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In silico Designing of Potential Drug Compounds against *Plasmodium falciparum*

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Authors' contributions

This work was carried out in collaboration between all authors. Author JK designed the study and supervised the work. Author FAN carried out all the computational work and performed the analysis. Authors JN and DK supervised the work. Author FAN wrote the first draft of the manuscript. Author JK edited the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Malaria is one of the most significant public health problems in the world today; with 97 countries having on-going transmission. Despite advancement in malaria research, the disease continues to be a global problem. This is attributed to inadequate knowledge of *Plasmodium falciparum's* physiopathology. This study employed *in-silico* approaches to design structure based potential drug candidates against *Plasmodium falciparum* malaria. The drug candidates in this study target proteins involved in parasite pyrimidine biosynthesis, type II fatty acid biosynthesis and detoxification of reactive oxygen species. Protein sequences were retrieved from PlasmoDB and the 3D structures of the target proteins were retrieved from PDB (RCSB Protein Data Bank- http://www.rcsb.org/pdb/home/home.do) and viewed using PyMOL program to identify the active sites. Structure prediction was done for targets with no available PDB 3D structure using PSvs2 (http://ps2.life.nctu.edu.tw). Ligand screening was done in PubChem databases. Docking and lead optimization was done using Autodock vina and lead molecules generated. The binding affinity analysis showed three lead molecules belonging to cyclopentane-diols and anilines

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with better docking scores of -10.49 kcal/mol, -10.3 kcal/mol and -12.96 kcal/mol. These molecules can further be tested and validated for their *in vitro* and *in vivo* efficacies as antimalarial drugs.

Keywords: Plasmodium falciparum; in silico; docking; drug candidates.

1. INTRODUCTION

Malaria is a vector-borne disease caused by protozoan parasite of the genus *Plasmodium* with the most serious form of the disease being caused by *P. falciparum* [1]. According to WHO's World malaria report 2015, there were 214 million cases and 438 000 deaths from malaria with almost half of the world population being at risk of malaria (3.2 billion) in the year 2015. As a result of a scale-up of malaria interventions, the global malaria mortality rate was reduced by 60% in 2015 as compared to the year 2000. Since the year 2000, malaria has cost sub-Saharan Africa US$ 300 million each year for case management alone and to achieve the 2030 targets for malaria control, an additional estimated US$ 1.2 trillion will be needed in endemic countries' economies [2]. This is unsustainable for the economies of the developing countries where malaria is endemic. The report also estimated that annual investments must be increased to US$ 6.4 billion by 2020, then US$ 7.7 billion by 2025, and finally $US 8.7 billion by 2030 in order to achieve a 90% malaria reduction. This global malaria situation is increasingly being exacerbated by the rapid emergence of drug resistance *P. falciparum* strains to most of the available antimalarials, necessitating search for novel drugs. The marked increases in the availability and use of artemisinin-based combination therapies, together with the increased use of insecticide-treated bed nets, have substantially reduced global morbidity and mortality from malaria. However, these gains, and the prospects for the elimination of malaria, are now threatened by the emergence of artemisinin resistance in *Plasmodium falciparum* [3]. However, factors leading to this resistance are still not well known owing to a lack of thorough understanding on the physio-pathogenic mechanisms of the disease [4]. One of the challenges of the future malarial chemotherapy is to develop compounds that are innovative with respect to the chemical scaffold and molecular target [5]. Computational biology offers several approaches which aid in designing structure-based candidate drug molecules that could be more effective in the fight against pathogens [6].

Plasmodium falciparum Aspartate carbamoyl-transferase (*Pf.* AT Case) is a highly regulated enzyme that catalyses the first committed step in pyrimidine biosynthesis, the condensation of aspartate and carbamoyl phosphate to form N-carbamoyl-L- aspartate and inorganic phosphate. Nucleic acids are the single most important means of coupling endergonic to exergonic reactions and store of genetic information in the form of DNA and RNA. The malarial parasite obtains preformed purines by the salvage pathway and synthesizes pyrimidines *de novo* [7]. The host can obtain both types of bases by either pathway thus the *de novo* biosynthesis of pyrimidines by the parasite is a good drug target. The parasite cannot utilize preformed pyrimidines and must synthesize them from bicarbonate and glutamine.

