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Sequence analysis, Homology Modeling, Docking and Pharmacophore Studies of Phosphocholine Cytidylyltransferase in *Plasmodium Falciparum*

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ABSTRACT

By virtue of the most fatal pandemic disease, Malaria, about a million individuals reach lethality globally every year and with ever consummating drug-resistant malarial parasite species, there occurred a coercive demand for the identification of incipient drug targets. Here we have evaluated a new drug target in phospholipid metabolic pathway such as Phosphocholine cytidylyltransferase (PfCCT) which is involved in the synthesis of Phosphatidylcholine, a class of phospholipids that significantly sways the developmental aspects of malarial parasite along with its replication and longevity within human red blood cells. The Objective of Present study is to identify potential lead molecule against PfCCT through docking with homology model of our target protein and common pharmacophore approach of our target inhibitor molecules. In this study, we computationally modeled the structure of PfCCT using Molsoft and validated by PROCHECK, ProSA and RMSD. With the finally refined target structure we performed docking using GOLD 3.1 and pharmacophore studies using Discovery Studio with 12 natural compounds. The predicted homology model of PfCCT is reliable. On the basis of the docking scores and pharmocophoric features, we have identified the compounds Amodiaquine and Quinidine showing better binding affinity towards PfCCT respectively with good fitvalues. In conclusion, the two compounds Amodiaquine and Quinidine shows potential inhibition against PfCCT respectively as targeted for malaria and also having better pharmacophoric features that could aid in the design of new lead molecules.

Keywords: Docking, Malaria, pharmacophore, Phosphatidylcholine, Phosphocholine cytidylyltransferase, Phospholipids.

INTRODUCTION

An inductive agent of the world's uttermost significant parasitic malady, Malaria, is an intraerythrocytic protozoan parasite belonging to the genus *Plasmodium* among which *Plasmodium falciparum* is found felonious for severe human malarial cases with death rates beyond 1 million every year [1,2].Advancements in strategies to encounter this disease has been made obligatory, in view of predicaments in the treatment and prophylaxis of malaria with an ever emerging drug resistant strains of *P. falciparum*, which paved the path for an incipient approach that suggests to target critical metabolic pathways known to be regulated parasite infection and transmission. Excellent targets have been provided by the recent studies on *P. falciparum* for lipid-based antimalarial therapy development involving the metabolic pathways which lead to the major *P. falciparum* phospholipids synthesis that requires enzymes, which are afflictive for a Brisky parasitic multiplication within human erythrocytes [3,4,5]. During the

course of intraerythrocytic longevity of the parasite, the *P. falciparum* takes up either of the two pathways for the production of phosphatidylcholine viz., The serine-decarboxylase phosphoethanolamine methyltransferase (SDPM) pathway and the CDP-choline pathway [4]. The SDPM pathway avails serine as a starting precursor either from human serum or from host hemoglobin degradation wherein, by the action of the parasite this serine gets decarboxylated with the aid of serine decarboxylase to form ethanolamine which is later phosphorylated by an ethanolamine kinase to form phosphoethanolamine (P-EA). This P-EA undergoes a three-step methylation to form phosphocholine [5,6,7] catalyzed by a parasite Sadenosylmethionine (SAM) -dependent methyltransferase, PfPMT, followed by its conversion into phosphatidylcholine (PtdCho) by the action of two parasite enzymes PfCCT and PfCEPT. As an alternative approach, CDP choline pathway might be employed for Phosphatidyl choline synthesis, which involves phosphorylation of choline to phosphocholine by a parasite-specific choline kinase (PfCK), that consequentially gets coupled to CTP to generate CDP-Cho by a CDP-choline cytidylytransferase (PfCCT).

On the general grounds that the three-dimensional (3D) structure of proteins determines its function, a manoeuvre has been attempted in the present study to generate three-dimensional (3D) structure of PfCCT from *Plasmodium falciparum* based on the available template (3HL4) structural homologue from Protein Data Bank with the aid of homology modelling, a predominantly esteemed technique which work towards foreboding the 3D structure of bio molecules, as the three- dimensional structures of PfCCT (Q9BMP5) from *Plasmodium falciparum* still remain uncharted and the model has been validated by PROCHECK & ProSA. In Further studies. The developed structures were subjected to docking studies with selected ligands to validate the efficiency against PfCCT.

