Medicinal Chemistry

Mothukuri et al., Med Chem (Los Angeles) 2019, 9:2
DOI: 10.4172/2161-0444.1000529

Research Article Open Access

Synthesis and Antibacterial Activity of Novel Benzimidazole Linked 1,3,4-Oxadiazole Derivatives

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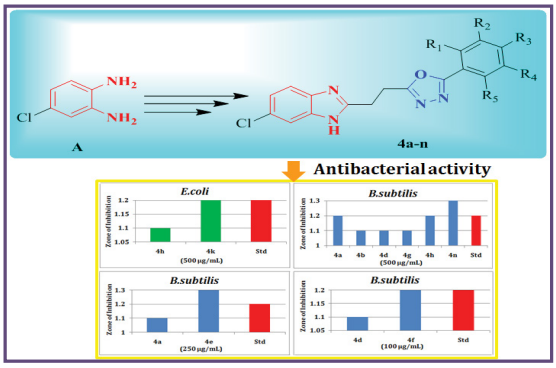
Abstract

With an intention to develop potent antimicrobial agents from the source of benzimidazole- 1,3,4-oxadiazole combined heterocyclic derivatives, novel 6-Chloro-2-(2-(5-(substituted phenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole derivatives were synthesized using condensation reaction of 3-(6-chloro-1H-benzo[d] imidazol-2-yl)propane hydrazide and benzoic acids as key step in presence of $POCl_3$. All newly synthesized target compounds (**4a-4n**) were characterized by ^1H NMR, Mass and IR spectral studies and were screened for their antibacterial activity with two bacterial pathogens (Gram positive: *Bacillus subtilis*, Gram negative: *Escherichia coli*) which confirmed that compounds **4a**, **4b**, **4d**, **4g**, **4h** and **4n** have potent activity against *B. subtilis* as compare with gentamicin at concentration 500 µg/mL. We hope that this study may helpful for further optimization in finding of lead antimicrobials from the origin of benzimidazole linked oxadiazole derivatives.



Keywords: Benzimidazole; 1,3,4-Oxadiazole; Antibacterial activity

Introduction

Now a day's diseases caused by microorganisms are increasing day by day along with population, if it is continued will become a serious threat to human's worldwide. Moreover, available antibiotics in the market facing considerable problems in cure of diseases caused microorganisms this could be due to the consequences of antibiotic mishandling, remarkable genetic plasticity of bacteria and a market failure of antibiotic development etc [1]. Hence, there was an urgent necessity in development of potent antimicrobial agents having varying degree of action and low side effects.

Heterocyclic compounds have immense significance in medical chemistry due to their broad spectrum of biological activities in treating of numerous diseases. Among them, benzimidazole derivatives exhibited huge importance in medicinal chemistry because of their broad variety of biological and pharmacological applications. The N-ribosyl demethylbenzimidazole is a prominent benzimidazole compound in nature, it exists in vitamin B12 through the connection of cobalt at axial position. Benzimidazole is a bicyclic organic compound consists the fusion of benzene and often called as 1,3-benzodiazole. They are efficient heterocycles in treating various diseases due to having of active sites [2]. At present, they have become an important target to current medicinal chemists and biologists in finding of proficient molecules possessing diverse biological activities. Compounds carrying benzimidazole nucleus are reported to exhibit antimicrobial [3], antitumor [4], antiviral [5], anti-inflammatory [6], antioxidant [7], antileishmanial [8] and antiproliferative [9] activities. Furthermore, some benzimidazole derivatives have been demonstrated to be inhibitors of MAO enzyme [10], angiotensin II receptor [11], enzyme topoisomerase I [12] and enzyme lipase [13] etc. Omeprazole, pantoprazole, lansoprazole and ofendazole like few drug molecules possessing benzimidazole core, these are already in use (Figure 1).

1,3,4-Oxadiazole ring is associated with numerous types of biological properties such as antitubercular [14], anti-HIV [15], anticancer [16], insecticidal [17], anti-inflammatory [18], 5-lipoxygenase inhibitor [19], antimicrobial [20] activities. It is an important core unit currently used in designing of potent bioactive molecules, the prominent pharmacological activity of 1,3,4-oxadiazole ring is due to the bearing of toxophoric –N=C-O- linkage in its structure. Some available 1,3,4-oxadiazole drugs currently in use are Raltegravir an antiretroviral, nesapidil an antihypertensive agent and Zibotentan an anticancer drug (Figure 1). Based on the above discussed literature survey and as part of

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Received January 09, 2019; **Accepted** February 20, 2019; **Published** February 27, 2019

Citation: Mothukuri BG, Settypalli T, Begari N, Dalavai VK, Chunduri VR (2019) Synthesis and Antibacterial Activity of Novel Benzimidazole Linked 1,3,4-Oxadiazole Derivatives. Med Chem (Los Angeles) 9: 014-019. doi: 10.4172/2161-0444.1000529

our ongoing synthesis and biological evaluation of novel heterocycles [21-29], in this study, we designed (Figure 2) and synthesized some benzimidazole linked 1,3,4-oxadiazole derivatives and examined their preliminary antibacterial activity.

