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## Synthesis, Molecular docking and evaluation of antifungal activity of some benzyl benzimidazole derivatives

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### ABSTRACT

A novel series of benzyl benzimidazoles substituted with piperazines were designed by study of active site of the fungal enzyme of cytochrome P 450 family lanosterol 14  $\alpha$  demethylase and synthesized. The molecular docking study of these compounds was performed at the active site of this enzyme. The synthesised compounds were characterised by spectral analysis (IR, <sup>1</sup>H NMR). The screening of the synthesised compounds for in vitro antifungal activity revealed activity against *Candida albicans* of some of the compounds comparable to that of ketoconazole.

**Keywords:** Benzimidazole, piperazine, antifungal, lanosterol 14  $\alpha$  demethylase

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### INTRODUCTION

Fungal infections are also known as mycoses. Superficial mycoses is localized to skin, hair nails while deep systemic mycoses involves vital organs like lungs, bones, central nervous system.

The incidences of systemic mycoses has been increasing rapidly due to an increase in number of immunocompromised hosts, such as patients undergoing anticancer chemotherapy or organ transplants and patients suffering from AIDS.[1]

Clinically candidosis, aspergillosis and cryptococcosis are three major fungal infections of immunocompromised patients.[2, 3]

Because of their high therapeutic index, azoles are first line drugs for treatment of invasive fungal infections. Unfortunately the broad use of azoles has led to development of severe resistance.[4, 5]

Azole antifungals act by competitive inhibition of the lanosterol 14 $\alpha$ -demethylase, key enzyme in sterol biosynthesis of fungi, selective inhibition of this enzyme would cause depletion of ergosterol and accumulation of lanosterol and other 14 methyl sterols resulting in inhibition of growth of fungal cell.[6]

From SAR study of azoles as antifungal agents basic structural requirement of azole class antifungal agents are

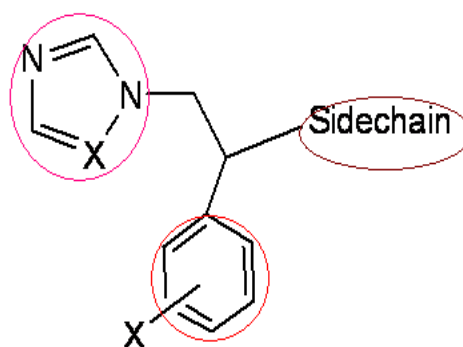


Figure 1 SAR study of azoles as antifungal agents

- i) weakly basic imidazole or 1,2,4 triazole ring bonded by nitrogen – carbon linkage to the rest of the structure .
- ii) Two or three aromatic rings, at least one of which is halogen substituted and other non polar functional groups [7,8,9]

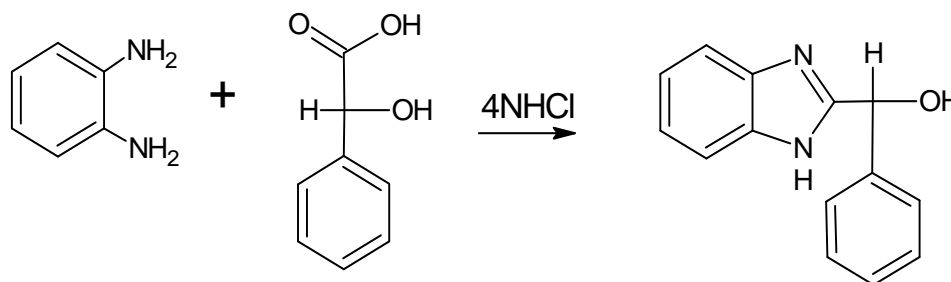
Study of three dimensional structure of lanosterol 14 $\alpha$  demethylase of *Candida albicans* for its active site revealed that active site has hydrophilic hydrogen bond binding site, core hydrophobic area above heme ring of enzyme, a narrow hydrophobic cleft and hydrophobic hydrogen bond binding site.[10]

