Synthesis and antimicrobial activity of some amino acids and sulfamoyl and pyrrole derivatives attached to 4-benzoimidazol-2-yl moiety

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Background and objectives
Benzoimidazole moiety is one of the heterocyclic compounds that plays a vital role in biological fields such as antioxidant, antidepressant, anticonvulsant, antimicrobial and anticancer. The aim of this study was to construct new compounds containing 4-(5-benzoyl-benzoimidazol-2) moiety incorporated into different amino acids and sulfamoyl and/or pyrrole analogues and to evaluate their antimicrobial activities.

Materials and methods
The starting material 4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzonitrile (**2**) was prepared through the reaction of (3,4-diamino-phenyl)-phenyl-methanone (**1**) with 4-cyanobenzaldehyde in absolute ethanol. Stirring compound **2** with 70% sulfuric acid gave benzoic acid derivative **3** followed by esterification and refluxing with hydrazine hydrate to form the corresponding 4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzoic acid hydrazide (**5**). A series of derivatives **6a–d** were prepared by coupling of benzoic acid derivative **3** with different amino acids ethyl ester. Reacting ester compound **4** with different amines and sulfa drugs led to the formation of the amides derivatives **7a,b** and **8a,b**, whereas on reacting the hydrazide compound **5** with different acid anhydrides, cyclohexane-1,4-dione and 5-nitroisatin compounds **9a–c**, **10** and **11** were obtained.

Results and conclusion
Most of the test compounds were found to be significantly effective against *Bacillus subtilis* and *Staphylococcus aureus* (gram-positive bacteria), *Escherichia coli* and *Pseudomonas aeuroginosa* (gram-negative bacteria) and *Candida albicans* and *Aspergillus niger* (fungi).

Keywords:
amino acids, antimicrobial activity, 4-benzoimidazol-2-yl, pyrrole, sulfamoyl

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Introduction

Infectious microbial diseases remain pressing problems worldwide, because resistance to a number of antimicrobial agents (β-lactam antibiotics, macrolides, quinolones and vancomycin) among variety of clinically significant species of microorganisms has become an important global health problem [1]. One way to battle with this challenge is the conscious usage of the currently marketed antibiotics; the other is the development of novel antimicrobial agents [2]. Hence, there will always be a vital need to discover new chemotherapeutic agents to avert the emergence of resistance and ideally shorten the duration of therapy.

Benzoimidazole is an important pharmacophore and privileged structure in medicinal chemistry. Literature survey shows that, among the benzoimidazole derivatives, 2-substituted ones are found to be pharmacologically more potent, and hence the design and synthesis of 2-substituted benzoimidazoles are the potential area of research [3]. Extensive biochemical and pharmacological studies have confirmed that its derivatives are effective against various strains of microorganisms [4–13]. The reason for a special interest of researchers towards benzoimidazole derivatives has been 5,6-dimethyl-benzoimidazole, which is a constituent of naturally occurring ring vitamin B_{12} [14]. Although vitamin B_{12} is capable of inducing the growth of bacteria, the benzoimidazole component and some of its derivatives repress the bacterial growth. Because of the structural similarity to purine, antibacterial ability of benzoimidazoles is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins [15,16].

Benzoimidazole derivatives possess antifungal activity, besides their antibacterial potential. They can be classified as the most important group of fungicides with systemic activity and are well known for their pronounced ability to control a large number of fungal diseases. Benomyl, thiabendazole and thiophnate methyl are some main examples of this fungicide class [17–19]. Owing to their systemic activity, they can help to control some infectious microbial diseases.

They are also used for the prevention of postharvest rots and as soil-drench treatments [20].

Looking at the antimicrobial importance of benzoimidazole, it was thought that it would be worthwhile to design and synthesize some new benzoimidazole derivatives bearing different functional groups and to evaluate their antibacterial and antifungal activities.

