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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NOVEL SULFAMOYL AND TRIAZOLE DERIVATIVES INCORPORATING A 4-BENZOIMIDAZOL-2-YL MOIETY

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Abstract

In this study 15 novel benzoimidazole compounds were synthesized in order to investigate their possible antibacterial and antifungal activity. Two different Gram-positive and two different Gram-negative bacterial strains were used in antibacterial activity tests. Antifungal activity tests were also performed against two different fungal strains. Most of the test compounds found to be significantly effective against Gram-positive, Gram-negative bacterial strains and antifungal strains.

Key words: Sulfamoyl, Triazole, 4-benzoimidazol-2-yl, Antimicrobial Activity.

Introduction

Benzimidazoles are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms [1-8]. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B_{12} [9]. This ring system is present in numerous antioxidant [10], antiparasitic [11], antiproliferative [12], anti-HIV [13], anticonvulsant [14], anti-inflammatory [15], antihypertensive [16], antineoplastic [17] and antitrichinellosis [18] compounds. Owing to the immense importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities. Looking at the antimicrobial importance of benzimidazole, it was thought that it would be worthwhile to design,

synthesize some new benzimidazole derivatives bearing different functional groups and screen them for potential antibacterial and antifungal activities.

Materials and Methods

Chemistry

Melting points (˚C) were determined in open capillary tubes using silicon oil on Gallen Kamp apparatus (Ultraporter Company, Walsall, United Kingdom). ^{1}H-NMR Spectra were measured in DMSO-d_6 on JEOL-270 MHz Spectrometer (JEOL, Canada) with tetramethylsilane as an internal standard. Mass Spectra were obtained with a Schimadzu GCS-QP1000EX Spectrometer (Schimadzu Scientific Instruments, Italy) at 70 ev. The IR Spectra were recorded with a Philips Infra cord Spectrophotometer Model PU 9712 (PerkinElmer, 940 Winter Street, Waltham, Masssachusetts 02451, USA) in KBr discs.

Elemental analysis was performed at the Micro analytical Laboratory of the National Research Center. All the reactions were monitored by thin layer chromatography (TLC) on silica gel with chloroform as mobile phase. The antimicrobial activity of the synthesized compounds was carried out at the National Research Center, Cairo, Egypt.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzonitrile (2):

4-Cyanobenzaldehye (0.6g, 0.21 mol) and (3,4-diaminophenyl)phenyl-methanone (**1**) (1g, 0.21 mol) were dissolved in EtOH. This mixture was heated under reflux for 5h and cooled to room temperature. Then, water was added slowly to the mixture with stirring. The suspension was maintained at -5˚C overnight. The product was washed repeatedly with ethanol-water (1:1) mixture and then recrystallized from acetone.

Yellowish white solid; yield = 1.3g (86%), mp 250-252˚C; IR (cm^{-1}) 3340 (NH), 2225 (CN), 1646 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 5.92 (s, 1H, N<u>H</u>, exchangeable with D_2O), 7.48-8.22 (m, 12H, Ar-H); MS *m/z* 323 (M^+, 100). Anal. Calcd for $C_{21}H_{13}N_3O$ (323.4): C, 78.0; H, 4.0; N, 13.0. Found: C, 78.0; H, 4.1; N, 13.0.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzoic acid (3):

A mixture of **2** (1g, 0.01 mol) and 30 mL 70% sulfuric acid was stirred in 100 ml three-necked flask at 140˚C for 5h, then suspended in 150 mL water and the resulting precipitate was filtered off. Recrystallization from diluted EtOH afforded yellow crystals. Yellowish white solid; yield = 0.95g (90%); mp 280-283˚C; IR (cm^{-1}) 3746 (NH), 3348 (OH), 1701

(CO), 1650 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 5.92 (s, 1H, NH, exchangeable with D_2O), 7.48-8.22 (m, 12H, Ar-H), 10.92 (s, 1H, OH, exchangeable with D_2O); MS *m/z* 342 (M^+, 60). Anal. Calcd for $C_{21}H_{14}N_2O_3$ (342.4): C, 73.7; H, 4.1; N, 8.2. Found: C, 73.7; H, 4.2; N, 8.2.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzoic acid ethyl ester (4):

To a solution of compound **3** (1g; 0.073 mol) in absolute EtOH, few drops of concentrated sulfuric acid were added and the mixture was refluxed for 4h. The crude product was filtered, air-dried and crystallized from EtOH. Yellow solid; yield = 0.9g (83%); mp 107-109˚C; IR (cm^{-1}) 3746 (NH), 3348 (OH), 1717 (CO), 1661 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.32 (t, *J* = 7.1 Hz, 3H, CH_3), 4.32 (q, 2H, CH_2), 5.92 (s, 1H, NH, exchangeable with D_2O), 7.48-8.22 (m, 12H, Ar-H); MS *m/z* 370 (M^+, 72). Anal. Calcd for $C_{23}H_{18}N_2O_3$ (370.4): C, 74.6; H, 4.9; N, 7.6. Found: C, 74.5; H, 4.9; N, 7.7.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzoic acid hydrazide (5):