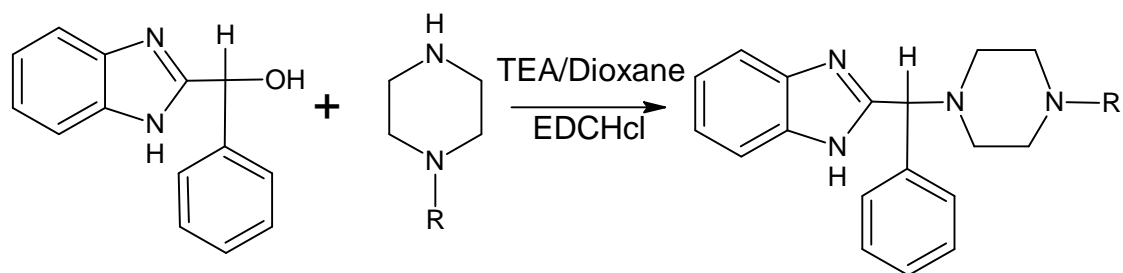
The increased intensity of life threatening fungal infections and development of resistance of currently used azoles as antifungal agents , warrant search for novel, alternative chemical moieties. In view of above considerations we designed and synthesized a series of benzyl-benzimidazole derivatives. The newly prepared derivatives were docked into the active site of homology modelled CYP 51 of *saccharomyces cerevisiae* using V life software ,MDS 4.3.The chemical structures of synthesized compounds were confirmed by spectral analysis. All compounds were investigated for in vitro antifungal activity against *Candida albicans*.

## MATERIALS AND METHODS

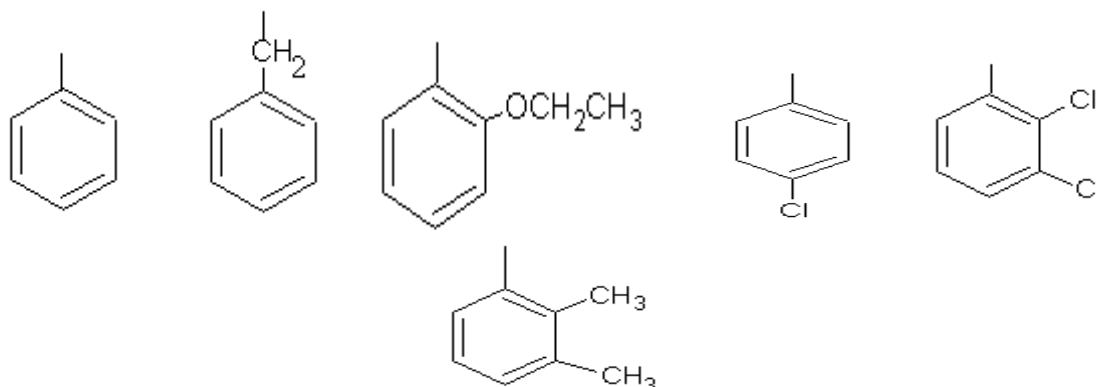
All materials were purchased from Sigma Aldrich and Merck specialties Pvt Ltd. (Mumbai,India). Solvents were dried and distilled before use. EDCHCl was obtained as gift sample from survival technologies Pvt.Ltd . Ankleshwar. Piperazines were procured from Cata -Pharma Sinnar as gift samples. Thin layer chromatography analysis were carried out on aluminium plates (Merck)precoated with silica gel G (Merck) and spots were visualised with U.V light and Iodine vapours. Melting points were taken in thiels tube in open glass capillary and were uncorrected. Infra red spectra were recorded on KBr pellets on Shimadzu 8400S FTIR spectrophotometer . Proton ( $^1\text{H}$ ) nuclear magnetic resonance (NMR) spectra of compound were recorded on at300MhZ using  $\text{CDCl}_3$  . Solvent in department of Chemistry Savitribai Phule Pune University, Pune. Chemical shifts ( $\delta$ ) were expressed in parts per million( $\delta$  ppm),downfield from TMS as an internal standard .

The method followed for synthesis of benzyl benzimidazole derivatives is described here. [11,12]





R= -CH<sub>3</sub>, -CH<sub>2</sub>-CH<sub>2</sub>OH, -CHO, -COCH<sub>3</sub>



Scheme1

#### Synthesis of 2-( $\alpha$ hydroxybenzyl)benzimidazole:

Ortho phenylene diamine (0.01 mol) and Mandelic acid (0.01mol) and 30ml of 4N HCl were placed in RBF. It was refluxed for 5 hours. The reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured in ice cold water. It was then basified with concentrated ammonia solution. Kept overnight. Solid precipitate was filtered, dried and recrystallized from ethanol. It was obtained as pale yellow solid.

