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Comparative modeling of *Plasmodium falciparam* Dihydropteroate Synthase 2 and docking study against compounds from anti-malarial plant *Carcia papaya* and *Swertia chirata*

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ABSTRACT

Malaria parasite *Plasmodium falciarum* are becoming drug resistant and the present drugs are becoming ineffective towards the disease. The *Plasmodium falciparum* causes the most malignant form of malaria highly prevalent to North–East India. In this investigation, a study was conducted to search and predict potent anti-malarial lead compounds from two traditionally important anti-malarial plant, *Carcia papaya* and *Swertia chirata*. Dihydropteroate synthase (DHPS) is an essential enzyme required for the folate biosynthesis in *Plasmodium falciparum*. Till date, three dimensional (3D) structure of DHPS is not yet elucidated and hence, homology model of DHPS was generated and docked with these plant derived compound. The study identified few phytochemical such as Atovquone, Nitroblue tetrazolium, Caffeic Acid and Kaempferol in terms of least docking and optimum ADME scores as a novel DHPS inhibitor.

Keywords: Malaria, *Plasmodium falciparum*, docking, homology modeling, ADME

INTRODUCTION

Malaria continues to cause morbidity and mortality on a large scale in tropical countries. The alarming rate at which the parasite, particularly *Plasmodium falciparum*, has developed resistance to currently used anti-malarial drugs makes it imperative to search for newer, more effective therapeutic agents[1]. Out of the 2.5 million reported cases in the South East Asia, the India alone contributes about 70% of the total cases. Currently, 80.5% of the total population of India lives in malaria risk areas. Of this, 4.2%, 32.5% and 43.8% live in areas of high, moderate and low risk to malaria respectively [2]. Around 3.96% of total population of India suffering from the *P. falciparum* malaria and 20% of death recorded in India. The Dihydropteroate synthase (DHPS) is an essential enzyme required for the biosynthesis of key folate coenzymes in *Plasmodium falciparum*, [3] required during the asexual division of merozoites in the infected RBCs during malarial infection. Inhibitors of DHPS are most often used in combination for a blissful effect and to slow down the development of drug resistance e.g. Fansidar [4]. The *Carcia papaya* and *Swertia chirata* used by traditional healers of the North-eastern region to treat 'recurrent fever''[1]. Herein, we retrieve few compounds of these plants from Pubmed database and performed *in silico* studies in order to develop best possible lead molecules for development of effective drug candidate for malaria.

MATERIALS AND METHODS

Homology Modeling: Modeller 9v8[5] , a comparative modelling software was used for dihydropteroate synthase 3D model generation. In this study, protein sequence having PDB id 1tx2 was chosen as best

template based on sequence identity and coverage from BLASTp result.

Model assessment: The generated model was tested with different methods to find out the errors in the model, disorder regions, and quality of the generated model. Herein, servers and tools such as Procheck [6] , Errat [7] , Verify-3D [8] , Ramachandran plot [9] , Q-Mean [10] , Prosa [11] were used to evaluate and validated the model.

Compound retrieving: A dataset of compounds was prepared by extensive literature study on traditionally important anti-malarial plants *Carcia papaya* and *Swertia chirata* specifically used in North-East India. More than thirty compounds were selected, where some of the available 3D structures were collected from PubChem (www.pubchem.ncbi.nlm.nih.gov) compounds database, and other compounds were sketched in Chem-BioDraw Ultra 12.0 [12].

Physiochemical property calculation and Optimization: Drug design requires some special physiochemical properties are calculate to assure that the compound should be an effective drug. The physiochemical properties calculation of the compounds was performed using Mol-Soft ICM Browser and Chem-Bio office. The PASS (Prediction of Activity Spectra for Substance) software [13] was used to predict the drug-likeness and toxicity properties of the selected compounds and finally the compounds were optimized using MM2 forcefield.

