International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:9, No:2, 2015

In silico Studies on Selected Drug Targets for Combating Drug Resistance in *Plasmodium falcifarum*

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Abstract**—**With drug resistance becoming widespread in *Plasmodium falciparum* infections, the development of the alternative drugs is the desired strategy for prevention and cure of malaria. Three drug targets were selected to screen promising drug molecules from the GSK library of 13469 molecules. Using an *in silico* structure-based drug designing approach, the differences in binding energies of the substrate and inhibitor were exploited between target sites of parasite and human to design a drug molecule against *Plasmodium*. The docking studies have shown several promising molecules from GSK library with more effective binding as compared to the already known inhibitors for the drug targets. Though stronger interaction has been shown by several molecules as compared to the reference, few molecules have shown the potential as drug candidates though *in vitro* studies are required to validate the results. In case of thymidylate synthase-dihydrofolatereductase (TS-DHFR), three compounds have shown promise for future studies as potential drugs.

Keywords**—**Drug resistance, Drug targets, *In silico* studies, *Plasmodium falciparum.*

I. Introduction

MALARIA cases, as reported in The World Malaria Report 2012, have been estimated at 219 million across the world with around 660000 reported deaths. There is a global effort being made to control malaria, but antimalarial drug resistance is an area of major concern. [1].

Artemisinin-based-combination therapies (ACTs) with its fast onset action are the current standard for uncomplicated malaria [2]. The ACTs seem to be relatively safe with few serious side effects [3]. The resistance of *Plasmodium falciparum* to artemisinins, for which there is currently no alternative, has been detected in various countries. Hence, the need to increase containment efforts becomes more urgent [1]. Most of the previous research work has focused on the use of artesunate combined with standard drugs, namely mefloquine, amodiaquine, sulfadoxine/ pyrimethamine and chloroquine [4]. Drug resistance to anti-malarial compounds remains a serious problem, with resistance to newer pharmaceuticals developing at an alarming rate [5] The main reason appears to be a number of drug resistance-associated mutations in malaria parasite genes (*dhfr*/*dhps*, *mdr1*, etc.) that have caused resistance to all known antimalarial compounds.

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The next generation of drugs is under prediction worldwide. The major drug discovery approaches that are being followed for exploring the availability of new antimalarials include using combination of current antimalarials, exploring natural sources, experimenting with drugs used for other diseases, chemically modifying currently used drugs, parasite- genome based discoveries and large scale screening of chemical libraries [6].

Plasmodium falciparum thymidylate synthase-dihydrofolatereductase (TS-DHFR) is an essential enzyme in folate biosynthesis, and a major malarial drug target. Point mutations in *P. falciparum* TS-DHFR have caused widespread global antifolate resistance. It is a bifunctional enzyme (in humans, these are two discrete enzymes) that presents different design approaches for developing novel inhibitors against drug-resistant mutants, developing active-site inhibitors equally effective against wild type and drug-resistant parasites, or targeting unique non-active site regions for parasite-specific inhibitors. A high-throughput *in silico* screen of a database of diverse, drug-like molecules against a non-active site pocket within the linker region of TS-DHFR identified three compounds which inhibited TS-DHFR in wild type and antifolate resistant *P. falciparum* but showed no inhibition to human DHFR enzyme [7]. A study of 58 cycloguanil (2,4-diamino-1,6-dihydro-1,3,5-triazine) derivatives was conducted to explore the relationship between various physicochemical properties. The reported binding affinity data on wild type and A16V +S108T mutant type of*Pf* (*Pf*DHFR-TS) led to investigation of FlexX, GOLD, Glide and Molegro virtual docking programs and 13 different scoring functions on 10 of the cycloguanil derivatives to evaluate which program was best for reproducing the experimental binding mode and correlating the docking scores with the reported binding affinity data. GOLD was identified using its GoldScore fitness function as the most accurate docking program for predicting binding affinity data of cycloguanil derivatives to DHFR. Molegro virtual docker with its template docking algorithm and MolDock [GRID] scoring function as most accurate for reproducing the experimental binding mode of a reference ligand that is structurally similar to the cycloguanil derivatives studied [8].