Mol. Formula - C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O Mol. wt - 224 M.P. -178-180<sup>0</sup>C

Yield: -71% , IR(KBr)- 3228(-OH), 3412(-NH), 2923.87, 2858.25(Ar-CH), 1590.55(C=N), 1428(C=C) cm<sup>-1</sup>.

<sup>1</sup>H NMR(300MHZ CDCl<sub>3</sub>)  $\delta$  ppm-7.11-7.4 (m, 9H, Ar-H) , 9.8 (bs, 1H, benzimidazole  $\text{N-H}$ ), 6.5(s, 1H, C-H) , 5.9 (s, 1H, OH)

#### Synthesis of 2-(4-methylpiperazin-1-yl) benz-1-yl benzimidazole

A solution of 2-( $\alpha$  hydroxybenzyl)benzimidazole (1.23gm 1.1mol) was prepared in 15ml dioxane. It was placed on magnetic stirrer with hot plate. Triethyl amine 0.55ml (1.1mol) was added to it with stirring. To this mixture EDCHCl was added 0.97gm (1.1mol) in portions and the temperature was raised upto 40-50<sup>o</sup>c. ,stirring was continued for about 2 hours. N-methyl piperazine ( 1.1mol) was dissolved in 5 ml of dioxane and added in portions to above reaction mixture. It was stirred for 2 hours the reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured in cold water. Solid precipitate obtained was filtered, dried and recrystallized from ethanol.

**2-(4-methylpiperazin-1-yl) benz-1-yl benzimidazole** Mol. Formula -C<sub>9</sub>H<sub>22</sub>N<sub>4</sub> Mol. wt-306

It was obtained as off whitesolid. M.P.-79-82<sup>o</sup>C, Yield-84.2% ,IR(KBr)- 3306(-NH), 2917.68(Ar-CH), 1652(C=N),

1426(C=C); 1256(C-N)Cm<sup>-1</sup>. <sup>1</sup>H NMR (300MHZ, CDCl<sub>3</sub>)  $\delta$ ppm , 13.08 (bs, 1H, benzimidazole  $\text{N-H}$ ) 7.19-7.6 (m, 9H, Ar-H) , 6.11(s, 1H, C-H), 5.9(s, 3H, -CH<sub>3</sub>) , 2.2-3.9(m, 8H, pip-H)

**2-[4-(2-hydroxyethyl) piperazin-1-yl] benz-1-yl benzimidazole** Mol. Formula C<sub>20</sub>H<sub>24</sub>N<sub>4</sub>O

Mol. wt-336. It was obtained as yellow solid M.P.- 115-118 <sup>o</sup>C yield -75.11% IR(KBr)- 3073(-NH) 3031(-OH), 2970(Ar-CH), 1653 (C=N), 1260, 1274(C-N); Cm<sup>-1</sup>, <sup>1</sup>H NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)  $\delta$  ppm , 9.8 (bs, 1H, benzimidazole

$\text{N-H}$ ), 8.9(s, 1H, OH), 7.2-7.8 (m, 9H, Ar-H) , 4.8(s, 1H, C-H) , 2.2-2.6(m, 8H, pip-H) 1-1.3(t, 4H, CH<sub>2</sub>CH<sub>2</sub>)

**2-(4-(2-ethoxyphenyl)piperazin-1-yl) benz-1-yl benzimidazole** Mol.Formula  $C_{26}H_{28}N_4O$  Mol.wt 417.52 It was obtained as pale yellow solid.M.P.-180-182 °C,yield -54.01% IR(KBr)-3416(-NH), 2935(Ar-CH), 1659(C=N),

1434(C=C), 1281,1234(C-N),  $Cm^{-1}$ ,  $^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>) 10. (bs,1H, bezimidazole  $\text{>N-H}$ ), 6.95-7.5 (m,14H, Ar-H), 4.8(s,1H, C-H),3.2(s,8H, pip-H),2.75(q,2H,-CH<sub>2</sub>),1.3(t,3H,-CH<sub>3</sub>)