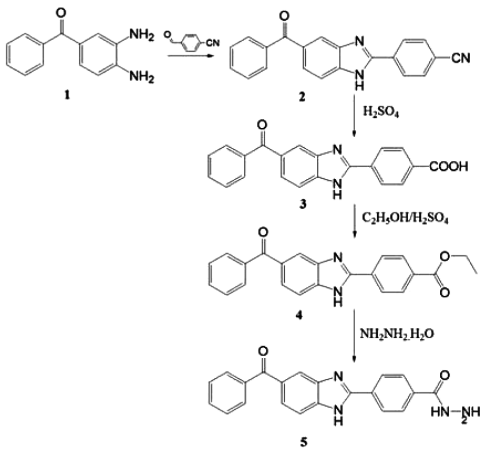
Chemistry

Melting points (°C) were taken 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, Massachusetts 02451, USA) in KBr discs. Elemental analysis was performed at the Microanalytical Laboratory of the National Research Center. The antimicrobial activity of the synthesized compounds was carried out at the National Research Centre, Giza, Egypt.

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

4-Cyanobenzaldehye (0.6 g, 0.21 mol) and (3,4-diamino-phenyl)-phenyl-methanone (**1**) (1 g, 0.21 mol) were dissolved in ethanol. This mixture was refluxed for 5 h and cooled to room temperature. Thereafter, 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 (Scheme 1). Yield = 1.3 g (86%), m.p. = 250–252°C. Analysis for $C_{21}H_{13}N_3O$ (323.4): Calcd.: C, 78.0; H, 4.0; N, 13.0; Fd.: C, 78.0; H, 4.1; N, 13.0. IR (cm^{-1}): 3340 (NH), 2225 (CN), 1705 (CO). MS: *m/z* (%): 323 (M^+, 100). ^{1}H-NMR: δ, ppm (DMSO-d_6); 5.92 (s, 1H, NH); 7.48–8.22 (m, 12H, Ar–H).

Scheme 1



Synthesis of 4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzoic acid hydrazide (5)

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

A mixture of **2** (1 g, 0.01 mol) and 30 ml 70% sulfuric acid was stirred in 100 ml three-necked flask at 140°C for 5 h, then suspended in 150 ml water and the resulting precipitate was filtered off. Recrystallization from diluted ethanol afforded yellow crystals (Scheme 1). Yield = 0.95 g (90%), m.p. = 280–283°C. Analysis for $C_{21}H_{14}N_2O_3$ (342.4): Calcd.: C, 73.7; H, 4.1; N, 8.2; Fd.: C, 73.7; H, 4.2; N, 8.2. IR (cm^{-1}): 3746 (NH), 3348 (OH), 1701 (COO), 1720 (CO). MS: *m/z* (%): 343 (M^+, 16); 342 (M^+, 60). ^{1}H-NMR: δ, ppm (DMSO-d_6); 5.92 (s, 1H, NH); 7.48–8.22 (m, 12H, Ar–H); 10.92 (s, 1H, OH).

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

To a solution of compound **3** (1 g; 0.073 mol) in absolute ethanol, few drops of concentrated sulfuric acid were added and the mixture was refluxed for 4 h. The crude product was filtered, air dried and crystallized from ethanol (Scheme 1). Yield = 0.9 g (83%), m.p. = 107–109°C. Analysis for $C_{23}H_{18}N_2O_3$ (370.4): Calcd.: C, 74.6; H, 4.9; N, 7.6; Fd.: C, 74.5; H, 4.9; N, 7.7. IR (cm^{-1}): 3746 (NH), 3348 (OH), 1717 (COO), 1705 (CO). MS: *m/z* (%): 371 (M^+, 32); 370 (M^+, 72). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.32 (t, 3H, CH_3); 4.32 (q, 2H, CH_2); 5.92 (s, 1H, NH); 7.48–8.22 (m, 12H, Ar–H).

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

To a solution of ester compound **4** (1 g; 0.033 mol) in ethanol, hydrazine hydrate (98%; 2 ml) was added and heated for 5 h on a water bath. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from ethanol (Scheme 1). Yield = 0.8 g (83%), m.p. = 73–78°C. Analysis for $C_{21}H_{16}N_4O_2$ (356.4): Calcd.: C, 70.8; H, 4.5; N, 15.7; Fd.: C, 70.7; H, 4.4; N, 15.8. IR (cm^{-1}): 3736 (NH), 3187 (NH_2), 1705 (CO), 1684 (CO). MS: *m/z* (%): 357 (M^+, 33); 356 (M^+, 49). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.96 (s, 2H, NH_2); 5.92 (s, 1H, NH); 7.48–8.22 (m, 12H, Ar–H); 10.45 (s, 1H, NH).

