



## Synthesis and In vitro Antifungal Evaluation of Benzoimidazolyl-piperazinyl-phenylmethanone Derivatives

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### ABSTRACT

Benzimidazole and piperazines are the important pharmacophores in the structures of many antifungal compounds. Further, the phenylmethanone are also a unique class of compounds whose antifungal profile is not much exploited. So to exploit their antifungal potential we have selected these three combinations and framed the novel parent structure for our research work. In this study a novel series of benzimidazoles derivatives was synthesized by microwave irradiation and characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, Infra Red (IR), and Mass Spectroscopy (MS), and by elemental analysis. The screening of compound for in vitro (turbidimetric method) antifungal activity against *C.albicans* revealed activity in many of the compounds as comparable to that of ketoconazole.

**Key words:** Antifungal activity, Benzimidazole, Piperazine and Phenylmethanone.

### INTRODUCTION

During the past two decades, the frequency of systemic infections has increased dramatically along with the number of invasive, mostly opportunistic, fungal species carrying infectious diseases<sup>1</sup>. In clinic, there are very few antifungal agents that can be used for life threatening fungal infections. Clinically available

antifungal agent include the following: polyenes (such as amphotericin B and nyastatin)<sup>2</sup> azoles (such as ketoconazole, fluconazole, itraconazole and voriconazole)<sup>3</sup> echinocandins (such as caspofungin and micafungin)<sup>4</sup>. None of the existing systemic antifungal satisfies medical need completely; there are weaknesses in spectrum, potency, safety and pharmacokinetic properties. Moreover, severe resistance has been observed

for triazoles<sup>5</sup>. Along with this the cost of treatment for such infections is also on a high. So an ideal, which has a broad spectrum of activity and has a low on side effects, is to be found having a cheaper cost of treatment.

Therefore, there is an emergent need to develop new antifungal drugs with novel chemical structures and novel modes of action. The field of antifungal development is wide open for extensive research work.

Benzimidazole derivatives possess great importance in medicinal chemistry due to wide variety of pharmacological activity<sup>6,7</sup> in controlling cardiovascular diseases<sup>8</sup>, anticancer properties<sup>9</sup>, antiinflammatory<sup>10</sup>, antibacterial<sup>11</sup>, antifungal<sup>12</sup>, antidiabetic<sup>13</sup> and anti HIV<sup>14</sup> activity and some benzimidazoles are antioxidant<sup>15-16</sup>. The special benzene-fused imidazole ring could readily interact with various active targets in biological system via diverse noncovalent interactions like hydrogen bonds, coordination, ion dipole, cation- $\pi$  stacking and hydrophobic effect as well as van der Waals force, therefore benzimidazole-based derivatives exhibit various bioactivities<sup>17</sup>. Benzimidazole and its derivatives are used in organic synthesis and vermicides or fungicides as they inhibit the action of certain microorganisms. Examples of

benzimidazole class fungicides include benomyl, carbendazim, chlorfenazole, cypendazole, debacarb, fuberidazole, furophanate, mecarbinzid, rabenzazole, thiabendazole, thiophanate. A perusal of literature revealed that benzimidazol, piperazine ring and phenylmethanone were significant structural moieties for antifungal activity<sup>18-21</sup>.

Considering the above scenario of biological profile we have designed, synthesized and evaluated for antifungal activity series of benzoimidazolyl-piperazinyl-phenylmethanone derivatives.

## RESULTS AND DISCUSSION

The syntheses of compounds **11a–11x** are outlined in Scheme 1.

The N1-methyl/ethyl-2-nitroaniline **2a-b** was synthesized by using 2-fluoronitrobenzene **1**, methylamine/ethylamine and DMF as solvent in microwave [22]. The nitro group of phenyl ring **2a-b** was further reduced to amino group in presence zinc and ammonium chloride to give **3a-b** [23]. Compounds **3a-b/4a-b** and lactic/mandelic acid **5a-b** dissolved in methanol and reflux in microwave to give **6a-f**. Then activation of carboxylic functional group of **6a-f** was carried out by using thionyl

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

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

(-)Indicates absence of growth; (+) Indicates presence of growth; K: Ketoconazole

chloride to give **7a-f**. Piperazine **8** and benzoyl chloride **9a-c** were dissolved in *N,N* dimethylformamide and reaction mixture stirred for 6 h at 80° C on a magnetic stirrer to give **10a-c**. Further **10a-c** condensed with **7a-f** to get target compounds **11a-x**.

