ORIGINAL ARTICLE

In Silico Evaluation of Inhibitory Potential of Sulfonamide Derivatives against Diadenosine Tetraphosphate Hydrolase as Antimalarial Agents

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Abstract

Aims: Malaria remains a worldwide health problem. Resistance to antimalarial drugs by the *Plasmodium falciparum* malaria parasite is posing a serious impediment in malaria control program. Hence continuous efforts on the development of new antimalarial are required. Being an important enzyme in parasite physiology diadenosine tetraphosphate (Ap4A) hydrolase may prove a good target for antimalarial drugs. The aim of this study was to explore the inhibitory potential of some sulfonamide derivatives against malaria parasite Ap4A hydrolase. **Materials and Methods:** The molecular three-dimensional structural data of malaria parasite Ap4A hydrolase were obtained from protein databank (PDB ID: 5CFI) and used as a drug target. The molecular docking approach was employed to find out the *in silico* inhibitory potential of the sulfonamide derivatives against Ap4A hydrolase and their relative stabilities were studied. **Results and Discussion:** All sulfonamide derivatives showed good binding affinity against the target protein. The binding free energy of compound 4-amino-N-(quinoxalin-2yl) benzenesulfonamide S6 (−8.43 Kcal/mole) showed it to be the most optimal sulfonamide derivative as inhibitor for Ap4A hydrolase enzyme. **Conclusions:** Evaluation of binding affinities using free energy simulations allowed establishing that sulfonamides having quinoxaline moiety is the highest quality compound of the series. The findings attained through this study on the molecular interaction mode of sulfonamide derivatives, and Ap4A hydrolase enzyme can be considered for further *in vitro* and *in vivo* validation for designing new potential antimalarial drugs.

Key words: Diadenosine tetraphosphate hydrolase, docking, sulfonamide

INTRODUCTION

Malaria, a most significant parasitic disease of humans, is one of the oldest and largest health challenges. The 2015 World Health Organization world malaria report found 214 million cases of malaria worldwide. This resulted in an estimated 438,000 deaths.[1] Malaria claims the lives of more children worldwide than any other infectious diseases. In India, malaria continues to pose a major public health threat in different parts of the country. According to the Annual Report of National Vector Borne Disease Control Programme, during 2014, 0.85 million cases and 316 deaths have been reported in India.[2] Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium* that is transmitted by *Anopheles* mosquitoes. Of the six malarial parasites, *Plasmodium falciparum* causes the most often fatal and medically severe form of disease. Roughly 50% of all malarial infections are caused by *P. falciparum*. Resistance to antimalarial drugs and insecticides, the decay of public health infrastructure, population movements, political unrest, and environmental changes are contributing to the spread of malaria.[3] Parasite resistance to artemisinin – The core

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compound of the best available antimalarial medicines has been reported.[4,5] The emergence and spread of drug-resistant parasites coupled with the absence of an effective vaccine makes malaria treatment more complicated, and thus the development of new antimalarial drugs is one of the urgent tasks in malaria research.

Diadenosine tetraphosphate (Ap4A) is a key signaling molecule present among eukaryotes, bacteria, archaea and viruses, and is well documented to participate in both intra- and extracellular signaling.[6] Ap4A and Ap5A molecules, chief substrates of Ap4AH, are key mediators of cellular communication and function through purinergic receptors. Hence, signaling mediated by these molecules within red blood cells is of special interest in malaria.[7] Purinergic signaling has been shown to play role in parasite invasion.[8] The malaria parasite enzyme Ap4A hydrolase regulates levels of signaling molecules like Ap4A by hydrolyzing them to adenosine triphosphate and adenosine monophosphate. Ap4A hydrolase in the parasite hints at a special role for this molecule in parasite physiology.[9,10]

Sulfonamides represent an important class of medically important compound, which is present in a number of biologically active molecules. In addition, sulfonamide derivatives are extensively used as antitumor,[11,12] antiviral,[13] antimalarial,[14,15] anti-inflammatory,[16] anticancer,[17] anti-carbonic anhydrase,[18] antidiabetic agents,[19] and in Alzheimer's diseases.[20]

Computer-aided structure-based rational drug designing have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Docking is a method that predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex in three-dimensional (3D) spaces. Knowledge of the preferred orientation, in turn, may be used to predict the strength of association or binding affinity between two molecules.[21-24] The aim of this study is to analyze by docking methods the interaction of 10 sulfonamide derivatives with the malaria parasite Ap4A hydrolase to characterize their potential as antimalarial agents.

