was predicted that the compound CID 867491 has least docking energy (-4.82 Kcal Mol^{-1}) among the 25 docked compounds, which inhibits *Pf*DHODH.

Results of hydrogen bond details: Details of hydrogen bond formation between each compound and *Pf*DHODH with atoms involved and their respective bond lengths are shown in Table 3.

Screening and molecular docking studies of benzamide analogues followed by hydrogen bonding formation suggested compound CID 867491 (N-(4-bromo-3-methylphenyl)-2-methyl-3-nitrobenzamide) as a potent compound for targeting *Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH). The novel findings based on an *in silico* approach may be significant for potent drug design against malaria.

The *Pf*DHODH is a protein drug target for drug discovery to combat malaria[15]. Flexible Molecular docking of ligands from chemical database to receptor target is an emerging approach and is widely used in drug discovery to reduce cost as well as time pertaining to wet laboratory experiments[16].

Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum

Table 2: Docking results of benzamide derivatives against *Pf*DHODH

CID No.	Binding energy	Intermol energy	Torsional energy	Internal energy	Docking energy
	(Kcal mol^{-1})				
798434	-3.6	-6.09	2.49	3.62	-2.47
878248	-3.35	-4.91	1.56	0.79	-4.11
878150	-2.84	-4.7	1.87	3.92	-0.78
878176	-3.31	-5.18	1.87	2.88	-2.3
870944	-4.03	-5.59	1.56	2.26	-3.33
596245	-1.17	-2.41	1.25	1.01	-1.41
796489	-4.35	-5.59	1.25	0.83	-4.76
796617	-3.45	-4.7	1.25	0.77	-3.93
803481	-3.36	-4.61	1.25	1.00	-3.61
803490	-3.18	-4.74	1.56	2.40	-2.34
796554	-3.32	-4.57	1.25	0.92	-3.65
874186	-2.77	-4.64	1.87	1.18	-3.46
843100	-4.53	-6.4	1.87	3.71	-2.68
878054	-3.49	-5.05	1.56	1.12	-3.93
878125	-3.85	-5.41	1.56	1.17	-4.23
779960	-3.49	-4.74	1.25	1.67	-3.07
779633	-3.84	-5.08	1.25	0.91	-4.18
779694	-3.53	-4.77	1.25	0.82	-3.95
779768	-3.96	-5.21	1.25	0.82	-4.38
779820	-4.52	-6.08	1.56	3.83	-2.25
761421	-3.44	-4.99	1.56	3.84	-1.15
867491	-4.43	-5.68	1.25	0.85	-4.82
870712	-4.03	-5.27	1.25	2.18	-3.09
743451	-1.84	-3.71	1.87	2.38	-1.34
755803	-4.11	-5.36	1.25	0.77	-4.59

Table 3: Hydrogen bonds and amino acid position of 25 benzamide derivatives *Pf*DHODH

Compound CID No.	Amino acid with position	Atom in amino acid	Atom in compound	Hydrogen bond length (Å)
798434	HIS185	N	O	2.788
878248	HIS185	N	O	2.983
878150	TYR528	H	O	2.532
878176	HIS185	N	O	2.755
870944	VAL532	N	O	2.925
596245	HIS185	N	O	2.874
796489	HIS185	N	O	2.998
796617	HIS185	N	O	2.823
803481	HIS185	N	O	2.929
803490	HIS185	N	O	2.903
796554	HIS185	N	O	2.837
874186	HIS185	N	O	2.858
843100	HIS185	N	O	2.79
878054	HIS185	N	O	2.774
878125	HIS185	N	O	2.886
779960	MET536	N	O	2.756
779633	HIS185	N	O	2.887
779694	HIS185	N	O	2.9
779768	HIS185	N	O	2.805
779820	HIS185	N	O	2.971
761421	HIS185	N	O	2.789
867491	HIS185	N	O	2.967
870712	ARG265	NH	O	2.68
743451	TYR528	H	OH	1.721
755803	HIS185	N	O	2.931

energy[17]. Auto Dock enables us to understand these molecular interactions between a ligand and corresponding protein in terms of binding and docking energy values, the lowest docking value is used to identify a possible drug candidate against target protein[18]. Therefore, optimal interactions and the best autodock score were used as criteria to interpret the best conformation, generated by AutoDock program[19]. The present study predicted the compound CID 867491 with least docking energy (-4.82 Kcal Mol^{-1}) which inhibits *Pf*DHODH (Fig. 1).

