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Microwave assisted synthesis, antifungal evaluation and moleculer docking of benzimidazole derivatives

Rani S. Kankate[*1], Parag S. Gide[1] and Deepak P. Belsare[2]

[1]*Department of Pharmaceutical Chemistry, Pune University, M.E.T's Institute of Pharmacy, Nashik, Maharashtra, India*
[2]*Department of Pharmaceutical Chemistry, Pune University, NDMVPS college of Pharmacy, Nashik, Maharashtra, India*

ABSTRACT

A novel series of biphenyl carbonyl piperazine moiety carrying benzimidazole derivatives, based on an initial design by molecular docking study of this scaffold at the active site of the fungal enzyme of cytochrome P450 family, lanosterol 14 α -demethylase (CYP51) was synthesized by microwave irradiation. The synthesized compounds were characterized by elemental and spectral analysis (IR, ^{1}H NMR and mass spectrometry).The screening of the synthesized compounds for invitro antifungal activity against Candida albicans revealed activity in many of the compounds as comparable to that of ketoconazole.

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Keywords: Benzimidazole, Biphenyl carbonyl chloride, Antifungal, Lanosterol14-α-demethylase, Microwave Irradiation, Docking.

INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound. The heterocyclic portion of the benzimidazole ring system; imidazole skeleton is found in several naturally occurring compounds which include amino acid histidine (a normal constituent of most proteins), histamines, the purines and biotin. The discovery that 5, 6-dimethyl-l-(α-ribofuranosyl) benzimidazole was an integral part of the chemical structure of vitamin B^{12} [1]. The 1, 2-disubstituted benzimidazole scaffold is a versatile pharmacophore in drug discovery due to wide range of biological activities exhibited by compounds bearing this structural unit [2-10]. Benzimidazole itself and 2-methyl benzimidazole has a good effect on Candida albicans and Aspergillus fumigates [11]. The benzimidazoles still remain one of the most versatile classes of compounds against fungi therefore, are useful substructures for further molecular exploration as an antifungal agent.

On the other hand, it has been observed that piperazine moiety has a great versatility in fusing to various ring systems and the N-bridged heterocycles derived from them are associated with different pharmacological activities. The Aryl, 1, 4-diaryl piperazines and carbonyl piperazine are common structural fragments of centrally acting antifungal drugs respectively. The incorporation of different substitutes in piperizine moiety is an significant synthetic strategy in medicinal chemistry due to its wide range of biological applications, proper alkality, solubility nature in water and physicochemical properties [12-13].

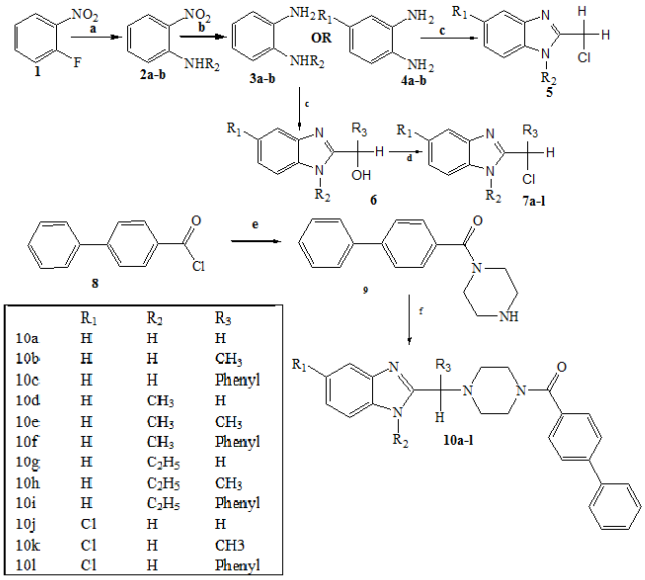
Given the worrying rise in fungal infections in the human population and decreasing success in the management of fungal infections; the present medical situation is by no means satisfactory with currently available antifungal therapy, therefore more effective and safe drugs are urgently needed.

In view of above consideration and based on our previous homology modeling and docking studies (reported in journal of pharmacy research, in press.), we designed and synthesized a series of benzimidazole derivatives using microwave irradiation. The newly prepared derivatives were docked into the active site of homology modeled CYP51 of *C.albicans*, using Vlife Software, MDS.4.3. The chemical structures of the new derivatives were confirmed by elemental and spectral (^{1}H-NMR and Mass) analyses. All compounds were investigated for *in vitro* antifungal activity against C.albicans.