**2-(4-formylpiperazin-1-yl) benz-1-yl benzimidazole**

: Mol.Formula  $C_{19}H_{20}N_4O$  Mol.wt 320.38 It was obtained as yellow solid. yield -17.54%, M.P.-119-120°C.IR(KBr)-3349.18(-NH), 2899(Ar-CH), 1656.37(C=N), 1434.7(C=C), 1278(C-N), 1710(C=O)  $Cm^{-1}$   $^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)  $\delta$  ppm - 7.26-7.9 (m,9H, Ar-H) , 5.9 (S, 1H,- CHO) , 4.8(s,1H, C-H) , 2.3-3.7(m, 8H, pip-H)

**2-(4-acetyl piperazin-1-yl) benz-1-yl benzimidazole**

Mol.Formula  $C_{20}H_{22}N_4O$  Mol.wt 334.41 It was obtained as pale yellow solid yield -73.01 % M.P.-110-115°C,IR(KBr) 3064(-N-H); 2821, 1457(-CH<sub>3</sub>); 2980, 745 (Ar-CH), 1653 (- C=N); 1252,1272 (-C-N) , 1700(C=O)  $^1H$  NMR (300MHZ CDCl<sub>3</sub>)  $\delta$  ppm-10 (bs

,1H,bezimidazole  $\text{>N-H}$ ), 7-8 (m,9H, Ar-H) , 4.8(s,1H, C-H) , 3.4-3.8(M, 8H, pip-H) ,2.3(s,3H,-CH<sub>3</sub>)

**2-(4-phenyl piperazin-1-yl) benz-1-yl benzimidazole**

Mol.Formula  $C_{24}H_{24}N_4$ Mol.wt-368.47 It was obtained as pale yellow solid . yield-%92.5 M.P.- 168-170 °C,IR(KBr)-3219 (-NH), 3059,3026,698 (Ar-CH), 1654(C=N), 1274,1291(C-N),  $Cm^{-1}$  NMR (300MHZ CDCl<sub>3</sub>)  $\delta$

ppm-9.8 (bs,1H, bezimidazole  $\text{>N-H}$ ) , 7.2-7.9 (m,14H, Ar-H) , 4.8(s, 1H,C-H) , 2.2-3.2(m, 8H, pip-H)

**2-(4-benzyl piperazin-1-yl) benz-1-yl benzimidazole**

Mol.Formula  $C_{25}H_{26}N_4$ Mol.wt 382.5. It was obtained as pale yellow solid. yield -86.75 M.P.-80-84°C IR(KBr)-3084 (-NH), 3060,3028 (Ar-CH), 2942(-CH<sub>3</sub>), 1656.37(C=N), 1434.7(C=C), CN1278(C-N),  $Cm^{-1}$

$^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)  $\delta$  ppm 9.8 (bs,1H, bezimidazole  $\text{>N-H}$ ) ,7.05-7.6 (m,14H, Ar-H), 4.8(s,1H, C-H), 2.2-2.8(m, 8H, pip-H),2.1(s,2H,-CH<sub>2</sub>)

**2-(4-(4-chlorophenyl)piperazin-1-yl) benz-1-yl benzimidazole** Mol.Formula  $C_{24}H_{23}ClN_4$  Mol.wt 402.91, It was obtained as pale yellow solid.M.P.-64-66°C yield -50.88% IR(KBr)- 3354 (-NH), 2919.60, 2850.36(Ar-CH), 1656.37(C=N), 1493.55,1446.19(C=C), 1227.7(C-N), 736.4 (-C-Cl)  $Cm^{-1}$

$^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)  $\delta$  ppm-13.08(bs, 1H bezimidazole  $\text{>N-H}$ ),7.16-7.52 (m,13H, Ar-H), 4.78(s,1H, C-H),3.19-3.70 (m, 8H, pip-H)

**2-(4-(2,3-dichlorophenyl)piperazin-1-yl) benz-1-yl benzimidazole** Mol.Formula.  $C_{24}H_{22}Cl_2N_4$  Mol.wt 437.36.It was obtained as pale yellow solid. M.P.-222-224°C,yield -66.25% IR(KBr)-3254 (-NH), 2876, 2816.6 (Ar-CH), 1659 (C=N), 1434.7(C=C), 1237(C-N), 733(-C-Cl)  $Cm^{-1}$   $^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)  $\delta$  ppm-9.6 (bs, 1H

bezimidazole  $\text{>N-H}$ ),6.8-7.6 (m,12H, Ar-H), 4.84(s,1H, C-H),3.2 (m, 8H, pip-H)

