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Homology Modeling and Docking Studies of Pfmc-2TM Maurer's cleft two trans-membrane protein –A Potential Drug Target in Malaria

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Abstract : Malaria is an important disease of the developing world. Around 1-3 million people die annually due to the disease. Due to multi-drug resistance exhibited by the malarial parasite, *Plasmodium falciparum*, there is an urgent need to explore new drug targets in malaria. Maurer's clefts are parasite-derived structures found in host cell cytoplasm and function as compartments between the parasite and the erythrocyte membrane. P*f*mc-2TM, Maurer's cleft two transmembrane protein, is present in the trans-membranous network of the infected erythrocyte. The aim of the work is to determine 3D structure of P*f*mc-2TM protein and to perform docking studies with natural molecules. Since the crystal structure of P*f*mc-2TM protein of P. falciparum is not yet available, its three-dimensional structure was elucidated by homology modeling using different modelling tools like Schrodinger, Swiss model and I-tasser softwares. The constructed model revealed appreciable measures when validated. The validation of the protein was done using Procheck and Errat. After the development of the model the structure was further subjected to virtual screening with the library of natural compounds present in the ZINC database. As the binding site of the target protein was not known, it was predicted using sitemap and the entire library was screened against the target. From the entire library of compounds, 276784 compounds were screened using HTVS for the first level of screening. The binding affinity of the compounds was studied using XP (Extra Precision) algorithm of Glide Docking. On the basis of docking scores, 164 compounds were selected for further analysis. The binding affinity was further calculated using MMGBSA. The interaction studies using molecular docking and MMGBSA revealed appreciable results. The high quality homology model of P*f*mc-2TM protein could be further exploited using structure based drug design techniques to impel the search of new and efficient ligands.
Keywords : Homology modelling, Virtual screening, Binding affinity.

1. INTRODUCTION

Malaria is most commonly caused by the parasite *Plasmodium falciparum* which obtains nutrients from the infected human erythrocyte and escapes the host immune system. During infection, the parasite transfers more than 10% of its protein in to the host cytosol. Maurer's clefts are the parasite derived membranous structure that comprise of this unfamiliar protein trafûcking system. Maurer's clefts are resultant structure that are present in the host cell cytoplasm of the parasite and its function is to categorize a partition in the parasite and the membrane of the erythrocyte.

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Plasmodium falciparum modifies the interior of the host cell after the incursion (1), forms a parasitophorus vacuole which is positioned beneath the infested erythrocyte membrane (2). These type of structures, recognized as Maurer's clefts, contribute in arrangement of proteins meant for distribution to the infested erythrocyte membrane and cytosol (4,5,7,8). Both vesicular transport and adjacent protein dispersion are used as a resource for the protein transport (6,9,11,12). Maurer's clefts have different morphological structures and are dispersed in the infested erythrocyte cytoplasm. Clefts are found in different positions in cytoplasm and allied by stalks and tethers with the membrane of the erythrocyte and and parasitophorus vacuole membrane (14). Maurer's clefts are evolutionarily conserved parasite-induced structures in infected erythrocytes (15).

Maurer's clefts are essential for host transformation and work as intermediate of transport in the erythrocyte cytoplasm for soluble proteins and membrane proteins. Protein trafficking system involves long-known parasite derived membranous structures in the host cell cytosol (16). The 130kD P*f*mc-2TM protein is an important component of the Maurer's cleft and inhibiting it can be important step for designing the new possible inhibitors for the malaria. In the present work, a homology model of the protein and a potential natural compound showing reasonable binding affinity to the binding site has been proposed.

2. MATERIAL AND METHODS

Homology Modelling and Validation of P*f*mc-2TM

3D structure of P*f*mc-2TM Maurer's cleft two transmembrane protein was derived using Prime (Schrodinger) software. Protein 3D model predicted from molecular modeling may be inaccurate. So it is very important to check the overall quality of the model, The predicted model was validated on the basis of energetic, stereochemical considerations and tertiary structure analysis. The model was validated using Procheck and Errat (17,18).

Preparation of protein

P*f*mc-2TM protein was prepared by using multistep protein preparation tool in Maestro. H- atoms were added and all water molecules were removed from the model using Maestro. Energy minimization was done upto 5000 steps using OPLS 2005 force field (19).

