IJCBS RESEARCH PAPER VOL. 2 [ISSUE 6] SEPTEMBER, 2015 ISSN: - 2349–2724

HOMOLOGY MODELLING AND DOCKING STUDIES FOR LANOSTEROL 14-ALPHA DEMETHYLASE OF CANDIDA ALBICANS AND 1,2,4-TRIAZOLE CLUBBED 1,3,4-OXADIAZOLE DERIVATIVES

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ABSTRACT: Candida albicans is one of the most common causes of invasive fungal infections. Newly synthesized Triazole-Oxadiazole derivatives acts as antifungal agents which inhibit the cytochrome P450 sterol 14-alpha-demethylase (CYP51). Lanosterol 14-alpha-demethylase is the target of azole antifungal agents. This study describes building 3D model structure of cytochrome P450 lanosterol 14-alpha demethylase of candida albicans from saccharomyces cerevisiae by using 3LD6 as a template. The reliability of the models was assessed by Ramachandran plots and Profile-3D analysis. Molecular docking identified the binding mode of the triazole-oxadiazole derivatives with modelled CACYP51. In docking studies N-4 nitrogen of the 1,2,4-triazole interacts with the heme portion of porphyrin ring of target receptor along with hydrogen bonding, pi-stacking, and hydrobhobic bonding. Among all derivatives APC-1, APC-3, APC-7 were found to have significant interactions with active site of receptor by calculating dock scores. Based on the results of this studies it can be concluded that structural model of candida albicans can be used in optimization and designing of newer antifungal agents.

Keywords: Homology modelling, Molecular Docking, Triazole-Oxadiazole derivatives, Lanosterol 14-alpha demethylase, Candida albicans.

1. INTRODUCTION

Over the past decade, fungal infections have become a major complication and cause of morbidity and mortality in immunocompromised individuals such as those suffering from tuberculosis, cancer, acquired immune deficiency syndrome (AIDS), and in organ transplant cases. [1-3] The azoles are a large and relatively new group of synthetic compounds. Imidazoles and triazoles are two azole derivatives employed in the treatment of systemic fungal infections as well as in the agriculture. [4-6] Azole antifungal agents inhibit the cytochrome P450 sterol 14 alpha-demethylase (14DM, CYP51) by a mechanism in which the heterocyclic nitrogen atom (N-4 of triazole) binds to the heme iron atom in the binding site of the enzyme. Lanosterol-14a-demethylase (CYP51) is a key enzyme of sterol biosynthesis in fungi. [7] The resulting ergosterol depletion and the accumulation of precursor 14 alpha-methylated sterols disrupt the structure of the plasma membrane, making it more vulnerable to further damage, and alter the activities of several membrane-bound enzymes. [8-9] The efficacy of azoles depends on the strength of the binding to heme iron as well as the affinity of the N-1 substituent for the cytochrome protein. [10] Because of the existence of CYP51 in fungi and mammals and the effects of these compounds on CYP3A4, the selective inhibition of 14 alpha-DM in the fungi is very important and results in an increased therapeutic index [11-14] However, the extensive use of azoles has led to the development of severe resistance [15-16] , which has greatly reduced their efficacy. In response to these limitations, the development of new drugs to optimally treat the fungal infection has been strongly advocated. Thus, the search for new antifungal drugs continues to be an active area of investigation in

medicinal chemistry. Docking studies are used at different stages in drug discovery such as in prediction of docked structure of ligand– receptor complex and to rank the ligand molecules based on their binding energy. Docking protocols aid in the elucidation of the most energetically favorable binding pose of a ligand to its receptor. Docking studies requirements are a model of the protein (receptor) and ligand. Due to the importance of cytochrome P450 sterol 14alpha-demethylase (14DM, CYP51) in antifungal drug studies, it is very important to know the three dimensional (3D) structures of 14DM, particularly, the CYP51s from pathogenic fungi. However, eukaryotic CYP51s are membrane-associated proteins and solving their crystal structures remains a challenge. Crystal structures of P450 proteins have been used as templates to construct the 3D models of fungal CYP51s. [17-24]

In the present study, we report the construction of the 3D structure of candida albicans by homology modeling using the sterol 14 alpha- demethylase (CYP51) from Saccharomyces cerevisiae 3LD6 as a template after that, We docked rationally designed triazole-oxadiazole derivatives (ligands) on model receptor, in order to clarify the binding mode, binding energies and the important residues involved in binding of receptor with the ligand.