### Reagents and conditions

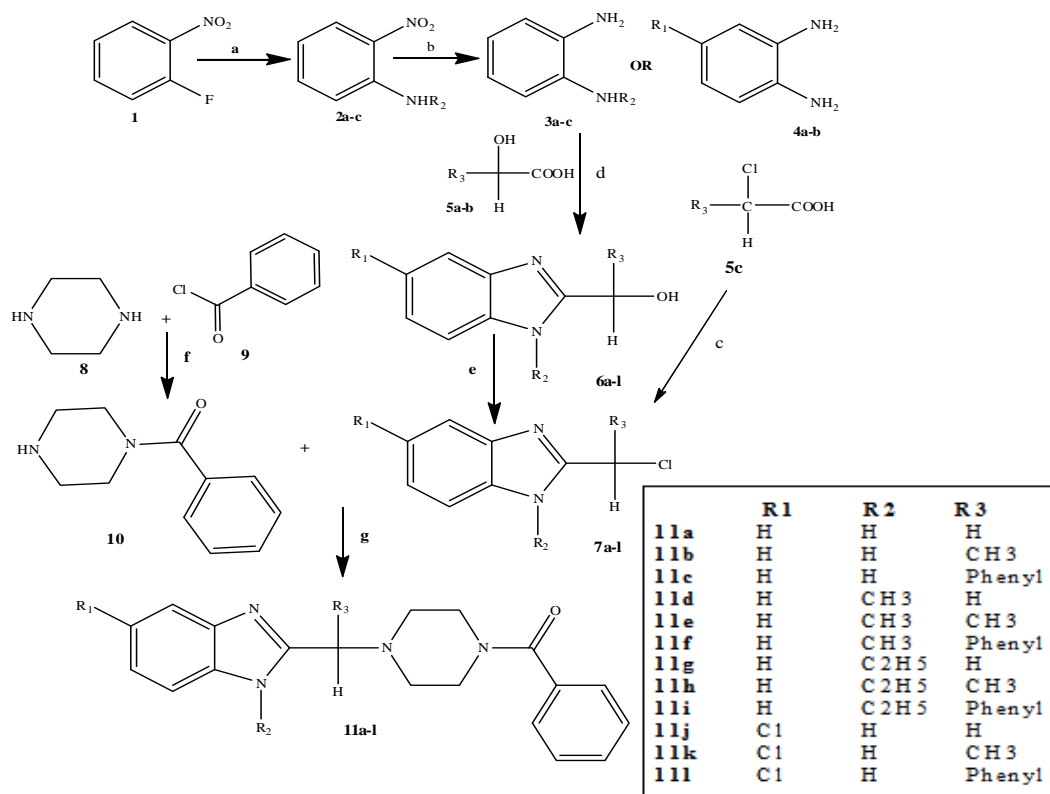
(a)  $K_2CO_3$ , DMF, alkylamine; (b) ammonium chloride, zinc dust; (c) mono chloro acetic acid, methanol, reflux; (d) lactic acid/mandelic acid, methanol, reflux; (e) thionyl chloride; (f) and (g) *N,N*-Dimethylformamide,  $K_2CO_3$ .

### Invitro Antifungal Activity

Antifungal activity of the synthesized compounds was tested against different fungal spores *in vitro*. *In vitro* antifungal activity was evaluated using the tube dilution method (turbidimetric method). The turbidimetric method [24] 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 MIC is the lowest concentration of an antimicrobial agent that inhibits the test organism. 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.

The antifungal activity clearly indicated that 5-chloro substituted benzimidazole derivatives **11l** has a good antifungal activity as compared with the other twelve compounds at 0.015  $\mu\text{mole/ml}$  which is closer to ketoconazole activity as far as the *in vitro* results are concerned. Compounds **11e** and **11k** demonstrated equivalent activity against *C. albicans* and had an MIC of 0.03  $\mu\text{mole/ml}$ . The other compounds showed decreased in antifungal activity those are lack of asymmetric center at C 2 and unsubstituted N-1 position of benzimidazole.



