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Research Article

IN SILICO STUDIES ON *NEISSERIA MENINGITIDIS* DIHYDROPTEROATE SYNTHASE AND PTERIN BASED INHIBITORS FOR MENINGITIS

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ABSTRACT

Meningitis, one of the dreadful disease of human which cause inflammation and swelling in the lining of brain and spinal cord. The infection caused by various routes like viral, bacterial and fungal. Among the microbes, the bacterial meningitis was primarily caused by the bacterium *Neisseria menigitidis*. Dihydropteroate synthase (DHPS) was assumed to be a novel drug target for the disease Meningitis based on the metabolic role in the bacterium. The pterin site was targeted for dihydropteroate synthase inhibition with pterin based inhibitors. The molecular docking analysis revealed the effective inhibition of *Neisseria menigitidis* dihydropteroate synthase with pterin based inhibitors. Our study constitutes a step towards anti-pterin pocket class of inhibition for dihydropteroate synthase which assumed to be therapeutic candidates for bacterial Meningitis treatment.

Keywords: Meningitis, *Neisseria meningitidis,* Dihydropteroate synthase, Pterin, Homology modeling, Docking.

INTRODUCTION

Meningitis, a disease mainly characterized by an inflammation of the meninges layer and caused by viral or bacterial infection[1]. Bacterial meningitis was characterized by life-threatening infection. The meningitis mortality rates around 20% to 25% for the bacterial infections[2]. The bacterial meningitis key infection mode was recruitment of highly activated leukocytes into the cerebrospinal fluid[3]. The bacterial meningitis with the symptoms of Seizures with fever was common type in childhood.

The recent report suggested that only 0.4–1.2% of children who have a seizure with fever will have unexpected acute bacterial meningitis (ABM)[4]. In case of potential bacterial inhabitants of the mucosa, the class of bacteria includes *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis*[5]. *Neisseria meningitidis* the potential cause of bacterial meningitis common in Northern Europe and the US. The infection primarily targeted the host inflammatory response and affect variety of detrimental pathophysiological changes in the brain, which includes increased blood–brain barrier permeability, increased CSF outflow resistance[6]. The bacterium was classified in to various serogroups based on capsular polysaccharide which the outermost structure on the meningococcal surface. Among the 12 serogroups identified on the basis of antigenic variation of the capsule, (A, B, C, W135, and Y) were more pathogenic of the invasive disease throughout world and considered to be an epidemic disease[7, 10].

The enzyme dihydropteroate synthase involved in the bacterial metabolism and play a critical role in the folate pathway, a novel drug target in the drug design of various bacterial infections, but it is not expressed in most eukaryotes including humans. Drug target dihydropteroate synthase (EC 2.5.1.15) catalyses the condensation of 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate to para-aminobenzoic acid to form 7,8-dihydropteroate[11].The crystallographic structure resolved in many bacterial species, including *Escherichia coli, Staphylococcus aureus* and *Mycobacterium tuberculosis*[8]. The enzyme has two substrates of a pteridine derivative and pABA. The pterin-binding pocket has been visualized in all the available crystal structures of DHPS and shown to be highly conserved. The pocket is located within the TIM barrel, directly below two flexible loops (loop1 and loop2) that are known to contain important elements of the active site, and is bounded by several key conserved residues that recognize the pterin-pyrophosphate substrate. The inhibitors of the constrained pterin binding pocket would be predicted to have a broad spectrum of activity against both Gram-positive and Gram negative bacteria, and also be less able to tolerate resistance mutations[9].