Materials and Methods

Chemistry

All the chemicals were obtained from Sigma Aldrich in synthetic grade. Reaction progress was monitored from time to time by analytical thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates. UV light (256 nm) sodium chamber was used for spots visualisation. Before reaction, all solvents were dried by appropriate drying agents based on Vogel's protocol. Purification was achieved with column chromatography using hexane and ethyl acetate as eluents. ACME grade silica gel (60-120 mesh) was used for column chromatography unless otherwise mentioned. The reagents were purified employing standard laboratory techniques. All the ^{1}H-NMR spectra have been recorded with Bruker 300 MHz instrument in $CDCl_3$ and DMSO-d_6 solvents. Chemical shifts reported were relative to TMS on the delta scale. The electron ionization mass spectra were recorded on Agilent 1100 series mass spectrometer. Melting points were determined in one ended capillaries on a Mel-temp apparatus and were uncorrected. IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR in KBr pellets.

General procedure for the synthesis of (6-chlo-1-phenyl)-1H-benzimidazol-2-yl) Propanoic acid (1): A mixture of compound 4-chlorobenzene-1,2-diamine (**A**, 10 g, 0.070 mol) and succinic acid (25 g, 0.211 mol) in dil HCl (100 mL) was stirred for 4 h at reflux condition and cooled to 0-5°C, filtered the solid and washed with 50 mL of water to get a solid (8.0 g, 50%); m.p. 176-178°C; IR (KBr ν cm^{-1}): 892, 961, 991, 1109 (C–O str), 1599, 1690 (C=O), 2995, 3203 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.78 (t, 2H, -$\underline{CH_2}CH_2$-), 3.01 (t, 2H, -$CH_2\underline{CH_2}$-), 7.12 (dd, 1H, *J*=8.7 and 1.8 Hz, Ar-H), 7.47 (d, 1H, *J*=8.4 Hz, Ar-H), 7.51 (d, 1H, *J*=1.8 Hz); MS (m/z): 223.04 (M-1) Anal. calcd.

General procedure for the synthesis of Ethyl 3-(6-chloro-1H-benzo[d]imidazol-2-yl)propanoate (2): Compound 1 (8.0 g, 0.0357 mol) was dissolved in 50 mL ethanol and placed in a cooling condition (0-5°C) by adding thionyl chloride (5.21 mL, 0.0714 mol) drop by drop over 30 min and refluxed for 2 h. It was cooled to room temperature and the excess ethanol was distilled using rotavap. The residue was stirred for 30 min with 100 mL cold water to get solid, filtered (8.8 g, 98%); m.p. 132-134°C; IR (KBr ν cm^{-1}): 892, 961, 991, 1128 (C–O str), 1599, 1710 (C=O), 2995, 3203 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 1.17 (t, 3H), 2.85 (t, 2H, -$\underline{CH_2}CH_2$), 3.06 (t, 2H, -$CH_2\underline{CH_2}$-), 4.06 (q, 2H, -CH_3), 7.14 (dd, 1H, *J*=8.4 and 1.8 Hz, Ar-H), 7.48 (d, 1H, *J* =8.4 Hz, Ar-H), 7.52 (d, 1H, *J* =1.5 Hz, Ar-H); MS (m/z): 274.98 (M+Na) Anal. calcd.