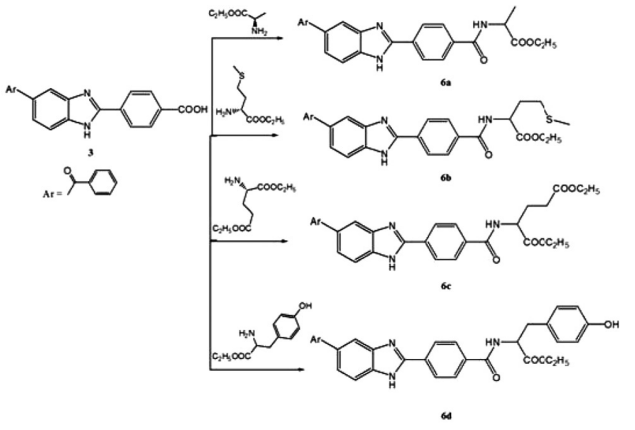
General procedure for the preparation of 2-[4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-substituted-ethyl ester (**6a–d**)

A stirring mixture of the respective amino acid ethyl ester (D-alanine, L-methionine, L-glutaminic or D-tyrosine ethyl ester hydrochloride) (0.038 mol) and compound **3** (1 g, 0.038 mol) in anhydrous methylene chloride (30 ml) was cooled to 0°C. Diisopropylethylamine (1.86 g, 0.14 mol) was then slowly added to the mixture, followed by the addition of the coupling reagent benzotriazol-1-yloxytris(dimethylamino) phosphonimhexafluorophosphate reagent (1.91 g, 0.042 mol) dissolved in 5 ml of anhydrous methylene chloride. The reaction was stirred for 12 h at 20°C. Ethyl acetate (50 ml) was added to the reaction mixture and the organic layer was successively washed with a 1 N hydrochloric acid solution (2 × 35 ml), a 20% sodium carbonate solution (2 × 30 ml) and brine. The organic layer was dried over magnesium sulfate and concentrated in vacuum. The crude residue was purified by chromatography on a silica gel column using ethyl acetate/petroleum ether as eluent (1 : 1) (Scheme 2).

2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-propionic acid ethyl ester (6a)

Yield = 1.1 g (85%); m.p. = 80–84°C. Analysis for $C_{26}H_{23}N_3O_4$ (441.5): Calcd.: C, 70.7; H, 5.2; N, 9.5; Fd.: C, 70.7; H, 5.3; N, 9.5. IR (cm^{-1}): 3371 (NH), 3187 (NH), 1714 (CO), 1705(CO), 1649 (CO). MS: *m/z* (%): 442 (M^+, 21); 441 (M^+, 29). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.04 (t, 3H, CH_3); 2.34 (d, 3H, CH_3); 3.08 (q, 2H, CH_2CH_3); 4.36 (q, 1H, CH); 5.92 (s, 1H, NH); 7.58–8.40 (m, 12H, Ar–H); 9.80 (s, 1H, NH). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 26 signals that correspond to the 26 different carbon groups; signals appeared at δ 13.9 (CH_3CH_2), 30.5 (CH_3CH), 60.9 ($CHCH_3$), 61.5 (CH_2CH_3), 122.5–130.7 (Ar–12CH), 134.7 (C = C), 138.1 (C–N), 142.4 (C–NH), 152.7 (C = N), 164.6 (C = C), 167.5 (2CO) and 171.5 (COO).

Scheme 2



Synthesis of 2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl) -benzoyl amino]-substituted-ethyl ester 6a–d

2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-4-methyl sulfanyl-butyric acid ethyl ester (6b)

Yield = 1.2 g (82%); m.p. = 105–109°C. Analysis for $C_{28}H_{27}N_3O_4$ (501.6): Calcd.: C, 67.0; H, 5.4; N, 8.4; Fd.: C, 66.9; H, 5.4; N, 8.5. IR (cm^{-1}): 3371 (NH), 3187 (NH), 1714 (CO), 1705(CO), 1649 (CO). MS: *m/z* (%): 502 (M^+, 21); 501 (M^+, 29). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.02 (t, 3H, CH_3); 1.05 (t, 2H, CH_2S); 1.33 (q, 2H, CH_2); 2.50 (s, 3H, CH_3); 3.33 (t, 1H, CH); 4.32 (q, 2H, CH_2CH_3); 5.92 (s, 1H, NH); 7.58–8.40 (m, 12H, Ar–H); 9.80 (s, 1H, NH). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 28 signals that correspond to the 28 different carbon groups; signals appeared at δ 14.1 (CH_3CH_2), 19.5 (CH_3S), 38.6 (CH_2CH), 40.2 (CH_2CH_2), 58.0 ($CHCH_2$), 61.1 (CH_2CH_3), 122.5–130.7 (Ar–12CH), 134.7 (C = C), 138.1 (C–N), 142.4 (C–NH), 152.7 (C = N), 164.6 (C = C), 167.5 (2CO) and 171.5 (COO).

