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Research Article

DOCKING STUDY OF FLUORO SUBSTITUTED IMIDAZOLE DERIVATIVES AS 14A-DEMETHYLASE INHIBITORS

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ABSTRACT

Imidazole is one of the most explored and marketed azole, used for the treatment of fungal infections. Lanosterol 14α-demethylase (Cytochrome P450DM) is the active target site for azole antifungals. This study involved the anti-*Candidal* evaluation of a series of fluorosubstituted imidazole analogues *via* molecular docking studies. Further the model was refined by molecular dynamic simulation. The imidazole analogues were prepared using Chem sketch and molecular docking was performed using Molergo Virtual Docker program. In order to accept a molecule as a drug, the molecule should have certain properties which were predicted through Lipinski rule of five and ADMET study which was carried out using Accelry's Accord for Excel programme. The docking study indicated that all the imidazole analogues (PN1-PN24) and standard drugs i.e., Ketoconazole, Miconazole and Clotrimazole possessed interaction with protein residue, heme cofactor and water molecule positioned above Heme cofactor of 14α-demethylase.

Keywords: Antifungal agents; ADME; 14α-demethylase; Imidazole; Lipinski rule; Molecular docking; Molecular dynamic simulation

INTRODUCTION

Within past few decades the risk of fungal infections had increased significantly. Furthermore, widespread use of antifungals has also resulted in the development of resistance among the pathogenic fungal species. Thus, created a demand for the development of new effective antifungals with low toxicity profile (Mamolo, et al. 2004; Menozzi, et al. 2004 and Rossello, et al. 2002). Various research groups are working on to develop potent antifungal agents with low toxicity profile and which can be administered *via* both oral and parenteral route. Till now only five classes of antifungal agents are known namely: polyenes, azoles, allylamines, thiocarbamates and fluoropyrimidines (Rossello, et al. 2002) and the most important amongst them are the azoles.

Imidazoles represents one of the most important class of azoles possessing antifungal potency against a variety of pathogenic fungi including *Candida spp*.. However, various imidazole containing drugs such as Ketoconazole, Miconazole, Clotrimazole, Tioconazole,

Econazole, Tinidazole, Enilconazole/Imazalil, Parconazole, Eberconazole,Lanoconazole, Fenticonazole, Bifonazole, Sulconazole, Lombazole, and Sertaconazole are some of the well-known marketed antifungal drugs. It possesses both fungistatic and fungicidal property at different concentrations i.e., fungistatic at low concentration and fungicidal at high concentrations (Khan, et al. 2000; Croxtall, et al. 2009; Sud, et al. 1981 and Rani, et al. 2013).

The azoles antifungal acts by inhibiting the cytochrome P450 dependent 14α-demethylase enzyme. The enzyme catalyzes the oxidative removal of 14α-methyl group of lanosterol during ergosterol biosynthesis leading to the depletion of ergosterol and accumulation of lanosterol and other 14α-methyl sterols and results in the inhibition of fungal cell growth (Rani, et al. 2013 and Sheng, et al. 2006).

The specific inhibition of cytochrome P450 enzyme of fungus rather than human being is the basis for clinically useful antifungal azoles which is in turn dependent upon the difference between the structure of active site of fungus and the host enzyme (Tafi, et al. 1996).

Computational methodologies have become a crucial component in the drug discovery programs which involves identification of target and lead along with their ADME and pharmacokinetic studies so as to obtain a potent lead. In recent years there is extensive research going on for the identification of target and the lead for the development of newer effective anti-*Candidal* agents. Molecular modeling is one of the *in silico* method used primarily as hit identification tool when only structure of target and its active site is available (Kitchen, et al. 2004). Docking method is an energy-based scoring function which identifies the energetically most favorable ligand conformation that binds to the target. According to general hypothesis lower the energy scores, the better protein-ligand binding and thus, molecular docking can be formulated as an optimization tool which is used to find the ligand-binding having the lowest energy (Thomsen, et al. 2006).

Further, computational modeling also provides an inexpensive and fast way to assess the ADME, toxicity and potential intestinal permeability profile of the molecules and thus helps in lead identification with good ADME profile, low toxicity and good intestinal absorption (Egan, et al. 2012).

Since several studies reported that the fluoro substituted azoles are capable of enhancing the antifungal potency and moreover, Fluconazole the most important and popular azole antifungal drug possesses fluoro group in its structure, so we selected a series of halogenated imidazoles from literature and evaluated them for antifungal potency (Menozzi, et al. 2004). Furthermore, due to the presence of higher homology between the 14α-demethylase of cytochrome P450 of *Mycobacterium tuberculosis* and *C. albicans*, the crystallographic structure of 14α-demethylase of *M. tuberculosis* was taken and 12 amino acids were replaced with that of *C. albicans*. The resulting protein structure was refined by Molecular dynamic simulation and the structure was validated by Ramachandran Plot and Sequence alignment followed by the calculation of interaction energy of azoles with the active site using Molergo Virtual Docker (MVD) and calculated the Lipinski Rule of Five and ADMET using Accelry's Accord for Excel (Pitchai, et al. 2012).

In view of this and in continuation of our work on imidazoles (Aggarwal, et al. 2007; Rani, et al. 2015; Gupta, et al. 2012; Rani, et al. 2015; Rani, et al. 2012; Rani, et al. 2011; Rani, et al. 2017; Rani, et al. 2013 and Rani, et al. 2017), we herein this article report the docking Study of Fluoro substituted imidazole derivatives as 14α-demethylase inhibitors to find new and more potent antimicrobial agent.

MATERIAL AND METHODS

Protein Preparation

The cytochrome P450 14α-demethylase from *C. albicans* P450DM was chosen for the study. Since the target enzyme is a membrane-bound enzyme and is difficult to crystallize from X-ray analysis, the crystallographic structure of cytochrome P450 14α-demethylase from *M. tuberculosis* (Mycobacterium P450DM) present in the Protein Data Bank with ID 1EA1 was chosen.

