Original Article

Drug Repurposing on Adenylosuccinate Synthetase of Plasmodium falciparum – A protein dynamics study

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Abstract

Malaria, a deadly disease in worldwide caused by female mosquitoes bite with the help of plasmodium parasites. In the current study, the major is to gain insights on inhibition of key malaria target *Plasmodium falciparum* adenylosuccinate synthetase (AS) using drug repurposing strategy. Using the experimental 3D structure of AS, rational drug design was performed using specific anti-malarial drugs by computational methods. Two phase virtual screening was carried out in AutoDock Vina and Autodock and top leads quinidine and quinine were screened based on respective binding energy evaluation and weak interactions in protein-lead complex. Molecular dynamics simulations of 10 ns were performed on lead complexes for evaluating the stability and behavior of the drug binding in the dynamic system. Overall, our study will be useful in studying the structural mechanism of adenylosuccinate synthetase inhibition with anti-malarial drugs in future treatment.

Keywords: Adenylosuccinate synthetase, Anti-malaria agents, Drug Repurposing, Molecular docking, Molecular dynamics simulation.

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INTRODUCTION

In Worldwide, malaria is a lethal disease caused by an infected female bite of Anopheles mosquito which carries the protozoan *Plasmodium* parasite. The parasite that can *transmit* malaria include *P. falciparum*, *P. vivax*, *P. ovale and P. malariae*. The major symptoms of malaria are fever, splenomegaly, anaemia, chills, nausea, vomiting and diarrhoea. The key severity is determined by factors like spontaneous bleeding, pulmonary oedema, liver dysfunction, haemoglobinuria, renal failure, respiratory distress, metabolic acidosis, hyper-parasitaemia, hypo-glycaemia and shock [1]. According to the extensive survey conducted on the Global Burden of Death (GBD), malaria is one of the top ten reasons for increased mortality rate [2]. Death due to malaria depends on various factors like vector control, proper medical facilities and the immunity levels of the people in the locality. Humidity and cleanliness are crucial to the transmission rate of the disease, thus countries like India have higher frequency of deaths due to malaria [3].

Malaria caused by *P. falciparum* alone affects 300 million people annually all over the world and 1-2 million of these incidences result in death [4]. Extensive research is being done to identify drugs and drug targets that are ideal for elimination of the disease. Till date, in the treatment of malaria the 5 namely Quinine, Quinoline and Artemisinin derivatives are the most common classes of anti-malarial drugs. But increasing resistance to artemisinin derivatives is a threat. Constant efforts are being made to identify novel targets and drugs in malaria for the successful treatment [5]. The purine salvage pathway is a popular target, as it is the only source of urines for the *Plasmodium* parasite. The critical conversion of adenosine to adenosine monophosphate (AMP) and Guanosine monophosphate (GMP) is involved in purine salvage pathway. As the genes encoding for the purine salvage pathway are absent in the parasite, it obtains adenosine derivative, hypoxanthine from the host and converts it to AMP using Adenylosuccinate synthetase and GMP using GMP synthetase. Adenylosuccinate synthetase also catalyses the reaction involved in hydrolysis of GTP to GDP in the presence of Mg^{2+} as co-factor [4]. On inhibiting Adenylosuccinate synthetase, it is observed that the erythrocytes are incapable of synthesize the purine ring by salvage pathway, resulting in apoptosis [6]. Adenylosuccinate synthetase has been isolated and

characterized from *Escherichia coli, Pyrococcus horikoshii, Cryptococcus neoformans, Dictyostelium discoideum, Saccharomyces cerevisiae* and various other model organisms [7 - 10].

MATERIALS AND METHODS

Dataset

The protein target used in this current computational study was adenylosuccinate synthetase from *Plasmodium falciparum*. The structural 3D model of target was retrieved from RCSB Protein Data Bank with PDB code 1P9B [4]. The protein was a polypeptide (L) chain consisting of 442 residues. The hadacidin (HDA) binding site was targeted and the residues involved were Thr308, Thr309 and Arg311.

Virtual screening

The phase I virtual screening was performed to obtain the top hits from the anti-malarial drugs in AutoDock Vina [11] using Pymol plugin [12]. The protein and ligand input files were converted to PDB format and saved. The parameter of docking was set with grid box volume 27000 Å and grid spacing 0.375 Å provide search space. The grid centre was assigned values x = 24.88, y = 87.67 and z = 29.45 with grid box size x = 60, y = 60 and z = 60 Å. Phase I was performed using molecular docking protocol of 100 runs. Hit compounds were screened using lowest binding affinity expressed in kcal/mol.

