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Synthesis, characterization and biological evaluation of new benzimidazoles

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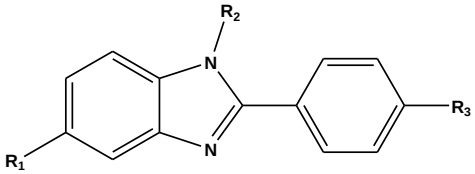
The benzimidazoles moieties play an important role in medical field with so many pharmacological activities such as antifungal activities. In the present study, we have reported the synthesis, spectral studies and antifungal evaluation of some novel benzimidazoles. The structures of all the synthesized 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 tested compounds displayed in vitro antifungal activity and Minimum Inhibitory Concentration (MIC) was determined for all compounds. The derivatives have shown moderate to good activity when compared with commercially available fungicides.

Keywords: benzimidazole, spectroscopic techniques, antifungal activity, fungicides..

INTRODUCTION

Benzimidazole is the heterocyclic compound formed from benzene and imidazole ring containing nitrogen, oxygen, sulphor and its derivatives are of wide interest because of their diverse biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. In fact the benzimidazoles moieties play an important role in medical field with so many pharmacological activities. The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds. 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 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 [18,19].

Due to great potential of the moiety, synthesis of benzimidazole derivatives was carried out to evaluate their antifungal. In generally benzimidazoles are readily formed by heating *o*-phenylenediamines with carboxylic acids or aldehydes. For example, benzimidazole itself is produced by heating *o*-phenylenedamine with 90% formic acid or formaldehyde [20]. In this work, is reported a study on synthesis of some novel brom derivatives of 2-bromomethyl-benzimidazole (Structures of **5-10** in Figure 1). These derivatives were screened for antifungal activity against against *Candida albicans*, *Candida glabrata*, and *Candida krusei.*



5) $R_1 = NO_2$ $R_2 = i\text{-}C_3H_7$ $R_3 = H$

6) $R_1 = NO_2$ $R_2 = i\text{-}C_3H_7$ $R_3 = Br$

7) $R_1 = NO_2$ $R_2 =$  $R_3 = Br$

8) $R_1 = NH_2$ $R_2 = i\text{-}C_3H_7$ $R_3 = H$

9) $R_1 = NH_2$ $R_2 = i\text{-}C_3H_7$ $R_3 = Br$

10) $R_1 = NH_2$ $R_2 =$  $R_3 = Br$

Fig.1. Chemical structures of chemical compound synthesized

The structures of the obtained compounds were characterized and the target compounds (**1c-9c**) were screened for their antibacterial activity against various strains of *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Candida albicans*.

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EXPERIMENTAL

Material and Equipment

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 isopropyl/cyclohexylamine (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 [21]. 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**)** [22, 23].

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

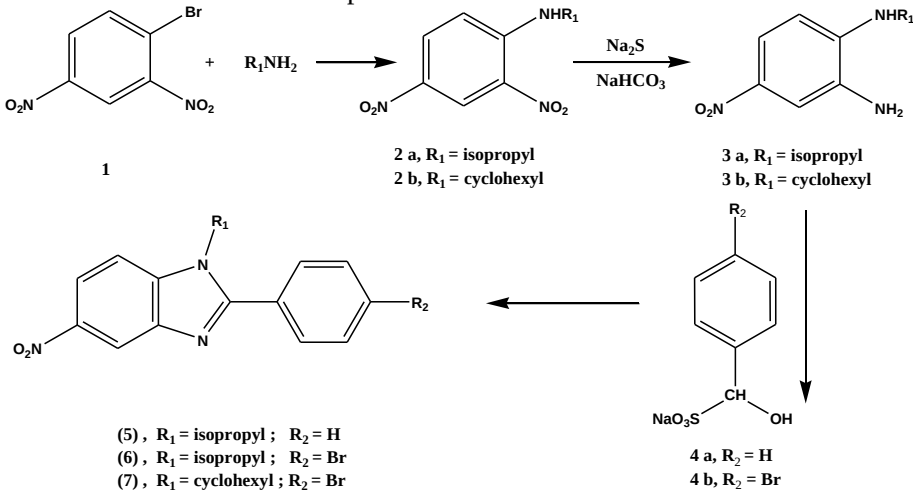
Cream powder; Yield 71%; m.p. 131-133 °C; IR (KBr, cm^{-1}): 2971 (CH), 1650 (N=C), 1302 (C-N stretching), 895 (C-C bonding aromatic). ^{1}H-NMR (δ/ppm): 1.33 (t, 6H, CH_3), 3.73 (m, 1H, CH), 7.21-7.59 (5H, m, Ar-benzimidazole), 8.01 (d, 1H, Jo= 8.8 Hz, H-7), 8.25 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.59 (d, 1H, Jm= 2 Hz, H-4). ^{13}C-NMR (δ / ppm): 22.6, 45.3, 112.1, 117, 119, 125.5, 128.5, 129.5, 130.0, 133.5, 137.1, 143.4, 146.7. Anal. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.32; H, 5.33; N, 14.95 %. Found: C, 67.91; H, 5.30; N, 15.08 %. MS (m/z, *regulatory intensity*, %): 281 (*100*), 282 (*18*).