MATERIALS AND METHODS

All computational studies were conducted using version 4.2 of AutoDock program suite of software which is used for simulating small molecule and macromolecule systems.[25] It combines a rapid energy evaluation through pre-calculated grids of affinity potentials with a variety of search algorithms to find suitable binding positions for a ligand on a given protein. In AutoDock, the protein is required to be rigid, but the program allows torsion in the ligand. Results differing by <2.0 Å in positional root-mean-square deviation were clustered together and represented the result with the most favorable free energy of binding.

Ligand preparation

The ligands (small molecules) studied are: 4-amino-N-(2-phenylpyrazol-3-yl)benzenesulfonamide (S1), 4-amino-N-(3,4-dimethyl-1,2-oxazol-5-yl)benzenesulfonamide (S2),4-amino-N-(3methoxypyrazin-2-yl)benzenesulfonamide (S3),4-amino-N-(4,6-dimethyl pyrimidin-2yl) benzenesulfonamide (S4), 4-amino-N-(6-chloropyridazin-3-yl)benzenesulfonamide (S5), 4-amino-N-(quinoxalin-2-yl)benzenesulfonamide (S6), 4-amino-N-(oxan-4-yl) benzenesulfonamide(S7),4-amino-N-(5-methyl-4,5-dihydro-1,3-thiazol-2-yl)benzenesulfonamide (S8), 4-amino-N-(5 methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (S9), and 4-amino-N-(furan-2ylmethyl)benzenesulfonamide (S10). Their structures were drawn using Chemsketch and are listed in Table 1.

Preparation of protein molecule

The experimental structure of malaria parasite Ap4A hydrolase protein (PDB ID: 5CFI) as shown in Figure 1 was retrieved from the RCSB protein databank in. pdb file format.

Molecular docking

Molecular docking is a frequently used tool in computer-aided structure-based rational drug design. Docking allows screening a database of compounds and calculating the strongest binders based on various scoring functions. AutoDock Tools are a program package of automated docking tools that is designed to predict how small molecules bind to a target protein of known 3D-structure. Besides generating binding energies in these docking studies, the position of the ligand in the host's binding site can be visualized. It can be useful for developing better drug candidates and also for understanding the nature of the binding. In this study, AutoDock 4.0 package software was used to investigate the affinity of sulfonamide derivatives to the binding pocket of Ap4A hydrolase. The receptor was used as a rigid molecule.

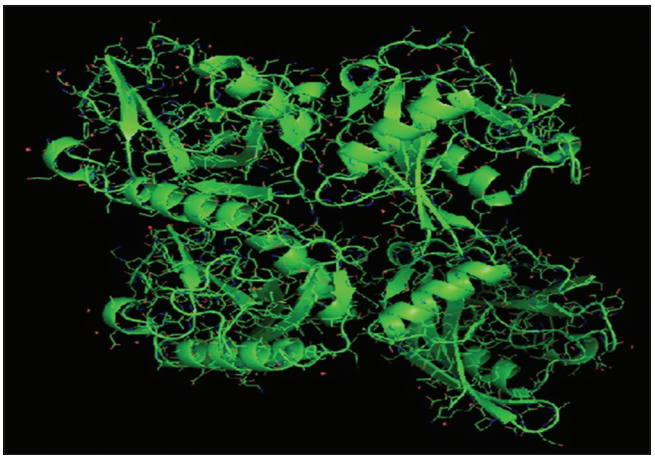
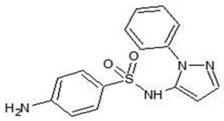
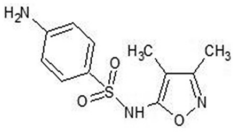
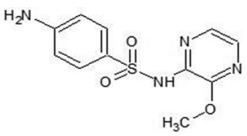
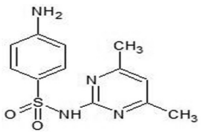
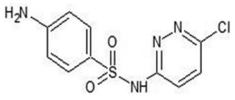
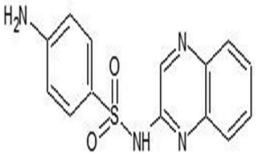
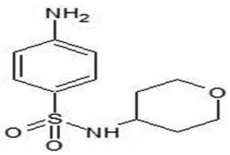
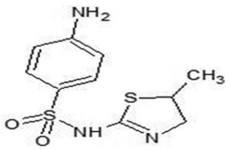
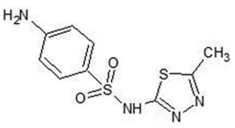
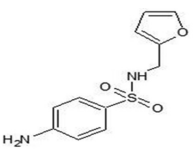