Re-docking

For re-docking, the hit compounds screened was docked again in Autodock [13] using Pymol plugin. The grid spacing parameters were similar to Vina docking**.** A default protocol was applied, with population size of 150 randomly placed individuals, a maximum number of 2.5 x 10^5 energy evaluations, and a maximum number of 2.7 x 10^4 generations, gene mutation rate of 0.02 and crossover rate of 0.8 were used. In Autodock, the docking run was set as 100. A hundred runs were performed for each docking state using Genetic Algorithms (GA-LS). The binding energy (kcal/mol), inhibition constant, hydrogen and hydrophobic interactions were assessed to determine the binding affinity of the docked ligands. The results were visualized using Ligplot+ [14] that projects the hydrogen and hydrophobic interactions between the ligand and the protein.

Molecular dynamics (MD) simulations

MD simulations were performed in GROMACS 4.5 [15] for refinement of binding affinity, analysis the stability of the complex and evaluate the conformational changes in target adenylosuccinate synthetase after ligand binding. The best binding conformation of two lead complexes from the AutoDock results were used as input for the MD simulations. The force field GROMOS96 43a1 [16] was used for all simulations and the energy minimization of protein complex was performed with steepest algorithm. Initially, the topology of ligands from the docked complex was generated using PRODRG server [17] and partial charges were added for the ligand preparation. After topology generation, the solvation of complex was performed in a dynamic system with cubic box size 1.0 nm and distance between periodic images with minimum of 2.0 nm. The specific water model spc216 was used for the aqueous environment in the dynamic system. The system was energy minimized by steepest descent minimization with emstep of 0.01, emtol of 1000 and steps 100 ps. The Verlet-leap-frog algorithm was used in numerical integration with a 1.0 fs time step length for minimization and 2.0 fs for dynamics. The neutralization of the system was done by adding eight chlorine ions and periodic boundary conditions were applied in all directions. Protein-ligand complex was well equilibrated by initial simulations in two phases namely NVT and NPT. In case of NVT, the complex was simulated at 300 K and with a coupling constant of 0.1 ps for duration 100 ps using leap-frog integrator. The cutoff range for short electrostatic and van der Waals interactions was set to 14 Å for both. All bond lengths and hydrogen bonds of the protein were constrained by LINCS algorithm [18] and geometry of water molecules were constrained by SETTLE algorithm [19]. After NVT, the complex was equilibrated with constant pressure of 1 bar was employed with a coupling constant of 5 ps with steps 100 ps using leap-frog integrator. Particle Mesh Ewald (PME) for long-range electrostatics interactions was set with order 4 and 0.16 fourier spacing. Finally, the production MD run was performed for duration 10 ns and all MD trajectories were analyzed. g_energy to evaluate the total energy, g_rms to analyze the structural deviation, g_hbond to evaluate inter-hydrogen bond interactions between two groups and g_sas to evaluate the surface area of the protein accessible to solvent. All plots from MD trajectories were plotted using Xmgrace tool.

RESULTS AND DISCUSSION

Virtual screening

In the lifecycle of parasite, purine salvage pathway was extremely important for the survival of the *Plasmodium*. Hence, several enzymes are used as potential drug targets for combating malaria. Anti-malarial drugs are classified into various groups like quinolone, artemisinin and anti-foliate drugs. In this computational study, we aimed at

identifying potent adenylosuccinate synthetase inhibitors using anti-malarial drugs based on rational drug design. The quinolone derivatives like amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, primaquine, quinidine and quinine were used. The artemisinin derivatives like artemether, artesunate and dihydroartemisinin were used. The anti-foliate derivatives like dapsone, pyrimethamine and sulfadoxine were used. The evaluation of feasible binding mechanism of quinolone, artemisinin and anti-foliate derivatives with adenylosuccinate synthetase was done by structure based virtual screening. Three dimensional structure of adenylosuccinate synthetase structure was complexed with 6-phosphoryl IMP, GDP, $Mg2^{+}$ and aspartate analogue hadacidin. In case of docking, the domain containing hadacidin binding pocket was used (Figure. 1). The target protein structure was crystallized at 2.0 Å resolution contains 3351 protein atoms, 68 hetero atoms and 278 solvent atoms. The protein was under structure classification of (a/b**)** and fold classification of P-loop containing nucleoside triphosphate hydrolases.

