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 and 10 are novel. The structures of all the synthesized compounds were deduced by elemental analysis and different spectroscopic techniques (IR, 1H- and 13C-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 1H- 13C-NMR spectra of the synthesized compounds were measured in DMSO-d6, CDCl3 solution and TMS as the internal standard using a V arian 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 El 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, was added a solution of 0.01 mole of Na2S2O5 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)
Mixtures of 5-Nitrobenzimidazole derivatives 5-7 (1 mmol) in 10 mL of hot EtOH and 10 mL of 6 N HCl were heated under reflux and then SnCl2.2H2O 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 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 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 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 (-N-). The structures of compounds 1 and 2 was reduced to 2-amino (-N-). The structures of compounds 1 and 2 was reduced to 2-amino (-N-). MS (m/z, (relative abundance, %): 346 (M +, 18), 317(23), 300 (20), 292 (100), 189 (65), 162 (90), 134 (100).

Yellow powder; Yield 85%; m.p. 172-173°C; Anal. Calcld. for C15H15N3O2: C, 75.92; H, 6.32; N, 17.62 %. IR (KBr, cm -1): 3162 (NH), 2988 (CH), 1620 (N=C), 1299 (C-N stretching), 895 (C-C bonding aromatic), 1381 (C=NO2), 142.2 (N-C=N). MS (m/z, (relative abundance, %): 368 (M +, 18), 317(23), 225 (32), 195 (42), 156 (100), 80 (65), 69 (100), 48 (48).
5-amino-2-(p-Bromophenyl)-1-ethyl benzimidazol (9)

Yellow powder; Yield 79%; m. p. 130-132°C; Anal.Calcd.
for C₉H₁₈BrN₃: 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 (CH₃), 1654 (N=C), 1292.4 (C-N stretching), 899 (C-C bonding (-N=), 114.0 (CH=), 115.1 (CH=), 117 (CH=), 125 (CH=), 127.0 (C-Br), 131.5 (4 CH=), 136 (C-CH₃), 139.5 (C=C-N), 142.2 (N=C-N), MS (m/z, relative abundance, %): 316 (M⁺, 28), 241 (22), 225 (19), 183 (16), 156 (12), 126 (12), 111 (20), 69 (100).

1H-NMR (400MHz, DMSO-d₆, δ/ppm): 0.7 (t, 3H, CH₃), 1.62-1.68 (m, 2H, CH₂), 2.55 (3H,s,CH₃ at C-2 of benzimidazole), 4.68-4.77 (4H, m, Ar-CH₂), 6.81 (s, 1H, H-4), 7.24-7.61 (4H, m, Ar-CH₃), 7.65-7.69 (m, 2H, H-2',6'); 13C-NMR (75.4 MHz, CDCl₃, δ/ppm): 24.7 (2 CH₂), 28.9 (2 CH₂), 45.1 (CH₂), 111.5 (C=C-N), 114.0 (CH=), 115.1 (CH=), 117 (CH=), 125 (CH=), 127.0 (C-Br), 131.5 (CH=), 136 (C-CH₃), 139.5 (C=C-N), 142.2 (C=N), MS (m/z, relative abundance, %): 356 (M⁺, 18), 241 (22), 225 (32), 183 (16), 156 (100), 126 (12), 111 (20), 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 10⁶ CFU/mL. The MICs were determined 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. kruusei (table 1).

Conclusions

A series of some novel Benzimidazole derivatives were successfully synthesized and characterized using IR, 1H- and 13C-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.

References

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Table 1

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