Synthesis, Characterization of Some Novel Benzimidazole Derivatives of 1-bromo-2,4-dinitrobenzene and Their Antifungal Activities

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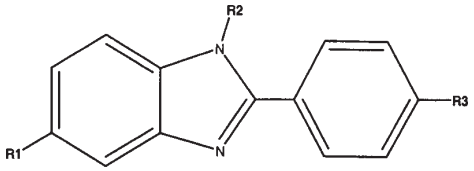
*Six benzimidazole derivatives, 5-Nitro-2-phenyl -1-ethyl benzimidazol (**5**), 2- (p-Bromophenyl)- 5-nitro- 1-ethyl benzimidazol (**6**), 2-(p-Bromophenyl-5-nitro-1–cyclopentyl benzimidazol (**7**), 2- (p-Bromophenyl)-5-nitro-1-cyclopentyl benzimidazol (**8**), 5-Amino-2-(p-Bromophenyl)-1-ethylbenzimidazol (**9**), and 5-Amino-2-(p-Bromophenyl)-1-cyclopentyl benzimidazol (**10**) were synthesized The compounds 6,8,9 and10 are novel. The structures of all the synthesized compounds were deduced by elemental analysis and different spectroscopic techniques (IR, ^{1}H- and ^{13}C-NMR and Mass Spectroscopy) and in vitro antifungal activities of these compounds tested against Candida albicans, Candida glabrata, and Candida krusei. The results showed that some of these compounds were found to be comparable commercially available fungicides with a minimum inhibitory concentration of 12.5 μg/mL.*

Keywords: benzimidazole, dinitrobenzene derivatives, antifungal activity, fungicides

Benzimidazole nucleus is an important heterocyclic ring, a wide variety of Benzimidazole derivatives are known for their chemotherapeutic importance and antimicrobial activities, [1-6] especially antifungal activity [7-9] anti-inflammatory, [10] and antioxidant [11-15]. In this context, it has been found that Benzimidazole derivatives to retard especial type of fungus that attack certain class of patients such as cancer chemotherapy and HIV patients. In particular, Candidiasis is the fungal infection that is most frequently associated with HIV-positive patients [16,17]. Benzimidazole derivatives were found to retard Cryptococcosis growth, which is the main cause of morbidity in AIDS patients. Benzimidazole fungicides are systemic pesticides widely used in agriculture for pre- and post-harvest treatment for control of a wide range of fungi [18-20]. The limited number of available antifungal compounds urges to synthesis new compounds with a potential use as fungicides, in particular, those attack people suppressed immune system e. g. In this work, six Benzimidazole derivatives of 1-bromo-2,4-dinitrobenzene (fig. 1) containing the above mentioned moieties for evaluation of their antifungal activities were synthesized and antifungal activities of these compounds were carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996,Vol II A-105) against *Candida albicans*, *Candida glabrata*, and *Candida krusei.*

Experimental part

All the chemicals and solvents were obtained from E-Merck (Darmstadt, Germany), and were used without further purification. All Melting points are uncorrected and were taken with an Electrothermal melting point apparatus. IR spectra were determinate in KBr on a Shimadzu Dr-8031 instrument. The ^{1}H- ^{13}C-NMR spectra of the synthesized compounds were measured in DMSO-d_6, $CDCl_3$ solution and TMS as the internal standard using a Varian Mercury 400, with 400 and 75 MHz respectively instrument. All Chemical shifts were reported as δ (ppm) values. The mass spectra were recorded on a LCQ ion trap mass spectrometer, equipped with an EI source. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400 and were within ± 0.4% of the theoretical values.



5	R1 = NO_2	R2 = C_2H_5	R3 = H
6	R1 = NO_2	R2 = C_2H_5	R3 = Br
7	R1 = NO_2	R2 = 	R3 = Br
8	R1 = NH_2	R2 = C_2H_5	R3 = H
9	R1 = NH_2	R2 = C_2H_5	R3 = Br
10	R1 = NH_2	R2 = 	R3 = Br

Fig.1. Chemical structures of chemical compound synthesized

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

To a mixture of the appropriate aldehyde derivative (1.5 mmol) in 5 mL of EtOH, was added a solution of 0.01 mole of $Na_2S_2O_3$ 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 **3** or **4** in 5 mL of DMF were heated under reflux for 8 hr, 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 and washed with water. The precipitate was recrystallized from ethanol-water mixture [21].