The high homology existing between these two enzymes suggested building a simple model having crystallographic structure of 1EA1in which the amino acid residues existing in the range of 7 Å from Fluconazole were substituted with those of Candida P450DM. Out of 449 amino acids only 12 substitutions listed in Table 1 were necessary, and only some of these were really important (Rossello, et al. 2002). Furthermore, missing atoms and residues were added to complete the protein chain.

Table 1: List of amino acid residues of *M. tuberculosis* P450DM substituted with that of Candida P450DM so as to obtain chimeric enzyme of P450 14α-demethylase of *C. albicans*.

***M. tuberculosis* P450DM**	**Chimeric enzyme of *C. albicans* P450**
Pro 77	Lys 77
Phe 78	His 78
Met 79	Leu 79
Arg 96	Leu 96
Met 99	Lys 99
Leu 100	Phe 100
Phe 255	Met 255
Ala 256	Gly 256
His 258	Gln 258
Ile 322	His 322
Ile 323	Ser 323
Leu 324	Ile 324

Molecular Dynamic Simulation

The prepared model was subjected to molecular dynamic (MD) simulation in order to obtain a stable low energy conformation. The MD simulation was carried out on Accelry's

Discovery Studio without harmonic restrains on the model. The energy of the model was applied using CHARMm force field and Momany-Rone partial charge. The heavy atom RMSD and Dreiding energy of the model was calculated and was found to be 0 and 28, 223.7 Kcal/mol respectively.

Model Validation

The prepared model was validated using Ramachandran plot and sequence determination. The Ramachandran plot for the model was produced by employing online software Rampage (http://modred.bioc.cam.ac.uk). For 14α-demethylase model, 93.9% of the residues were in favored region, 4.5% in allowed region and 1.6% in outlier region. Thus, 98.4% of the residues of the modeled 14α-demethylase after MD simulation were in allowed region, which indicated that the backbone dihedral angles ϕ and ψ were reasonable in the model.

The structure of the model was further validated by sequence determination employing Accelry's Discovery Studio and final structure is represented in Figure 1

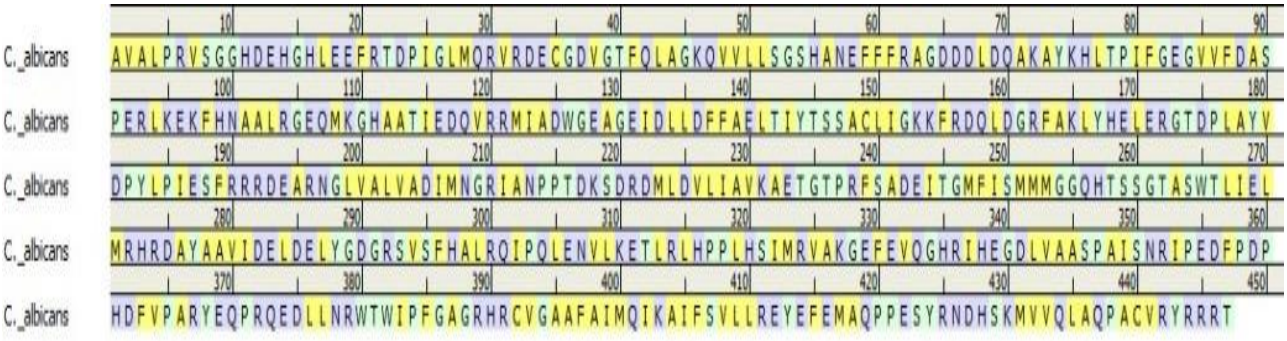


Figure 1: Protein sequence of cytochrome P450 14α-demethylase of *C. albicans* (PDB: 1EA1). Yellow region indicates Hydrophobic region, Violet color: Hydrophilic and Green color: Neutral.

Docking Protocol

Ligand preparation: A set of 24 fluorosubstituted imidazole analogues (Figure 2, Table 2) were selected from literature (Rani, et al. 2015; Khosropour, et al. 2008; Husain, et al. 2009; Zarghi, et al. 2012; Zhu, et al. 2008; Ermolatev, et al. 2009; Dietrich, et al. 2010; Saadeh, et al. 2009 and Domling, et al. 2007). The imidazole analogues along with three reference compounds i.e., Ketoconazole (**PN-25**), Miconazole (**PN-26**) and Clotrimazole (**PN-27**) have been generated in MDL mol files (V2000) using Chem Sketch (Version 12.01) for molecular docking study and in MDL SD file using Chem Draw ultra (Version 11.0) for the prediction of ADMET study.

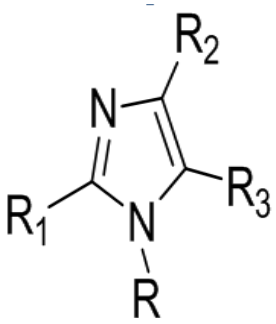
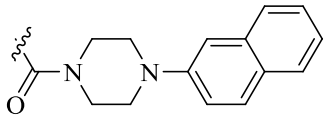
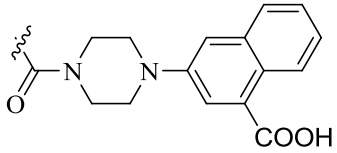
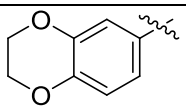
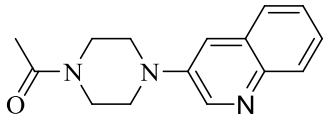
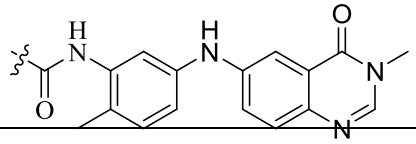
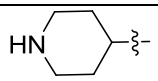
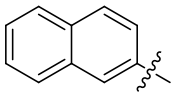
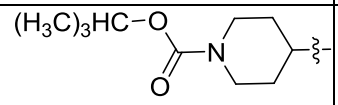


Figure 2: General Structure of Trisubstituted Imidazole.