Scheme 1: Synthesis of tertiary amine type of substituted benzimidazole derivatives

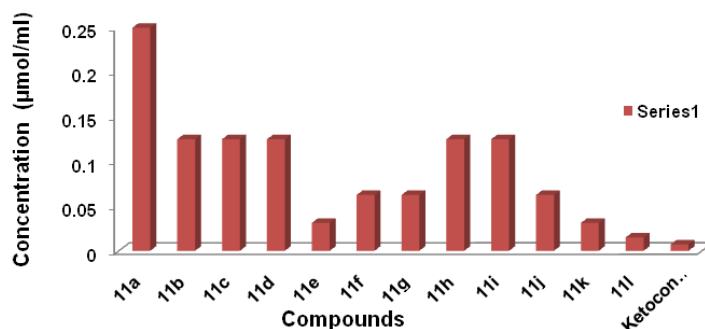


Fig. 1: In vitro Antifungal activity against *Candida albicans*

## EXPERIMENTAL

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  $I_2$ . 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 Elico 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  $4000-200\text{ cm}^{-1}$ , Resolution 2.0 with number of scan - 45. Apodization; Happ-Genzel. Proton ( $^1\text{H}$ ) Nuclear Magnetic Resonance (NMR) and  $^{13}\text{C}$  NMR spectra of compounds were recorded on Bruker Advance II 400 NMR Spectrophotometer using  $\text{CDCl}_3$  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  $\pm 0.4\%$  of theoretical values.

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

N1-methyl/ethyl-2-nitroaniline (2) was synthesized by using 25ml (33.5 gm, 0.24 mol) of 2-

fluronitrobenzene (1) dissolved in DMF and anhydrous  $\text{K}_2\text{CO}_3$  was added to it. 115 ml (6 mol) of methylamine/ethylamine 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 (3a, 3b)

N<sup>1</sup>- 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-H/Chloro-1-alkyl-2-(a-hydroxyethyl/benzylo)benzo[d]imidazoles (6a-l)

In a typical experiment, (0.163 m) 3a-b or 4a-b, (0.170 m) of lactic/mandelic 5a-b / monochloroacetic acid 5c 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 5-H/Chloro-1-alkyl-2-( $\alpha$ -chloroethyl/benzyl)benzo[d]imidazoles (6a-l)**

The activation of carboxylic function was carried out by using an excess of thionyl chloride and catalytic amount of dimethyl formamide placed in an ice cold water bath. To this mixture, 15 gm 2- $\alpha$ -hydroxy benzyl/ethyl-N<sup>1</sup>-alkyl-benzimidazole was added slowly with occasional stirring. This mixture was then placed in microwave irradiation at 250W for 10 min. Excess thionyl chloride was recovered under vacuum on a water bath. To the residue dry dioxane was added and stirred for 30 min. Dioxane was recovered under vacuum to get the final product.

#### **Synthesis of phenyl(piperazine-1-yl)methanone from anhydrous Piperazine and Benzoyl chloride 10.**

A solution of Benzoyl chloride **9** (8.26 g, 0.058mol) and Piperazine **8** (10.12 g, 0.117mol) in N, N dimethylformamide was taken in a RBF K<sub>2</sub>CO<sub>3</sub> (3gm) was added to the reaction mixture. The reaction mixture was stirred for 8 hr at 80°C on a magnetic stirrer (heat + 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 **10**. Recrystallized with chloroform.

#### **Synthesis of Substituted Benzimidazole Derivatives Bearing 1-[(4-phenyl)carbonyl]piperazinyl alkyl benzo(d)imidazole derivatives 11a-l**

A solution of 2-chloroalkyl/aryl substituted with or without N-substitution as well as with or without 5 and/or 6-substituted benzimidazole derivative (1.75 g, 0.01051mol) and 1-[(4-phenyl)

carbonyl]piperazine (3g, 0.0105mol) in N, N dimethylformamide was taken in a RBF. K<sub>2</sub>CO<sub>3</sub> (2gm) was added to the reaction mixture. The reaction mixture was stirred for 8h at 80°C on a magnetic stirrer (heat + 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. **11a-l** Recrystallized with various solvent like chloroform, ethanol, methanol.