*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 6 N 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

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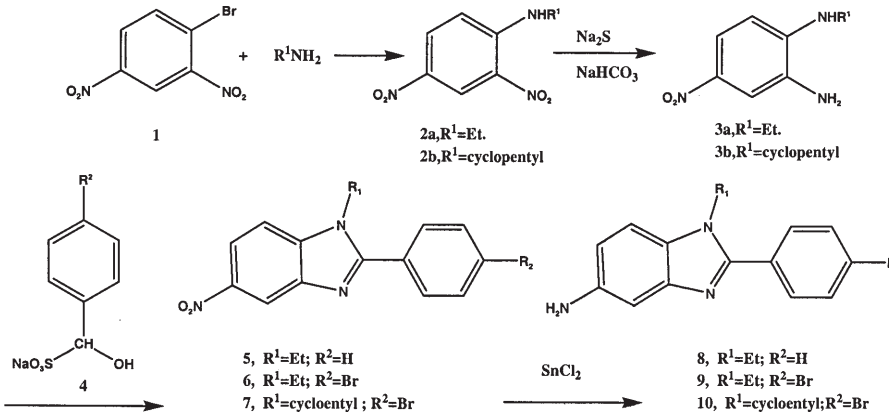

Scheme1. Preparation rout of the compounds

made alkaline with KOH, then, extracted with EtOAc and washed with water. EtOAc was evaporated slowly and the precipitate recrystallized from ethanol [21].

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 *p*H 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 discussions

Compounds **1** and **2** were prepared from 1-Bromo-2,4-dinitrobenzene by reaction with ethyl/cyclopentylamine in DMF according to the literature [22].The 2-nitro group of compounds 1 and 2 was reduced to 2-amino (**3** and **4**) by using $Na_2S/NaHCO_3$ in methanol [22]. Condensation of o-phenylenediamines (**3** and **4**) with the $Na_2S_2O_5$ adduct of appropriate benzaldehydes in DMF [23] gave **5-7**. Reduction of compounds **5-7** with $SnCl_2.2H_20$ produced **8-10** (scheme 1). The structures of ***5, 6, 7, 8, 9*** and ***10*** were deduced from their elemental analysis, mass spectrometric data, and their ^{1}H- and ^{13}C- NMR, and IR spectral data, given below.

*5-Nitro-2-phenyl -1-ethyl benzimidazol **(5)***

Cream powder; Yield 65%; m.p. 123-124 °C; Anal. Calcd. for $C_{15}H_{13}N_3O_2$: C, 67.42; H, 4.87; N, 15.73 %. Found: C, 67.31; H, 4.81; N, 15.62 %. IR (KBr, cm^{-1}): 2995 (CH), 1645 (N=C), 1292.4 (C-N stretching), 892.1 (C-C bonding aromatic). ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 0.73 (t, 3H, CH_3), 1.66-1.71 (m, 2H, CH_2), 2.5 (3H,s,CH_3 at C-2 of benzimidazole), 4.37 (t, 2H, CH_2), 7.21-7.59 (4H, m, Ar-benzimidazole), 7.62-7.65 (m, 3H, H-3',4',5'), 7.81-7.98 (m, 2H, H-2',6'), 7.96 (d, 1H, Jo= 8.8 Hz, H-7), 8.24 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.59 (d, 1H, Jm= 2 Hz, H-4), 12.5(1H,s,-NH-Benzimidazole).^{13}C-NMR (75.4 MHz. $CDCl_3$, δ / ppm): 12.2 (CH_3), 45.1 (CH_2),111.5 (C=**C**-N=), 115 (CH=), 122.5(CH=), 125.5 (CH=), 128.5 (2 CH=),129.5 (CH=), 130.0 (2 CH=),133.5 (CH=), 138 (=C-NO_2), 142.2 (N-C=N), 145.0 (C=**C**-N-). MS (m/z, (relative abundance, %): 267 (M^+, 22), 189 (58), 161 (39), 152 (32), 123 (23), 115 (32), 106 (47), 77 (100), 46 (56), 29 (100).