Table 2: Data Set of Imidazole analogues used for the generation of pharmacophore for cytochrome P450 14α-demethylase from *C. albicans* P450DM.

S. No.	Code	R	R_1	R_2	R_3
1	PN-1	p-FC_6H_4	SH	C_6H_5	-
2	PN-2	p-FC_6H_4	SH	p-FC_6H_4	-
3	PN-3	H	p-FC_6H_4	C_6H_5	C_6H_5
4	PN-4	C_6H_5	p-FC_6H_4	p-ClC_6H_4	-
5	PN-5	H	p-FC_6H_4	p-$CH_3SO_2C_6H_4$	C_6H_5
6	PN-6	m-$OEtC_6H_4$	p-FC_6H_4		-
7	PN-7	m-$OEtC_6H_4$	o,p-$F_2C_6H_3$		-
8	PN-8	m-$OEtC_6H_4$	p-FC_6H_4		-
9	PN-9	m-$OEtC_6H_4$	o,p-$F_2C_6H_3$		-
10	PN-10		o,p-$F_2C_6H_3$		-
11	PN-11		p-FC_6H_4		-
12	PN-12		p-FC_6H_4		-
13	PN-13	m-$OEtC_6H_4$	o,p-$F_2C_6H_3$		-
14	PN-14	C_6H_5	SH	p-FC_6H_4	-
15	PN-15	p-ClC_6H_4	SH	p-FC_6H_4	-
16	PN-16	p-$CH_3OC_6H_4$	SH	p-FC_6H_4	-
17	PN-17	H	NH_2	p-FC_6H_4	C_6H_5NHCO
18	PN-18	H	CF_3		m-CH_3-p-FC_6H_3
19	PN-19	H	CF_3		m-Cl-p-

					FC_6H_3
20	PN-20	H	CF_3		m,p-$F_2C_6H_3$
21	PN-21	CH_3	H	NO_2	p-FC_6H_4
22	PN-22		H	m,p-$(CH_3O)_2C_6H_4$	m,p-$F_2C_6H_3$
23	PN-23		H		m-Br-p-FC_6H_4
24	PN-24		H	m,p-$(CH_3O)_2C_6H_4$	m,p-$F_2C_6H_3$

Protein selection: The prepared model of cytochrome 14α-demethylase (1EA1) was chosen for the study. The protein was imported along with crystallographic ligand (Fluconazole, a triazole) and water molecules and the molecular docking study was performed using MVD 2010.4.1.0. The active site of the protein was identified and the site which was involved in binding with the imported ligand was selected and considered as the best region for docking. The Fluoro substituted imidazoles were docked into the active site. The resulting conformation having lowest energy was selected as bioactive compound (Thomsen, et al. 2006).

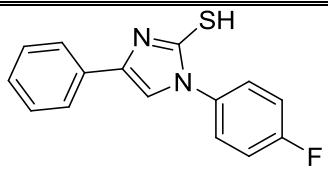
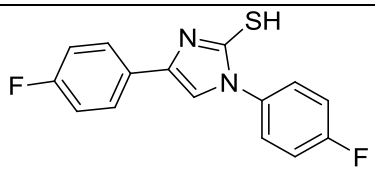
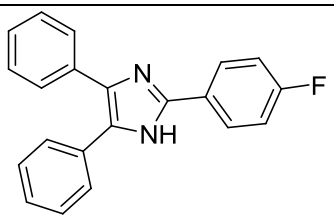
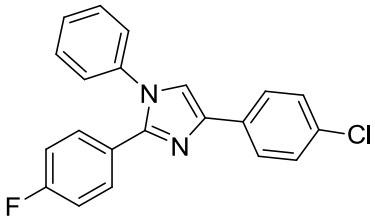
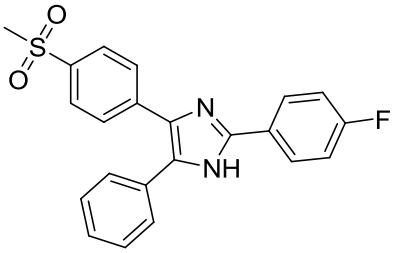
ADMET and lipinski rule: The molecules generated in SD file were used for ADMET study. The ADMET study was performed with Accord for Excel programme (Version 7.1.5) developed by Accelry's Discovery Studio. The predictions were carried out by inserting the ligands in the software and the desired properties i.e., Human Intestinal absorption (HIA), Blood Brain Barrier (BBB) penetration, Aqueous solubility, plasma protein binding, Hepatotoxicity and CYP2D6 were selected for the determination of ADME. However, for the prediction of Oral Bioavailability M logP, Molecular weight, Hydrogen bond donor, H Bond Acceptor and Lipinski rule of five alerts were determined.

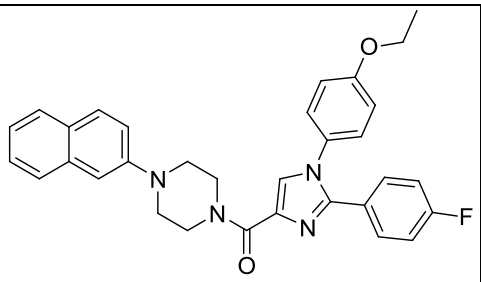
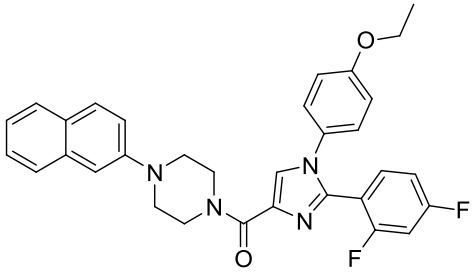
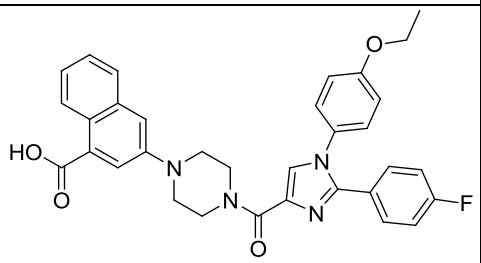
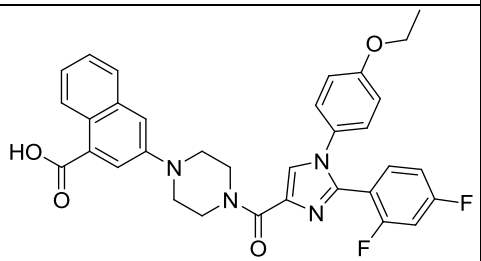
RESULTS AND DISCUSSION