MATERIALS & METHODS

Construction of 3D model and validation

For the drug target enzyme *Neisseria meningitidis* dihydropteroate synthase, the experimental 3D structure was not available. The homology modeling methods[15] was implemented for 3D structure prediction for the drug target *Neisseria meningitidis* dihydropteroate synthase. The protein sequence of *Neisseria meningitidis* serogroup – A dihydropteroate synthase (283 amino acids) was retrieved from SWISSPROT database (Accession No: Q9JT70). NCBI BLAST[16] (www.ncbi.nlm.nih.gov/blast/) tool was used to identify the template for 3D structural modeling. The results showed dihydropteroate synthase from *Thermus thermophilus* Hb8(PDB ID: 2DQW) with a resolution of 1.65 Å had sequence similarity of 49% with target sequence. The E- value of 8e-62 revealed 2DQW was a suitable template for the in silico 3D structural modeling. Modeller9v10[17] was used to perform 3D structure prediction by homology modeling methods. The script files "align2d.py" and "model-default.py" has been used, based on the "mol pdf" values, the best model was determined. The modeled structure of the *Neisseria meningitidis* dihydropteroate synthase was visualized in Pymol[18] to identify the structure features.

The modeled target structure of dihydropteroate synthase was evaluated by PROCHECK[19] (http://nihserver.mbi.ucla.edu/SAVES/) using the Phi/Psi conformation in Ramachandran plot. The PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analysing its overall and residue-by-residue geometry. The modeled target structure showed significant sterochemical quality in Ramachandran plot. The 3D structural superimposition of the template dihydropteroate synthase from *Thermus thermophilus* Hb8 (2DQW) and modeled *Neisseria meningitidis* dihydropteroate synthase was performed in SPDBV[20] based on Cα trace. RMS was calculated to evaluate the modeling based on checking fit between two molecules. RMS value of 0.55Å justified the perfect modeling through structural similarity. The quality of model was further validated by ProSA[21] which reveals crossly misfolded structures in PDB file (https://prosa.services.came.sbg.ac.at/prosa.php). The results showed *Neisseria meningitidis* dihydropteroate synthase folded correctly based on the Z score -5.9.

Conformation of binding by molecular docking

Molecular docking analysis was carried out by predicting active sites for the target protein and selection of ligands. The possible active sites of target were searched using QSiteFinder[22] (www.modelling.leeds.ac.uk/qsitefinder/). Ten binding sites were obtained and the best site was selected based on the algorithm of pocket and ligand binding site. The pterin based inhibitors[12] were

selected and assumed as ligands for the drug target dihydropteroate synthase were namely compound 1: (2-amino-6-(methylamino)-5-nitropyrimidin-4(3H)-one) and compound 2: 2-amino-6-(methylamino)-5-nitroso-1H-pyrimidin-4-one, analog of compound 1 were retrieved from pubchem database[23] (http://pubchem.ncbi.nlm.nih.gov/) with Lipinski's rule of five properties[24] based on the previous studies on enzyme dihydropteroate synthase[25]. The ligand structure 2D and 3D drawing was done in ACD-Chemsketch[26]. The OPEN BABEL[27] (www.vcclab.org/lab/babel/start.html) was used to convert mol format to PDB format. The molecular docking studies for drug target *Neisseria meningitidis* dihydropteroate synthase with two ligands was performed based on flexible docking in Autodock[28].

RESULTS & DISCUSSION

In homology modeling, the structure of unknown protein is solved based on the structure of known, structurally related, template proteins. From the BLAST against PDB alignment, the template was selected based on identity, score and E-value. The template for the homology modeling of Neisseria *meningitidis* dihydropteroate synthase was 2DQW. The 3D structure of dihydropteroate synthase showed the protein was under classification of classic beta/alpha which contains 8 helices, 8 strands and 28 turns. The primary secondary structure of alpha helices showed in red color and bets sheets showed in yellow color. The secondary structure elements loops and turns showed in green color.