2. EXPERIMENTAL

Homology modeling:

The 3D model of cytochrome P450 Lanosterol 14alpha-demethylase of *C.albicans* (CACYP51) was built using homology modeling. Amino acid sequence of enzyme was obtained from the Universal Protein Resource (http://www.uniprot.org/) (Accession Code: P10614), and sequence homologous was obtained from Protein Data Bank (PDB) using Blast search. In literature, the structure of cytochrome P450 lanosterol 14alpha-demethylase was developed homologically using crystal structure of lanosterol 14alpha-demethylase from Saccharomyces cerevisiae (S288C) as template (619 amino acid residues). Based on the result of blast search, we used the crystal structure of Saccharomyces cerevisiae (S288C) lanosterol 14 alpha-demethylase (CYP51) with intact transmembrane domain bound to Ketoconazole as a template for homology modeling (PDB ID,3LD6, RESOLUTION 2.8A^0). These procedures are performed by VLife MDS 4.3 software.

Model Validation:

The chosen model was subjected to energy minimization and molecular dynamics simulations to obtain a stable and low energy conformation. The quality of the final refined model was assessed by a series of tests for its internal consistency and reliability. Finally, the best quality model of C. albicans S288C was subjected to further calculations and molecular modeling studies. The final refined model of CACYP51 is validated and evaluated by calculating the Ramachandran plot, and RMSD. Further then, by analysing ramachandran plot core region has obtained.

Root mean square deviation (RMSD) is obtained by superimposition of 3LD6 (template) and modelled CACYP51 using VLife MDS 4.3.

Docking tool and algorithm

Molecular Docking studies and conformational analysis were performed by using the Molecular Design Suite (VLife MDS software package, version 4.2 ; from VLife Sciences, Pune, India). The docking algorithm Biopredicta is based on a genetic algorithm which offers a successful strategy for globally searching the docked conformer's space. Genetic algorithms allow a population of solutions to exist and in each 'generation' these can evolve by processes such 'breeding' and 'mutation'. Poor solutions are killed off, while good ones leave their offspring in future generations. Such algorithms may typically reach an excellent solution is a few tens of generations.

Ligand generation and Optimization

Structures of compounds were sketched using the 2D structure draw application Vlife2Ddraw and converted to 3D structures. All the structures were minimized and optimized with the AMBER method taking the root mean square gradient (RMS) of 0.01 kcal/mol A° and the iteration limit to 10,000. Conformers for each structure were generated using Monte Carlo be applying AMBER force field method and least energy conformer was selected for further study.

3. RESULTS AND DISCUSSION

Homology Modeling of Cytochrome P450 Lanosterol 14a- demethylase of *C. albicans*

Accuracy and presicion of homology model is closely related to the degree of sequence identity and likeliness between template 3LD6 and target i.e. lanosterol 14 alpha demethylase. Selection of suitable template and an optimal sequence alignment leads to success of homology model. Sequence alignment of 3LD6 (Chain A & B) was done by using VLife MDS 4.3. It shows 39% identity, 64% positives and 8% gaps with target sequence. Furthermore, protein energy minimization and loop refinement of developed homology model was carried out by applying MMFF force fields and smart minimization algorithms followed by conjugate gradient algorithms until convergence gradient was satisfied.

Fig.1. Shows alignment between query and template sequence, Gaps are represented by dashes. Numbers to the right and left of the sequences are the amino acid numbers.

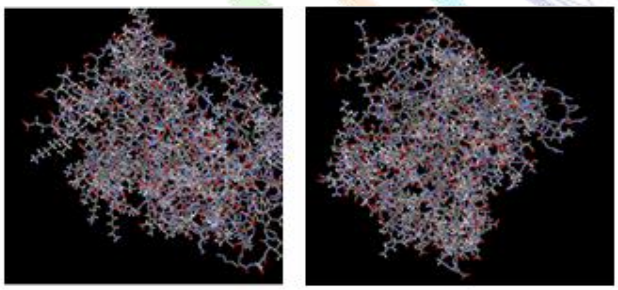
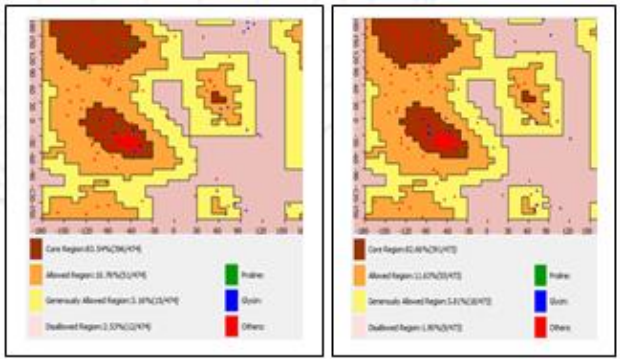


Fig. 2. Homology Model of Lanosterol 14- alpha demethylase of *Candida albicans for* 3LD6 Chain A and Chain B.