2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-pentanedioic acid diethyl ester (6c)

Yield = 1.3 g (84%); m.p. = 110–113°C. Analysis for $C_{30}H_{29}N_3O_6$ (527.6): Calcd.: C, 68.3; H, 5.5; N, 8.0; Fd.: C, 68.4; H, 5.6; N, 7.9. IR (cm^{-1}): 3371 (NH), 3187 (NH), 1714 (CO), 1705(CO), 1689 (CO), 1649 (CO). MS: *m/z* (%): 527 (M^+, 39). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.32 (m, 6H, $2CH_3$); 2.25 (t, 2H, CH_2); 2.29 (q, 2H, CH_2); 4.12 (m, 4H, $2CH_2CH_3$); 4.42 (t, 1H, CH); 5.92 (s, 1H, NH); 7.58–8.40 (m, 12H, Ar–H); 9.80 (s, 1H, NH).

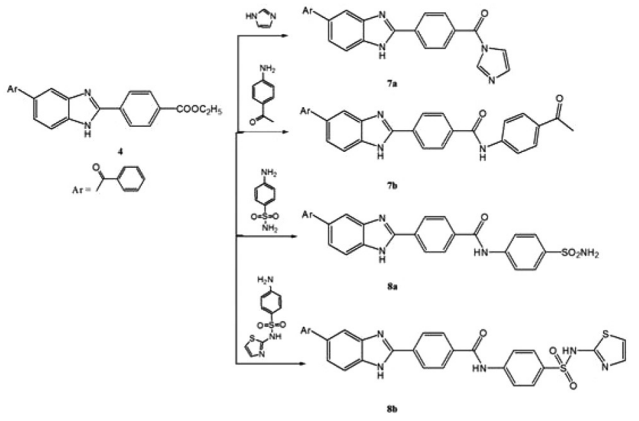
2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-3-(4-hydroxy-phenyl)-propionic acid ethyl ester (6d)

Yield = 1.3 g (83%); m.p. = 127–129°C. Analysis for $C_{32}H_{27}N_3O_5$ (533.6): Calcd.: C, 72.0; H, 5.1; N, 7.9; Fd.: C, 72.0; H, 5.1; N, 7.9. IR (cm^{-1}): 3374 (NH), 3254 (NH), 3182 (OH), 1714 (CO), 1705(CO), 1689 (CO). MS: *m/z* (%): 533 (M^+, 27). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.30 (t, 3H, CH_3); 3.48 (d, 2H, CH_2); 4.15 (q, 2H, CH_2CH_3); 4.81 (t, 1H, CH); 5.19 (s, 1H, OH, exchangeable with D_2O); 5.92 (s, 1H, NH, exchangeable with D_2O); 6.68–8.23 (m, 16H, Ar–H); 9.80 (s, 1H, NH, exchangeable with D_2O).

General procedure for the preparation of [4-(5-benzoyl-1H-benzoimidazol-2-yl)]-substituted-benzamide (**7a,b**)

A mixture of compound **4** (1 g, 0.002 mol), the respective amine (1H-imidazole or 4-aminoacetophenone) (0.002 mol) and 20 ml of ethanol were heated under reflux for 10 h. The reaction mixture was then cooled and the precipitate was filtered off, dried and recrystallized from methanol (Scheme 3).