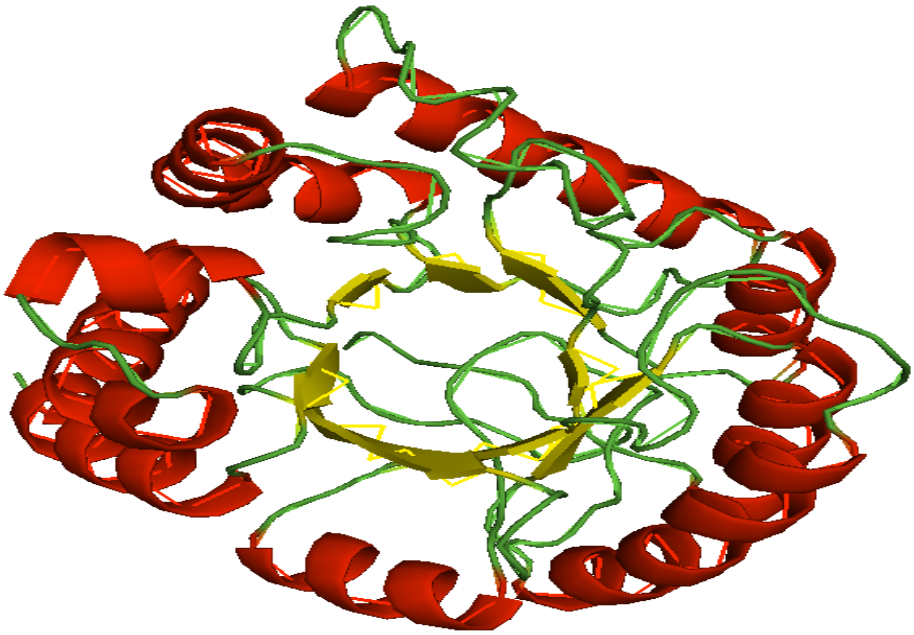
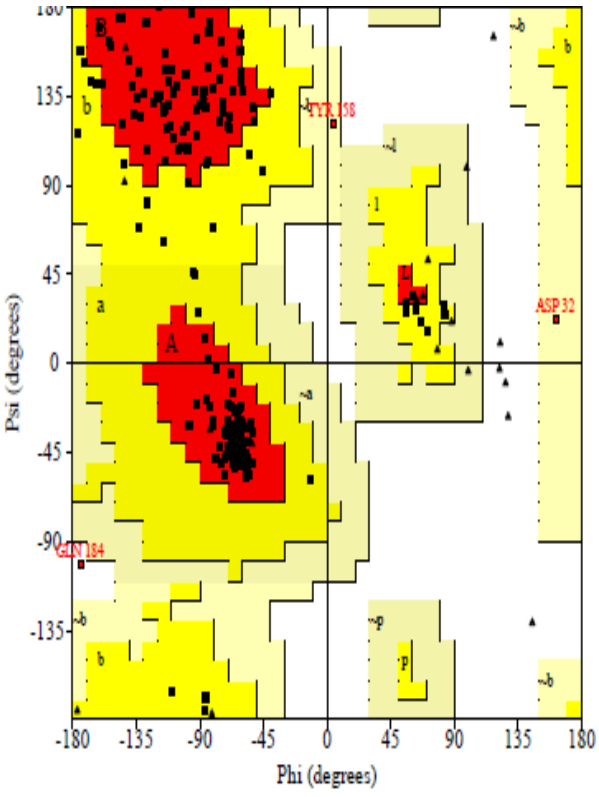
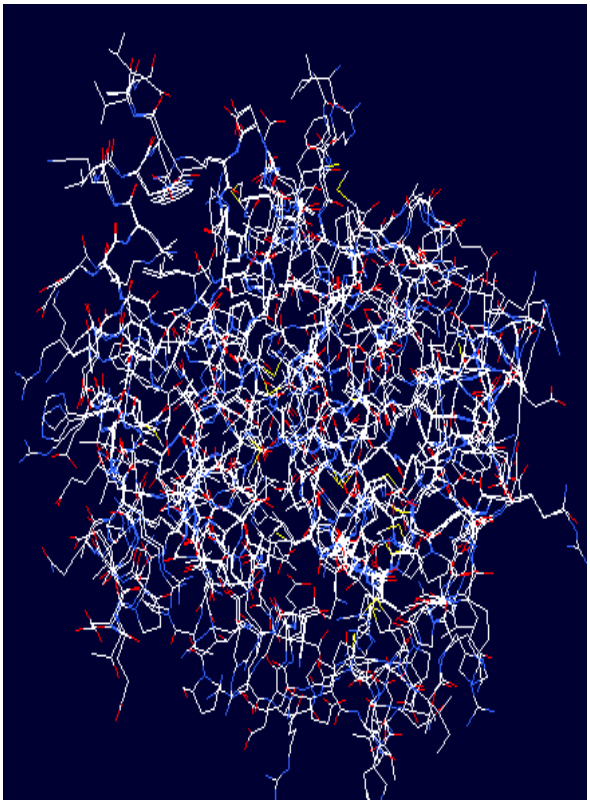


