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Homology Modeling and Docking Study on Plasmodium Protein Farensyl Transferase: A Viable Target to Combat Malaria

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ABSTRACT

The contemporary therapeutic demand is to develop a new antimalarial agent with a novel mode of action. Recently, the *Plasmodium* protein farensyl transferase (Pf-PFT) has been suggested as a viable target to develop new and alternative antimalarial agent. The present work describes the homology modeling of Pf-PFT and docking study to provide a structural basis for the design of new Pf-PFT inhibitors and antimalarial agents. Homology model of Pf-PFT was developed using X-ray structure of Rat-PFT. The sequence alignment was done using ClustalW. The homology model of Pf-PFT was generated using Modeller 9v7. The outliers were identified using PROCHECK and were minimized by energy minimization. The docking study of two potent Pf-PFT inhibitors (BMS-214662 and SCH66336) were performed using GOLD5.0.1 software. The Pf-PFT is a heterodimeric protein which consist of α and β chains. The sequences of target and template were aligned using ClustalW and refined manually. Twenty five models of Pf-PFT were developed and the model with least objective function was selected for validation with PROCHECK and WHATIF. The docking of BMS-214662 and SCH66336 at substrate binding site of Pf-PFT identified important ligand-receptor interactions. Both inhibitors coordinated with zinc cofactor and adopt the conformations sufficient to inhibit substrate-Pf-PFT binding. A 3D-conformation of Pf-PFT was developed and validated by standard protocol. The docking study highlighted the crucial amino acid residues involved in drug-receptor interactions. The Pf-PFT model may be useful to design and develop new Pf-PFT inhibitors and anti-malarial agents.

1. INTRODUCTION

Malaria is a vector borne parasitic disease, mainly transmitted by *Anopheles* species of female mosquitoes. It is prevalent in tropical and sub-tropical regions like sub-Saharan Africa, South Asia, and parts of South America, and kills approximately 800,000 people every year.[1] Malaria is both curable and preventable but still it is 5th leading cause of death from infectious diseases worldwide. The most clinical cases of malaria have been observed due to the infection with *Plasmodium falciparum.* Early diagnosis and treatment with effective antimalarial drugs not only shorten the duration of malaria but also reduce the incidence of complications and risk of death. The diverse classes of compounds like quinines, artemesinins, 4-aminoquinolines, sulphonamides and antifolates etc. have been used in alone or in combination for effective treatment of malaria. The irrational use of antimalarial drugs led to development of drug resistance strains of *Plasmodium* parasite. This greatly reduced the effectiveness of antimalarial chemotherapy. Therefore alternative drugs which act via novel targets are required to control the morbidity and mortality caused by malaria.

In last decade, the *Plasmodium falciparum* Protein farnesyltransferase (Pf-PFT) has been emerged as a potential target for inhibition of malaria parasite. The Pf-PFT catalyzes the transfer of the 15-carbon farnesyl group from farnesyl-pyrophosphate (FPP) and while another enzyme,

protein geranyl-geranyltransferase-1 (PGGT-I) catalyzes the transfer of the 20-carbon geranyl group from geranyl-pyro phosphate (GPP) to the SH group of the tetrapeptide motif CaaX (C is cysteine, aa is usually but not necessarily an aliphatic amino acid, and X is a variety of amino acids) present at the C-terminus of many essential signal transduction proteins, including members of the Ras superfamily.[2] If X is Ser, Met, Ala, or Gln, the protein is processed by PFT while Leu at this positions directs modification by PGGT-I. PGGT-I attaches geranylgeranyl groups to two C-terminal cysteines in Ras-related GTPases of a single family, the Rab family (in lower eukaryotes) that terminates in Cys-Cys or Cys-X-Cys motifs.[3]

The PFT and PGGT-I are zinc metalloenzymes. The zinc is required for substrate binding in both PFT and PGGT-I.[4] The PFT is involved in many vital functions including cell cycle, apoptosis, glycogen metabolism and cellular framework.[5-7] The studies have shown that PFT activity is present in *P. falciparum* while the PGGT-1 activity is absent.[8] This has led to identification of Pf-PFT as a viable target for the development of new antimalarial agents. The 3D structure of the Pf-PFT is not available from Protein Data Bank (www.pdb.org) which hampers the structure based design of selective Pf-PFT inhibitors.[9] In this view, the present work was undertaken to develop the 3D structure model of Pf-PFT. The model will serve as a prototype for the docking study to develop new Pf-PFT inhibitors and antimalarial agents.

