COMPUTER ASSISTED DRUG DESIGN (CADD) AGAINST *PLASMODIUM FALCIPARUM*

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Abstract

Despite advancement in malaria research, it continues to be a global problem. Of the estimated 655,000 malaria deaths occurring 2011, about 90% occurred in Africa and mainly in children under the age of 5 years. The current WHO first-line treatment for malaria is the artemisinin based combination therapies (ACTs). With increased reports of reduced susceptibility of *Plasmodium falciparum* to ACTs, the search for new drugs is vital. The aim of this study was to screen Plasmodium *falciparum* genome for possible drug targets and model novel drug compounds by use of bioinformatics approaches. Plasmodium *falciparum* genome sequence data was downloaded and screened using GenScan™ for potential drug targets. Target sequences were validated using sequence motif database ScanProsite for identification of specific residues likely to be involved in function. The uniqueness of the target proteins was underpinned by use of homology search algorithms specifically BLASTp. Some of the target proteins identified included glutathione reductase (E.C 1.8.1.7), Enoyl Acyl Carrier Protein reductase (E.C 1.3.1.9). 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 RasMol program to identify the active sites. Docking and lead optimization was done using Arguslab software and lead molecules generated. The drug relevant properties of the lead molecules were predicted using OSIRIS property explorer. cDNA synthesis was done to determine the expression of the target genes.

Key words: *Plasmodium falciparum*, antimalarial resistance, *In silico* drug design, docking

1 Introduction
1.1 Malaria, Its Epidemiology and Economic Impact
Malaria is a vector-borne infectious disease caused by protozoan parasite of the genus *Plasmodium* with the most serious form of the disease being caused by *P. falciparum* (Greenbaum, 2008). In 2009, about 3.3 billion people - half of the world's population - were at risk of malaria (WHO, 2010). It is widespread in tropical and sub-tropical regions, including parts of the America, Asia and Africa. The disease has been associated with major negative economic effects in regions where it is widespread. All over Sub-Saharan Africa, already struggling economies are being battered by the economic burden of the disease, which is estimated to cost Africa $12 billion a year (World Malaria Report, 2011). Reduced sensitivity of the parasite to ACTs was reported on the Cambodian-Thailand border urging resistance monitoring plans for 2008-2009 (WHO, 2008). This resistance to the only drugs that can beat severe malaria (ACTs) poses a real threat to the realization of malaria elimination globally and maintains pressure on the pharmaceutical companies to keep up their research into new malaria treatments (World Malaria Report, 2011). Reducing the impact of malaria is key to the achievement of the Millennium Development Goals, which are geared towards not only combating the disease itself, but the improvement of women's and children's rights to health, access to education and the reduction of extreme poverty (World Malaria Report, 2011).

1.2 *Plasmodium falciparum* Resistance to Antimalarials
Resistance to anti-malarials is due to mutations on the target gene, drug modifications and transport. Chloroquine (4-Aminoquinoline) resistance is mediated by a parasite food vacuole membrane transporter encoded by the gene *Pf* crt on chromosome 7 and a second transporter encoded by the *Pf* mdr-1 gene on chromosome 5 of the parasite (Bradley *et al.*, 2000). Resistance to anti-folates is due to several structural mutations in each of the genes encoding dihydrofolate reductase on chromosome 4 and dihydropteroate synthase on chromosome 8 (White, 2004). Quinine derived Atovaquine targets the parasite's mitochondrial cytochrome b. Treatment failures are due to point mutations of cytochrome b gene at key positions such as codon 268 where different mutations have occurred. Mefloquine resistance is due to amplification of gene copy number of *Pf* mdr1. Treatment failures occurred with drugs involved in redox reactions such as metronidazole and other nitronidazoles (Lell *et al.*, 2003). Artemisinin based combination therapies (ACTs) has been advocated as the therapy of choice to handle widespread drug resistance in *P. falciparum* malaria, at the same time preventing recrudescence due to artemisinin monotherapy.