In the present study three plasmodium targets were selected to identify the drug molecule against them. The binding affinities of drug molecules were tested by performing molecular docking procedures. The targets selected were Plasmepsin, TS-DHFR and Phospho-ethanolamine methyl transferase. These targets were selected on the basis of that they are essential for *Plasmodium*, absent in the host system,

have dIndex between 0.2 to 1 and their 3-D structure is available. These targets were virtually screened against the GSK library with the filteration of 2um IC50 value. In case of thymidylate synthase-dihydrofolatereductase (TS-DHFR), three compounds have shown promise for future studies as potential drugs.

II. Methodology

The essential pathways in malaria parasite *Plasmodium falciparum* were studied which included various metabolic pathways. The initial list of 546 proteins was selected on the basis of literature study and most of them were taken from Medicine for Malaria Venture (MMV), www.mmv.org/, based on relevant information of the listed targets, specifically their 3-D structures in ".pdb" format, essential function, active sites, active site residues, available ligands & inhibitors, bioinformatics software involved and binding energy value. The targets were short listed on the basis of their availability of crystal structure on PDB database and minimal sequence homology with humans. Information of their structural, biological and biochemical interactions with inhibitors was also taken as a basis of their selection by using various databases such as PDB, uniPROT, pubmed and plasmoDB. As the next step, the putative targets from 546 proteins were eliminated and 37 targets were short listed for studying homology with human targets using BLAST. Nine targets with minimal homology, less than 40%, were selected for further study. The shortlisted drug targets which are part of major metabolic pathways of *Plasmodium falciparum* include Dihydrofolatereductase (DHFR), Choline kinase, N-methyl transferase, Plasmepsin 2, Peptide deformylase, Enoyl acyl carrier protein reductase, M1 family aminopeptidase, uridinephosphorylase, and orotatephosphoribosyltransferase. Three targets were finally selected to perform docking studies with 13469 leads predicted by GSK against malaria from ChemBL and TDR targets database v5 using DiscoveryStudio 2.0. Finally top hit compounds were validated by AutoDock 1.5.4.

Compounds of the GSK library were extracted from CHEMBL with the link https://www.ebi.ac.uk/chemblntd/download/#tcams_dataset'GSK ChEMBL-NTD contributed data set' file was downloaded in ".txt" format using chemblntd_gsk.txt.gzlink. Opening the file in Microsoft Excel was followed by copying the first and the third row in another excel file keeping the SMILES string in the first row followed by the GSK number in the second. The new excel file was saved in tab delimited format with the name "GSK.smi". The double inverted commas are necessary while saving the file in 'smi format' or else the file won't get saved in SMILES format. The header line was also removed before saving. Screening of the lead compounds extracted from CHEMBL was done using Discovery Studio 2.0 for DHFR. The top hit compounds were selected on the basis of the LigScore 1 and 2. Validation of the top hit compounds recorded from Discovery Studio was done using AutoDock tools. The softwares for validation procedures were **Pymol**: to view the 3D structure of the protein and prediction of binding site, **Chemsketch**: to draw the structure of the ligand/molecule, **Open Babel**: to convert the format of the file from .MOL to .PDB file, **Autodock1.5.4**: Docking software used for validation, **Cygwin**: to create .glg and .dlg file by running docking algorithm and **UCSF Chimera**: to visualize and analyze H-bonds.

The XRay crystal structures of pfDHFR were co-complexed with their inhibitors and cofactors were obtained from pdb. All the docking procedures (validation and library screening) were done using Discovery Studio. The pdb files of DHFR were taken and their ligand (inhibitors and substrates) was extracted and water molecules were removed. Hydrogens were added and force field was applied until the binding constants of inhibitors matched their reported value. The receptor was minimized and the binding site was identified by extracting the ligand in the pymol and then validating the docking with the same interacting residues. The protein with the characterized binding site was taken for further docking procedures for screening the library.

