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Synthesis, characterization and biological evaluation of some novel Benzimidazole derivatives

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The benzimidazole nucleus has a significant importance in medicinal chemistry and many benzimidazole-containing compounds exhibit important biological activities. In the present study, synthesis, spectral studies and biological evaluation of nine novel benzimidazole derivatives were investigated. The structures of the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy and CHN elemental analyzer and the target new synthesized compounds (**1c**-**9c**) screened for antibacterial activity against various strains of *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Candida albicans*.

Keywords: Benzimidazole, Antibacterial activity, Antifungal activity, Gram-positive bacteria, Gram-negative bacteria.

INTRODUCTION

Infectious microbial diseases remain pressing problems worldwide, because resistance to a number of antibiotics agents among variety of clinically significant species of microorganisms has become an important global health problem. One way to battle with this challenge is the conscious usage of the currently marketed antibiotics and the other is the development of novel drugs. They are natural and synthetic heterocyclic compounds and classified by medicinal chemists as the privileged sub-structures for drug design. In light of the affinity, they display towards a variety of enzymes and protein receptors [1] i.e., as selective neuropeptide YY1 receptor antagonists [2–5], 5-lipoxygenase inhibitors for use as novel anti-allergic agents [6], factor Xa (FXa) inhibitors [7], poly (ADP-ribose) polymerase (PARP) inhibitors [8] and as human cytomegalovirus (HCMV) inhibitors [9]. A wide variety of Benzimidazole derivatives are known for their chemotherapeutic importance and antimicrobial [10-15], antifungal [16-18], anti-inflammatory [19] and antioxidant [20-24] activities in this context. Because of their wide range of pharmacological activities and industrial and synthetic applications, several methods have been reported in the literature for their synthesis and biological evaluations. Traditionally, the synthesis of benzimidazoles involves the condensation of *o*-phenylenediamine with aldehydes and carboxylic acids or their derivatives. These methods include cyclo-condensation reaction of *o*-phenylenediamines with carboxylic acids or derivatives [25-29]. In view of these valid observations and as a continuation of our work, prompted us to synthesize new 2-substituted benzimidazole derivatives (**1b-9b** and **1c-9c**) (Figure 1) and the structures of the synthesized compounds were characterized by analysis techniques and the target synthesized compounds (**1c-9c**) were screened for their antibacterial activity against various strains of *Escherichia coli* and *Staphylococcus aureus* and antifungal activities against *Candida albicans*.

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 determined 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.

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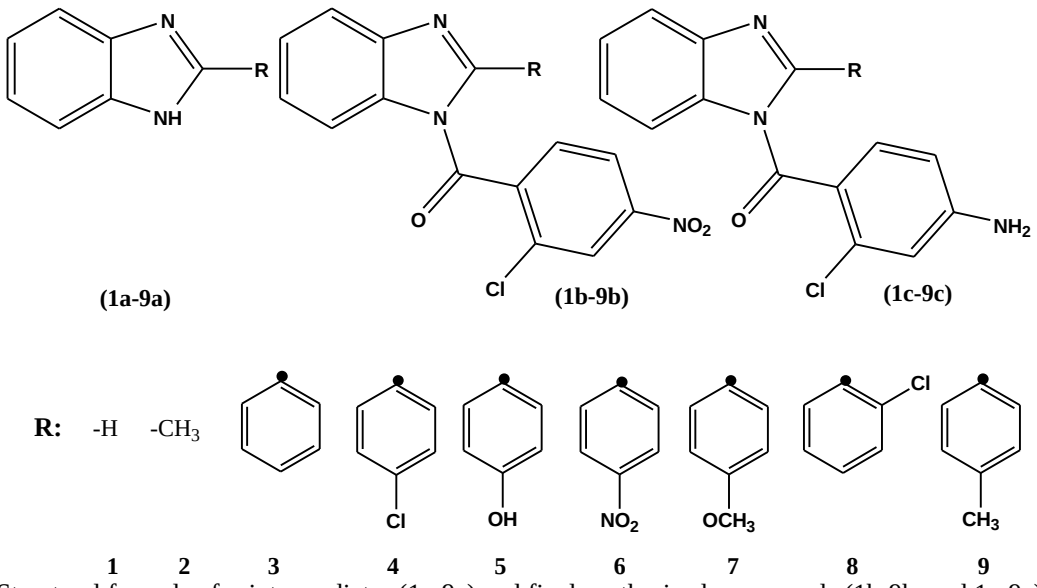


Figure1. Structural formulae for intermediates (1a-9a) and final synthesized compounds (1b-9b, and 1c-9c).

Synthesis of Compounds

These compounds (**1a-9a**) were prepared based on a known methods with some modification [30,31].

1*H*-benzimidazole (1a):

o-phenylenediamine (5.4 g, 0.25 mole) and formic acid 90% (3.2 g, 0.34 mole) were heated on water bath for 2 h at 100 ^{o}C . The mixture was slowly cooled, basified with aqueous sodium hydroxide and the solid compound was obtained, filtered and re-crystallized from boiling water (Scheme 1).

