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Research Article

Synthesis and antimicrobial evaluation of selected new benzimidazole-acetamido conjugates

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We dedicate this paper to the memory of the late Professor Mohsen Daneshtalab of the School of Pharmacy, Memorial University of Newfoundland.

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Abstract

A simple and convenient syntheses and characterization of selected amino acid esters, 1,2,4-triazaoles, thiadiazoles and oxadiazoles in good yields using simple conditions are described. The antimicrobial activities of these new benzimidazole-acetamido conjugates are described.

Keywords: Organic synthesis; benzimidazole, acetamido, amino acid esters, antimicrobials.

1. Introduction

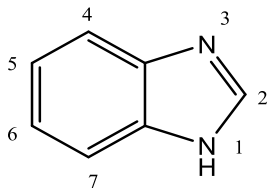

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Figure 1: **Benzimidazole** (**1**)

The benzimidazole (**1**) **(**Figure 1) molecular motif is found extensively in natural products including, importantly, a functionalized derivative, 5,6-dimethyl-1-(α-D-ribofuranosyl)-benzimidazole which is a component of vitamin B_{12}. Due to its importance as a significant pharmacophore [1] there have been many reports on the synthesis and biological activities of a vast number and variety of benzimidazole derivatives.[1] A recent review on the patent literature from 2013-2014 concerning substituted benzimidazoles [2] attests to the growing importance of this structural motif in new drug design. Biological activities of benzimidazole derivatives include anticancer, antihypertensive, antibacterial, antifungal, antiparasitic, proton pump inhibitors, analgesic and anti-inflammatory agents.[2]

Benzimidazole itself is commercially-available and it, as well as many of its derivatives, can be synthesized by several different methods, [1] including the direct condensation of *o*-phenylenediamine with aromatic or aliphatic carboxylic acids, aldehydes, [1,3] or amides. [4] It has also recently been reported that benzimidazole derivatives can be prepared from the reaction of substituted *o*-nitroanilines with substituted benzyl alcohol derivatives, [5] or by a base-mediated intramolecular amination reaction of an iodophenylbenzamidine using simple basic conditions.[6] A recent paper by Ghosh highlighted the use of $MgCl_2$-catalyzed syntheses of 2-substituted-1*H*-benzimidazoles.[7]

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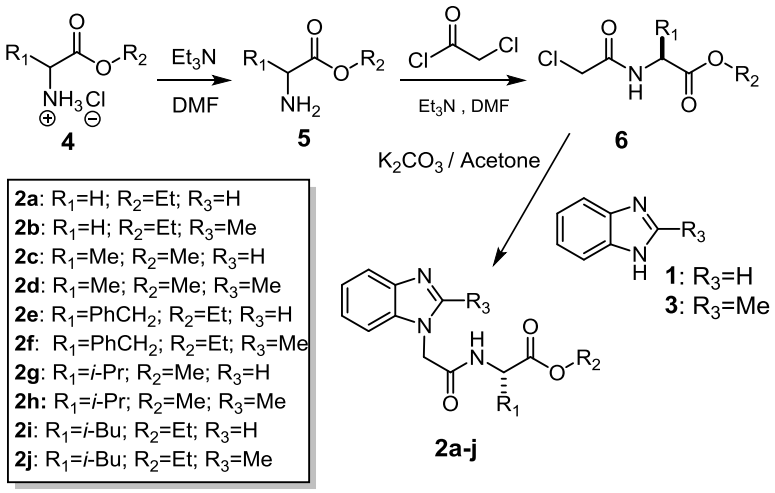
Most of the derivatives which have been reported have various substituents on the phenyl ring, and/or on the C-2 position with various aryl or aliphatic substituents. In addition to these substituents, the secondary amine also has a large variety of both simple, and more complex, substituents. Nevertheless, in pharmaceutical and medicinal chemistry, there is always a need for new compounds to be synthesized and tested for their potential bioactivity. Therefore, as part of our on-going interest in producing new benzimidazoles which are functionalized at the secondary, or N-1 position, which may have potential antimicrobial properties, we report herein our studies on the synthesis and antimicrobial evaluation of several new N-1 acetamido derivatives **2a-j** using the methyl or ethyl esters of the amino acids glycine, alanine, phenylalanine, valine and leucine with benzimidazole (**1**) or 2-methylbenzimidazole (**3**) under conditions summarized in **Scheme 1**, *via* compounds **4**-**6**.

Futhermore, the antimicrobial evaluation of the corresponding thiadiazoles **7a-c**, triazoles **8a-c** and oxadiazoles **9a-c** which were synthesized starting from the corresponding compounds **2a, 2b** or **2g**, via the corresponding intermediates **10a-c** and **11a-c**, as outlined in **Scheme 2**, are also reported.

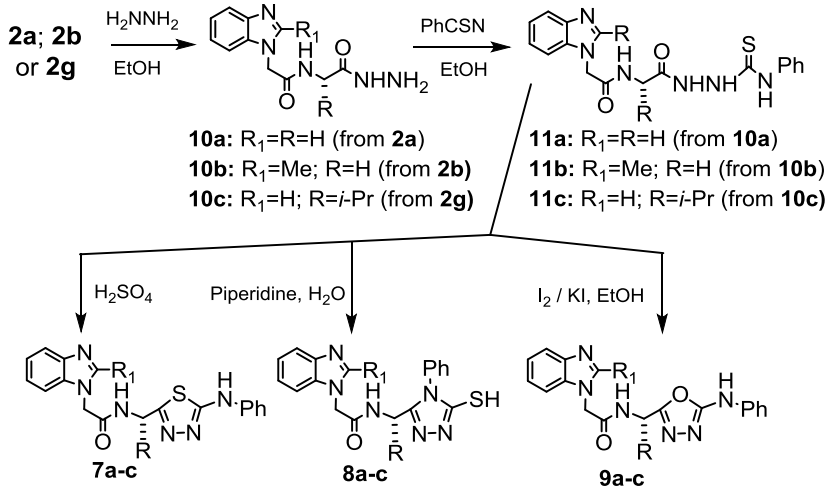
2. Materials and Methods

2.1 Experimental

All reagents including the amino acid methyl or ethyl ester hydrochlorides **4** were purchased from Alfa-Aesar or Sigma-Aldrich and were used without further purification. Melting points were determined with a MPA 100 Optimelt automated melting point system and are uncorrected. ^{1}H NMR (300MHz) and ^{13}C NMR (75 MHz) spectra were recorded in DMSO-d_6 on a Bruker AVANCE III 300MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. APCI mass spectra (in positive mode) were recorded on LC/MSD (Trap) Agilent 1100 series SL. APPI HRMS (+mode) were recorded on a LC/MSD -TOF Agilent 6200 series instrument.



Scheme 1: Synthesis of benzimidazole-acetamido compounds 2a-j.



Scheme 2: Synthesis of benzimidazole-acetamido compounds 7a-c; 8a-c; 9a-c.