To a solution of ester compound **4** (1g; 0.033 mol) in EtOH, hydrazine hydrate (98%; 2 mL) was added and heated for 5h on a water-bath. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from ethanol. Yellow solid; yield = 0.8g (83%); mp 73-78˚C; IR (cm^{-1}) 3736 (NH), 3187 (NH_2), 1705 (CO), 1684 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.96 (s, 2H, NH_2, exchangeable with D_2O), 5.92 (s, 1H, NH, exchangeable with D_2O), 7.48-8.22 (m, 12H, Ar-H), 10.45 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 356 (M^+, 49). Anal. Calcd for $C_{21}H_{16}N_4O_2$ (356.4): C, 70.8; H, 4.5; N, 15.7. Found: C, 70.7; H, 4.4; N, 15.8.

General procedure for the preparation of (un)substituted-benzoic acid N'-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoyl] hydrazide (6a-c):

Compound **5** (1g, 0.016 mol) was dissolved in dry acetone (50 mL). Triethylamine (2.5 mL) was added to this solution. Then a solution of benzoyl chloride, 4-methylbenzoyl chloride and/or 4-nitrobenzoyl chloride (0.016 mol) in dry acetone was added and the mixture was stirred for 2h at room temperature. The solid formed was filtered off and the solvent was removed from the clear solution under reduced pressure. The crude product was purified by crystallization from DMF.

Benzoic acid N'-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoyl] hydrazide (6a):

Yellow solid; yield = 1.2g (93%); mp 80-84˚C; IR (cm^{-1}) 3736 (NH), 3420 (NH), 3187 (NH), 1705 (CO), 1684 (CO), 1678 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.24 (s, 1H, NH, exchangeable with D_2O), 7.28-7.85 (m, 17H, Ar-H), 8.78

(s, 2H, 2NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6) δ 122.4-132.7 (Ar-17CH), 131.2 (C-CO), 138.2 (C-N), 134.7 (C=C), 137.0 (C-CO), 139.0 (C-CO), 142.1 (C-NH), 153.9 (C=N), 165.7 (C=C), 164.2 (2C=O) and 195.9 (C=O); MS *m/z* 460 (M^+, 27). Anal. Calcd for $C_{28}H_{20}N_4O_3$ (460.5): C, 73.0; H, 4.4; N, 12.2. Found: C, 73.0; H, 4.5; N, 12.2.

4-Methylbenzoic acid N'-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoyl] hydrazide (6b):

Yellow solid; yield = 1.3g (98%); mp 92-94˚C; IR (cm^{-1}) 3736 (NH), 3420 (NH), 3187 (NH), 1705 (CO), 1684 (CO), 1678 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.51 (s, 3H, CH_3), 4.24 (s, 1H, NH, exchangeable with D_2O), 7.28-7.85 (m, 16H, Ar-H), 8.78 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z* 474 (M^+, 29). Anal. Calcd for $C_{29}H_{22}N_4O_3$ (474.5): C, 73.4; H, 4.7; N, 11.8; Fd.: C, 73.3; H, 4.7; N, 11.9.

4-Nitro-benzoic acid N'-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoyl] hydrazide (6c):

Orange solid; yield = 1.3g (92%); mp 52-54˚C; IR (cm^{-1}) 3736 (NH), 3420 (NH), 3187 (NH), 1705 (CO), 1684 (CO), 1678 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.24 (s, 1H, NH, exchangeable with D_2O), 7.28-7.85 (m, 16H, Ar-H), 8.78 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z* 505 (M^+, 61). Anal. Calcd for $C_{28}H_{19}N_5O_5$ (505.5): C, 66.5; H, 3.8; N, 13.9. Found: C, 66.5; H, 3.9; N, 13.9.

General procedure for the preparation of 4-(5-benzoyl-1*H*-benzoimidazol-2-yl)-N-substituted-benzamide (7a-c):

Compound **5** (1g, 0.016 mol) was dissolved in dry acetone (50 mL). Triethylamine (2.5 mL) was added to this solution. Then a solution of benzene sulfonyl chloride, toluene sulfonyl chloride and/or camphor-10-sulfonyl chloride (0.016 mol) in dry acetone was added and the mixture was stirred for 8h at room temperature.