MATERIALS AND METHODS

All materials were procured from Sigma Aldrich and Merck specialties Pvt. Ltd. (Mumbai. India). Solvents were dried and distilled being used. Anhydrous sodium sulphate was used to dry the solvents. Thin Layer Chromatography (TLC) analyses were carried out on aluminum plates (Merck) precoated with silica gel 60 F254 (0.2 mm), and spots were visualized with UV light and I2. Liquid intermediates were checked for purity using Gas chromatography (Pack column SE-30, OV-101, and capillary column BP-5). Gravity column chromatography was performed using silica gel (Merck 60). Melting points were taken in open glass capillary using OMEGA melting point apparatus and were uncorrected. Infra red (IR) spectra were recorded on KBr pellets on a Shimadzu 1000 FTIR spectrometer in the range of 4000-200 cm-1, Resolution 2.0 with number of scan - 45. Apodization; Happ-Genzel. Proton (1H) Nuclear Magnetic Resonance (NMR) spectra of compounds were recorded on Bruker Advance II 400 NMR Spectrophotometer using $CDCl_3$ solvent, at SAIF, Punjab University, Chandigarh. Mass spectra of compounds were recorded on API 4000 Q TRAP LC/MS/MS system using electron spray ionization positive ion mass spectrometric technique, at NHRDF, Chitegaon, Nashik. Elemental analyses were performed on a Perkin-Elmer 2400 Analyser and are within ±0.4% of theoretical values.

The method followed for the synthesis of benzimidazole derivatives has been elaborated here:



	R_1	R_2	R_3
10a	H	H	H
10b	H	H	CH_3
10c	H	H	Phenyl
10d	H	CH_3	H
10e	H	CH_3	CH_3
10f	H	CH_3	Phenyl
10g	H	C_2H_5	H
10h	H	C_2H_5	CH_3
10i	H	C_2H_5	Phenyl
10j	Cl	H	H
10k	Cl	H	CH3
10l	Cl	H	Phenyl

Scheme 1: Synthesis of benzimidazole derivatives

Reagents and conditions: *(a) K_2CO_3, DMF, alkylamine; (b) ammonium chloride, zinc dust; (c) K_2CO_3, methanol, reflux; (d)Thionyl chloride (e) and (f) N,N-Dimethylformamide, K_2CO_3.*

Synthesis of N1-methyl/ethyl/propyl-2-nitroaniline[14]**(2a, 2b).**

N1-methyl/ethyl-2-nitroaniline **(2)** was synthesized by using (0.24 mol) of 2-fluronitrobenzene **(1)** dissolved in DMF and anhydrous K_2CO_3 was added to it.Methylamine/ethylamine(6 mol) was slowly added it through dropping

funnel in cold condition. After complete addition of methylamine/ethylamine solution the reaction mixture was kept in microwave for 10 min. The completion of reaction was checked by monitoring TLC. After completion, the reaction mixture was poured into ice cold water with stirring and extracted with the product with ether, the ether layer was separated and dried with sodium sulphate, and then ether was removed by distillation to get products **2a, 2b**.

Synthesis of N1- methyl/ethyl-o-Phenylenediamine[15] **(3a, 3b):**
N^1- methyl/ethyl-o-Phenylenediamine (5 mM), Zinc dust (0.25 gm) and ammonium chloride (10 mM) in 5 ml water were mixed thoroughly in a small beaker (25 ml). The reaction solid mixture was placed in a microwave oven (300Watt) at 50% power level for 8 to 15 min; the progress of the reaction was monitored by checking the solubility of the solid reaction mixture product in dilute HCl, when the entire organic solid dissolved it was filtered to separate Zinc dust, and neutralized with aqueous solution. The solid was separated by filtration and recrystallized from an appropriate solvent (aqueous Ethanol).

Synthesis of 5-substituted-1-alkyl-2-(α-chloromethyl)benzo[d] imidazoles (7a-l):
In a typical experiment, (0.163 m) **3a-b** or **4a-b**, (0.170 m) of monochloroacetic acid/lactic/mandelic acid, K_2CO_3 and 25 ml of methanol was taken in a RBF and then placed in microwave irradiation at 350 W for 15 min. The reaction was monitored by TLC. A test portion was added in water and basified with ammonia solution. The solid was extracted with ether and TLC of this ether extract was done to check for completion of reaction. After completion, the reaction mixture was poured in ice-cold water. It was then basified with concentrated ammonia solution. The solid precipitate was filtered immediately and dried.

Synthesis of 1-[(4-phenylphenyl)carbonyl]piperazine from anhydrous piperazine and biphenyl carbonyl chloride 9.
A solution of Biphenyl carbonyl chloride (10 g, 55.50 mmol) , Piperazine (5.26 g, 61.05 mmol), and K_2CO_3 (23.01 g, 166.5 mmol) in N, N dimethylformamide was taken in a RBF and then placed in microwave irradiation at 400 W for 45 min. with continue stirring.The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of the reaction, water was added to the reaction mixture and the product extracted by shaking the reaction mixture with dichloromethane in a separating funnel.The dichloromethane layer was washed successively with water and brine, dried over anhydrous sodium sulfate. Evaporation of the solvent gave the product **8a-l**. Recrystallized with chloroform.

