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ORIGINAL ARTICLE

Synthesis, antimicrobial and cytotoxic activity of novel azetidine-2-one derivatives of 1*H*-benzimidazole

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Abstract A series of 1-methyl-*N*-[(substituted-phenylmethylidene)-1*H*-benzimidazol-2-amines **(4a–4g)** were prepared via the formation of 1-methyl-1*H*-benzimidazol-2-amine **(3)**, which was prepared by the cycloaddition of *o*-phenylenediamine **(1)** with cyanogen bromide in the presence of aqueous base followed by N-methylation with methyl iodide in the presence of anhydrous potassium carbonate in dry acetonitrile. Moreover, the four-membered β-lactam ring was introduced by the cycloaddition of **4a–4g** and chloroacetyl chloride in the presence of triethylamine catalyst to give 3-chloro-1-(1-methyl-1*H*-benzimidazol-2-yl)-(4′-substituted)-phenylazetidin-2-one **5a–5g**. A total of 14 compounds were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral technique, in addition they were evaluated for anti-bacterial and cytotoxic properties. Among the chemicals tested **4a**, **4b**, **5a**, **5b**, **5g** exhibited good antibacterial activity and **5f**, **5g** shown good cytotoxic activity in vitro.

1. Introduction

Despite numerous attempts to search and develop new structural prototype as effective antimicrobials, benzimidazoles still remain as potential class of compounds. Recently, the chemistry and biological profiles of various pharmacophores of N-1 substituted and 2-substituted benzimidazole derivatives have been worked out (Ansari and Lal, 2008). Effect of substituents on the benzimidazole ring exhibited correlated structure–activity relationship (Powers et al., 2006). Incorporation of an imidazole nucleus, a biologically active pharmacophore, in the benzimidazole molecule has made it a versatile heterocycle with wide spectrum of biological activity. Moreover, benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allow them to interact easily with the biophores (Starcevic et al., 2007). Therefore, numerous biological activities of benzimidazoles derivatives have been described; antimicrobial (Kus et al., 2009), anticancer (Thimmegowda et al., 2008), anti inflammatory (Mader et al., 2008), antiviral (Vazquez et al., 2001),

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antiparasitic (Kazimierczuk et al., 2002), antiprotozoal (Gomez et al., 2008), antihelminitics (Dahiya and Pathak, 2007), protein kinase inhibitors (Bernatowicz et al., 2009) and H^+/K^+ ATPase inhibitors (Cho et al., 2001). Polyfunctionality of 2-aminobenzimidazole molecule resulting from the cyclic guanidine moiety has made it a building block for the synthesis of a large number of derivatives of pharmacological interest (Mavrova et al., 2007). Different substituted 2-aminobenzimidazoles have been found to possess in vivo and in vitro growth inhibition activity against various strains of bacteria, fungi and yeast.

On the other side, literature survey revealed that 2-azetidinones are associated with various pharmacological activities. A large number of 3-chloro monocyclic β-lactam having substitution at positions 1 and 4 possess powerful anti-bacterial, anti-microbial, sedative, anti-fungal and anti-tubercular activity (Kagan and Luche, 1968; Mohamed et al., 2006). With the above facts and in continuation of our research for newer anti-cancer and anti-microbial agents in the present study, we report here the synthesis of 2-azetidinone derivatives of 2-amino-1*H*-benzimidazoles to develop potential drug candidates of this class (Manjula et al., 2009; Badiger et al., 2006). These molecules operate by forming a covalent adduct with the membrane-bound bacterial transpeptidase, also known as penicillin binding proteins (PBPs), involved in the biosynthesis of cell walls. These mechanism-based inhibitors prevent the construction of cell wall and eventually lead to cell lysis and death (Halve et al., 2007).

2. Material and method

2.1. Chemistry

All chemicals and solvents were supplied by Merck, S.D. Fine-chem limited, Mumbai. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF_{254} silica gel, 0.2 mm layer thickness (E. Merck). Various solvent systems used for developing the chromatograms were (a) chloroform/methanol (9:1), (b) chloroform/methanol (9.5:0.5), (c) ethyl acetate/pet-ether (2:1) and (d) chloroform/benzene (1:1). Columns of different sizes packed with G60 (70–230 mesh) silica gel was used for purification. Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was acquired on a Shimadzu Infra Red Spectrometer, (model FTIR-8400S). ^{1}H NMR spectra of the synthesized compounds were performed in DMSO with a Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Punjab University (Chandigarh). ^{13}C NMR spectra of the synthesized compounds were scanned with a Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in Regional Research Laboratory (RRL), Jammu Tawi. Chemical shifts are reported in δ scale (ppm). Mass spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University, Chandigarh and Regional Research Laboratory (RRL), Jammu Tawi.

