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Targeting resistant *Plasmodium* through *In silico* approach

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ABSTRACT

With the emergence of widespread drug resistance in *Plasmodium falciparum* infections, there is an urgent need of developing an alternative drug to combat malaria. Phosphoethanolaminemethyltransferase is reported in this study to be a promising drug target to combat drug resistant *Plasmodium*, as selected from a large number of drug targets shortlisted on the basis of virtual screening of databases and literature study of their characteristics. 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. These can act as potential drugs but further lead optimization strategies and *in vitro* studies are required to validate the results. From amongst thousands of shortlisted molecules from GSK library, thirteen compounds have shown promise for future studies as potential drugs.

Key Words-drug resistance, drug targets, *In silico* studies, *Plasmodium falciparum*

I. INTRODUCTION

The World Malaria Report 2014 has estimated 198 million malaria cases worldwide (range 124–283 million) in 2013, and an estimated 584 000 deaths (range 367 000–755 000) [1]. The treatment and prevention of this disease is primarily based on anti-malarial drug administration and anti-vector measures respectively [2]. Malaria is caused by a protozoan called *Plasmodium* that has four species namely *P.falciparum, P.ovale, P.malariae and P.vivax.* Out of these four species, *Plasmodium falciparum* causes the most lethal type of malaria [3]. The parasite has evolved drug resistance against almost all known anti-malarial chemotypes. The efficacy of anti-malarial drugs is diminishing due to the ability of *Plasmodium* species to develop drug resistance. *Plasmodium falciparum* drug-resistant malaria originates from mutation in genes. Mechanisms of resistance against various drugs is analyzed using molecular, genetic and biochemical approaches which have shown that

(i) mutations of the Pfmdr1, Pfcg2 and Pfcrt genes has led to the impairment of chloroquine uptake by the parasite vacuole; (ii) one to four point mutations of dihydrofolate reductase (DHFR), the enzyme target of antifolates (pyrimethamine and proguanil) produce a moderate to high level of resistance to these drugs; (iii) mutations related to dihydropteroate synthase (DHPS) is the mechanism of resistance to sulfonamides and sulfones; (iv) treatment with sulphadoxine-pyrimethamine selects for DHFR variants Ile(51), Arg(59), and Asn(108) and for DHPS variants Ser (436), Gly (437), and Glu (540); (v) clones that were resistant to some traditional antimalarial agents acquire resistance to new ones at a high frequency (accelerated resistance to multiple drugs, ARMD) [4].

In recent years, parasite resistance to artemisinin, drug that is currently used in the frontline treatment, has been detected in five countries of the Greater Mekong sub region [1]. Thus there is an urgent need for discovery of new and more effective anti-malarial drugs. *In sillico* approach to drug design is one of the cost effective and less time consuming method. It involves virtual screening of large database against target proteins to get lead compounds which can be further studied in order to obtain promising drug molecules. It also consists of *in silico* ADMET prediction and predicting the proteins-ligand interactions i.e. structure based drug designing. It would be nearly impossible to test millions of compounds against target proteins in the lab which has been made possible with the virtual screening using in computational software resulting in small set of lead compounds which can be tested in labs [5].

In search for therapeutics, the study of effective targets by way of essential pathways is a critical step. Parasite undergoes a stage of rapid division within the erythrocytes, during their lifetime. In this stage, there is a significant requirement of phospholipids for membrane biogenesis; therefore, this pathway can be an effective target for therapeutics [6]. In mammals, dietary choline is converted to phosphocholine (Kennedy pathway), which is further converted to phospholipid via CDP intermediates or by conversion of phosphatidylethanolamine to phosphatidylcholine through the Bremer-Greenberg pathway. In plants, phosphoethanolamine is converted to phosphocholine which then proceeds through Kennedy pathway to give phospholipids. *Plasmodium* uses similar pathway as used by plants for phospholipid biosynthesis. It involves S-adenosylmethionine(AdoMet)-dependent phosphoethanolamine methyltransferase to catalyse the reaction of conversion of phosphoethanolamine to phospho-monomethylethanolamine (pMME), pMME to phospho-dimethylethanolamine [7], [8].