**2-(4-(2,3-dimethylphenyl)piperazin-1-yl) benz-1-yl benzimidazole** Mol.Formula  $C_{26}H_{28}N_4$  Mol.wt 396.52, It was

obtained as pale yellow solid. M.P.-74-76°C,yield -73 % IR(KBr)- 3349.18 (-NH), 2899(Ar-CH), 1587 (C=N), 1446 (C=C), 1274.13 (C-N) $Cm^{-1}$   $^1H$  NMR (300MH<sub>2</sub> CDCl<sub>3</sub>)

$\delta$  ppm-9.6 (bs bezimidazole  $\text{>N-H}$ ) , 7.2-7.5 (m,12H, Ar-H) , 6.9(s,1H, C-H) , 2.59-2.93(m, 8H, pip-H)

**Docking tool and algorithm**

Molecular docking [13-14] was completed using V life MDS version 4.3 . The structures were drawn in 2D and converted to 3D and were optimized for docking study. The docking algorithm biopredicta B based on genetic algorithm which offers a successful strategy for searching the docked conformer's space.

The genetic algorithm method was performed to study and predict binding mode of newly synthesised compound with target enzyme ( homology modelled) cytochrome P450 lanosterol 14 $\alpha$  demethylase of *saccharomyces cerevisiae*.

**Invitro antifungal activity**

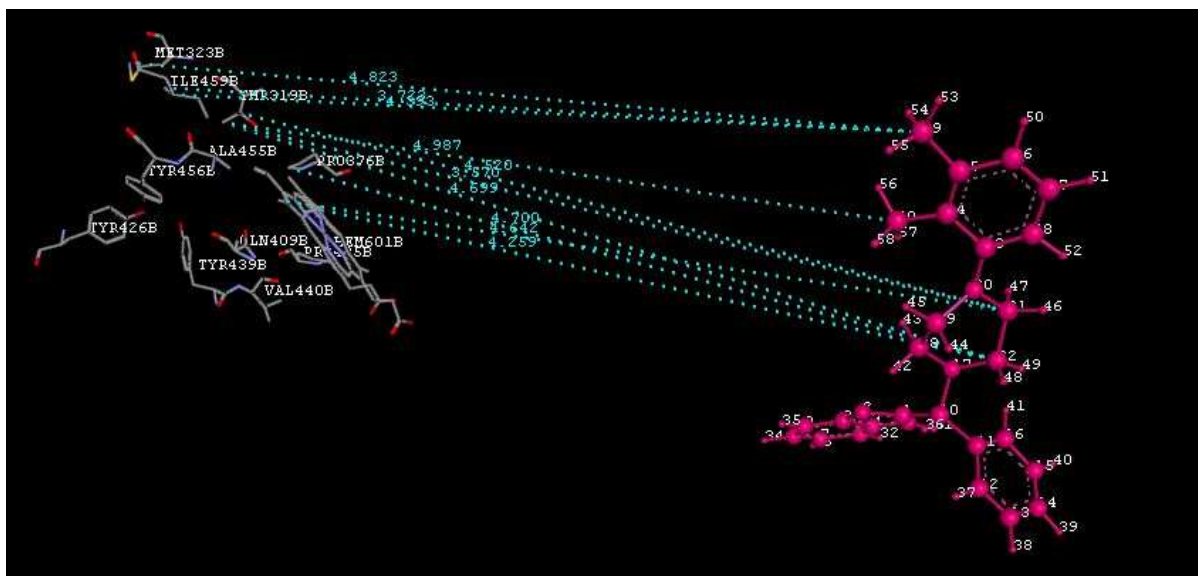
Antifungal activity of the synthesized compounds was tested against *Candida albicans* (ATCC2091). Invitro antifungal activity was evaluated using tube dilution (turbidimetric method). In this method minimal inhibitory concentration (MIC) of the antifungal agent was determined. The growth in the tubes was observed visually for turbidity and inhibition was determined by absence of growth. Ketoconazole and dimethyl sulphoxide were used as standard and solvent respectively.

Compound X has good antifungal activity 0.007 $\mu$  mole/ml which is closer to activity of ketoconazole.