Scheme 3



Synthesis of compounds 7a, 7b, 8a and 8b

[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-phenyl]-imidazol-1-yl-methanone (7a)

Yield = 0.9 g (85%), m.p. = 220–223°C. Analysis for $C_{24}H_{16}N_4O_2$ (392.4): Calcd.: C, 73.5; H, 4.1; N, 14.3; Fd.: C, 73.4; H, 4.1; N, 14.4. IR (cm^{-1}): 3182 (NH), 1672 (CO), 1633 (CO). MS: *m/z* (%): 393 (M^+, 13); 392 (M^+, 25). ^{1}H-NMR: δ, ppm (DMSO-d_6); 5.92 (s, 1H, NH); 7.10 (d, 2H, 2CH); 7.47–8.33 (m, 12H, Ar–H); 8.71 (s, 1H, CH). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 24 signals that correspond to the 24 different carbon groups; signals appeared at δ 118.7 (C = C–N), 120.7 (C = C–N), 122.5–130.7 (Ar–12CH), 134.7 (C = C), 138.1 (C–N), 142.4 (C–NH), 152.7 (C = N), 164.6 (C = C) and 167.5 (2CO).

***N*-(4-Acetylphenyl)-4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzamide (7b)**

Yield = 1.1 g (89%), m.p. = 250–253°C. Analysis for $C_{29}H_{21}N_3O_3$ (459.5): Calcd.: C, 75.8; H, 4.6; N, 9.1; Fd.: C, 75.8; H, 4.7; N, 9.1. IR (cm^{-1}): 3406 (NH), 3222 (NH), 1711 (CO), 1672 (CO), 1633 (CO). MS: *m/z* (%): 460 (M^+, 9); 459 (M^+, 13). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.40 (s, 3H, CH_3); 5.87 (s, 1H, NH); 7.47–8.33 (m, 16H, Ar–H); 8.71 (s, 1H, NH).

General procedure for the preparation of 4-(5-benzoyl-1H-benzoimidazol-2-yl)-*N*-(4-sulfamoylphenyl)/[4-(thiazol-2-yl-sulfamoyl)-phenyl]-benzamide (8a,b)

Compound **4** (1 g, 0.0034 mol) was dissolved in ethanol (20 ml) and 10% aqueous sodium hydroxide solution; the sulfa drug (sulfanilamide or sulfathiazole) (0.0034 mol) was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The mixture was then poured onto ice/cold water and neutralized with dilute hydrochloric acid. The crude product was filtered and crystallized from petroleum ether (Scheme 3).

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-(4-sulfamoylphenyl)-benzamide (8a)

Yield = 1.1 g (82%), m.p. = 210–213°C. Analysis for $C_{27}H_{20}N_4O_4S$ (496.5): Calcd.: C, 65.3; H, 4.1; N, 11.3; S, 6.4; Fd.: C, 65.1; H, 4.0; N, 11.3; S, 6.5. IR (cm^{-1}): 3406 (NH_2), 3322 (NH), 3222 (NH), 1692 (CO), 1668 (CO), 1394 (SO_2). MS: *m/z* (%): 497 (M^+, 7); 496 (M^+, 10). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.66 (s, 2H, NH_2); 5.87 (s, 1H, NH); 7.47–8.40 (m, 16H, Ar–H); 8.71 (s, 1H, NH).

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-[4-(thiazol-2-yl-sulfamoyl)-phenyl]-benzamide (8b)

Yield = 1.2 g (77%), m.p. = 232–235°C. Analysis for $C_{30}H_{21}N_5O_4S_2$ (579.7): Calcd.: C, 62.2; H, 3.7; N, 12.1; S, 11.1; Fd.: C, 62.1; H, 3.7; N, 12.0; S, 11.2. IR (cm^{-1}): 3406 (NH), 3322 (NH), 3222 (NH), 1692 (CO), 1668 (CO), 1375 (SO_2). MS: *m/z* (%): 580 (M^+, 9); 579 (M^+, 12). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.66 (s, 1H, NH, exchangeable with D_2O); 5.87 (s, 1H, NH, exchangeable with D_2O); 7.47–8.40 (m, 16H, Ar–H); 8.01 (d, 1H, CH); 8.09 (d, 1H, CH); 8.71 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 30 signals that correspond to the 30 different carbon groups; signals appeared at δ 108.7 (C = C–S), 115.5–132.7 (Ar–16CH), 134.7 (C = C), 138.1 (C–N), 138.7 (C = C–N), 142.4 (C–NH), 152.7 (C = N), 164.6 (C = C), 164.9 (CO), 167.5 (CO) and 171.7 (N = C–S).