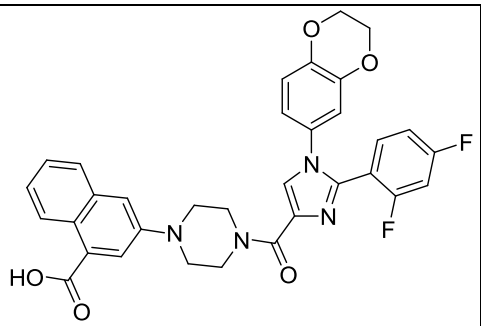
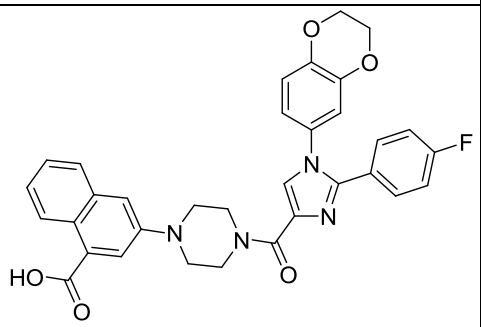
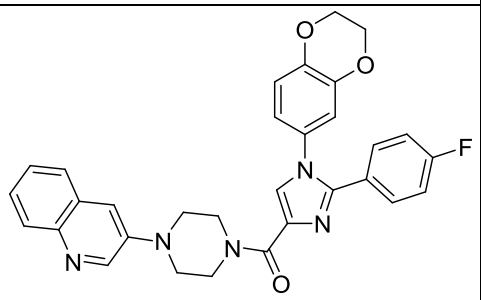
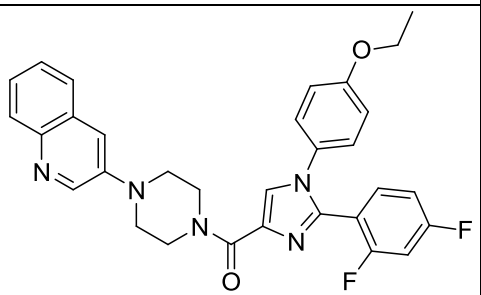
Molecular modeling is one of the most useful techniques for the identification of the novel substrates and is used for the structure based drug design. It screens the molecules by orienting and screening the molecules in the binding site of the protein and the results of the ligand-protein interaction are obtained in the form of scoring functions (Pitchai, et al. 2012). In this study the Fluoro substituted imidazole analogues were evaluated for the antifungal potency against cytochrome P450 14α-demethylase using MVD 2010.4.1.0. Mol Dock is a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm and involves automatic preparation of protein and ligand which makes it easy to fully automate the entire bench marking process. Further, it involves automatic energy minimization of found poses which further increases the docking accuracy (Thomsen, et al. 2006). In order to assign the perfect grid to each ligand docking was performed while

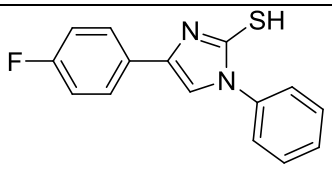
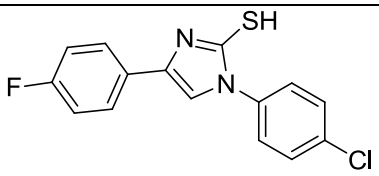
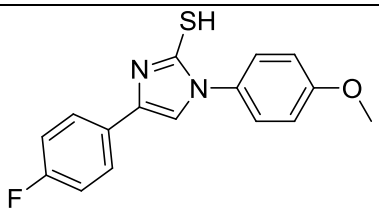
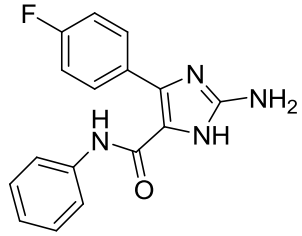
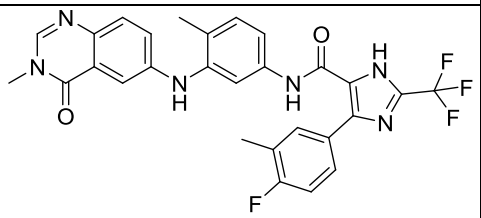
minimizing the energy using MVD with 10 independent docking runs for each imidazole analogue. Flexible docking of all data sets used for the computational study was carried out on the active site of 14α-demethylase. The docking study revealed that all the molecules were docked into the same active site of the enzyme where Fluconazole molecule was present. Furthermore, three reference compounds i.e., Ketoconazole, Miconazole, and Clotrimazole was taken as standard in order to decrease the chances of error. The predicted binding energy which is given by docking score and other docking results of the imidazole analogues are listed in Table 3.