#### **(4-((1H-benzo[d]imidazol-2-yl) methyl) piperazin-1-yl)(Phenyl) methanone (11a)**

Compound 11a was obtained as a white solid (yield: 74.35%; MP:224-226; IR (KBr): 3220 (N-H), 2365 (C-H), 1670(C=O), 1604(C=N), 1556(C=C), 1232 (C-N) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 3.45-3.51(m, 4H, PIP-H), 4.34(s, 2H, -CH<sub>2</sub>-), 4.79(s, 1H, N-H), 7.30-7.45 (m, 4H, Ar -H of Benzim) 7.89-8.07 (4H, Ar -H of benzoyl); <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>, TMS):  $\delta$  52.14 (CH<sub>2</sub>), 55.4(CH<sub>2</sub>), 61.7 (CH<sub>2</sub>), 117(arom. CH), 124 (arom. CH), 129 (arom. CH), 133.5 (quat. C), 139.8 (quat. C), 145.6 (quat. C), 171.6 (carbonyl C). ESI-MS m/z: 320, 243, 189, 131. Anal. Calcd. for C<sub>19</sub>H<sub>20</sub>N<sub>4</sub>O (320): C, 71.23; H, 6.29; N, 17.49; O, 4.99. Found: C, 71.10; H, 6.23; N, 17.43; O, 4.91.

#### **(4-(1-(1H-benzo[d]imidazol-2-yl) ethyl) piperazin-1-yl)(Phenyl) methanone (11b)**

Compound 11b was obtained as a white solid (yield: 71.25%; MP:234-236; IR (KBr): 3227 (N-H), 2361 (C-H), 1674(C=O), 1601(C=N), 1550(C=C), 1239 (C-N) cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 1.24(d, 1H, -CH-), 3.25-3.31(m, 4H, PIP-H), 4.13(q, 3H, -CH<sub>3</sub>), 5.09(s, 1H, N-H), 7.28-7.35 (m, 4H, Ar -H of Benzim) 7.79-8.01 (4H, Ar -H of benzoyl); <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>, TMS):  $\delta$  14.8 (CH<sub>3</sub>), 51.11 (CH<sub>2</sub>), 53.2(CH<sub>2</sub>), 61.5 (CH<sub>2</sub>), 115(arom. CH), 123 (arom. CH), 128 (arom. CH), 131.6 (quat. C), 135.5 (quat. C), 144.2 (quat. C), 172.5 (carbonyl C); ESI-MS m/z: 334, 319, 257, 145, 117. Anal. Calcd. for C<sub>20</sub>H<sub>22</sub>N<sub>4</sub>O (334): C, 71.83; H, 6.63; N, 16.75; O, 4.78. Found: C, 71.80; H, 6.61; N, 16.79; O, 4.73.

**(4-((1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)(Phenyl) methanone (11c)**

Compound 11c was obtained as a white solid (yield: 65.45%; MP:284-286; IR (KBr): 3219 (N-H), 2359 (C-H), 1671(C=O),1605(C=N), 1548(C=C), 1236 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm:3.10-3.14(m, 4H,PIP-H), 4.89(s, 1H,N-H), 5.04(s, 1H, C-H), 7.28-8.05 (Complex m, 4H, Ar -H);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  50.11 ( $\text{CH}_2$ ), 52.4( $\text{CH}_2$ ), 71.5 (CH), 118(arom. CH), 125 (arom. CH), 130 (arom. CH), 131.2 (arom. CH), 132.8 (quat. C), 136.5 (quat. C), 141.9 (quat. C), 144.3 (quat. C), 169.5; (carbonyl C); ESI-MS m/z:396,319, 291,279,207. Anal. Calcd. for  $\text{C}_{25}\text{H}_{24}\text{N}_4\text{O}$  (396): C, 75.69; H, 6.14; N, 14.11; O, 4.08 Found: C, 75.73; H, 6.10; N, 14.13; O, 4.04.

**(4-((1-methyl-1H-benzo[d]imidazol-2-yl) methyl)piperazin-1-yl)(Phenyl) methanone (11d)**

Compound 11d was obtained as a white solid (yield: 68%; MP:212-215; IR (KBr): 3225 (N-H), 2354 (C-H), 1668(C=O),1608(C=N), 1546(C=C), 1230 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm:3.01-3.05(m, 4H,PIP-H), 3.19(s, 3H, $\text{N-CH}_3$ ), 5.07(s, 1H, C-H), 7.28-7.35 ( m, 4H, Ar -H), 7.88-7.95 ( m, 4H, Ar -H);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  30.8 ( $\text{CH}_3$ ), 51.11 ( $\text{CH}_2$ ), 55.2( $\text{CH}_2$ ), 60.5 ( $\text{CH}_2$ ), 119(arom. CH), 122.5 (arom. CH), 128.7 (arom. CH), 132.6 (quat. C), 135.4 (quat. C), 144.5 (quat. C), 173.5 (carbonyl C); ESI-MS m/z: 334,319,229,203,145. Anal. Calcd. For  $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}$  (334): C, 71.83; H, 6.63; N, 16.75; O, 4.72 Found: C, 71.79; H, 6.61; N, 16.70; O, 4.74.