2.1.1. Synthesis of 2-aminobenzimidazoles (**2**)

o-Phenylenediamine **1** (43.2 g, 0.4 mol) was stirred with water (400 mL) in an ice-bath and cyanogen bromide (35.5 g, 0.333 mol) was added drop wise during 1 h. The mixture was stirred for 3 h, filtered, and made basic with aqueous sodium hydroxide, the base being precipitated and filtered off. The solid obtained was filtered, and crystallized from water to give **2**, yield 70.1%, mp 226–228 °C, λ_{max} (methanol) 212 nm; IR (KBr, *v*, cm^{-1}): 3383 and 3060 (–NH, –NH_2), 1168 and 1568 (NH bend), 1269 and 1313 (–C–N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.6 (s, 2H, NH_2), 6.7 (s, 1H, NH), 7.10–7.28 (m, 4H, Ar-H); ^{13}CNMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [154.63 (C), 138.48 (C), 134.89 (C), 122.67 (CH), 116.29 (CH), 118.54 (CH)]; HRMS calcd for $C_3H_7N_3$: 133.1511, found 133.1592.

2.1.2. Synthesis of 1-methyl-1H-benzimidazol-2-amine (**3**)

Compound **2** (2.6 g, 0.02 mol) and potassium carbonate (2.6 g, 0.02 mol), acetonitrile (30 mL) methyl iodide (0.75 mL, 0.01 mol) were refluxed under nitrogen overnight at 50 °C then added water and extracted with ethyl acetate. Dried with $MgSO_4$ and recrystallized from ethanol to give compound **3**, yield 78%, mp 201–203 °C (in water), 209–210 °C (in acetone), λ_{max} (methanol) 211 nm; IR (KBr, *v*, cm^{-1}): 3374 (–NH, –NH_2), 2921 and 2849 (C–CH_3), 1314 and 1270 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.9 (s, 2H, NH_2), 3.5 (s, 3H, CH_3), 7.20–7.48 (m, 4H, Ar-H); ^{13}CNMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [153.67 (C), 139.47 (C), 132.27 (C), 121.34 (CH), 116.29 (CH), 111.25 (CH)], 27.89 (N–CH_3); HRMS calcd for $C_8H_9N_3$: 147.1771, found 147.1751

2.1.3. General procedure for the synthesis of compounds (**4a–4g**)

The 2-amino-1-methyl benzimidazole **3** (0.01 mol) was added to a solution of the different substituted benzaldehydes (0.012 mol) in dry ethanol 40 mL in RBF. Dry benzene (10 mL) and two drops of glacial acetic acid were also added to the above mixture. The mixture was refluxed for 8–20 h and at the end of the reaction; solvents were partially evaporated then poured into water. The precipitates were collected by filtration, washed with ether, dried and compounds **4a–4g** were synthesized and recrystallized from the appropriate solvent like ethanol or ethanol–water.

*2.1.3.1. N-[(4-Chlorophenyl) methylidene]-1-methyl-1H-benzimidazol-2-amine (**4a**).* This was prepared and purified as per the above mentioned procedure: yield 58%, mp 216–218 °C, λ_{max} (methanol) 208.4 nm; IR (KBr, *v*, cm^{-1}): 1432 (–N=CH), 1092 (Ar-Cl), 2925 and 2869 (N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.46 (s, 3H, N–CH_3), 7.35–7.82 (4H, Ar-H), 7.11–7.30 (m, 4H, Ar-H), 9.3 (s, 1H, N=CH); ^{13}CNMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.14 (C), 140.47 (C), 134.2 (C), 120.98 (CH), 114.25 (CH), 111.25 (CH)], Arom-C: [135.66 (C), 133.57 (C), 131.67 (CH), 130.76 (CH)], 156.67 (N=CH), 26.18 (N–CH_3); HRMS calcd for $C_{15}H_{12}ClN_3$: 269.7291, found 269.7234.

*2.1.3.2. 1-Methyl-N-[(4-nitrophenyl) methylidene]-1H-benzimidazol-2-amine (**4b**).* This was prepared and purified as per the above mentioned procedure: yield 58.9%, mp 248–250 °C, λ_{max} (methanol) 208.5 nm; IR (KBr, *v*, cm^{-1}): 1432 (N=CH), 1515 (–N=O), 1342 (–N=O), 855 and 834 (–C–NO), 2929 and 2849 (N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.42 (s, 3H, –N–CH_3), 7.62–8.34 (4H, Ar-H), 7.14–7.43 (m, 4H, Ar-H), 9.53 (s, 1H, –N=CH); ^{13}C NMR (400 MHz, DMSO-d_6) δ

(ppm): Benzimidazole C: [151.34 (C), 149.41 (C), 132.27 (C), 120.76 (CH), 116.24 (CH), 110.78 (CH)], Arom-C: [136.2 (C), 131.57 (C), 126.88 (CH), 124.98 (CH)], 156.15 (N═CH), 26.87 (N–CH_3); HRMS calcd for $C_{15}H_{12}N_4O_2$: 280.2831, found 280.2842.

*2.1.3.3. 1-Methyl-N-[(2-nitrophenyl) methylidene]-1H-benzimidazol-2-amine (**4c**).* This was prepared and purified as per the above mentioned procedure: yield 61.4%, mp 149–151 °C, λ_{max} (methanol) 252 nm; IR (KBr, *v*, cm^{-1}): 1429 (–N═CH), 1519 (–N═O), 1342 (–N═O), 854 (–C–NO), 2831 and 2881 (–N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, N–CH_3), 7.12–8.30 (m, 8H, Ar-H), 9.54 (s, 1H, –N═CH); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.76 (C), 140.46 (C), 136.23 (C), 120.80 (CH), 114.21 (CH), 110.78 (CH)], Arom-C: [135.81 (C), 130.41 (C), 133.98 (CH), 130.93 (CH), 128.17 (CH), 124.32 (CH)], 156.74 (N═CH), 26.24 (N–CH_3); HRMS calcd for $C_{15}H_{12}N_4O_2$: 280.2821, found 280.2823.