Hence, this approach was considered in the present study for further research as its extensively worked on and the crystal structure is easily available in online databases. Structural, biological, biochemical and inhibition properties of selected targets and validated the crystal structure of the reference PDB ID were used for docking studies on the same. PMII shows 31% identity with human protein arginine N- methyltransferase, therefore it was assumed to be an ideal drug target for our studies.

II. METHODOLOGY

Specific essential pathways were studied in reference to the malaria parasite *Plasmodium falciparum* which included various metabolic pathways. The initial list of selected proteins was based on literature study and most of them were taken from Medicine for Malaria Venture (MMV), www.mmv.org/. Literature citations and research papers available for these proteins were studied thoroughly. The compilation of 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 was done. 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 that was explored using various databases such as PDB, UniPROT, Pubmed, PlasmoDB etc. After eliminating the putative targets, thirty seven proteins were short listed. From these short listed targets, nine targets with minimal homology (NCBI BLAST) to human proteins (less than 48%) were selected for further study. These 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, uridine phosphorylase, and orotate phosphoribosyltransferase. The vital enzyme , N-methyl transferase was finally selected for further studies.

The X-RAY crystal structure of was downloaded from RCSB PDB and was then validated using both DS 2.0 and AutoDock Tools. The validated crystal structure was taken to screen the GSK antimalarial lead compounds (having more than 80% inhibition) which were extracted from ChEMBL using the link [9] 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.gz link. Screening of the lead compounds extracted from ChEMBL was done using Discovery Studio 2.0. 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[9]

RESULTS AND DISCUSSION

The proteins selected through literature study were present in the essential metabolic pathways of Plasmodium *falciparum*. These proteins were further shortlisted based on literature study, essentiality for survival of the parasite, structural and functional data available online. These proteins were subjected to a sequence alignment tool (NCBI BLAST) to select proteins with minimal sequence homology with human i.e. upto only 40%. *In silico* procedures were then performed on these selected proteins resulting in a final list of thirteen potential drug targets . Table I lists the protein biology and structure characteristics of Phosphoethanolamine-N-methyl transferase

Table I- Attributes of Phosphoethanolamine-N-methyl transferase used *in silico* Studies

Protein Structure & biology	Description
Uniprot ID	Q6T755
X-RAY/NMR/Model	X-RAY
PDB ID	3UJ8
Organism name	Plasmodium falciparum
Protein name	Phosphoethonalamine N- Methyltransferase

Protein function	Phosphoethonalamine N Methyltransferase () Catalyzes the methylation of phosphoethonalamine to phosphocholine in membrane biogenesis (1*)
Length of protein	258 aa (1*, 3*)
Key active site residues	Tyr 19 and His 132 (1*)
Name of the ligand if present	Phosphate Ion (1*)
Number of binding sites	10 (2*)
Link of paper where structure reported	**PMID:22117061**
Resolution	1.35 A (1* , 2*)
No. of subunits	1
No. of binding site	10
Name of natural substrates	Phosphoethonalamine (1*)
Total no. of ligands	1
Name of the bound inhibitor	SINEFUNGIN
Name of coenzyme/ prosthetic group	Phosphate Ion (1*)
Interacting target residues	Try 19 and His 132 (1*)
No. of subunits	2
Functional unit	1
Name of the coenzyme/prosthetic groups	*S*-adenosylmethionine
Protein function	Phospholipid biosynthesis
Turnover no.	1.82
Action mechanism	random bi bi mechanism
Interacting partners	protein-protein
Type of interaction	Hydrogen bonds ,non bonded contacts

Table II- Protein Inhibitor Interaction of Protein

Protein ID	3UJ8
Inhibitor ID	65482
Ligand SMILE	C1=NC2=C(C(=N1)N)N=CN2C3C(C(C(O3)CC(CCC(C(=O)O)N)N)O)O
Inhibitor name	sinefungin
Ligand InChi	InChI=1S/C15H23N7O5/c16-6(1-2-7(17)15(25)26)3-8-10(23)11(24)14(27-8)22-5-21-9-12(18)19-4-20-13(9)22/h4-8,10-11,14,23-24H,1-3,16-17H2,(H,25,26)(H2,18,19,20)/t6-,7-,8+,10+,11+,14+/m0/s1
MolWt(Da)	381.38702
Mechanism of Inhibition	competitive
Binding with	Active

target(active/allosteric)	