*2- (p-Bromophenyl) - 5-nitro- 1-ethyl benzimidazol **(6)***

Yellow powder; Yield 62%, m.p. 157-158 °C; Anal. Calcd. for $C_{15}H_{12}BrN_3O_2$: C, 52.04; H, 3.46; N, 12.13 %. Found: C, 52.00; H, 3.40; N, 12.03 %. IR (KBr, cm^{-1}): 2995 (CH), 1655 (N=C), 1291 (C-N stretching), 895 (C-C bonding aromatic), 667 (C-Br); ^{1}H-NMR (400 MHz DMSO-d_6, δ / ppm): 0.73 (t, 3H, CH_3), 1.65-1.71 (m, 2H, CH_2), 2.5 (3H,s,CH_3 at C-2 of benzimidazole), 4.35 (t, 2H, CH_2), 7.22-7.65 (4H, m, Ar-benzimidazole), 7.45-7.49 (m, 2H, H-3',5'), 7.88-7.91 (m, 2H, H-2',6'), 7.96 (d, 1H, Jo= 8.8 Hz, H-7), 8.22 (d, 1H, Jo= 8.8 Hz, H-6), 8.58 (s, 1H, H-4). 12.8(1H, s,-NH-Benzimidazole); ^{13}C-NMR (75.4 MHz. $CDCl_3$, δ/ppm): 12.2 (CH_3), 45.1 (CH_2),111.5 (C=**C**-N=), 115.0 (CH=),122.5 (CH=), 125 (CH=), 125.5 (CH=), 127.0 (C-Br), 131.5 (4 CH=),138 (=C-NO_2), 142.2 (N-C=N), 145.0 (C=**C**-N-). MS (m/z, (relative abundance, %): 346 (M^+, 18), 317(23), 300 (20), 266 (56), 189 (58), 156 (100), 155 (18), 80 (65), 46 (48), 29 (100).

*2- (p-Bromophenyl)-5-nitro-1- cyclopentyl benzimidazol **(7)***

Yellow powder; Yield 85%; m.p. 172-173 °C; Anal. Calcd. For $C_{18}H_{16}BrN_3O_2$: C, 55.97; H, 4.14; N, 10.87 %, Found: C, 55.90; H, 4.11; N, 10.81 %. IR (KBr, cm^{-1}): 2923 (CH), 1624 (N=C), 1291 (C-N stretching), 901 (C-C bonding aromatic), 681 (C-Br); ^{1}HNMR (400 MHz, DMSO-d_6, δ / ppm): 1.68-2.16 (m, 8H, CH_2), 2.5 (3H,s,CH_3 at C-2 of benzimidazole), 4.85-4.89 (m, 1H, CH), 7.20-7.60 (4H, m, Ar-benzimidazole)7.45-7.49 (m, 2H, H-3',5'), 7.78-7.82 (m, 2H, H-2',6'), 7.89 (d, 1H, Jo= 9.2 Hz, H-7), 8.17 (dd, 1H, Jo=9.2 Hz, Jm= 2 Hz, H-6), 8.58 (d, 1H, Jm= 1.6 Hz, H-4), 12.9(1H,s,-NH-Benzimidazole); ^{13}C-NMR (75.4 MHz. $CDCl_3$, δ/ppm): 24.7 (2 CH_2), 28.9 (2 CH_2), 62.5 (CH-N),111.5 (C=**C**-N=), 115.0 (CH=),122.5 (CH=), 125 (CH=), 125.5 (CH=), 127.0 (C-Br), 131.5 (4 CH=),138 (C-NO_2), 142.2 (N-C=N), 145.0 (C=**C**-N-) MS (m/z, (relative abundance, %): 386 (M^+, 18), 271 (22), 225 (32), 195 (42), 156 (100), 80 (65), 69 (100), 46 (48).

*5-amino-2-phenyl -1-ethyl benzimidazol **(8)***

Cream powder;Yield 71%; m. p. 199-201°C; Anal. Calcd. for $C_{15}H_{15}N_3$: C, 75.92; H, 6.32; N, 17.70 %. Found: C, 75.85; H, 6.29; N, 17.62 %. IR (KBr, cm^{-1}):3162 (NH), 2988 (CH), 1620 (N=C), 1299 (C-N stretching), 895 (C-C bonding aromatic); ^{1}H-NMR (400MHz ,DMSO-d_6, δ / ppm): 0.73 (t, 3H, CH_3), 1.56-1.61 (m, 2H, CH_2), 2.55 (3H,s,CH_3 at C-2 of benzimidazole), 4.22 (t, 2H, CH_2), 7.25-7.69 (4H, m, Ar-benzimidazole),7.52-7.65 (m, 3H, H-3',4',5'), 7.89-7.96 (m, 2H, H-2',6'), 7.98 (d, 1H, Jo= 8.8 Hz, H-7), 8.11 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.46 (d, 1H, Jm= 2 Hz, H-4). 13.05(1H, s,-NH-Benzimidazole); ^{13}C-NMR (75.4 MHz. $CDCl_3$, δ / ppm): 12.2 (CH_3), 45.1 (CH_2),111.5 (C=**C**-N=), 114 (CH=), 115(CH=), 117 (CH=), 128.5 (2 CH=),129.5 (CH=), 130.0 (2 CH=),133.5 (CH=),136 (C-NH_2), 139.5 (C=**C**-N-), 142.2 (N-C=N). MS (m/z, (relative abundance, %): 237 (M^+, 24), 122 (38), 106 (47), 77 (100), 29 (100), 16 (100).

