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Synthesis and Antifungal Properties of Some Benzimidazole Derivatives

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Some benzimidazole derivatives were synthesized and their in vitro antifungal activities were tested against *Candida albicans, Candida glabrata* and *Candida krusei*. Compounds **6** and **9** possessed activity comparable to fluconazole against *C. albicans* with a minimum inhibitory concentration of 12.5 μg/mL.

Key Words: Benzimidazole, thiourea, antifungal.

Introduction

Life-threatening infections caused by pathogenic fungi are becoming increasingly common, especially in individuals with suppressed immune systems such as cancer chemotherapy or AIDS patients. However, there are only a limited number of antifungal compounds available for such infections, which leads to a strong need to develop new classes of compounds having antifungal activities. In particular, Candidiasis is the fungal infection most frequently associated with HIV-positive patients, and Cryptococcosis, which is the cause of morbidity and mortality in AIDS patients, is caused by *Cryptococcus neoformans*.

Compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities such as antitubercular[1], anticancer[2,3], anthelmintic[4], antiallergic[5,6], antioxidant[7−9], antihistaminic[10] and antimicrobial[11−17]. In addition, some thiourea derivatives have been reported as antimycobacterial[18] and antimicrobial[19]. In our previous studies we reported the synthesis and antimicrobial[20−23] activities of a large series of benzimidazole derivatives. On the basis of these reports and as a continuation of our research program on benzimidazole derivatives, we report here the synthesis of novel benzimidazole derivatives to evaluate their antifungal properties.

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Experimental

Chemistry

Melting points were determined with an Electrothermal (Electrothermal Eng. Ltd., Essex, UK) melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured with a Varian Mercury 400, 400 MHz instrument (California, USA) using TMS internal standard and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. ESMS were obtained with a Waters ZQ Micromass LC-MS spectrometer (Milford, MA, USA) with positive electrospray ionization. Elemental analyses (C, H, N, S) were determined on a Leco CHNS 932 instrument (St. Joseph, MI, USA), and were within ± 0.4% of the theoretical values. All instrumental analyses were performed at the Scientific and Technical Research Council of Turkey and Ankara University, Faculty of Pharmacy, Central Laboratory. The chemical reagents used in the synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA). Compound 8 was used as a crude product for further reaction.

General procedure for the preparation of the compounds 5-7

The appropriate aldehyde derivative (1.5 mmol) was dissolved in 5 mL of EtOH. Then 0.160 g of $Na_2S_2O_5$ in 5 mL of water was added 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 6 mL of DMF were heated at 110 °C for 5 h. At the end of this period the reaction mixture was cooled, and poured into water. The precipitate was collected and recrystallized from ethanol-water.

5-Nitro-2-phenyl-1-propylbenzimidazol (5)

Anal. Calcd. For $C_{16}H_{15}N_3O_2 0.5H_2O$: C, 66.19; H, 5.55; N, 14.47. Found: C, 65.97; H, 5.18; N, 14.49; Yield % (56), ES (+) 282 (M+H); ^{1}H NMR (DMSO-d_6) δ 0.73 (t, 3H, CH_3), 1.66-1.71 (m, 2H, CH_2), 4.37 (t, 2H, CH_2), 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).

2-(p-Fluorophenyl)-5-nitro-1-propylbenzimidazol (6)

Anal. Calcd. For $C_{16}H_{14}FN_3O_2$: C, 64.20; H, 4.71; N, 14.03. Found: C, 63.93; H, 4.38; N, 14.25; Yield % (42), ES (+) 300 (M+H); ^{1}H NMR (DMSO-d_6) δ 0.73 (t, 3H, CH_3), 1.65-1.71 (m, 2H, CH_2), 4.35 (t, 2H, CH_2), 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).

2-(p-Fluorophenyl 5-nitro-1-cyclopentylbenzimidazol (7)

Anal. Calcd. For $C_{18}H_{16}FN_3O_2$: C, 66.45; H, 4.95; N, 12.91. Found: C, 66.86; H, 5.24; N, 12.57; Yield % (75), ES (+) 326 (M+H); ^{1}H NMR (DMSO-d_6) δ 1.68-2.16 (m, 8H, CH_2), 4.85-4.89 (m, 1H, CH), 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).