The crystal structure of N-methyl transferase was then validated by docking the protein with known inhibitor using both DS 2.0 and AutoDock Tools and RMSD values were confirmed as < 2, thus validating these computational tools. The details of known inhibitor i.e. sinefungin are mentioned in Table (II). The GSK compounds were screened against the protein in Discovery Studio 2.0. Ligands with the best hit and docking score with this protein were selected. LigScore 1 was set as standard score due to higher accuracy in predicting ligand-protein interaction energy for different types of proteins [12]. For Phosphoethanolamine-N-methyl transferase, thirteen compounds were found to have more effective binding than the already bound inhibitor at the same binding site.

TABLE III- Hits with higher score than reference compound

Compound ID	LigScore
TCMDC 140069	6.67
TCMDC 139925	6.59
TCMDC 133212	6.34
TCMDC 138710	6.33
TCMDC 137264	6.32
TCMDC 139789	6.29
TCMDC 140015	6.27
TCMDC 135164	6.19
TCMDC 138191	6.13
TCMDC 137246	6.07
TCMDC 139964	6.05
TCMDC 134290	5.96
TCMDC 134290	5.95
TCMDC 139964	5.88

The table III shows different poses of thirteen major lead compounds identified against Phosphoethanolamine-N-methyl transferase.

Validation of docking

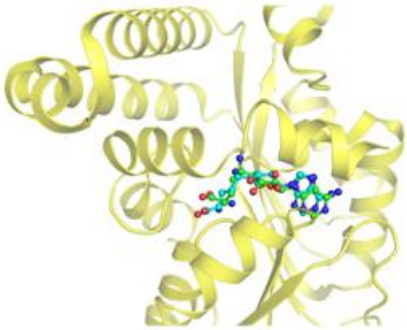

Figure1. Comparison between binding interactions of reference compound and the compound identified with Phosphoethanolamine-N-methyl transferase.

In case of Phosphoethanolamine-N-methyl transferase, there were thirteen top hits. Reference compound PDB id is 3UJ8 for Phosphoethanolamine-N-methyl transferase with sinefungin which is the known inhibitor. The interacting energy as well as interacting residues were also reported (Figure 2)

Compound	Lig Score	Interaction energy			Interacting Residues
		Total	vdW	Electrostatics	
Reference	5.27	-67.74	-49.14	-18.60	Leu14, Tyr19, Asn34,Tyr35,Ile36 **Ser37**,Ile62,**Ser64**, leu66, Ile84,Asp85, Ile87, **Asp110**,Ile111,**Arg127**,**Asp128**
TMDC – 140069	6.67	-76.45	-58.00	-18.45	Leu14, Gln18, Tyr19, Tyr27, **Ile36**, Seer37, **Asp85**, Ile87, **Asp110**, Ile111, Arg127, **Asp128**, Leu131, His132, Leu133, Tyr160, Tyr181

Figure 2. Overall Comparison of identified and reference compound

The Figure 2 shows that the lead compound identified against Phosphoethanolamine-N-methyl transferase showed better LigScore and more negative interaction energy than the reference compound. It gives a comparison between interacting residues of Phosphoethanolamine-N-methyl transferase with both compounds.

Reference compound had total interaction energy at -67.74 kcal/mol, which was less negative than the total interaction energy of the lead compounds. This was as a result of the greater interaction of the lead compound with the protein. This comparison shows that the lead compounds identified against malaria had better inhibition than already known inhibitor present in the crystal structure of Phosphoethanolamine-N-methyl transferase (PDB ID 3UJ8).