Synthesis of Substituted Benzimidazole Derivatives Bearing 1-[(4-phenylphenyl)carbonyl]piperazinyl alkyl/aryl benzo(d) imidazole derivatives 10a-l.
A solution of 5-substituted-1-alkyl-2-(α-chloromethyl/ethyl/phenyl)benzo[d] imidazoles **(5a-l)** (1.75 g,0.01051mol) and1-[(4-phenylphenyl)carbonyl]piperazine(3g,0.0105mol) in N, N dimethylformamide was taken in a RBF K_2CO_3 (2gm) was added to the reaction mixture. Then placed in microwave irradiation at 450 W for 56 min. with continue stirring.The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of the reaction, water was added to the reaction mixture and the product extracted by shaking the reaction mixture with dichloromethane in a separating funnel.The dichloromethane layer was washed successively with water and brine, dried over anhydrous sodium sulfate. Evaporation of the solvent gave the product. **10a-l** Recrystallized with various solvent like chloroform, ethanol, methanol.

(4-((1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)([1,1'-biphenyl]-4-yl)methanone.10a
Compound **10a** was obtained as off white solid (yield: 66.12%; MP:186-188^{0}C); IR (KBr): 3222 (N-H), 2260 (C-H), 1668(C=O),1600(C=N), 1552(C=C), 1230 (C-N) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.64(s,3H,-CH_3), 3.5-3.95 (m, 8H,PIP-H), 3.993 (s, 2H, -CH_2-),7.30-7.71 (m, 13H, Ar -H); ESI-MS m/z: 396,229,181.Anal. Calcd. For C25H24N4O (396.20): C, 76.07; H, 6.38; N, 13.65; O, 3.90. Found C, 75.01; H, 6.32; N, 11.62; O, 2.99.

[1, 1'-biphenyl]-4-yl (4-((1-ethyl-1H-benzo[d]imidazol-2-yl) methyl) piperazin-1-yl) methanone.10b
Compound **10b** was obtained as off white solid (yield: 62.71%; MP:193-195^{0}C); IR (KBr): 3218 (N-H), 2265 (C-H), 1663(C=O),1602(C=N), 1558(C=C), 1235 (C-N) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.10(t,2H,-CH_2), 4.44(q,3H,-CH3), 3.3-3.75 (m, 8H,PIP-H), 4.881 (s, 2H, -CH_2-),7.25-7.89 (m, 13H, Ar -H); ESI-MS m/z:410,395,243.Anal. Calcd. For C26H26N4O (410): C, 76.39; H, 6.65; N, 13.20; O, 3.77. Found C, 76.34; H, 6.41; N, 12.87; O, 3.72.

(4-((1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)([1,1'-biphenyl]-4-yl)methanone.10c
Compound **8c** was obtained as off white solid (yield: 57.12%; MP:243-245^{0}C); IR (KBr): 3223 (N-H), 2267 (C-H), 1663(C=O),1604(C=N), 1560(C=C), 1233 (C-N) cm-1; 1H NMR (300 MHz, $CDCl_3$) δ ppm:1.15(t,2H,-CH2),1.99(m,5H,CH3,CH2) ,4.58(t,2H,-CH2), 3.6-3.85 (m, 8H,PIP-H), 5.12 (s, 2H, -CH2-),7.15-7.79 (m, 13H, Ar -H); ESI-MS m/z: 472,257,181.Anal. Calcd. For C31H28N4O (472): C, 76.68; H, 6.89; N, 12.78; O, 3.65. Found C, 76.62; H, 6.73; N, 12.72; O, 3.68.

[1,1'-biphenyl]-4-yl(4-((1-methyl-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)methanone.10d
Compound **8d** was obtained as off white solid (yield: 50.88%; MP:194-196^{0}C); IR (KBr): 3221 (N-H), 2264 (C-H), 1659(C=O),1601(C=N), 1558(C=C),1228 (C-N) cm^{-1}; 1H NMR (300 MHz, CDCl3) δ ppm: 1H NMR (300 MHz, CDCl3) δ ppm:1.59(s,3H,-CH3), 3.2-3.81 (m, 8H,PIP-H), 3.993 (s, 2H, -CH2-),7.27-7.68 (m, 12H, Ar -H);; ESI-MS m/z: 410,395,265.Anal. Calcd. For C26H26N4O (410): C, 68.56; H, 5.53; N, 15.37; O, 10.54. Found C, 68.41; H, 5.49; N, 15.28; O, 10.50.

[1,1'-biphenyl]-4-yl(4-(1-(1-methyl-1H-benzo[d]imidazol-2-yl)ethyl)piperazin-1-yl)methanone.10e
Compound **10e** was obtained as off white solid (yield: 48.52%; MP:203-205^{0}C); IR (KBr): 3218 (N-H), 2265 (C-H), 1663(C=O),1602(C=N), 1558(C=C), 1235 (C-N) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.18(t,2H,-CH_2), 4.39(q,3H,-CH3), 3.42-3.81 (m, 8H,PIP-H), 4.72 (s, 2H, -CH_2-),7.24-7.85 (m, 12H, Ar -H); ESI-MS m/z:424,395,288,181.Anal. Calcd. For C27H28N4O (424): C, 69.07; H, 5.80; N, 14.92; O, 10.22. Found C, 69.01; H, 5.78; N, 14.81; O, 10.03.