Fig. 1: **Modeled 3D structure of *Neisseria meningitidis* dihydropteroate synthase visualized in Pymol.**





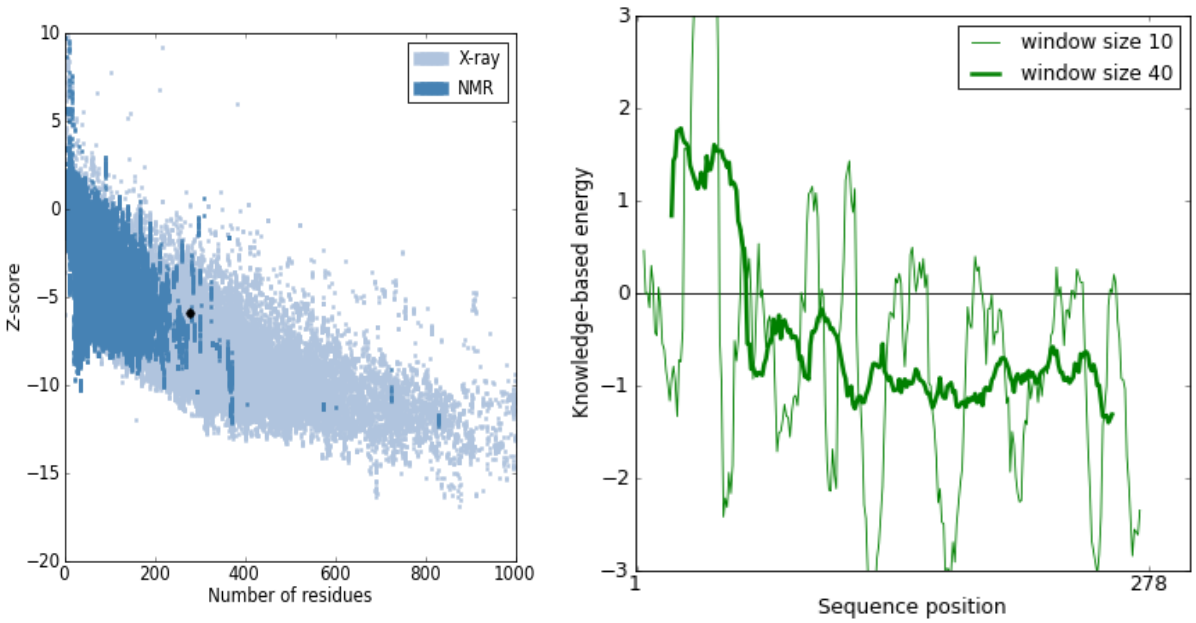
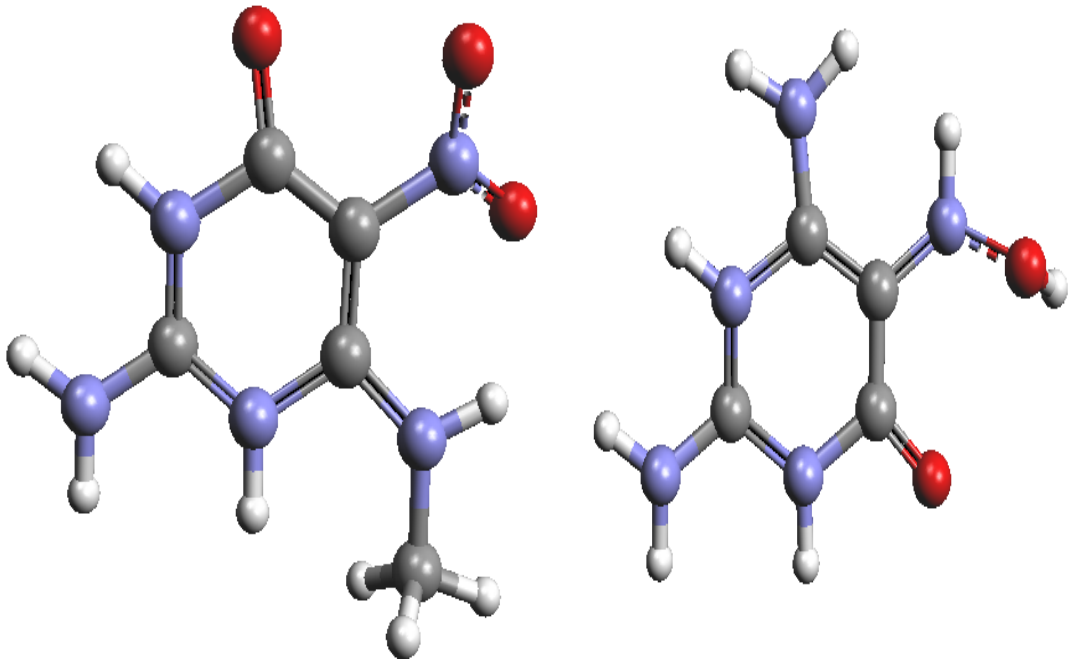