General procedure for the preparation of 2-chloroacetamido acid esters (6): A mixture of 12.5 mmol of the amino acid ester hydrochloride and DMF (10.0 mL) was stirred in a 100–mL round–bottom flask under N_2 for 2 min. Triethylamine (1.80 mL, 12.5 mmol) was added to the mixture and was stirred for 1 h to form the free amino acid ester. The mixture was filtered to remove the formed solid. Triethylamine (1.80 mL, 12.5 mmol) and chloroacetylchloride (1.00 mL, 12.5 mmol) was added to the filtrate which was cooled in an ice-bath. The mixture was stirred under N_2 until the disappearance of the starting material. After reaction completion the solid was separated by filtration. To the filtrate brine (100 mL) was added and the mixture was then extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with brine (100 mL) and then dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated on a rotavap at room temperature to afford the crude product.

Ethyl 2-(2-chloroacetamido) acetate (6: R_1 = H; R_2 = Et).

Yellowish-white solid, 1.6 g (71 %): mp 51 ^oC (Lit.[14b] 45-50 ^oC). ^1H NMR (300 MHz, $CDCl_3$): δ 7.17 (s, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 4.14–4.04 (m, 4H), 1.30 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 166.4, 61.8, 42.4, 41.6, 14.1. HRMS (+APPI): *m/z* calcd for $[C_6H_{10}ClNO_3]$: 179.0349; found 179.0337.

Methyl 2-(2-chloroacetamido)propanoate (6: R_1 = R_2 = Me).

Yellow viscous oil, 3.00 g (contains residual DMF): ^1H NMR (300 MHz, $CDCl_3$): δ 7.18 (s, 1H), 4.61 (p, *J* = 7.2 Hz, 1H), 4.07 (s, 2H), 3.78 (s, 3H), 2.97 (s, 4H), 1.47 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 165.7, 52.7, 48.4, 41.2,42.4, 18.2. HRMS (+APPI): *m/z* calcd for $[C_6H_{10}ClNO_3]$: 179.0349; found 179.035.

Ethyl-2-(2-chloroacetamido)-3-phenylpropanoate (6: R_1 = CH_2Ph; R_2 = Et).

Brownish-red solid, 2.02 g (71%); mp 64 ^oC; ^1H NMR (300 MHz $CDCl_3$) δ 7.40–7.23 (m, 3H), 7.21–7.10 (m, 2H), 7.02 (d, *J* = 7.8 Hz, 1H), 4.88 (dt, *J* = 7.8, 5.9 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.06 (s, 2H), 3.19 (dd, *J* = 5.9, 1.6 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 165.5, 135.4, 129.3, 128.7, 127.3, 61.8, 53.4, 42.4, 37.9, 14.1. HRMS (+APPI): *m/z* calcd for $[C_{13}H_{16}ClNO_3]$: 269.0819; found 269.0824.

Methyl 2-(2-chloroacetamido)-3-methylbutanoate (6: R_1 = *i*-Pr; R_2 = Me).

Violet oil, 3.20 g (contains residual DMF). ^1H NMR (300 MHz, $CDCl_3$): δ 7.05 (br, *J* = 8.8 Hz, 1H), 4.55 (dd, *J* = 8.8, 4.9 Hz, 1H), 4.09 (s, 2H), 3.77 (s, 3H), 2.22 (pd, *J* = 6.9, 4.9 Hz, 1H), 0.96 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7, 165.9, 57.4, 52.3, 42.6, 31.3, 18.9, 17.7. HRMS (+APPI): *m/z* calcd for $[C_8H_{14}ClNO_3]$: 207.0662; found 207.649.

Ethyl 2-(2-chloroacetamido)-4-methylpentanoate.(6: R_1 = *i*-Bu; R_2 = Et).

Yellow viscous oil, 2.80 g (contains residual DMF). ^1H NMR (300 MHz, $CDCl_3$): δ 6.99 (d, *J* = 8.4 Hz, 1H), 4.69–4.55 (m, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.08 (s, 2H), 1.77–1.55 (m, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.03–0.89 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 165.8, 61.6, 51.2, 42.4, 41.4, 24.9, 22.8, 21.9, 14.1. HRMS (+APPI): *m/z* calcd for $[C_{10}H_{18}ClNO_3]$: 235.0975; found 235.0963.

General procedure for the preparation of 2-(1*H*-benzo[d]-imidazole-1-yl)acetamido acid esters 2a-j: A mixture of benzimidazole **1** or **4** (4.20 mmol) and potassium carbonate (1.10 mmol) in acetone (10.0 mL) was added to an acetone solution of the respective crude 2-chloroacetamido products **6**, obtained in the previous reaction steps. The reaction mixture was heated at reflux and monitored by TLC for the disappearance of the starting material. When completed, the reaction mixture was filtered and the acetone was evaporated on a rotavap. The product was purified by column chromatography (80:20 hexanes: ethanol).

Ethyl 2-(2-(1*H*-benzo[d]imidazole-1-yl)acetamido)acetate (2a).

Yellowish-white solid, 657 mg (60%); mp 97.8 ^oC. ^1H NMR (300 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.50 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.38 – 7.09 (m, 4H), 4.79 (s, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.93 (d, *J* = 5.7 Hz, 2H), 1.16 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3, 167.1, 143.4, 143.4, 133.5, 123.8, 123.1, 120.1, 109.7, 61.6, 48.1, 41.2, 14.1. In DMSO-*d*6: ^1H NMR (300 MHz, DMSO-d_6) δ 8.81 (t, *J* = 5.9 Hz, 1H), 8.20 (s, 1H), 7.75 – 7.64 (m, 1H), 7.53 – 7.42 (m, 1H), 7.33 – 7.17 (m, 2H), 5.05 (s, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.93 (d, *J* = 5.8 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.5, 167.2, 144.8, 143.2, 134.2, 122.3, 121.5, 119.3, 110.3, 60.5, 46.6, 40.8, 14.0. MS (+APCI): *m/z* = 262.1 $[M + H]^+$.

Ethyl 2-(2-(2-methyl 1*H*-benzo[d]imidazole-1-yl)acetamido) acetate (2b).

Colourless solid, 624 mg (54%); mp 148.9 ^oC. ^1H NMR (300 MHz, $CDCl_3$): δ 7.37 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.25–7.13 (m, 2H), 7.08 (ddd, *J* = 7.8, 6.5, 1.9 Hz, 1H), 7.00 (t, *J* = 5.7 Hz, 1H), 4.70 (s, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.94 (d, *J* = 5.8 Hz, 2H), 2.41 (s, 3H), 1.17 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 167.2, 151.9, 142.4, 134.8, 122.9, 122.8, 119.0, 108.8, 61.6, 46.9, 41.1, 14.1, 13.6. In DMSO-*d*6: ^1H NMR (300 MHz, DMSO-*d*6)

δ 8.72 (t, *J* = 5.9 Hz, 1H), 7.58 – 7.46 (m, 1H), 7.46 – 7.35 (m, 1H), 7.22 – 7.07 (m, 2H), 4.93 (s, 2H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.88 (d, *J* = 5.9 Hz, 2H), 1.17 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ 169.56, 167.47, 152.79, 141.35, 135.22, 121.94, 121.73, 117.91, 109.56, 60.85, 45.33, 40.74, 37.97, 13.84, 12.98. MS (APCI+): *m/z* = 276.1 [M + H]+. HRMS (+APPI): *m/z* calcd for [$C_{14}H_{17}N_3O_3$]: 275.127; found 275.1275.