The solid formed was filtered off and the solvent was removed from the clear solution under reduced pressure. Purification of the product was carried out by preparative thin-layer chromatography using ethyl acetate as eluent. The products were recrystallized from EtOH.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl)-N-(sulfamoyl phenyl) benzamide (7a):

Yellowish white solid; yield = 1.3g (94%); mp 230-234˚C; IR (cm^{-1}) 3536 (NH), 3420 (NH), 3187 (NH), 1684 (CO), 1668 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.24 (s, 1H, NH, exchangeable with D_2O), 4.78 (s, 1H, NH, exchangeable with D_2O), 7.28-7.85 (m, 17H, Ar-H), 8.78 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 496 (M^+, 37). Anal. Calcd for $C_{27}H_{20}N_4O_4S$ (496.5): C, 65.3; H, 4.1; N, 11.3; S, 6.5. Found: C, 65.3; H, 4.1; N, 11.3; S, 6.5.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl)-N-(4-methylsulfamoyl phenyl) benzamide (7b):

Brown Solid; yield = 1.3g (91%); mp 215-218˚C; IR (cm^{-1}) 3536 (NH), 3420 (NH), 3187 (NH), 1684 (CO), 1668 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.24 (s, 1H, NH, exchangeable with D_2O), 2.35 (s, 3H, CH_3), 4.78 (s, 1H, NH, exchangeable with D_2O), 7.28-7.85 (m, 16H, Ar-H), 8.78 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 510 (M^+, 32). Anal. Calcd for $C_{28}H_{22}N_4O_4S$ (510.5): C, 65.9; H, 4.3; N, 11.0; S, 6.3. Found: C, 65.9; H, 4.4; N, 11.0; S, 6.3.

4-(5-Benzoyl-1*H*-benzoimidazol-2-yl)-N-(sulfonyl-methyl-7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-yl)benzamide (7c):

Brown Solid; yield = 1.5g (94%); mp 282-285˚C; IR (cm^{-1}) 3536 (NH), 3420 (NH), 3187 (NH), 1711 (CO), 1684 (CO), 1668 (CO), 1377 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.24 (s, 3H, H_h), 1.27 (s, 3H, H_g), 1.75-2.87 (m, 7H, $H_{c\text{-}f}$), 2.24 (s, 1H, NH, exchangeable with D_2O), 3.22 (d, *J* = 6.8 Hz, 1H, H_a), 3.31 (d, *J* = 6.8 Hz, 1H, H_b), 4.41 (s, 1H, NH, exchangeable with D_2O), 7.47-8.16 (m, 12H, Ar-H), 8.78 (s, 1H, NH, exchangeable with D_2O) as shown in figure **1**; MS *m/z* 570 (M^+, 48). Anal. Calcd for $C_{31}H_{30}N_4O_5S$ (570.6): C, 65.3; H, 5.3; N, 9.8; S, 5.6. Found: C, 65.4; H, 5.4; N, 9.7; S, 5.6.

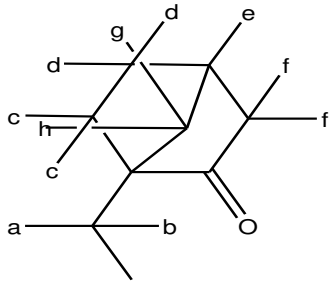


Figure 1

General procedure for the preparation of [4-(5-benzoyl-1*H*-benzoimidazol-2-yl)benzoyl]-4-(4-chlorophenyl) semi carbazide /4-phenylthiosemicarbazide (8a,b):

A mixture of **5** (1g, 0.01 mol) and 4-chloroarylisocyanate or arylisothiocyanate (0.01 mol) in dry benzene was heated under reflux for 6h. The solid material obtained on cooling was filtered off and recrystallized from MeOH.

[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzoyl]-4-(4-chlorophenyl) semicarbazide (8a):

Brown solid; yield = 1.3g (91%); mp 260-263˚C; IR (cm^{-1}) 3496 (NH), 3420 (NH), 3320 (NH), 3187 (NH), 1711 (CO), 1684 (CO), 1668 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.78 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H,

Ar-H), 8.91 (s, 1H, NH, exchangeable with D_2O), 9.02 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z* 511 (M^+, 3.3), 509 (M^+, 10). Anal. Calcd for $C_{28}H_{20}ClN_5O_3$ (509.9): C, 65.9; H, 4.0; N, 13.7. Found: C, 66.0; H, 4.0; N, 13.6.

[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) benzoyl]-4-phenylthiosemicarbazide (8b):

Brown solid; yield = 1.2g (88%); mp 282-4˚C; IR (cm^{-1}) 3496 (NH), 3420 (NH), 3320 (NH), 3187 (NH), 1684 (CO), 1668 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.78 (s, 1H, NH, exchangeable with D_2O), 4.02 (s, 1H, NH, exchangeable with D_2O), 5.05 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 17H, Ar-H), 8.91 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 491 (M^+, 30). Anal. Calcd for $C_{28}H_{21}N_5O_2S$ (491.6): C, 68.4; H, 4.3; N, 14.3; S, 6.5. Found: C, 68.4; H, 4.4; N, 14.1; S, 6.6.