General procedure for the preparation of the compounds 9-10

5-Nitro benzimidazole derivatives **5-7** (1 mmol) in 10 mL of hot EtOH and 10 mL of 6 N HCl were refluxed and $SnCl_2.2H_2O$ was added in portions until the starting material was completely exhausted. The ethanol was removed and the residue was made alkaline with KOH, extracted with EtOAc, and washed with water. EtOAc was evaporated and the residue crystallized from EtOH.

5-Amino-2-(p-Fluorophenyl)-1-propylbenzimidazol (9)

Anal. Calcd. For $C_{16}H_{16}FN_3$: C, 71.35; H, 5.98; N, 15.60. Found: C, 70.95; H, 5.73; N, 15.36; Yield % (67), M.p. 127-129 °C; ES (+) 270 (M+H); ^{1}H NMR (DMSO-d_6) δ 0.7 (t, 3H, CH_3), 1.62-1.68 (m, 2H, CH_2), 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), 7.29 (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′).

5-Amino-(2-(p-Fluorophenyl)-1-cyclopentylbenzimidazol (10)

Anal. Calcd. For $C_{18}H_{18}FN_3.0.1\ H_2O$: C, 72.75; H, 6.77; N, 14.13. Found: C, 73.06; H, 6.42; N, 13.76; Yield % (85), M.p. 196 °C; ES (+) 296 (M+H); ^{1}H NMR (DMSO-d_6) δ 1.63-2.15 (m, 8H, CH_2), 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), 7.36-7.40 (m, 2H, H-3′,5′), 7.65-7.69 (m, 2H, H-2′,6′). ^{13}C NMR (DMSO-d_6) δ 25.19, 30.45, 57.74, 103.39, 112.74, 116.22, 116.43, 125.99, 128.31, 132.16, 132.25, 144.83, 145.37, 152.46, 162.06, 164.52.

General procedure for the preparation of compounds 11-14

5-Amino benzimidazole derivatives (0.25 mmol) and appropriate phenylisothiocyanate (0.375 mmol) were refluxed in 5 mL of absolute ethanol for 8 h. The reaction mixture was poured into water. The precipitate was collected, washed with ether and recrystallized from ethanol.

N^1-(2-phenyl-1-propyl-benzimidazol-5-yl)-N^3-phenylthiourea (11)

Anal. Calcd. For $C_{23}H_{22}N_4S.\ 1.1\ H_2O$: C, 67.98; H, 6.00; N, 13.78; S, 7.89. Found: C, 67.52; H, 5.71; N, 13.41; S, 7.67; Yield % (26), M.p. 140 °C; ES (+) 387 (M+H); ^{1}H NMR (DMSO-d_6) δ 0.74 (t, 3H, CH_3), 1.69-1.71 (m, 2H, CH_2), 4.27 (t, 2H, CH_2), 7.12-7.76 (m, 13H, Ar-H), 9.71 (s, 1H, NH), 9.85 (s, 1H, NH).

N^1-[2-(p-Fluorophenyl)-1-propyl-benzimidazol-5-yl]-N^3-(p-chlorophenyl)-thiourea (12)

Anal. Calcd. For $C_{23}H_{20}ClFN_4S.\ 1.1\ H_2O$: C, 60.21; H, 4.87; N, 12.21; S, 6.98. Found: C, 60.01; H, 4.62; N, 12.21; S, 6.88; Yield % (27), M.p. 136 °C; ES (+) 439 (M+H); ^{1}H NMR (DMSO-d_6) δ 0.72 (t, 3H, CH_3), 1.68-1.70 (m, 2H, CH_2), 4.29 (t, 2H, CH_2), 7.29-7.83 (m, 11H, Ar-H), 9.76 (s, 1H, NH), 9.94 (s, 1H, NH).^{13}C NMR (DMSO-d_6) δ 11.58, 23.26, 46.41, 111.40, 115.63, 116.47, 116.69, 121.33, 126.06, 127.44, 128.80, 128.87, 132.15, 132.23, 134.03, 134.49, 139.38, 142.63, 153.30, 162.35, 164.80, 180.80.