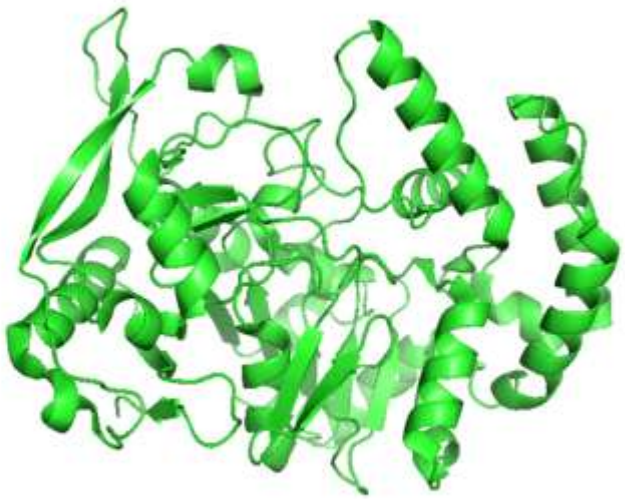

Figure 1 Experimental structure of Plasmodium falciparum adenylosuccinate synthetase.

The conformation of hadacidin, containing nitrogen with planar geometry is stabilized by hydrogen bonding interactions with the protein residues, Asp26, Thr307, Thr308, Thr309, Arg311 and Arg313. The Thr307 residue forms hydrogen bonds with hadacidin, and interaction stabilized by L-aspartate. The target region for inhibitor design was contributed mainly by the key residues Thr308, Thr309, Arg309 and Arg311. The anti-malarial drugs were retrieved from Pubchem database and depend on the drug-likeness, fourteen drugs were selected for virtual screening.

The results obtained after virtual screening concluded that the anti-malarial drugs docked well with adenylosuccinate synthetase. The strong binding was observed with least binding affinity of -9.2 kcal/mol in quinidine complex. The top two ligands quinidine and quinine from quinolone derivatives were chosen as top hits with the effective binding affinities (Table 1). Also, quinine was proved to be more useful in treatment of severe malaria in adults [20] and children [21]. In most of African countries, quinine was still used as monotherapy, contrary to the WHO recommendation [22, 23]. Thus, the virtual screening method proved to be extremely efficient in identifying the hits from the anti-malarial drugs.

Table 1. Virtual screening results of ligand dataset against Plasmodium falciparum Adenylosuccinate synthetase from AutoDock Vina.

Compound	**Binding affinity (kcal/mol)**
Quinidine	-9.2
Quinine	-9.0
Artesunate	-8.9
Mefloquine	-8.6
Amodiaquine	-8.2
Dapsone	-8.0

Dihydroartemisinin	-7.7
Sulfadoxine	-7.7
Pyrimethamine	-7.6
Chloroquine	-7.5
Primaquine	-7.3
Halofantrine	-6.8
Artemether	-6.6
Lumefantrine	-5.7

Re-docking

After the identification of hits, re-docking was performed in AutoDock for identification of the lead compounds [24]. Docking run of 100 conformations was generated for each hits and the binding mode with least binding energy was selected. The docking parameters like favorable energy, low inhibition constant and weak interactions involved between protein/ligand complexes were used to evaluate post docking analysis. The energies calculated were binding energy, final intermolecular energy, electrostatic energy, van der Waals and desolvation energy. Hydrogen and hydrophobic interactions, are weak interactions plays an important role in recognition of ligand and maintaining stability of protein post binding in the protein-ligand complex. From post docking analysis (Table 2), it was observed that two hits quinidine and quinine showed binding energy in range of -8 kcal/mol with effective docked complex. After ligand binding, changes were induced in the protein and subsequent rearrangements occurred, thus the binding energy was more reasonable. Also, the effective interaction was further supported by the final intermolecular energy, electrostatic energy, van der Waals energy and desolvation energy. The energy analysis revealed that the inhibition of adenylosuccinate synthetase by anti-malarial drugs occurred mainly by means of hydrogen and hydrophobic bond interactions.