Lipids are a major component of membranes. The rapidly growing parasite requires large amounts of lipids for this increase in parasite surface area and volume of internal membranes. This huge demand for lipids makes lipid metabolism an attractive target for anti-malarial drugs [8]. Inhibition of type II fatty acid biosynthesis (FAS-II) appears to hold significant promise in devising novel antimalarials. This dissociative FAS-II pathway, which occurs in the plastid-like apicoplast organelle, is composed of four separate enzymes. Thus it is different from the associative FAS-I multifunctional polypeptide present in mammalian cells. The final reaction in the FAS-II pathway is catalyzed by the enzyme *Pf* Eonyl acyl carrier protein reductase (*Pf*. EACP) which mediates the NADH-dependent reduction of *trans*-2-enoyl-ACP to acyl-ACP [9]. The parasite exhibits a high rate of glycolysis and utilizes up to 75 times more glucose than uninfected erythrocytes. Most of the glucose is converted to lactate and the high LDH activity functions in the regeneration of NAD+ from NADH which is produced earlier in the glycolytic pathway by glyceraldehyde-3-phophate dehydrogenase.

P. falciparum metabolism such as the digestion of oxy-hemoglobin releases a lot of reactive oxygen intermediates (ROI) such as superoxide,

hydroxyl radical and hydrogen peroxide. These ROI can damage lipids, proteins and nucleic acids and therefore need to be oxidized to oxygen and water [10]. *Pf.* Glutathione reductase, a homodimeric flavoenzyme which catalyzes the reduction of glutathione disulfide is important to the malaria parasite antioxidant defense and + NADPH + H+ ↔ 2 GSH + NADP+), that is, it keeps the cellular concentration of the reduced form of glutathione (GSH) high and that of its oxidized form (GSSG) low. Due to its central function in cellular redox metabolism, inhibition of *P. falciparum* glutathione reductase represents an important approach to antimalarial drug development. Glutathione reductase is essential for parasite survival within the human erythrocytes [10].

2. METHODOLOGY

The protein sequences and 3D structures of the target proteins were retrieved from PlasmoDB (http://plasmodb.org/) and protein data bank (http://www.rcsb.org/pdb/home/home.do) respectively. For the proteins with no available PDB structures, the structures were predicted using $(PS)^2$ Version 3.0 [11]. *Pf.* Enoyl Acyl Carrier Protein reductase (PDB ID: 2o2y) and glutathione reductase (PDB ID: 1onf) structures were retrieved while *Pf.* Aspartate Carbamoyl-transferase 3D structure was predicted using (PS) v2 (http://ps2.life.nctu.edu.tw) [11]. The 3D structures were saved in PDB format and viewed using PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC [12]. The program reads in molecular coordinate files and interactively displays the molecule on the screen in a variety of representations and colour schemes. These structures were used for molecular docking in AutoDock vina [13] which employs the Lamarckian genetic algorithm. Using AutoDock tools, the receptor (protein) and ligand (which were obtained through virtual screening in PubChem databases) files were prepared in PDBQT format. Then, a text file for the binding site was also prepared. Molecular docking and lead optimization was performed and the possible binding geometries of the protein and the ligand/ lead molecule were shown on an output file with the best binding geometry based on energies of the protein-ligand complexes and RMSD topping the list. The lead (candidate) molecules were selected based on their binding energies, that is, lowest energy hence high affinity and lowest root-mean-square deviation (RMSD) of atomic positions which measures the average distance between the atoms (usually the backbone atoms) of superimposed proteins. The ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the compounds were determined by OSIRIS Property Explorer [14].

3. RESULTS AND DISCUSSION

The target proteins employed in this study were chosen for their uniqueness in aiding parasite survival during the disease process in the mammalian host. From their physicochemical parameters, both the instability and aliphatic indices showed that the targets are fairly stable proteins (Table 1).