Figure 1: Protein structure 5CFI

Table 1: Chemical structure of synthesized compounds

Compounds	Name	Chemical structure	Molecular formula	Molecular weight (g/mol)
S1	4-amino-N-(2-phenylpyrazol-3-yl) benzenesulfonamide		$C_{15}H_{14}N_4O_2S$	314.36
S2	4-amino-N-(3,4-dimethyl-1,2-oxazol-5-yl) benzenesulfonamide		$C_{11}H_{13}N_3O_3S$	267.30
S3	4-amino-N-(3-methoxypyrazin-2-yl) benzenesulfonamide		$C_{11}H_{12}N_4O_3S$	280.30
S4	4-amino-N-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide		$C_{12}H_{14}N_4O_2S$	278.33
S5	4-amino-N-(6-chloropyridazin-3-yl) benzenesulfonamide		$C_{10}H_9ClN_4O_2S$	284.72
S6	4-amino-N-quinoxalin-2-ylbenzen esulfonamide		$C_{14}H_{12}N_4O_2S$	300.33
S7	4-amino-N-(oxan-4-yl) benzene sulfonamide		$C_{11}H_{16}N_2O_3S$	256.32
S8	4-amino-N-(5-methyl-4,5-dihydro-1, 3-thiazol-2-yl) benzenesulfonamide		$C_{10}H_{13}N_3O_2S_2$	271.35
S9	4-amino-N-(5-methyl-1,3,4-thiadiazol-2-yl) benzenesulfonamide		$C_9H_{10}N_4O_2S_2$	270.33
S10	4-amino-N-(furan-2-ylmethyl) benzenesulfonamide		$C_{11}H_{12}N_2O_3S$	252.28

Water molecules were removed, and hydrogen atoms were added to the protein amino acids. The grid maps representing the protein in the actual docking process were calculated with AutoGrid. The grids were chosen to be sufficiently large to include not only the active site but also significant portions of the surrounding surface. During the docking, the grid dimensions were 100 Å × 100 Å × 100 Å with points separated by 0.375 Å. The X, Y, and Z coordinates were specified as −18.15, 0.356, and 18.844, respectively. Lamarckian Genetic Algorithm was employed as the docking algorithm with 10 runs, 150 population size, 2,500,000 maximum numbers of energy evaluations, and 27,000 maximum numbers of generations.

RESULTS AND DISCUSSION

The interaction of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and also evaluated their binding affinities using free energy simulations.[26-28] A docking method estimates the forces involved in the protein-ligand interactions, *viz.,* electrostatic, Van der Waal's and hydrogen bonding and place the ligands appropriately in the active site. A drug's effectiveness depends on the structural interaction with the receptor or target molecule. The docking simulations in the active sites of 5CFI were performed by the AutoDock program, which has been shown to successfully reproduce experimentally observed binding modes in terms of the lowest docking energy. Docking results show that there is a positive correlation between the binding free energy and the inhibition of malaria parasite Ap4A hydrolase protein receptor. In this study, all the 10 organic compounds were docked with 5CFI malaria parasite Ap4A hydrolase which is an important target of antimalarial drugs. All the compounds gave good docking results. Compound 4-amino-N-(quinoxalin-2-yl)benzenesulfonamide (S6) was found to have the greatest affinity to Ap4A hydrolase having binding energy, i.e., −8.43 Kcal/mole followed by compound 4-amino-N-(6-chloropyridazin-3-yl)benzenesulfonamide (S5), 4-amino-N-(5-methyl 4,5-dihydro-1,3-thiazol-2-yl) benzenesulfonamide (S8), and 4-amino-N-(4,6-dimethyl pyrimidin-2yl)benzenesulfonamide (S4) with binding energy −8.41 Kcal/mole, −7.81 Kcal/mole, and −7.79 Kcal/mole, respectively. This provides the evidence that the ligand molecules have more affinity to the active site of protein and can be used as an efficient and potential inhibitor or drug molecule. Compound 4-amino-N-(3-methoxypyrazin-2-yl) benzenesulfonamide (S3) showed the least affinity to the receptor protein with binding energy −6.95 Kcal/mole. Thus, molecular docking studies showed compound 4-amino-N-quinoxalin-2-yl) benzenesulfonamide (S6) can be considered to be a better inhibitor with stronger activities with malaria parasite Ap4A hydrolase. The best possible binding modes of the compound S6, S5, and S8 at targeted protein's active sites are displayed in Figures 2-4. The binding free energy of the docked compounds is listed in Table 2.