EXPERIMENTAL SECTION

Primary and secondary structure analysis of PfCCT

Physicochemical characterization, theoretical isoelectric point (pi), the total number of positive and negative residues, extinction coefficient [8], instability index [9], half life time, aliphatic index [10] and grand average hydropathy (GRAVY) [11] were computed using Expasy's Prot-Param server [12]. Secondary structure analysis was performed with the aid of PSI-PRED server.

Sequence Alignment of PfCCT

The FASTA sequence of PfCCT from *Plasmodium falciparum* was fetched from the Swiss-prot database which has a length of 370 amino acids (Accession No: Q9BMP5 Protein name: Phosphocholine cytidylyltransferase (PfCCT). Customarily, Comparative modelling starts with the search for known protein structures in the PDB taking the target sequence for query [13] which is attained by comparing the target sequence with the sequences of the structures already present in the database using the Basic Local Alignment Search Tool (BLAST) [14] against PDB. The BLAST results thus obtained yielded X-ray structure of 3HL 4 of *Mammalian* ctp: Phosphocholine cytidyltransferase that showed 47% identity to PfCCT from Plasmodium falciparum.

Homology Modelling of PfCCT

The theoretical structure of PfCCT from Plasmodium falciparum is generated using Molsoft ICM v3. 5 software by comparative modeling of protein structure prediction. In ICM-Homology modeling algorithm after the initial placement of the aligned polypeptide chain onto the template structure, the side-chain torsion angles are predicted by simultaneous global optimization of the energy for all non-identical residues.

Methodology for conformational modeling of protein side chains and loops, implemented in ICM, relies on the internal coordinate definition of the molecular object combined with computationally efficient ICM Biased Probability Monte Carlo (BPMC) optimization [15] An extended force field includes surface terms, electrostatics with the boundary element solution of the Poisson equation [16], side chain entropy terms, and a fast algorithm for calculating molecular surfaces.

Validation of of PfCCT Model

The developed model is further evaluated by Procheck, ProSA and RMSD. The validation of protein structure model is carried out by means of the Procheck [17] which was employed in verifying the Ramachandran plot quality and the Protein Structure Analysis program (ProSA) which was used for comparing the Z-scores of the target and the template structures wherein the Z-scores of a model is the measure of compatibility between its sequence and structure [18]. Root Mean Squared Deviation (RMSD) which was calculated by SPDBV is customarily exercised to ascertain the distance between two objects.

Active site Identification

As the final model is obtained, the probable binding sites of PfCCT is positioned pertaining to the structural comparison of the template and the model built with the aid of CASTP server [19].

Ligand preparation and Optimization

By the agency of ACD/ ChemSketch (12.0), 12 natural compounds were drawn and saved [20,21] which later were imported into the Argus Lab and were minimized after adding hydrogen bonds. The 3D structures of the compounds are shown in Figure 1.

Docking studies

All the possible conformations of the drug binding to the protein were generated by means of the Genetic algorithm GOLD v 3.0.1 [22] wherein the population size-100, the number of islands-5, the niche size-2, the selection pressure-1.1, the migrate-2, the number of operators-100,000, the mutate-95, and the cross over-95 were employed as the working parameters for the docking process [23], considering a maximum of 10 different conformations for the drug. The conformer that yielded the highest binding score was used for further analysis [24]. The possible active site co-ordinates for PfCCT is identified using Discovery Studio [25]. It is found that the active site pocket one has X= -7.707200,Y=44.167400,Z=3.714400 coordinate values for PfCCT.