N^1-[2-(p-Fluorophenyl)-1-cyclopentyl-benzimidazol-5-yl]-N^3-phenylthiourea (13)

Anal. Calcd. For $C_{25}H_{23}FN_4S$: C, 69.74; H, 5.38; N, 13.01; S, 7.44. Found: C, 70.12; H, 5.13; N, 12.78; S, 7.43; Yield % (18), M.p. 138-139 °C; ES (+) 431 (M+H); ^{1}H NMR (DMSO-d_6) δ 1.68-2.13 (m, 8H, CH_2),

4.83 (m, 1H, CH), 7.27-7.75 (m, 12H, Ar-H), 9.75 (s, 1H, NH), 9.96 (s, 1H, NH).

N^1-[2-(p-Fluorophenyl)-1-cyclopentyl-benzimidazol-5-yl]-N^3-(p-chlorophenyl)-thiourea (14)

Anal. Calcd. For $C_{25}H_{22}ClFN_4S$. 0.8 H_2O: C, 62.63; H, 4.96; N, 11.68; S, 6.68. Found: C, 62.58; H, 4.79; N, 11.46; S, 6.29; Yield % (34), M.p. 138 °C; ES (+) 465 (M+H); ^{1}H NMR (DMSO-d_6) δ 1.68-2.19 (m, 8H, CH_2), 4.83 (m, 1H, CH), 7.34-7.79 (m, 11H, Ar-H), 9.78 (s, 1H, NH), 9.94 (s, 1H, NH).

Antifungal activity assay

The yeasts were maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1 °C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the 2-fold dilution was applied. A set of tubes containing only inoculated broth were kept as controls. After incubation for 48 h at 25 ± 1 °C, the last tube with no yeast growth was recorded to represent minimum inhibitory concentration (MIC), expressed in μg/mL.