General procedure for the synthesis of 3-(6-chloro-1H-benzo[d] imidazol-2-yl) propane hydrazide (3): A mixture of 2 (8 g, 0.031 mol) and hydrazine hydrate (7.8 mL, 0.155 mol) in toluene (15 mL) was refluxed for 2 h and it was cooled filtered to get a white solid (5 g, 66%); m.p. 180-182°C; IR (KBr ν cm^{-1}): 1138, 1225, 1307, 1343, 1395, 1449, 1599, 1670 (C=O), 2965, 3206 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.57 (t, 2H,-$\underline{CH_2}CH_2$-), 3.00 (t, 2H, -$CH_2\underline{CH_2}$), 3.61 (s, 2H, -NH_2), 7.14 (dd, 1H, *J*=8.7 and 1.8 Hz, Ar-H), 7.47 (d, 1H, *J*= 8.7 Hz, Ar-H), 7.51 (d, 1H, *J*=1.5Hz, Ar-H), 9.10 (s, 1H, NH); ^{13}CNMR (DMSO-d_6) δ (ppm): 24.30, 30.97, 114.35, 115.37, 121.34, 125.53, 137.07, 140.13, 155.90, 170.39 (aromatic and quaternary carbons); MS (m/z): 238.8 (M+1) Anal. calcd.

General procedure for the synthesis of 6-chloro-2-(5-(substituted phenyl)-(1,3,4)oxadiazol-2,4^{1}methyl)-1-phenyl-1H-benzimidazole (4a-4n): A mixture of acid hydrazide 3 (500 mg, 0.0021 mol) and substituted benzoic acid (0.0021 mol) was added $POCl_3$ (3 mL) and refluxed for 2 h. The total reaction mixture was cooled to 0-5C and water was added at pH 9. Filtered the solid and recrystalized in methanol:water (70:30).

6-Chloro-2-(2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4a): Yellow colour solid (646 mg, 86%); m.p. 168-170°C; IR (KBr ν cm^{-1}): 1023, 1190, 1482, 1640, 2829 (C-H str), 3060 (=CH str), 3210 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.37 (t, 2H,

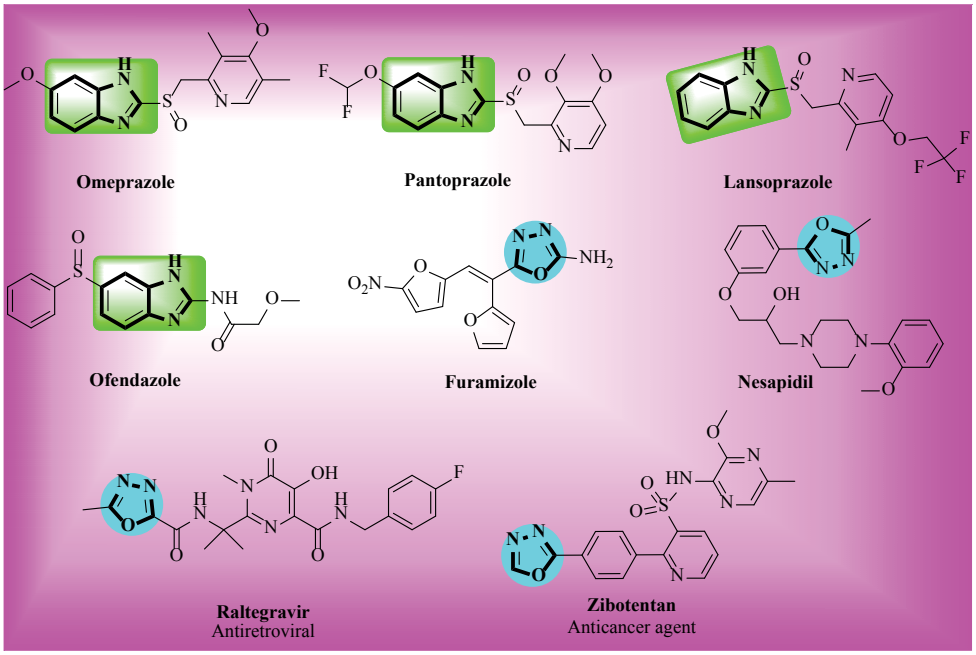


Figure 1: Some available drugs containing benzimidazole and 1,3,4-oxadiazole moieties.

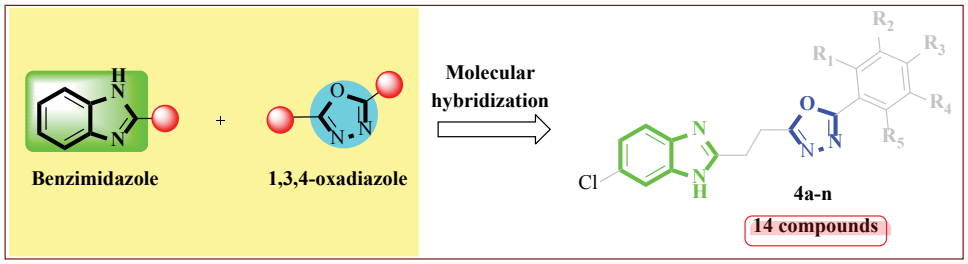


Figure 2: Designed pathway of compounds 4a-n.