Generation of pharmacophore models: common feature based approach

Common feature based pharmacophore modeling is performed with a set of highly active inhibitors of our target protein PfCCT which utilizes the common chemical features present in the most active compounds for the generation of pharmacophore. Common feature hypotheses are generated by the HipHop algorithm of catalyst program in Accerlys Discovery Studio. To derive the best featured model, conformers for each compound are generated within DS Diverse Conformation Generation protocol using the Best Conformation Method with a value of 255 as the maximum number of conformers for each compound and an energy threshold of 20 kcal/mol. All the possible pharmacophore feature mappings with desired chemical groups were identified for the generated conformers of all the compounds with Feature Mapping module. Based on the feature mapping results, using the "common feature pharmacophore generation"protocol, a pharmacophore query is created with chemical features like hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), hydrophobic (HY), ring aromatic (RA) and positive ionizable with a minimum of 0 to a maximum of 5 features to include in the generated pharmacophore. Multiple common-feature pharmacophore generation runs were carried out with a principal value of '2', maximum omit the feature value of '0' and minimum inter feature distances of '2.97 Å' that ensures that all the chemical features in the molecules were considered to build the pharmacophore space and must map to the compounds. Also the parameters such as maximum pharmacophores as 10, Number of Leads That May Miss and Feature Misses, were used as a value of '1' to allow that one of the compounds may not contain all the features when building hypothesis space. The Complete Misses option is set to '0', which is used for specifying the number of compounds that do not have to map to any features in the hypothesis. With the result of common feature pharmacophore model generation, 10 possible pharmacophore hypotheses having a different arrangement of constituent features are generated and the best one is selected based on the ranking score of the hypothesis. To the best featured hypothesis , compounds were screened for mapping on to the pharmacophore model and analysed the best mapped compound is selected based on the fitvalues and aligned pharmacophoric features.

RESULTS AND DISCUSSION

Primary and secondary structure analysis

The predicted physicochemical characteristics showed that the molecular formula of PfCCT is C1866H2926N506O622S8. The amino acid composition of PfCCT has71 (Asp+Glu) and 53(Arg+Lys).The computed pI values of Modeled PfCCT is less than 7 (pI<7) indicating that target protein is considered as acidic. The computed GRAVY of PfCCT is -0.990, which implied that the solubility of protein is supported by their hydrophilic nature. This has the potentiality to solve the major issue with the isolation of soluble protein by acquiring high-concentrations of soluble proteins which still remains an intensive experimental challenge [26].

Homology modelling of pfcct

Homology modelling used to predict the 3D structure of PfCCT, (target) based on its alignment with the identified templates. The PfCCT sequence is having 47% amino acid sequence identity with the crystal structure of 3HL4 (Mammalian ctp: phosphocholine cytidyltransferase). Based on the crystal structures of the template, the 3D structures of PfCCT is modelled using Molsoft ICM and visualized in Rasmol as shown in Figure 2.

Validation of modeled protein structures

The Ramachandran plot analysis was carried out for evaluating the stereo chemical quality and accuracy of the predicted model of PfCCT after the refinement process by means of the PROCHECK program which revealed the contribution of the phi and psi angles in conformation of amino acids excluding glycine and proline with the results displaying 94.5% residues in the core region, 4.0% in the allowed region, 1.5% in the generous region. The Ramachandran's map for PfCCT model and statistics is represented in Figure 3 and Table 1.