Ligand preparation

Zinc database is a freely available database of commercially available compounds that can be used for virtual screening. A library of biogenic compounds of 1,89466 compounds was chosen. Bond order was assigned to each structure using Ligprep. Different tautomeric forms were produced from all input structures. All possible stereoisomers for each ligand were generated by the program. With the help of Ligprep, a low energy conformation for each structure was produced. Molecular mechanics energy minimization was done for all the structures using Impact .Using 2005 OPLS force field all the structures were optimized which were further used for Glide docking.

Molecular docking

At the centroid of P*f*mc-2TM, binding site receptor grid file was generated using Glide. For P*f*mc-2TM, a box of size 10 Å x 10 Å x 10 Å was defined in the center of binding site for the binding of docked ligand. To occupy all the atoms of the docked poses one more enclosing box of 10 Å x 10 Å x 10 Å was also defined. Using Glide XP (extra precision) all the prepared ligands were docked and then evaluated using Glide XP Scoring.

3. RESULTS AND DISCUSSION

Homology modeling of P*f*mc-2TM

As the x-ray crystal structure of P*f*mc-2TM was not available, it was generated using homology modelling. The constructed model of P*f*mc-2TM was prepared using PRIME module of Schrodinger suits (Figure 1). The crystal structure of sugar phosphate isomerase/epimerase (3L23_A) with 1.7 Å resolution was used as the template for homology modelling. Using homology building method, the missing amino acids were filled in the template structure.

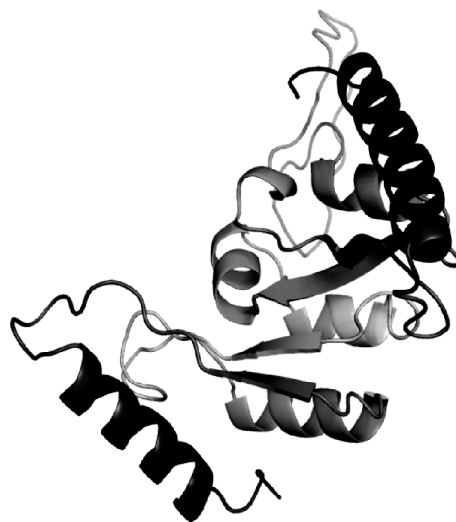

Fig. 1. Homology model of P*f*mc-2TM .

Validation of the predicted model

By using PROCHECK and ERRAT the validation of the predicted model was carried out. The PROCHECK results represent 85.5% amino acids in allowed regions. Ramachandran plot showed 0.6 residue in the disallowed region, 12.1% residues in additionally allowed regions and 1.8 % residues in generously allowed regions as shown in figure 2. The ERRAT score was 92.121% (as shown in figure3). Thus the overall quality of the P*f*mc-2TM model generated by homology modelling was very good.