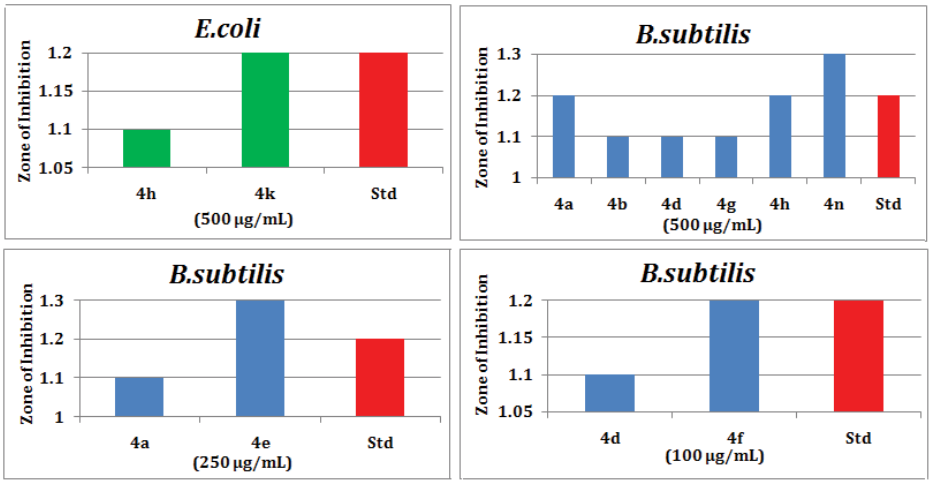


Figure 3: Antibacterial activity of potent active compounds (Here Std=Gentamicin ZOI: 1.2 cm at 10 µg/mL).