*2.1.3.4. 1-Methyl-N-[(3-nitrophenyl) methylidene]-1H-benzimidazol-2-amine (**4d**).* This was prepared and purified as per the above mentioned procedure: yield 62.5%, mp 180–182 °C, λ_{max} (methanol) 209.2 nm; IR (KBr, *v*, cm^{-1}): 1427 (–N═CH), 1518 (N═O), 1342 (–N═O), 846 (–C–NO), 2935 and 2850 (–N–CH_3); ^{1}H NMR (DMSO): 2.44 (s, 3H, –N–CH_3), 7.20–7.40 (m, 4H, Ar-H), 7.82–8.24 (m, 3H, Ar-H), 9.20 (s, 1H, –N═CH); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.56 (C), 139.49 (C), 133.27 (C), 120.98 (CH), 118.21 (CH), 110.78 (CH)], Arom-C: [132.45.0 (C), 131.18 (C), 129.78 (CH), 128.67 (CH), 126.72 (CH), 124.96 (CH)], 158.89.0 (N═CH), 26.23 (N–CH_3); HRMS calcd for $C_{15}H_{12}N_4O_2$: 280.2812, found 280.2818.

*2.1.3.5. N-{[4-(Dimethylamino) phenyl] methylidene}-1-methyl-1H-benzimidazol-2-amine (**4e**).* This was prepared and purified as per the above mentioned procedure: yield 51%, mp 120–124 °C, λ_{max} (methanol) 343 nm; IR (KBr, *v*, cm^{-1}): 1440 (–N═CH), 2949 and 2834 (–N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.35 (s, 3H, N–CH_3), 2.90 (s, 6H, N(CH_3)$_2$, 7.10–7.56 (m, 8H, Ar-H), 9.34 (s, 1H, N═CH); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.78 (C), 139.4 (C), 132.2 (C), 120.89 (CH), 118.21 (CH), 110.78 (CH)], Arom-C: [131.64 (C), 125.99 (C), 127.37 (CH), 110.19 (CH)], 158.89.0 (N═CH), 26.34 (N–CH_3), 40.34 [N–(CH_3)$_2$]; HRMS calcd for $C_{17}H_{18}N_4$: 278.3522, found 278.3541.

*2.1.3.6. N-[(2,5-Dimethoxyphenyl) methylidene]-1-methyl-1H-benzimidazol-2-amine (**4f**).* This was prepared and purified as per the above mentioned procedure: yield 53.5%, mp 157–159 °C, λ_{max} (methanol) 343 nm; IR (KBr, *v*, cm^{-1}): 1440 (–N═CH), 2904 and 2820 (–N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, N–CH_3), 3.48 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3), 7.12–7.45 (m, 4H, Ar-H), 7.51 (m, 2H, Ar-H), 7.67 (s, 1H, Ar-H), 9.38 (s, 1H, N═CH); ^{13}CNMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.16 (C), 138.42 (C), 136.25 (C), 120.76 (CH), 115.23 (CH), 110.89 (CH)], Arom-C: [134.23 (C), 132.49 (C), 130.71 (C), 117.63 (CH), 115.34 (CH), 112.14 (CH)], 158.34 (N═CH), 26.89 (N–CH_3), 45.8 (OCH_3); HRMS calcd for $C_{17}H_{17}N_3O_2$: 295.3362, found 295.3321.

*2.1.3.7. N-[(2-Chlorophenyl) methylidene]-1-methyl-1H-benzimidazol-2-amine (**4g**).* This was prepared and purified as per the above mentioned procedure: yield 55.5%, mp 235–237 °C, λ_{max} (methanol) 252.6 nm; IR (KBr, *v*, cm^{-1}): 1430 (–N═CH), 1091 and 822 (Ar-Cl), 2925 and 2868 (N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, N–CH_3), 7.15–7.80 (m, 8H, Ar-H), 9.58 (s, 1H, N═CH); ^{13}CNMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [151.21 (C), 141.54 (C), 132.62 (C), 120.23 (CH), 114.2 (CH), 110.34 (CH)], Arom-C: [132.91 (C), 131.41 (C), 130.43 (CH), 128.31 (CH), 126.24 (CH), 122.98 (CH)], 158.12 (N═CH), 26.98 (N–CH_3); HRMS calcd for $C_{15}H_{12}ClN_3C_{l2}$: 269.2791, found 269.2762.

*2.1.4. General procedure for the synthesis of compounds (**5a–5g**)* Schiff bases **4a–4g**, (0.04 mol) and triethylamine (0.02 mol) in dioxan (20 mL) at 0–5 °C mixture was stirred for 5 h. During stirring, chloroacetyl chloride (0.01 mol) in dioxan (10 mL) was added dropwise. The mixture was refluxed for 2 h and kept for two days on room temperature. The resulting mixture was poured in the water and the solid was separated out. Recrystallization was done with ethanol–water or chloroform–water to give the azetidine-2-one, **5a–5g** compounds.