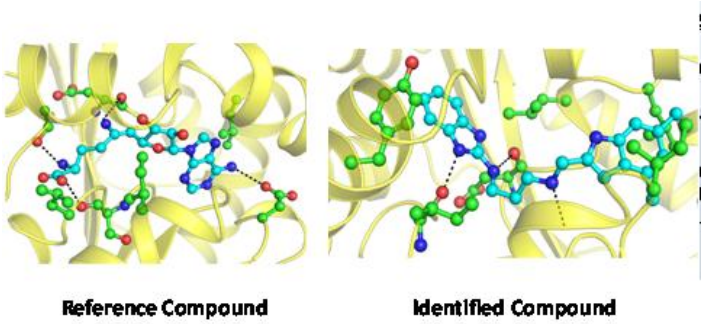

Fig. 3 Interaction pattern of docked conformation in reference and identified compound

The Figure 3 shows the comparison between the docked conformations of reference and identified compound with Phosphoethanolamine-N-methyl transferase and their interaction patterns with different residues at same binding pocket.

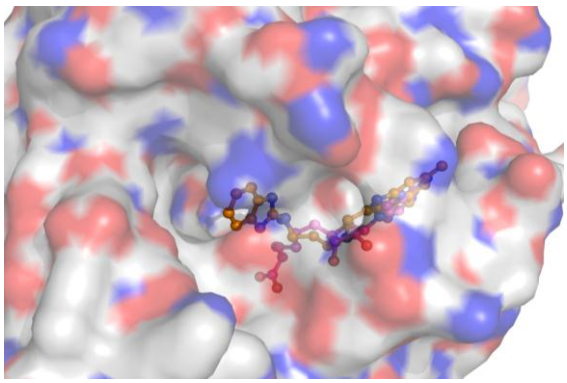

Figure 4- Docked conformation of reference and best identified compound in binding pocket

Figure 4 shows alignment of both compounds together and the difference between in their interaction with Phosphoethanolamine-N-methyl transferase. The top hits obtained, were validated by performing docking procedures using AutoDock. PDB file of protein were extracted form Protein Data Bank and ligands, wates molecules and inhibitors were removed form the structure and then, docked with different poses of major lead compounds. RMSD (Root Mean Square Deviation) values of many lead compound were obtained in permissible range i.e. < 2. For Phosphoethanolamine-N-methyl transferase (3UJ8), the reference PDB id was docked with best-hit compounds and the major results i.e. best run ,binding enrgy, RMSD value are reported in Table IV.

TABLE IV- AutoDock results of docking of N-methyl transferase with top hits

Top hits	Best Run	RMSD value	Binding energy(kcal/mol)
TCMDC 140069	7	1.47	-8.24
TCMDC 134290	1	1.85	-8.39
TCMDC 137246	9	1.58	-7.05
TCMDC 133212	4	4.06	-11.81
TCMDC 136090	7	1.72	-8.65
TCMDC 139964	8	6.39	-7.14
TCMDC 140015	4	8.11	-8.06
TCMDC 138710	8	4.59	-5.40
TCMDC 139925	5	3.97	-9.16
TCMDC 137264	9	2.68	-10.16
TCMDC 138191	1	4.36	-8.07
TCMDC 135164	7	1.72	-7.83

Figure 5 represents hydrogen bonds formed between Phosphoethanolamine-N-methyl transferase and the top hits.

