INTERACTION STUDIES TO EVALUATE PYRIMETHAMINE DERIVATIVES AS INHIBITORS OF *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE

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ABSTRACT

The rapid emergence of *Plasmodium falciparum* malaria resistant to currently available antifolate antimalarial drugs has added a further serious concern, making the provision of malarial treatment increasingly difficult and costly. So, there is an urgent need to search for new antimalarials which are both effective and cheap, to combat the emerging drug resistant parasites. The dihydrofolate reductase (DHFR) domain of *P. falciparum* bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a validated target for antifolate antimalarials. Inhibitors of DHFR disrupted the folate biosynthesis pathway. In this study, Molecular modeling techniques will perform to design pyrimethamine derivatives of *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) and candidates were shortlisted on the basis of docking scores and binding affinity. The docking results of Pyrimethamine and their twenty six derivatives with *Pf*DHFR predicted that compound CID 10476801 had the lowest docking energy (-11.48 kcal/mol) and stabilized by hydrogen bonds and could be a drug candidate, which inhibit PfDHFR structure. Yet pharmacological studies have to confirm it.

Keywords: Pyrimethamine, *Pf*DHFR, Malaria, AutoDock, *Plasmodium falciparum*.

INTRODUCTION

Malaria is a major public health problem all around the world mostly in developing and under developed countries, accounting for sizeable morbidity, mortality and economic loss. Apart from preventive measures, early diagnosis and complete treatment are the important modalities that have been adopted to contain the disease. Four species of *Plasmodium* commonly infects human, but most severe form of the disease is caused by *Plasmodium falciparum*. The World Malaria Report 2011 summarizes that there were 216 million cases of malaria in 2010 in which 81% of these were in the WHO African region. An estimated 655 000 persons died of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African region.

Antifolate antimalarial drugs interfere with folate metabolism, a pathway essential to malaria parasite survival. Unfortunately, the antifolates have proven susceptible to resistance in the malaria parasite. Resistance is caused by point mutations in dihydrofolate reductase, the key enzyme in the folate biosynthetic pathway that is targeted by the antifolates. Disruption of folate synthesis by DHFR inhibitor leads to decreased levels of fully reduced tetrahydrofolate, a necessary cofactor in important one-carbon transfer reactions in the purine, pyrimidine, and amino acid biosynthetic pathways (Ferone, 1977). The lower levels of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methionine synthesis, and lower thymidylate levels with a subsequent arrest of DNA replication (Schellenberg and Coatney, 1961; Gutteridge and Trigg, 1971; Newbold et al., 1982; Gritzmacher and Reese, 1984; Triglia and Cowman, 1999).

In this work, we have computationally design analogues of Pyrimethamine and dock with *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) protein using AutoDock (Morris et al., 1998). Interaction between the *Pf*DHFR protein with Pyrimethamine derivatives were analysed through Python Molecular Viewer software (Sanner, 1999).

MATERIALS AND METHODS

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Receptor x-ray structure

The 3D coordinates of the crystal structure of Quadruple mutant (N51I+C59R+S108N+I164L) *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS) complexed with WR99210, NADPH, and dUMP (PDB id: 1J3K) was retrieved from PDB (http://www.rcsb.org) and taken as the receptor model in flexible docking program.

Active site analysis

The active site residues of Quadruple mutant (N51I+C59R+S108N+I164L) *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS) was taken from the PDBSUM entry of 1J3K having binding site residues ASP54, CYS15, ILE14, LEU164, ASN108, PHE58, PRO113, ILE112 and MET55 for inhibitor WRA(6,6-dimethyl-1-[3-(2,4,5-trichlorophenoxy)propoxy]-1,6-dihydro-1,3,5-triazine-2,4-diamine).

Inhibitors Dataset

26 analogues of Pyrimethamine with experimentally derived quadruple mutant *Pf*DHFR pKi values were obtained from the literature (Kamchonwongpaison et al., 2004). The 3D structures of known inhibitors were downloaded in .sdf format from pubchem compound database. They were later converted in .pdb format by the help of open babel (Boyle et al., 2011) software.

Molecular docking

Docking of 26 analogues of Pyrimethamine screened from literature against *Plasmodium falciparum* DHFR structure was done using molecular docking program AutoDock (Morris et al., 1998). Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool (http://autodock.scripps.edu/resources/adt). Kollman charges and the solvation term were then added to the protein structure using the same. We have made the grid and adjusted the number of points in X, Y, Z-axis so that the entire active site residue (ASP54, CYS15, ILE14, LEU164, ASN108, PHE58, PRO113, ILE112 and MET55) of the DHFR is covered. The Lamarckian genetic algorithm implemented in Autodock was used. Docking parameters were 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, Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock program. Further the best-docked complexes were analyzed through Python Molecular Viewer (Sanner, 1999) software for their interaction studies.