*2.1.4.1. 3-Chloro-4-(4-chlorophenyl)-1-(1-methyl-1H-benzimidazol-2-yl) azetidin-2-one (**5a**).* This was prepared and purified as per the above mentioned procedure: yield 47.7%, mp 160–162 °C, λ_{max} (methanol) 203.8 nm; IR (KBr, *v*, cm $^{-1}$): 2925 and 2852 (N–CH_3), 1685 (C═O), 813 and 741 (Cl–C), 1094 (Ar-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.12 (s, 1H, –CH), 2.43 (s, 3H, N–CH_3), 6.72 (s, 1H, –CH), 7.14–7.34 (m, 4H, Ar-H), 7.41–8.47 (4H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [155.93 (C), 137.21 (C), 135.16 (C), 123.41 (CH), 116.23 (CH), 111.45 (CH)], Arom-C: [140.18 (C), 130.13 (C), 126.35 (CH), 125.48 (CH)], 29.45 (N–CH_3), β-lactam C: [164.42 (C═O), 62.53 (CHCl), 64.61 (CH)]; LC–MS *m*/*z* (% RA): M + 1 = 347.3 (35%), 331.3 (55%), 305.2 (33%), 274.0 (25%), 174.2 (87%), 122.0 (19%), 106.0 (45%), 104.0 (100%) and HRMS calcd for $C_{17}H_{13}ON_3C_{l2}$: 346.2106, found 346.2112.

*2.1.4.2. 3-Chloro-1-(1-methyl-1H-benzimidazol-2-yl)-4-(4-nitrophenyl) azetidin-2-one (**5b**).* This was prepared and purified as per the above mentioned procedure: yield 51.96%, mp 195–197 °C, λ_{max} (methanol) 204 nm; IR (KBr, *v*, cm^{-1}): 1682 (–C═O), 2921 and 2849 (–N–CH_3), 1520 (–N═O), 1344 (–N═O), 864 and 816 (–C–NO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.11 (s, 1H, –CH), 2.48 (s, 3H, N–CH_3), 6.75 (s, 1H, –CH), 7.10–8.47 (2H, Ar-H); 7.10–7.28 (m, 4H, Ar-H), 7.42–8.49 (4H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [155.78 (C), 136.43 (C), 132.65 (C), 123.63 (CH), 114.62 (CH), 112.16 (CH)], Arom-C: [148.71 (C), 146.79 (C), 122.14 (CH), 122.23 (CH)], 29.64 (N–CH_3), β-lactam C: [164.55 (C═O), 62.31 (CHCl), 63.35 (CH)]; LC–MS *m*/*z* (% RA): M + NH_3 = 373.2 (41%), 353.3 (30%), 331.3 (54%), 284.4 (22%), 174.2 (25%), 158.1 (14%), 102.1 (100%) and HRMS calcd for $C_{17}H_{13}O_3N_4Cl$: 356.7631, found 356.7625.

*2.1.4.3. 3-Chloro-1-(1-methyl-1H-benzimidazol-2-yl)-4-(2-nitrophenyl) azetidin-2-one (**5c**).* This was prepared and purified as

per the above mentioned procedure: yield%, mp 168–170 °C, λ_{max} (methanol) 248.6 nm; IR (KBr, ν, cm^{-1}): 1682 (–C═O), 2921 and 2849 (N–CH_3), 1520 and 1344 (–N═O), 864 and 816 (C–NO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.17 (s, 1H, –CH), 2.41 (s, 3H, N–CH_3), 6.67 (s, 1H, –CH), 7.13–8.22 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [154.12 (C), 136.43 (C), 134.21 (C), 123.12 (CH), 116.51 (CH), 110.12 (CH)], Arom-C: [146.21 (C), 136.15 (C), 134.16 (CH), 126.48 (CH), 125.37 (CH), 124.46 (CH)], 29.36 (N–CH_3), β-lactam C: [167.24 (C═O), 62.12 (CHCl), 63.15(CH)]; DIPMS m/z: 356.76 M^+ and HRMS calcd for $C_{17}H_{13}O_3N_4Cl$: 356.7631, found 356.7627.

*2.1.4.4. 3-Chloro-1-(1-methyl-1H-benzimidazol-2-yl)-4-(3-nitrophenyl) azetidin-2-one (**5d**).* This was prepared and purified as per the above mentioned procedure: yield 49.7%, mp 132–134 °C, λ_{max} (methanol) 204.6 nm; IR (KBr, ν, cm^{-1}): 1677(–C═O), 2953 and 2874 (–N–CH_3), 1536 (–N═O), 1347 (–N═O), 810 (–C–NO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.15 (s, 1H, –CH), 2.44 (s, 3H, N–CH_3), 6.63 (s, 1H, –CH), 7.18–7.78 (m, 4H, Ar-H), 7.84–8.43 (m, 3H, Ar-H), 8.82 (s, 1H, Ar-H);^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [155.18 (C), 137.24 (C), 135.25 (C), 123.45 (CH), 112.15 (CH), 111.24 (CH)], Arom-C: [145.57 (C), 138.45 (C), 130.30 (CH), 129.34 (CH), 121.49 (CH), 118.55 (CH)], 29.12 (N–CH_3), β-lactam C: [167.52 (C═O), 62.12 (CHCl), 63.14 (CH)]; DIPMS m/z: 356.76 M^+ and HRMS calcd for $C_{17}H_{13}O_3N_4Cl$: 356.7631, found 356.7630.