General procedure for the preparation of 5-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl)phenyl]-4-(4-chlorophenyl)-[1,2,4]-triazol-3-one /4-phenyl-[1,2,4]-triazol-3-thione (9a,b):

A stirring mixture of compound **8a** or **8b** (0.01mol) and NaOH (40mg, 2N solution) was heated under reflux for 4h. After cooling, the solution was acidified with HCL and the precipitate was filtered. The product was then crystallized from MeOH.

5-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-2,4-dihydro-[1,2,4]-triazol-3-one (9a):

Brown solid; yield = 0.8g (83%); mp 250-252˚C; IR (cm^{-1}) 3296 (NH), 3228 (NH), 1669 (CO), 1652 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H), 9.07 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 493 (M^+, 3.3), 491 (M^+, 10). Anal. Calcd for $C_{28}H_{18}ClN_5O_2$ (491.9): C, 68.4; H, 3.7; N, 14.2. Found: C, 68.4; H, 3.7; N, 14.2.

Phenyl-{2-[4-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-[1,2,4]-triazol-3-yl)phenyl]-1*H*-benzoimidazol-5-yl}-methanone (9b):

Brown solid; yield = 0.85g (89%); mp 277-279˚C; IR (cm^{-1}) 3296 (NH), 3228 (NH), 1664 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 5.02 (s, 1H, NH, exchangeable with D_2O), 7.05 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 17H, Ar-H); MS *m/z* 473 (M^+, 27). Anal. Calcd for $C_{28}H_{19}N_5OS$ (473.6): C, 71.0; H, 4.0; N, 14.8; S, 6.8. Found: C, 71.0; H, 4.2; N, 14.8; S, 6.8.

General procedure for the preparation of 5-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-2-substituted-2,4-dihydro-[1,2,4]-triazol-3-one (10a-e):

A mixture of compound 9a (2g; 0.067 mol), appropriate alkyl halide (methyliodide, ethylchloroformate, methyl-2-bromoacetate, ethyl-2-bromoacetate or ethyl-2-bromopropanoate) (0.067 mol), anhydrous sodium carbonate (4g) and acetone (30 mL) was heated under reflux for 8h. Most of the solvent was distilled off, the residue was diluted with water and the obtained product was collected.

5-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-2-methyl-2,4-dihydro-[1,2,4]-triazol-3-one (10a):

Brown solid; yield = 0.8g (78%); mp 242-245˚C; IR (cm^{-1}) 3296 (NH), 1685 (CO), 1666 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 5.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H); MS *m/z* 507 (M^+, 33), 505 (M^+, 100). Anal. Calcd for $C_{29}H_{20}ClN_5O_2$ (505.9): C, 68.8; H, 4.0; N, 13.8. Found: C, 68.8; H, 4.1; N, 13.8.

3-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazole-1-carboxylic acid ethyl ester (10b):

Brown solid; yield = 0.9g (79%); mp 265-268˚C; IR (cm^{-1}) 3296 (NH), 1705 (CO), 1690 (CO), 1656 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.31 (t, J = 7.1 Hz, 3H, CH_3), 4.11 (q, 2H, CH_2), 5.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H); MS *m/z* 566 (M^+, 33), 564 (M^+, 100). Anal. Calcd for $C_{31}H_{22}ClN_5O_4$ (564.0): C, 66.0; H, 3.9; N, 12.4. Found: C, 66.0; H, 3.9; N, 12.5.

{3-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl}acetic acid methyl ester (10c):

Brown solid; yield = 0.95g (83%); mp 295-298˚C; IR (cm^{-1}) 3296 (NH), 1705 (CO), 1690 (CO), 1656 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.31 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 5.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H); MS *m/z* 566 (M^+, 3.3), 564 (M^+, 10). Anal. Calcd for $C_{31}H_{22}ClN_5O_4$ (564.0): C, 66.0; H, 3.9; N, 12.4. Found: C, 66.0; H, 4.0; N, 12.5.

{3-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl}acetic acid ethyl ester (10d):

Brown solid; yield = 1.0g (83%); mp 283-285˚C; IR (cm^{-1}) 3296 (NH), 1705 (CO), 1690 (CO), 1656 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.31 (t, J = 7.2 Hz, 3H, CH_3), 4.11 (q, 2H, CH_2), 4.14 (s, 2H, CH_2), 5.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H); MS *m/z* 580 (M^+, 3.3), 578 (M^+, 10). Anal. Calcd for $C_{32}H_{24}ClN_5O_4$ (578.0): C, 66.5; H, 4.2; N, 12.1. Found: C, 66.5; H, 4.2; N, 12.1.