1.3 *In Silico* Drug Design
Bioinformatics offers several approaches for the prediction of structure and function of proteins on the basis of sequence similarities hence can help in designing structure-based drugs that can fight malaria effectively (Bikadi *et al.*, 2008). *In silico* approaches are both time and cost saving (Gail, 2006) when compared to pharmaceutical industries which take approximately 12-14 years and costing up to $1.2 - $1.4 billion dollars to discover and market a drug (Ayma and Masood, 2011). Through structure-based drug design, interactions between a potential drug and its proposed target can be visualized and this helps the drug development companies to refine a promising molecule to enhance its characteristics, thus improving efficacy, specificity or pharmacokinetic properties (Gail, 2006). This approach has been used in the development of many drugs and some of them include the HIV1 protease inhibitors, Captopril (for treating high blood pressure, heart failure and for preventing kidney failure due to high blood pressure and diabetes), anti-bacterial agent (Norfloxacin).

2 Materials and Methods
2.1 Screening of *Plasmodium falciparum* Genome
The *P. falciparum* genome sequence data was downloaded from ftp: /ftp.sanger.ac.uk/projects /P.falciparum (Hall *et al.*, 2002, Gardner *et al.*, 2002). GenScan[TM] (Burge and Karlin, 1997) was used to screen the entire *P. falciparum* genome for genes encoding specific proteins identified as

potential drug targets. *P. falciparum* consists of fourteen linear chromosomes ranging from 0.643 to 3.29 Mb. Only chromosome one and two are less one million base pairs long thus were uploaded directly on GenScan^{TM}. The larger chromosomes were divided into sections which were each uploaded separately onto GenScan^{TM} by copy pasting the nucleotide sequence. GenScan^{TM} screened the nucleotide sequence giving results inform of peptide sequences, indicates the region of the chromosome expressing that particular peptide sequence and calculates the probability of each predicted exon thus giving a guide to the likelihood that a given exon is correct (Burge and Karlin, 1997).

2.2   Characterization of Target Sequences
2.2.1   Physico-chemical Parameters and Target Validation
The physico-chemical parameters of each target protein sequence, amino acid and atomic compositions, isoelectric point and extinction coefficient were determined using Protparam tool which is an on-line tool (Gattiker *et al.*, 2002) from Expasy (Gasteiger *et al.*, 2003) . ScanProsite tool (Sigrist *et al.*, 2005) was used to scan the target sequence against PROSITE or a pattern against the UniProt Knowledge base (Wu *et al.*, 2006) to identify their pattern and profile. The uniqueness of the candidate targets was underpinned by use of homology search algorithms specifically BLAST (Altschul *et al.*, 1990).

2.2.2   Molecular Docking and Lead Optimization
The 3D structure of the target proteins were   retrieved from the PDB (Berman *et al.*, 2000). The program Rasmol program (Sayle and Bisell, 1992) was downloaded from (http://rasmol.org/) and used to visualize structures. 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 Arguslab^{TM} software. The docking process is divided into two; search algorithm which finds the possible binding geometries of the protein and the ligand and the scoring function which ranks the search results and selects out the best binding geometry based on energies of the complexes. The selected ligands were modified through lead optimization in Arguslab^{TM} software. The optimized molecules were then represented on ChemSketch software (Gunda, 2006) from ACD labs which also generated their names automatically. Through ChemSketch, a search on optimized molecules was performed on Drug bank (Wishart *et al.*, 2006), emolecules (Gubernator, James and Mistry, 2006), Pubchem databases (Iberri, 2007) hosted in NCBI Web server to find out any documented studies and bioactivity assays on the molecules.