Table 2. Re-docking results of top hits against *Plasmodium falciparum* Adenylosuccinate synthetase from AutoDock.

Compound	**Binding energy (kcal/mol)**	**Inhibition constant (um)**	**Final intermolecular energy (kcal/mol)**	**Electrostatic energy (kcal/mol)**	**Vdw+ hbond+ desolv energy (kcal/mol)**
Quinidine	-8.40	701.86	-9.91	+0.34	-10.25
Quinine	-8.46	627.28	-9.69	-0.10	-9.60

The hydrogen bond interactions protein-quinidine complex was evaluated using Ligplot+. The results displayed three hydrogen bonds between the protein and ligand atoms. The interaction residues include Val281, Thr308 and Thr309 that are involved in the inhibition of adenylosuccinate synthetase. The binding pose of quinidine clearly suggested effective competitive inhibition of hadacidin ligand. The ‘O1’ atom of quinidine interacts with ‘O’ atom of Val281, the ‘O2’ atom forms two hydrogen bonds with the ‘N’ atom of Thr308 and Thr309 (Figure. 2a). In case of protein-quinine complex, the results displayed three hydrogen bonds between the protein and ligand atoms. The interaction residues include Asn232 and Arg280. The ‘O1’ atom of quinine forms a hydrogen bond interaction with ‘ND2’ atom of Asn232. The ‘O2’ atom is involved in a hydrogen bond interaction with the ‘NH2’ atom and ‘NE’ atom of Arg280 (Figure. 2b). Thus, from the re-docking results quinidine and quinine was identified as lead compounds for adenylosuccinate synthetase with potential binding.

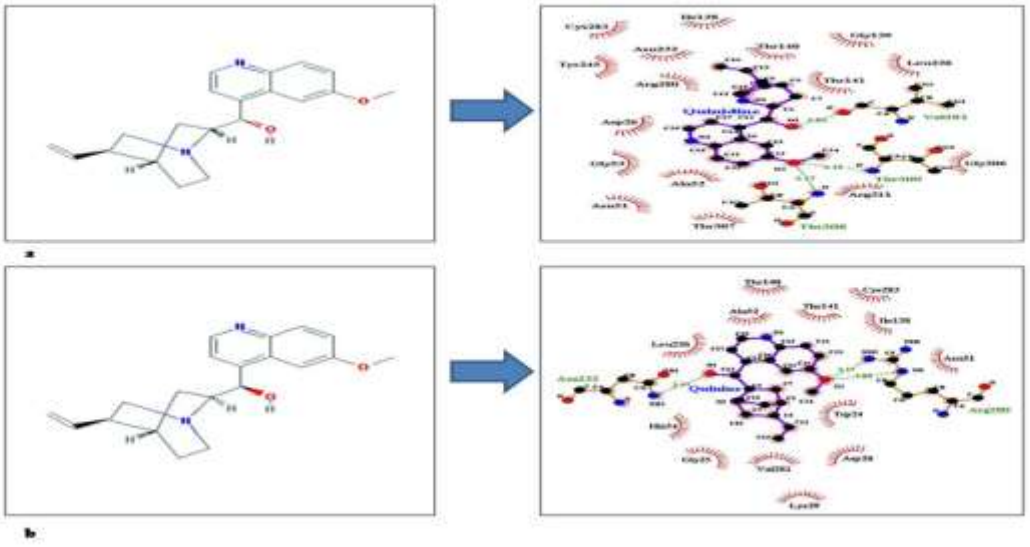

Figure 2 AutoDock results of lead candidates visualized in LigPlot+. Hydrogen bonds showed in green color dots and hydrophobic contacts in red color arc. **a** Quinidine complex **b** Quinine complex.

Molecular dynamics simulation

Molecular dynamics simulations, a powerful method in investigate the stability of protein-lead complex in dynamic system [25]. Total energy was calculated for the lead complexes and the evaluated the protein stability after lead binding. The total energy of quinidine complex was -631800 kJ/mol and quinine complex was -632458 kJ/mol respectively showed the stable conformation of adenylosuccinate synthetase (Figure. 3a.) The comparison of total energy reported that both complexes possessed the favorable energy during the complex formation in the dynamic system.