(**1a:** White powder, m.p. 169-171 ^{o}C, 92.8% yield).

2-Methyl-1*H*-benzimidazole (2a):

o-phenylenediamine dihydrochloride (5.43 g, 0.03 mole) in 20 ml of water and (5.4 g, 0.09) of acetic acid were heated under reflux for 45 min. The mixture was slowly cooled, basified with ammonia solution and the solid compound filtered and re-crystallized from ethanol (Scheme 1).

(**2a:** Light beige to brown powder, m.p. 175–177 ^{o}C, 91.8% yield)

General procedure for the preparation of the compounds (3a-9a):

o-phenylenediamine (0.055 mol), appropriate benzoic acid (0.05 mol) and HCl (4N, 25 ml) were refluxed for 2 h. The reaction mixture was cooled, poured into crushed ice and the product was re-crystallized in boiled water (Scheme 1).

2-Phenyl-1H-benzimidazole (3a):

(**3a:** White to beige-grey powder, m.p. 293–296 ^{o}C, 89.1% yield)

IR (KBr, cm^{-1}): 3345, 3060, 2925, 1671, 1444, 1276, 922, 744, 696; ^{1}H NMR ($CDCl_3$, δ/ppm): 7.22 (m,2H), 7.48 (m, 5H), 7.58 (s, 1 H), 8.04 (dd, 2H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.6 (benzimidazole, CH=), 122.1 (benzimidazole, CH=), 135.4 (benzimidazole, C), 149.3 (N-C=N), 134.3 (phenyl, C), 126.8, 129.4 (phenyl, CH=); Anal. Calcd. for $C_{13}H_{10}N_2$: C, 80.33; H, 5.15; N, 14.42 %. Found: C, 80.12; H, 5.08; N, 14.22%; MS, *m/z*: 195 $[M+H]^{+}$.

2-(4-Chlorophenyl)-1*H*-benzimidazole (4a):

(**4a:** White powder, m.p. 301–303 ^{o}C, 90.7% yield)

IR (KBr, cm^{-1}): 3369, 2918, 1604, 1515, 1341, 855, 745, 710; ^{1}H NMR (DMSO-d_6, δ/ppm): 12.70 (br s, 1H), 8.08 (d, 2H), 7.59 (m, 1H), 7.26 –7.20 (m, 3H), 7.18–7.04 (m, 2H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.5 (benzimidazole, CH=), 119.1 (benzimidazole, CH=), 136.7 (benzimidazole, C), 142.1 (N-C=N), 132.4 (phenyl, C), 130.9 (phenyl, Cl), 128.8, 129.8 (phenyl, CH=); Anal. Calcd. for $C_{13}H_9ClN_2$: C, 68.21; H, 3.93; N, 12.24 %. Found: C, 68.12; H, 3.89; N, 12.18%; MS, *m/z*: 229 $[M+H]^{+}$.

2-(4-Hydroxyphenyl)-1H-benzimidazole (5a):

(**5a:** White powder, m.p. 240-242 ^{o} C, 91.1% yield)

IR (KBr, cm^{-1}): 3420, 2921, 1610, 1447, 1280, 838, 746; ^{1}H NMR (DMSO-d_6, δ/ppm): 8.16–8.22 (m, 2H), 7.21–7.73 (m, 6H), 4.60 (d, 1H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.6 (benzimidazole, CH=), 120.2 (benzimidazole, CH=), 137.8 (benzimidazole, C), 144.1 (N-C=N), 151.9 (phenyl, OH), 128.4 (phenyl, C), 127.8, 117.8 (phenyl, CH=); Anal. Calcd. for $C_{13}H_{10}ON_2$: C, 74.28; H, 4.76; N, 13.33 %. Found: C, 74.01; H, 4.65; N, 13.18%; MS, *m/z*: 211 $[M+H]^{+}$.

2-(4-Nitrophenyl)-1*H*-benzimidazole (6a):

(**6a:** Cream powder, m.p. 312-314 ^{o}C, 88.4% yield)

IR (KBr, cm^{-1}): 3369, 3058, 1604, 1515, 1341, 855, 745; ^1H NMR (DMSO-d_6, δ/ppm): 8.00–8.08 (m, 2H), 7.20–7.60 (m, 6H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.2 (benzimidazole, CH=), 119.2 (benzimidazole, CH=), 138.6 (benzimidazole, C), 143.5 (N-C=N), 150.1 (phenyl, NO_2), 144.4 (phenyl, C), 125.8, 128.6 (phenyl, CH=); Anal. Calcd. for $C_{13}H_9O_2N_3$: C, 65.27; H, 3.76; N, 17.57 %. Found: C, 65.14; H, 3.69; N, 17.43%; MS, *m/z*: 240 $[M+H]^+$.

