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

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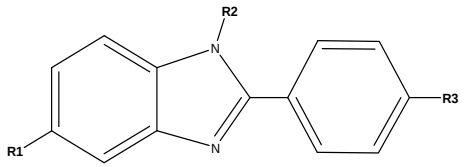
Abstract: Six novel benzimidazole derivatives, 5-nitro-2-phenyl -1-ethyl benzimidazol (**5**), 2- (*p*-bromophenyl)- 5-nitro- 1-ethyl benzimidazol (**6**), 2- (*p*-bromophenyl-5-nitro-1–cyclopentyl benzimidazol (**7**), 2- (*p*-bromophenyl) -5-nitro-1-cyclopentyl benzimidazol (**8**), 5-amino-2-(*p*-bromophenyl)-1-ethylbenzimidazol (**9**) and 5-amino-2-(*p*-bromophenyl)-1-cyclopentyl benzimidazol (**10**) were synthesized . The structures of all the synthesized compounds were elucidated by using elemental analysis and different spectroscopic techniques (IR, NMR and mass spectroscopy). Some of these compounds showed potential antifungal activities. The biological activity of these compounds as fungicides was tested against Candida albicans, patient isolate Candida glabrata and Candida krusei. The biological activity of four compounds was found to be comparable to that of the commercially available fungicides with a minimum inhibitory concentration of 12.5 µg/mL.

Keywords: Benzimidazole, 1-Bromo-2, 4-Dinitrobenzene, Antifungal activity, Fungicides

Introduction

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, candidiasies is the fungal infection most that is 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.* candidiasies is the fungus infection that is most frequently associated with HIV-positive patients. We report in this work, synthesis of a six benzimidazoles compounds (Figure 1) of the title structure type containing the above mentioned moieties for evaluation of their antifungal activity. Four of the six investigated compounds showed antifungal properties.



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

Figure 1. Chemical structures of chemical compound synthesized

Experimental

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 (Electrothermal Eng. Ltd, Essex, UK). IR spectra were determinate in KBr on a Shimadzu Dr-8031 instrument. The ^{1}H NMR spectra 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 model 2400 and were within ±0.4% of the theoretical values.

*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, 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 **3** or **4** in 5 mL of DMF were heated under reflux for 8 h, 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[21].

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

Yield 65%, m. p: 123-124 ^{o}C; IR (KBr, cm^{-1}): 2995 (CH), 1645 (N=C), 1292.4 (C-N stretching), 892.1 (C-C bonding aromatic); ^{1}H NMR (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-bbenzimidazole), 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). 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; Mass spectra, m/z=267.20 (100%).

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

Yield 62%, m. p: 157-158 ^oC; IR (KBr, cm^{-1}): 2995 (CH), 1655 (N=C), 1291 (C-N stretching), 895 (C-C bonding aromatic), 667 (C-Br); ^{1}HNMR (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-Bbenzimidazole), 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). 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; Mass spectra, m/z=346.10 (100%).

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

Yield 85%, m. p.: 172-173 ^oC; IR (KBr, cm^{-1}): 2923 (CH), 1624 (N=C), 1291 (C-N stretching), 901 (C-C bonding aromatic), 681 (C-Br); ^{1}HNMR (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-bbenzimidazole)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, J m= 1.6 Hz, H-4), 12.9(1H,s,-NH-benzimidazole) 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; Mass spectra, m/z=386.20 (100%).

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

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

Yield 71%; m. p.: 199-201 ^oC; IR (KBr, cm^{-1}): 3162 (NH), 2988 (CH), 1620 (N=C), 1299 (C-N stretching), 895 (C-C bonding aromatic); ^{1}HNMR (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-bbenzimidazole),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). 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; Mass spectra, *m/z*=237.30 (100%).

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

Yield 75%, m. p. 130-132 ^oC; IR (KBr, cm^{-1}): 3300 (NH), 2923 (CH), 1624 (N=C), 1281 (C-N stretching), 901 (C-C bonding aromatic), 685 (C-Br) ; ^{1}HNMR (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-bbenzimidazole) 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). 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; Mass spectra, *m/z*=316.2 (100%).

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

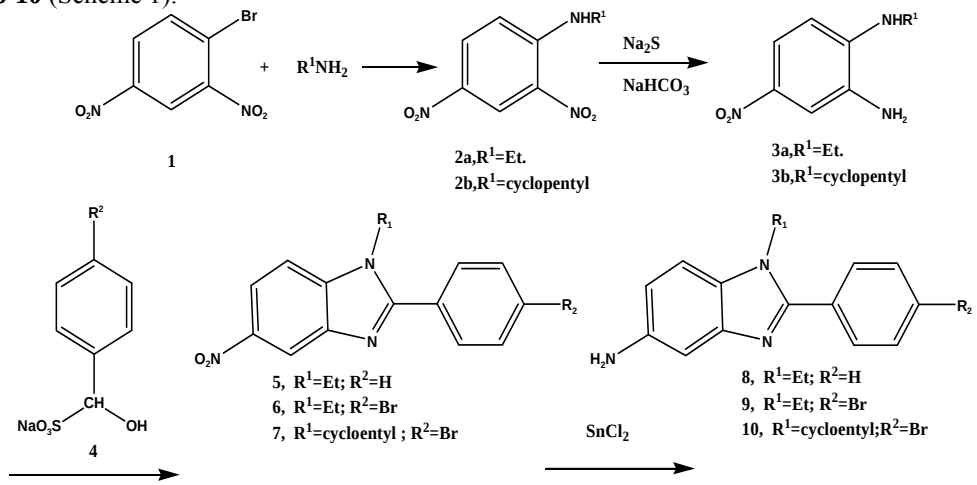
Yield 82%, m. p. 193-195 ^{o}C; IR (KBr, cm^{-1}):3162 (NH), 2986 (CH), 1654 (N=C), 1292.4 (C-N stretching), 899 (C-C bonding aromatic), 695 (C-Br) ^{1}HNMR (DMSO-d_6, δ ppm) 1.63-2.15 (m, 8H, CH_2), 2.55 (3H,s,CH_3 at C-2 of benzimidazole), 4.68-4.77 (m, 1H, CH), 4.83 (s, 2H, NH_2), 6.61 (d, 1H, Jo =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-bbenzimidazole), 7.36-7.40 (m, 2H, H-3',5'), 7.65-7.69 (m, 2H, H-2',6'); 13.06(1H,s,-NH-Benzimidazole). 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; Mass spectra, *m/z*=356.10 (100%).

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 antifugal 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

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 $Na_2S_20_5$ 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).



Scheme 1. Preparation rout of the compounds

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 10^5CFU/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.

Table 1. Antifungal activities of the synthesized compounds

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

Conclusion

A series of novel benzimidazole derivatives were successfully synthesized and characterized using IR and ^{1}H-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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