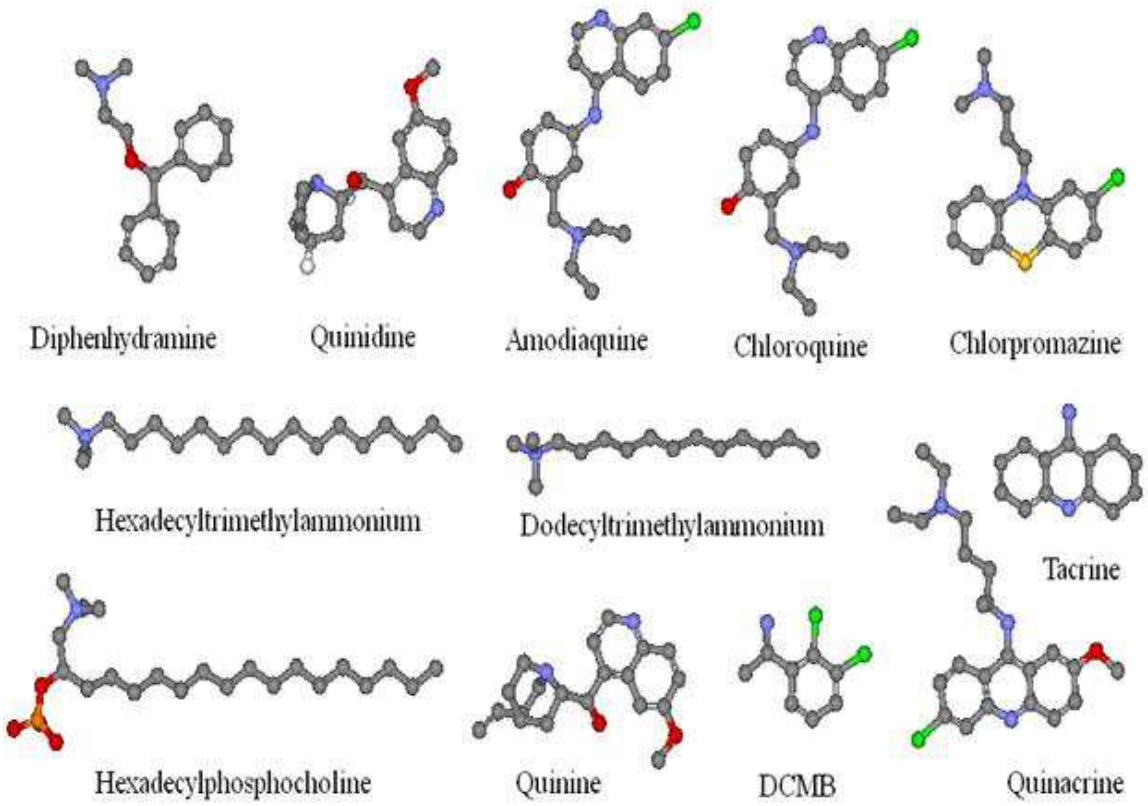


Figure 1: The three dimensional structures of selected ligand molecules represented in ball-stick

Figure 2. Modeled structure of PfCCT from Plasmodium falciparum as visualized in Rasmol