2.2.3   ADMET Properties Predictions
This was done using OSIRIS Property explorer (Sander, 2009) which is an online tool. It lets one draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior. It determines water solubility of a substance (log S). More than 80% of the drugs on the market have estimated log S value greater than -4. It determines partition coefficient of a substance (c log P). c Log P is the logarithm of a compound's partition coeffient between n- octanol and water log ($c_{octanol}$/c_{water}). It has been shown for compounds to have a reasonable probability of being well absorbed their c log P value must not be greater than 5.0. Molecular weight, more than 80 % of all traded drugs have a molecular weight below 500 g/mol. Through this, the generated molecules were filtered out further by selecting only the molecules whose drug relevant properties conformed to those of most traded drugs.

3   Results
3.1   Identification of Drug Targets
After screening the entire *P. falciparum* genome, the following proteins were identified as potential drug targets based on their importance in disease process and specificity in expression; *Pf* ATCase

(Aspartate Carbomoyl transferase), *Pf* ENR (Enoyl Acyl Carrier Protein Reductase), *Pf* AMA 1 and *Pf* Glutathione reductase (Table 1).

3.2   Characterization of Target Sequences

The physico-chemical parameters of each target protein sequence determined using Protparam tool. The molecular weights of the proteins ranged from about 42250 Da to 106600 Da with pI ranging from about 5 to 8. The amino acid length ranged from 368 to 1299 amino acids with instability index all being below 40 showing that the proteins are stable (Table 2).

3.3   Structures of Target Proteins

The structures of the target proteins are shown below as viewed using Rasmol program on Arguslab™ software. They show the amino acid chains and the ligand binding site.
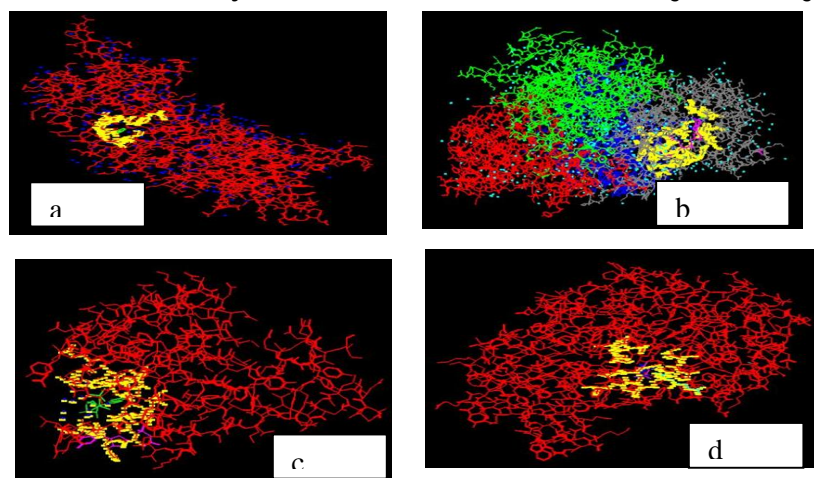

Figure 1.1 (a) is the structure of *Pf* AMA 1; the yellow region is the binding site, red region is the amino acid chain A, green represent the ligand, blue are the water molecules. (b) is the structure of *Pf* ENR; the yellow region is the binding site, red region is the amino acid chain A, blue amino acid chain B, green amino acid chain C, grey is the amino acid chain D, purple represents the ligand, dots represent the water molecules. (c) is the structure for *Pf* glutathione reductase; the yellow region is the binding site, red region is the amino acid chain A, green represents the ligand, purple is NAD molecule, dots represent the water molecules. (d) is the structure for *Pf* ATCase, the yellow region is the binding site, red region is the amino acid chain A, purple represent the ligand, blue are the water molecules.

