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New Benzimidazoles Derivatives: Synthesis, Characterization and Antifungal Activities

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Abstract

One of the most important goals in medicinal chemistry is the development of new heterocyclic compounds with pharmaceutical activity. Thus, a novel series of the derivatives of benzimidazole were synthesized and the structures of all the synthesized compounds have been confirmed by IR, ^{1}H- and ^{13}C-NMR, Mass Spectroscopy and elemental analysis.

The title compounds have been evaluated for antifungal activities against *Candida albicans, Candida glabrata*, and *Candida krusei*. Some of these compounds have been found to exhibit moderate to good antifungal activity when compared with commercially available fungicides.

Keywords: *Benzimidazole, Spectroscopic techniques, Antifungal activity, Fungicides.*

Introduction

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The usual synthesis involves condensation of o-phenylenediamine with formic acid [1], or the equivalent trimethyl orthoformate:

$$C_6H_4(NH_2)_2+HC(OCH_3)_3 \rightarrow C_6H_4N(NH)CH+3\ CH_3OH$$

By altering the carboxylic acid used, this method is generally able to afford 2-substituted benzimidazoles.[1] Benzimidazole has fungicidal properties [2-4]. It acts by binding to the fungal microtubules and stopping hyphal growth. It also binds to the spindle microtubules and blocks nuclear division. Due to great potential of the moiety, in this work, is reported a study on synthesis of some novel derivatives of 2-bromomethyl-benzimidazole (Structures of **5-10** in Figure 1). These derivatives were screened for antifungal activity against *Candida albicans, Candida glabrata*, and *Candida krusei*.

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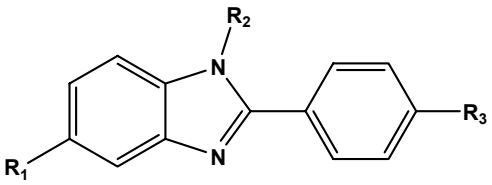


5)	**$R_1 = NO_2$**	**$R_2 = CH_3$**	**$R_3 = H$**
6)	**$R_1 = NO_2$**	**$R_2 = CH_3$**	**$R_3 = Br$**
7)	**$R_1 = NO_2$**	**$R_2 =$**	**$R_3 = Br$**
8)	**$R_1 = NH_2$**	**$R_2 = CH_3$**	**$R_3 = H$**
9)	**$R_1 = NH_2$**	**$R_2 = CH_3$**	**$R_3 = Br$**
10)	**$R_1 = NH_2$**	**$R_2 =$**	**$R_3 = Br$**

Figure 1. Chemical structures of chemical compound synthesized.

Experimental

Material and Equipments

All chemicals and solvents were obtained from E-Merck and Sigma-Aldrich and used without further purification. All melting points are uncorrected and taken with an Electrothermal melting point apparatus (Electrothermal Eng. Ltd, Essex, UK). IR spectra were determinate in KBr on a Shimadzu Dr-8031 instrument. The ^{1}H and ^{13}C-NMR spectrums of the synthesized compounds were measured in DMSO-d_6 or $CDCl_3$ solution and TMS as the internal standard using a Varian Mercury 400, 400MHz instrument. All Chemical shifts were reported as δ (ppm) values. The Mass Spectra were recorded on a LCQ ion trap mass spectrometer (Thermo Fisher. San Jose.CA, USA), equipped with an EI source. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer.

Synthesis of Compounds

*General procedure for the preparation of the compounds (**5-7**)*

1-Bromo-2,4-dinitrobenzene (2 mmol, 0.5 gr) is mixed with DMF (5 ml) and methyl/cyclobutylamine (2.2 mmol). The mixture is heated at reflux for 12 hrs then cooled and concentrated under vacuum (Intermediates **2a** and **2b**). The 2-nitro group of compounds **2a** and 2b was reduced to 2-amino (**3a** and **3b**) by using $Na_2S/NaHCO_3$ in methanol according to Willitzer et al. method [5]. To a mixture of the appropriate benzaldehyde derivative (4a and 4b) (1.5 mmol) in 5 mL of EtOH, then was added a solution of 0.01 mole of $Na_2S_2O_5$ in 5 ml of water in portions to the cooled ethanolic solution. The precipitate formed was filtered off and dried. A total of 1.2 mmol of this precipitate and 1.2 mmol of compound 3a or 3b in 5 ml of DMF were heated under reflux

for 8 hr, and then it was concentrated. At the end of this period the reaction mixture was cooled and poured into water and the resulting solid was collected, washed with water. The precipitate re-crystallized from ethanol-water mixture (Scheme 1) [6, 7].