Molecular Docking Studies: The molecular docking study was carried out to find the best binding interaction (hydrogen bonds and hydrophobic interactions) for each plant compounds with active sites of modeled DHPS2 protein. A licensed version of MVD-Molegro Virtual Docker (2010.4.0.2)[14] based on new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm was used for docking studies. The MVD has special features for detecting the cavities in the sense of active sites of the model protein. On the basis of Re-rank score [15] and Mol-Dock score [14] best poses were taken to annotate their H-bond interaction in terms of lowest energy scores.

ADME prediction: ADME (Absorption, Distribution, Metabolism, Excretion) prediction was performed using online sever based program Pre-ADMET [16]. Pre ADME like properties HIA (Human Intestinal Absorption), MDCK (Madin-Darby Canine Kidney) cell permeability, CACO-2 cell permeability, Skin permeability, Blood Brain Barrier permeability and Plasma Protein Binding values were calculated to find out the best possible anti-malarial inhibitors.

RESULTS

Homology modeling: Basic model building step was performed to modeling of DHPS protein using Modeler 9v8 tool. The 1tx2A was chosen as best template protein that having good sequence coverage of 49%, seq. identity of 67%, low E-value and R-factor of 1.8. Five models were predicted for their DOPE Score [17] and GA341 score [18] (table-1). The model DHPS.B99990002.pdb has found least Dope Score of -36663.41016 and chosen as a best predicted model (fig-1).

Model Assessment: The ERRAT results provided the overall quality factor of the generated model is 46.398 (fig. 2). The results from dDFIRE[19] and DFIRE2 [20] calculated the total energy to be (-730.81 and -592.839). VARIFY 3D estimated that the atom no 187 had the highest compatibility of 0.69 with 3D-1D profile score. The PROSA analysis result predicted Z-score for the protein was -5.07 as shown in table-2.

Docking Study: In docking procedure, the potential binding sites were predicted using MVD docking software. In our study, MVD predicted five cavities for model DHPS among which the cavity volume 130.56 Å was selected for docking study. After completion of docking procedure different energy values and H-bond interaction has observed and few top scoring compounds were selected and identified (table- 3).

ADME Analysis: The pre-ADMET server analysis showed that compounds from the *Caria papaya* and *Swertia chirata* have good absorption, permeability etc that essential for pharmacodynamics regulation. The ADME results of compounds are presented in the table-4.

DISCUSSION

Prediction of a drug by wet lab methods like NMR, Mass spectrometry and raw data collection etc are more time consuming process and needs high effort and intensive care. But the introduction of CADD approach makes it very easy in some part by reducing time and cost[21]. As this days the malarial parasites are growing highly drug resistant as a result there needs of new effective anti-malarial drugs to overcome these resistances. In this present work, 3D model for the protein DHPS was generated and dock with the compounds of two traditionally important anti-

malarial plants *Caria papaya* and *Swertia chirata* to available in North East India [1; 22; 23]. The plant compounds were retrieved from pubchem databases. 3D structure of the protein DHPS was generated in Modeller9v8 [24] using the template of 1tx4 (PDB ID) on the basis of optimum query coverage and sequence similarity score (table-1). During model generation, five models were generated having suitable Dope scores as shown in Table-1. The DHPS.B99990002.pdb has been predicted lowest Dope score, and hence it was chosen as best model. Predicted model was validated using Procheck[11], Prosa, Errat, dDfire and QMean [10], and found optimum score as shown in the table-2. The Prochek analysis showed that 85.7% are in favoured region 12.8% in allowed region and only 1.5% in disallowed region. Docking study reveals 10 compounds with good interactions with the receptor and each forming more than five hydrogen bonds (table-3 and figure-2). The docking results were analyzed on the basis of Mol-dock and Re-rank Score. Amarogentin (Re-rank score = -110.792) and Benzyl Glucosinolate (Re-rank score = -78.6031) of *Swertia chirata* and *Carcia papaya are* respectively predicted as best lead molecule for the degradation of DHPS protein (table-3, figure-3 and 4). The prediction for distribution, permeability, excretion of these compounds are study was carried out at Pre-ADMET server and seven compounds namely Amarogentin, Benzyl Glucosinolate , Atovquone, Nitroblue tetrazolium, Caffeic Acid, Kaempferol and p-coumaric acid had showed acceptable range of ADME scores.