[1,1'-biphenyl]-4-yl(4-((1-methyl-1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)methanone.10f
Compound **8f** was obtained as off white solid (yield: 57.12%; MP:243-245^{0}C); IR (KBr): 3223 (N-H), 2267 (C-H), 1663(C=O),1604(C=N), 1560(C=C), 1233 (C-N) cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) δ ppm:1.09(t,2H,-CH2),1.94(m,5H,CH3,CH2) ,4.53(t,2H,-CH2), 3.4-3.75 (m, 8H,PIP-H), 5.02 (s, 2H, -CH2-),7.10-7.74 (m, 12H, Ar -H); ESI-MS m/z: 486,440,437,302.Anal. Calcd. For C32H30N4O (486): C, 69.55; H, 6.04; N, 14.48; O, 9.93.Found C, 69.52; H, 6.01; N, 14.42; O, 9.89.

[1,1'-biphenyl]-4-yl(4-((1-ethyl-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)methanone.11g
Compound **8g** was obtained as off white solid (yield: 59%; MP:205-207^{0}C); IR (KBr): 3222 (N-H), 2263 (C-H), 1661(C=O),1602(C=N), 1558(C=C), 1230 (C-N)cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.41(s,3H,-CH_3), 3.2-3.82 (m, 8H,PIP-H), 3.47 (s, 2H, -CH_2-),7.32-7.76 (m, 12H, Ar -H); ESI-MS m/z:424,395,263,181.Anal. Calcd. For C27H28N4O (424): C, 70.18; H, 5.66; Cl, 7.97; N, 12.59; O, 3.60Found C, 70.14; H, 5.61; Cl, 7.92; N, 12.54; O, 3.58.

[1,1'-biphenyl]-4-yl(4-(1-(1-ethyl-1H-benzo[d]imidazol-2-yl)ethyl)piperazin-1-yl)methanone.10h
Compound **8h** was obtained as off white solid (yield: 61.45%; MP:197-199^{0}C); IR (KBr): 3218 (N-H), 2265 (C-H), 1663(C=O),1607(C=N), 1563(C=C), 1235 (C-N)cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.19(t,2H,-CH_2), 4.24(q,3H,-CH3), 3.45-3.98 (m, 8H,PIP-H), 3.99 (s, 2H, -CH_2-),7.32-7.91 (m, 12H, Ar -H); ESI-MS m/z:438,409,365,173.Anal. Calcd. For C28H30N4O (438): C, 76.39; H, 6.65; N, 13.20; O, 3.77. Found C, 76.31; H, 6.38; N, 12.85; O, 3.71.

[1,1'-biphenyl]-4-yl(4-((1-ethyl-1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)methanone.10i
Compound **10i** was obtained as off white solid (yield: 53%; MP:232-234^{0}C); IR (KBr): 3217 (N-H), 2254 (C-H), 1659(C=O),1609(C=N), 1567(C=C), 1238 (C-N)cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) δ ppm:1.20(t,2H,-CH2),2.12(m,5H,CH3,CH2) ,4.45(t,2H,-CH2), 3.1-3.84 (m, 8H,PIP-H), 4.98(s, 2H, -CH2-),7.23-7.81 (m, 12H, Ar -H); ESI-MS m/z: 500,423265,77.Anal. Calcd. For C33H32N4O (500): C, 71.10; H, 6.18; Cl, 7.50; N, 11.84; O, 3.38. Found C, 71.12; H, 6.12; Cl, 7.56; N, 11.83; O, 3.32.

[1,1'-biphenyl]-4-yl(4-((5-chloro-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)methanone.10j
Compound **10j** was obtained as off white solid (yield: 58.45%; MP:200-202^{0}C); IR (KBr): 3225 (N-H), 2261 (C-H), 1664(C=O),1609(C=N), 1555(C=C), 1234(C-N), 729(C-Cl) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.51(s,3H,-CH3), 3.21-3.92 (m, 8H,PIP-H), 4.13 (s, 2H, -CH2-),7.39-7.91 (m, 13H, Ar -H); ESI-MS m/z:430, 395,265,181.Anal. Calcd. For C25H23ClN4O (430): C, 72.88; H, 5.88; F, 4.43; N, 13.08; O, 3.69 Found C, 72.58; H, 5.88; F, 4.39; N, 13.05; O, 3.71.

[1,1'-biphenyl]-4-yl(4-(1-(5-chloro-1H-benzo[d]imidazol-2-yl)ethyl)piperazin-1-yl)methanone.10k
Compound **10k** was obtained as off white solid (yield: 49%; MP:197-199^{0}C); IR (KBr): 3215 (N-H), 2247 (C-H), 1651(C=O),1614(C=N), 1549(C=C), 1238 (C-N),721(C-Cl) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.17(t,2H,-CH_2), 4.88(q,3H,-CH3), 3.47-3.99 (m, 8H,PIP-H), 4.21 (s, 2H, -CH_2-),7.33-7.92 (m, 13H, Ar -H); ESI-MS m/z:444,413,394,181.Anal. Calcd. For C26H25ClN4O (444): C, 73.28; H, 6.15; F, 4.29; N, 12.66; O, 3.62 Found C, 73.25; H, 6.21; F, 4.15; N, 12.66; O, 3.59.