2-(4-Methoxyphenyl)-1*H*-benzimidazole (7a):

(**7a:** White powder, m.p. 225–226 °C**,** 85.9% yield)

IR (KBr, cm^{-1}): 3675, 3346, 1650, 1548, 1420, 1182, 812, 742; ^1H NMR (DMSO-d_6, δ/ppm): 8.00 – 8.08 (m, 2H), 7.20–7.60 (m, 6H), 3.52 (m, 3H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 56.7 (CH_3), 111.7 (benzimidazole, CH=), 118.9 (benzimidazole, CH=), 136.9 (benzimidazole, C), 142.3 (N-C=N), 161.1 (phenyl, OMe), 129.3 (phenyl, C), 113.8, 127.9 (phenyl, CH=); Anal. Calcd. for $C_{14}H_{12}ON_2$: C, 75.02; H, 5.36; N, 12.51 %. Found: C, 74.91; H, 5.30; N, 12.43%; MS, *m/z* 224 $[M]^+$.

2-(2-Chlorophenyl)-1*H*-benzimidazole (8a):

(**8a:** White powder, m.p. 232-234 °C, 86.3% yield)

IR (KBr, cm^{-1}): 3372, 2922, 1599, 1521, 1348, 850, 739, 712; ^1H NMR (DMSO-d_6, δ/ppm): 8.00–8.08 (m, 2H), 7.20–7.60 (m, 6H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.3 (benzimidazole, CH=), 121.1 (benzimidazole, CH=), 135.9 (benzimidazole, C), 140.9 (N-C=N), 139.4 (phenyl, C), 134.6 (phenyl, Cl), 127.8, 128.8, 130.1 (phenyl, CH=); Anal. Calcd. for $C_{13}H_9ClN_2$: C, 68.21; H, 3.93; N, 12.24 %. Found: C, 68.14; H, 3.87; N, 12.15%; MS, *m/z*: 228 $[M]^+$.

2-*p*-Tolyl-1*H*-benzimidazole (9a):

(**9a:** White to beige-grey powder, m.p. 268-270 °C, 84.9% yield)

IR (KBr, cm^{-1}): 3392, 2919, 1616, 1515, 1384, 1274, 821, 747; ^1H NMR ($CDCl_3$, δ/ppm): 8.00-8.08 (m, 2H), 7.20–7.60 (m, 6H), 2.60 (m, 3H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 21.3 (CH_3), 112.4 (benzimidazole, CH=), 119.9 (benzimidazole, CH=), 138.3 (benzimidazole, C), 145.1 (N-C=N), 138.9 (phenyl, Me), 134.7 (phenyl, C), 128.8, 130.1 (phenyl, CH=); Anal. Calcd. for $C_{14}H_{12}N_2$: C, 80.77; H, 5.77; N, 13.46 %. Found: C, 80.65; H, 5.59; N, 13.37%; MS, *m/z*: 209 $[M+H]^+$.

General procedure for the preparation of the compounds (1b-9b):

A solution of 2-Chloro-4-nitrobenzoyl chloride (2.2 g, 0.01 mol) in acetone (2.5 ml) was drop wise added to the solution (25 ml) of benzimidazoles (**1a-9a**) (0.01 mol) in NaOH (1g). The mixture was stirred and heated under reflux for 2 h. After completion, the reaction was slowly cooled, water added and the solid compound obtained, filtered, dried and re-crystallized from THF (Scheme 1) [30].

Benzimidazole-1-yl-(2-chloro-4-nitrophenyl)-methanone (1b):

(**1b**: Yellowish powder, m.p. 137-142 °C, 86.3% yield)

IR (KBr, cm^{-1}): 3068, 2973, 1731, 1571, 1501, 1285, 684; ^1H-NMR ($CDCl_3$, δ/ppm): 7.40-7.75 (m, 4H, benzimidazole), 8.01(s, 1H), 7.95-8.21 (m,3H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.9-135.2 (benzimidazole, C), 120.6-151.9 (Phenyl, C), 145.1 (N-C=N), 187.6 (C=O). Anal. Calcd. for $C_{14}H_8ClN_3O_3$: C, 55.74; H, 2.67; N, 13.93 %. Found: C, 55.68; H, 2.60; N, 13.85 %. MS, *m/z*: 301$[M]^+$.

(2-Chloro-4-nitrophenyl)-(2-methyl-benzimidazole-1-yl)-methanone (2b):

(**2b**: Pale cream powder, m.p. 145-147 °C, 88.9% yield)

IR (KBr, cm^{-1}): 3059, 2963, 1745, 1580, 1483, 1270,825, 684; ^1H-NMR ($CDCl_3$, δ/ppm): 2.52 (s,3H), 7.38-7.80 (m, 4H, benzimidazole), 7.95-8.21 (m,3H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 17.9 (CH_3), 116.1-136.2 (benzimidazole, C), 121.6-154.3 (Phenyl, C), 144.3 (N-C=N), 191.1 (C=O). Anal. Calcd. for $C_{15}H_{10}ClN_3O_3$: C, 57.06; H, 3.19; N, 13.31 %. Found: C, 57.01; H, 3.05; N, 13.22 %. MS, *m/z*: 315$[M]^+$.