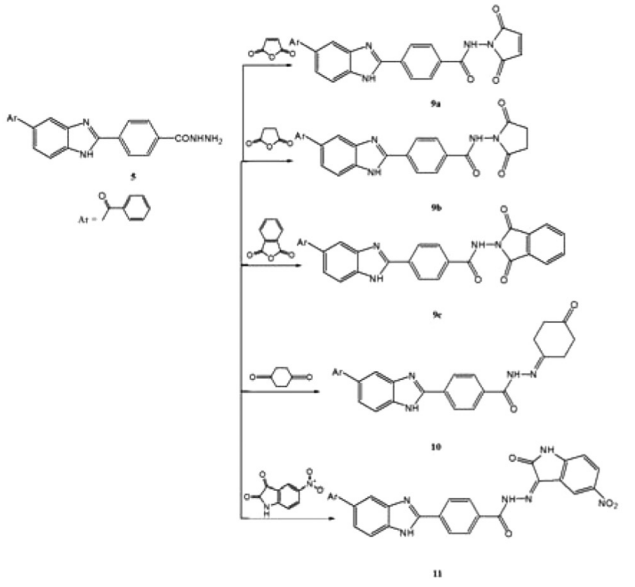
General procedure for the preparation of 4-(5-benzoyl-1H-benzoimidazol-2-yl)-*N*-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)/(2,5-dioxo-pyrrolidin-1-yl)/(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-benzamide (**9a–c**)

To a stirred solution of compound **5** (1 g; 0.017 mol) in glacial acetic acid (10 ml), acid anhydride (maleic anhydride, succinic anhydride or phthalic anhydride) (0.0348 mol) was added. The mixture was heated under reflux with stirring for 8 h. The precipitate formed was filtered, washed with water and the crude product was crystallized from ethanol (Scheme 4).

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-benzamide (9a)

Yield = 1.1 g (90%); m.p. = 292–295°C. Analysis for $C_{25}H_{16}N_4O_4$ (436.4): Calcd.: C, 68.8; H, 3.7; N, 12.8; Fd.: C, 68.8; H, 3.8; N, 12.8. IR (cm^{-1}): 3288 (NH), 3198 (NH), 1734 (CO), 1692 (CO), 1668 (CO), 1649 (CO). MS: *m/z* (%): 437 (M^+, 30); 436 (M^+, 33). ^{1}H-NMR: δ, ppm (DMSO-d_6); 6.06 (s, 1H, NH); 6.18 (d, *J* = 6.9 Hz, 2H, 2CH =); 7.21–8.06 (m, 12H, Ar–H); 8.71 (s, 1H, NH).

Scheme 4



Synthesis of compounds 9a-c, 10 and 11

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-(2,5-dioxo-pyrrolidin-1-yl)-benzamide (9b)

Yield = 1.1 g (89%); m.p. = 270–275°C. Analysis for $C_{25}H_{18}N_4O_4$ (438.4): Calcd.: C, 68.5; H, 4.1; N, 12.8; Fd.: C, 68.4; H, 4.2; N, 12.9. IR (cm^{-1}): 3288 (NH), 3198 (NH), 1734 (CO), 1692 (CO), 1668 (CO), 1649 (CO). MS: *m/z* (%): 439 (M^+, 30); 438 (M^+, 33). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.34 (t, 2H, CH_2); 2.41 (t, 2H, CH_2); 6.06 (s, 1H, NH, exchangeable with D_2O); 7.21–8.06 (m, 12H, Ar–H); 8.71 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 25 signals that correspond to the 25 different carbon groups; signals appeared at δ 29.7 ($2CH_2$), 118.5–132.7 (Ar–12CH), 134.7 (C = C), 138.1 (C–N), 142.4 (C–NH), 152.7 (C = N), 164.6 (C = C), 164.9 (CO), 167.5 (CO) and 171.7 (2CO).

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-benzamide (9c)

Yield = 1.2 g (88%); m.p. = 287–289°C. Analysis for $C_{29}H_{18}N_4O_4$ (486.5): Calcd.: C, 71.6; H, 3.7; N, 11.5; Fd.: C, 71.6; H, 3.7; N, 11.5. IR (cm^{-1}): 3288 (NH), 3198 (NH), 1734 (CO), 1692 (CO), 1668 (CO), 1649 (CO). MS: *m/z* (%): 486 (M^+, 33). ^{1}H-NMR: δ, ppm (DMSO-d_6); 6.06 (s, 1H, NH); 7.21–8.06 (m, 16H, Ar–H); 8.71 (s, 1H, NH).