3.4   Docking Results for Ligands Using ArgusDock

ArgusDock was used to select the best ligands for the target proteins and their chemical structures based on ligand poses during docking are shown in Figure 1.2.
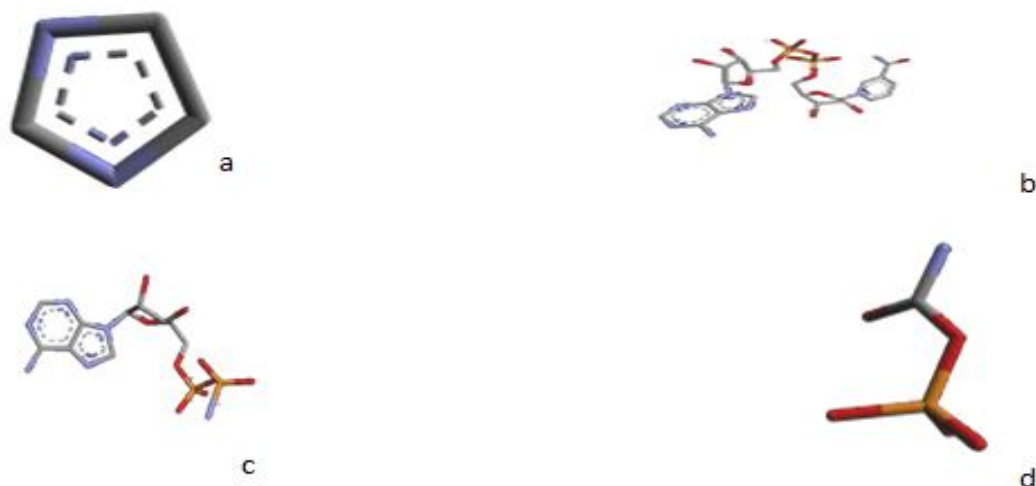
Figure 1.2: (a) to (d) are best ligands for selected drug targets (Table 3). The molecules are coloured by Rasmol program according to their atomic number. The purple colour represent Nitrogen atoms, grey colour represent Carbon atoms, red represent oxygen while yellow represent phosphorous atoms.

3.5 Redocking of Optimized Structures into the Binding Site of Target Proteins

Lead optimization of the generated structures was done. This entails modeling of the drug candidate molecule by adding and / or changing functional groups and / or molecules to the different ligands. The optimized structures were docked into the binding site to determine the best ligand pose, binding energy and RMSD. The generated structures were then filtered out based on their binding energy which was expected to be lower than that of the original ligand for higher affinity and RMSD of ≤ 3.5 Angstroms.
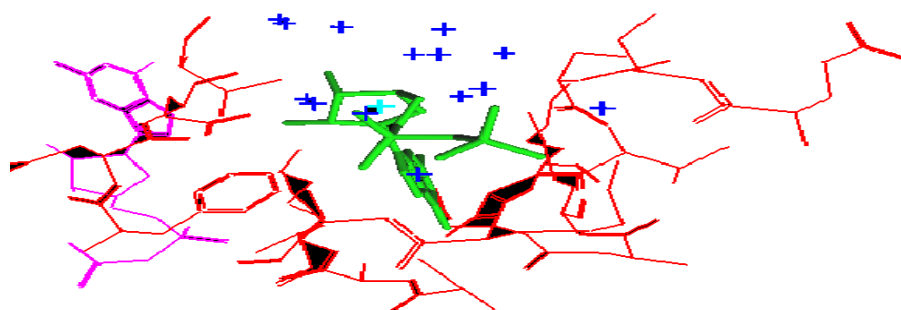