(2-Chloro-4-nitrophenyl)-(2-phenyl-benzimidazole-1-yl)-methanone (3b):

(**3b**: Cream powder, m.p. 155-157 °C, 90.7% yield)

IR (KBr, cm^{-1}): 3033, 2955, 1756, 1570, 1496, 1263,840, 675; ^1H-NMR ($CDCl_3$, δ/ppm): 7.30-7.75 (m, 4H, benzimidazole), 7.45-8.52 (m,8H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 113.1-135.7 (benzimidazole, C), 124.5-133.4 (Phenyl, C), 146.7 (N-C=N), 190.8 (C=O). Anal. Calcd. for $C_{20}H_{12}ClN_3O_3$: C, 63.59; H, 3.20; N, 11.12 %. Found: C, 63.51; H, 3.16; N, 11.02 %. MS, *m/z*: 377$[M]^+$.

(2-Chloro-4-nitrophenyl)-[2-(4-chlorophenyl)-benzimidazole-1-yl]-methanone (4b):

(**4b**: Yellow powder, m.p. 160-162 °C, 93.1% yield)

IR (KBr, cm^{-1}): 3044, 2966, 1766, 1578, 1501, 1272,851, 669; ^1H-NMR ($CDCl_3$, δ/ppm): 7.32-7.80 (m, 4H, benzimidazole), 7.51-8.48 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.4-136.9

(benzimidazole, C), 130.5-157.4 (Phenyl, C), 142.5 (N-C=N), 191.3 (C=O). Anal. Calcd. for $C_{20}H_{11}Cl_2N_3O_3$: C, 58.27; H, 2.69; N, 10.19 %. Found: C, 58.19; H, 2.60; N, 10.10 %. MS, *m/z*: 411$[M]^+$.

(2-Chloro-4-nitrophenyl)-[2-(4-hydroxyphenyl)-benzimidazole-1-yl]-methanone (5b):

(**5b**: White-grey powder, m.p. 175-178 °C, 88.1% yield)

IR (KBr, cm^{-1}): 3430, 3015, 2940, 1750, 1540, 1478, 1233,902, 701; ^{1}H-NMR ($CDCl_3$, δ/ppm): 5.44 (s, 1H, OH), 7.30-7.75 (m, 4H, benzimidazole), 7.44-8.56 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.9-137.3 (benzimidazole, C), 130.5-148.4 (Phenyl, C), 160.3 (Phenyl, C-OH), 147.5 (N-C=N), 195.3 (C=O). Anal. Calcd. for $C_{20}H_{12}ClN_3O_4$: C, 61.01; H, 3.08; N, 10.67 %. Found: C, 60.92; H, 3.01; N, 10.58 %. MS, *m/z*: 393$[M]^+$.

(2-Chloro-4-nitrophenyl)-[2-(4-nitrophenyl)-benzimidazole-1-yl]-methanone (6b):

(**6b**: White-grey powder, m.p. 175-178 °C, 88.1% yield)

IR (KBr, cm^{-1}): 3022, 2970, 1801, 1555, 1465, 1238,889, 707; ^{1}H-NMR ($CDCl_3$, δ/ppm): 7.25-7.75 (m, 4H, benzimidazole), 7.90-8.45 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.8-138.8 (benzimidazole, C), 129.8-143.4 (Phenyl, C), 154.3 (Phenyl, C-NO_2), 145.5 (N-C=N), 194.4 (C=O). Anal. Calcd. for $C_{20}H_{11}ClN_4O_5$: C, 56.82; H, 2.62; N, 13.25 %. Found: C, 56.75; H, 2.53; N, 13.11 %. MS, *m/z*: 422$[M]^+$.

(2-Chloro-4-nitrophenyl)-[2-(4-methoxyphenyl)-benzimidazole-1-yl]-methanone (7b):

(**7b**: White-cream powder, m.p. 163-167 °C, 90.8% yield)

IR (KBr, cm^{-1}): 3053, 2975, 1781, 1563, 1488, 1247,1115, 877, 705; ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.69 (s, 3H, CH_3), 7.33-7.75 (m, 4H, benzimidazole), 7.01-8.22 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm):59.6 (CH_3) 115.8-137.6 (benzimidazole, C), 114.8-140.4 (Phenyl, C), 167.3 (Phenyl, O-CH_3), 144.1 (N-C=N), 192.2 (C=O). Anal. Calcd. for $C_{21}H_{14}ClN_3O_4$: C, 61.85; H, 3.46; N, 10.30 %. Found: C, 61.79; H, 3.40; N, 10.22 %. MS, *m/z*: 407$[M]^+$.

(2-Chloro-4-nitrophenyl)-[2-(2-chlorophenyl)-benzimidazole-1-yl]-methanone (8b):

(**8b**: Pale yellow powder, m.p. 160-162 °C, 93.1% yield)

IR (KBr, cm^{-1}): 3044, 2966, 1766, 1578, 1501, 1272,851, 669; ^{1}H-NMR ($CDCl_3$, δ/ppm): 7.28-7.85 (m, 4H, benzimidazole), 7.19-8.40 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 113.8-137.9 (benzimidazole, C), 131.5-149.4 (Phenyl, C), 146.3 (N-C=N), 190.9 (C=O). Anal. Calcd. for $C_{20}H_{11}Cl_2N_3O_3$: C, 58.27; H, 2.69; N, 10.19 %. Found: C, 58.22; H, 2.63; N, 10.12 %. MS, *m/z*: 413$[M+2H]^+$.