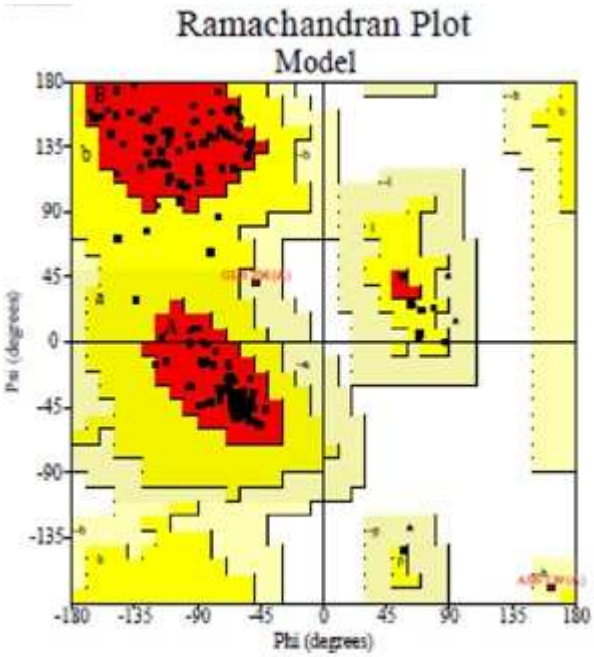


Figure 3:Ramachandran's Plot of PfCCT Model

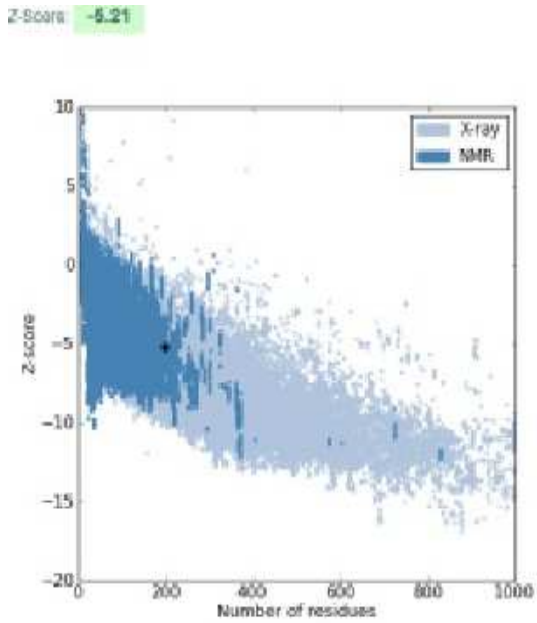


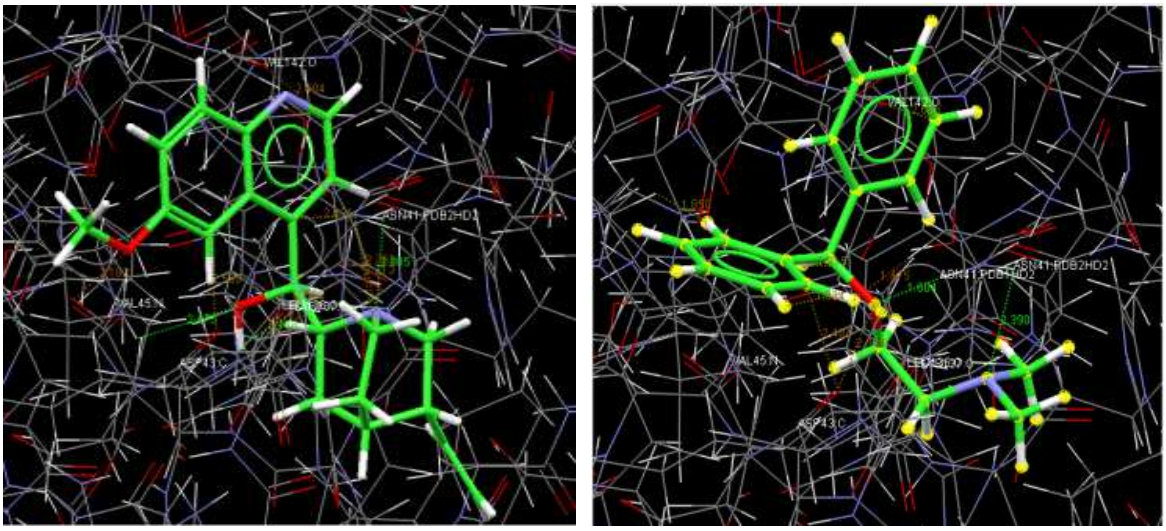
Figure 4: The plot of the Z-Score shows spot for Z score value of protein that is determined by NMR (represented in dark blue colour) and x ray (represented in light blue colour) with the aid of ProSA program. The black dot encircled by orange circles represent Z-Scores of our model PfCCT (-5.21) which indicates the overall quality of the modeled 3D structures of PfCCT

ProSA was employed to analyse energy criteria comparing with Z scores between the modeled protein and 3D template structure. The Z-scores of the obtained modeled Structure of PfCCT is -5.21 (shown in Figure 4) located within the space of proteins determined by X ray crystallography derived structures. The RMSD value calculated based on Alpha carbon atoms by superimposing template and modeled structure is calculated to be 1.12 A^o for PfCCT using SPDBV.

Docking Studies

Docking of 12 drawn ligands is studied against Modeled PfCCT using GOLD. The best docking solutions were analysed with the assistance of GOLD scores generated for all the 12 compounds. Comparison of Gold scores of 12 compounds with PfCCT is shown in Figure 5. Among all the docked complexes of PfCCT with the 12 ligands, Quinidine shows highest binding affinity towards PfCCT with a Gold score of 36.61 forming interactions with the amino acids Asp 41, Lys137, Val 45, and Val42 respectively. Diphenhydramine is shown the next highest docking score with PfCCT having a fitness score of 36.38 respectively. The interaction patterns of Quinidine and

Diphenhydramine with PfCCT is shown in (Figure 5). The docking results of all the 12 compounds with PfCCT along with their Fitness scores are shown in Table 2.



(a) Quinidine **(b) Diphenhydramine**

Figure 5: Shows H-Bond interactions of (a) Quinidine and (b) Diphenhydramine with PfCCT