After that, analysis by ramachandran plot for developed homology model of lanosterol 14-alpha demethylase was done for 3LD6 (Chain A And Chain B) [core regions-83.54% and 82.66% for chain A & B resp.] **(Table 1)** This was calculated between the main chain atom of model and template. It showed close homology. This ensured the reliability of the model. These score percentage show the overall quality of the modelled structure of Lanosterol 14-alpha demethylase of candida albicans. and it can be concluded that this model can be apply for further docking programme.

Table 1. Ramachandran Analysis of developed homology model of lanosterol 14-alpha demethylase by using template 3 LD6 chain A and Chain B showing results of Core region , Allowed region, Generously allowed region, and Disallowed region .

Sr.no.	Ramachandran analysis window	[lanosterol 14-alpha demethylase] (3LD6)	
	Regions	CHAIN A (%)	CHAIN B (%)
1.	Core region	83.54	82.66
2.	Allowed region	10.76	11.63
3.	Generously allowed region	3.16	3.81
4.	Disallowed region	2.53	1.90



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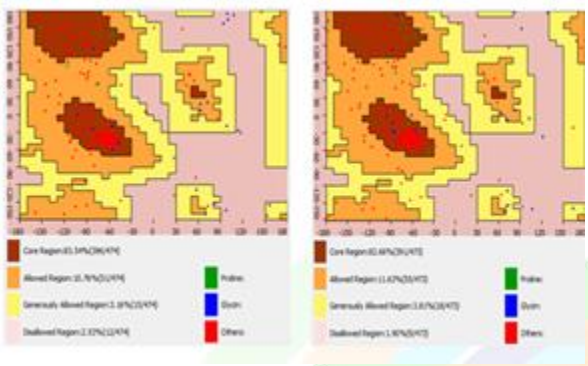

Fig. 3. Ramachandran Plot for developed homology model of lanosterol 14-alpha DM 3LD6 Chain A and chain B.

Molecular Docking

The genetic algorithm method was performed to study and predict the binding of newly synthesized compounds with the target enzyme (homology modeled) cytochrome P450 lanosterol 14 alpha-demethylase of *C. albicans*. All compounds showed binding in the active site of the receptor which reveals novel set of information. The results of the docking analysis of most active antifungal compounds and their interactions with the selected receptor proteins are discussed in the following sections.

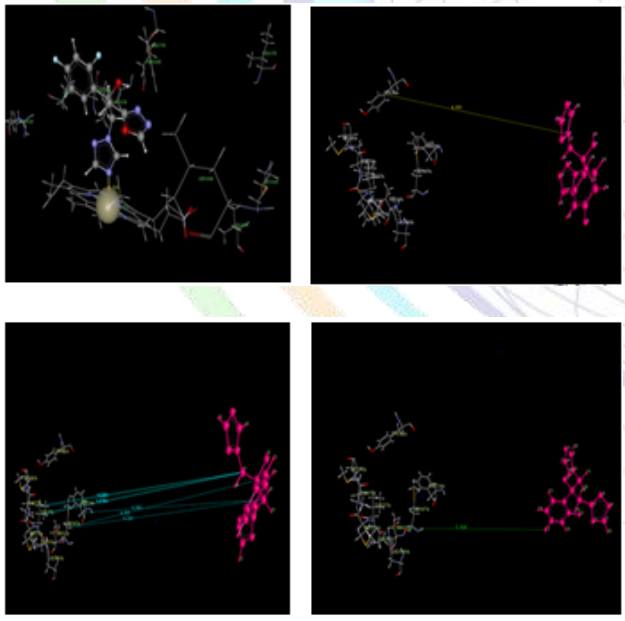

Fig. 4. Best docking poses of APC-1 on homology model of cytochrome p450 of *Candida albicans*

In figure 4,

A. Triazole ring of molecule APC-1 is positioned perpendicular to the porphyrin plane, with a ring nitrogen (N-4) atom co-ordinated to the heme iron of cytochrome p450 of *Candida albicans.*