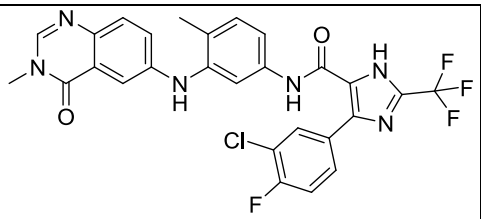
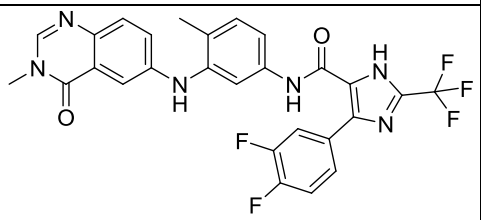
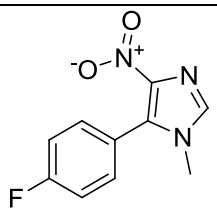
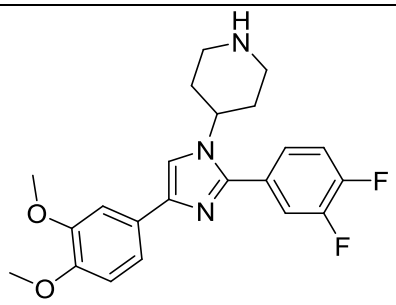
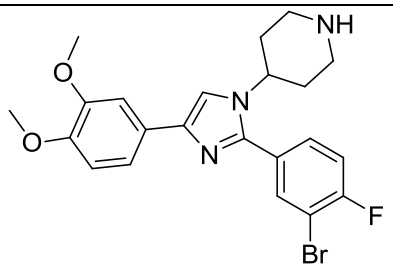
Table 3: Compounds with Mol. Dock Score, Interaction Data and Distance between the protein residues.

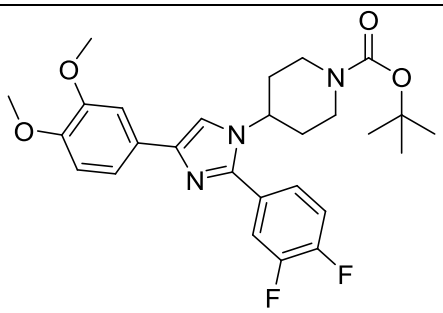
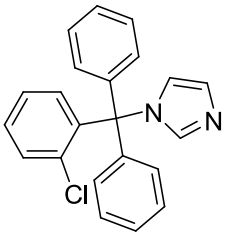
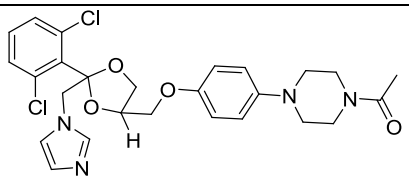
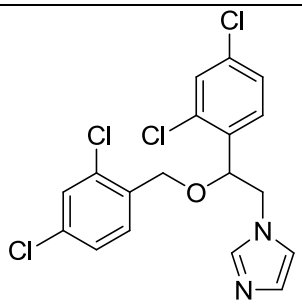
Code	Structure	M. Dock Score	Interaction Data		Distance Annotation
			Protein Residue	Ligand atom	
PN-1		-96.0332	H_2O 175 (O)	SH (S)	2.60 Å
PN-2		-101.116	-	-	-
PN-3		-100.631	H_2O 175 (O)	Imidazole (N1)	3.05 Å
PN-4		-104.649	H_2O 175 (O) H_2O 175 (O)	Imidazole (N1) Imidazole (N3)	3.32 Å 3.10 Å
PN-5		-103.312	H_2O 175 (O) H_2O 87 (O) Thr 260 (O)	Imidazole (N3) SO_2 (O) SO_2 (O)	3.08 Å 2.42 Å 3.10 Å

PN-6		-154.59	Gln 72 (O) H_2O 175 (O)	Imidazole (N3) Piperazine (N2)	3.20 Å 3.32 Å
PN-7		-157.106	Gln 72 (N) H_2O 122 (O) H_2O 174 (O) H_2O 175 (O) H_2O 175 (O)	CO (O) OEt (O) OEt (O) Imidazole (N1) Imidazole (N3)	2.80 Å 3.30 Å 3.16 Å 3.09 Å 3.08 Å
PN-8		-153.877	Thr 80 (O) Gln 72 (N) H_2O 175 (O) H_2O 175 (O) H_2O 174 (O) H_2O 122 (O)	COOH (O) CO (O) CO (O) Imidazole (N3) OEt (O) OEt (O)	2.92 Å 2.62 Å 3.50 Å 3.01 Å 3.21 Å 2.98 Å
PN-9		-155.676	Gln 72 (N) Thr 80 (N) H_2O 122 (O) H_2O 174 (O) H_2O 175 (O) H_2O 175 (O)	CO (O) COOH (O) OEt (O) OEt (O) Imidazole (N1) Imidazole (N3)	2.83 Å 2.99 Å 3.30 Å 3.06 Å 3.06 Å 3.10 Å

PN-10		-159.577	Gln 72 (N) H_2O 122 (O) H_2O 174 (O) H_2O 175 (O) H_2O 175 (O)	CO (O) Benzodioxine (O) Benzodioxine (O) Imidazole (N1) Imidazole (N3)	2.97 Å 3.51 Å 2.81 Å 3.10 Å 3.10 Å
PN-11		-156.218	Gln 72 (N) H_2O 122 (O) H_2O 174 (O) H_2O 175 (O) H_2O 175 (O)	CO (O) Benzodioxine (O) Benzodioxine (O) Imidazole (N1) Imidazole (N3)	2.98 Å 3.54 Å 2.69 Å 3.06 Å 3.10 Å
PN-12		-161.929	Gln 72 (N) H_2O 87 (O) H_2O 175 (O) H_2O 175 (O)	CO (O) Benzodioxine (O) Imidazole (N1) Imidazole (N3)	2.81 Å 3.44 Å 3.20 Å 3.35 Å
PN-13		-145.661	Gln 72 (N) Thr 80 (O) H_2O 122 (O) H_2O 174 (O) H_2O 175 (O) H_2O 175(O)	CO (O) COOH (O) OEt (O) OEt (O) Imidazole (N1) Imidazole (N3)	2.83 Å 2.99 Å 3.30 Å 3.06 Å 3.06 Å 3.10 Å