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

Light yellow powder; Yield 69%, m.p. 155-157 °C; IR (KBr, cm^{-1}): 2983 (CH), 1663 (N=C), 1288 (C-N stretching), 879 (C-C bonding aromatic), 673 (C-Br); ^{1}H-NMR (δ/ppm): 1.41 (t, 6H, CH_3), 3.85 (m, 1H, CH), 7.30-7.50 (4H, m, Ar-benzimidazole), 7.80 (d, 1H, Jo= 8.8 Hz, H-7), 8.12 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.60 (d, 1H, Jm= 2 Hz, H-4); ^{13}C-NMR (δ/ppm): 19.7, 40.3, 114.5, 118, 121, 127.5, 130.5, 133.5, 134.0, 136.5, 139.2, 146.1, 149.2. Anal. Calcd. for $C_{16}H_{14}BrN_3O_2$: C, 53.30; H, 3.88; N, 11.66 %. Found: C, 53.35; H, 3.83; N, 11.61 %. MS (m/z, *regulatory intensity*, %): 359 (100), 361(98), 360 (20).

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

Yellow powder; Yield 88%; m.p. 181-183 °C; IR (KBr, cm^{-1}): 2936 (CH), 1664 (N=C), 1285 (C-N stretching), 910 (C-C bonding aromatic), 675 (C-Br); ^{1}H-NMR (δ/ppm): 1.54-2.86 (m, 10H, CH_2), 3.25 (1H,s,CH, Cyclohexyl), 7.21-7.44 (4H, m, Ar-benzimidazole), 7.70 (d, 1H, Jo= 8.8 Hz, H-7), 8.23



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

(dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.59 (d, 1H, Jm= 2 Hz, H-4); ^{13}C-NMR (δ/ppm): 26.5, 29.5, 65.5, 113.5, 118.0, 121.5, 122, 124, 126.5, 132.5, 136.5, 141.3, 148.0. Anal. Calcd. For $C_{19}H_{18}BrN_3O_2$: C, 56.96; H, 4.50; N, 10.50 %, Found: C, 56.90; H, 4.46; N, 10.41 %. MS (m/z, *regulatory intensity*, %): 401 (100), 399 (100), 400 (25).

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 $SnCl_2.2H_2O$ 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) [21-23].

***1- Isopropyl-2-pjenyl-1H-benzimidazole-5-ylamine* (8)**

Cream powder; Yield 75%; m. p. 191-193 °C; IR (KBr, cm^{-1}):3155 (NH), 2981 (CH), 1628 (N=C), 1285 (C-N stretching), 887 (C-C bonding aromatic); ^{1}H-NMR (δ/ppm): 1.43 (t, 6H, CH_3), 3.95 (m, 1H, CH), 4.8 (s, 2H, NH_2), 6.95-7.69 (3H, m, Ar-Bbenzimidazole), 7.60 (d, 1H, Jo= 8.8 Hz, H-7), 8.11 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.44 (d, 1H, Jm= 2 Hz, H-4); ^{13}C-NMR (δ/ppm): 24.2, 48.1,112.5, 115, 117, 119, 129.7 ,130.8, 131.0,134.5, 137, 139.8 , 145.2 . Anal. Calcd. for $C_{16}H_{17}N_3$: C, 76.46; H, 6.82; N, 16.72 %. Found: C, 76.85; H, 6.79; N, 16.62 %. MS (m/z, *regulatory intensity*, %): 251 (100), 252 (18).