Methyl 2-(2-(1*H*-benzo[d]imidazole-1-yl)actamido)propanoate (2c).

Off-white solid, 657 mg (60 %); mp 94.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 – 7.60 (m, 2H), 7.37–7.15 (m, 3H), 6.92 (d, *J* = 7.6 Hz, 1H), 4.77 (s, 2H), 4.55 (p, *J* = 7.3 Hz, 1H), 3.61 (s, 3H), 1.28 (d, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.7, 166.2, 143.6, 143.4, 133.6, 123.8, 123.0, 120.4, 109.6, 52.6, 48.3, 48.1, 17.8. MS (+): *m/z* =262.1 $[M + H]^+$. HRMS (+APPI) : *m/z* calcd for [$C_{13}H_{15}N_3O_3$] : 261.1113; found 261.1113.

Methyl 2-(2-(2-methyl-1*H*-benzo[d]imidazole-1-yl)actamido) propanoate (2d).

Colourless solid, 704 mg (61%); mp 149.1°C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.57 (dp, J = 5.3, 1.9 Hz, 1H), 7.22–7.16 (m, 3H), 6.55 (d, *J* = 7.4 Hz, 1H), 4.68 (s, 2H), 4.55 (p, *J* = 7.3 Hz, 1H), 3.62 (s, 3H), 2.46 (s, 3H), 1.26 (d, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.5 , 166.4, 151.9, 142.5, 134.9, 122.9, 122.8, 119.3, 108.8, 52.6 , 48.2, 46.9, 17.8, 13.6. MS (+APCI): *m/z* = 276.1 $[M + H]^+$.

Ethyl 2-(2-(1*H*-benzo[d]imidazole-1-yl)acetamido-3-phenyl-propanoate (2e).

Colourless solid, 929 mg (63%); mp 133.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.73–7.68 (m, 2H), 7.28–7.02 (m, 5H), 6.75–6.72 (m, 2H), 6.20 (d, *J* = 8.0 Hz, 1H), 4.75 (dt, *J* = 8.0, 6.1 Hz, 1H), 4.70 (d, *J* = 3.7 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.94 (qd, *J* = 14.0, 6.1 Hz, 2H), 1.13 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 170.7, 166.1, 143.7, 143.2, 135.2, 133.5, 128.9, 128.6, 127.2, 123.9, 123.0, 120.6, 109.4, 61.8, 53.1, 48.0, 37.3, 14.1. MS (APCI+): *m/z* = 352.2 $[M + H]^+$. HRMS (+APPI): *m/z* calcd for [$C_{20}H_{21}N_3O_3$]: 351.1583; found 351.1585.

Ethyl 2-(2-(2-methyl-1*H*-benzo[d]imidazole-1-yl)acetamido-3-phenylpropanoate (2f).

Brownish white solid: 1.04 g (68%); mp 160.5 °C ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.76–7.66 (m, 1H), 7.27 (td, J = 6.3, 5.3, 1.6 Hz, 3H), 7.22–7.04 (m, 4H), 6.82–6.72 (m, 2H), 5.79 (d, *J* = 8.0 Hz, 1H), 4.80 (td, *J* = 7.6, 5.5 Hz, 1H), 4.69 (s, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.04 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.98–2.83 (m, 1H), 2.45 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 170.5, 166.2, 151.7, 142.7, 135.0, 134.8, 128.8, 128.7, 127.3, 123.0, 122.8, 119.5, 108.7, 61.8, 52.9, 46.8, 37.3, 14.1, 13.5. MS (APCI+): *m/z* = 366.2 $[M + H]^+$. HRMS (+APPI): *m/z* calcd for [$C_{21}H_{23}N_3O_3$]: 365.1739; found 365.1747.

Methyl 2-(2-(1*H*-benzo[d]imidazole-1-yl)acetamido)-3-methylbutanoate (2g).

Colourless solid, 728 mg (60%); mp 122.0 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.90 – 7.78 (m, 1H), 7.44 – 7.27 (m, 3H), 6.19 (d, *J* = 8.7 Hz, 1H), 4.90 (s, 2H), 4.53 (dd, *J* = 8.7, 4.9 Hz, 1H), 3.67 (s, 3H), 2.08 (m, *J* = 6.9, 5.0 Hz, 1H), 0.82 (d, *J* = 6.9 Hz, 3H), 0.68 (d, J = 6.9 Hz, 3H).). 13C NMR (75 MHz, CDCl3): δ 171.6, 166.4, 143.7, 143.4, 133.6, 123.9, 123.1, 120.7, 109.5, 57.4, 52.3, 48.1, 30.8, 18.94, 17.6. In DMSO-*d*6: ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.77 (d, *J* = 8.2 Hz, 1H), 8.17 (s, 1H), 7.70 – 7.60 (m, 1H), 7.48 – 7.38 (m, 1H), 7.31 – 7.14 (m, 2H), 5.15 – 4.95 (m, 2H), 4.23 (dd, *J* = 8.2, 6.1 Hz, 1H), 3.65 (s, 3H), 2.08 (h, *J* = 6.7 Hz, 1H), 0.91 (dd, *J* = 11.5, 6.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-*d*6): δ 171.7, 167.0, 144.8, 143.2, 134.1, 122.3, 121.5, 119.4, 110.1, 57.5, 51.8, 46.5, 30.0, 18.9, 18.1. MS (APCI+): m/z = 290 $[M + H]^+$. HRMS (+APPI): *m/z* calcd for [$C_{15}H_{19}N_3O_3$]: 289.1426; found 289.1431.

Methyl 2-(2-(2-methyl-1*H*-benzo[d]imidazole-1-yl)acetamido)-3-methylbutanoate (2h).

Colourless solid, 776 mg (61%); mp 141.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.73–7.61 (m, 1H), 7.27–7.13 (m, 2H), 5.87 (d, *J* = 8.8 Hz, 1H), 4.73 (s, 1H), 4.44 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.59 (s, 2H), 2.54 (s, 2H), 2.00 (pd, *J* = 6.9, 4.9 Hz, 1H), 0.74 (d, *J* = 6.8 Hz, 2H), 0.55 (d, *J* = 6.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 171.5, 166.6, 151.8, 142.7, 134.8, 123.0, 122.9, 119.6, 108.7, 57.2, 52.3, 46.9, 30.7, 19.0, 17.4, 13.70. MS (APCI+): *m/z* = 304.2 [M + H]+. HRMS (+APPI): *m/z* calcd for [$C_{16}H_{21}N_3O_3$]: 303.1583; found 303.159.