PN-14		-99.222	Ser 252 (O)	SH (S)	3.56 Å
PN-15		-89.1859	H_2O 175(O) H_2O 175(O)	Imidazole (N1) Imidazole (N3)	3.24 Å 3.22 Å
PN-16		-100.186	H_2O 87(O) Thr 260 (O)	OCH_3 (O) OCH_3 (O)	3.29 Å 3.44 Å
PN-17		-118.985	H_2O 87 (O) H_2O 87 (O) H_2O 90 (O) Gly 256 (O) Thr 260 (O) Pyrrole (N16) Pyrrole (N24) Pyrrole (N24) Pyrrole (N32)	Imidazole (N1) NH_2 (N) NH_2 (N) NH_2 (N) NH_2 (N) NH_2 (N) NH_2 (N) Imidazole (N3) Imidazole (N3)	3.32 Å 3.17 Å 3.46 Å 2.91 Å 2.43 Å 3.05 Å 3.10 Å 3.37 Å 3.57 Å
PN-18		892.931	Tyr 76 (O) Thr 80 (O) H_2O 87 (O) H_2O 87 (O) Pyrrole (N32)	Imidazole (N3) Imidazole (N1) Quniazoline (O) Quinazoline (N3) Quinazoline (N1)	3.38 Å 2.62 Å 3.35 Å 3.28 Å 3.11 Å

PN-19		880.346	Ala 73 (O) Thr 80 (O) H_2O 175 (O)	Quinazoline (N1) Quinazoline (N3) CO (O)	3.01 Å 3.00 Å 2.23 Å
PN-20		894.341	Thr 80 (O) H_2O 87 (O) H_2O 87 (O) Pyrrole (N32)	Imidazole (N1) Quinazoline (N3) Quinazoline (O) Quinazoline (N1)	2.68 Å 3.38 Å 3.45 Å 3.15 Å
PN-21		-108.814	H_2O 175 (O) H_2O 175 (O) H_2O 175 (O) Pyrrole (N5)	NO_2 (O1) NO_2 (O2) NO_2 (N) Imidazole (N3)	2.96 Å 2.31 Å 3.02 Å 3.19 Å
PN-22		-125.265	Lys 99 (N) H_2O 174 (O) H_2O 175 (O)	OCH_3 (O) Piperidine (N) Imidazole (N1)	3.10 Å 3.10 Å 3.57 Å
PN-23		-126.548	H_2O 174 (O) H_2O 175 (O)	Piperidine (N) Imidazole (N3)	3.26 Å 3.01 Å

PN-24		-130.503	Gln 72 (N)	Imidazole (N3)	3.44 Å
PN-25		-1003.68	H_2O 175(O)	Imidazole (N1)	3.57 Å
PN-26		-71.393	Thr 80 (O) H_2O 175(O) H_2O 175(O)	Piperazine (N1) Imidazole (N1) Dioxolane (O)	3.23 Å 3.07 Å 2.85 Å
PN-27		-128.501	H_2O 175 (O) H_2O 87 (O) Gly 256 (O) Thr 260 (O) Pyrrole (N16) Pyrrole (N24)	OCH_3 (O) Imidazole (N3) Imidazole (N3) Imidazole (N3) Imidazole (N3) Imidazole (N3)	3.10 Å 3.13 Å 3.36 Å 2.72 Å 3.16 Å 3.43Å

- No interaction

The predicted binding energy E_{score}, is defined as the sum of intermolecular energy (E_{inter})and the internal energy of the ligand (E_{intra}) and is given below (Thomsen, et al. 2006):

$$E_{score} = E_{inter} + E_{intra}$$

A compound can only be accepted in the form of drug if it possesses certain properties which are determined by Lipinski rule of five and ADMET study. An important parameter that a drug must fulfill is the good permeability across the cell membrane which is in turn predicted by Lipinski rule of five. According to Lipinski rule the molecule must have molecular weight below 500 g/mol, partition coefficient less than 5, not have more than five hydrogen bond donors and not more than ten hydrogen bond acceptors. If any molecule

crosses any one of the parameter, it results in poor permeability (Egan, et al. 2012) and this parameter was evaluated *via* using Accelrys Accord for excel (Version 7.1.5) and the results obtained are given in Table 4.

If the molecule confirms the rule, i.e., the Lipinski rule of five, alert comes out to be true while if the molecule doesn't confirms the rule, the value of alert comes out to be false. Further, ADMET study helps in the prediction of other parameters such as plasma protein binding, HIA, aqueous solubility, BBB penetration, hepatotoxicity and CYP2D6 inhibition property.

Table 4: Lipinski Rule of five.

Code	Chemistry. Mlogp	Chemistry. Weight	Lipinski.Hbond. Donor	Lipinski.Hbond. Acceptor	Lipinski. Rule. Of.Five.Alert
PN-1	3.923844	270.329	0	2	FALSE
PN-2	4.315735	288.319	0	2	FALSE
PN-3	4.565503	314.363	1	2	FALSE
PN-4	5.318285	348.808	0	2	FALSE
PN-5	4.078646	392.452	1	4	FALSE
PN-6	4.535552	520.608	0	6	TRUE
PN-7	4.627991	538.598	0	6	TRUE
PN-8	3.957356	564.617	1	8	FALSE
PN-9	4.048691	582.607	1	8	FALSE
PN-10	3.309683	596.59	1	9	FALSE
PN-11	3.218348	578.6	1	9	FALSE
PN-12	2.841482	535.579	0	8	FALSE
PN-13	3.683405	539.586	0	7	FALSE
PN-14	3.923844	270.329	0	2	FALSE
PN-15	4.438087	304.774	0	2	FALSE
PN-16	3.602307	300.355	0	3	FALSE
PN-17	2.744054	296.305	4	5	FALSE
PN-18	3.808929	550.516	3	8	FALSE
PN-19	3.808929	570.934	3	8	FALSE
PN-20	3.712867	554.479	3	8	FALSE
PN-21	3.028413	221.191	0	5	FALSE
PN-22	3.481726	399.441	1	5	FALSE
PN-23	3.692863	460.352	1	5	FALSE
PN-24	3.7105	499.558	0	7	FALSE
PN-25	4.889229	344.845	0	2	FALSE
PN-26	3.368171	531.44	0	8	FALSE
PN-27	4.862379	416.135	0	3	FALSE

According to ADMET the molecule must possess good absorption, human intestinal absorption, aqueous solubility, blood brain penetration without hepatotoxicity. The ADMET study was carried out *in silico* using Accelry's Accord for Excel (Version 7.1.5). The data for ADMET study is given in Table 5 and the HIA and BBB penetration plots are shown in Figure 3 and 4 respectively.