Root mean square deviation (RMSD) was evaluated for the convergence of the protein structure towards an equilibrium state after lead binding. From protein RMSD plot based on backbone atoms, quinidine complex showed till 2 ns the structure was equilibrated well and started to converge with RMSD range near to 0.15 nm. The RMSD value of quinidine complex was less and clearly explained the less structural deviation after ligand binding. From the RMSD plot of quinine complex, the structure was equilibrated till 2 ns and then started to converge near to 0.2 nm. Comparatively, the RMSD of two lead complexes showed the less structural deviation after ligand binding and obtain the stability till the end of 10 ns simulation (Figure. 3b).

Inter-hydrogen bond interactions between protein and ligands were evaluated for the protein-lead complexes. In case of quinidine complex, NH plot results showed range of one to two hydrogen bond interactions were observed throughout 10 ns simulation and maximum of three hydrogen bonds. NH analysis confirmed strong inhibition of adenylosuccinate synthetase by quinidine in dynamic system same as docking results inferred with three hydrogen bonds. In case of quinine complex, the results showed with range one to three hydrogen bond interactions were found throughout 10 ns simulation and maximum of three hydrogen bonds same as docking results (Figure. 3c). The inter-hydrogen bond interactions pattern confirmed the binding mode of lead candidates with adenylosuccinate synthetase favored the inhibition.

Solvent accessible surface area (SASA) was the property of protein cover the region that is accessible to solvent. From SASA plot, quinidine complex showed SASA value of 105-110 nm^2 and quinine complex showed 102-115 nm^2 confirmed the appropriate change in SASA due to ligand binding (Figure. 3d). The results confirmed the solvent accessibility in the protein was decreased due to ligand binding and favored the closure of binding cavity.

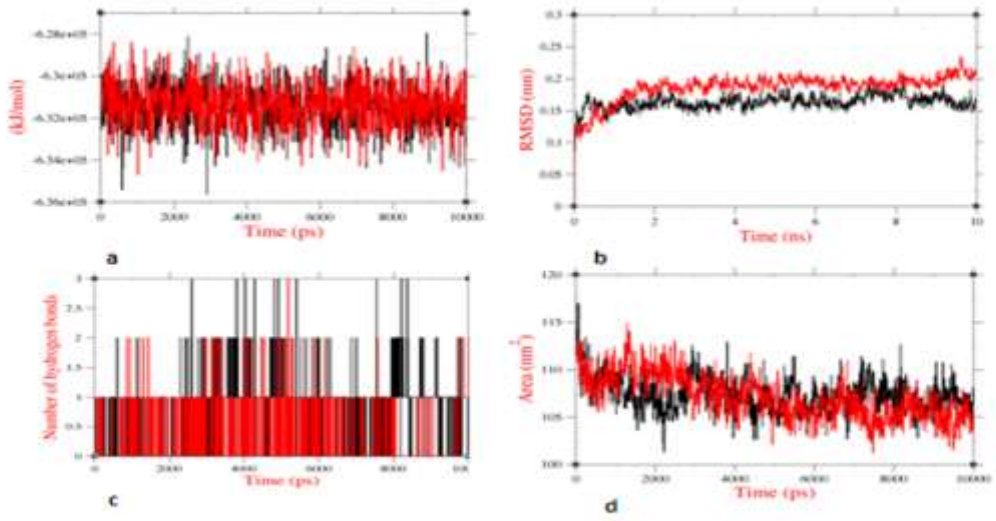

Figure 3 a Total energy **b** RMSD of protein backbone atoms **c** Inter-hydrogen bond interactions **d** Solvent accessible surface area. Color representation: Quinidine complex in black and Quinine complex in red.

CONCLUSION

In this computational study, inhibition of *Plasmodium falciparum* adenylosuccinate synthetase was well studied with anti-malarial drugs. The virtual screening identified the best lead candidates from the ligand dataset. quinidine and quinine compounds were identified as most potential inhibitors against adenylosuccinate synthetase than artemisinin derivatives and anti-foliate derivatives. Both lead complexes were stabilized with hydrogen bonds and hydrophobic interactions that favored the strong affinity. Furthermore, molecular dynamics simulation results confirmed the protein-lead complexes was more stable and formed hydrogen bond interactions in atomic level. In conclusion, the drug repurposing strategy favored the structural mechanism of quinidine and quinine with adenylosuccinate synthetase that can be future anti-malarial agents.

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