CONCLUSION

The study revealed compounds reported from *Carcia papaya* and *Swertia chirata* has affinity to inhibit dihydropteroate synthase of *Plasmodium falciparum*. Therefore, further investigation in this aspect may be carried out to design novel antimalarial candidate drug compound especially Amarogentin, Benzyl Glucosinolate , Atovquone, Nitroblue tetrazolium, Caffeic Acid, Kaempferol and p-coumaric acid as potent DHPS inhibitor.

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Table-1: Dope score of predicted model.

Filename	Molpdf score	DOPE score	GA341 score
DHPS.B99990001.pdb	2745.2971	-35756.546	1.000
DHPS.B99990002.pdb	2755.333	-36663.410	1.000
DHPS.B99990003.pdb	3294.354	-34761.777	1.000
DHPS.B99990004.pdb	2971.378	-35305.375	1.000
DHPS.B99990005.pdb	3297.815	-34790.296	1.000

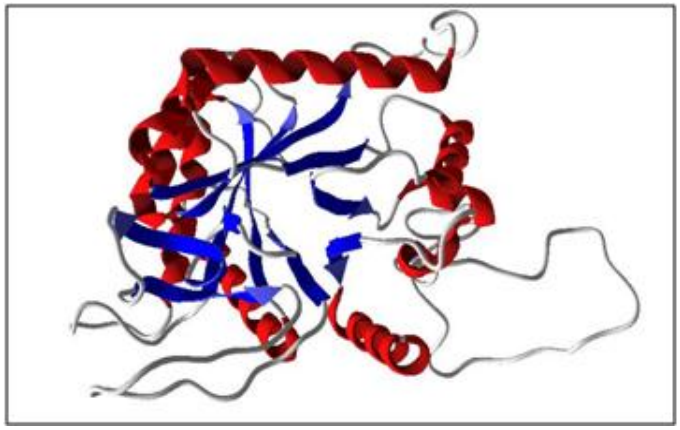

Fig-1: Generated Model of DHPS by MODELER 9v8

Table-2: Model assessment results of the best model.

Errat value	Prosa z-score	dDfire energy	QMEAN score	Z-Score	Rampage result (%)		
					F.R	A.R	D.R
46.39	-5.07	-730.81	0.436	-3.84	85.7	2.8	1.5

