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-yethyl)-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 POCl₃. All newly synthesized target compounds (4a-4n) were characterized by 'H 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 dimethylbenzimidazole 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 to be inhibitors of MAO enzyme [10], angiotensin II receptor [11], enzyme topoisomerase I [12] and enzyme lipase [13] etc. Omeprazole, pantoprazole, lansoprazole and omeprazole 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, nesaprid 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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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 with column chromatography using hexane and ethyl acetate as solvents. Chemical shifts reported were relative to TMS on the delta scale. The electron ionization mass spectra were determined in one ended capillaries on a Mel-temp apparatus 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%); IR (KBr ν cm -1): 1138, 1225, 1307, 1343, 1395, 1449, 1599, 1670 (C=O), 2965, 3206 (NH); 'HNMR (DMSO-d_6) δ (ppm): 2.80 (t, 2H, -CH_2CH2-), 3.00 (t, 2H, -CH_2CH2), 3.61 (s, 2H, -NH2), 7.14 (dd, 1H, J=8.4 and 1.8 Hz, Ar-H), 7.47 (d, 1H, J=1.5 Hz, Ar-H), 7.51 (d, 1H, J=1.5 Hz, Ar-H), 9.10 (s, 1H, NH); '13CNMR (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-(1H-benzimidazol-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); 'HNMR (DMSO-d_6) δ (ppm): 2.57 (t, 2H, -CH_2CH2-), 3.00 (t, 2H, -CH_2CH2), 3.61 (s, 2H, -NH2), 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=1.5 Hz, Ar-H), 9.10 (s, 1H, NH); '13CNMR (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-(5-(substituted phenyl)-(1,3,4)oxadiazol-2,4-1methyl)-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 0-5°C and water was added at pH 9. Filtered the solid and recrystallized in methanol:water (70:30).

6-Chloro-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-1H-benzimidazole (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); '13CNMR (DMSO-d_6) δ (ppm): 3.63 (t, 2H, J=8.7 Hz, Ar-H), 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=1.5 Hz, Ar-H), 9.10 (s, 1H, NH); '1HNMR (DMSO-d_6) δ (ppm): 2.80 (t, 2H, -CH_2CH2-), 3.00 (t, 2H, -CH_2CH2), 3.61 (s, 2H, -NH2), 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=1.5 Hz, Ar-H), 9.10 (s, 1H, NH); '13CNMR (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.
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); ¹HNMR (DMSO-d₆) δ (ppm): 3.42 (t, 2H, -CH₂CH₂-), 3.54 (t, 2H, -CH₂CH₂-), 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 (dd, 1H, J=8.7, 1.8 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. 194-196°C; IR (KBr ν cm⁻¹): 1041, 1105, 1533, 1641, 2832 (C-H str), 3058 (=CH str), 3312 (NH); ¹HNMR (DMSO-d₆) δ (ppm): 3.35 (t, 2H, -CH²CH₂-), 3.46 (t, 2H, -CH²CH₂-), 3.83 (s, 3H, -OCH₃), 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-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); ¹HNMR (DMSO-d₆) δ (ppm): 2.40 (s, 3H, -CH₃), 3.39 (t, 2H, -CH₂CH₂-), 3.50 (t, 2H, -CH₂CH₂-), 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): 1048, 1115, 1545, 1639, 2825 (C-H str), 3058 (=CH str), 3312 (NH); ¹HNMR (DMSO-d₆) δ (ppm): 3.35 (t, 2H, -CH₂CH₂-), 3.46 (t, 2H, -CH₂CH₂-), 3.83 (s, 3H, -OCH₃), 7.09-7.12 (m, 3H, Ar-H), 7.48 (d, 1H, J=8.4 Hz, Ar-H), 7.51 (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.

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).
2-(2-(5-(5-Bromo-2-methylphenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4h): Red colour solid (372 mg, 88%); m.p. 188-190°C; IR (KBr ν cm⁻¹): 1041, 1109, 1546, 1635, 2829 (C-H str), 3056 (CH str), 3302 (NH); 1HNMR (DMSO-d₆) δ (ppm): 2.49 (s, 3H), 3.40 (t, 2H, -CH₂CH₂-), 3.53 (t, 2H, -CH₂CH₂-), 7.18 (dd, 1H, J=8.8 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): 3050 (=CH str), 3308 (NH); 1HNMR (DMSO-d₆) δ (ppm): 2.49 (s, 3H), 3.40 (t, 2H, -CH₂CH₂-), 3.53 (t, 2H, -CH₂CH₂-), 7.18 (dd, 1H, J=8.8 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), 7.56-7.60 (m, 2H, Ar-H), 7.75 (d, 1H, J=7.8 Hz, Ar-H), 7.96 (d, 2H, J=7.8 Hz, Ar-H); MS (m/z): 415.20 (M+1) Anal. calcd.

6-Chloro-2-(2-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)ethyl)-1H-benzo[d]imidazole (4j): Off white colour solid (363 mg, 86%); m.p. 248-250°C; IR (KBr ν cm⁻¹): 1039, 1128, 1710 cm⁻¹, 1713 cm⁻¹, 3493 cm⁻¹. Appearance of two triplets at δ 2.78 and 3.01 ppm in the 1H NMR spectrum. 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 'H NMR spectrum. The obtained ester (2) was then treated with hydrazine hydrate to get the corresponding hydrazide (3). Appearance of additional -NH₂ group signal in the 'H NMR spectrum corroborated with the structure of compound (3). Further cyclodehydration reaction of hydrazide (3) with various benzoic acids in the presence of POCl₃ gave the corresponding oxadiazole derivatives (4a-n) (Table 1; Scheme 1).

**Results and Discussion**

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 '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 'H NMR spectrum. The obtained ester (2) was then treated with hydrazine hydrate to get the corresponding hydrazide (3). Appearance of additional -NH₂ group signal in the 'H NMR spectrum corroborated with the structure of compound (3). Further cyclodehydration reaction of hydrazide (3) with various benzoic acids in the presence of POCl₃ gave the corresponding oxadiazole derivatives (4a-n) (Table 1; Scheme 1).

**Chemistry**

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, POCl₃, 100 °C.
considerable activity against at concentration Escherichia coli µg/mL exhibited good activity against 4a, with gentamicin (ZOI: 1.2 cm).

antibacterial activity at highest concentration 500 µg/ml as compare (ZOI: 1.1 cm), than have good activity with Bacillus subtilis Escherichia coli zone around each disc was measured (Table 2).

mL), they were incubated at 37°C for 12 h. After that formed inhibition agar medium using forceps along with gentamicin standard disk (10 µg/ using micropipette. After placing of test samples loaded disks on the test sample (50, 100, 250, 500 µg/mL) was loaded on 6 mm sterile disk was swabbed on Mueller Hinton Agar plate. 20 µL of serially diluted 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 concentrations using disk diffusion method [31]. DMSO was used for their research facilities. Author Triloknadh S was expressed his gratitude to 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 500 µg/mL 250 µg/mL 100 µg/mL 50 µg/mL 500 µg/mL 250 µg/mL 100 µg/mL 50 µg/mL for their research facilities. Author Triloknadh S was expressed his gratitude to developing of lead antimicrobial agents. 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 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. 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 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

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Table 1: Various substituents of 4a-n.

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Table 2: Antibacterial activity of 4a-n.

*ZOI: Zone of inhibition
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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