Figure 1.3: A docking shot of an optimized molecule interacting at the binding site of *Pf* glutathione reductase. The bold green molecule is the optimized molecule, outline red molecules are amino acids, the blue crosslike structures represent water molecules and the purple molecule is NAD (Table 3).
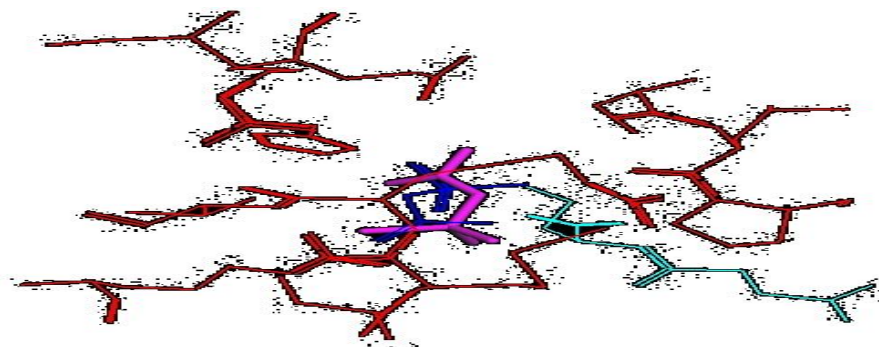
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Figure 1.4: A docking shot of an optimized molecule interacting at the binding site of *Pf* ATCase. The bold purple molecule is the optimized molecule and the outline red molecules are amino acids (Table 3).
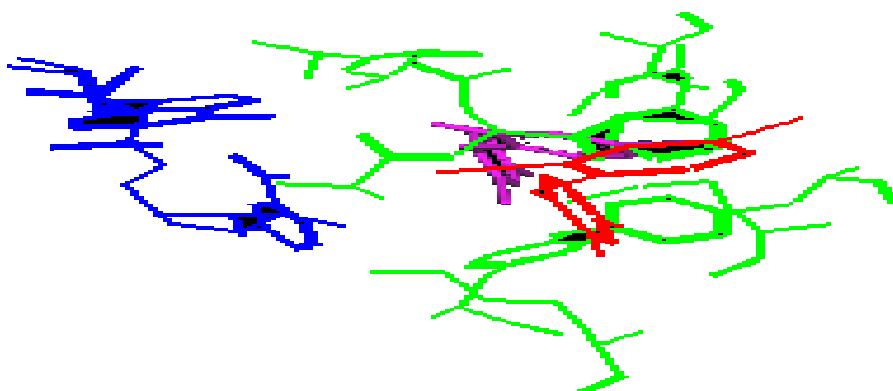

Figure 1.5: A docking shot of an optimized molecule interacting at the binding site of *Pf* ENR reductase. The red molecule is the optimized molecule, the blue molecule is NAD and the green molecules are amino acids (Table 3).
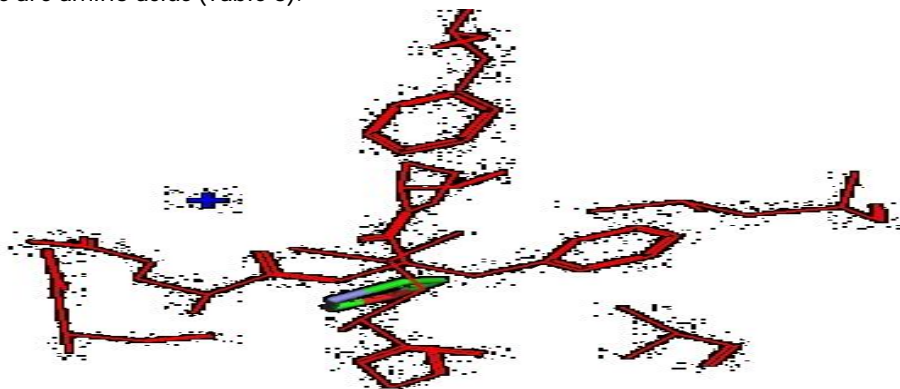

Figure1.6: A docking shot of an optimized molecule interacting at the binding site of *Pf* AMA 1. The bold molecule is the optimized molecule, the red outline molecules are amino acids and the blue crosslike structures represent water molecules (Table 3).

3.6   Lead Compounds and their ADMET Analysis Results

The drug relevant properties of the lead compounds were determined using OSIRIS property predictor. The chemical structures of the generated molecules and their ADMET properties are shown as in table 4. The drug score ranged from 0.54 to 0.89 while log S ranged from -0.85 to -5.45. Log S value for most traded drugs range from -10 to 2. Log P ranged from -0.18 to 3.9. This lies between the required range of not greater than 5.