1-methyl-5-nitro-2-phenyl-1H-benzimidazole (5)

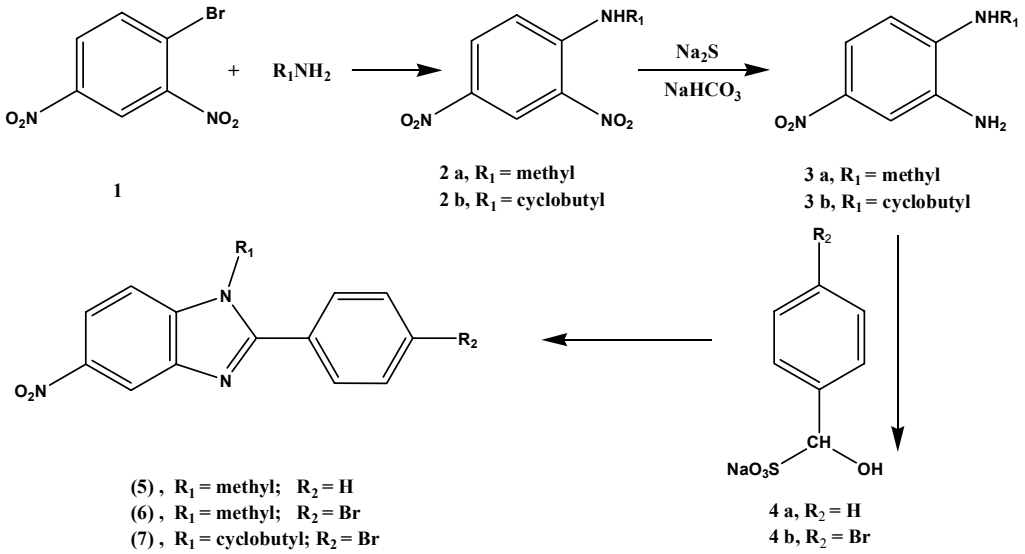
White powder; Yield 75%; m.p. 125-127 oC; IR (KBr, cm^{-1}): 2965 (CH), 1655 (N=C), 1313 (C-N stretching), 889 (C-C bonding aromatic). ^{1}H-NMR (δ/ppm): 3.68 (t, 3H, CH_3, 7.24-7.63 (5H, m, Ar-benzimidazole), 7.95 (d, 1H, Jo= 8.8 Hz), 8.25 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz), 8.69 (d, 1H, Jm= 2 Hz). ^{13}C-NMR (δ / ppm): 32.1, 115.1, 118, 129.5, 130.5, 133.5, 136.0, 137.5, 137.9, 144.4, 148.7. Anal. Calcd. for $C_{14}H_{11}N_3O_2$: C, 64.40; H, 4.38; N, 14.59 %. Found: C, 64.51; H, 4.30; N, 14.48 %. MS (m/z, *regulatory intensity*, %): 253 (100), 2254 (16).

2-(4-Bromophenyl)-1-methyl-5-nitro-1H-benzimidazol (6)

Light yellow powder; Yield 70%, m.p. 158-160 oC; IR (KBr, cm^{-1}): 2975 (CH), 1671 (N=C), 1294 (C-N stretching), 885 (C-C bonding aromatic), 679 (C-Br); ^{1}H-NMR (δ/ ppm): 3.41 (t, 3H, CH_3), 7.32-7.52 (4H, m, Ar-benzimidazole), 7.85 (d, 1H, Jo= 8.8 Hz), 8.22 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz), 8.71 (d, 1H, Jm= 2 Hz); ^{13}C-NMR (δ/ppm): 39.1, 115.6, 119, 123, 129.5, 131.5, 135.6, 137.0, 138.5, 139.2, 146.5, 149.7. Anal. Calcd. for $C_{14}H_{10}BrN_3O_2$: C, 50.62; H, 3.03; N, 12.65 %. Found: C, 50.65; H, 3.08; N, 12.61 %. MS (m/z, *regulatory intensity*, %): 331 (100), 332(16), 322 (98).