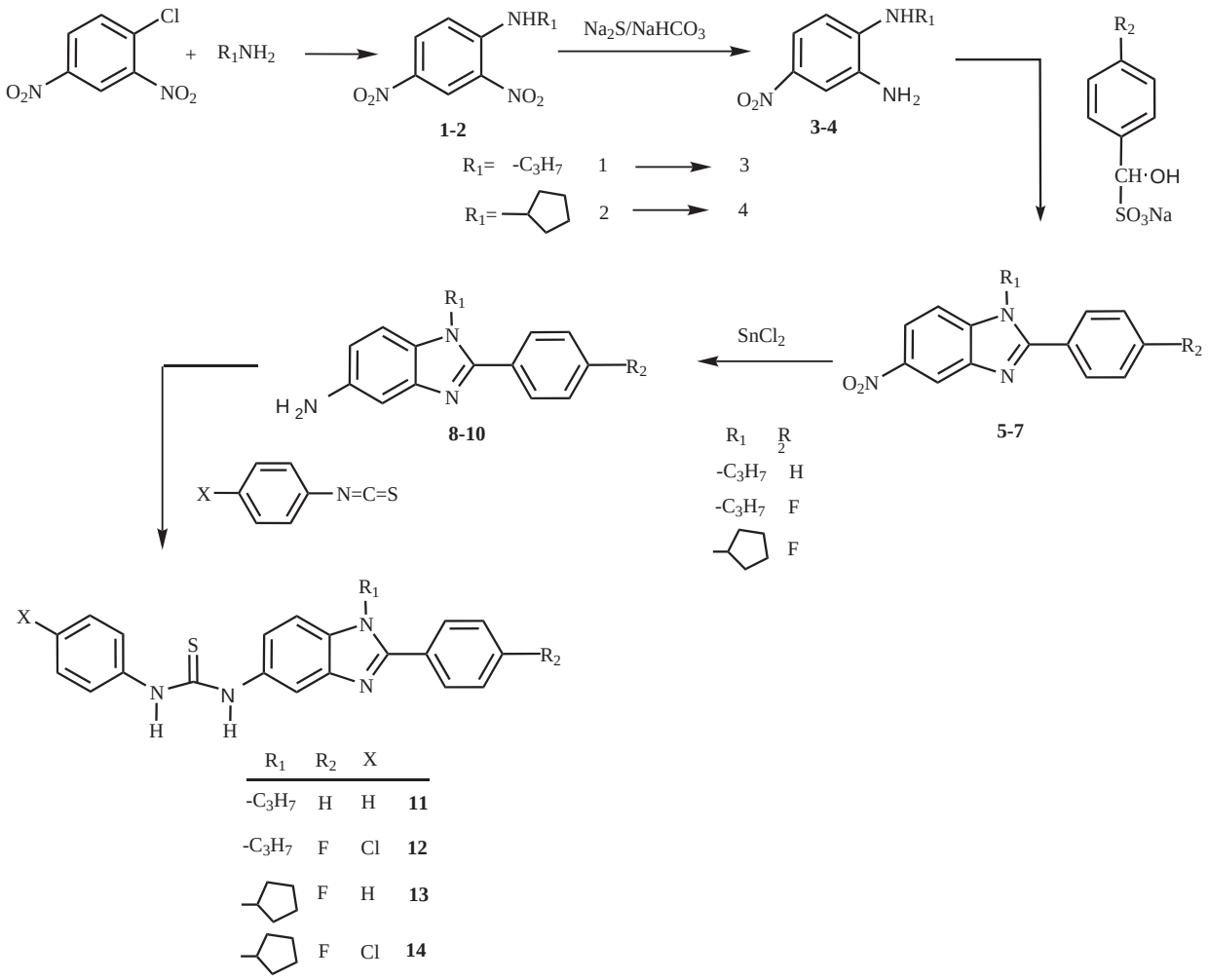
Results and Discussion

Compounds **1** and **2** were prepared from 1-chloro-2,4-dinitrobenzene by reaction with propyl/cyclopentylamine in DMF according to the literature[24]. 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[24]. Condensation of o-phenylenediamines **(3** and **4)** with the $Na_2S_2O_5$ adduct of appropriate benzaldehydes in DMF[25] gave **5-7**. Reduction of compounds **5-7** with $SnCl_2.2H_2O$ produced **8-10**. Thiourea compounds **11-14** were obtained with the reaction of compounds **8-10** with appropriate phenylisothiocyanates in absolute ethanol (Scheme).

The in *vitro* antifungal activity of the compounds was tested by the tube dilution technique[26]. Each of the test compounds and standards miconazole and fluconazole were dissolved in 12.5% 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, 3.12, 1.56, 0.78 μg/mL concentrations. The final inoculum 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* ATCC 10231, patient isolate *C. glabrata* and *C. krusei* ATCC 6258 (Table).

Compounds **6** and **9** possessed comparable activity to fluconazole against *C. albicans* with a MIC of 12.5 μg/mL. Compounds **6, 9** and **12** were more effective against *C. krusei* (6.25 μg/mL) compared with the other derivatives. However, none of the compounds was superior to the standards used against any fungi. Generally, the thiourea compounds (**12-14**) were less active than their amine counterparts (**9** and **10**), with the exception of compound **12** against *C. krusei*.



R_1	R_2	X	
$-C_3H_7$	H	H	**11**
$-C_3H_7$	F	Cl	**12**
	F	H	**13**
	F	Cl	**14**

Scheme. Synthetic route of the compounds.

Table. The in vitro antifungal activity of the synthesized compounds (MIC, μg/mL).

Compound	C. albicans	C. glabrata	C. krusei
5	25	25	12.5
6	12.5	6.25	6.25
9	12.5	12.5	6.25
10	25	25	12.5
11	25	25	12.5
12	25	25	6.25
13	25	25	25
14	50	25	25
Fluconazole	12.5	3.125	3.125
Miconazole	6.25	3.125	1.56