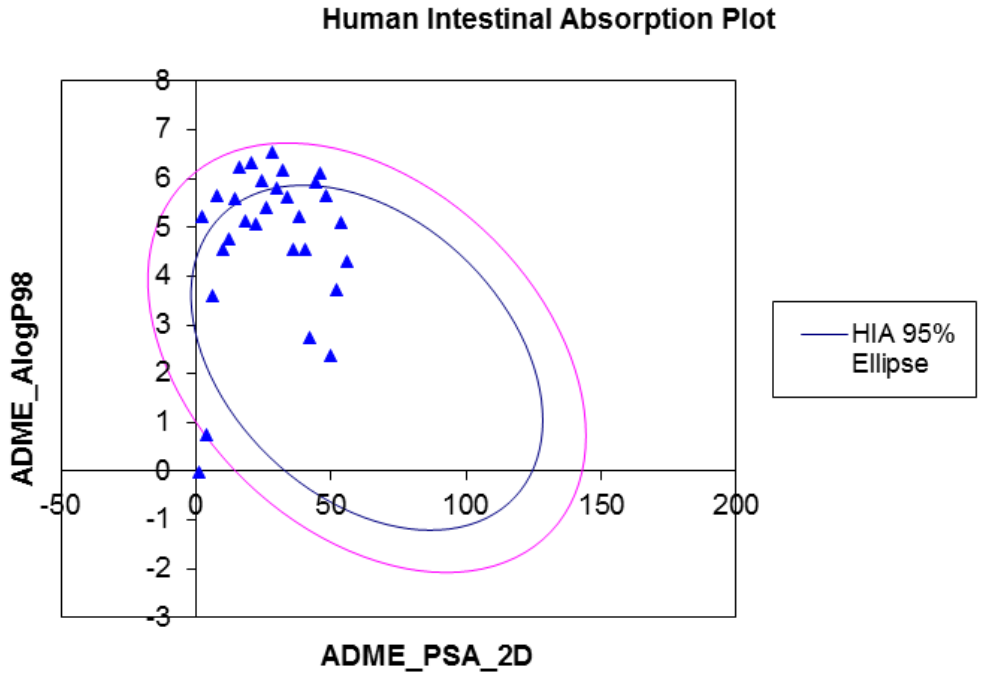


Figure 3: Human Intestinal Absorption plot. Small blue colored triangles represent compounds under study. Most of the compounds as shown in Figure lies between the two circles represent that these molecules have moderate absorption.

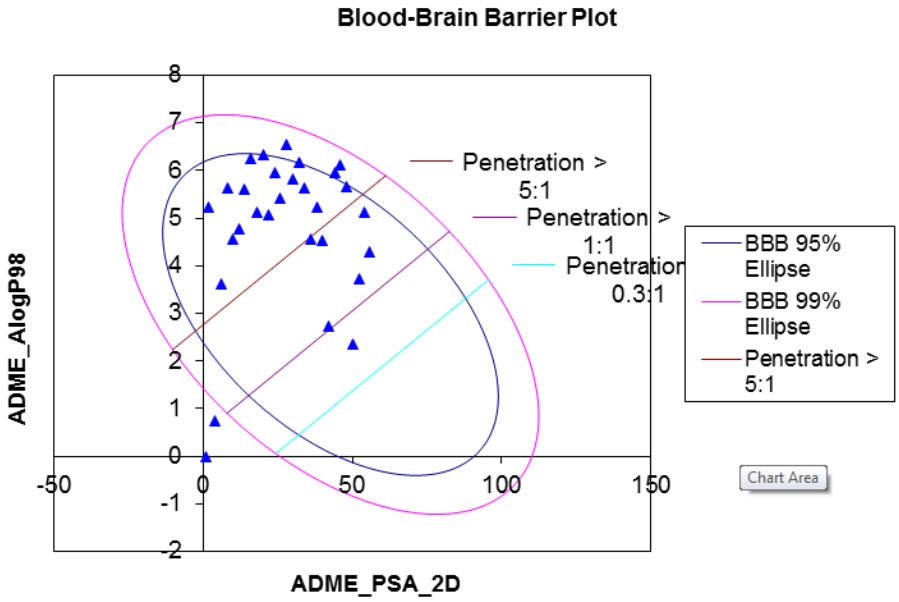


Figure 4: Blood Brain Barrier penetration plot. The molecules are represented in blue triangles and the Figure depicts that most of the molecules (present in portion zero) have high blood brain barrier penetration property.

Table 5: ADMET Study Data.