III. Results and Discussion

The first step of protein based docking was target selection. Based on the parameters suggested in methodology, 546 proteins were identified as potential target proteins. These proteins were present in the metabolic pathways of *Plasmodium falciparum.*

TABLE I
TS-DHFR Attributes Used *in silico* Studies [9], [10]

Protein Biology and Structure	Description
TS-DHFR	Protein name- Bifunctional dihydrofolate reductase-thymidylate synthase
Protein ID	1J31
Uniprot ID	P13922
Subcellular location	Cytoplasm
Protein family	bifunctional, Oxidoreductase, Transferase
No. of subunits	Two
Protein function	Bifunctional enzyme. Involved in *de novo* dTMP biosynthesis. Key enzyme in folate metabolism. Catalyzes an essential reaction for *de novo* glycine and purine synthesis, DNA precursor synthesis, and for the conversion of dUMP to dTMP
Name of the coenzyme/prosthetic groups	NDP NADPH dihydro-nicotinamide-adenine-dinucleotide phosphate UMP 2'-Deoxyuridine 5'-Monophosphate WRA 6,6-Dimethyl-1-[3-(2,4,5-Trichlorophenoxy)propoxy]-1,6-dihydro-1,3,5-triazine-2,4-diamine C14 H18 Cl3 N5 O2
Essential for survival or virulence?	Essential for survival
Type of interaction	Protein-protein
X-ray/NMR	X-RAY
Protein name	Bifunctional dihydrofolate reductase-thymidylate synthase
Length of protein	1216
Number of binding sites	11
Name of natural substrates	None
Total number of ligands	Three
Liganded/Unliganded	Liganded

These proteins were further shortlisted to 37 proteins based on literature study, essentiality for survival of the parasite, structural and functional data available online. Putative targets and proteins with unknown structures were removed in further shortlisting. These 37 proteins were tested for homology with similar human targets using NCBI Blast. Proteins with homology up to only 40% were selected for further *in silico* procedures. According to the results obtained only 9 proteins were considered as potential drug targets.

Table I lists the protein biology and structure characteristics of TS-DHFR [9], [10]. The docking methodology used in the work was first tested on the known inhibitors for DHFR at their established binding site. After the docking score (delta G and Ki values) was validated with the already published data for the inhibitors for the proteins, the GSK library with 13469 chemically synthesized compounds was screened against the 3D structures of the proteins. These compounds have more than 80% inhibition for *Plasmodium falciparum* were downloaded from https://www.ebi.ac.uk/chemblntd in SMILES format. Prior to the screening of compounds, target proteins were again validated using Discovery Studio and RMSD values were confirmed as < 2. After this, the GSK compounds were made to run against the protein in discovery studio for their screening. Leads or ligands with the best hit and docking score with these proteins were selected (Table-II). Lig Score 1 was set as standard score due to higher accuracy in predicting ligand- protein interaction energy for different types of proteins.

TABLE II
LEADS FOR TS-DHFR

Compound ID	LigScore
TCMDC-137540	5.9
TCMDC-137540	5.95
TCMDC-137978	6.11
TCMDC-131700	6.46
TCMDC-131700	6.62
TCMDC-137978	6.74

TABLE III
PROTEIN INHIBITOR DATA OF TS-DHFR [11]-[13]

Protein ID	Inhibitor ID	Inhibitor's Name	SMILE
1J3I	WRA	6,6-Dimethyl-1-[3-(2,4,5-Trichlorophenoxy)Propoxy]-1,6-Dihydro-1,3,5-Triazine-2,4-Diamine	CC1(C)N=C(N)N=C(N)N1OCCCOc1cc(Cl)c(Cl)cc1Cl
4DPH	BME	Beta-mercaptoethanol	C(CS)O
KMO	CP6	Pyrimethamine	CCC1=C(C(=NC(=N1)N)N)C2=CC=C(C=C2)Cl
4DPD	DHF	Dihydrofolic Acid	C1C(=NC2=C(N1)NC(=NC2=O)N)CNC3=CC=C(C=C3)C(=O)NC(CCC(=O)O)C(=O)O
3JSU	KA5	-Chloro-N~6~-(2,5-Dimethoxybenzyl)Quinazoline-2,4,6-Triamine	COC1=CC(=C(C=C1)OC)CNC2=C(C3=C(C=C2)N=C(N=C3N)N)Cl
2BLA	MES	2-(N-Morpholino)-Ethanesulfonic Acid	C1COCC[NH+]1CCS(=O)(=O)[O-]
4DP3	MMV	-(2-{3-[(2,4-Diamino-6-Ethylpyrimidin-5-Yl)Oxy]Propoxy}Phenyl)Propanoic Acid	CCC1=C(C(=NC(=N1)N)N)OCCCOC2=CC=CC=C2CCC(=O)O
4DPD	NAP	NADPNicotinamide-Adenine-Dinucleotide Phosphate	C1=CC(=C[N+](=C1)C2C(C(C(O2)COP(=O)([O-])OP(=O)(O)OCC3C(C(C(O3)N4C=NC5=C4N=CN=C5N)OP(=O)(O)O)O)O)O)C(=O)N
4DP3	NDP	NadphDihydro-Nicotinamide-Adenine-Dinucleotide Phosphate	NC(=O)C1=CN(C=CC1)[C@@H]1O[C@H](CO[P@@](O)(=O)O[P@](O)(=O)OC[C@H]2O[C@H]([C@H](OP(O)(O)=O)[C@@H]2On2cnc3c(N)ncnc23)[C@@H](O)[C@H]1O
4DPH	P65	2,4-Diamino-6-Methyl-5-[3-(2,4,5-Trichlorophenoxy)Propyloxy]Pyrimidine	CC1=C(C(=NC(=N1)N)N)OCCCOC2=CC(=C(C=C2Cl)Cl)C
4DPH	PO4	Phosphate Ion	[O-]P(=O)([O-])[O-]
3DGA	RJ1	N-[2-Chloro-5-(Trifluoromethyl)Phenyl]ImidodicarbonimidicDiamide	C1=CC(=C(C=C1C(F)(F)F)N=C(N)N=C(N)N)Cl
3DG8	RJ6	N-(3,5-Dimethoxyphenyl)ImidodicarbonimidicDiamide	COC1=CC(=CC(=C1)N=C(N)N=C(N)N)OC
4DPD	UMP	2'-Deoxyuridine 5'-Monophosphate	C1C(C(OC1N2C=CC(=O)NC2=O)COP(=O)(O)O)O