Fig. 2: a) Ramachandran Plot by PROCHECK server b) Superimposed view of target and template in SPDBV c) Protein quality check analysis in ProSA

The 3D structural evaluation was done in PROCHECK by constructing Ramachandran plot. The protein *Neisseria meningitidis* dihydropteroate synthase which modeled by Modeller9V10 was evaluated which contains core region of Ramachandran plot: 89.5% core, 9.3% additional allowed, 0.8% generously allowed and 0.4% disallowed regions. The superimposition was done in SPDBV by superimpose target *Neisseria meningitidis* dihydropteroate synthase and template 2DQW and calculate RMS for the fit. The RMS calculation of target and template showed 0.55 Å which is less than 1Å confirms the modeled structure was good. The results of ProSA showed the Z score -5.9 for the overall quality and modeling near to X –ray methods. The local quality of the modeled protein showed good with range 0- 1.5 of knowledge based energy.

The active sites of the modeled drug target *Neisseria meningitidis* dihydropteroate synthase was predicted by using Q-sitefinder and active sites were analysed. The sites included ILE22, ASP59, ASP98, ASN118, ASP119, VAL120, ALA121, MET143, MET145, ASP189, GLY191, PHE192, PHE194, ILE221, GLY222, VAL223, SER224, LYS226, ARG261, VAL262, and HIS263. The 2D and 3D drawing for pterin based inhibitors compound 1 and compound 2 was done by using ACD- Chemsketch. The file conversion was done in OPEN BABEL. The carbon atom showed in grey color, nitrogen atom in blue color, oxygen atom in red color and hydrogen atoms in white color.



Compound 1 **Compound 2**

Fig. 3: Ligand 3D structure of pterin based inhibitors

Table 1: Lipinski's rule of 5 for ligands

S. No	Ligand	Molecular Weight[g/mol]	XLogP3-AA	H-Bond Donor	H-Bond Acceptor
1	Compound 1 (CID 448810)	185.14078	-0.5	3	4
2	Compound 2 (CID 298670)	169.14138	-1	3	4

The ligands compound 1 and 2 used for the in silico studies followed the rules of Lipinski's rule of 5. The H - bond donor and H – bond acceptor favored the binding with the inhibitor molecules[13]. Molecular docking, a method of predicting the orientation of one molecule to a second molecule when docked. The orientation was the justification parameter used to predict the strength of binding affinity of two docked molecules. The binding orientation of small molecules to the drug targets leads to a drug design[14].