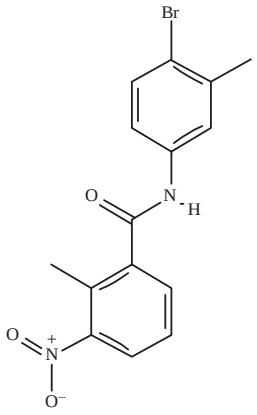

Fig. 1: Chemical structure of the best compound CID 867491

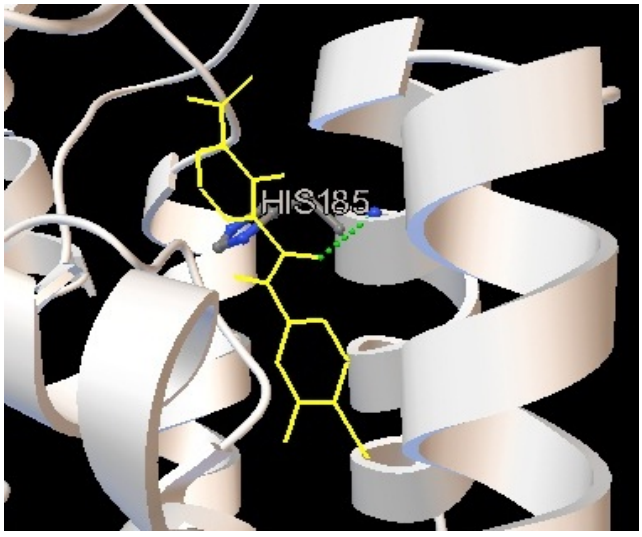

Fig. 2: Docked complex of *Plasmodium falciparum* dihydroorotate dehydrogenase with compound CID 867491. One H-bond was formed between amino acid HIS185 and compound CID 867491. Compound is represented by yellow lines and amino acid as sticks and ball. Hydrogen bond is represented by green dotted spheres

Hydrogen bonding plays an important role in the inhibition of a complex molecule by providing structural and functional stability[20]. The details of atoms in the formation of hydrogen bonds with the bond lengths may provide useful information for in-depth understanding binding mechanism of the compound to the active site of the protein[21]. The Compound having CID: 867491 was found to have hydrogen bond formation with HIS185 residue.

A close view of the Docked complex of *Plasmodium falciparum* dihydroorotate dehydrogenase with compound CID 867491 was analyzed through Python Molecular Viewer shown in Fig. 2. Compound is represented by yellow colour lines, amino acid as sticks in and ball in blue colour while hydrogen bond is represented by green colour dots.

On the basis of present study the molecule CID 867491 (N-(4-bromo-3-methylphenyl)-2-methyl-3-nitrobenzamide) was found to have lowest docking energy (docking energy = -4.82 Kcal mol^{-1}) and hydrogen bond formation of compound CID 867491 with active site residues HIS 185 of *Pf*DHODH, these *in silico* findings validates the structural and functional stability of ligand and receptor protein complex and suggested CID 867491 molecule to be a suitable antimalarial drug candidate.

Data obtained from the present study provide new insights into the identification and validation for a new specific antimalarial drug candidate. However, it is required to test and validate the identified target in relevant wet (Biochemistry and Molecular Biology) laboratories prior to be successfully brought into practice in view as an active antimalarial drug molecule.

CONCLUSION

The *Plasmodium falciparum* dihydroorotate dehydrogenase is a drug targeting protein for the drug discovery to combat against malaria. Auto-Dock is a popular non-commercial docking program and an emerging approach widely used in drug discovery for docking ligand from chemical database with target protein which helps in reducing the cost and time for drug discovery process which otherwise takes many years. Information acquired from the present *in silico* study give new bits of knowledge for the identification and validation of a new inhibitor against a specific drug target. However, other preclinical , *in vitro* and *in vivo* testings, with other important relevant wet laboratories experiments are needed to be performed to test and validate the computationally identified molecule prior, to be successfully brought into practice in view as an active antimalarial drug agent.

SIGNIFICANCE STATEMENTS

Drug design and development is not only a costly procedure but also time-consuming. Therefore, computational approaches and methodologies can be of significance for pharmacophore generation in a drug-discovery procedure. The present study revealed potent antimalarial drug candidate employing computational tools. Besides, this molecule can be

validated and tested in wet labs prior to be successfully brought into practice in view of active antimalarial drug compound application and *in vivo* drug trials on mammalian system followed by approaching the relevant drug for human system.

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