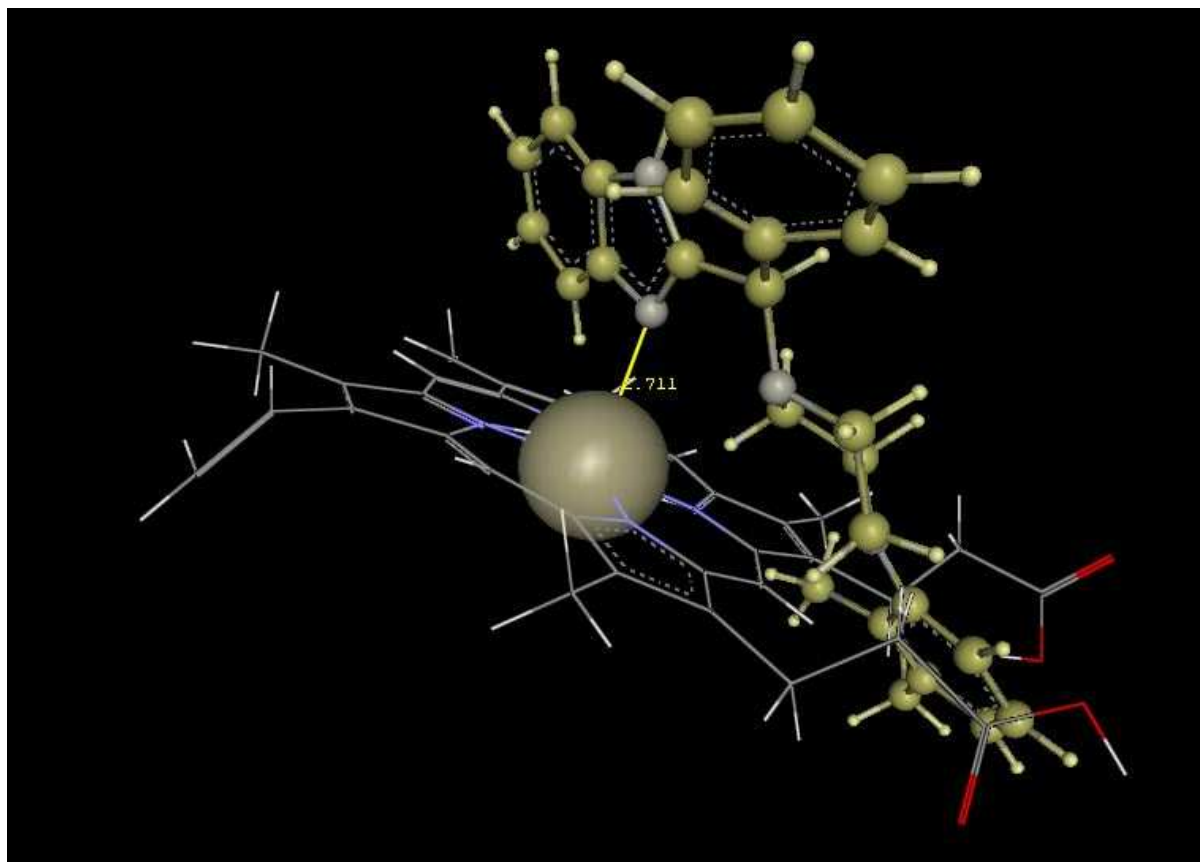
**RESULTS AND DISCUSSION****Molecular Docking**

All compounds showed binding interaction in the active site of the enzyme. The benzimidazole ring (compound X) is almost perpendicular to the porphyrin plane, with a ring nitrogen (N-3) atom co-ordinated to the heme iron. In particular, all hydrophobic substituents find location in hydrophobic subsite above heme ring. The  $\pi$  stacking. Hydrophobic interaction of 2,3 dimethyl phenyl side chain of X was observed with Threonine 319 in chain B, Isoleucine 459 in chain B, Methionine 323 in chain B. Hydrophobic interaction of piperazine side chain of 10 was observed with Proline 076 in chain B of lanosterol 14  $\alpha$  demethylase. Vander waal interaction was observed with amino acids Isoleucine 459 in chain B, valine 410 in chain B and Proline 076 in chain B.