Code	BBB penetration*	Aqueous Solubility**	Protein Binding	HIA Level***	CYP2D6****	Hepato-toxicity*****
PN-1	0	2	>= 95%	0	1	1
PN-2	0	2	>= 95%	0	1	1
PN-3	0	1	>= 95%	0	1	1
PN-4	0	1	>= 95%	1	1	1
PN-5	1	1	>= 95%	0	1	1
PN-6	0	1	>= 95%	1	1	0
PN-7	4	1	>= 95%	1	1	1
PN-8	4	1	>= 95%	2	1	1
PN-9	4	1	>= 95%	2	1	1
PN-10	4	1	>= 95%	2	0	1
PN-11	4	1	>= 95%	2	0	1
PN-12	1	1	>= 95%	0	1	1
PN-13	1	1	>= 95%	1	1	1
PN-14	0	2	>= 95%	0	1	1
PN-15	0	1	>= 95%	0	1	1
PN-16	0	2	>= 95%	0	1	1
PN-17	3	2	>= 95%	0	0	1
PN-18	4	1	>= 95%	2	0	1
PN-19	4	0	>= 95%	2	0	1
PN-20	4	0	>= 95%	2	0	1
PN-21	2	3	< 90%	0	0	1
PN-22	1	2	>= 95%	0	1	0
PN-23	1	2	>= 95%	0	1	0
PN-24	1	2	>= 95%	0	1	0
PN-25	0	1	>= 95%	0	1	1
PN-26	2	2	>= 95%	0	0	0
PN-27	0	1	>= 95%	0	1	1

(* 0, 1, 2, 3 and 4 indicates very high, high, medium, low and undefined BBB penetration respectively; ** 0, 1, 2, 3 and 4 indicates extremely low, very low, low, good and optimal aqueous solubility respectively; ***0, 1, 2, 3 indicates good, moderate, low and very low HIA respectively; ****0,1 indicates Non-inhibitor and inhibitor respectively and *****0,1 indicates non-toxic and toxic respectively)

The molecular docking study revealed that most of the compounds under the study were present in the hydrophobic pocket of the protein and within the active site of the enzyme cytochrome P450 14α-demethylase. Most of the molecules have interaction with the protein residues and heme cofactor. However, the active site residues interacting with the ligands were same as those interacting with the standard. The study revealed that about 5 protein residues were important for ligand binding namely Thr 80, Gly 256 and Th 260. However, some of the ligands also exhibited interaction with nitrogen atom of Heme (Figure 5).

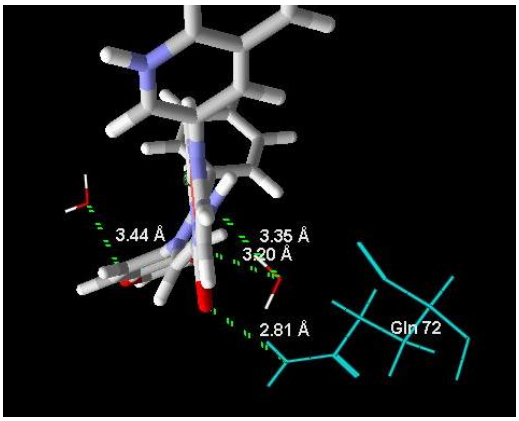


Figure 5: Binding mode of **PN-12** within the active site of Cytochrome P450 14α-demethylase of *C. albicans*. Four hydrogen bonds of their interaction (One with Gln 72 (N) and three with Water molecule) were presented along with the distance.

Further, most of the molecules exhibited interaction with the water molecule present above the surface of Heme cofactor which bridges the interaction with the active site (Figure 6). It is suggested that the water molecules present in the active site mediated the interaction between the hydroxyl group and vicinal H310 which is a highly conserved residue in CYP51 family (Sheng, et al. 2006). The docking score of some of the ligands was better than to that of standards. However, lower docking score depicts stronger ligand-protein interaction (Thomsen, et al. 2006). The lower docking score obtained in the study depicted the stronger ligand-protein binding affinity between the ligands with the receptor.

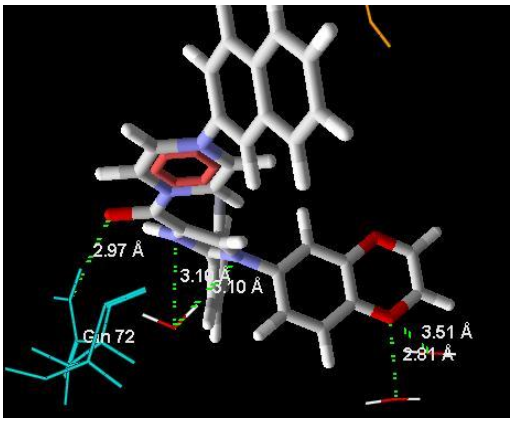


Figure 6: Binding mode of **PS-10** within the active site of Cytochrome P450 14α-demethylase of *C. albicans*. Five hydrogen bonds of their interaction (Four with water and one with Gln 72) were presented along with the distance. Gln 72 is indicated in blue color.

The study also depicted that imidazole with p-Fluoro phenyl (**PN-12**), has good docking score and strong interaction was most effective. Furthermore, o,p-difluorophenyl imidazoles (**PN-7, PN-10**) were also good due to their good docking score and strong interaction.

From Table 4, it is clear that most of the compounds do not follow the Lipinski rule of five and are thus impermeable to the cell membrane. Thus are less absorbed and have low bioavailability. Further most of the compounds can easily cross the blood brain barrier with low solubility. The compounds possessed good HIA. Irrespective of this the compounds are CYP2D6 inhibitors and thus led to unwanted side-effects and also resulted in drug-drug interaction and caused hepatotoxicity.

CONCLUSION

A 3D model of lanosterol 14α-demethylase of *C. albicans* was built and molecular simulation was applied so as to refine the structure. The structure was validated by Ramachandran plot and sequence determination which depicted that the insertions made in the structure were similar to those in *C. albicans*. Molecular docking results for fluoro substituted imidazoles toward the model suggested the possible binding mode of the compounds. The predicted binding modes of the fluoro substituted imidazoles will be helpful for the better understanding of the mechanism of action of the compounds and for lead optimization. Further, the ADMET study carried out depicted the pharmacokinetic and toxicity profile of the compounds, which could be an effective way to find novels for the development of antifungal drugs with good pharmacokinetic profile and low toxicity.

LIST OF ABBREVATIONS

ADME: Absorption, Distribution, Metabolism and Excretion
ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity
BBB: Blood Brain Barrier
CYP: Cytochrome P450
HIA: Human Intestinal Absorption
MVD: Molergo Virtual Docker

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