Ethyl 2-(2-(1*H*-benzo[d]imidazole-1yl)acetamido)-4-methyl-pentanoate (2i).

Colourless solid, 772 mg (58%); mp 83 °C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.81 (s, 1H), 7.76–7.64 (m, 1H), 7.43–7.23 (m, 3H), 6.89 (d, *J* = 8.3 Hz, 1H), 4.87 (d, *J* = 1.9 Hz, 2H), 4.63 (td, *J* = 8.7, 5.1 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 1.63–1.40 (m, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.85 (dd, *J* = 14.7, 6.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.3, 166.4, 145.2, 143.4, 133.6, 123.8, 123.1, 120.3, 109.6, 61.5, 51.1, 48.2, 40.6, 24.8, 22.8, 21.6, 14.1. MS (APCI+): *m/z* = 318.2 $[M + H]^+$. HRMS (+APPI): *m/z* calcd for [$C_{17}H_{23}N_3O_3$]: 317.1739; found 317.1748.

Ethyl 2-(2-(2-methyl-1*H*-benzo[d]imidazole-1yl)acetamido)-4-methylpentanoate (2j).
Colourless solid, 834 mg (60%); mp 107.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.54 (m, 1H), 7.19 (dd, *J* = 3.5, 1.3 Hz, 3H), 6.13 (d, *J* = 8.5 Hz, 1H), 4.70 (s, 2H), 4.54 (ddd, *J* = 9.4, 8.4, 4.8 Hz, 1H), 4.05 (q, *J* = 7.1 Hz, 2H), 2.48 (s, 3H), 1.55–1.42 (m, 1H), 1.42–1.26 (m, 2H), 1.15 (t, *J* = 7.1 Hz, 3H), 0.77 (dd, *J* = 14.9, 6.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 166.6, 151.9, 142.6, 134.9, 122.9, 122.8, 119.4, 108.8, 61.5, 51.0, 46.9, 40.6, 24.9, 22.7, 21.6, 14.10, 13.7. MS (APCI+): *m/z* = 332.2 $[M + H]^+$. HRMS (+APPI): *m/z* calcd for $[C_{18}H_{25}N_3O_3]$: 331.1896; found 331.1903.

General procedure for the preparation of 7a-c: Concentrated H_2SO_4 (10.0 mL) was cooled in and ice-bath and with stirring 1.30 mmol of (**11a**, **11b** or **11c**) was added gradually then the solution was allowed to stir at room temperature for 4hr then it was neutralised using aqueous 30% ammonia solution. The formed solid was separated by filtration and washed with water and recrystallized from mixture of DMF and water.

2-(1*H*-Benzo[*d*]imidazol-1-yl)-*N*-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)methyl)acetamide (7a).
Colourless solid, 464 mg (98%);198 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (t, *J* = 5.9 Hz, 1H), 8.22 (s, 1H), 7.73 – 7.64 (m, 1H), 7.64 – 7.51 (m, 3H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.30 – 7.17 (m, 3H), 7.16 (s, 1H), 7.01 (t, *J* = 7.3 Hz, 1H), 5.06 (s, 2H), 4.57 (d, *J* = 5.9 Hz, 2H).^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2, 165.1, 164.9, 157.4, 157.1, 144.8, 143.2, 141.7, 140.7, 140.6, 134.2, 129.1, 126.5, 122.4, 121.8, 121.6, 119.4, 117.3, 116.2, 110.2, 46.6, 38.0. MS (APPI+): *m/z* = 365.11 $[M+1]^+$. HRMS (+APPI): *m/z* calcd for $[C_{18}H_{16}N_6OS]$: 364.1106, found 364.1119.

2-(2-Methyl-1*H*-benzo[*d*]imidazol-1-yl)-*N*-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)methyl)acetamide (7b).
Colourless solid, 481 mg (98%); 245 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (d, *J* = 5.6 Hz, 1H), 7.56 (qd, *J* = 7.8, 6.8, 2.2 Hz, 5H), 7.48 – 7.27 (m, 2H), 7.26 – 7.13 (m, 4H), 4.98 (s, 2H), 4.56 (dd, *J* = 5.9, 2.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2, 165.0, 164.8, 157.5, 157.2, 141.5, 140.7, 140.6, 129.0, 126.5, 121.8, 121.6, 117.8, 117.3, 116.2, 109.7, 45.61, 38.1, 13.3. MS (+APPI): *m/z* = 379.133$[M+1]^+$. HRMS (+APPI): *m/z* calcd for $[C_{19}H_{18}N_6OS]$: 378.1263, found 378.1265.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(2-methyl-1-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)pro-pyl)acetamide (7c).
Colourless solid, 507 mg (96%) 200 °C.^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (d, *J* = 8.6 Hz, 1H), 8.19 (s, 1H), 7.70 – 7.49 (m, 4H), 7.48 – 7.16 (m, 5H), 7.13 (s, 1H), 5.16 – 4.86 (m, 3H), 2.33 – 2.15 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6, 164.4, 164.2, 144.9, 140.7, 129.1, 126.6, 122.4, 121.8, 121.6, 119.3, 117.3, 116.2, 110.1, 54.6, 46.7, 32.1, 19.3, 18.5. MS (APPI+): *m/z* = 407.1656$[M+1]^+$. HRMS (+APPI): *m/z* calcd for $[C_{21}H_{22}N_6OS]$:406.1583, found 406.1583.

General procedure for the preparation of 8a-c: A solution of 1.30 mmol of **11a** (or **11b** or **11c**) in 5.0 mL water and 0.50 mL piperidine was heated at reflux for 7 h and after cooling was acidified with acetic acid. The formed solid was filtered and washed with water and dried before using in the next steps.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)acetamide (8a).
Colourless solid, 263 mg (55.5%) 295 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.89 (s, 1H), 8.79 (t, *J* = 5.2 Hz, 1H), 8.08 (s, 1H), 7.70 – 7.60 (m, 1H), 7.52 (qd, *J* = 4.5, 1.6 Hz, 3H), 7.45 – 7.33 (m, 3H), 7.33 – 7.14 (m, 2H), 4.84 (s, 2H), 4.19 (d, *J* = 5.2 Hz, 2H).^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 166.6, 149.1, 144.6, 142.5, 133.8, 133.2, 129.5, 129.4, 127.9, 122.7, 121.9, 119.1, 110.3, 46.2, 43.6, 34.2. MS (+APPI): *m/z* = 365.11$[M+1]^+$. HRMS (+APPI): calcd for $[C_{18}H_{16}N_6OS]$: 364.1106, found 364.1117.