a) Hydrophobic interactions of 2,3 dimethyl phenyl side chain of X with THR 319B, ILE 459B, MET 323B and piperazine side chain of 10 with PRO 076B with lanosterol 14 $\alpha$  demethylase enzyme



b)The benzimidazole ring of 10 is positioned almost perpendicular to the porphyrin plane, with a ring nitrogen(N-3) coordinated to the heme iron of the enzyme lanosterol 14 $\alpha$  demethylase



c)Vanderwaals interaction of 10 with amino acids ILE 459B, PRO 0760, VAL 410B of enzyme lanosterol 14 $\alpha$  demethylase

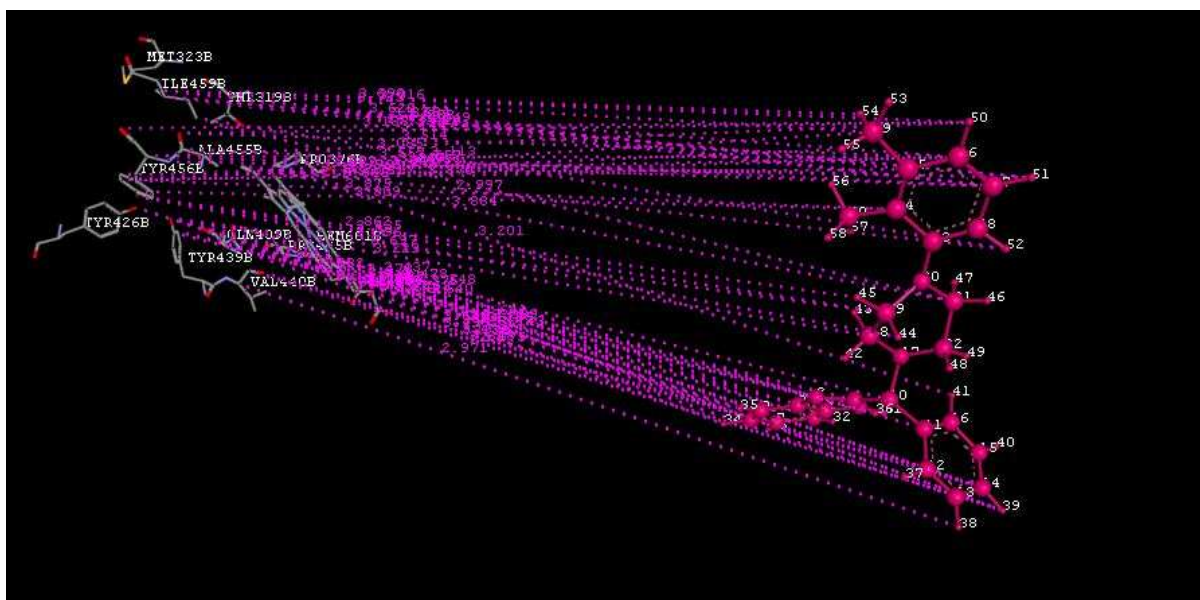
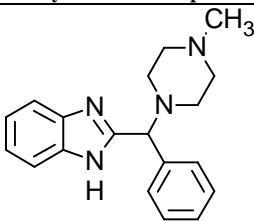
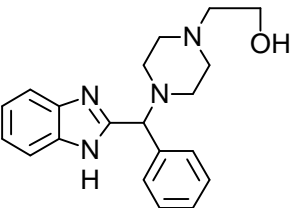
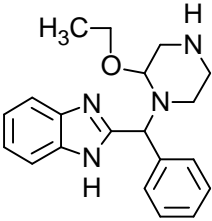
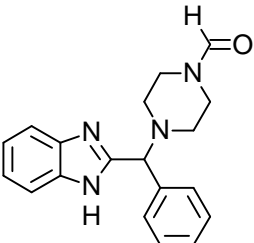
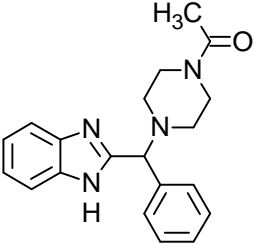
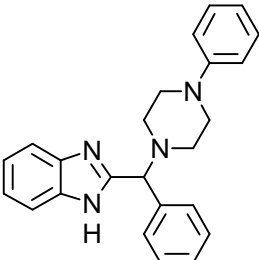
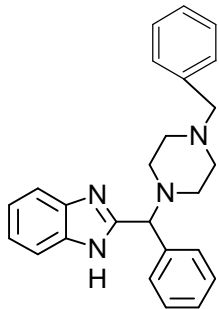
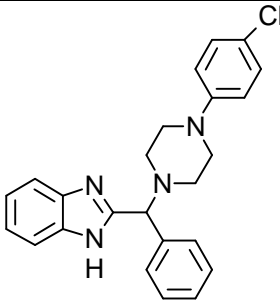
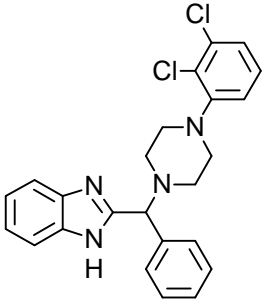
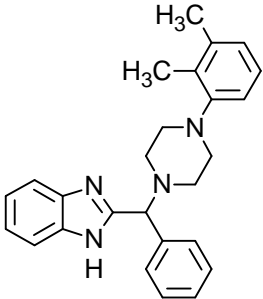
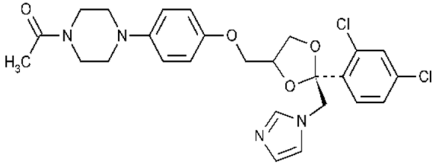