2-(4-Bromophenyl)-1-cyclobutyl-5-nitro 1H-benzimidazol (7)

Light yellow powder; Yield 85%; m.p. 191-193 oC; IR (KBr, cm^{-1}): 2952 (CH), 1672 (N=C), 1293 (C-N stretching), 918 (C-C bonding aromatic), 681 (C-Br); 1H-NMR (δ/ ppm): 2.05 (m, 2H, CH_2), 2.65 (4H,s,CH_2, Cylobutyl),5.2 (1H,s, CH,Cylobutyl) 7.41-7.64 (4H, m, Ar-benzimidazole), 7.76 (d, 1H, Jo= 8.8 Hz,), 8.42 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz), 8.75 (d, 1H, Jm= 2 Hz); ^{13}C-NMR (δ/ ppm): 21.5, 29.5, 66.5, 117.5, 119.0, 123.5, 126, 128, 129.5, 134.5, 136.8, 143.3, 149.4. Anal. Calcd. For $C_{17}H_{14}BrN_3O_2$: C, 54.86; H, 3.79; N, 11.29 %, Found: C, 54.90; H, 3.76; N, 11.33 %. MS (m/z, *regulatory intensity*, %): 371 (100), 373 (97), 372 (25).



Scheme 1. Schematic synthesis of intermediates and new compounds (**5-7**).

*General procedure for the preparation of the compounds (**8-10**)*

Mixture of 5-Nitrobenzimidazole derivatives **5-7** (1 mmol) in 10 mL of hot EtOH and 10 mL of 6N HC1 were heated under reflux and then $SnCl_2.2H_20$ was added in portions until the starting material was completely exhausted. The ethanol was decanted; the residue was made alkaline with KOH, then, extracted with EtOAc, and washed with water. EtOAc was evaporated slowly and the precipitate re-crystallized from ethanol (Scheme 2) [5-7].

*1-Methyl-2-phenyl-1H-benzimidazole-5-ylamine (**8**)*

White cream powder; Yield 79%; m. p. 181-183 oC; IR (KBr, cm^{-1}):3175 (NH), 2991 (CH), 1633 (N=C), 1289 (C-N stretching), 892 (C-C bonding aromatic); ^{1}H-NMR (δ/ ppm): 1.48 (t, 3H, CH_3), 4.75 (s, 2H, NH_2), 6.98-7.71 (3H, m, Ar-Bbenzimidazole), 7.63 (d, 1H, Jo= 8.8 Hz), 8.14 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz), 8.49 (d, 1H, Jm= 2 Hz); ^{13}C-NMR (δ/ppm): 38.1, 113.5, 115.5, 118.5, 119.5, 129.7 ,132.8, 133.0,134.5, 137.5, 139.8, 145.8. Anal. Calcd. for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82 %. Found: C, 75.35; H, 5.81; N, 18.72 %. MS (m/z, regulatory intensity, %): 223 (100), 224 (18).

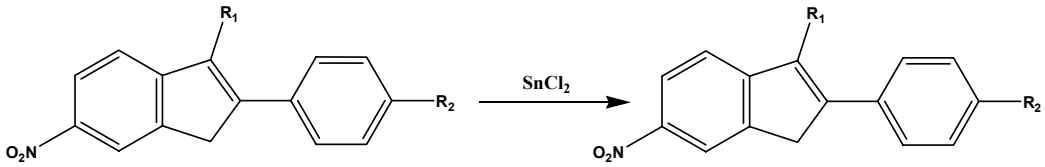
*2-(4-Bromo-phenyl)-1-methyl-1H-benzimidazole-5-ylamine (**9**)*

Light yellow powder; Yield 81%, m. p. 147-149^{o}C; IR (KBr, cm^{-1}): 3335 (NH), 2955 (CH), 1642 (N=C), 1281 (C-N stretching), 918 (C-C bonding aromatic), 695 (C-Br); ^{1}H-NMR (δ/ppm): ^{1}H-NMR (δ/ppm): 1.45 (t, 3H, CH_3), 4.71 (s, 2H, NH_2), 6.91-7.68 (3H, m, Ar-Bbenzimidazole), 7.65 (d, 1H, Jo= 8.8 Hz), 8.21 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz),

8.52 (d, 1H, Jm= 2 Hz); ^{13}C-NMR (δ/ppm): 31.2, 111.5, 116.5, 119.5, 123, 127.5, 133.8, 135.3, 137.2, 138.2, 139.8, 148.3. Anal.Calcd. for $C_{14}H_{12}BrN_3$: C, 55.65; H, 4.00; N, 13.91 %. Found: C, 55.60; H, 4.05; N, 13.86 %. MS (m/z, *regulatory intensity*, %): 301 (100), 303 (97), 302 (20).