2. MATERIALS AND METHODS

The primary amino acid sequence of the Pf-PFT was obtained from the central repository of protein sequence, Uniprot (www.uniprot.org) having accession code: A0FDU3 for α-chain and A0FDU2 for β-chain. The obtained sequences of Pf-PFT was characterized using Expasy-ProtParam tool.[10]

Template identification: Basic local alignment search tool (BLAST) was used to explore proteins similar to the Pf-PFT in Protein Data Bank (PDB).[11] The X-ray structure of Rat-PFT (PDB ID: 1JCR; resolution 2.0Å)[12] was identified and selected as a template for development of homology model of Pf-PFT (Target).
Sequence alignment: The sequence alignment of target and template was performed using ClustalW module of Bioedit software using default parameters and refined manually.[13,14] ClustalW utilize the profile-based progressive alignment procedure for the alignment of the sequences. The alignment used for the model generation step is shown in Figure 1.

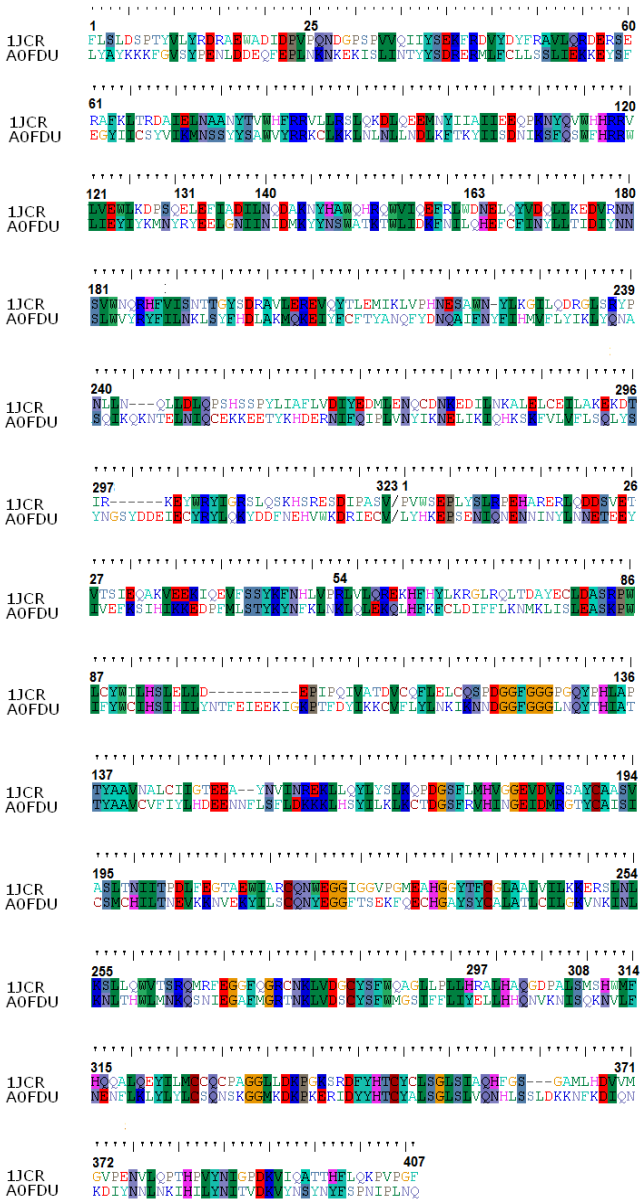

Figure 1. Sequence alignment of Rat-PFT (1JCR) and Pf-PFT (A0FDU)

2.1 Model generation

The model of Pf-PFT was developed using Modeller 9v7.[15] Modeller is rigorously used program for homology modeling of proteins. Modeller automatically derives the restraints from the provided known related structures and their alignment with the target sequence and thus it satisfies the distances, angles, dihedral angles and other spatial features of atoms. The generated model was validated by PROCHECK and WHATIF programs.[16,17] The model was refined by energy minimization by standard procedure as defined in Molecular Operating Environment (MOE 2010.10).[18]

2.2 Molecular docking study

The site finder module of MOE was used for the identification of the substrate-binding site. The two potent Pf-PFT inhibitors and antimalarials agents (BMS214662 and SCH66336) were selected for docking study using GOLD (Genetic Optimization of Ligand Docking) software.[19] The GOLD uses a genetic algorithm for the generation of different conformations of a ligand. The Goldscore was used for the ranking of docked conformations of inhibitors.[20] The Goldscore is force field based scoring function comprises of four components such as external hydrogen bond energy, external van der Waals (vdw) energy, ligand internal vdw energy and ligand internal torsional strain energy. The search algorithm was kept slow to get accurate results. For each inhibitor, ten binding conformations were generated. The conformation with highest Goldscore was selected as the optimum binding pose (best fit conformation). The optimum binding pose was selected on the basis of pose reproducibility and visual inspection.