***N*-((5-Mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)-2-(2-methyl-1*H*-benzo[d]imidazol-1-yl)acetamide (8b).**
Colourless solid, 260 mg (53%) 282 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.83 (s, 1H), 8.77 (t, *J* = 5.2 Hz, 1H), 7.59 – 7.35 (m, 6H), 7.34 – 7.22 (m, 1H), 7.22 – 7.06 (m, 2H), 4.75 (s, 2H), 4.17 (d, *J* = 5.2 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.1, 166.7, 152.5, 149.2, 141.8, 135.4, 133.2, 129.6, 129.4, 127.9, 121.6, 121.4, 118.0, 109.5, 45.2, 34.5, 13.3. MS (APPI+): *m/z* =379.133 $[M+1]^+$. HRMS (+APPI): calcd for $[C_{19}H_{18}N_6OS]$: 378.1268, found 378.1274.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(1-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)-2-methylpropyl)acetamide (8c).
Colourless solid, 274 mg (52%) mp 171-173 °C.^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (d, *J* = 8.3 Hz, 1H), 8.11 (d, *J* = 1.1 Hz, 1H), 7.70 – 7.58 (m, 1H), 7.49 (dd, *J* = 5.4, 2.0 Hz, 3H), 7.36 (ddd, *J* = 14.4, 6.9, 1.6 Hz, 3H), 7.23 (m,

2H), 4.91 (q, *J* = 16.6 Hz, 2H), 4.42 (t, *J* = 7.8 Hz, 1H), 2.07 (h, *J* = 6.8 Hz, 1H), 0.82 (dd, *J* = 24.2, 6.7 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 166.5, 151.8, 144.8, 142.88, 133.9, 133.2, 129.6, 129.4, 128.3, 122., 121.7, 119.3, 110.1, 50.4, 46.3, 30.7, 19.3, 18.1. MS (APPI+): *m/z* = 407.1664 [M+1]$^+$. HRMS (+APPI): *m/z* calcd for [$C_{21}H_{22}N_6OS$]: 406.1576, found 406.1591.

General procedure for the preparation of 9a-c: A solution of I_2/KI was added to a stirred ethanolic solution of 1.00 mmol of (**11a**, **11b** or **11c**) and 1.0 mL of aqueous 10% NaOH at room temperature until the colour of the iodine persisted. The mixture was then allowed to stir overnight. The formed solid was filtered, washed with water and ethanol to dryness.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-((5-(phenylamino)-1,3,4-oxadiazol-2-yl)methyl)acetamide (9a).

White solid 208.8 mg (60%) 225 ^{o}C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.06 (t, *J* = 5.6 Hz, 1H), 8.18 (s, 1H), 7.72 – 7.60 (m, 1H), 7.60 – 7.52 (m, 2H), 7.52 – 7.39 (m, 1H), 7.39 – 7.26 (m, 2H), 7.22 (ddd, *J* = 6.8, 4.5, 1.7 Hz, 2H), 7.06 – 6.93 (m, 1H), 5.03 (s, 2H), 4.49 (d, *J* = 5.5 Hz, 2H).^{13}C NMR (75 MHz, DMSO-d_6) δ 167.1, 160.0, 156.9, 144.8, 143.2, 138.7, 134.2, 129.0, 122.4, 121.8, 121.5, 119.3, 116.9, 110.2, 46.6, 33.6. MS (+APPI): *m/z* = 349.14 [M+1]$^+$. HRMS (+APPI): *m/z* calcd for [$C_{18}H_{16}N_6O_2$]: 348.1335, found 348.1341.

2-(2-Methyl-1*H*-benzo[d]imidazol-1-yl)-*N*-((5-(phenylamino)-1,3,4-oxadiazol-2-yl)methyl)-acetamide (9b).

Colourless solid, 220 mg (61%) 246-247 ^{o}C (decomp.).^{1}H NMR (300 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.10 (t, *J* = 5.7 Hz, 1H), 7.54 (t, *J* = 9.0 Hz, 3H), 7.35 (qd, *J* = 11.2, 10.0, 3.4 Hz, 4H), 7.22 – 7.08 (m, 3H), 7.00 (t, *J* = 7.4 Hz, 1H), 4.96 (s, 2H), 4.50 (d, *J* = 5.1 Hz, 2H), 2.49 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2, 160.0, **156.98**, 152.5, 142.2, 138.7, 135.7, 129.0, 121.8, 121.5, 121.2, 118.1, 116.9, 109.5, 45.6, 33.6, 13.5. MS (+APPI): *m/z* =363.15[M+1]$^+$. HRMS (+APPI): calcd for [$C_{19}H_{18}N_6O_2$]: 362.1491, found 362.1498.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(2-methyl-1-(5-(phenylamino)-1,3,4-oxadiazol-2-yl)propyl)acetamide (9c).

Colourless solid, 332 mg (85%) mp165 ^{o}C.^{1}H NMR (300 MHz, DMSO-d_6) δ 10.49 (s, 1H) 9.06 (d, *J* = 8.5 Hz, 1H), 8.19 (s, 1H), 7.73 – 7.29 (m, 5H), 7.21 (p, *J* = 7.2 Hz, 3H), 7.11 (s, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 5.21 – 4.96 (m, 2H), 4.88 (t, *J* = 7.9 Hz, 1H), 2.32 – 2.05 (m, 1H), 0.96 (dt, *J* = 24.6, 9.5 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.8, 159.8, 158.8, 144.9, 143.2, 138.7, 134.2, 129.1, 122.3, 121.8, 121.5, 119.4, 116.9, 110.1, 50.6, 30.6, **18.97**, 18.4. MS (APPI+): *m/z* = 391.1880 [M+1]$^+$. HRMS (+APPI): *m/z* calcd for [$C_{21}H_{22}N_6O_2$]:390.1804, found 390.1808.

General procedure for the preparation of 10a-c: A solution of 12.0 mmole **2a** (**2b** or **2g**) and 12.0 mmol of hydrazine hydrate in (20.0 mL) ethanol was heated at reflux. The reaction was monitored by TLC until the disappearance of the starting material. The mixture was cooled and the formed solid was filtered and washed with ethanol and water.

2-(2-(1*H*-Benzo[d]imidazol-1-yl)acetamido)acetohydrazide (10a).

Colourless solid, 2.52 g (85%) mp 222-223 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 8.63 (t, *J* = 5.8 Hz, 1H), 8.18 (s, 1H), 7.72 – 7.61 (m, 1H), 7.57 – 7.43 (m, 1H), 7.30 – 7.14 (m, 2H), 5.01 (s, 2H), 3.75 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 167.0, 144.9, 143.2, 134.3, 122.3, 121.5, 119.3, 110.4, 46.7, 40.9. MS (APPI+): *m/z* = 248.11 [M+1]$^+$. HRMS (+APPI): calcd for [$C_{11}H_{13}N_5O_2$]; 247.1069, found 247.1058.

2-(2-(2-Methyl-1*H*-benzo[d]imidazol-1-yl) acetamido)acetohydrazide (10b).

Colourless solid, yield, 2.60 g (83%) mp 221.5 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.59 (t, *J* = 5.8 Hz, 1H), 7.59 – 7.49 (m, 1H), 7.49 – 7.36 (m, 1H), 7.17 (qd, *J* = 7.4, 3.6 Hz, 2H), 4.94 (s, 2H), 4.26 (s, 2H), 3.74 (d, *J* = 5.7 Hz, 2H), 2.50 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 167.1, 152.5, 142.2, 135.7, 121.4, 121.1, 118.1, 109.6, 45.6, 40.81, 13.47. MS (+APPI): *m/z* = 262.13 [M+1]$^+$. HRMS (+APPI): calcd for [$C_{12}H_{15}N_5O_2$]: 261.1226, found 261.1228.