B. Hydrogen bonding of carbonyl group of molecule APC-1 with THR318A.

C. Hydrophobic interaction of molecule APC-1 with ILE 377A, ALA 370A, MET 491A of cytochrome p450.

D. pi- Stacking of phenyl ring of molecule APC-1 with TYR 131A of cytochrome p450.

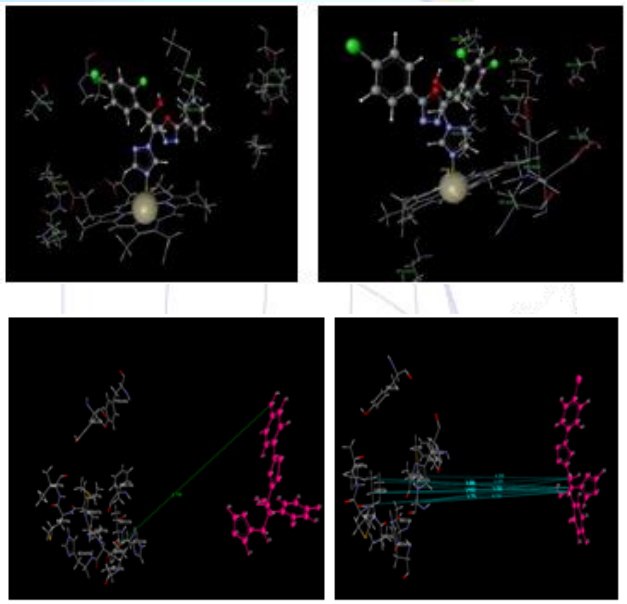

Fig. 5 . Best docking poses of APC-3 on homology model of cytochrome p450 of *Candida albicans.*

In figure 5,

A. Triazole ring of APC-3 is positioned perpendicular to the porphyrin plane, with a ring nitrogen (N-4) atom co-ordinated to the heme iron of cytochrome p450 of *Candida albicans*.

B. Hydrogen bonding of carbonyl group of APC-3 with HIS 489A.

C. Hydrophobic interaction showing side chain of APC-3 with ALA 371A, PHE 372A, MET 273A of cytochrome p450.

D. Pi- stacking of phenyl ring of APC-3 with TYR 131A of cytochrome p450.

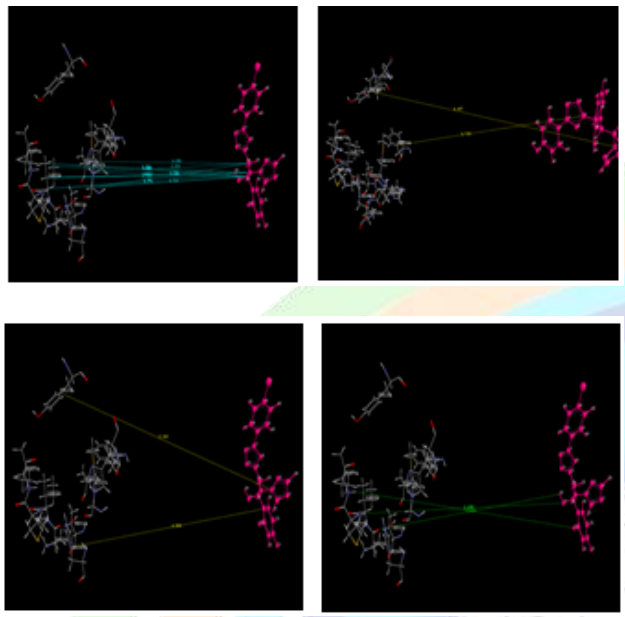

Fig. 6. Best docking poses of APC-7 on homology model of cytochrome p450 of *Candida albicans*.

In figure 6,

A. Triazole ring of APC-7 is positioned almost perpendicular to the porphyrin plane, with a ring nitrogen (N-4) atom co-ordinated to the heme iron of cytochrome p450 of *Candida albicans*.

B. Hydrogen bonding of carbonyl group of **APC-7** with PRO 376A, MET 378A, ILE 379A.

C. Hydrophobic interaction with side chain of **APC-7** with ILE 377A, MET 487A, LEU 574A, PRO 475A of cytochrome p450.

D. Pi- Stacking of phenyl ring of **APC-7** with TYR 131A and HIS 489 A of cytochrome p450.

CONCLUSION

A homologous 3D model of lanosterol 14a-demthylase from *C. Albicans* was built on the basis of the crystal coordinates of sterol 14a-demethylase from *Saccharomyces cerevisiae* S288C complex with ketoconazole. The reliability of the models was assessed by Ramachandran plots. The overall structures of the resulting CACYP51 model are similar to those of the template structures. In the docking studies, we confirmed that all nine compounds interact with the CACYP51, and triazole (N-4) - heme coordination, hydrogen bonding, pi- stacking and hydrophobic interactions. Compounds **APC-1, APC-3, APC-7** showed comparable interaction pattern as that of fluconazole. The most potent and novel 1,2,4-triazole clubbed with 1,3,4-oxadiazole derivatives resulted in this study can be subjected to synthesis and pharmacological evaluations to develop potent antifungal agent.

ACKNOWLEDGEMENT

The authors are very thankful to the management of MET'S Institute of Pharmacy for providing infrastructural facilities to carry out the research work. The authors are thankful to Dr. Kundan Ingle of Vlife sciences for his help and guidance for homology modelling and docking analysis.

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