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4   Discussion

The malaria parasite exhibits a rapid growth and multiplication rate during many stages of its life cycle. This necessitates that the parasite, like all other organisms, acquires nutrients and metabolize these various biological molecules in order to survive and reproduce. The host-parasite interactions are further complicated by the complex life cycle of the parasite involving vertebrate and invertebrate hosts as well as different physiology within each of these hosts (Lauer *et al.*, 1997). *In silico* approach to drug design embraced in this research work is based on the hypothesis that modulation of a specific biological target may have therapeutic value.

From this study, *Pf* ATCase, *Pf* Glutathione reductase, *Pf* ENR and *Pf* AMA 1 were identified as drug targets and these targets were verified through TDR (Tropical Diseases Research) website. *Pf* ATCase catalyses the first committed step in pyrimidine biosynthesis. The malarial parasite obtains preformed purines by the salvage pathway and synthesizes pyrimidines *de novo* (Gutteridge *et al.*, 1979). The host can obtain both types of bases by either pathway. Through molecular docking, the ligand generated from was carbamoyl phosphate and through lead optimization of the ligand, a molecule belonging to dichloroamino acid derivatives was generated. Its drug relevant properties conformed to those of most traded drugs. However, its *in vitro* and *in vivo* efficacy has not been tested.

The rapidly growing parasite requires large amounts of lipids (Mitamura and Palacpac, 2003). Type II fatty acid biosynthesis occurs in the apicoplast and the final reaction is catalyzed by the *Pf* ENR which mediates the NADH-dependent reduction of *trans*-2-enoyl-ACP to acyl-ACP (Muench *et al.*, 2007). The compound from this target identified as critical through docking was NAD which acts as a coenzyme in redox reactions which are involved in the release of energy in the blood stage parasite (Muench *et al.*, 2007). A compound belonging to chlorinated aromatic compounds which have functional groups representative of both ethers and phenols was modeled. Its ADMET properties conformed to those of most traded drugs. However, *in vitro* and *in vivo* efficacy has not been tested.

P. falciparum metabolism such as the digestion of oxy-hemoglobin releases a lot of reactive oxygen intermediates which can damage lipids, proteins and nucleic acids. (Sarma *et al.*, 2003). *Pf* Glutathione reductase catalyzes the reduction of glutathione disulfide important to the malaria parasite antioxidant defense and repair mechanisms (Sarma *et al.*, 2003). The ligands from this target identified as critical for catalytic activity through docking include NADPH, Flavin and GDP. In the binding pocket of NADPH, there is the side chain of Tyr-197. The compound modeled had the negative charges of the phosphate groups replaced with pentyl groups and the side chains replaced with chlorine molecules. This prevents the formation of the redox-active disulfide Cys-58: Cys-63 bridge which contacts the flavin ring near to its C4a atom. This binding therefore may affect the activity of glutathione reductase and disrupts the malaria parasite antioxidant defense. Its drug relevant properties conformed to those of most traded drugs. However, its *in vitro* and *in vivo* efficacy has not been tested.

Pf AMA1 is involved in merozoite invasion of red blood cells hence essential to the proliferation and survival of the malarial parasite (David *et al.*, 2006). Although it is a surface protein and much work has been done on vaccine development, this study modeled a structure based candidate drug molecule which would interfere with the maintenance of the three dimensional peptide structure necessary for erythrocyte binding. Its drug relevant properties conformed to those of most traded drugs. However, its *in vitro* and *in vivo* efficacy has not tested.