The gene ID shown is enough for retrieving the target proteins from PlasmoDB.

Table 1. Selected physicochemical parameters of the target proteins

Parameter	*Pf.* ATCase	*Pf.* EACP	*Pf.* GR
Gene ID	PF3D7_1344800	PF3D7_0615100	PF3D7_1419800.1
Component	Apicoplast	Apicoplast	N/A
Sequence length	375	432	500
Molecular weight (Da)	43252	49763	56493
Isoelectric point	8.13	9.69	8.04
Half- life (Hrs)	20	4.4	100
Instability index	32.4	29.57	35.3
Aliphatic index	96.88	41.72	101.92
Grand average of hydropathicity (GRAVY)	-.243	0.796	0.688

Cavities and binding pockets for the ligands along the chains were predicted by PyMOL Molecular Graphics System, Version 1.8 Schrödinger as shown in Fig. 1. Ligand screening, molecular and lead optimization generated several molecules which were filtered out based on their ADMET properties (Table 2). These molecules also were able to bind with a lower energy thus high affinity and lower RMSD than the protein substrates hence may act as competitive inhibitors (Table 2).

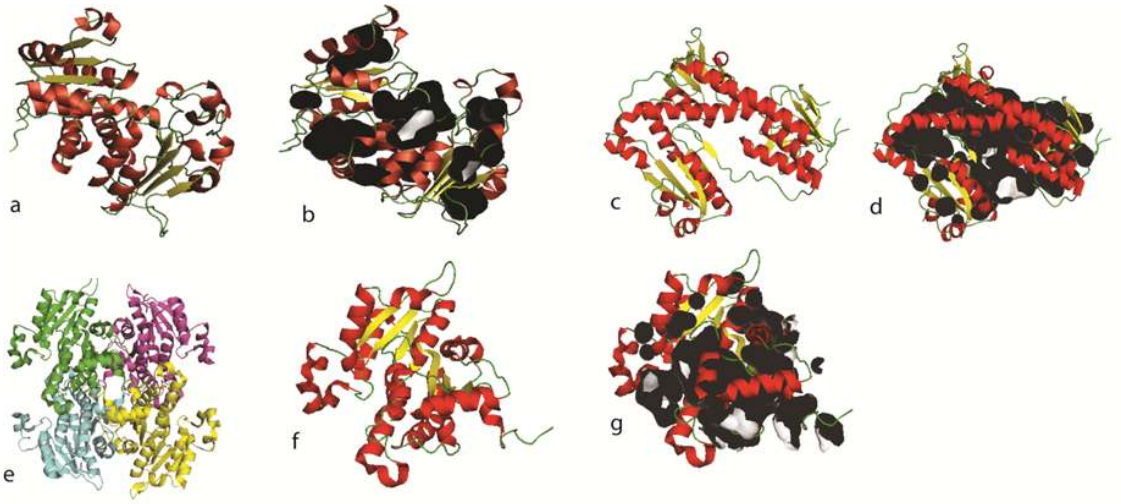
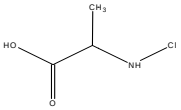
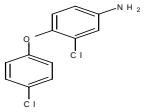
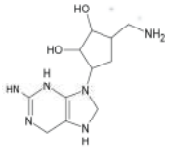


Fig. 1. The 3D structures of the target proteins; a and b: Pf ATCase, c and d: Pf glutathione reductase, where b and d: were surface representations showing cavities and pockets only. The chains were coloured by secondary structure. Pf EACP were represented by e, f: and g where e: showed the four chains, f: represented the monomer which was coloured by secondary structure and g: was a surface representation showing cavities and pockets only