**(4-(1-(1-methyl-1H-benzo[d]imidazol-2-yl) ethyl)piperazin-1-yl)(Phenyl) methanone (11e)**

Compound 11e was obtained as a white solid (yield: 70.15%; MP:222-224; IR (KBr): 3205 (N-H), 2355 (C-H), 1663(C=O),1610(C=N), 1541(C=C), 1235 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 1.20(d,1H,-CH-), 3.22-3.29(m, 4H,PIP-H), 3.83(s, 3H,  $\text{N-CH}_3$ ), 4.16(q, 3H,-CH3), 7.28-7.35 (m, 4H, Ar -H of Benzim) 7.79-8.01 (4H, Ar -H of benzoyl);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  21.4 ( $\text{CH}_3$ ), 33.8 ( $\text{CH}_3$ ),54.2( $\text{CH}_2$ ), 59.8 ( $\text{CH}_2$ ), 62.10 (CH), 115(arom. CH), 121.9 (arom. CH), 127.9 (arom. CH), 134.5(quat. C), 136.6 (quat. C), 147.6 (quat. C), 171.2 (carbonyl C); ESI-MS m/z: 348,333, 319,217. Anal. Calcd. For  $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}$  (348): C, 72.39; H, 6.94; N, 16.08; O, 4.59 Found: C, 72.34; H, 6.97;

N, 16.05; O, 4.62.

**(4-((1-methyl-1H-benzo[d]imidazol-2-yl)(phenyl)methyl)piperazin-1-yl)(Phenyl) methanone (11f)**

Compound 11f was obtained as a white solid (yield: 66.55%; MP:291-293; IR (KBr): 3219 (N-H), 2359 (C-H), 1671(C=O),1605(C=N), 1548(C=C), 1236 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm:3.14-3.19(m, 4H,PIP-H), 3.83(s, 3H, $\text{N-CH}_3$ ), 5.01(s, 1H, C-H), 7.39-8.13 (Complex m, 4H, Ar -H);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  35.4 ( $\text{CH}_3$ ), 51.15 ( $\text{CH}_2$ ), 54.7( $\text{CH}_2$ ), 74.5 (CH), 116(arom. CH), 126 (arom. CH), 132.3 (arom. CH), 135.6 (arom. CH), 133.1 (quat. C), 137.4 (quat. C), 142.8 (quat. C), 143.2 (quat. C), 168.3; (carbonyl C); ESI-MS m/z: 410,395,333,305,207. Anal. Calcd. For  $\text{C}_{26}\text{H}_{26}\text{N}_4\text{O}$  (410): C, 76.07; H, 6.38; N, 13.65; O, 3.90 Found: C, 76.04; H, 6.35; N, 13.57; O, 3.87.

**(4-((1-ethyl-1H-benzo[d]imidazol-2-yl) methyl)piperazin-1-yl)(Phenyl) methanone (11g)**

Compound 11g was obtained as a white solid (yield: 74.35%; MP:224-226; IR (KBr): 3220 (N-H), 2365 (C-H), 1670(C=O),1604(C=N), 1556(C=C), 1232 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm:1.23(t,2H, $\text{N-CH}_2$ -), 3.35-3.41(m, 4H,PIP-H), 4.10(q, 3H,  $-\text{CH}_3$ ), 4.31(s, 2H,  $-\text{CH}_2$ -), 7.35-7.42 (m, 4H, Ar -H of Benzim) 7.84-8.05 (4H, Ar -H of benzoyl);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  14.6 ( $\text{CH}_3$ ), 38.6( $\text{CH}_2$ ), 49.3( $\text{CH}_2$ ), 55.4 ( $\text{CH}_2$ ), 56.7 ( $\text{CH}_2$ ), 118(arom. CH), 122.8 (arom. CH), 130.8 (arom. CH), 132.7(quat. C), 138.6 (quat. C), 149.5 (quat. C), 174.3 (carbonyl C); ESI-MS m/z: 348,319,243,159. Anal. Calcd. For  $\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}$  (348): C, 72.39; H, 6.94; N, 16.08; O, 4.59 Found: C, 72.15; H, 6.81; N, 16.05; O, 4.53.