*2.1.4.5. 3-Chloro-4-[4-(dimethylamino) phenyl]-1-(1-methyl-1H-benzimidazol-2-yl) azetidin-2-one (**5e**).* This was prepared and purified as per the above mentioned procedure: yield 44.5%, mp 114–116 °C, λ_{max} (methanol) 239.5 nm; IR (KBr, ν, cm^{-1}): 1677 (–C═O), 2955 and 2839 (–N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.21 (s, 1H, –CH), 2.46 (s, 3H, N–CH_3), 6.62 (s, 1H, –CH), 7.10–7.56 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [155.18 (C), 136.23 (C), 135.25 (C), 121.33 (CH), 115.53 (CH), 111.45 (CH)], Arom-C: [146.51 (C), 130.67 (C), 128.21 (CH), 112.17 (CH)], 29.13 (N–CH_3), β-lactam C: [169.12 (C═O), 62.12 (CHCl), 63.11 (CH)], 35.31 [N(CH_3)$_2$]; LC–MS m/z (% RA): M + 2 = 352.8 (58%), 318.7 (32%), 286.7 (13%), 262.8 (22%), 171.8 (45%), 128.8 (38%), 103.3 (100%) and HRMS calcd for $C_{19}H_{19}ON_4Cl$: 354.8334, found 354.8330.

*2.1.4.6. 3-Chloro-4-(2,5-dimethoxyphenyl)-1-(1-methyl-1H-benzimidazol-2-yl) azetidin-2-one (**5f**).* This was prepared and purified as per the above mentioned procedure: yield 50.23%, mp 158–160 °C, λ_{max} (methanol) 240.7 nm; IR (KBr, ν, cm^{-1}): 1677 (C═O), 2955 and 2839 (N–CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.17 (s, 1H, –CH), 2.48 (s, 3H, N–CH_3), 6.63 (s, 1H, –CH), 7.06–7.42 (m, 4H, Ar-H), 7.55 (m, 2H, Ar-H), 7.78 (s, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [146.23 (C), 136.24 (C), 132.12 (C), 122.34 (CH), 115.56 (CH), 113.12 (CH)], Arom-C: [145.76 (C), 143.48 (C), 128.45 (C), 116.31 (CH), 115.67 (CH), 113.19 (CH)], 29.59 (N–CH_3), β-lactam C: [168.65 (C═O), 62.53 (CHCl), 64.23 (CH)], 51.95 (OCH_3), 51.41 (OCH_3); DIPMS m/z: 371.10 M^+ and HRMS calcd for $C_{19}H_{18}O_3N_3Cl$: 371.1037, found 371.1035.

*2.1.4.7. 3-Chloro-4-(2-chlorophenyl)-1-(1-methyl-1H-benzimidazol-2-yl) azetidin-2-one (**5g**).* This was prepared and purified as per the above mentioned procedure: yield 53.5%, mp 130–132 °C, λ_{max} (methanol) 248.6 nm; IR (KBr, ν, cm^{-1}): 2923, 2848 (N–CH_3), 1684 (–C═O), 812 and 741 (Cl–C–); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.25 (s, 1H, –CH), 2.53 (s, 3H, N–CH_3), 6.72 (s, 1H, –CH), 7.23–7.92 (m, 8H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): Benzimidazole C: [154.89 (C), 136.26 (C), 134.27 (C), 123.45 (CH), 116.25 (CH), 111.23 (CH)], Arom-C: [140.45 (C), 134.52 (C), 130.65 (CH), 128.53 (CH), 128.14 (CH), 125.63 (CH)], 29.78 (N–CH_3), β-lactam C: [167.42 (C═O), 62.18 (CHCl), 64.32 (CH)]; DIPMS m/z: 346.21 M^+ and HRMS calcd for $C_{17}H_{13}ON_3C_{l2}$: 346.2106, found 346.2114.

2.2. Antimicrobial activity

2.2.1. Sample preparation

Each of the test compounds and standard ampicillin was dissolved in DMSO, at concentrations of 1000 μg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5 and 6.5 μg/mL.