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoic acid-(4-oxo-cyclohexylidene)-hydrazide (10)

To the hydrazide **5** (1 g; 0.018 mol) dissolved in absolute ethanol (30 ml), cyclohexane-1,4-dione (0.31 g; 0.018 mol) and few drops of glacial acetic acid were added, then the mixture was refluxed for 6 h. Solvent was distilled off and solid product was crystallized from ethyl acetate/petroleum ether (95 : 5) (Scheme 4). Yield = 1.1 g (87%), m.p. = above 300°C. Analysis for $C_{27}H_{22}N_4O_3$ (450.5): Calcd.: C, 72.0; H, 4.9; N, 12.4; Fd.: C, 72.0; H, 5.0; N, 12.4. IR (cm^{-1}): 3284 (NH), 3198 (NH), 1704 (CO), 1692 (CO), 1668 (CO). MS: *m/z* (%): 450 (M^+, 33). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.90 (m, 4H, $2CH_2$); 2.49 (m, 4H, $2CH_2$); 6.06 (s, 1H, NH); 7.21–8.06 (m, 12H, Ar–H); 8.71 (s, 1H, NH).

1-(4-Benzothiazol-2-yl-benzoyl)-5-(5-nitro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-1H-pyrazole-4-carbonitrile (11)

To a solution of compound **5** (1 g; 0.018 mol) in absolute ethanol (30 ml), 5-nitroisatin (0.54 g, 0.018 mol) and few drops of glacial acetic acid were added, then the mixture was refluxed for 6 h. Solvent was distilled off and solid product was crystallized from ethyl acetate/petroleum ether (95 : 5) (Scheme 4). Yield = 1.3 g (87%); m.p. = 237–239°C. Analysis for $C_{29}H_{18}N_6O_5$ (530.5): Calcd.: C, 65.7; H, 3.4; N, 15.8; Fd.: C, 65.7; H, 3.4; N, 15.8. IR (cm^{-1}): 3348 (NH), 3288 (NH), 3198 (NH), 1734 (CO), 1692 (CO), 1668 (CO). MS: *m/z* (%): 531 (M^+, 35); 530 (M^+, 37). ^{1}H-NMR: δ, ppm (DMSO-d_6); 6.06 (s, 1H, NH, exchangeable with D_2O); 7.21–8.06 (m, 15H, Ar–H); 10.05 (s, 1H, NH, exchangeable with D_2O); 11.62 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 29 signals that correspond to the 29 different carbon groups; signals appeared at δ 117.1 (C–C = N), 118.5–142.7 (Ar–15CH), 132.7 (C = N), 134.7 (C = C), 138.1 (C–N), 142.4 (C–NH), 152.7 (C = N), 153.4 (C–NH), 164.6 (C = C), 164.9 (CO) and 167.5 (2CO).

Antimicrobial activity test

The *in-vitro* antimicrobial activity was performed 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), *Candida albicans* NRRL Y-477 and *Aspergillus niger* NRRL 599 (fungi), by agar diffusion method [21]. A suspension of the organisms was 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 and 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 preincubation 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) were 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 were dispensed into each sterile Petri dish. Thereafter, 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 MIC of the test compounds against that bacteria or fungi on the plate.

Results and discussion

Chemistry

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

2-[4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoyl amino]-substituted-ethyl ester **6a–d** were prepared by coupling 4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzoic acid **(3)** with the appropriate amino acid ethyl ester (d-alanine, l-methionine, l-glutaminic or d-tyrosine ethyl ester hydrochloride, respectively) in presence of coupling reagent benzotriazol-1-yloxytris(dimethylamino) phosphonimhexafluorophosphate reagent and diisopropylethylamine as represented in Scheme 2; their structures were confirmed by ^{1}H-NMR and ^{13}C-NMR.

By reacting **4** with different amines, [4-(5-benzoyl-1H-benzoimidazol-2-yl)]-substituted-benzamide **7a,b** were obtained. In addition, by reacting **4** with sulfanilamide or sulfathiazole in presence of sodium hydroxide aqueous solution and ethanol, the sulfa derivatives **8a,b** were obtained, respectively, as illustrated in Scheme 3.