(2-Chloro-4-nitrophenyl)-(2-*p*-tolyl-benzimidazole-1-yl)-methanone (9b):

(**9b**: White powder, m.p. 170-172 °C, 88.3% yield)

IR (KBr, cm^{-1}): 3044, 2983, 1786, 1575, 1492, 1265,1133, 881, 693; ^{1}H-NMR ($CDCl_3$, δ/ppm): 2.83 (s, 3H, CH_3), 7.25-7.80 (m, 4H, benzimidazole), 7.10-8.30 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm):30.6 (CH_3) 114.4-135.8 (benzimidazole, C), 118.7-142.5 (Phenyl, C), 142.1 (N-C=N), 193.1 (C=O). Anal. Calcd. for $C_{21}H_{14}ClN_3O_3$: C, 64.37; H, 3.60; N, 9.05 %. Found: C, 64.28; H, 3.52; N, 8.95 %. MS, *m/z*: 391$[M]^+$.

General procedure for the preparation of the compounds (1c-9c):

Reduction of 2-substituted-1H-benzoyl imidazol-1-yl (2-Chloro-4-nitrophenyl) methanone

Synthesized products (**1b-9b**) (0.1 mol), tin powder (30 g, 0.25 mol) and conc. HCl solution (15 ml) were refluxed for 1 h. The reaction mixture was then cooled; added water and ammonia solution, heated on water bath for 30 min, filtered and wash with hot water for obtaining the liquid compound, acidified with glacial acetic acid, evaporated, filtered and dried (Scheme 1) [30].

(4-Amino-2-chloro-phenyl)-(benzimidazol-1-yl)-methanone (1c):

(**1c**: Pale brown powder, m.p. 270-272 °C, 79.3% yield)

IR (KBr, cm^{-1}): 3304,3298, 2956, 2983, 1645, 1601, 1482, 1275, 890, 705; ^{1}H-NMR (DMSO-d_6, δ/ppm): 4.40 (br s, 2H, NH_2), 7.05-7.60 (m, 4H, benzimidazole), 6.69-7.68 (m, 3H, Ar-H), 8.01 (s,1H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 115.4-131.98 (benzimidazole, C), 123.7-140.8 (Phenyl, C), 143.8 (N-C=N),166.3 (Phenyl, C-NH_2), 189.1 (C=O). Anal. Calcd. for $C_{14}H_{10}ClN_3O$: C, 61.89; H, 3.71; N, 15.47 %. Found: C, 61.78; H, 3.66; N, 15.39 %. MS, *m/z*: 271$[M]^+$.

(4-Amino-2-chloro-phenyl)-(2-methyl-benzimidazol-1-yl)-methanone (2c):

(**2c**: Brown powder, m.p. 282-285 °C, 76.8% yield)

IR (KBr, cm^{-1}): 3245,3198, 2920, 2893, 1678, 1586, 1469, 1315, 907, 659; ^{1}H-NMR (DMSO-d_6, δ/ppm): 2.11 (s, 3H, CH_3), 5.20 (br s, 2H, NH_2), 7.12-7.65 (m, 4H, benzimidazole), 6.45-7.68 (m,

3H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 12.8 (CH_3), 116.4-132.8 (benzimidazole, C), 126.7-142.8 (Phenyl, C), 146.2 (N-C=N),159.8 (Phenyl, C-NH_2), 190.8 (C=O). Anal. Calcd. for $C_{15}H_{12}ClN_3O$: C, 63.05; H, 4.23; N, 14.71 %. Found: C, 62.92; H, 4.06; N, 14.62 %. MS, *m/z*: 285$[M]^+$.

(4-Amino-2-chloro-phenyl)-(2-phenyl-benzimidazol-1-yl)-methanone (3c):

(**3c**: Pale brown powder, m.p. 298-301 °C, 75.6% yield)

IR (KBr, cm^{-1}): 3225, 3075,3171, 2938, 2893, 1671, 1595, 1455, 1323, 875, 708; ^{1}H-NMR (DMSO-d_6, δ/ppm): 5.10 (br s, 2H, NH_2), 7.18-7.85 (m, 4H, benzimidazole), 6.95-7.55 (m, 8H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.9-129.5 (benzimidazole, C), 122.7-146.8 (Phenyl, C), 142.2 (N-C=N),156.5 (Phenyl, C-NH_2), 188.1 (C=O). Anal. Calcd. for $C_{20}H_{14}ClN_3O$: C, 69.07; H, 4.06; N, 12.08 %. Found: C, 68.92; H, 3.98; N, 11.95 %. MS, *m/z*: 347$[M]^+$.