Table 2. Drug candidate molecules and their ADMET properties

Target	***Pf.* ATCase**	***Pf.* EACP**	***Pf.* GR**
IUPAC name	2-(Chloroamino)propionic acid	3-Chloro-(4-chlorophenoxy) analine	3-amino-5-(6-amino-purin-9-yl) cyclopentane-1,2-diol
Other names	N-chloro-L-analine	2,4'-Dichloro-4-aminodiphenyl ether	
PubChem CID	13743745	90644	490430
Molecular structure			
Log S (Aqueous solubility)	-0.85	5.45	-2.45
Log P (Partition Coefficient)	-1.55	3.9	-0.18
Polar surface area	49.2 A^2	35.2 A^2	136 A^2
Drug likeness	1.03	1.47	0.92
Molecular weight	123.53824g/mol	254.11196g/mol	250.2570g/mol
Molecular formula	$C_3H_6ClNO_2$	$C_{12}H_9Cl_2NO$	$C_{10}H_{11}N_6O$
Binding energy (Kcal/mol)	-10.49	-10.3	-12.96
RMSD (Angstroms)	0.67	2.7	2.40
Hydrogen bond donor	2	1	4
Hydrogen bind acceptor	3	1	2
Toxicity risk assessment	Low	Low	Low
Drug score	0.86	0.54	0.82