2-(2-(1*H*-Benzo[d]imidazol-1-yl)acetamido)-3-methylbutanehydrazide (10c).

Colourless solid, 3.92 g (84%) mp 218-219 ^{o}C .^{1}H NMR (300 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.54 (d, *J* = 9.0 Hz, 1H), 8.16 (s, 1H), 7.70 – 7.59 (m, 1H), 7.51 – 7.40 (m, 1H), 7.22 (pd, *J* = 7.2, 1.4 Hz, 2H), 5.15 – 4.98 (m, 2H), 4.26 (s, 1H), 4.11 (dd, *J* = 9.0, 7.2 Hz, 1H), 1.95 (hept, *J* = 6.8 Hz, 1H), 0.85 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.8, 166.4, 144.9, 143.2, 134.2, 122.3, 121.5, 119.3, 110.1, 56.6, 46.6, 30.7, 19.1, 18.3. MS (+APPI): *m/z* = 290.1603 [M+1]$^+$. HRMS (+APPI): *m/z* calcd for [$C_{14}H_{19}N_5O_2$]: 289.1539, found 289.1533.

General procedure for the preparation of 11a-c: A mixture of 8.00 mmole of **10a** (or **10b** or **10c**) in 20.0 mL ethanol and 8.00 mmole of phenylisothiocyanate was heated at reflux and the reaction was minitored by TLC. The formed solid was filtered, washed with ethanol and water, and dried in air.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(2-oxo-2-(2-(phenylcarbamothioyl)hydrazinyl)ethyl)-acetamide (11a).

Colourless solid, 2.72 g (89%) 235-238 ^{o}C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.20 (s, 1H), 9.71 (s, 1H), 8.78 (t, *J* = 5.4 Hz, 1H), 8.18 (s, 1H), 7.67 (dd, *J* = 6.6, 2.2 Hz, 1H), 7.55 – 7.39 (m, 1H), 7.39 – 7.18 (m, 6H), 7.13 (q, *J* = 8.4, 7.1 Hz, 1H), 5.06 (s, 2H), 3.89 (d, *J* = 5.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.7, 168.1, 144.7, 142.4, 138.3, 133.9, 128.1, 125.3, 124.9, 122.9, 122.1, 119.1, 110.4, 46.4. 41.4. MS (APPI+): *m/z* =383.127 $[M+1]^{+}$. HRMS (+APPI): calcd for $[C_{18}H_{18}N_6O_2S]$: 382.1212, found 382.1195.

2-(2-Methyl-1*H*-Benzo[d]imidazol-1-yl)-*N*-(2-oxo-2-(2-(phenylcarbamothioyl)hydra-zinyl)ethyl)acetamide (11b).

Colourless solid, yield, 2.34 g (74%) 260-263 ^{o}C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.20 (s, 1H), 9.72 (s, 1H), 9.36 – 9.29 (m, 1H), 8.77 (t, *J* = 5.4 Hz, 1H), 7.58 – 7.48 (m, 1H), 7.47 – 7.30 (m, 3H), 7.24 (t, *J* = 7.6 Hz, 3H), 7.14 (dt, *J* = 9.0, 3.7 Hz, 3H), 4.98 (s, 2H), 3.88 (d, *J* = 5.3 Hz, 2H), 2.47 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 167.9, 152.7, 141.7, 138.5, 135.4, 128.1, 125.0, 124.8, 121.8, 121.5, 118.0, 109.6, 45.4, 41.3, 13.2. MS (+APPI): *m/z* =397.144$[M+1]^{+}$. HRMS (+APPI): calcd for $[C_{19}H_{20}N_6O_2S]$: 396.1367, found 396.1368.

2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(3-methyl-1-oxo-1-(2-(phenylcarbamothioyl)hydrazinyl)butan-2-yl)acet-amide (11c).

Colourless solid, 2.19 g (64.7%) 202 ^{o}C (decomp).^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (s, 1H), 9.74 (s, 1H), 8.82 (s, 1H), 8.17 (s, 1H), 7.74 – 7.60 (m, 1H), 7.55 – 7.42 (m, 1H), 7.28 – 7.14 (m, 4H), 7.11 (s, 1H), 7.04 (dd, *J* = 8.1, 5.8 Hz, 2H), 5.10 (d, *J* = 3.2 Hz, 2H), 3.34 (s, 1H), 2.08 (h, *J* = 6.6 Hz, 1H), 0.98 (dd, *J* = 17.4, 6.7 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.3, 144.8, 143.2, 138.7, 134.3, 127.9, 124.5, 123.7, 122.4, 121.5, 119.3, 110.2, 56.0, 46.4, 29.6, 19.0, 18.6. MS (APPI+): *m/z* = 425.1748 $[M+1]^{+}$. HRMS (+APPI): *m/z* calcd for $[C_{21}H_{24}N_6O_2S]$:424.1681, found 424.1677.

2.2 Sample Preparation and Antimicrobial Assays

The *in vitro* antimicrobial activities of the compounds reported herein were evaluated using the agar well diffusion method [8] against two Gram positive bacteria (*S. pneumoniae* and *B. subtilis*), two Gram negative bacteria (*P. aeruginosa* and *E. coli*), using nutrient agar medium. Antifungal activity was determined against two fungi (*Aspergillius fumigatus* and *C. albicans*). Sabouraud Dextrose Agar medium. ampicillin, ciprofloxacin and amphotricin B were used as the standard drugs for the Gram positive, Gram negative and antifungal activities respectively. DMSO was used as the solvent control. The compounds were tested at concentrations of 5.0 mg/mL against both the bacterial and fungal strains.

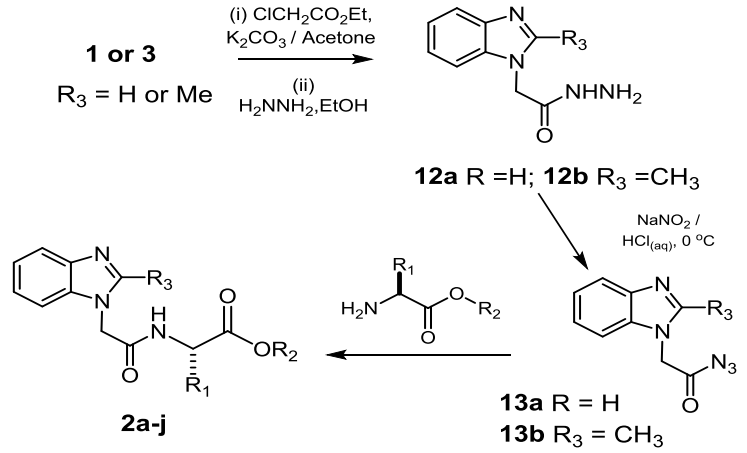
The sterilized media was poured into sterilized Petri dishes (20 mL/petri dish) and allowed to solidify. Wells of 6-mm diameter were made in the solidified media with the help of a sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of the solidified media, and solutions of the tested samples were micropippetted into each well. The plates were incubated at 37 °C for 24 h for the antibacterial assays and 48 h at 25°C for the antifungal assays. The experiments were carried out in triplicate and each of the zones of inhibition were measured. The results for the observed mean zones of inhibition (mm) are summarized in Table 1. None of the compounds showed any activity against *P. aeruginosa* and are therefore not included in the Table.