2.2.2. Culture of microorganisms

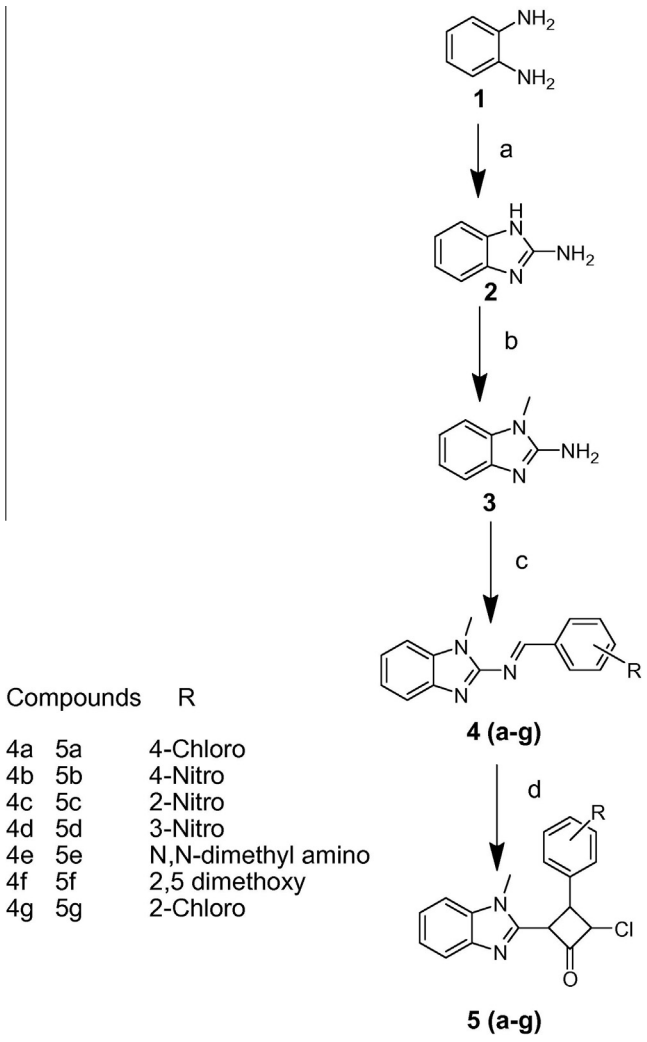
Four strains of bacteria were used as test microorganisms. The bacterial strains included Gram-positive *Staphylococcus aureus* (ATCC 6538), *Bacillus pumilus* (ATCC 14884) and Gram-negative *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 25619). All microorganisms were obtained from the microbiological section of Shree Dhanvantary Pharmaceutical Analysis and Research Centre (SDPARC), Kim (Surat). The cultures were carefully identified as authentic using standard microbiological methods.

2.2.3. Assay for in vitro antimicrobial activity

The in vitro antimicrobial activity of compounds was tested by agar diffusion assay (Ella et al., 1996; Katritzky and Rachal, 1994). The agar diffusion assay was followed to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. Nutrient agar was employed as a culture medium for antibacterial activity. The sterilized (autoclaved at 120 °C for 30 min) medium at 40–50 °C was inoculated with (0.001 mL/mL of medium) of suspension of microorganism and poured into a petri dish to give a depth of 3–4 mm and was allowed to solidify (Fioravanti et al., 1995). Bores were made on the medium using a sterile borer. Test solution (0.1 mL) and standard solution (100 μg/mL in dimethyl sulfoxide) were taken. The plates were incubated at 37 °C for 24 h. Following which the zone of inhibition was measured, to compare the potency of test with that of standard. The minimal inhibitory concentrations (MIC) of the compounds were determined by the agar diffusion assay.

2.3. In vitro cytotoxic activities (MTT assay)

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system (Abdel-Hafez, 2007). In brief, exponentially growing cells were plated in 96-well plates (10^4 cells/well in 100 μL of medium)



Scheme 1 Reactants: (a) CNBr, THF, aq. NaOH; (b) CH_3I, K_2CO_3, acetonitrile; (c) C_2H_5OH, substituted aldehydes, CH_3COOH; (d) $ClCH_2COCl$, $N(C_2H_5)_3$, dioxane.

and incubated for 24 h. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and were diluted with the medium. The cells were then exposed to different concentrations of the drugs (1–100 μM) at a volume of 100 μL/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and the cell cultures were incubated with 100 μL MTT reagent (1 mg/mL) for 4 h at 37 °C. The formazan crystals produced by the viable cells were solubilized by the addition of 100 μL DMSO. The suspension was placed on micro-vibrator for 5 min and the absorbance was recorded at 540 nm by the ELISA reader. The experiment was performed in triplicate. The percentage cytotoxicity was calculated using the formula

$$\%\text{Cytotoxicity} = \frac{(\text{Control abs} - \text{Blank abs}) - (\text{Test abs} - \text{Blank abs})}{(\text{Control abs} - \text{Blank abs})} \times 100$$

For MTT time course study, MCF-7 cells (5×10^3 cells/well) seeded in 96-well plates were exposed to different concentrations of test compounds (1–50 μM). The percentage cytotoxicity and IC_{50} values were determined at 24, 48 and 72 h of drug incubation.

3. Result and discussion

In the present work, benzimidazole derivatives containing azetidine-2-one moiety were prepared according to reaction Scheme 1. 2-Amino-1*H*-benzimidazole **(2)** was prepared by the cycloaddition of *o*-phenylenediamine **1** with cyanogen bromide in presence of aqueous base.

The IR spectra of compound **2** shown two sharp absorption bands, one of which, appearing at 3383 cm^{-1}, was attributed to the NH function of 2-aminobenzimidazoles and other, observed 3060 cm^{-1}, was assigned to NH_2 stretching frequency. The ^1H NMR spectrum of compound **2** exhibited two different signals at 5.6 ppm (NH_2) and 6.7 ppm (NH) integrating for 2 and 1 proton, respectively. The signals of aromatic carbon atom of compound were observed at 116.29–154.63 ppm.

2-Amino-1*H*-benzimidazole **2** on N-methylation with methyl iodide in the presence of anhydrous potassium carbonate in dry acetonitrile gave 1-methyl-1*H*-benzimidazole-2-amine **(3)** which on condensation with various selected aromatic aldehydes in the presence of benzene, ethanol and few drops of acetic acid formed Schiff bases, (1-methyl-*N*-[(substituted) phenylmethylidene]-1*H*-benzimidazol-2-amine **(4a–4g))**.