*2-(4-Bromo-phenyl)-1-cyclobutyl-1H-benzimidazole-5-ylamine (**10**)*

White yellow powder; Yield 86%, m. p. 166-168 oC; IR (KBr, cm^{-1}): 3158 (NH), 2997 (CH), 1668 (N=C), 1301 (C-N stretching), 915 (C-C bonding aromatic), 702 (C-Br); ^{1}H-NMR (δ/ppm): 2.20 (m, 2H, CH2), 3.25 (m, 4H, CH2),4.5 (s, 1H, CH), 4.88 (s, 2H, NH2), 6.93-7.68 (3H, m, Ar-Bbenzimidazole), 7.75 (d, 1H, Jo= 8.8 Hz), 8.48 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz), 8.67 (d, 1H, Jm= 2 Hz). ^{13}C-NMR (δ/ppm): 19.7, 30.9, 118.5, 119.0,120, 122.5, 126.6, 129.8, 134.5, 139.5, 142.5, 146.1. Anal. Calcd. For $C_{17}H_{16}BrN_3$: C, 59.66; H, 4.71; N, 12.28 %. Found: C, 59.62; H, 4.69; N, 12.20 %. MS (m/z, *regulatory intensity*, %): 341 (100), 343 (98), 344 (21).



(5) , R_1 = methyl; R_2 = H
(6) , R_1 = methyl; R_2 = Br
(7) , R_1 = cyclobutyl; R_2 = Br

(8) , R_1 = methyl; R_2 = H
(9) , R_1 =methyl; R_2 = Br
(10) , R_1 = cyclobutyl ; R_2 = Br

Scheme 2. Schematic synthesis of new compounds (**8 - 10**).

Antifungal activity assay

The yeasts Candida albicans, patient isolate Candida glabrata and Candida krusei were grown on Sabouraud Dextrose Broth (Difco); the yeasts were incubated for 48 h at 25.91°C. The antifungal activity tests were carried out at pH 7.4 in Sabouraud Dextrose Broth and the 2-fold dilution was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25.91°C, the last tube with no yeast growth was recorded to represent minimum inhibitory concentration (MIC), expressed in μg/mL.

Results and discussion

Chemistry

In continuation of our interest to investigate of new pharmaceutical potential compounds, the syntheses of biologically active benzimidazole derivatives were carried out in this study. To materialize the proposed project, initially, intermediates were synthesized from 1-Bromo-2,4-dinitrobenzene by reaction with methyl/cyclobutylamine in DMF according

to the literature [5]. The 2-nitro group of compounds was reduced to 2-amino by using $Na_2S/NaHCO_3$ in methanol [5]. Condensation of o-phenylenediamines with the $Na_2S_2O_5$ adduct of appropriate benzaldehydes in DMF [8] gave **5-7**. Reduction of compounds **5-7** with $SnCl_2.2H_{20}$ produced **8-10**. The structures of **5-10** were deduced from their elemental analysis, mass spectrometric data, ^{1}H-and ^{13}C-NMR, and IR spectral data, given in Experimental section.

Antifungal activity

The in vitro antifungal activity of the compounds was tested by the tube dilution technique [9]. Each of the test compounds and standards Miconazole, Fluconazole and Cotrimoxazole were dissolved in 10% DMSO, at concentrations of 100 µg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities of 50, 25, 12.5, 6.25, 3.125, 1.5 and 0.75 µg/mL concentrations. The final inoculums size was 105 CFU/ml. The MICs were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antifungal activity against any of the test microorganisms. All the compounds were tested for their in vitro growth inhibitory activity against C. albicans, patient isolate C. glabrata and C. krusei (Table 1). Compounds **5, 7, 8** and **10** possessed comparable activity to fluconazole and cotrimoxazole against C. albicans with a MIC of 12.5 µg/mL. However none of the compounds was superior to the standards used against any fungi.

Table 1. Antifungal activities of the synthesized compounds (MIC, µg/ml).

Compound	C.albicans	C.glabrata	C.krusei
5	12.5	6.25	6.25
6	25	25	12.5
7	12.5	25	6.25
8	12.5	25	12.5
9	25	25	6.25
10	12.5	12.5	12.5
Fluconazole	12.5	3.125	3.125
Miconazole	6.25	3.125	1.5
Cotrimoxazole	12.5	3.125	3.125

Conclusion

A series of some novel Benzimidazole derivatives were successfully synthesized and characterized using IR, ^{1}H- and ^{13}C-NMR, mass spectroscopy and elemental analysis. Our studies clearly demonstrate that novel Benzimidazole derivatives had significant antifungal activity against different fungi

species. As a consequence, we can conclude that newly synthesized Benzimidazole derivatives can be used for the development of new fungicide.

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