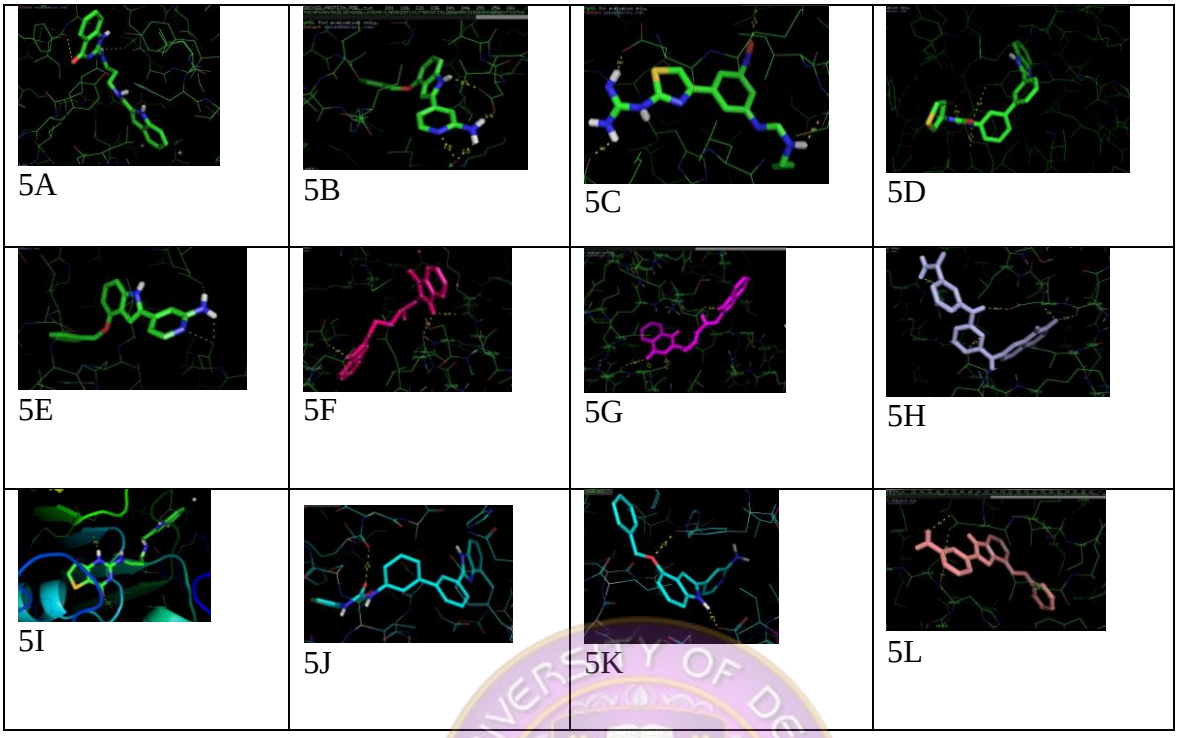

Figure 5 Hydrogen bonds formed after docking between Phosphoethanolamine-N-methyl transferase and (A) TCMDC-140069 (B) TCMDC- 134290 (C) TCMDC- 137246 (D) TCMDC- 133212 (E) TCMDC- 136090 (F) TCMDC- 139964 (G) TCMDC- 140015 (H) TCMDC- 138710 (I) TCMDC- 139925 (J) TCMDC- 137264 (K) TCMDC- 138191 (L) TCMDC- 135164

DISCUSSION

For comparison of affinity of top hit compounds and reference compounds i.e., known inhibitors with N-methyl transferase. LigScore was used as a standard score due to higher accuracy in predicting ligand protein interaction energy for different types of proteins. Ligscore higher than the reference compound indicates a better fit of ligand for the target site. For Phosphoethanolamine methyl transferase, the LigScore of reference compound was 5.27 and total interaction energy was found to be -67.74 which is lower than the total interaction energy of top hits, for e.g. TCMDC 140069 was found to have total interaction energy of -76.47 and LigScore 6.67.

Validation of top hits in AutoDock tools- 13 Top hits obtained from DiscoveryStudio were validated on AutoDock. Top hit compounds with RMSD less than or equal to the reference compound indicate a better affinity for the protein. For Phosphoethanolamine methyl transferase, RMSD of reference compound was 1.23. Five of the top hit compounds of Phosphoethanolamine methyl transferase had RMSD values< 2. These were TCMDC – 140069, TCMDC –134290, TCMDC –137246, TCMDC – 136090, TCMDC – 135164. These top hit compounds can be considered as positive results and can be subjected to *in vitro* studies.

The top five hits obtained after validation using AutoDock can be used for lead optimization strategies that will include ADMET studies, 2D Visualization etc. and then subjected to *in vitro* studies which includes techniques like assaying enzyme activity in presence of these inhibitors and then further conducting clinical trials. The remaining seven drug targets out of ten can be screened against GSK library followed by the similar steps of lead generation and optimization as performed in this case.

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