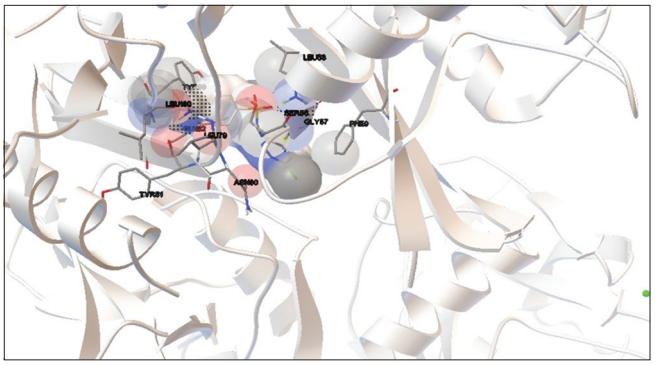


Figure 2: Binding of the high ranking generated conformers for compounds S6 inside the binding pocket of diadenosine tetraphosphate hydrolase

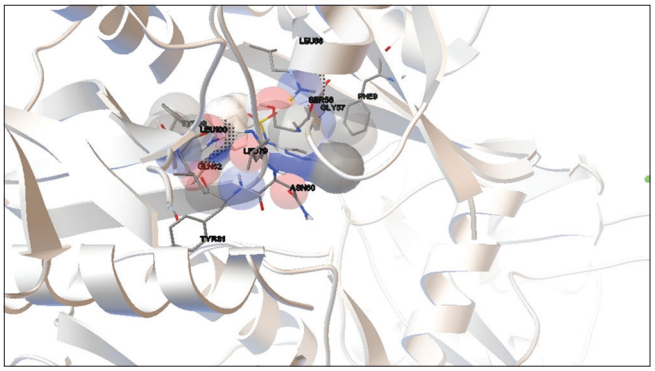


Figure 3: Binding of the high ranking generated conformers for compound S5 inside the binding pocket of diadenosine tetraphosphate hydrolase

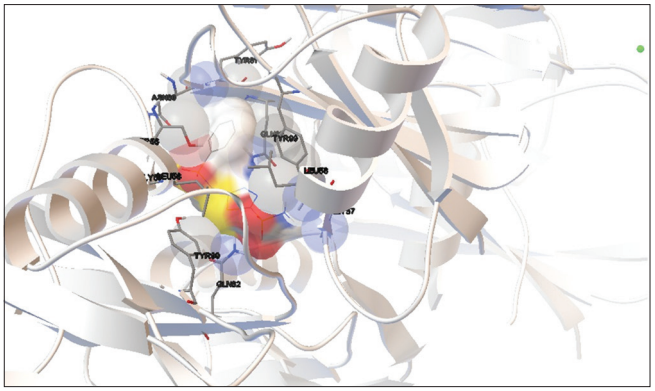


Figure 4: Binding of the high ranking generated conformers for compound S8 inside the binding pocket of diadenosine tetraphosphate hydrolase

CONCLUSION

Molecular docking continues to hold great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. In this investigation, *in silico* docking studies sulfonamide scaffold bearing various heterocyclic rings such as pyrazole, oxazole, pyrazine, pyrimidine, pyridazine, quinoxaline, oxan, thiazole, oxazole and furan as central ring scaffold were performed using the crystal structure of malaria

Table 2: Binding free energy of docked compounds

Compounds	Binding free energy (Kcal/mol)	Inhibition constant Ki (μM)
S1	−7.42	3.64
S2	−7.60	2.66
S3	−6.95	8.1
S4	−7.79	1.96
S5	−8.41	0.679
S6	−8.43	0.656
S7	−7.36	4.01
S8	−7.81	1.89
S9	−7.62	2.62
S10	−7.23	5.01

parasite Ap4A hydrolase protein (5CFI) to recognize the hypothetical binding mode of the 10 sulfonamide derivatives (ligands) with the receptor. The results show that the binding affinity of the sulfonamide derivatives significantly depends on the kind of heterocyclic skeleton. Sulfonamide bearing quinoxaline moiety is shown optimal binding site interaction. The study of dataset allowed establishing that compound 4-amino-N-quinoxalin-2-ylbenzenesulfonamide (S6) is the highest quality compound of the series. The antimalarial activity of these potential candidates may be ascertained by *in vitro* and *in vivo* antimalarial activity study. Further optimization of this lead compound may provide a more potent and selective Ap4A hydrolase inhibitor, potentially with better druggability. The results obtained will be helpful in designing of new series of highly effective and potential drugs for the resistant malaria parasite.

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