4-(5-Benzoyl-1H-benzoimidazol-2-yl)-*N*-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)/(2,5-dioxo-pyrrolidin-1-yl)/(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-benzamide **9a–c** were formed by stirring of hydrazide **5** with the appropriate acid anhydride (maleic anhydride, succinic anhydride or phthalic anhydride) in glacial acetic acid. 4-(5-Benzoyl-1H-benzoimidazol-2-yl)-benzoic acid-(4-oxo-cyclohexylidene)-hydrazide **(10)** was prepared through the reaction of hydrazide **5** with cyclohexane-1,4-dione. The indole derivative **11** was achieved by reacting the hydrazide derivative **5** with 5-nitroisatin dissolved in absolute ethanol in the presence of few drops of glacial acetic acid as illustrated in Scheme 4.

Antimicrobial activity test

The results revealed that compounds **4**, **7a**, **7b**, **9a**, **9c** and **11** showed the best antimicrobial activity against the tested microorganisms. Compound **2** displayed less activity against all the tested microorganisms. Compounds **3**, **5**, **6a** and **8a** showed moderate activity. The antifungal activities depicted in Table 1 revealed that compounds **4**, **7a** and **7b** showed good activity against *C. albicans* and *A. niger* followed by compounds **5**, **9a**, **9c** and **11**, which were moderately active, whereas compounds **2**, **3**, **6a** and **8a** exhibited the lowest antifungal activities.

The MIC of synthesized compounds, which are shown in Table 2, are in accordance with the results obtained in the primary screening. The structure–activity relationship indicated that the attachment of imidazole and 4-acetylphenyl moieties through a methanone and amide linkage (**7a**,**b**), respectively, resulted in a marked increase in inhibition activity. In addition, compound **7a** showed higher activity than ciprofloxacin and fluconazole, which were used as standard for antibacterial and antifungal activity, respectively. Furthermore, the ethyl ester **4** displayed the highest activities. This ethyl ester showed high activity against gram-positive bacteria (*B. subtilis* and *S. aureus*), gram-negative bacteria (*E. coli* and *P. aeuroginosa*) and yeast-like pathogenic fungus (*C. albicans* and *A. niger*). In addition, the antibacterial activity observed for the 2-[4-(5-benzoyl-1H-benzoimidazol-2-yl)-benzoyl-amino]-propionic acid ethyl ester **6a** indicated the importance of the free carboxylic group, as the activity was reduced when this group was replaced with propionic acid ethyl ester.

Table 1 Inhibition zone (mm) as a criterion of antibacterial and antifungal activities of the newly synthesized compounds

Compounds	Microorganism inhibition zone diameter (mm)					
	Gram-positive bacteria		Gram-negative 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	17	16	13	15	14	–
7a	27	26	24	25	27	20
7b	23	22	19	20	22	16
8a	13	12	13	12	12	–
9a	20	19	24	23	20	14
9c	21	20	20	20	17	11
11	21	19	20	18	20	15
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.

Table 2 MIC (μmol/ml) of the newly synthesized compounds against microorganisms

Compounds	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Aspergillus niger*
4	1.43	1.51	1.08	1.54	1.3	1.62
7a	1.15	1.28	1.4	1.28	1.28	1.53
7b	1.2	1.3	1.3	1.3	1.2	1.52
9a	1.12	1.15	0.92	1.24	1.24	1.6
9c	1.02	1.1	1.13	1.13	1.13	1.44
11	0.2	0.75	1.13	0.28	1.13	1.51
Ciprofloxacin	0.15	0.15	0.15	0.15	–	–
Fluconazole	–	–	–	–	0.16	0.16

MIC, minimum inhibitory concentration.

Moreover, the antibacterial activity observed for the pyrrole **9a** and isoindole **9c** derivatives was relatively higher than that of the indole **11**.

The antifungal activity results revealed that the ethyl ester **4**, imidazole **7a** and 4-acetylphenyl **7b** showed the highest antifungal activities. Furthermore, the results indicated that the activities of the indole **11** were higher than those attached to the pyrrole **9a** and isoindole **9c** moieties. It is also obvious that the free carboxylic group was highly active than the corresponding propionic acid ethyl ester **6a**.

Conclusion

Several substituted benzimidazoles **2–11** 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 imidazole and 4-acetylphenyl moieties through a methanone and amide linkage (**7a**,**b**), respectively, resulted in a marked increase in inhibition activity. In addition, the antimicrobial activity observed for the pyrrole **9a**, isoindole **9c** and indole **11** derivatives was relatively high. 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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Conflicts of interest

There are no conflicts of interest.

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