The spectral data of **4a–4g** shown IR band at 1432 cm^{-1} confirming the formation of imine (HC═N–). Further ^1H NMR of compounds **4a–4g** shown the presence of a singlet between δ 8.90–9.54 ppm indicating the formation of imine (>HC═N–). A Peak at δ 2.40–2.45 ppm shown the presence of >N–CH_3, δ 7.10–8.30 ppm shown the presence of aromatic protons. Syntheses of the compounds **3a–3g** were also confirmed by ^{13}C NMR data, peaks at δ 26.23 ppm and 158.89 ppm have confirmed the formation of methyl carbon (N–CH_3) and imine (>HC═N–), respectively.

The four-membered β-lactam ring was introduced by the cycloaddition of **4a–4g** and chloroacetyl chloride in the presence of triethylamine catalyst to give 3-chloro-1-(1-methyl-1*H*-benzimidazol-2-yl)-(4′-substituted)-phenylazetidin-2-one **5a–5g**.

Title compounds **5a–5g** shown IR bands at 1677–1685 cm^{-1} which confirmed the formation of azetidine-2-one ring which was further substantiated with the help of ^1H NMR data with the peaks at 1.17–1.26 ppm and δ 6.65–6.67 ppm for the protons of lactam ring. It is further confirmed by the absence of peak at δ 8.90–9.54 ppm for –CH═N–. A peak was observed for δ 2.45 ppm for N–CH_3 group, peaks between δ 7.20–8.70 ppm for respective aromatic protons. Synthesis of final compounds was also confirmed by ^{13}C NMR data, peaks between δ 165–169 ppm, δ 63.52 ppm and 61.23 ppm have confirmed the formation of lactam ring. Peaks between δ 115–150 ppm and δ 29.3 confirmed the presence of aromatic carbons and methyl carbon, respectively. Title compounds were further confirmed by mass spectral data, molecular ion M^+ peak observed for compounds **5a–5g** were *m*/*z* 344.5, 355.5, 355.76, 345.5, 353.83, 370.81, 355.76 and HRMS data.

The synthesized compounds **4a–4g**, **5a–5g** were screened for their in vitro antimicrobial activity against Gram +ve bacteria (*Staphylococcus aureus*, *Bacillus pumillus*) and Gram −ve bac-

Table 1 In vitro antimicrobial activity of the synthetic compounds **4a–4g**, **5a–5g** at a concentration of 100 μg/mL (zone of inhibition in mm) and minimum inhibitory concentration (MIC).

Compound	R	Zone of Inhibition in mm				Minimum Inhibitory Concentration (μg/mL)			
		S. aureus	*B. pumillus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. pumillus*	*E. coli*	*P. aeruginosa*
3a	4′-Chloro	12.4	10.2	11.9	10.6	50	75	50	50
3b	4′-Nitro	12.9	11.3	11.8	10.8	50	75	50	50
3c	2′-Nitro	11.8	11.5	NA	11.3	25	50	50	75
3d	3′-Nitro	12.2	10.8	12.6	10.5	75	50	50	50
3e	4′-Dimethylamin	11.3	10.2	10.8	10.6	50	75	75	50
3f	2,5-Dimethoxy	10.9	10.5	11.2	11.0	75	75	50	50
3g	2′-Chloro	12.6	11.4	12.2	11.3	25	50	50	75
4a	4′-Chloro	13.2	11.2	13.6	10.9	25	50	50	50
4b	4′-Nitro	13.6	12.4	14.4	11.7	25	25	50	50
4c	2′-Nitro	12.9	11.8	12.3	12.2	50	75	50	50
4d	3′-Nitro	12.2	11.4	11.7	NA	50	50	75	75
4e	4′-Dimethylamino	11.6	11.4	12.2	NA	50	50	50	50
4f	2,5′-Dimethoxy	NA	10.6	12.5	11.4	50	75	50	50
4g	2′-Chloro	13.2	11.5	12.8	11.3	25	25	50	75
Std.	Ampicillin	14.8	12.8	15.2	13.4	6.5	12.5	25	25

NA means no activity.

teria (*Pseudomonas aeruginosa*, *Escherichia coli*) by measuring the zone of inhibition at concentration 100 μg/mL (mm), minimum inhibitory concentration (μg/mL) and % inhibition shown in Table 1, respectively. Compounds **4a** (12.4, 10.2, 11.9 and 10.8 mm), **4b** (12.9, 11.3, 11.8 and 10.8 mm), **5a** (13.2, 11.2, 13.6, 11.4 mm), **5b** (13.6, 12.4, 13.4 and 11.7 mm) and **5g** (13.2, 11.5, 12.8, 11.3 mm) against *S. aureus*, *Bacillus pumillus*, *E. coli* and *Pseudomonas aeruginosa* compared with the standard Ampicillin (13.8, 11.8, 13.2 and 12.4 mm) shown good antimicrobial activities, respectively. Compounds **4g**, **5a**, **5b** and **5g** shown 25 μg/mL MIC against *S. aureus* and compounds **5b** and **5g** also shown 25 μg/mL MIC against *Bacillus pumillus* compared with standard Ampicillin (6.5, 12.5, 22, 25 μg/mL) against *S. aureus*, *Bacillus pumillus*, *E. coli* and *Pseudomonas aeruginosa*, respectively. %Inhibition calculated based on zone of inhibition, compound **5b** shown maximum % inhibition (52.78%) compared with standard Ampicillin 55.26% against *Escherichia coli* shown in Table 1.