The autodock program followed the parameter setup and prepares the macromolecules by adding essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of autodock tools. The autodock tools assigns atomic solvation parameters based on atomic occupancies for use in protein simulations. The grid map was constructed for the affinity analysis with grid maps of 63· 41·55 grid points and 0.375 Å spacing were generated using the autogrid program, and maps were centered on the protein center. The searching calculation was done in the autogrid step. For the scoring calculation, docking simulations were performed using the Lamarckian genetic algorithm (LGA). Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 2,500,000 energy evaluations. The population size was set to 200. Based on the least binding free energy of 10 different conformations, the best conformation was selected.

Docking between compound 1 with dihydropteroate synthase

Compound 1 docked with the drug target *Neisseria meningitidis* dihydropteroate synthase, conformational pose 9 was best with lowest binding energy of -5.39 Kcal/mol. Compound 1 showed six H bond interaction with active site residues ASN24, ASP59 and ASP98. The H bond interactions showed in dashed yellow lines between the ligand atoms and drug target residue atoms confirmed the inhibition of *Neisseria meningitidis* dihydropteroate synthase.

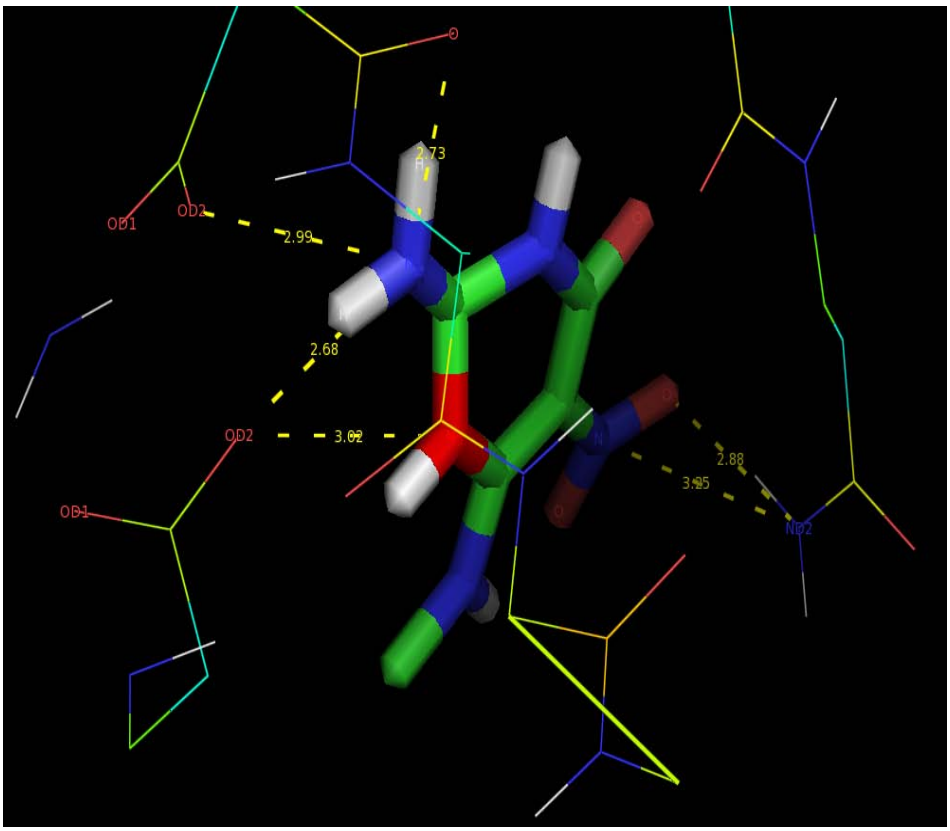


Fig. 4: **Docked pose of compound 1 with dihydropteroate synthase**

Table 2: H-bond interaction of compound 1 with dihydropteorate synthase

S. No	Atom in ligand	Atom in protein	H-Bond Distance (Å)
1	H	ASP59(O)	2.73
2	N	ASP59(OD2)	2.99
3	H	ASP98(OD2)	2.68
4	N	ASP98(OD2)	3.02
5	N	ASN24(ND2)	3.25
6	O	ASN24(ND2)	2.88