Table III lists already known inhibitors of TS-DHFR [11]-[13] with their inhibitor ID, name and SMILE structure. The study is relevant for understanding the molecular behavior of the target. Table III shows different poses of three major lead compounds identified against DHFR-TS. Three compounds were found to have more effective binding than the already bound inhibitor at the same binding site and higher score than reference compounds. The top hits screened in Discovery Studio were validated in AutoDock by performing docking procedures. For that separate PDB of the best hit compounds in its best pose has to be docked with the reference protein with no bound ligand using AutoDock tools. Validation of AutoDock tools was done using reference PDB IDs (For DHFR-TS it is 1J3I) of the respective selected target proteins.

The reference PDB structures of proteins were downloaded with already attached ligands from Protein Data Bank. In the first step of the process the attached ligand was removed from downloaded PDB file and the ligand was saved as a separate PDB file. This separated PDB files were docked using AutoDock tools and results were analyzed. Docked confirmation showed Root Mean Square Deviation (RMSD) Values < 2. These values show the particular deviation of the docked ligand in target with respect to ligand bound to the target in the original conformation (as downloaded from Protein Data Bank). These values were considered appropriate thus validating AutoDock tools 1.5.6.

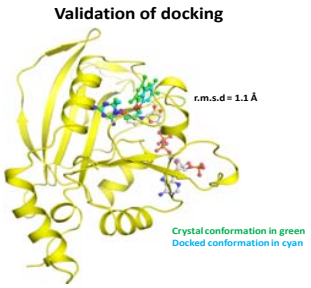

Fig. 1 Comparison between binding interactions of reference compound and the compound identified with TS-DHFR

In case of DHFR there were three top hits. Reference compound PDB id is 1J3I for DHFR with WRA that is the known inhibitor. The interacting energy as well as interacting residues was also reported (Fig. 2)

Compound	LigScore	Interaction Energy			Interacting Residues
		Total	vdW	Electro-statics	
Reference	5.87	-81.52	-43.39	-38.13	Ile14, Cys15, Ala16, Val45, Leu46, **Asp54**, Met55, Phe57, Ser111, Ile112, Pro113, Ile163, **Tyr170**, Thr185
TCMDC-137978	6.74	-94.62	-51.37	-43.25	Cys15, Ala16, Leu46, Trp48, **Asp54**, Met55, Phe57, Cys58, Ser108, **Ser111**, Ile112, Pro113, Phe116, Leu119, **Ser120**, **Arg122**, Ile163, Thr185

Fig. 2 Overall comparison of identified and reference compound

Fig. 2 shows that the lead compound identified against DHFR-TS showed better LigScore and more negative interaction energy than the reference compound. Also it gives a comparison between interacting residues of DHFR-TS with both compounds.