(4-Amino-2-chloro-phenyl)-[2-(4-chloro-phenyl)-benzimidazol-1-yl]-methanone (4c):

(**4c**: Pale yellow powder, m.p. 288-291 °C, 74.7% yield)

IR (KBr, cm^{-1}): 3346, 3267,3028, 2946, 2812, 1680, 1611, 1501, 1312, 885, 720; ^{1}H-NMR (DMSO-d_6, δ/ppm): 4.90 (br s, 2H, NH_2), 7.22-7.75 (m, 4H, benzimidazole), 6.85-7.75 (m, 7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 113.1-128.6 (benzimidazole, C), 125.7-139.8 (Phenyl, C), 145.2 (N-C=N),160.5 (Phenyl, C-NH_2), 189.4 (C=O). Anal. Calcd. for $C_{20}H_{13}Cl_2N_3O$: C, 62.84; H, 3.43; N, 10.99 %. Found: C, 62.78; H, 3.38; N, 10.91 %. MS, *m/z*: 383$[M+2]^+$.

(4-Amino-2-chloro-phenyl)-[2-(4-hydroxy-phenyl)-benzimidazol-1-yl]-methanone (5c):

(**5c**: Pale orange powder, m.p. 302-304 °C, 71.9% yield)

IR (KBr, cm^{-1}): 3448, 3367,3128, 2956, 2832, 1690, 1641, 1511, 1332, 890, 672; ^{1}H-NMR (DMSO-d_6, δ/ppm): 4.95 (br s, 2H, NH_2), 7.12-7.65 (m, 4H, benzimidazole), 6.65-7.85 (m, 7H, Ar-H) 11.95 (br s,1H,OH) ; ^{13}C-NMR ($CDCl_3$, δ/ppm): 114.1-129.8 (benzimidazole, C), 128.7-142.8(Phenyl, C), 141.9 (N-C=N),161.1 (Phenyl, C-NH_2),164.2 (Phenyl, C-OH) 181.4 (C=O). Anal. Calcd. for $C_{20}H_{14}ClN_3O_2$: C, 66.03; H, 3.88; N, 11.55 %. Found: C, 65.95; H, 3.82; N, 11.49 %. MS, *m/z*: 363$[M]^+$.

(4-Amino-2-chloro-phenyl)-[2-(4-nitro-phenyl)-benzimidazol-1-yl]-methanone (6c):

(**6c**: Orange powder, m.p. 300-302 °C, 72.5% yield)

IR (KBr, cm^{-1}): 3342,3136, 2948, 2844, 1685, 1652, 1493, 1312, 897, 721; ^{1}H-NMR (DMSO-d_6, δ/ppm): 4.60 (br s, 2H, NH_2), 7.13-7.80 (m, 4H, benzimidazole), 6.51-8.35 (m, 7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 115.3-134.6 (benzimidazole, C), 126.3-143.5(Phenyl, C), 143.6 (N-C=N), 164.3 (Phenyl, C-NH_2),178.4 (C=O). Anal. Calcd. for $C_{20}H_{13}ClN_4O_3$: C, 61.16; H, 3.34; N, 14.26 %. Found: C, 61.10; H, 3.28; N, 14.19 %. MS, *m/z*: 392$[M]^+$.

(4-Amino-2-chloro-phenyl)-[2-(4-methoxy-phenyl)-benzimidazol-1-yl]-methanone (7c):

(**7c**: Cream powder, m.p. 283-286 °C, 75.8% yield)

IR (KBr, cm^{-1}): 3203, 2948, 1745, 1602, 1542, 1321,1175, 802, 715; ^{1}H-NMR ($CDCl_3$, δ/ppm): 2.29 (s, 3H, CH_3), 5.34 (br s, 2H, NH_2), 7.18-7.65 (m, 4H, benzimidazole), 6.53-7.42 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm):54.7 (CH_3) 116.8-138.1 (benzimidazole, C), 122.8-148.4 (Phenyl, C), 147.1 (N-C=N), 169.5 (Phenyl, O-CH_3), 179.2 (C=O). Anal. Calcd. for $C_{21}H_{16}ClN_3O_2$: C, 66.76; H, 4.27; N, 11.12 %. Found: C, 66.69; H, 4.20; N, 11.02 %. MS, *m/z*: 377$[M]^+$.