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

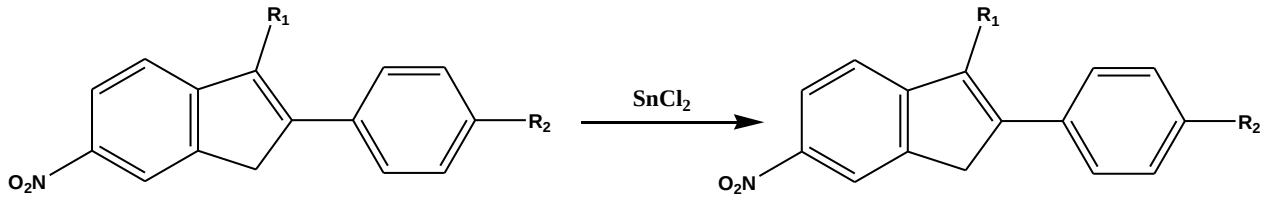
Light Yellow powder; Yield 78%, m. p. 137-139°C; Anal.Calcd. for $C_{16}H_{16}BrN_3$: C, 58.19; H, 4.88; N, 12.72 %. Found: C, 58.05; H, 4.90; N, 12.76 %. IR (KBr, cm^{-1}): 3330 (NH), 2945 (CH), 1632 (N=C), 1288 (C-N stretching), 912 (C-C bonding aromatic), 691 (C-Br); ^{1}H-NMR (δ/ppm): 1.56 (t, 6H, CH_3), 4.22 (m, 1H, CH), 5.1 (s, 2H, NH_2), 6.95-7.69 (3H, m, Ar-Bbenzimidazole), 7.75 (d, 1H, Jo= 8.8 Hz, H-7), 8.25 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.56 (d, 1H, Jm= 2 Hz, H-4). ^{13}C-NMR (δ/ppm): 21.2, 42.1, 109.5, 114, 118, 120, 127.5, 131.8, 132.3, 135.2, 138.2, 139.8, 147.3. Anal.Calcd. for $C_{16}H_{16}BrN_3$: C, 58.19; H, 4.88; N, 12.72 %. Found: C, 58.05; H, 4.90; N, 12.76 %. MS (m/z, *regulatory intensity*, %): 329 (100), 331 (97), 332 (20).

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

Yellow powder; Yield 85%, m. p. 189-191 °C; IR (KBr, cm^{-1}): 3155 (NH), 2991 (CH), 1661 (N=C), 1296 (C-N stretching), 905 (C-C bonding aromatic), 699 (C-Br);^{1}H-NMR (δ/ppm): 1.69-2.20 (m, 10H, CH_2), 4.65-4.75 (m, 1H, CH), 4.85 (s, 2H, NH_2), 6.91-7.64 (3H, m, Ar-Bbenzimidazole), 7.71 (d, 1H, Jo= 8.8 Hz, H-7), 8.42 (dd, 1H, Jo =8.8 Hz, Jm= 2 Hz, H-6), 8.62 (d, 1H, Jm= 2 Hz, H-4). ^{13}C-NMR (δ/ppm): 29.7, 30.9, 61.5, 113.5, 114.0,116, 117.5, 125.6, 127.8, 132.5, 138.5, 140.5, 143.1. Anal. Calcd. For $C_{19}H_{20}BrN_3$: C, 61.63; H, 5.44; N, 11.35 %. Found: C, 61.66; H, 5.39; N, 11.46 %. MS (m/z, *regulatory intensity*, %): 369 (100), 371 (95), 370 (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.



(5) , R_1 = isopropyl ; R_2 = H
(6) , R_1 = isopropyl ; R_2 = Br
(7) , R_1 = cyclohexyl ; R_2 = Br

(8) , R_1 = isopropyl ; R_2 = H
(9) , R_1 = isopropyl ; R_2 = Br
(10) , R_1 = cyclohexyl ; R_2 = Br

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

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 isopropyl/cyclo-hexylamine in DMF according to the literature [21]. The 2-nitro group of compounds was reduced to 2-amino by using $Na_2S/NaHCO_3$ in methanol [21]. Condensation of o-phenylenediamines with the $Na_2S_2O_5$ adduct of appropriate benzaldehydes in DMF [24] 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 [25]. 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 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 **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.

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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СИНТЕЗА, ОХАРАКТЕРИЗИРАНЕ И БИОЛОГИЧНА ОЦЕНКА НА НОВИ БЕНЗИМИДАЗОЛИ

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(Резюме)

Бензимидазолевото ядро е от голямо значение в медицинската химия и много бензимидазол-съдържащи съединения проявяват значителна биологична активност. В настоящата работа са изследвани синтезите, спектралните характеристики и биологичните свойства на девет нови производни на бензимидазола. Структурите на синтезираните съединения са охарактеризирани чрез IR, 1H-NMR, 13C-NMR, мас-спектроскопия and CHN-елементен анализ. Антибактериалната активност на съединенията е оценена спрямо щамовете *Candida albicans, Candida glabrata, and Candida krusei.* Изследваните съединения показват ин витро противогъбично действие и минималната инхибираща концентрация (MIC) е определена за всички съединения. Производните показват от умерена до добра активност в сравнение с наличните в търговската мрежа фунгициди.