[1,1'-biphenyl]-4-yl(4-((5-chloro-1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)methanone.10l
Compound **10l** was obtained as off white solid (yield: 48%; MP:218-220^{0}C); IR (KBr): 3225 (N-H), 2261 (C-H), 1660(C=O),1608(C=N), 1567(C=C), 1230(C-N), 732(C-Cl) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ ppm:1.25(t,2H,-CH2),1.88(m,5H,CH3,CH2) ,4.12(t,2H,-CH2), 3.1-3.68 (m, 8H,PIP-H), 4.98 (s, 2H, -CH2-),7.12-7.87 (m, 13H, Ar -H); ESI-MS m/z: 507,471,429,393,.Anal. Calcd. For C31H27ClN4O (507): C, 73.28; H, 6.15; F, 4.29; N, 12.66; O, 3.62. Found C, 73.18; H, 6.02; F, 4.14; N, 12.45; O, 3.59.

***Invitro* Antifungal Activity [**16]
The stock solution of (1µ mole/ml) compounds (equimolar mixture) was prepared in DMSO and water. To each tube containing sterilized sabouraud's liquid medium (2 ml), 2 ml of drug solution were added. Each tube was inoculated with the microorganism and was kept at 30^{0}C for 14 days. The solutions were first tested at the concentration of 0.5 µmole/ml. The sets, which are found active at this concentration, were again tested at concentration of 0.25 µmole/ml. The groups, which were found active, were subjected to serial dilutions. The serial dilutions were made to obtain concentrations 0.5, 0.25, 0.125, 0.0625, 0.0314,0.0152 and 0.0076 µ mole/ml. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared. Each tube was inoculated with the microorganism. All the tubes were incubated at 30^{0}C for 14 days. The readings were taken as expressed as (-) if inhibition of growth is seen and (+) if inhibition of growth is not seen. [Table 1]

Docking tool and algorithm
Molecular docking [17-18] was completed using VLife MDS version 4.3. The structures were drawn in 2D and converted to 3D and were finally optimized for docking using VLife MDS.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.The genetic algorithm method was performed to study and predict the binding mode of newly synthesized compounds with the target enzyme (homology modeled) cytochrome P450 lanosterol 14-α-demethylase of *C. albicans*.

RESULTS AND DISCUSSION

Invitro Antifungal Activity
Antifungal activity of the synthesized compounds was tested against candida albicans *in vitro*. *In vitro* antifungal activity was evaluated using the tube dilution method (turbidimetric method). The turbidimetric method depends on the inhibition of growth in a microbial culture of uniform solution containing the drug in fluid medium favorable for rapid growth. In this method, minimal inhibitory concentration (MIC) of the antifungal agent was determined. The growth in the tube was observed visually for turbidity and inhibition was determined by the absence of growth. In the present study, *C.albicans* (ATCC10235) was used to investigate the activity. Ketoconazole and Dimethyl sulphoxide were used as standard and solvent respectively.

10f and 10l has a good antifungal activity as compared with the other twelve compounds at 0.0312 µmole /ml which is closer to ketoconazole activity as far as the *in vitro* results are concerned. Compounds.Figure.1

Table 1: Effect of synthesized compounds against *C.albicans* by serial dilution method

Sr. No.	Compounds	Concentration of compound required for inhibition (µmol/ml)						
		0.5	0.25	0.125	0.0625	0.0312	0.015	0.007
1.	10a	-	-	+	+	+	+	+
2.	10b	-	-	+	+	+	+	+
3.	10c	-	-	-	+	+	+	+
4.	10d	-	-	-	+	+	+	+
5.	10e	-	-	-	+	+	+	+
6.	10f	-	-	-	-	-	+	+
7.	10g	-	-	-	+	+	+	+
8.	10h	-	-	-	+	+	+	+
9.	10i	-	-	-	-	+	+	+
10.	10j	-	-	-	-	+	+	+
11.	10k	-	-	-	-	+	+	+
12.	10l	-	-	-	-	-	+	+
13.	Ketoconazole	-	-	-	-	-	-	-