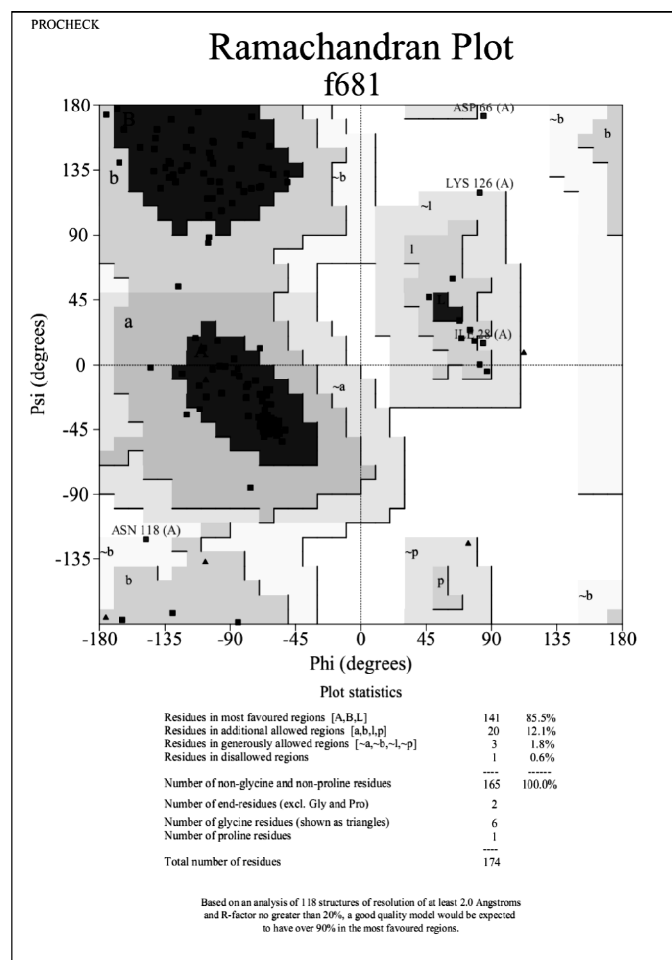

Fig. 2. Ramachandran plot for the validation of modelled P*f*mc-2TM protein

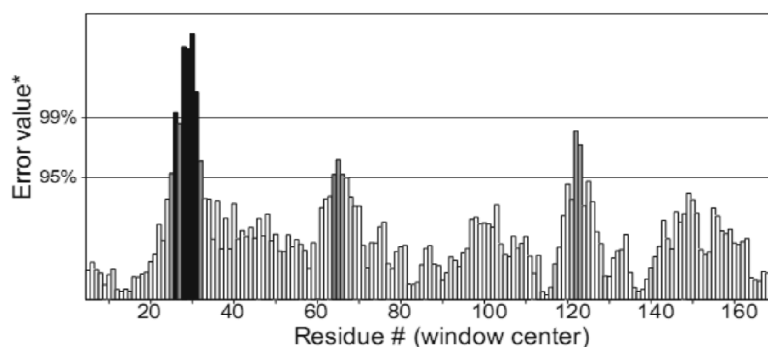

Fig. 3. ERRAT graph for the validation of modelled P*f*mc-2TM protein

Binding site prediction

The binding site of the P*f*mc-2TM protein was predicted using Sitemap, based on several physico-chemical properties. Site1 with the best sitemap score was selected. The residues predicted by Sitemap are PHE-42, MET-44, LYS-60, ASN-61, THR-62, LEU-63, LEU-64, ASN-68, ASN-94, GLU-95, GLU-96. These residues were present in the binding site of the Pfmc-2TM.

Binding affinity of P*f*mc-2TM protein

The binding site was studied for interaction with the entire library of compounds present in ZINC database of natural compounds using Glide XP docking. The whole library was screened against the target. From the entire library of compounds, 276784 compounds were screened using HTVS for the first level of screening. The binding affinity of the compounds was further studied using XP (Extra Precision) algorithm of Glide Docking. On the basis of docking scores, 164 compounds were selected for further analysis. The interaction studies using molecular docking revealed appreciable docking scores The 10 best compounds showing promising leads with appreciable docking scores in the range of -7.561 to -5.328 as shown in Table 1.

Table 1. Table showing Glide energy and Docking score and MMGBSA score.

S. No.	*ZINC ID*	*GLIDE energy*	*Docking Score*	*MMGBSA*
1.	ZINC12503216	-43.937	-5.581	-81.06
2.	ZINC64220137	-36.907	-5.122	-76.913
3.	ZINC12496233	-35.241	-7.427	-71.645
4.	ZINC12496087	-43.708	-5.547	-73.639
5.	ZINC02566274	-47.18	-7.561	-72.61
6.	ZINC04096987	-34.835	-6.461	-70.852
7.	ZINC64220136	-34.502	-6.02	-76.416
8.	ZINC77257360	-33.881	-6.53	-66.951
9.	ZINC33955230	-37.125	-6.095	-79.038
10.	ZINC15111008	-33.255	-5.328	-77.526

Binding affinity calculation

The binding affinity was further calculated using MMGBSA. The interaction studies using molecular docking and MMGBSA revealed appreciable docking scores and ÄG_{bind}. The 10 best compounds demonstrated appreciable ÄG_{bind} score from -81.06 to -77.526 kcal/mol. Analysis of the binding mode of Pfmc-2TM showed change in H-bond pattern in the binding pocket of all ligands. H-bond has been observed between the ZINC12503216 and ASN-94, ARG-43 and GLY-39 in the binding pocket of P*f*mc-2TM as shown in figure 4 *a*,*b*.