3. RESULTS AND DISCUSSION

The α-chain contains 521 amino acid residues with an estimated molecular weight (MW) and isoelectric point (Pi) 63880.3D and 5.29 respectively. The β-chain contains 923 amino acid residues with MW and Pi 109815.6D and 6.93 respectively. The Grand average of hydropathy (GRAVY) index values of α-chain and β-chain were found to be -0.661 and -0.597 respectively, which show the hydrophilic nature of the protein.

3.1 Sequence alignment

The accuracy of the homology model depends upon the extent of sequence identity and similarity between the target and template sequences. A large difference was observed in the number of amino acid of Pf-PFT and Rat-PFT. The α and β chain of Rat-PFT contains 377 and 437 residues respectively. The sequences of α and β chains of the Pf-PFT (Target) was aligned with respective chains of the Rat-PFT (Template). Only regions with reasonable reliability of the alignment were used in model building (Table 1). The level of sequence identity (similarity) between Pf-PFT and Rat-PFT in these regions amount to 21%(45%) for the α -chain and 34%(55%) for the β -chain. The identity in binding region is relatively high i.e. 42% (Figure 1). The alignment used in study is shown in Figure 1.

3.2 Model development

The Modeller program builds a 3D structure model of proteins by satisfying their spatial restraints. The initial Pf-PFT model was prepared which contains α and β chains, a metal atom zinc, a Farensyl Pyrophosphate (FPP) and six structurally conserved water molecules. The model was subjected to the loop optimization using Modeller. The loop optimization method relies on an atomistic distance-dependent statistical potential of mean force for non-bond interactions.[21] The Modeller generates a define number of loop optimized models. Each loop model takes the initial loop conformation and randomizes it by ±5Å in the Cartesian directions. The model is then optimized thoroughly twice, initially considering the loop atoms only and then with these atoms "feeling" the rest of the system. Total twenty five models were generated by loop modeling procedure. The model with Least Objective Function (LOF =2447.11) was selected for validation.

Table No. 1. Regions of Rat-PFT and Pf-PFT sequence which were used to develop homology model of Pf-PFT

α- chain		β- chain	
Rat-PFT	**Pf-PFT**	**Rat-PFT**	**Pf-PFT**
1-25	6-30	1-26	324-349
26-131	65-170	27-54	380-407
132-140	269-277	55-297	421-675
141-163	311-333	298-308	770-780
164-323	346-515	309-407	801-902

A Ramachandran plot of the model showed 11 residues in disallowed region (11 outliers). These outliers were selected and energy minimized using Assisted Molecular Binding with Energy Refinement (AMBER99) forcefield in MOE software. The refined model left with only 7 outliers (Figure 2). The calculated Cα-RMSD with respect to template was found to be 0.547Å (Figure 3).

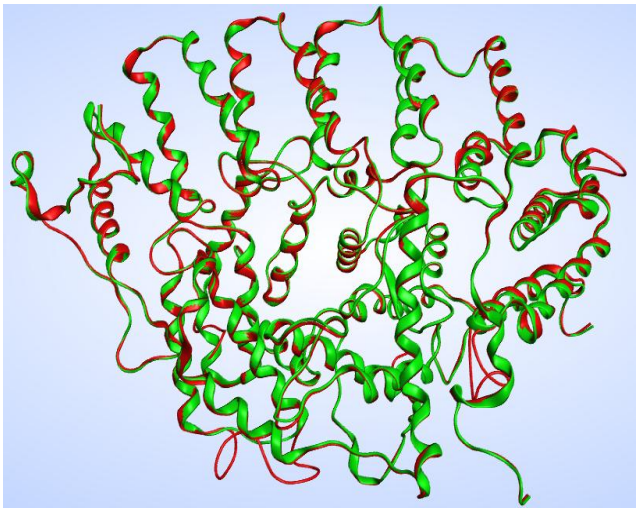

Figure 2. Ramachandran Plot of the Pf-PFT model

3.3 Structure validation

The model was validated for geometry, stereoquality and electronic distribution. The PROCHECK assess the stereochemical aspects of the energy refined model. The Ramachandran plot showed that 86.5%, 11.7%, 1.0%, and 0.8% of residues are present in most favored region, additionally allowed, generously allowed and disallowed region respectively (Figure 2). In total, 99.2% of the residues are in the most favored and allowed regions. In WHATIF, the RMS Z-score of various geometric features of the model were near to 1.0, which ensures geometric reliability of the model (Table 2). The Z-score value (Z-score = -3.67) reflects the good overall structural integrity of the model. The model has almost normal distribution of residue types over the inside and the outside of the protein. The validated model was used in molecular docking study.