-$\underline{CH_2}CH_2$-), 3.48 (t, 2H, -$CH_2\underline{CH_2}$-), 7.15 (d, 1H, *J*=7.5 Hz, Ar-H), 7.49-7.58 (m, 2H, Ar-H), 7.66 (d, 2H, *J*=8.4 Hz, Ar-H), 7.95 (d, 2H, *J*=8.4 Hz, Ar-H), 12.58 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.95, 25.04, 121.37, 121.55, 122.30, 125.75, 128.15, 128.52, 128.57, 129.35, 129.54, 136.55, 154.60, 163.19, 166.12 (aromatic and quaternary carbons); MS (m/z): 359.50 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(2-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d] imidazole (4b): Yellow colour solid (347 mg, 82%); m.p. 180-186°C; IR (KBr ν cm^{-1}): 1027, 1181, 1302, 1473, 1648, 2830 (C-H str), 3050 (=CH str), 3203 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.42 (t, 2H, -$\underline{CH_2}CH_2$-), 3.54 (t, 2H, -$CH_2\underline{CH_2}$-), 7.17 (dd, 1H, *J*=8.7 and 1.8 Hz, Ar-H), 7.50 (d, 1H, *J*=8.7 Hz, Ar-H), 7.55 (d, 1H, *J*=1.5 Hz, Ar-H), 8.02 (d, 1H, *J*=8.7 Hz, Ar-H), 8.24 (dd, 1H, *J*=8.4 and 1.8 Hz, Ar-H), 8.57 (d, 1H, *J*=1.8 Hz, Ar-H), 12.56 (s, 1H, NH); MS (m/z): 404.00 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(3,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d] imidazole (4c): Off white colour solid (685 mg, 85%); m.p. 194-196C; ^{1}HNMR (DMSO-d_6) δ (ppm): 3.45 (t, 2H, -$\underline{CH_2}CH_2$-), 3.54 (t, 2H, -$CH_2\underline{CH_2}$-), 3.79 (s, 6H, -OCH_3), 6.72 (t, 1H, *J*=2.1 Hz, Ar-H), 7.01 (d, 2H, *J*=2.1 Hz, Ar-H), 7.24 (dd, 1H, *J*=8.4 and 1.8 Hz, Ar-H), 7.56 (d, 1H, *J*=8.4 Hz, Ar-H), 7.62 (d, 1H, *J*=1.8 Hz, Ar-H); MS (m/z): 385.10 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4d): Pale yellow colour solid (323 mg, 86%); m.p. 200-202°C; IR (KBr ν cm^{-1}): 1043, 1113, 1546, 1646, 2825 (C-H str), 3055 (=CH str), 3305 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.46 (t, 2H, -$\underline{CH_2}CH_2$-), 3.56 (t, 2H, -$CH_2\underline{CH_2}$-), 7.24 (d, 1H, *J*=8.4 Hz, Ar-H), 7.43-7.68 (m, 5H, Ar-H), 7.90 (d, 1H, *J*=7.2 Hz, Ar-H); MS (m/z): 359.00 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-p-tolyl-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4e): Reddish colour solid (610 mg, 86%); m.p. 183-188°C; IR (KBr ν cm^{-1}): 1041, 1110, 1542, 1643, 2825 (C-H str), 3054 (=CH str), 3307 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.40 (s, 3H, -CH_3), 3.39 (t, 2H, -$\underline{CH_2}CH_2$-), 3.50 (t, 2H, -$CH_2\underline{CH_2}$), 7.15 (d, 1H, *J*=8.1 Hz, Ar-H), 7.38 (d, 2H, *J*=7.8 Hz, Ar-H), 7.44 (d, 1H, *J*=8.4 Hz, Ar-H), 7.54 (s, 1H, Ar-H), 7.81 (d, 2H, *J*=7.8 Hz, Ar-H), 12.63 (s, 1H, NH); MS (m/z): 339.00 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl) ethyl)-1H-benzo[d]imidazole (4f): Pale yellow colour solid (319 mg, 86%); m.p. 196-200°C; IR (KBr ν cm^{-1}): 1041, 1105, 1533, 1641, 2832 (C-H str), 3058 (=CH str), 3312 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.35 (t, 2H, -$\underline{CH_2}CH_2$-), 3.46 (t, 2H,-$CH_2\underline{CH_2}$-), 3.83 (s, 3H, -OCH_3), 7.09-7.12 (m, 3H, Ar-H), 7.48 (d, 1H, *J*=8.4 Hz, Ar-H), 7.51 (s, 1H, Ar-H), 7.87 (d, 2H, *J* =8.4 Hz, Ar-H), 12.52 (s, 1H, NH); MS (m/z): 355.08 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4g): Red colour solid (337 mg, 87%); m.p. 268-270°C; IR (KBr ν cm^{-1}): 1048, 1115, 1545, 1639, 2835 (C-H str), 3052 (=CH str), 3312 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.42 (t, 2H, -$\underline{CH_2}CH_2$), 3.56 (t, 2H, -$CH_2\underline{CH_2}$-), 7.18 (d, 1H, *J*=9.3 Hz, Ar-H), 7.48 (d, 1H, *J*=8.4 Hz, Ar-H), 7.58 (s, 1H, Ar-H), 8.22 (d, 2H, *J* =9 Hz, Ar-H), 8.47 (d, 2H, *J*=9 Hz, Ar-H), 12.59 (s, 1H, NH); MS (m/z): 370.14 (M+1) Anal. calcd.

2-(2-(5-(5-Bromo-2-methylphenyl)-1,3,4-oxadiazol-2-yl)ethyl)-6-chloro-1H-benzo[d] imidazole (4h): Red colour solid (372 mg, 85%); m.p. 210-212°C; IR (KBr ν cm^{-1}): 1043, 1110, 1540, 1642, 2825 (C-H str), 3056 (=CH str), 3302 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.49 (s, 3H), 3.40 (t, 2H, -$CH_2$$CH_2$), 3.53 (t, 2H, -$CH_2$$CH_2$), 7.18 (dd, 1H, *J*=8.7 and 1.8 Hz, Ar-H), 7.40 (d, 1H, *J*=8.4 Hz, Ar-H), 7.51 (d, 1H, *J*=8.4 Hz, Ar-H), 7.56 (d, 1H, *J*=1.5 Hz, Ar-H), 7.68 (dd, 1H, *J*=8.1 and 1.8 Hz, Ar-H), 7.90 (d, 1H, *J*=2.1 Hz, Ar-H), 12.70 (s, 1H, NH); MS (m/z): 417.01 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(4-iodophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4i): Red colour solid (396 mg, 84%); m.p. 188-190°C; IR (KBr ν cm^{-1}): 1041, 1109, 1546, 1635, 2829 (C-H str), 3050 (=CH str), 3308 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.38 (t, 2H, -$CH_2$$CH_2$-), 3.47 (t, 2H, -$CH_2$$CH_2$-), 7.13 (d, 1H, *J*=8.1 Hz, Ar-H), 7.48 (d, 1H, *J*=8.7 Hz, Ar-H), 7.52 (s, 1H, Ar-H), 7.70 (d, 2H, *J*=7.8 Hz, Ar-H), 7.96 (d, 2H, *J*=7.8 Hz, Ar-H); MS (m/z): 449.10 (M-1) Anal. calcd.