Table 2 In vitro % viability of compounds (**5a–5g**) by MTT assay.

Conc. (μM)	**5a**	**5b**	**5f**	**5g**	Cyclophosphamide
5.0	83.1	76.2	51.3	57.8	81.7
10.0	55.5	52.9	40.2	51.6	44.1
20.0	43.1	38.5	35.5	37	40.4
40.0	26.7	22.1	23.5	30.8	35.4

Table 3 In vitro % inhibitions of compounds (**5a–5g**) by MTT assay (MCF-7).

Conc. (μM)	**5a**	**5b**	**5f**	**5g**	Cyclophosphamide
5.0	16.9	23.8	48.7	42.2	18.3
10.0	44.5	47.1	59.8	48.4	55.9
20.0	56.9	61.5	64.5	63	59.6
40.0	73.3	77.9	76.5	69.2	64.6

The selected title compounds **5a**, **5b**, **5f** and **5g** were subjected to in vitro cytotoxic activity. The results of the MTT assay percentage viability, percentage inhibition and IC_{50} values are shown in Tables 2–4 and Figs. 1 and 2. Compound **5f** has shown promising cytotoxic activity with percentage viability of 51.3% inhibition of 48.7% at 5.0 μM solution. The IC_{50} value

Table 4 The in vitro cytotoxic activity of compounds (**5a–5g**) by MTT assay (MCF-7).

Compound	IC_{50}[a] value (μM)
4a	13.0
4b	11.5
4f	6.0
4g	11.0
Cyclophosphamide	8.7

[a] IC_{50}: the concentration that causes a 50% reduction of the cell growth.

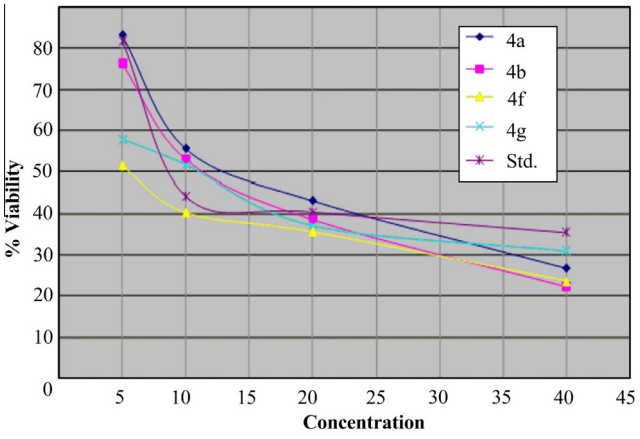


Figure 1 Graph of concentration v/s %viability of compound (**5a–5g**) by MTT assay.

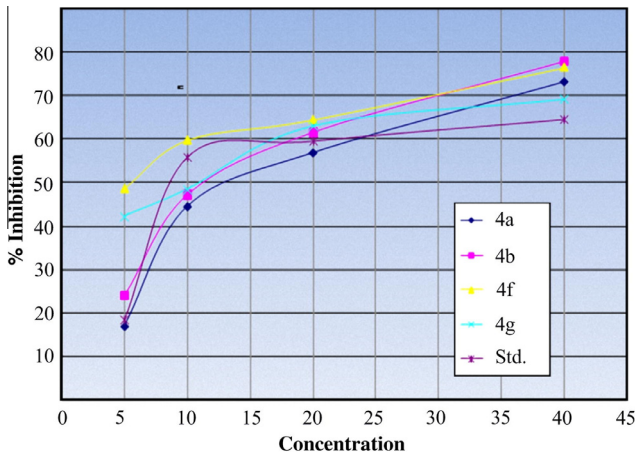


Figure 2 Graph of concentration v/s %inhibition of compound (**5a–5g**) by MTT assay.

shown by compound **5f** was 6.0 μM in comparison with standard cyclophosphamide at 8.7 μM.

4. Conclusion

A series of 1-methyl-*N*-[(substituted-phenylmethylidene)-1*H*-benzimidazol-2-amine **(4a–4g)** were prepared via the formation of 1-methyl-1*H*-benzimidazol-2-amine **(3)**, which was prepared by the cycloaddition of *o*-phenylenediamine **(1)** with cyanogen bromide in the presence of aqueous base followed by N-methylation with methyl iodide in the presence of anhydrous potassium carbonate in dry acetonitrile. Moreover, the four-membered β-lactam ring was introduced by the cycloaddition of **4a–4g** and chloroacetyl chloride in the presence of triethylamine catalyst to give 3-chloro-1-(1-methyl-1*H*-benzimidazol-2-yl)-(4′-substituted)-phenylazetidin-2-one **5a–5g**. Among the chemicals tested **4a**, **4b**, **5a**, **5b**, **5g** exhibited good antibacterial activity and **5f**, **5g** shown good cytotoxic activity in vitro. Finally it is conceivable that further derivatization of such compounds will be of interest with hope to get more selective agents.

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