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

Table 1
ANTIFUNGAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS (MIC, μ/mL)

5-amino-2- (p-Bromophenyl) -1- ethyl benzimidazol ***(9)***

Yellow powder; Yield 75%, m. p. 130-132°C; Anal.Calcd. for $C_{15}H_{14}BrN_3$: C, 56.97; H, 4.46; N, 13.28 %. Found: C, 56.88; H, 4.41; N, 13.01 %. IR (KBr, cm^{-1}): 3300 (NH), 2923 (CH), 1624 (N=C), 1281 (C-N stretching), 901 (C-C bonding aromatic), 685 (C-Br) ; ^{1}H-NMR (400MHz ,DMSO-d_6, δ / ppm): 0.7 (t, 3H, CH_3), 1.62-1.68 (m, 2H, CH_2), 2.55 (3H,s,CH_3 at C-2 of benzimidazole), 4.12 (t, 2H, CH_2), 4.8 (s, 2H, NH_2), 6.63 (d, 1H, Jo= 8.4 Hz, H-6), 6.79 (s, 1H, H-4), 7.23-7.62 (4H, m, Ar-benzimidazole) 7.29 (d, 1H, Jo= 8.4 Hz, H-7), 7.36-7.40 (m, 2H, H-2',6'), 7.74-7.78 (m, 2H, H-3',5'), 13.06(1H,s,-NH-Benzimidazole).; ^{13}C-NMR (75.4 MHz. $CDCl_3$, δ/ppm): 12.2 (CH_3), 45.1 (CH_2),111.5 (C=**C**-N=), 114.0 (CH=),115 (CH=), 117 (CH=), 125 (CH=), 127.0 (C-Br), 131.5 (4 CH=),136 (C-NH_2), 139.5 (C=**C**-N-), 142.2 (N-C=N). MS (m/z, (relative abundance, %): 316 (M^+, 22), 185 (32), 156 (100), 125 (18), 80 (65), 29 (100).

5-amino- 2- (p- Bromophenyl)- 1- cyclopentyl benzimidazol ***(10)***

Yellow powder ;Yield 82%, m. p. 193-195°C; Anal. Calcd. For $C_{18}H_{18}BrN_3$: C, 60.71; H, 5.05; N, 11.79 %. Found: C, 60.66; H, 5.00; N, 11.66 %. IR (KBr, cm^{-1}):3162 (NH), 2986 (CH), 1654 (N=C), 1292.4 (C-N stretching), 899 (C-C bonding aromatic), 695 (C-Br) ;

^{1}H-NMR (400MHz ,DMSO-d_6, δ / ppm) 1.63-2.15 (m, 8H, CH_2), 2.55 (3H,s,CH_3 at C-2 of benzimidazole), 4.68-4.77 (m, 1H, CH), 4.83 (s, 2H, NH_2), 6.61 (d, 1H, Jo =8.8 Hz, H-6), 6.81 (s, 1H, H-4), 7.28 (d, 1H, Jo= 8.8 Hz, H-7), 7.24-7.61 (4H, m, Ar-benzimidazole), 7.36-7.40 (m, 2H, H-3',5'), 7.65-7.69 (m, 2H, H-2',6'); 13.06(1H,s,-NH-Benzimidazole) ;

^{13}C-NMR (75.4 MHz. $CDCl_3$, δ/ppm): 24.7 (2 CH_2), 28.9 (2 CH_2), 62.5 (CH-N),111.5 (C=**C**-N=), 114.0 (CH=),115 (CH=), 117 (CH=), 125 (CH=), 127.0 (C-Br), 131.5 (4 CH=),136 (C-NH_2), 139.5 (C=**C**-N-),142.2 (N-C=N). MS (m/z, (relative abundance, %): 356 (M^+, 18), 241 (22), 225 (32), 165 (28), 156 (100), 69 (100).

The in vitro antifungal activity of the compounds was tested by the tube dilution technique [24]. 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 0.78 μg/mL concentrations. The final inoculums size was 10^5 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 **6, 7, 8** and **9** 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.

Conclusions

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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