5   Conclusion and Recommendations

From this study, some of the compounds modeled had chloro - anilines pharmacophores, cyclopenten-diol pharmacophores which have recently been shown to have antimalarial activity. Other drug compounds were of the methamine family and had good drug relevant properties with

high binding affinities while another one was a dichloroamino acid. The drug compounds identified in this study have potential as antimalarial drugs. This study therefore recommends that the drug compounds undergo *in vitro* and *in vivo* assays to test their efficacy.

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List of Tables

Table 1: Targets selected from genome screening

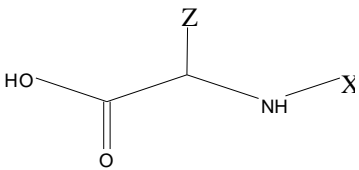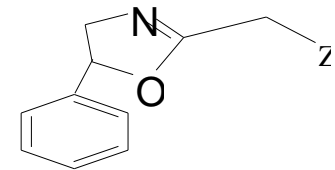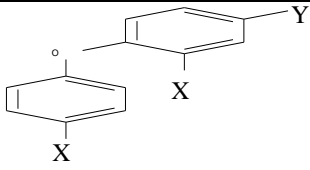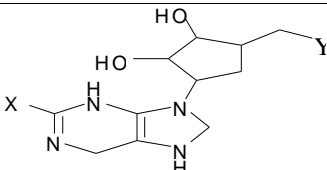| Selected Target	Chromosome number	Gene ID	Nucleotide location	Role in the malaria disease process
Pf Aspartate carbamoyl transferase	13	Mal 13P1.221	1792612 - 1794047 (+1)	Catalyses the first step in pyrimidine biosynthesis
Pf Apical Membrane Antigen 1	11	PF 11-0344	1293854 - 1295722 (+1)	Merozoite invasion of red blood cells
Pf Glutathione reductase	14	PF 14-0192	822418 – 824191(-1)	Detoxification of reactive oxygen species
Pf Enoyl acyl carrier protein reductase	6	PFF 0730c	623018 – 624316 (+1)	Catalyses the last step in type II fatty acid biosynthesis in apicoplast.

Table 2: Physicochemical parameters of the target proteins

Parameter	*Pf* AMA1	*Pf* ATCase	*Pf* ENR	*Pf* Glutathione Reductase
No. Amino acids	621	368	1299	499
Molecular Weight	71910.6 Da	42253.7 Da	106604.2 Da	56360.8
Isoelectric point	5.36	8.13	5.13	8.04
-vely charged residues (Asp+Glu)	99	46	0	65
+vely charged residues(Arg+ Lys)	77	48	0	67
Extinction coefficient (Cys form cystines)	92110	30050	10250	34185
Extinction coefficient (Cys are reduced)	91110	29800	0	33810
Half life	1 hr	20 hrs	4.4 hrs	100 hrs
Instability Index	37.19	32.40	29.57	35.30
Aliphatic Index	62.35	96.88	41.72	101.92
GRAVY	-0.812	-0.243	0.796	0.688

Table 3: Binding energies and RMSD of the ligands

	Target		Binding energ (K...)	RMSD (Angstroms)
a	*Pf* Apical Membrane Antigen 1	Ligand: Imidazole	-4.63	1.69
		Optimized	-5.63	0.43
b	*Pf* Enoyl acyl carrier protein reductase	NAD	-10.055	3.402
		Optimized	-10.3	2.7
c	*Pf* glutathione reductase	GDP	-9.457	3.038
		Optimized	-12.96	2.4
d	*Pf* Aspartate carbamoyl transferase	Carbamoyl phosphate	-9.64	3.25
		Optimized	-10.49	0.69

Table 4: ADMET Properties of the candidate drug molecules

Compound	Log S	Log P	Drug score	Mwt (g/mol)	Toxicity Risk Assessment
	-0.85	-1.55	0.86	123	Low risk
	-2.15	1.73	0.89	175	Low risk
	-5.45	3.9	0.54	253	Low risk
	-2.45	-0.18	0.82	156	Low risk

The symbols X, Y and Z represent different functional groups.