References

1. V.L. Khairnar, S.R. Lockhande, M.R. Patel and B.G. Khadse, **Chemical Abstract 95**, 203833h (1981).
2. L.L. Kruse, D.L. Ladd, P.B. Harrsch, F.L. McCabe, S.M. Mong, L. Faucette and R. Johnson, **J. Med. Chem. 32,** 409-417 (1989**)**.
3. I. Islam, E.B. Skibo, R.T. Dorr and D.S. Alberts, **J. Med. Chem**. **34,** 2954-2961 (1991).
4. V.J. Habernickel, **Drugs made in Germany**, **35**, 97 (1992).
5. T. Fukuda, T. Saito, S. Tajima, K. Shimohara and K. Ito, **Arzneim.-Forsch./Drug Res. 34,** 805-810 (1984).
6. H. Nakano, T. Inoue, N. Kawasaki, H. Miyataka, H. Matsumoto, T. Taguchi, N. Inagaki, H. Nagai and T. Satoh, **Chem. Pharm. Bull**. **47**, 1573-1578 (1999).
7. B. Can-Eke, M.O. Puskullu, E. Buyukbingol and M. İşcan, **Chemico-Biological Interactions 113**, 65-77 (1998).
8. C. Kuş, G. Ayhan-Kılcıgil**,** B. Can-Eke and M. İşcan, **Arch. Pharm. Res. 27**, 156-163 (2004).
9. G. Ayhan-Kılcıgil, C. Kuş, T. Çoban, B. Can-Eke and M. İşcan, **Journal of Enzyme Inhibition and Medicinal Chemistry 19**, 129-135 (2004).
10. H. Göker, G. Ayhan-Kılcıgil, M. Tunçbilek, C. Kuş, R. Ertan, E. Kendi, S. Özbey, M. Fort, C. Garcia and A.J. Farre, **Heterocycles 51**, 2561-2573 (1999).
11. A.E. Abdel-Rahman, A.M. Mahmoud, G.M. El-Naggar and H.A. El-Sherief, **Pharmazie 38**, 589-590 (1983).
12. F.S.G. Soliman, S.M. Rida, E.A.M. Badawey and T. Kappe, **Arch. Pharm**. **317**, 951-958 (1984).
13. R.A. Coburn, M.T. Clark, R.T. Evans and R.J. Genco, **J. Med. Chem**. **30**, 205-208(1987).
14. N.S. Habib, S. Abdel-Hamid and M. El-Hawash, **Farmaco**, **44**, 1225-1232 (1989).
15. H. Göker, C. Kuş, D.W. Boykin, S. Yıldız and N. Altanlar, **Bioorg. Med. Chem. 10,** 2589-2596 (2002).
16. S. Özden, H. Karataş, S. Yıldız and H. Goker, **Arch. Pharm. Pharm. Med. Chem. 337**, 556-562 (2004).
17. S. Özden, D. Atabey, S.Yıldız and H. Göker, **Bioorg. Med. Chem. 13**, 1587-1597 (2005).
18. I. Küçükgüzel, G. Küçükgüzel, S. Rollas and M. Kiraz, **Bioorg. Med. Chem. Letters 11,** 1703-7007 (2001).
19. M.S. El-Gaby, J.A. Micky, N.M. Taha and M.A.M. El-Sharief, **J. Chin. Chem. Soc. 49,** 407-414 (2002).
20. C. Kuş, H. Göker, G. Ayhan, R. Ertan, N. Altanlar and A. Akın, **Il Farmaco 51,** 413-417 (1996).
21. H. Göker, M. Tunçbilek, G. Ayhan and N. Altanlar, **Il Farmaco 53**, 415-420 (1998).
22. G. Ayhan-Kılcıgil, M. Tunçbilek, N. Altanlar and H. Göker, **Il Farmaco 54**, 562-565 (1999).
23. G. Ayhan-Kılcıgil and N. Altanlar, **Il Farmaco 58**, 1345-1350 (2003).
24. H. Willitzer, D. Brauniger, D. Engelmann, D. Krebs, W. Ozegowski and M. Tonew, **Pharmazie 33**, 30-38 (1978).
25. H.F. Ridley, R.G.W. Spickett and G.M.J. Timmis, **Heterocyclic Chem**. **2,** 453-456 (1965).
26. D.F. Sahm and J.A. Washington, "**Antibacterial Susceptibility Tests: Dilution Methods**", in Manual of Clinical Microbiology, 5^{th} ed., eds. A. Balowes, W.J. Hausler, K.L. Hermann and H.D. Shadomy, pp. 1105-1116, American Society for Microbiology, Washington DC, 1991.