2-{3-[4-(5-Benzoyl-1*H*-benzoimidazol-2-yl) phenyl]-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl} propionic acid ethyl ester (10e):

Brown solid; yield = 0.85g (71%); mp 285-289˚C; IR (cm^{-1}) 3296 (NH), 1705 (CO), 1690 (CO), 1656 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.31 (t, J = 7.1 Hz, 3H, CH_3), 1.73 (d, J = 6.9 Hz, 3H, CH_3), 4.15 (q, 2H, CH_2), 4.61 (q, 1H, CH), 5.19 (s, 1H, NH, exchangeable with D_2O), 7.29-8.30 (m, 16H, Ar-H); MS *m/z* 594 (M^+, 3.3), 592 (M^+, 10). Anal. Calcd for $C_{33}H_{26}ClN_5O_4$ (592.0): C, 67.0; H, 4.4; N, 11.8. Found: C, 66.9; H, 4.4; N, 11.9.

Antimicrobial activity test

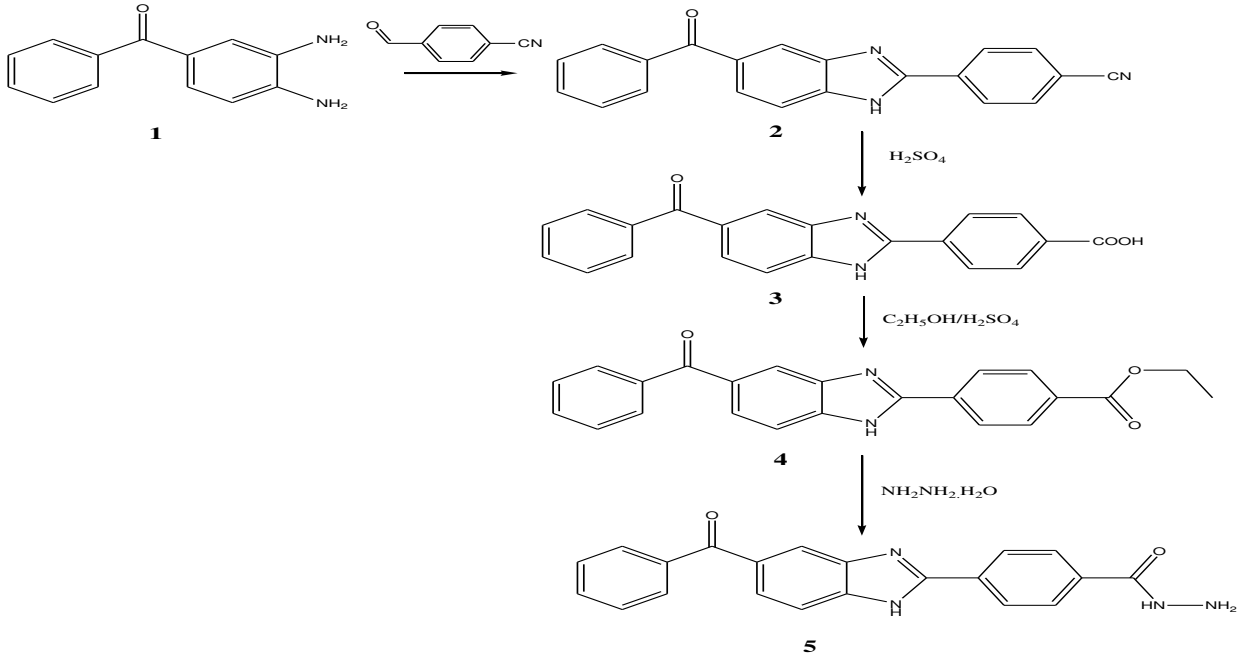
The *in vitro* antimicrobial activity was performed against *Bacillus subtilis* NRRL 543, *Staphylococcus aureus* NRRL B-313 (Gram-positive bacteria), *Escherichia coli* NRRL B-210 and *Pseudomonas aeuroginosa* NRRL B-23 (Gram-negative bacteria), *Candida albicans* NRRL Y-477 and *Aspergillus niger* NRRL 599 (Fungi), by agar diffusion method [19]. A suspension of the organisms were added to sterile nutrient agar media at 45˚C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer an amount of 0.1 mL of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37˚C for 24 h and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin and Fluconazole were used as standard for antibacterial and antifungal activity, respectively. To evaluate the activity of synthesized compounds against bacteria and fungi, minimum inhibitory

concentrations (MIC) was determined by agar streak dilution method, 100 mg/mL stock solution of the synthesized compounds was made using DMSO as the solvent. From this stock solution, a range of concentration from (5 to 0.05 mg/mL) of the tested compounds solutions was mixed with the known quantities of molten sterile agar media aseptically. About 20 mL of nutrient agar medium for bacteria and Sabouraud dextrose agar medium for fungi containing the tested compound under study was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 30˚C for 24 h / 48 h for bacteria and fungi respectively. Then the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate.