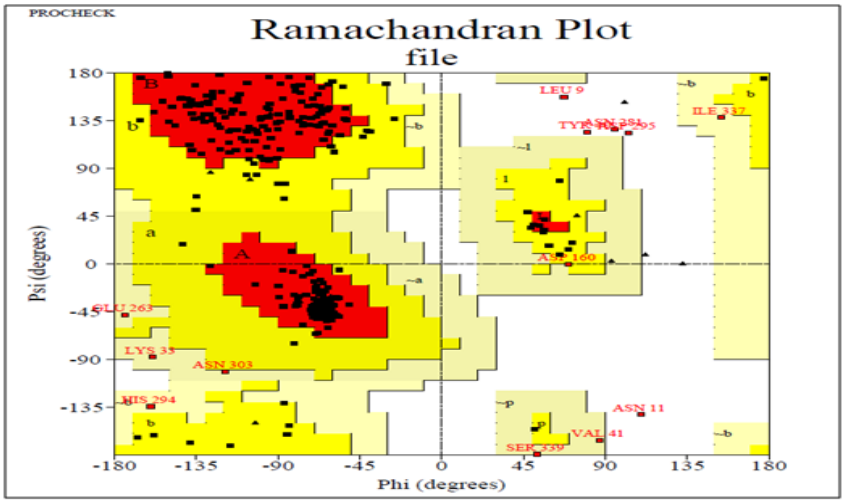

Fig-2: Ramachandran plot for model protein

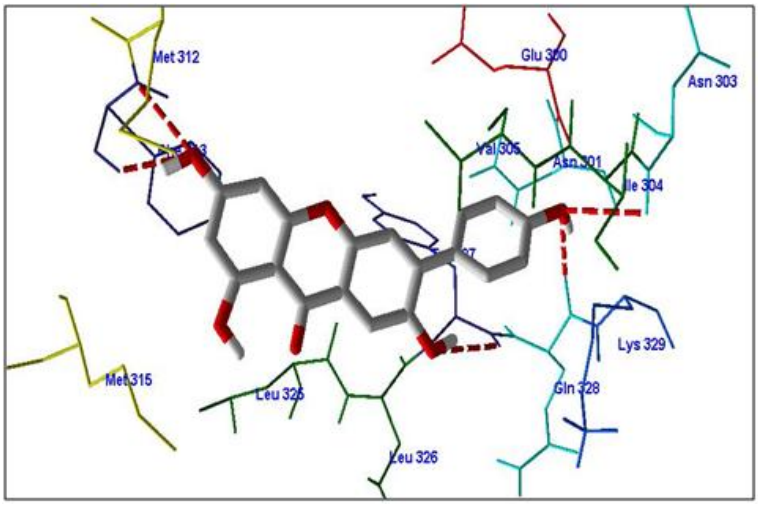

Fig-3: H-bond interaction (Red dotted line) of Kaempferol with model protein.

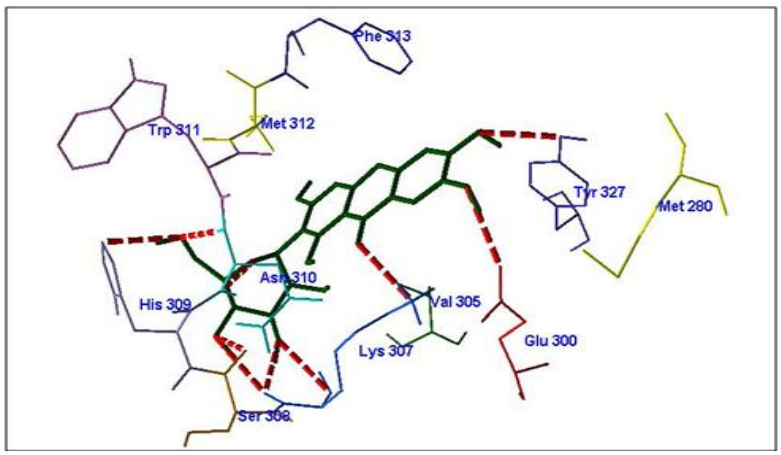

Fig-4: H-bond interaction (Red dotted line) of Mangiferin with annotation.

Table-3: Selected compounds after docking with scores.

Ligands	**Mol-dock Score**	**Re-rank Score**	**H-bond energy**	**H-Bond**
Swertia chirata				
Amarogentin	-137.78	-110.792	-9.72173	9
Amaroswerin	-140.397	-110.809	-9.66261	4
Atovquone	-76.9644	-65.9448	-2.63038	5
Nitroblue tetrazolium	-132.609	-103.921	-6.54185	6
Mangiferin	-69.7826	-70.2931	-13.2641	10
Swertiamarin	-78.9093	-79.3773	-11.2982	6
Carcia papaya				
Benzyl Glucosinolate	-108.393	-78.6031	-6.10615	5
Caffeic Acid	-78.2929	-55.7468	-4.76694	5
Chlorogenic Acid	-117.04	-82.3613	-9.99469	6
Kaempferol	-92.2391	-61.6437	-9.227	7
P-coumaric acid	-71.0436	-46.6267	-6.13287	5
Quercetin	-92.8664	-72.555	-9.30737	6

Table- 4: Table of the compounds that satisfies the ADME property

Compound Name/ ID	HIA (%)	Caco-2 (nm/sec)	MDCK (nm/sec)	Log kp (cm/hour)	PPB(%)	BBB (C.brain/C.blood)
Atovquone	96.013	22.323	10.711	-2.463	99.709	1.935
Nitroblue tetrazolium	99.463	19.403	0.0430	-2.308	100.00	0.946
Caffeic Acid	82.301	21.107	109.433	-2.669	40.290	0.497
Kaempferol	85.667	18.045	2.228	-4.026	100.00	0.511
P-coumaric acid	92.095	21.109	75.059	-1.707	63.055	0.694

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