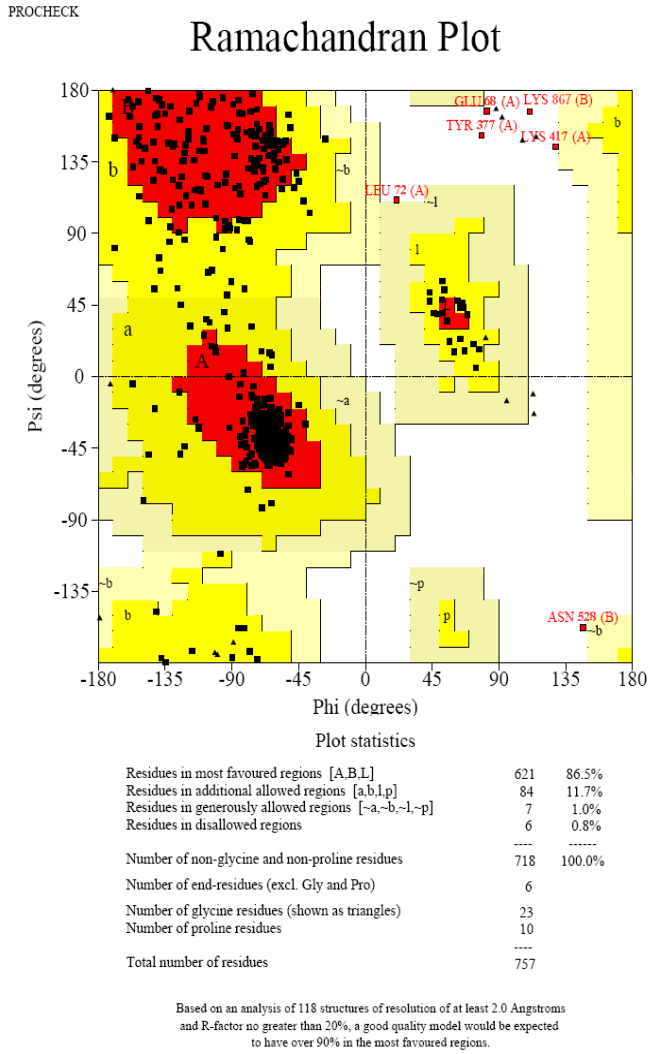

Figure 3. Overlay of 3D conformation of Pf-PFT model (red) and template 1JCR (green)

Table No 2. RMS Z-score of the different geometric features of the model

Geometric feature	RMS Z-score	Geometric feature	RMS Z-score
Backbone-backbone contacts	–0.249	Bond angles	1.416
Backbone-sidechain contacts	–0.255	Omega angle restraints	1.011
Sidechain-backbone contacts	–0.670	Side chain planarity	0.544
Side chain-sidechain contacts	–0.559	Improper dihedral distribution	1.251
Bond lengths	0.896	Inside/outside distribution	1.220

3.4 Pf-PFT binding site

The Pf-PFT contains a well defined binding cavity which is composed of four sub-pockets. The first pocket contains Zn^{++} ion coordinated with Cys659, Asp657 and His838. The second pocket is a hydrophobic cavity formed by Trp456, Trp452 and Tyr837. The third pocket is formed by Lys314 and Tyr316. The fourth pocket is a hydrophilic domain consists of three water molecules which make hydrogen bonds with Ser449. The Pf-PFT homology model was used in docking study with known potent Pf-PFT inhibitors, BMS214662 and SCH66336 (Figure 4).