Results and Discussion

Chemistry

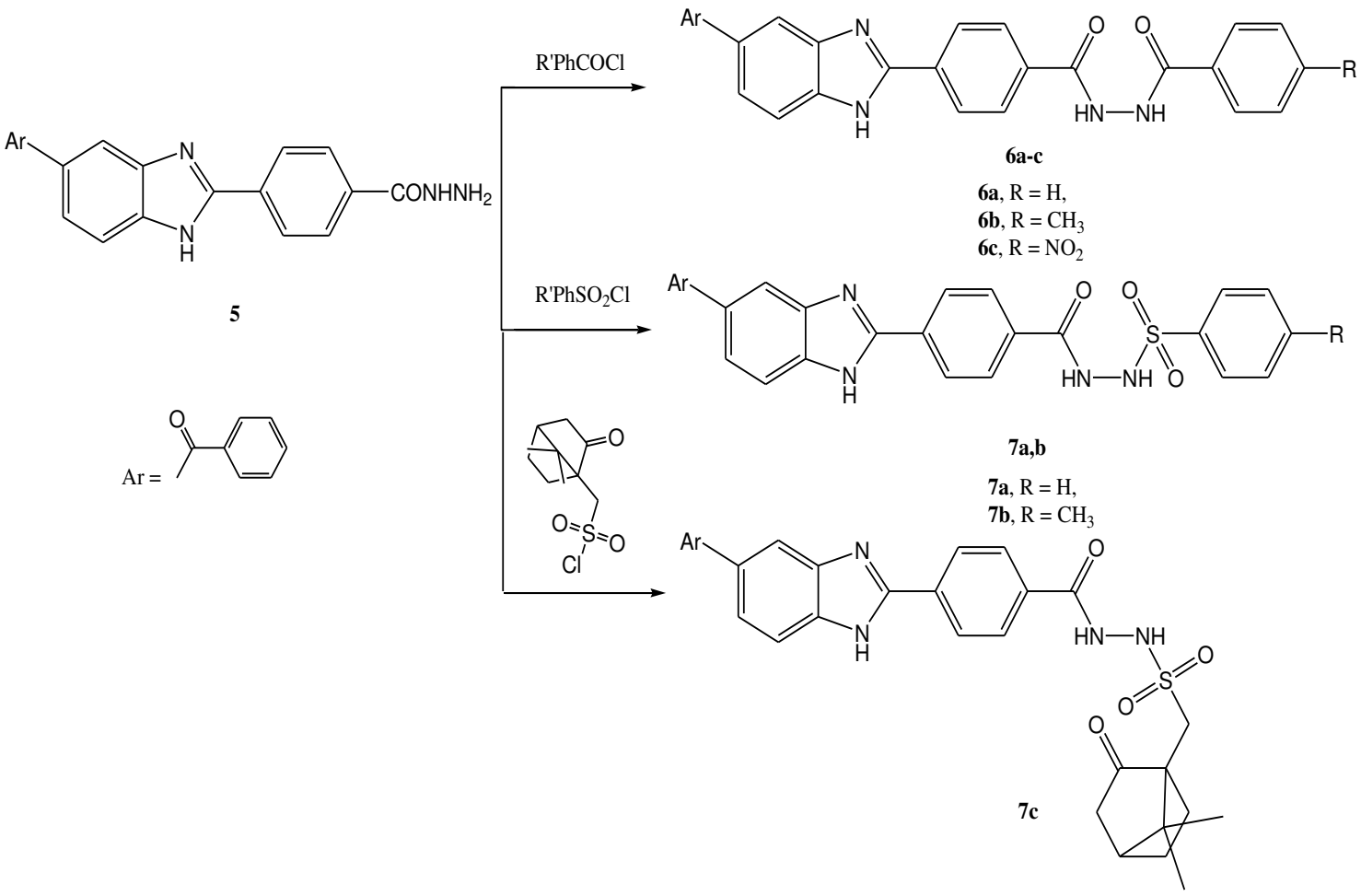
The starting material 4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzonitrile (**2**) was prepared through the reaction of (3,4-diaminophenyl)phenyl-methanone (**1**) with 4-cyanobenzaldehyde in EtOH. Acid oxidation of carbonitrile group by stirring with 70% sulfuric acid to give benzoic acid derivative **3** followed by esterification and heating under reflux with hydrazine hydrate to form the corresponding 4-(5-benzoyl-1*H*-benzoimidazol-2-yl)benzoic acid hydrazide (**5**) (Scheme **1**).