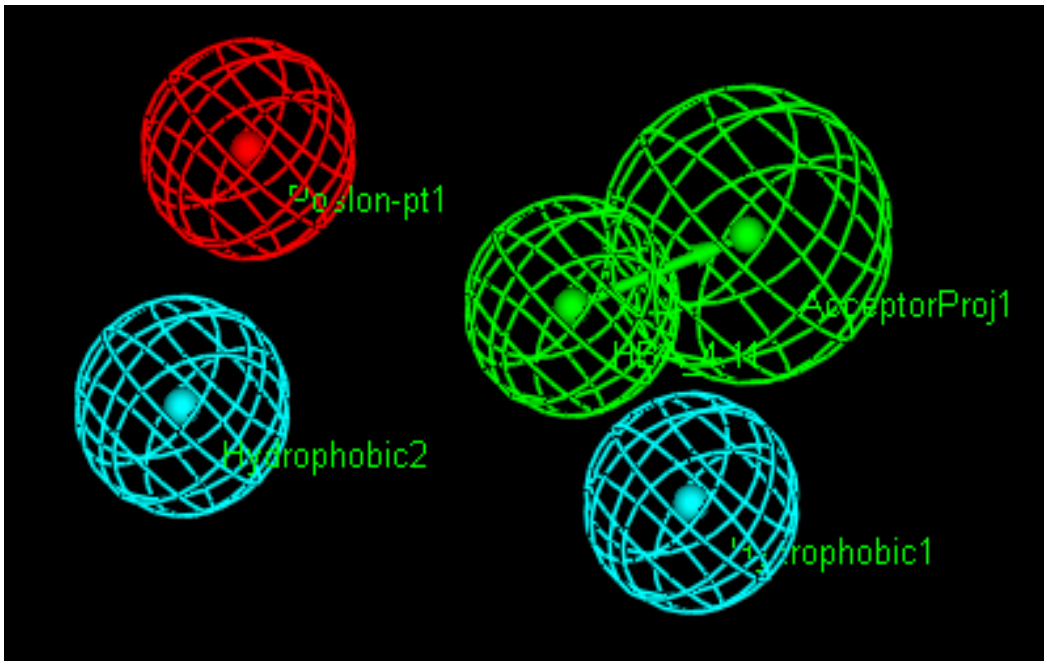


Figure 6: Best DS catalyst pharmacophore model (Hypo 1) illustrating the hydrophobic regions (light blue), the hydrogen bond acceptor (green) and the positive ionizable region (red)

Pharmacophore model generation and validation

Important common pharmacophore hypotheses are computed by HipHop algorithm for the selected inhibitors of pfpmt and pfcct, inorder to find the chemical features shared by them and also for assuming the relative alignment of the compounds with the best derived common feature pharmacophore model.By selecting the features obtained through the results of Feature Mapping protocol, the pharmacophore generation run was performed along with 902 diverse conformers of all the active inhibitor molecules. The common pharmacophoric features are obtained as top 10 hypotheses with their ranking scores ranging from 65.093 to 57.783 (Table 3). Table 3 reports the top 10 pharmacophore models along with the displayed features and maxFit values. Among the generated hypotheses, Hypo1 to 8 consisting of four features, one positive ionizable, one hydrogen bond acceptor and two hydrophobic features while the Hypo9 and 10 containg three features, one ring aromatic one positive ionizable and one hydrophobic features.The pharmacophore which scored the highest ranking score is chosen as the most accurate pharmacophore hypothesis (Hypo 1) (Figure 6). The top ranking pharmacophore model Hypo 1 was used to

determine the mapping of the most active compounds and estimate the fit values for each compound. The predicted fitvalues of the active compounds from the most accurate hypothesis Hypo 1 is given in Table 4. The fit values of all the compounds molecules were ranging from 3.99 to 0.674 of highly mapped molecule to molecule showing low mapping. Remarkably, the highest active compounds Quinidine mapped well on all the chemical features of the Hypo1 model with good fitting scores of 3.786 indicating that the Hypo1 model provides reasonable pharmacophoric characteristics of PfCCT inhibitors for components of their activities. The features mapping of high active molecule Quinidine on the generated best pharmacophore Hypo 1 is shown in Figures 8.

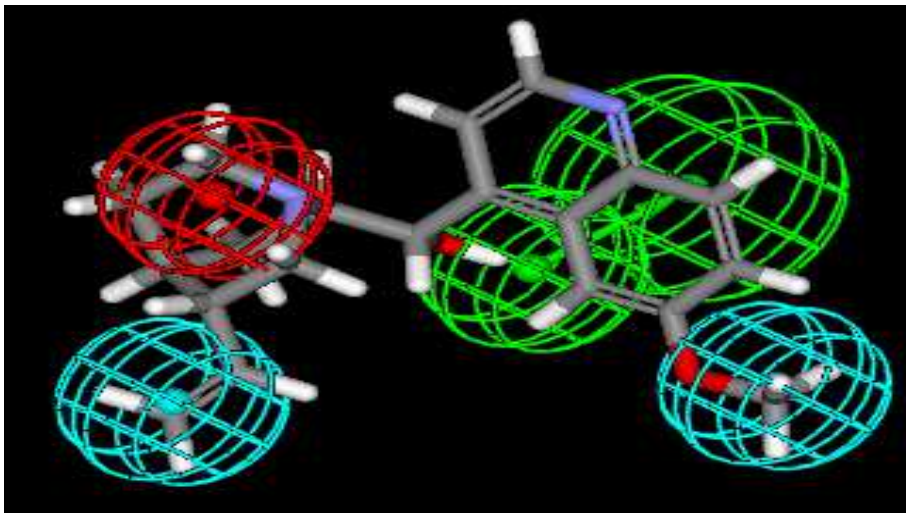


Figure 7: The most active compound Quinidine mapping the common feature pharmacophore Hypo 1