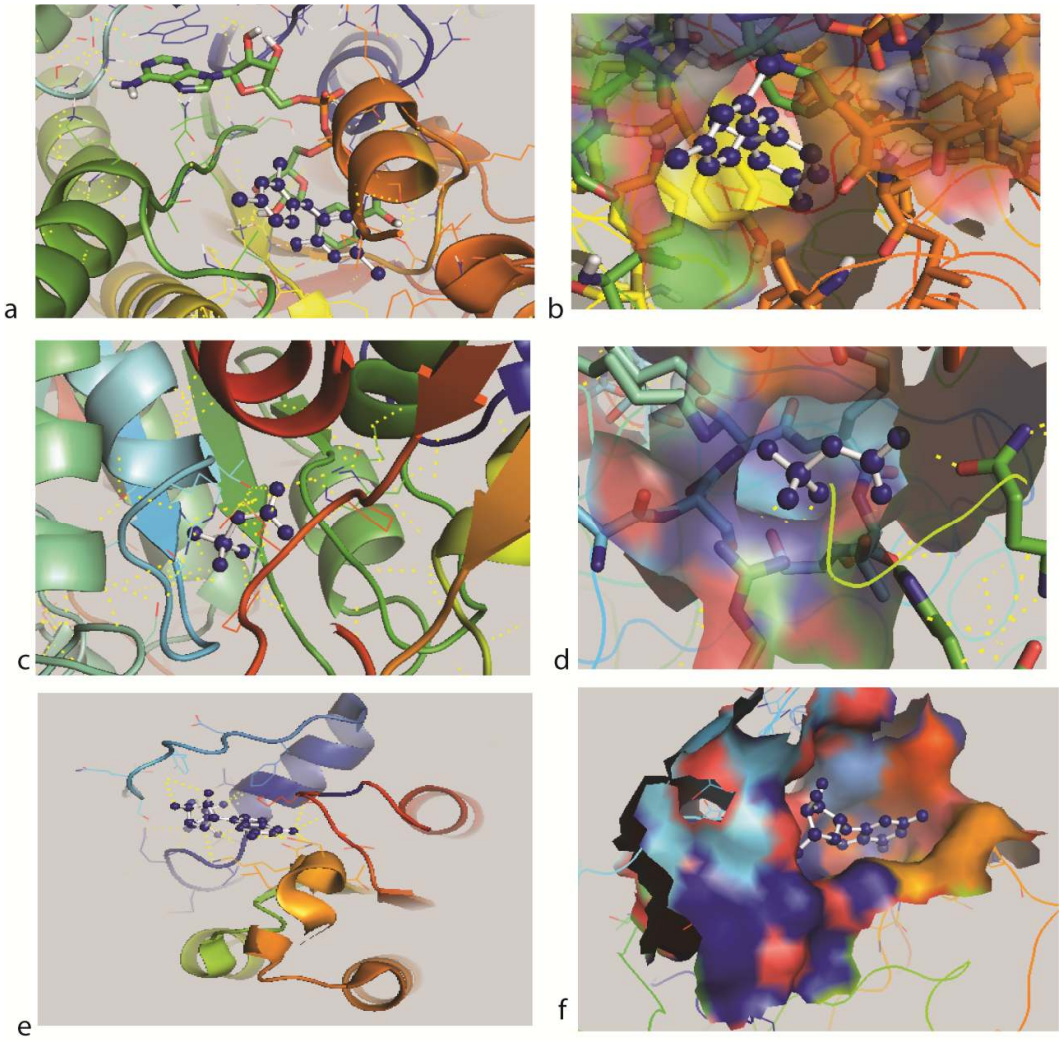


Fig. 2. The potential drug compounds interactions at the active site of the target proteins. The drug compounds were represented as ball and stick

Key: a and b: show the ligand site and surface representation of the binding pocket with the potential drug compound for Pf EACP. The same representation was done for Pf ATCASE and Pf GR as shown in c, d: and e, f respectively

The drug score combines drug likeness, cLogP, logS, molecular weight and toxicity risks and it judges the compound's overall potential to qualify for a drug. The value should range from 0 to 1 qualifying all molecules in this study.

Pf. ATCase, 2-(Chloroamino)propanoic acid would compete for the receptor of carbamoyl phosphate. Studies have shown that the active site of *Pf.* ATCase is a highly positively charged pocket and one of the most critical side-chains is from Arg54, which interacts with terminal oxygen and the anhydride oxygen of carbamoyl phosphate, stabilizing the negative charge of the leaving phosphate group. Arg105, His134, and Thr55 help to increase the electrophilicity of the carbonyl carbon by interacting with the carbonyl oxygen. Thus, enhancement of its activity is achieved by orientation and stabilization of substrates, intermediates, and products rather than by direct involvement of amino acid residues in the catalytic mechanism [15]. Therefore, the binding of 2 - (chloroamino) propanoic acid would result in terminal chloride ion and the hydride oxygen interacting with Arg 54. However, the carbonyl carbon, which is attached to a more stable ethylamine group, does not leave thus resulting in the

destabilization and disorientation of substrates, intermediates and products. This has a potential of disrupting pyrimidine biosynthesis in the parasite.

Pf. EACP, 3-chloro-4-(4-chlorophenoxy) analine targets residues next to the inhibitor binding site of the enzyme (Asn 218, Met 281 and Met 368). One ring of 3-chloro-4-(-chlorophenoxy) aniline interacts with pyrophosphate and nicotinamide moieties of NAD^+ by peptide backbone residues and by side chains of Asn-218, Val-222, Tyr-277 and Met-281 while the other ring associates with the side chain of Phe-368 through Van der Waals interactions. These interactions increase the affinity of the enzyme for NAD^+ resulting in the formation of a stable ternary complex of enzyme-NAD^+-R (where R is the drug candidate molecule). This stable ternary complex may disrupt type II fatty acid biosynthesis in apicoplast.

Pf. Glutathione reductase is less compact and has nine extra segments compared to human host enzyme. The major differences occur at the ligand binding site, that is, the dinucleotide binding motif (Gly –X- Gly- X-X-Gly) and NADH binding is via induced fit in the parasite protein. In the human host enzyme, the terminal Gly is replaced by an Ala and position 374 contains Glu residue instead of the expected basic side chain Arg required for fixing the α-carboxylate of the central glutathione moety [16]. These differences in binding orientations result into selectivity of the drug candidate molecules. Studies have shown that the stable form of the enzyme contains an open redoxactive disulfide bridge Cys-58: Cys-63 which lies between flavin and glutathione disulfide (Bohme et al. [16]). In 3-amino-5-(6-amino-purin-9-yl) cyclopentane-1, 2-diol, pentyl groups on the side chains are likely to interfere with the formation of the redox-active disulfide Cys-58: Cys-63 bridge which will destabilize the protein structure hence disrupting its antioxidant defense.

4. CONCLUSION

This study concludes that the designed molecules have a potential of being drug candidates against *P. falciparum* malaria based on their binding affinity and ADME properties thus could be considered as drug molecules for *in vitro and in vivo* analysis and validation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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