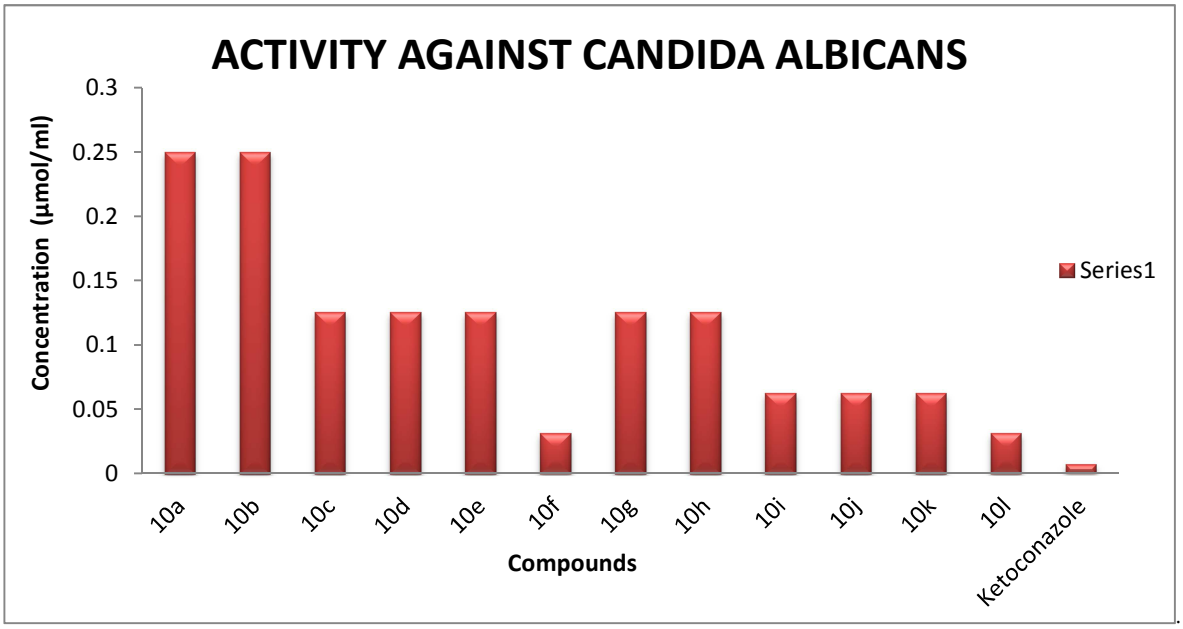


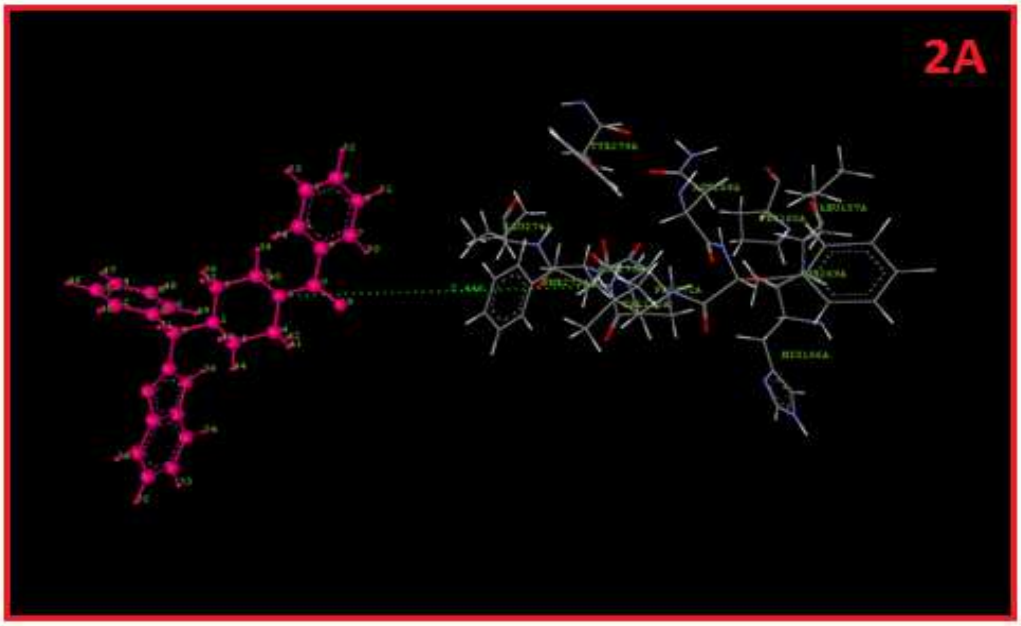
Figure.1. In vitro Antifungal activity against Candida albicans

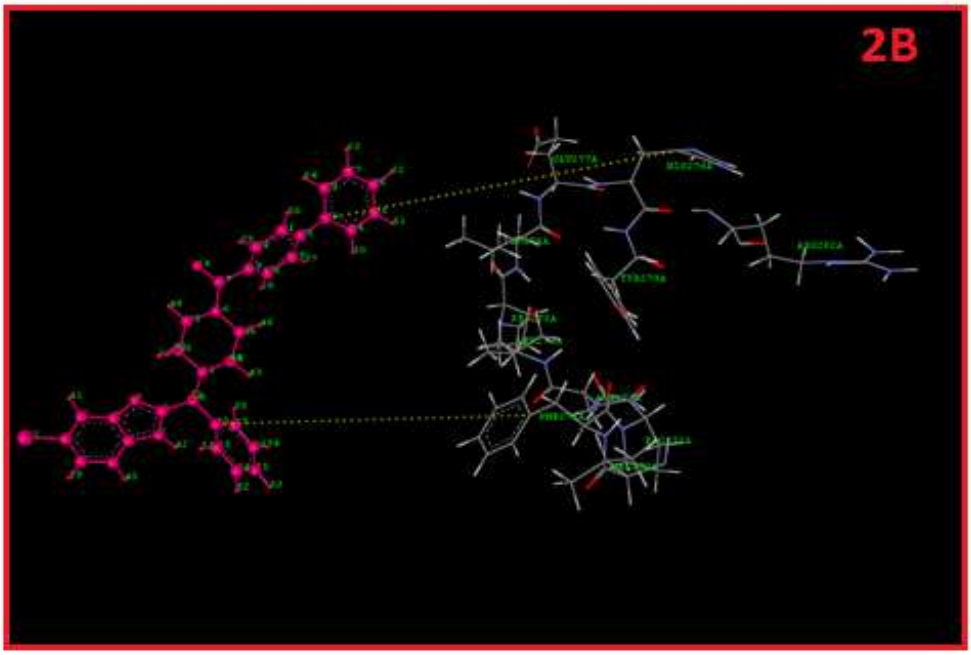
Docking results of antifungal activity:

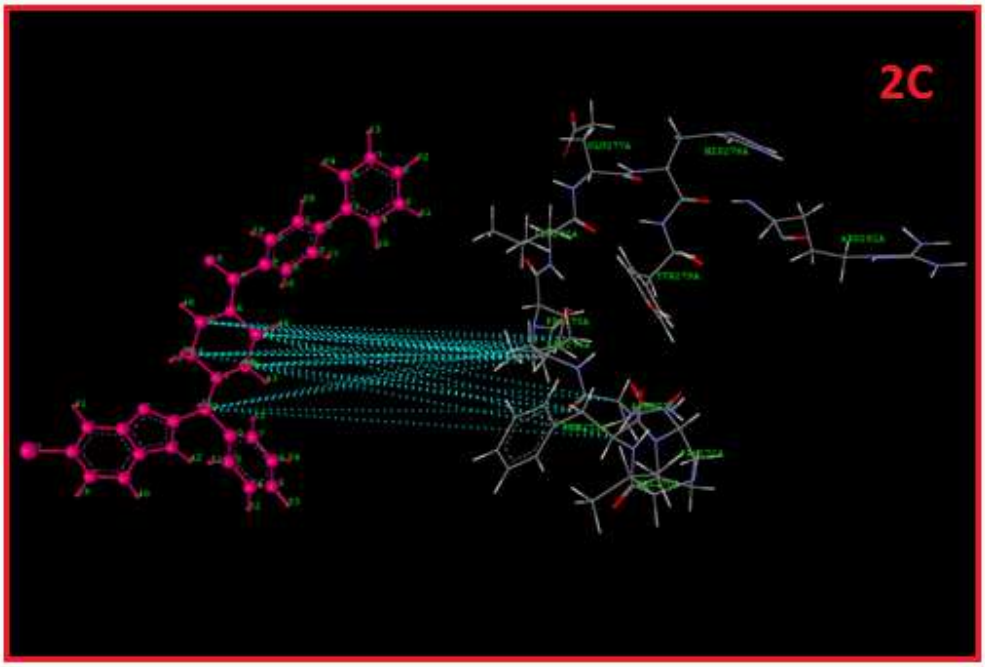
The genetic algorithm method was performed to study and predict the binding mode of newly synthesized compounds with the target enzyme (homology modeled) cytochrome P450 lanosterol 14-α-demethylase of C. albicans. All compounds showed binding in the active site of the enzyme. The benzimidazole ring (compounds **10a**-**l**) is positioned 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 a hydrophobic sub-site above the heme ring.The π- stacking, Hydrogen bonding, hydrophobic and vander walls interactions of the ligands with receptor proteins were analyzed which reveals novel set of information. The results of the docking analysis of most active antifungal compound (**10l**) and their interactions with the selected receptor proteins are discussed in the following sections **Figure.2**

Table.2.Dock scores and energy calculation for grip docking of ketoconazole and synthesized compounds

Ligand code	Dock score	Energy of Ligand after optimization G_{Ligand} (Kcal) Before docking	Energy of complex after optimization $G_{Complex}$(Kcal) after docking	$\Delta G_{Binding} = G_{Complex} - (G_{Protein} + G_{Ligand})$ (Kcal)
10a	-2.02	40.69	7756.21	-170.69
10b	-3.00	73.39	7770.82	-188.57
10c	-3.19	96.33	7795.03	-187.33
10d	-1.88	97.09	7839.44	-143.65
10e	-2.07	69.89	7818.97	-136.92
10f	-4.23	69.23	7792.42	-162.81
10g	-3.89	83.14	7790.66	-178.48
10h	-3.02	98.17	7804.86	-180.31
10i	-3.42	125.91	7907.31	-104.60
10j	-3.17	97.18	7827.24	-156.18
10k	-3.56	101.06	7854.92	-132.14
10l	-3.92	42.89	7773.58	-155.31
Ketoconazole	-3.10	97.18	7827.24	-156.18