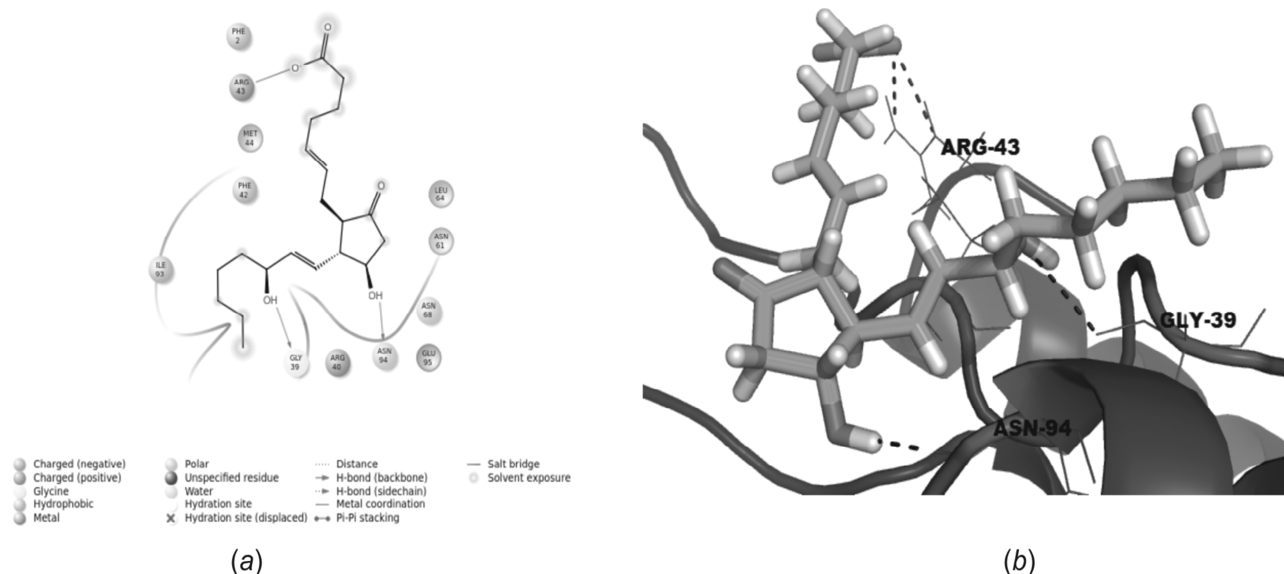

Fig. 4. Ligand interaction with P*f*mc-2TM protein (*a*) 2-Dimensional (*b*) 3-Dimensional ligand interaction.

Per residue energy contribution

For further studies about the interaction of Pfmc-2TM with ZINC12503216 ligand, per residue energy contribution van der waals (E_{vdw}) and electrostatic (E_{ele}) energy was calculated. Active site residues showed significant contribution to the E_{vdw} as well as E_{ele} energy in ligands as shown in Table 2 Specifically MET-44, PHE42 and ASN-94 amino acids have an appreciable E_{vdw} energycontribution and ARG-43, ARG-40 and LYS-65 amino acids have an E_{ele} energy contribution as shown in fig 5 (*a*), (*b*). The specificity of P*f*mc-2TM with different ligands was demonstrated by binding affinity and interaction pattern of P*f*mc-2TM.

Table 2. Table showing per residue energy contribution for van der waals (E_{vdw}) and electrostatic (E_{ele}) within 12 Å of docked ligands.

Residues	*E_{vdw} (kcal/Mol)*	*Residues*	*E_{ele} (kcal/Mol)*
ASN94	-2.273	LYS100	-5.574
ASN68	-0.537	LYS69	-11.787
ASN61	-1.233	LYS65	-13.486
MET44	-3.446	LYS60	-10.931
PHE42	-2.765	ARG43	-53.361
ARG44	-1.974	ARG40	-17.932