Minimum inhibitory concentrations (MICs) summarized in Table 2 were determined by the broth micro dilution method using 96-well micro-plates [9]. The inoculates of the microbial strains were prepared from the 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (1.0 mg) was dissolved in DMSO (1.0 mL) to obtain 1000 µg/mL stock solutions. A number of wells were reserved in each plate for the positive and negative controls. Sterile broth (100 µL) was added to the well from rows B to H. The stock solutions of samples (100 µL) were added to the wells in rows A and B. Then, the mixture of samples and sterile broth (100 µL) in row B were transferred to each well in order to obtain a two-fold serial dilution of the stock samples (concentrations of 500, 250, 125, 62.5, 31.3, 15.6 and 7.81, 3.9, 1.95, 0.98 and 0.49 µg/mL). The inoculums (100 µL) were added to each well and final volumes of 200 µL were obtained in each well. Plates were incubated at 37 °C for 24 h for the antibacterial assays and for 48 h at 25 °C for the antifungal assays. Microbial growth was indicated by the presence of turbidity of the well. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

3. Results and Discussion

3.1 Synthesis

Initially, attempts were undertaken to form the desired benzimidazole-acetamido derivatives **2a-j** using either **13a** or **13b** [10a,b], by analogy with the Curtius rearrangement [11] methodology used by Abdel-Rahman *et al.* [12] for their elaboration of the indole N atom with amino acid esters in which the appropriate amino acid esters were coupled to the indole nucleus via the *N*-acetamidohydrazide (Scheme 3).



Scheme 3: Synthesis scheme via Curtius rearrangement of 13a or **13b**

Using the procedure of Abdel-Rahman *et al.* the corresponding azides **13a/b** were obtained from **12a/b** using $NaNO_2$ and hydrochloric acid, at 0 ^oC (Scheme 3). The azide intermediate was reacted with the free amino acid esters but only small amounts of the desired products were obtained from complex mixtures which formed. This methodology required a high degree of temperature control since the azides are unstable above 5 ^oC, and long reaction times of up to 24 h to produce **2a-j** from the reaction of the amino acid esters with the precursor azides.

Since we encountered difficulties in our attempts using the hydrazide approach, we investigated alternative methodologies.[13] Chloroacetyl chloride [10a,b; 14a,b] was then employed to produce the corresponding chloroacetamido amino acid esters without first needing to protect the nitrogen atoms of the amino acid esters. Schotten-Baumann conditions were not suitable with the corresponding amino acid esters and led to the formation of complex product mixtures. The free amino acid esters from the corresponding hydrochloride salts therefore were first generated in DMF. After filtering off the precipitated triethylammonium chloride the filtrate was worked-up in the usual manner by washing with brine solution and extracting with ethyl acetate. The organic extracts were dried and evaporated to dryness, and the residue (which contained traces of DMF, especially in the case of the glycine ethyl ester) dissolved in dioxane and added to benzimidazole or 2-methylbenzimidazole. The use of dioxane, with trimethylamine, to effect the coupling to the N-1 position of the benzimidazoles failed to afford the desired product(s) cleanly and only complex mixtures were once again formed requiring tedious purification.

The use of potassium carbonate in dry acetone, however, proved effective for the desired coupling step which afforded the target benzimidazole-acetamido derivatives **2a-j** in good and convenient isolated yields. Typically, glycine ethyl ester hydrochloride (**5**: R_1 = H; R_2 = Et) was first converted to its corresponding free amino acid methyl ester **6** (R_1 = H; R_2 = Et) by treatment with triethylamine in DMF for 1 h. After filtration, triethylamine was added to the filtrate and after cooling on an ice-bath, chloroacetyl chloride was added in one portion. The mixture was stirred for 15 min with ice-bath cooling and then was allowed to warm to room temperature and stirred for to a further 1-2 h, as monitored by TLC. The mixture was then quenched with brine and extracted with ethyl acetate; the combined organic layers were dried and filtered in the usual manner, and the solvent evaporated on a rotavap. The residue was dissolved in dry acetone and then added to a mixture of anhydrous K_2CO_3 and benzimidazole in dry acetone. When the reaction was finished as ascertained by TLC monitoring, the mixture was filtered and the filtrate evaporated to dryness on a rotavap. The resulting mixture was purified by flash column chromatography on silicagel using hexane:ethanol 80:20 as eluant to afford the respective products, **2a** from benzimidazole (**1**); and **2b** when 2-methyl benzimidazole (**3**) was used.

No apparent potential epimerization was detected with the respective products. This was checked by testing, for example, an equimolar amount of **2g** with Pirkle's NMR shift reagent.[15] There were no changes in the

resulting ^{1}H-NMR spectra that could be attributed to the presence of any epimerization or racemization. Only shifts in the position of the N–H and N–CH_2– protons could be observed.

Subsequent examination of the literature revealed that there were only two previous examples that had been reported in which the secondary amine of benzimidazole itself had been elaborated with an acetamido substituent. Jung and coworkers reported the reaction of benzimidazole with methyl 2-(2-bromoacetamido)-3-methylbutanoate derived from valine methyl ester, under strongly basic KOH conditions in DMF at room temperature, over 16 h to form **2g**. [16] Their product was used only as a precursor to produce a Pd(II) complex as a catalyst for a three-component Strecker reaction, although they did not describe the procedure they used for the synthesis of the methyl 2-(2-bromoacetamido)-3-methylbutanoate. When we repeated Jung's procedure using the corresponding methyl 2-(2-chloroacetamido)-3-methylbutanoate **7** (R_1 = *i*-Pr; R_2 = Me) intermediate, ~ 50% yields of **2g** were obtained which was comparable to our method described herein. In the only other example, Sakaguchi's group reported the corresponding benzimidazole serine methyl ester conjugate as a precursor to a chiral ligand with $Cu(OTf)_2$ in conjugate addition reactions of dialkylzinc reagents to cyclic enones. [17] Neither authors however reported on the potential biological properties of their specific products.

Since thiadiazoles [18a,b], triazoles [19] and oxadiazoles [20] have reported extensive biological properties [21] it was of interest to extend our methodology to incorporating these individual motifs into the benzimidazole-acetamido framework. Therefore, compounds **7a-c**, **8a-c** and **9a-c** were synthesized as shown in Scheme 3 and their antimicrobial activities were evaluated.