Figure 2 Best docking poses of X(active compound) on homology model of cytochrome P450 lanosterol 14 $\alpha$  demethylase of *saccharomyces cerevisiae*

Table 1: Synthesized compounds, Dock score and its antifungal activity

Code	Synthesised comp.	Dock score	MIC $\mu\text{mol/ml}$ against <i>Candida albicans</i>
1		-4.46	0.031
2		-4.82	0.031
3		-4.09	0.031
4		-4.04	0.031
5		-4.16	0.125
6		-4.19	0.062

7		-3.11	0.062
8		-5.34	0.125
9		-4.43	0.125
10		-4.81	0.015
Standard		-3.02	0.007



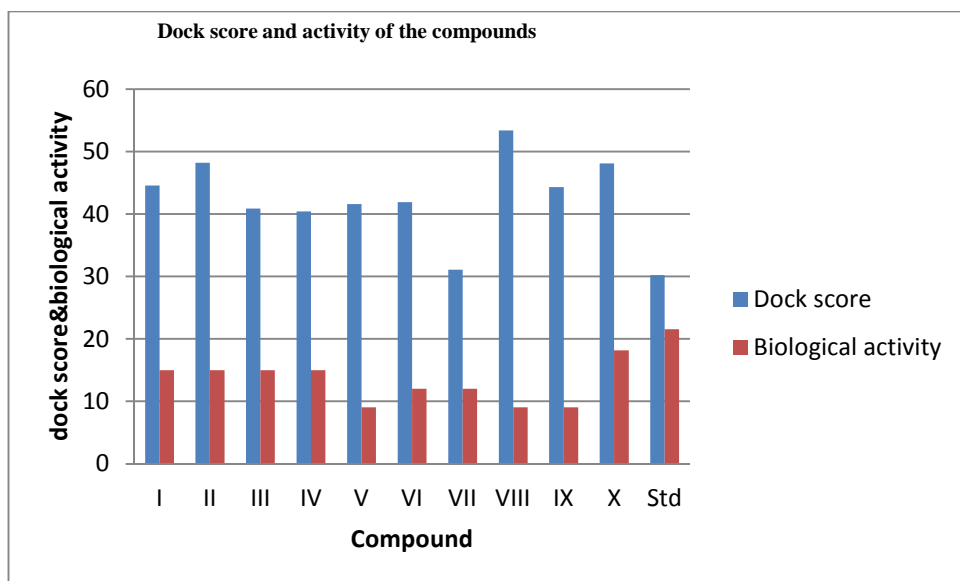


Figure3: Dock score &amp; activity of compounds

### CONCLUSION

A new series of benzyl benzimidazole derivatives have been synthesised and characterised by IR,<sup>1</sup>HNMR. In vitro antifungal activity of all synthesised compounds carried out and reported. Docking score and antifungal activity of these compounds have been justified by investigating their interaction with (homology modelled) cytochrome P450 lanosterol 14 $\alpha$  demethylase of *saccharomyces cerevisiae*. The compound X showed comparable activity with ketoconazole.

### Acknowledgements

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