RESULTS AND DISCUSSION

Docking studies predicted the interaction of ligands with protein and residues involved in this complex. For such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best autodock score were used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock program. The docking results of twenty six compounds and one known inhibitor Pyrimethamine with PfDHFR were shown in table 1. Among the above docked compounds CID 10476801 had the lowest docking energy with PfDHFR than other docked compounds. Therefore it was predicted that compound CID 10476801 has lowest docked energy (-11.48 kcal/mol) with protein was a drug candidate, which inhibit *Pf*DHFR structure.

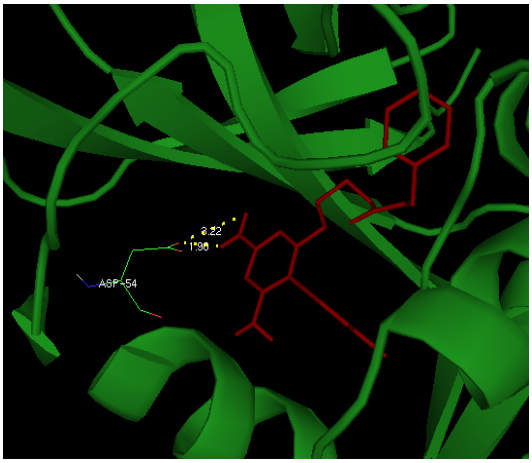

Figure 3.4: Docked complex of *Plasmodium falciparum* dihydrofolate reductase with compound (CID 10476801). Two H-bonds are formed between amino acid ASP54 (O) and compound CID: 10476801 (H).Hydrogen bond is represented by dotted yellow line.

Docking pose of the best conformation of compound CID 10476801 in the binding site of PfDHFR protein was shown in figure 1. Residues of PfDHFR protein involved in the formation of hydrogen bonds with compound CID 10476801 is ASP54. Hydrogen bonding plays an important role for the structure and function of biological molecules, especially for inhibition in a complex.

Table 1: The docking results of the twenty seven compounds with *Pf*DHFR.

Sl. No.	CID No.	Binding Energy (Kcal/mol)	Intermol Energy (Kcal/mol)	Torsional Energy (Kcal/mol)	Internal Energy (Kcal/mol)	Docking Energy (Kcal/mol)
1.	25099455	-8.84	-9.15	0.31	0.2	-8.96
2.	23423608	-7.38	-8.32	0.93	-0.31	-8.63
3.	10090457	-9.5	-11.05	1.56	-0.07	-11.12
4.	9974420	-9.28	-10.52	1.25	-0.71	-11.23
5.	10018953	-7.5	-9.37	1.87	-0.17	-9.54
6.	23423607	-8.71	-9.33	0.62	-0.2	-9.52
7.	11463215	-8.59	-10.46	1.87	-0.65	-11.11
8.	10476801	-9.15	-11.64	2.49	0.17	-11.48
9.	9814965	-9.47	-10.71	1.25	-0.42	-11.13
10.	10902094	-8.78	-9.4	0.62	-0.22	-9.62
11.	11152240	-8.75	-10.62	1.87	-0.44	-11.06
12.	13926968	-8.15	-8.15	0.0	-0.08	-8.23
13.	10266000	-7.05	-8.92	1.87	-0.37	-9.3
14.	11369668	-7.47	-9.03	1.56	-0.14	-9.17
15.	11290186	-6.81	-8.36	1.56	-0.02	-8.38
16.	93114	-7.65	-7.96	0.31	-0.11	-8.07
17.	29142	-8.53	-8.84	0.31	-0.09	-8.93
18.	11369471	-7.61	-8.55	0.93	-0.48	-9.02
19.	11121319	-9.54	-10.78	1.25	-0.55	-11.33
20.	11020649	-7.69	-8.0	0.31	-0.1	-8.1
21.	134626	-8.61	-8.92	0.31	-0.02	-8.94
22.	11369471	-9.0	-9.94	0.93	-0.19	-10.13
23.	10426185	-7.42	-9.29	1.87	-0.29	-9.57
24.	13926986	-8.21	-8.83	0.62	0.15	-8.68
25.	10927461	-6.95	-9.75	2.8	0.29	-9.46
26.	10060600	-8.56	-8.87	0.31	-0.09	-8.96
27.	4993	-8.3	-8.61	0.31	-0.07	-8.68

CONCLUSION

The *Plasmodium falciparum* dihydrofolate reductase is a drug targeting protein for the drug discovery fighting with the malaria. Flexible docking of ligand from chemical database to receptor is an emerging approach and is widely used in drug discovery to reduce the cost and time. Docking study predicted that compound CID 10476801 has lowest docked energy with *Pf*DHFR and interaction is stabilizes by hydrogen bonding.

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