The syntheses of **7a-c** were achieved by treating the corresponding intermediates **11a-c** with concentrated sulfuric acid. The HRMS data of all of the new compounds were consistent with the expected structures and with the exception of **7a** all of the NMR spectra of **7b** and **7c** were straightforward. In DMSO-*d*6 at ambient temperature the ^{1}H NMR of **7a** revealed, among other signals which could be assigned, a broad doublet centred at δ = 10.33 ppm, each corresponding to 0.5 of a proton, which could be assigned to the phenyl-N–H signal. Upon heating to 40 ^{o}C these two signals merged into a broad singlet, clearly suggesting that a dynamic equilibrium was occurring between presumably the amine and imine tautomeric forms. [20a,b]

The syntheses of **8a-c** [18] and **9a-d** [19] were also conducted using standard methods and are described in the Experimental. All of the analytical spectral and mass data were consistent with the expected structures.

3.2 Antimicrobial Screening of compounds 2a-j; 7a-c, 8a-c and 9a-c

Compounds **10a** and **10c** showed antimicrobial activity against the two Gram positive and Gram negative bacteria *E coli*, and also the two fungi. The 2-methylbenzimidazole analogue **10b** however only showed activity against the two Gram positive bacteria. In general, with the exceptions noted below, most of the derivatives that contain the benzimidazole ring showed antimicrobial activity in terms of greater zones of inhibition than the corresponding 2-methylbenzimidazole derivatives.

With thiadiazoles 7**b** and **7c**, phenylcarbamothioyl-hydrazinyl compounds **11b** and **11c**, and with the alanine, phenylalanine and valine ester derivatives of 2-methylbenzimidazole *i.e.* **2d**, **2f** and **2h**, (unlike the corresponding benzimidazole derivatives **2c**, **2e** and **2f**) no inhibition of microbial growth was observe. In the case of **8b** it was found that this 2-methylbenzimidazole derivative was more active than the corresponding benzimidazole analogue **8a**. In both benzimidazole **2i** and 2-methylbenzimidazole **2j** leucine ester derivatives, similar antimicrobial activities were seen. Whereas **2a** showed activity against all of the tested microbes (except with *P. aeruginosa*), the corresponding 2-methylbenzimidazole:ethyl gycinyl ester **2b** showed inhibition of the microbial growth of only the Gram positive bacteria (*S. pneumoniae* and *B. subtilis*) and the fungus *Aspergilius fumigatus*. MICs (Table 2) were measured for the five compounds namely, **2g**, **8b**, **8c**, **10a** and **10c** which showed the largest zones of inhibition of microbial growth. Compounds **2g, 2b** and **10c** were able to inhibit the growth of the *E. coli* at MICs of 0.98 µg/mL which is the same MIC for the Ciprofloxacin under the same testing conditions employed herein.

In conclusion, we have demonstrated herein that the benzimidazole-amino acid ester conjugates **2a-j** can be prepared by a simple, inexpensive and reproducible method which does not require the protection of the nitrogen atom of the amino acid esters used. As well, a series of thiadiazoles, triazoles and oxazoles were prepared and some of their antimicrobial properties were examined. We plan to extend this general methodology to generate other benzimidazole-based derivatives for evaluation of their potential biological properties.

Table 1: Mean diameters ± SDs (mm) of the inhibition zones against the corresponding standard strains of the different organisms. All assays were conducted in triplicate.

Compounds	Gram positive bacteria		Gram negative bacteria	Fungi	
	S. pneumoniae RCMB 010010	*B. subtilis* RCMB010067	*E. coli* RCMB 010052	*Aspergillius fumigatus* RCMB 02568	*C. albicans* RCMB 05036
2a	18.3±1.2	19.4±1.5	11.4±0.63	20.3±1.5	14.3±0.63
2b	15.1±0.44	19.8±0.58	-	15.8±0.58	-
2c	11.9±0.63	15.4±1.2	11.4±0.63	17.9±1.2	13.9±1.5
2d	-	-	-	-	-
2e	20.0±1.5	21.7±0.53	18.3±1.2	21.3±0.58	17.2±1.2
2f	-	-	-	-	-
2g	**22.3±0.44**	25.6±.063	**22.1±0.19**	**22.6±0.58**	21.3±0.58
2h	-	-	-	-	-
2i	16.3±0.38	18.8±0.17	14.3±0.42	16.7±0.23	15.3±0.27
2j	16.2±0.25	16.9±0.44	12.5±0.19	16.8±0.38	16.2±0.19
7a	16.2±1.2	16.9±1.5	12.5±0.63	16.8±0.63	14.4±0.58
7b	-	-	-	-	-
7c	-	-	-	-	-
8a	17.3±0.56	18.4±0.67	17.3±0.72	15.6±0.63	12.2±0.36
8b	21.3±0.44	23.4±0.53	19.3±0.23	19.3±0.53	17.4±0.44
8c	21.5±0.36	23.7±0.58	21.3±0.68	20.3±0.25	17.9±0.44
9a	16.4±1.5	18.5±0.63	19.1±0.58	15.7±0.19	13.6±1.2
9b	15.3±0.44	15.6±0.63	12.4±1.5	14.2±0.58	12.6±1.2
9c	20.3±1.5	21.3±0.63	20.6±1.5	17.8±1.2	14.9±0.58
10a	**23.1±1.5**	26.3±1.2	**22.4±0.58**	**23.3±0.58**	21.4±1.2
10b	9.3±0.58	11.4±0.44	-	-	-
10c	**22.3±0.44**	24.3±0.28	**21.7±0.44**	**21.6±0.58**	20.3±1.5
11a	19.3±0.63	20.1±0.58	21.2±1.2	17.1±1.2	19.9±1.5
11b	-	-	-	-	-
11c	-	-	-	-	-
Amphotericin B	-	-	-	23.7±1.2	25.4±0.58
Ampicillin	23.8±1.2	32.4±2.1	-	-	-
Ciprofloxacin	-	-	23.4±0.63	-	-
DMSO	-	-	-	-	-

Table 2: Antimicrobial activity as MICs (µg/mL) of tested samples against tested microorganisms

Supplementary data

^{1}H and ^{13}C-NMR spectra of all compounds, MS/HRMS of all new compounds described in this article can be found on the online version at **doiXXXXXX** or can be requested directly from the authors.

Compounds	Gram positive bacteria		Gram negative bacteria	Fungi	
	S. pneumoniae RCMB 010010	*B. subtilis* RCMB010067	*E. coli* RCMB 010052	*Aspergillius fumigatus* RCMB 02568	*C. albicans* RCMB 05036
2g	0.98	0.49	**0.98**	0.98	1.98
8b	0.98	0.49	**0.98**	0.98	1.98
8c	1.95	0.49	1.95	3.9	7.81
10a	1.95	0.98	3.9	3.9	15.63
10c	0.98	0.49	**0.98**	3.9	1.95
Amphotericin B	-	-	-	0.49	0.49
Ampicillin	0.49	0.29			
Ciprofloxacin	-	-	**0.98**	-	-
DMSO	-	-	-	-	-

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