2-(2-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-6-chloro-1H-benzo[d]imidazole (4j): Off white colour solid (363 mg, 86%); m.p. 248-250°C; IR (KBr ν cm^{-1}): 1039, 1120, 1541, 1636, 2835 (C-H str), 3049 (=CH str), 3315 (NH): ^{1}HNMR (DMSO-d_6) δ (ppm); 3.46 (t, 2H, -$CH_2$$CH_2$-), 3.48 (t, 2H, -$CH_2$$CH_2$-), 7.14 (d, 1H, Ar-H), 7.51-7.56 (m, 2H, Ar-H), 7.80 (d, 2H, *J*=7.5 Hz, Ar-H), 7.87 (d, 2H, *J*=7.5 Hz, Ar-H), 12.56 (s, 1H, NH); MS (m/z): 401.10 (M-1) Anal. calcd.

6-Chloro-2-(2-(5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl) ethyl)-1H-benzo[d]imidazole (4k): Red colour solid (365 mg, 84%); m.p. 260-262°C ; IR (KBr ν cm^{-1}): 1047, 1118, 1548, 1638, 2838 (C-H str), 3059 (=CH str), 3318 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.54 (t, 2H, -$CH_2$$CH_2$-), 3.56 (t, 2H, $CH_2$$CH_2$-), 7.15 (d, 1H, *J*=7.2 Hz, Ar-H), 7.53 (m, 2H, Ar-H), 8.94 (s, 1H, Ar-H), 8.98 (s, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 12.63 (s, 1H, NH); MS (m/z): 415.20 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4l): Brown colour solid (289 mg, 85%) ; m.p. 166-168°C; IR (KBr ν cm^{-1}): 1043, 1117, 1544, 1632, 2830 (C-H str), 3054 (=CH str), 3310 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm); 3.47 (t, 2H, -$CH_2$$CH_2$-), 3.56 (t, 2H, -$CH_2$$CH_2$-), 7.15 (d, 1H, Ar-H), 7.59 (m, 5H, Ar-H), 7.92 (d, 2H, Ar-H), 11.32 (s, 1H, NH); MS (m/z): 325.00 (M+1) Anal. calcd.

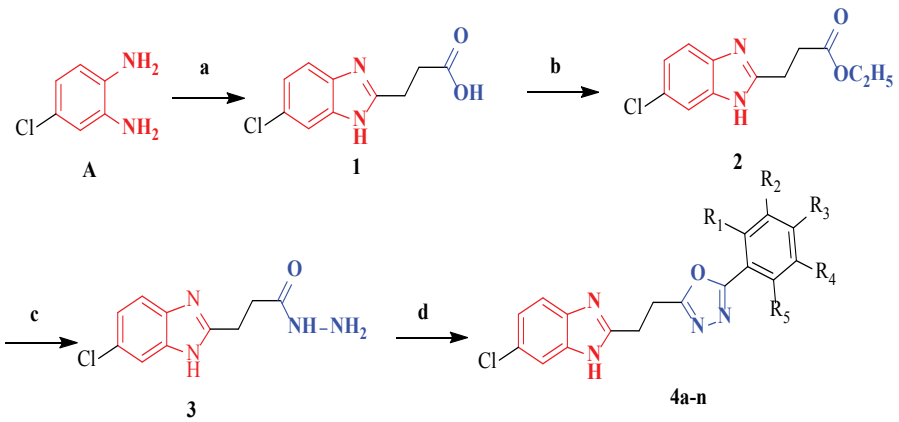
6-Chloro-2-(2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-yl) ethyl)-1H-benzo[d]imidazole (4m): Off white colour solid (319 mg, 86%); m.p. 80-82°C; IR (KBr ν cm^{-1}): 1041, 1119, 1548, 1633, 2835 (C-H str), 3057 (=CH str), 3316 (NH); ^{1}HNMR (DMSO-d_6) δ (ppm): 3.37 (t, 2H, -$CH_2$$CH_2$-), 3.47 (t, 2H, -$CH_2$$CH_2$-), 3.82 (s, 3H, -$OCH_3$), 7.06-7.16 (m, 2H, Ar-H), 7.24 (d, 1H, *J*=8.1 Hz, Ar-H), 7.50 (d, 1H, *J*=8.4 Hz, Ar-H), 7.55-7.60 (m, 2H, Ar-H), 7.75 (d, 1H, *J*=7.2 Hz, Ar-H), 11.52 (s, 1H, NH); MS (m/z): 355.00 (M+1) Anal. calcd.