**(4-(1-(1-ethyl-1H-benzo[d]imidazol-2-yl) ethyl)piperazin-1-yl)(Phenyl) methanone (11h)**

Compound 11h was obtained as a white solid (yield: 64%; MP:275-277; IR (KBr): 3227 (N-H), 2361 (C-H), 1674(C=O),1601(C=N), 1550 (C=C), 1239 (C-N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm:1.22-1.27(m,3H,  $-\text{CH}_3$ ), 3.23-3.28(m, 4H,PIP-H), 4.10(q, 3H,- $\text{CH}_3$ ), 5.02(s, 1H, N-H), 7.26-7.32 (m, 4H, Ar -H of Benzim) 7.95-8.01 (4H, Ar -H of benzoyl);  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  14.6 ( $\text{CH}_3$ ), 20.8 ( $\text{CH}_3$ ), 48.7( $\text{CH}_2$ ), 51.2( $\text{CH}_2$ ), 53.1 ( $\text{CH}_2$ ), 60.2 (CH), 120(arom. CH), 125.8 (arom. CH),

132(arom. CH), 133(quat. C), 134.5(quat. C), 150.6 (quat. C), 171.2 (carbonyl C); ESI-MS *m/z*: 362,347,333,319,257,145. Anal. Calcd. For  $C_{22}H_{26}N_4O$  (362): C, 72.90; H, 7.23; N, 15.46; O, 4.41 Found: C, 72.92; H, 7.20; N, 15.49; O, 4.39.

**(4-((1-ethyl-1H-benzo[d]imidazol-2-yl) (phenyl) methyl) piperazin-1-yl)(Phenyl) methanone (11i)**

Compound 11i was obtained as a white solid (yield: 67%; MP:258-260; IR (KBr): 3219 (N-H), 2359 (C-H), 1671(C=O),1605(C=N), 1548(C=C), 1236 (C-N)  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  ppm:1.24(t,2H,N- $CH_2$ -),3.12-3.16(m, 4H,PIP-H), 3.81(q, 3H,C- $CH_3$ ), 4.98(s, 1H, C-H), 7.49-8.11 (Complex m, 4H, Ar -H);  $^{13}C$ -NMR (400 MHz,  $CDCl_3$ , TMS):  $\delta$  16.1 ( $CH_3$ ), 39.10 ( $CH_2$ ), 52.09 ( $CH_2$ ), 53.2( $CH_2$ ), 68.2 (CH), 116(arom. CH), 124 (arom. CH), 131 (arom. CH), 133.1 (arom. CH), 134.5 (quat. C), 138.6(quat. C), 147. (quat. C), 142.6 (quat. C), 165.5; (carbonyl C);  $^{13}C$ -NMR (400 MHz,  $CDCl_3$ , TMS):  $\delta$  49.05 ( $CH_2$ ), 53.2( $CH_2$ ), 63.1 ( $CH_2$ ), 115(arom. CH), 125.8 (arom. CH), 130.4 (arom. CH), 128.3 (quat. C), 135.9 (quat. C), 141.5 (quat. C), 147.3 (quat. C), 175.3 (carbonyl C) ; ESI-MS *m/z*:424,395,319,235,181. Anal. Calcd. For  $C_{27}H_{28}N_4O$  (424): C, 76.39; H, 6.65; N, 13.20; O, 3.77 Found: C, 76.39; H, 6.65; N, 13.20; O, 3.77.