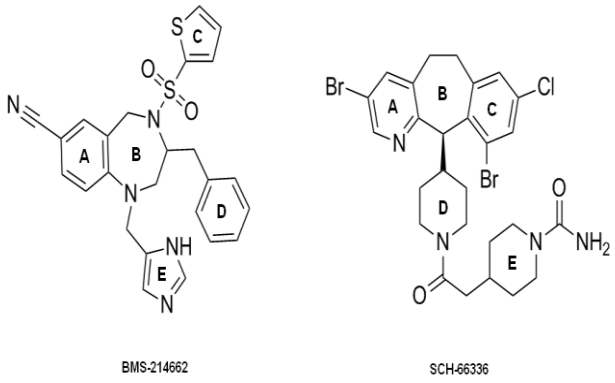

Figure 4. Pf-PFT inhibitors used in docking

3.5 Docking validation

The docking protocol was validated to determine the effectiveness of docking procedure. The X-ray conformation of

BMS-214662 complexed in Rat-PFT was extracted and docked at the Rat-PFT binding site (PDB: 1SA5). The GOLD software reproduced the conformation of BMS-214662 with RMSD = 0.400 (Figure 5).

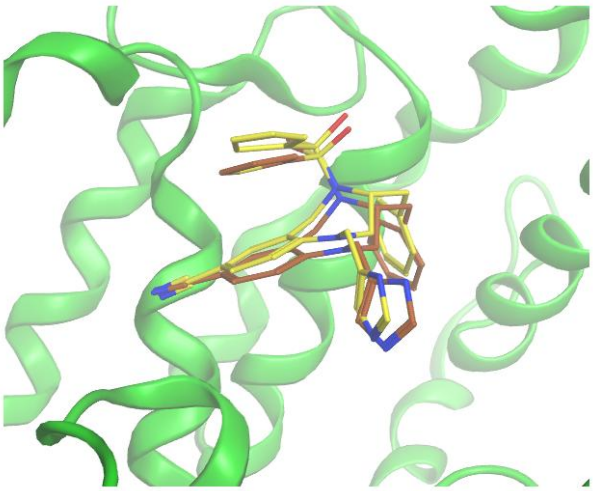

Figure 5. Overlay of complexed (yellow) and docked (brown) conformations of BMS-214662

The BMS-214662 and SCH66336 (potent Pf-PFT inhibitors) were docked in Pf-PFT model. In docked conformation of BMS-214662, the nitrogen of imidazole ring (ring E) was coordinated with Zn^{++} ion (Figure 6a). The phenyl ring (ring B) gets oriented in a hydrophobic cavity formed by Trp456, Trp452 and aliphatic chain of FPP. A π-π stacking interaction (face to face) was observed between ring A and Tyr837. The sulphonyl group positioned in a solvated region formed by structurally conserved water molecules (HOH761 and HOH762).

In the binding conformation of SCH66336, the nitrogen atom of piperidine (ring E) was coordinated with Zn^{++} ion. The bromo pyridyl (ring A) occupied the hydrophobic cavity created by Trp456, Trp452 and Tyr837. The Cl group of ring C act as H-bond acceptor and involved in H-bond interactions with HOH758 (Figure 6b).

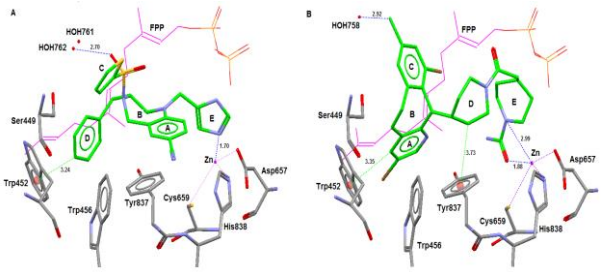

Figure 6: Docked conformation (green) of (a) BMS-214662 and (b) SCH66336

4. SUMMARY AND CONCLUSION

In summary, a 3D structure model of Pf-PFT was developed through homology modeling using X-ray structure of Rat-PFT and validated by PROCHECK and WHATIF. A flexible docking of potent Pf-PFT inhibitors i.e. BMS-214662 and SCH66336 was performed on Pf-PFT model using GOLD software. The inhibitors occupied well at the substrate binding site in Pf-PFT. Both inhibitors were found coordinated with Zn^{++} ion. The hydrophobic part of inhibitors (ring B of BMS-214662 and ring A of SCH66336) gets positioned in a hydrophobic cavity formed by Tyr837, Trp452 and Trp456. The conserved water residues, HOH758 and HOH762 have shown hydrogen bond interaction with inhibitors. This developed model can be used in structure based drug-design of new Pf-PFT inhibitors. Furthermore, it may be helpful in virtual screening to identify new leads/compounds as Pf-PFT inhibitors.

5. ACKNOWLEDGEMENT

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