2-(2-(6-Chloro-1H-benzo[d]imidazol-2-yl)ethyl)-5-(4-chloro-3-nitrophenyl)-1,3,4-oxadiazole (4n): Brown colour solid (347 mg, 82%); m.p. 180-186°C ; IR (KBr ν cm^{-1}): 1027, 1181, 1302, 1473, 1647, 2998 (C-H str), 3052 (=CH str), 3203 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.42 (t, 2H, -$CH_2$$CH_2$-), 3.54 (t, 2H, -$CH_2$$CH_2$-), 7.17 (dd, 1H, *J*=8.4 and 1.8 Hz, Ar-H), 7.51 (d, 1H, *J*=8.7 Hz, Ar-H), 7.55 (d, 1H, *J*=1.2 Hz, Ar-H), 8.01 (d, 1H, *J*=8.7 Hz, Ar-H), 8.24 (dd, 1H, *J*=8.4 and 1.8 Hz, Ar-H), 8.56 (d, 1H, *J*=1.8 Hz, Ar-H); MS (m/z): 404.00 (M+1) Anal. calcd.

Results and Discussion

Chemistry

The synthetic route of final target compounds (**4a-n**) was depicted in Scheme 1. Benzimidazole propionic acid (**1**) was used as synthetic intermediate for the preparation of 1,3,4-oxadiazoles by exploiting the acid functionality. The compound (**1**) was prepared by the reaction of *o*-phenylenediamine (**A**) with succinic acid in the presence of dil HCl [30]. Appearance of two triplets at δ 2.78 and 3.01 ppm in the ^{1}H NMR spectrum of **1** corroborated with ethylene linkage between benzimidazole and carboxylic acid group and the signals at δ 7.12, 7.47 and 7.51 ppm were due to aromatic ring protons. Next for acid to ester conversion, the compound **1** was treated with thionylchloride in ethanol to yield **2**. Its IR spectrum showed bands at 1128, 1710 cm^{-1} corresponding to the ester function and confirmed by the appearance of a triplet at δ 1.17 ppm and quartet at δ 4.06 in the ^{1}H NMR spectrum. The obtained ester (**2**) was then treated with hydrazine hydrate to get the corresponding hydrazide (**3**). Appearance of additional -NH_2 group signal in the ^{1}H-NMR spectrum corroborated with the structure of compound (**3**). Further cyclocondensation reaction of hydrazide (**3**) with various benzoic acids in the presence of $POCl_3$ gave the corresponding oxadiazole derivatives (**4a-n**) (Table 1; Scheme 1).



Regents and conditions: (a) Succinic acid, dil HCl, reflux, 4 h; (b) Thionyl chloride, ethanol, 2 h, reflux; (c) Hydrazine hydrate, toluene, 2 h, reflux; (d) R-COOH, $POCl_3$,100 ^{0}C.

Scheme 1: Synthesis of new benzimidazole linked 1,3,4-oxadiazoles.

S No	Compounds	R_1	R_2	R_3	R_4	R_5
1	**4a**	-H	-H	-Cl	-H	-H
2	**4b**	-H	-H	$-NO_2$	-H	-Cl
3	**4c**	-H	$-OCH_3$	-H	$-OCH_3$	-H
4	**4d**	-Cl	-H	-H	-H	-H
5	**4e**	-H	-H	$-CH_3$	-H	-H
6	**4f**	-H	-H	$-OCH_3$	-H	-H
7	**4g**	-H	-H	$-NO_2$	-H	-H
8	**4h**	-H	-Br	-H	-H	$-CH_3$
9	**4i**	-H	-H	-I	-H	-H
10	**4j**	-H	-H	-Br	-H	-H
11	**4k**	-H	$-NO_2$	-H	$-NO_2$	-H
12	**4l**	-H	-H	-H	-H	-H
13	**4m**	$-OCH_3$	-H	-H	-H	-H
14	**4n**	-H	-H	-Cl	$-NO_2$	-H

Table 1: Various substituents of 4a-n.