**(4-((5-chloro-1H-benzo[d]imidazol-2-yl) methyl) piperazin-1-yl)(Phenyl) methanone (11j)**

Compound 11j was obtained as a white solid (yield: 52%; MP:293-295; IR (KBr): 3224 (N-H), 2361 (C-H), 1672(C=O),1607(C=N), 1553(C=C), 1235 (C-N)  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  ppm: 3.39-3.41(m, 4H,PIP-H), 4.28(s, 2H, - $CH_2$ -), 4.82(s, 1H, N-H), 7.25-7.85 (m, 3H, Ar -H of Benzim) 7.98-8.12 (4H, Ar -H of benzoyl) ;  $^{13}C$ -NMR (400 MHz,  $CDCl_3$ , TMS):  $\delta$  54.8 ( $CH_2$ ), 56( $CH_2$ ), 65.2 ( $CH_2$ ), 119(arom. CH), 121.3 (arom. CH),127.5 (arom. CH), 138.5 (quat. C), 140.8 (quat. C), 147.1 (quat. C),172.4 (carbonyl C).ESI-MS *m/z* : 354,319,203,151. Anal. Calcd. For  $C_{19}H_{19}ClN_4O$  (354): C, 64.31; H, 5.40; Cl, 9.99; N, 15.79; O, 4.51 Found: C, 64.28; H, 5.42; Cl, 9.95; N, 15.74; O, 4.48.

**(4-(1-(5-chloro-1H-benzo[d]imidazol-2-yl)ethyl)piperazin-1-yl)(Phenyl) methanone (11k)**

Compound (11k) was obtained as a white solid (yield: 56%; MP:245-246; IR (KBr): 3209 (N-H), 2358 (C-H), 1667(C=O),1611(C=N),

1557(C=C), 1235 (C-N)  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  ppm:1.22(d,1H,-CH-), 3.24-3.29(m, 4H,PIP-H), 4.09(q, 3H,- $CH_3$ ), 5.14(s, 1H, N-H), 7.25-7.48 (m, 4H, Ar -H of Benzim) 7.89-8.20 (4H, Ar -H of benzoyl) ;  $^{13}C$ -NMR (400 MHz,  $CDCl_3$ , TMS):  $\delta$  18.9 ( $CH_3$ ), 50 ( $CH_2$ ), 52.4( $CH_2$ ), 63.4 (CH), 118(arom. CH), 121.6 (arom. CH), 127.5 (arom. CH), 133.5 (quat. C), 136.2 (quat. C), 143.2 (quat. C), 174.5 (carbonyl C); ESI-MS *m/z*:368,319,257, 145,117. Anal. Calcd. For  $C_{20}H_{22}N_4O$  (368): C, 65.12; H, 5.74; Cl, 9.61; N, 15.19; O, 4.34. Found: C, 65.09; H, 5.72; Cl, 9.59; N, 15.17; O, 4.30.

**(4-((5-chloro-1H-benzo[d]imidazol-2-yl) (phenyl) methyl) piperazin-1-yl)(Phenyl) methanone (11l)**

Compound (11l) was obtained as a white solid (yield: 54%; MP:214-216; IR (KBr): 3214 (N-H), 2352 (C-H), 1668(C=O),1613(C=N), 1539(C=C), 1236 (C-N)  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  ppm:3.19-3.28(m, 4H,PIP-H), 4.78(s, 1H,N-H), 5.94(s, 1H, C-H), 7.25-8.12 (Complex m, 4H, Ar -H);  $^{13}C$ -NMR (400 MHz,  $CDCl_3$ , TMS) : $\delta$  48.05 ( $CH_2$ ), 51.4( $CH_2$ ), 74(CH), 116(arom. CH), 127 (arom. CH),131.5 (arom. CH), 134.3 (arom. CH), 134.8 (quat. C), 139 (quat. C), 145.5 (quat. C), 147.2 (quat. C),170(carbonyl C); ESI-MS *m/z*:430,395,325, 245. Anal. Calcd. For  $C_{25}H_{23}ClN_4O$  (430): C, 69.68; H, 5.38; Cl, 8.23; N, 13.00; O, 3.71 Found: C, 69.71; H, 5.40; Cl, 8.21; N, 13.11; O, 3.69.

**Invitro Antifungal Activity**

The stock solution of (1m 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°C for 14 days.

The solutions were first tested at the concentration of 0.5 mmole/ml. The sets, which are found active at this concentration, were again tested at concentration of 0.25 mmole/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 m 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°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]

### CONCLUSION

In conclusion, we have synthesized series of benzimidazole derivatives and confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, Infra Red (IR), Mass Spectroscopy (MS), and by elemental analysis. These novel compounds were evaluated for in vitro antifungal activity against *C. albicans* strains using turbidimetric method. Benzimidazole derivative with N-1 methyl, C2 as asymmetric center and C5 chlorine substitutions showed good antifungal activity. The structural difference of these compounds

from the Ketoconazole can make them less prone to the development of resistance by pathogenic fungal strain against them. The present investigation opens a new lead for antifungal agents and there is a wide scope for future investigation.

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