Reference compound had total interaction energy at -81.52 kcal/mol which was greater than the total interaction energy of the lead compounds. This was a result of the greater interaction of the lead compound with the protein.

The comparison shows that the lead compounds identified against malaria had better inhibition than already known inhibitor present in the crystal structure of DHFR-TS (PDB ID IJ3I).

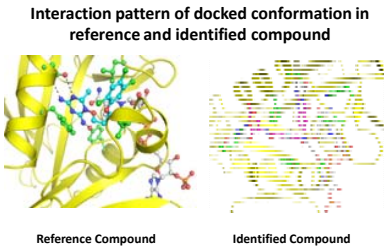

Fig. 3 Interaction pattern of docked conformation in reference and identified compound

Fig. 3 shows the comparison between the docked conformations of reference and identified compound with TS-DHFR and their interaction patterns with different residues at same binding pocket.

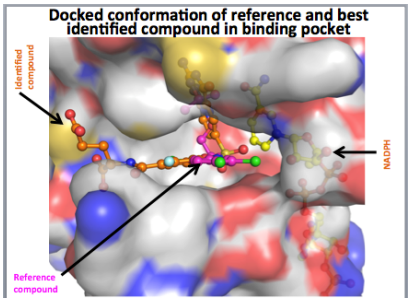

Fig. 4 Docked conformation of reference and best identified compound in binding pocket

Fig. 4 shows alignment of both compounds together and the difference between in their interaction with DHFR-TS.

The top hits received through Discovery Studio were validated by Auto Dock tools. For DHFR 1J3I, the reference PDB id was docked with best-hit compounds. For, DHFR with TCMDC – 131700, the Best Run was 3 with binding energy -3.89 and RMSD value at 2.98.

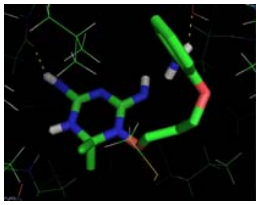

Fig. 5 Hydrogen bonds formed TCMDC-131700 and TS- DHFR after docking

For, DHFR with TCMDC – 137978, the Best Run was 7 with binding energy -1.34 and RMSD value at 4.95.

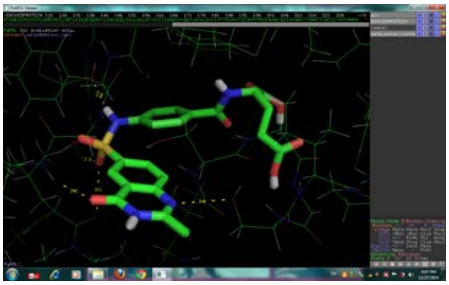

Fig. 6 Hydrogen bonds formed after docking between TCMDC-137978 and TS- DHFR

Lig Score 1 was set as standard score due to higher accuracy in predicting ligand protein interaction energy for different types of proteins. Comparison of top hit compounds was done with reference compounds, that is, known inhibitors for each of the three proteins. Lig Score greater than the reference compounds indicates a better fit of ligand/affinity for the target site. Comparison of identified and reference compound was done for DHFR and phosphoethanolamine methyl transferase. For DHFR-TS, the Lig Score of reference compound was 5.87 and total interaction energy was found to be 81.52 which is lower than the total interaction energy of top

hits, for e.g. TCMDC 137978 was found to have total interaction energy of -94.62.

Validation of top hits in AutoDock tools – AutoDock tools give the protein- Ligand interaction in terms of Root Mean Square Deviation (RMSD) and Binding Energy. Top hits from Discovery Studio were validated on AutoDock and comparison of results was done with data of reference compound. Top hit compounds with RMSD less than or equal to the reference compound indicate a better affinity for the protein. RMSD value equal to 2 was taken as the maximum value for selection of compounds. For DHFR-TS, RMSD of reference compound was 0.2 though none of the top hits selected for DHFR-TS had RMSD less than or equal to 2 but many of them had better binding energy. Many of the top hits compounds of DHFR did not follow the criteria of selection (RMSD less than or equal to 2.0). These top hit compounds can be considered as positive results and are susceptible for *in vitro* studies [14]-[16]. In future we can screen similar leads for the remaining proteins and also work on the lead generation and lead optimization strategies that will include ADMET studies, 2D Visualization etc.

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