Compounds	Antibacterial activity (ZOI)[a]							
	E. coli (MTCC-1668)				*B. subtilis* (MTCC-1133)			
	500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL
4a	-	-	-	-	**1.2**	**1.1**	0.8	0.7
4b	0.7	0.7	0.7	0.6	**1.1**	0.9	0.9	0.8
4c	0.8	-	-	-	0.9	0.8	0.7	0.7
4d	-	-	-	-	**1.1**	1.0	**1.1**	1.0
4e	-	0.6	-	-	1.0	**1.3**	1.0	0.8
4f	-	0.8	0.7	-	1.0	0.7	**1.2**	0.7
4g	0.7	0.8	0.6	0.6	**1.1**	0.9	0.7	0.7
4h	**1.1**	0.7	0.6	-	**1.2**	0.9	0.8	0.8
4i				-	-	0.9	0.8	0.7
4j	0.9	0.7	0.7	0.8	0.8	0.9	0.8	0.6
4k	**1.2**	1.0	0.8	0.7	0.9	0.7	0.7	0.6
4l	-	0.6	-	-	0.9	0.7	0.6	0.6
4m	-		-	-	1.0	0.9	0.9	0.8
4n	0.9	0.6	0.5	-	**1.3**	1.0	**1.2**	0.9

Table 2: Antibacterial activity of 4a-n.

[a]ZOI: Zone of inhibition

Biological assay

Antibacterial activity: The antibacterial activity of compounds **4a-n** was performed with two microorganisms such as *Escherichia coli* (MTCC-1668) and *Bacillus subtilis* (MTCC-1133) at various concentrations using disk diffusion method [31]. DMSO was used for dissolve the sample which has no effect in the experiment and Gentamicin was chosen as standard drug with inhibition zone 1.2 cm at 10 µg/mL. A fixed inoculum ($1\text{-}2 \times 10^7$ cfu/mL) of each bacterial strain was swabbed on Mueller Hinton Agar plate. 20 µL of serially diluted test sample (50, 100, 250, 500 µg/mL) was loaded on 6 mm sterile disk using micropipette. After placing of test samples loaded disks on the agar medium using forceps along with gentamicin standard disk (10 µg/mL), they were incubated at 37°C for 12 h. After that formed inhibition zone around each disc was measured (Table 2).

The antibacterial activity data in Table 2 indicated that derivatives **4a-n** have good activity with *Bacillus subtilis* than *Escherichia coli*. Compounds **4a** (ZOI: 1.2 cm), **4b** (ZOI: 1.1 cm), **4d** (ZOI: 1.1 cm), **4g** (ZOI: 1.1 cm), **4h** (ZOI: 1.2 cm) and **4n** (ZOI: 1.3 cm) exhibited good antibacterial activity at highest concentration 500 µg/ml as compare with gentamicin (ZOI: 1.2 cm). **4a**, **4e** at 250 µg/mL and **4d**, **4f** at 100 µg/mL exhibited good activity against *Bacillus subtilis*, but **4h**, **4k** have considerable activity against *Escherichia coli* at concentration 500 µg/mL (Figure 3). The results revealed that various substituents present on the phenyl ring of 1,3,4-oxadiazole were responsible for activity, since **4a** (R_3=-Cl), **4b** (R_3=-NO_2, R_5=-Cl) are more active than **4c** (R_2=-OCH_3, R_4=-OCH_3) and have comparable activity with **4g** (R_3=-NO_2), **4h** (R_2=-Br, R_5=-CH_3), but **4j-m** are lees active than **4n** (R_3=-Cl, R_4=-NO_2). According to activity results, either strong (-NO_2) or weak (-Cl) electron withdrawing groups on the phenyl ring of oxadiazole are suitable for potent activity at 500 µg/mL with *Bacillus subtilis*.

Conclusion

A series of new benzimidazole liked 1,3,4-oxadiazole derivatives were synthesized from 4-chlorobenzene-1,2-diamine (**A**) via four step synthetic path way and tested for their antibacterial activity with two bacterial pathogens. Among the synthesized derivatives (**4a-n**), compounds **4a**, **4b**, **4d**, **4g**, **4h** and **4n** showed potent activity against *B. subtilis* at the highest concentration 500 µg/mL, while **4a**, **4e** and **4d**, **4f** were active at 250 µg/mL and 100 µg/mL respectively. These results indicated that compounds **4a-n** may be helpful for further studies in developing of lead antimicrobial agents.

Acknowledgements

One of the authors Bala guraiah M is thankful to S.V. University, Tirupati for their research facilities. Author Triloknadh S was expressed his gratitude to

University Grants Commission (UGC), New Delhi for their financial assistance (JRF and SRF fellowship, Award Letter no. F.17-142/98(SA-I)).

Conflicts of Interest

The authors declare no conflict of interest.

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