Scheme 1

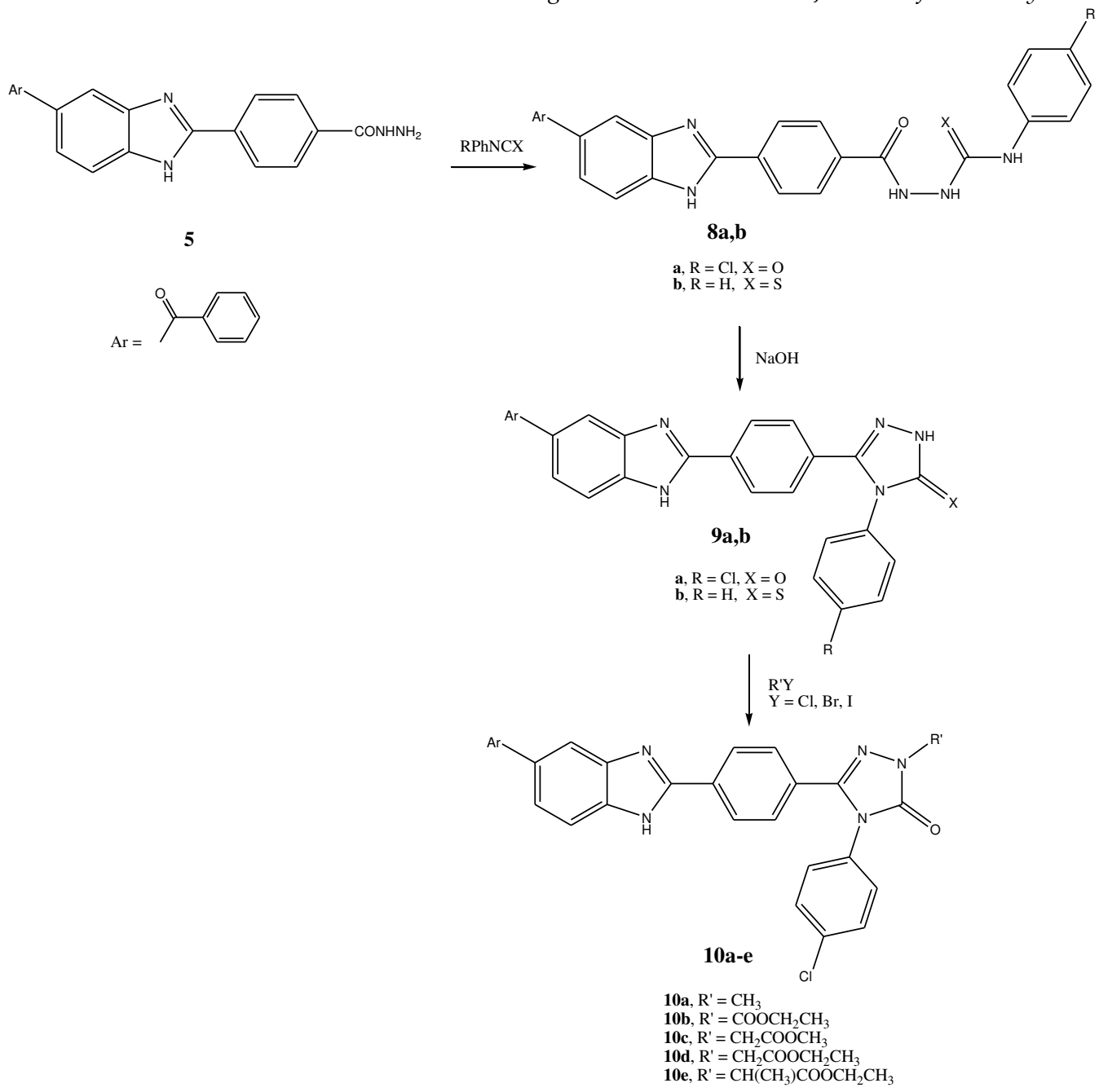
Scheme

The reaction of 4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoic acid hydrazide (**5**) dissolved in 10 mL dry benzene and few drops of triethylamine with benzoyl chloride, 4-methylbenzoyl chloride and/or 4-nitrobenzoyl chloride, gave the corresponding benzamides **6a-c**. In addition, stirring compound **5** with sulfonyl chloride, toluene sulfonyl chloride and/or camphor-10-sulfonyl chloride in dry acetone and triethylamine yielded the corresponding sulfonamides **7a-c** as illustrated in Scheme **2**.



Scheme 2

Compounds **8a**,**b** were synthesized *via* refluxing of hydrazide **5** with 4-chloroarylisocyanate or arylisothiocyanate in dry benzene followed by heating under reflux the mixture of compound **8a** or **8b** and sodium hydroxide for 4h, afforded the corresponding triazole **9a,b**. Condensing **9a** with the required alkylhalide (methyliodide, ethylchloroformate, methyl-2-bromoacetate, ethyl-2-bromoacetate or ethyl-2-bromopropanoate) in the presence of anhydrous sodium carbonate as acid halide abstract and acetone as solvent. The products **10a-e** was obtained mostly in pure state (Scheme **3**).