Docking between compound 2 with dihydropteroate synthase

Compound 2 docked with the drug target *Neisseria meningitidis* dihydropteroate synthase , conformational pose 1 was best with lowest binding energy of -4.88 Kcal/mol. Compound 2 showed five H bond interaction with active site residues ASN24, ASP59, ASP98 and VAL23.

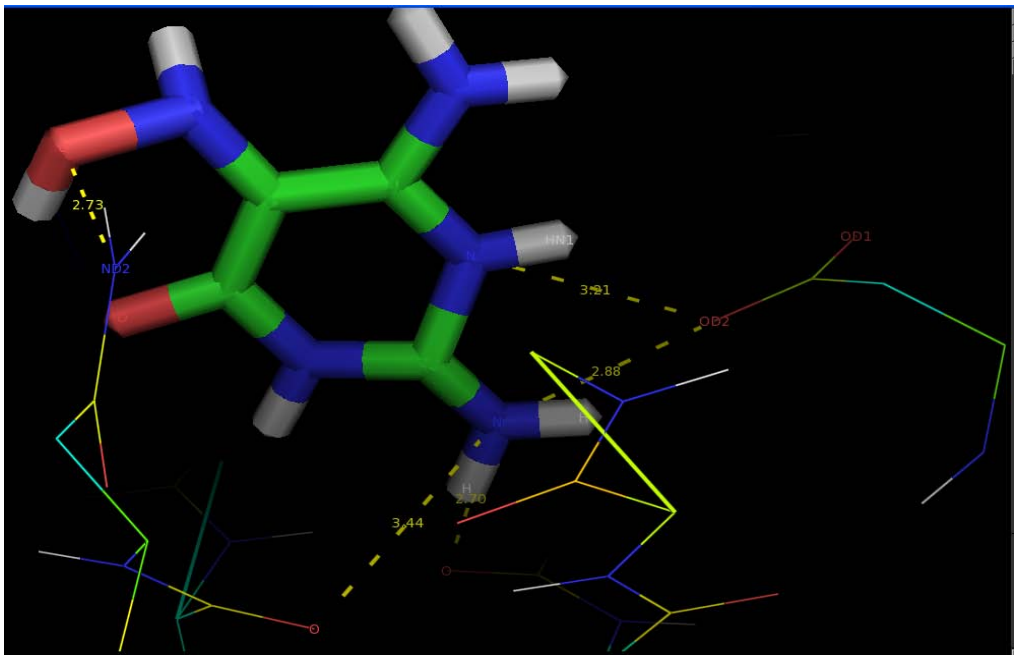


Fig. 5: **Docked pose of compound 2 with dihydropteroate synthase**

Table 3: H-bond interaction of compound 2 with dihydropteorate synthase

S. No	Atom in ligand	Atom in protein	H-Bond Distance (Å)
1	O	ASN24(ND2)	2.73
2	N	ASP98(OD2)	3.21
3	N	ASP98(OD2)	2.88
4	H	ASP59(O)	2.70
5	H	VAL23(O)	3.44

CONCLUSION

In our present study on *Neisseria meningitidis* dihydropteroate synthase, the homology modeling and molecular docking revealed the binding affinity of compound 1 and 2. The analysis evaluated by binding free energy calculations for the ligands. The investigation on potential binding modes of compound 1 and compound 2 showed significant interactions with the active sites of the drug target *Neisseria meningitidis* dihydropteroate synthase to confirm the inhibitory activity. The results obtained by our study may be valuable for future computational drug design of potent inhibitors for *Neisseria meningitidis* dihydropteroate synthase as promising anti-meningitis therapeutics.

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