Table 1: Percentage of the residues in the core region of the Ramachandran Plot

Structure	Modeled *PfCCT*
Core	94.5
Allowed	4.0
Generous	1.5
Disallowed	0.0

Table2: Gold Scores and interaction of 12 ligands with PfCCT

Compound	Fitness score	S(hb_ext)	S(Vdw_ext)	S(hb_int)	S(int)	Interacting atoms	H-Bond Distance
Diphenhydramine	36.38	0.0	37.60	0.0	-91.67	Asn41:ND2…N10(L)	1.875
Quinidine	36.61	0.0	32.19	0.0	-7.66	Asp41 :ND2...N3(L) Asp41 :ND2...C14(L) Lys137:OH...O1H(L) Val 45: NH..O1H(L) Val142:OH…C20(L)	2.085 2.631 2.448 2.475 2.484
Amodiaquine	29.70	0.28	37.60			Val 45: NH...O1H(L) Asp41 :ND2...N3(L)	2.475 2.085
Chloroquine	23.77	0.0	29.87	0.0	-19.52	Arg140:HA…C3(L) Pro46:HD1…H35(L)	1.875 1.307
Chlorpromazine	34.17	2.80	31.16	0.0	-.9.54	Asp41:ND2..N10(L)	2.397
DCMB	31.36	0.0	29.98	0.0	-4.84	Asp41 :ND2...N3(L) Lys137:OH...O1H(L)	1.821 1.479
Dodecyltrimethyl Ammonium	31.98	0.0	29.63	0.0	-16.68	Lys137:OH...O1H(L) Val 45: NH..O1H(L)	2.448 2.475
Hexadecylphosphocholine	26.43	0.0	46.81	0.0	-33.34	-	
Hexadecyltrimethylammonium	23.43	0.0	31.11	0.0	-17.21	-	
Quinacrine	26.72	0.0	36.78	0.0	-27.58	Arg140: HA...C3(L)	1.875
Quinine	34.73	0.0	29.90	0.0	-7.37	Val142:O…N15(L) Lys187:O…C5(L)	1.977 2.376
Tacrine	32.90	5.78	19.73	0.0	0.0	Asp43:O…..N10(L) Asn41:ND2…N10(L) Arg140:HA..C3(L) Val142:O…N15(L)	2.288 2.397 1.875 1.977

Table 3: Features shared by top 10 hypotheses in common feature pharmacophore generation

Hypo	Features	Rank	Direct Hit	Partial Hit	Max Fit
01	PHHA	65.093	1111111	0000000	4
02	PHHA	64.240	1111111	0000000	4
03	PHHA	62.002	1111111	0000000	4
04	PHHA	60.719	1111111	0000000	4
05	PHHA	60.156	1111111	0000000	4
06	PHHA	59.228	1111111	0000000	4
07	PHHA	58.553	1111111	0000000	4
08	PHHA	58.403	1111111	0000000	4
09	RPH	57.834	1111111	0000000	3
10	RPH	57.783	1111111	0000000	3

Table 4: Predicted fit values of the active compounds from the hypothesis Hypo 1

Compound name	Fit value	Pharmprint
Amodiaquine	3.999	'1111'
Quinidine	3.786	'1111'
Chloroquine	3.703	'1111'
Quinacrine	2.443	'1111'
Quinine	1.907	'1111'
Chloropromazine	0.793	'1111'
Diphenhydramine	0.674	'1111'

CONCLUSION

In the present study, based on the selected template 3HL4, the 3D structural model for PfCCT is predicted and validated. Further molecular docking of 12 natural compounds with our modeled target structure reveals that the binding affinity of compound Quinidine with PfCCT is high showing significant interactions suggesting their inhibitory activity. The pharmacophore results also indicate that the compound Quinidine is well matched with the obtained pharmacophore model features with good fitvalues. This study reveals that Quinidine is a potential inhibitors of PfCCT respectively as targeted for malaria, to act as a drug candidate. Yet pharmacological study will confirm it to be promising in future.

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