(4-Amino-2-chloro-phenyl)-[2-(2-chloro-phenyl)-benzimidazol-1-yl]-methanone (8c):

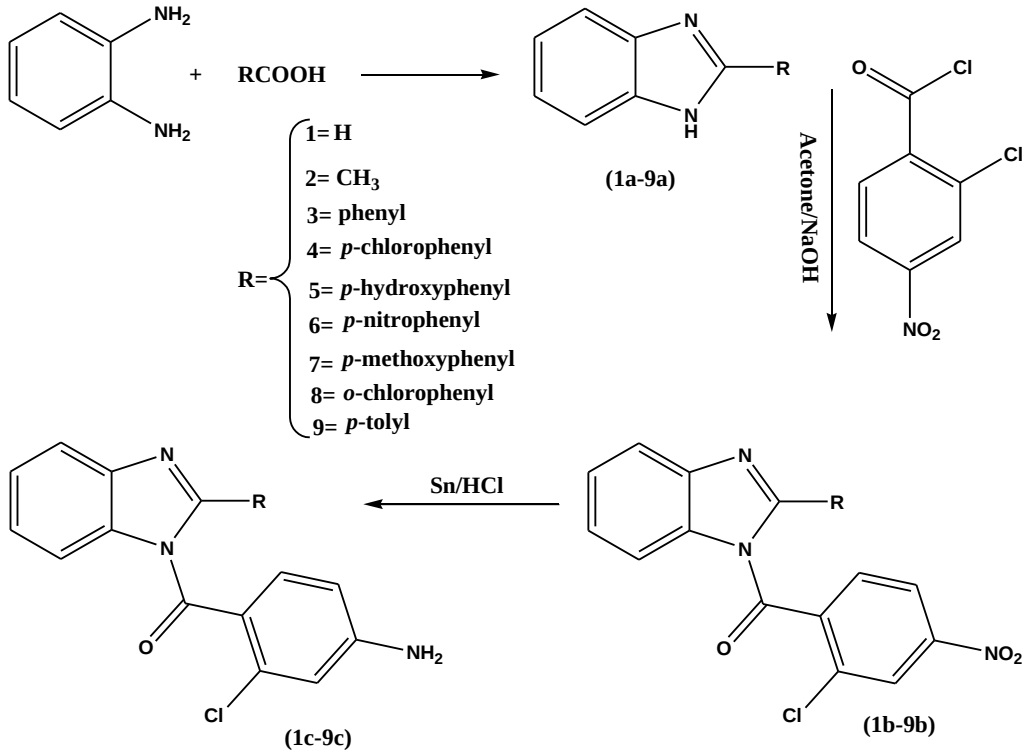
(**8c**: Yellow powder, m.p. 275-278 °C, 73.4% yield)

IR (KBr, cm^{-1}): 3332, 3222, 2931, 2825, 1692, 1601, 1495, 1344, 875, 707; ^{1}H-NMR (DMSO-d_6, δ/ppm): 4.93 (br s, 2H, NH_2), 7.23-7.78 (m, 4H, benzimidazole), 6.88-7.83 (m, 7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 113.5-128.2 (benzimidazole, C), 125.1-139.3 (Phenyl, C), 144.8 (N-C=N),162.5 (Phenyl, C-NH_2), 180.1 (C=O). Anal. Calcd. for $C_{20}H_{13}Cl_2N_3O$: C, 62.84; H, 3.43; N, 10.99 %. Found: C, 62.76; H, 3.36; N, 10.90 %. MS, *m/z*: 383$[M+2]^+$.

(4-Amino-2-chloro-phenyl)-(2-p-tolyl-benzimidazol-1-yl)-methanone (9c):

(**9c**: White-grey powder, m.p. 268-272 °C, 74.8% yield)

IR (KBr, cm^{-1}): 3033, 2975, 1778, 1582, 1465, 1287,1167, 892, 722; ^{1}H-NMR ($CDCl_3$, δ/ppm): 2.78 (s, 3H, CH_3), 5.22 (br s, 2H, NH_2), 7.20-7.85 (m, 4H, benzimidazole), 7.14-8.20 (m,7H, Ar-H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 32.1 (CH_3), 115.8-136.5 (benzimidazole, C), 120.1-145.5 (Phenyl, C), 144.1 (N-C=N), 189.8 (C=O). Anal. Calcd. for $C_{21}H_{16}ClN_3O$: C, 69.71; H, 4.46; N, 11.61 %. Found: C, 69.66; H, 4.39; N, 11.55 %. MS, *m/z*: 361$[M]^+$.



Scheme 1. Schematic synthesis of intermediates (1a-9a) and new compounds (1b-9b and 1c-9c).

In Vitro Biological Evaluation Test

The antibacterial and antifungal screening of newly synthesized compounds (**1c-9c**) were tested by filter paper disc method. The antibacterial activity of test compounds were evaluated against Gram-positive bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli*. Antifungal activity was screened against fungal strain, *Candida albicans*. The antimicrobial activity was performed at concentrations 50 & 100 µg/ml. Mueller hinton agar (MHA) & Potato dextrose agar (PDA) were employed as culture medium and DMSO was used as solvent control for antimicrobial activity. Ciprofloxacin and Amphotericin B were used as standard for antibacterial and antifungal activities respectively. The potato dextrose agar (PDA) media dissolved in distilled water then was sterilized by autoclaving at 121^{o}C for 20 min at the appropriate pressure. The media removed and cooled at 40-45 ^{o}C. Whatman filter paper-1 discs (6 mm) were sterilized by dry heat were saturated with test solution and placed on (PDA) media in Petri dishes in triplicate. The Petri dishes were covered and set aside for an hour, and then incubated at 37 ^{o}C for 48 hrs. After incubation, the zones of inhibition around the disc were observed. Results were interpreted in terms of diameter (mm) of zone of inhibition. The zones of inhibition were measured and the average of three readings was calculated [32-34].