Scheme 3

Antimicrobial evaluation

The antibacterial activity of the synthesized compounds was tested against *Bacillus subtilis* NRRL 543 and *Staphylococcus aureus* NRRL B-313 (Gram-positive bacteria), *Escherichia coli* NRRL B-210 and *Pseudomonas aeuroginosa* NRRL B-23 (Gram-negative bacteria) using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* NRRL Y-477 and *Aspergillus niger* NRRL 599 using Sabouraud dextrose agar medium.

The results revealed that compounds **4**, **6a**, **6b**, **6c** and **9a** showed the best antimicrobial activity against the tested microorganisms. Compound **2** displayed less activity against all the tested microorganisms. Compounds **3**, **5**, **7b**, **8a**, **9b** and **10b** showed moderate activity. The antifungal activities depicted in Table **1** revealed that compounds **4** and **6a** showed good activity against *Candida albicans* and *Aspergillus niger* followed by compounds **5**, **6b**, **6c**, **9a**, **9b** and **10b** were moderately active while compounds **2**, **3**,**7b** and **8a** exhibited the lowest antifungal activities.

Table-1: **Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds.**

Compds.	Microorganism inhibition zone diameter (mm)					
	Gram +ve bacteria		Gram –ve bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Aspergillus niger*
2	-	-	11	-	13	-
3	20	19	15	17	18	-
4	16	17	23	20	22	17
5	17	16	17	15	19	14
6a	27	26	24	25	27	20
6b	21	19	20	18	20	15
6c	20	19	24	23	20	14
7b	13	11	12	12	12	-
8a	14	15	15	15	15	-
9a	21	20	20	20	17	11
9b	19	18	17	18	18	12
10b	13	12	15	14	17	12
Ciprofloxacin	22	24	24	23	-	-
Fluconazole	-	-	-	-	22	24

Highly active = (inhibition zone > 20 mm); Moderately active = (inhibition zone 15 - 20 mm)

Slightly active = (inhibition zone 11 - 14 mm); Inactive = (inhibition zone < 11 mm)

The MIC of synthesized compounds which are shown in Table **2** was in accordance with the results obtained in the primary screening. The structure activity relationship indicated that the attachment of (un)substituted benzoic acid groups (**6a-c**) to the hydrazide **5** resulted in a marked increase in inhibition activity. Furthermore, benzoic acid N'-[4-(5-benzoyl-

1*H*-benzoimidazol-2-yl) benzoyl] hydrazide **6a** displayed higher activities than Ciprofloxacin and Fluconazole which were used as standard for antibacterial and antifungal activity, respectively. Additionally, the antibacterial activity observed for 4-(5-benzoyl-1*H*-benzoimidazol-2-yl)-N-(4-methylsulfamoyl-phenyl) benzamide (**7b**) indicated the importance of the carbonyl group as the activity was reduced when this group was replaced with the sulphonyl group. Moreover, the antibacterial activity observed for the 1,2,4-triazol-3-one **9a** derivative was relatively higher than that of the corresponding semicarbazide **8a**.

Table-2: **MIC in mg/mL of the newly synthesized compounds against microorganisms.**

Compds	Gram +ve bacteria		Gram –ve bacteria		Fungi	
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeuroginosa***	***Candida albicans***	***Aspergillus niger***
4	0.53	0.56	0.4	0.57	0.48	0.6
6a	0.45	0.5	0.55	0.5	0.5	0.6
6b	0.1	0.4	0.6	0.15	0.6	0.8
6c	0.49	0.5	0.4	0.57	0.54	0.7
9a	0.5	0.53	0.55	0.55	0.55	0.7
Ciprofloxacin	0.05	0.05	0.05	0.05	-	-
Fluconazole	-	-	-	-	0.05	0.05

The antifungal activity results revealed that the ethyl ester **4** and benzoic acid N'-[4-(5-benzoyl-1*H*-benzoimidazol-2-yl) benzoyl] hydrazide **6a** showed the highest antifungal activities. Furthermore, the results indicated that the activities of the 1,2,4-triazol-3-one **9a** were higher than that of the corresponding semicarbazide **8a**. It is also obvious that the unsubstituted benzoic acid **6a** were highly active then the corresponding substituted benzoic acid analogs **6b,c**.

Conclusions

In conclusion, several substituted benzimidazoles **6a-11** were synthesized. The pharmacological study was undertaken to evaluate the effects of substituents on the antibacterial and antifungal activities. Most of the synthesized compounds exhibited good antibacterial activity towards Gram-positive bacteria and Gram-negative bacteria and some of the synthesized compounds showed good to moderate antifungal activity.

The presence of (un) substituted benzoic acid (**6a-c**) resulted in a marked increase in inhibition activity. Also, the antimicrobial activity observed for the 1,2,4-triazol-3-one **9a** was relatively high than the semicarbazide **8a**. Findings

from SAR have encouraged us to make some modifications on basic structure of the obtained compounds to achieve selective and more active derivatives in ongoing studies.

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Conflict of Interest

The authors have declared no conflicts of interest.

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