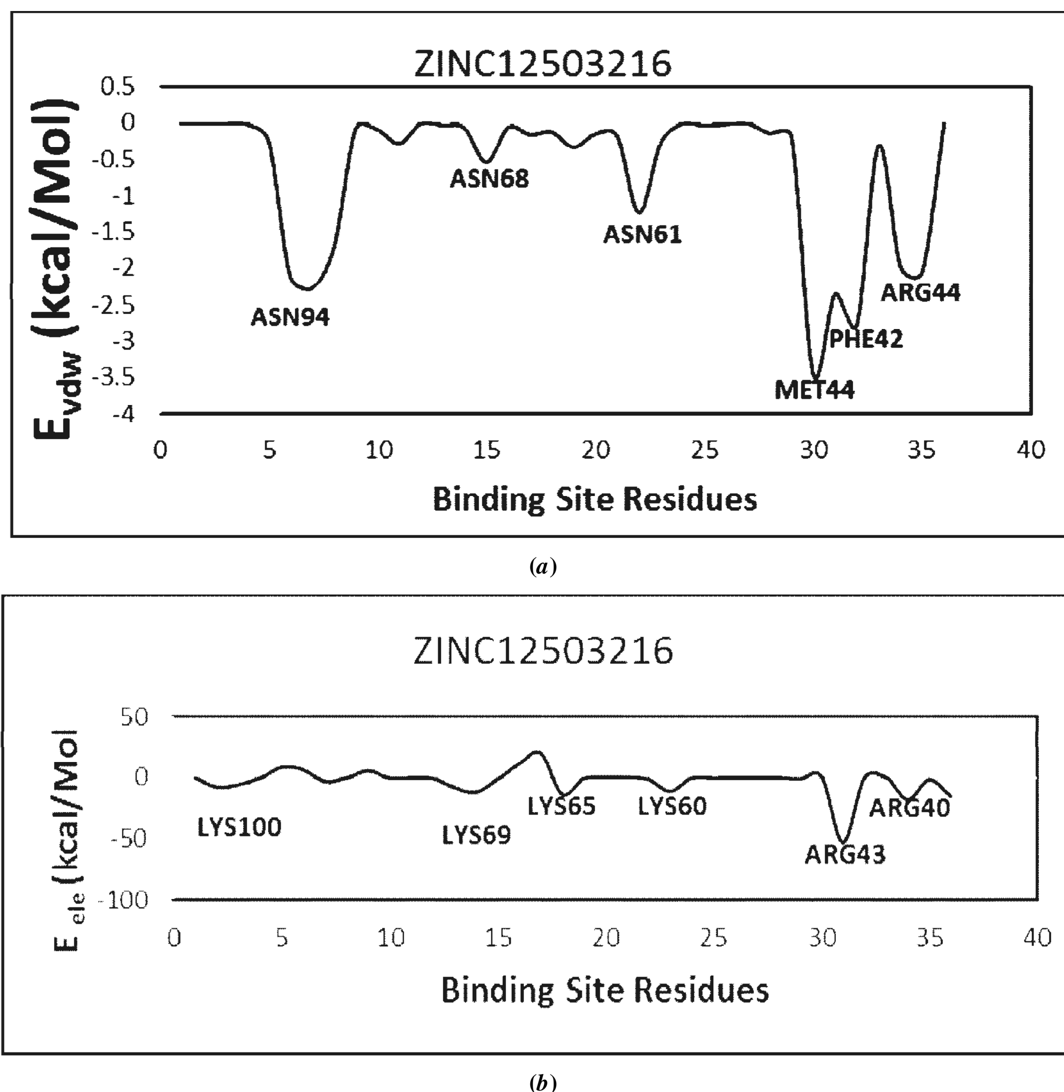

Fig. 5. Table showing per residue energy (*a*) Evdw and (*b*) Eele

5. CONCLUSION

In this study, the 3D structure of Pfmc-2TM protein was elucidated using various homology modelling software. Homology model was validated to ensure the quality by Procheck and Errat, both of which reported a high quality model. Virtual screening of the biogenic compounds of ZINC database was performed. Extra precision docking of a few selected compounds yielded appreciable docking score and binding affinity. The interaction studies using molecular docking and MMGBSA revealed appreciable docking scores and ÄG $_{bind}$. The per residue van der Waals (E_{vdw}) and electrostatic (E_{ele}) energy contribution of the binding site residues depicted important interactions between Asn 94, Met 44, Phe 42 and Arg 43 of the protein and various positions on the ligand. Our data thus show compelling indication that the purposed ligands hold considerable potential for further experimental evaluation.

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