RESULTS AND DISCUSSIONS

Chemistry

In continuation of our interest to investigate of new pharmaceutical potential compounds, the syntheses of biologically active 2-substituted benzimidazole derivatives were carried out in this study. To materialize the proposed project, initially, some intermediates were synthesized (**1a-9a**) in good yields by coupling of *o*-phenylenediamine dihydrochloride with some carboxylic acids such as formic, acetic, benzoic, 4-chloro-benzoic, 4-hydroxy-benzoic, 4-nitro-benzoic, 4-methoxy-benzoic, 2-chloro-benzoic and 4-methyl-benzoic respectively based on a known method with some modification for increasing yields. Benzoylation of the 2-substituted benzimidazoles were applied in presence of 2-Chloro-4-nitrobenzoyl chloride yield 2-substituted-1*H*-benzoyl imidazol-1-yl (2-Chloro-4-nitrophenyl) methanones (**1b-9b)**. Reductions of these compounds by tin powder produce the final products (**1c-9c**). TLC was used for monitoring the progress of the reaction and the structures of new compounds were assessed by interpretation of obtained spectra (IR, ^{1}H-NMR,^{13}C-NMR, Mass Spectra and CHN analysis).

Biological Evaluation

For evaluation of the biological activities, the synthesized compounds (**1c-9c)** were screened for their in vitro antibacterial activity against

Table1. Zone of inhibition (mm±S.D) of test sample and Standard Drug (Ciprofloxacin and Amphotericin B) against *S. Aureus, E. coli* and *C. albicans*

Test samples	Diameter of zone of inhibition in mm [mean ± SD (n=3)]					
	S. Aureus		*E.coli*		*C. albicans*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1c	9.52±0.42	12.281±0.42	8.42±0.50	11.60±0.32	N/A	N/A
2c	9.90±1.30	12.80±0.30	8.15±0.50	11.22±0.60	N/A	N/A
3c	9.70±0.75	12.95±0.20	8.10±0.64	11.25±0.15	N/A	N/A
4c	9.75±0.44	12.96±0.40	8.40±0.25	11.86±0.36	N/A	N/A
5c	9.58±0.71	12.64±1.30	8.86±1.11	11.10±0.46	N/A	N/A
6c	9.32±0.36	12.88±0.32	8.88±0.78	11.66±0.35	N/A	N/A
7c	9.85±0.66	13.10±0.68	8.76±0.38	11.20±0.45	N/A	N/A
8c	9.45±0.60	13.25±0.20	8.26±0.30	11.20±0.25	N/A	N/A
9c	9.35±0.60	13.15±0.20	8.46±0.40	11.30±0.40	N/A	N/A
Ciprofloxacin	14.36±0.36	19.24±0.35	11.36±0.44	16.45±0.28	-	-
Amphotericin B	-	-	-	-	9.35±0.24	14.56±0.45

"N/A" = Not Active, " – " = Not Applicable

Staphylococcus aureus and *Escherichia coli* and antifungal activity against *Candida albicans* by measuring the zone of inhibition in mm in comparison with those of the standard drugs Ciprofloxacin and Amphotericin B. The preliminary screening results for the compounds (**1c-9c)** established that the newly synthesized compounds have not shown antifungal activity against *Candida albicans*. But the antibacterial activity data reveals that the compounds (**1c-9c)** exhibited good antibacterial activity against various strains of bacteria as compared to standard Ciprofloxacin. The results are tabulated in Table 1.

CONCLUSION

A series of some novel 2-Substituted benzimidazole derivatives were synthesized and evaluated for their potential antimicrobial and antifungal activities. Based on results, it can be concluded that all the target synthesized compounds (**1c-9c**) showed good to moderate antimicrobial activities. The results indicated that new antimicrobial compounds could be prepared by changing of different substrates on various benzimidazole derivatives. Although further pharmacological activities of these new compounds could be evaluated in future studies.

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СИНТЕЗА, ХАРАКТЕРИСТИКИ И БИОЛОГИЧНА ОЦЕНКА НА НЯКОИ НОВИ ПРОИЗВОДНИ НА БЕНЗИМИДАЗОЛА

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(Резюме)

Бензимидазоловото ядро има важно значение в медицинската химия, а много съединения, съдържащи бензимидазолово ядро проявяват важни биологично активни свойства. В настоящата работа се изследва синтезата, спектралните свойства и се оценява биологичната активност на девет нови произвдодни на бензимидазола. Структурите на синтезираните съединения са охарактеризирани чрез ИЧ-спектроскопия, ^{1}H-NMR, ^{13}C-NMR, мас-спектроскопия и CHN – елементен анализ. Целевите нови съединения (**1c-9c**) са скринирани за антибактериална активност срещу щамове на *Escherichia coli и Staphylococcus aureus,* антигъбичната активност срещу *Candida albicans*.