2B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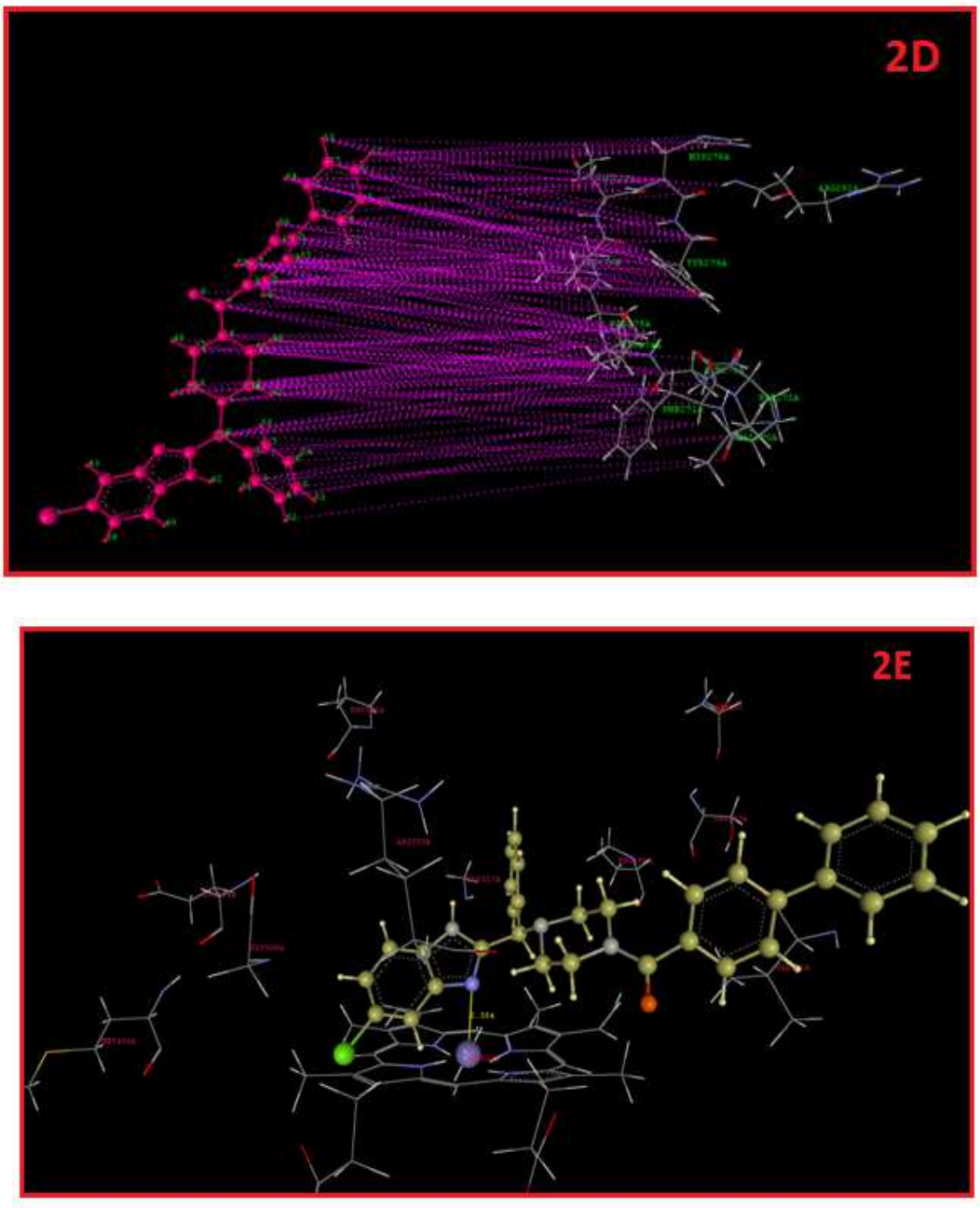

Fig.2.Best docking poses of 10l (active compound) on homology model of cytochrome p450 of *candida albicans.*

A. *Hydrogen bonding of carbonyl group of* ***10l*** *with LEU 276A.*
B. *Π- stacking of phenyl ring of* ***10l*** *with PHE 271 of cytochrome p450.*
C. *Hydrophobic interaction piperazine side chain of* ***10l*** *with PHE 271A, PRO 272A, ASN 273A of cytochrome p450.*
D. *Vander waal interaction with amino acids.*
E. *The Benzimidazole ring of* ***10l*** *is positioned almost perpendicular to the porphyrin plane, with a ring nitrogen (N-3) atom co-ordinated to the heme iron of cytochrome p450 of candida albicans.*

CONCLUSION

A new series of Benzimidazole derivatives have been synthesized and characterized fully by IR, MASS, ^{1}HNMR and Elemental analysis. In vitro antifungal activity of all the synthesized compounds carried out and reported. Docking scores and antifungal activity of these compounds have been justified by investigating their interaction at the active site of CYP51. The compounds **10l** (having 5-chloro and C2- Phenyl substitution on benzimidazole) showed comparable antifungal antifungal activity with ketoconazole.The present investigation opens a new lead for antifungal agents and there is a wide scope for future investigation.

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