Synthesis, Characterization, and Antimicrobial Activities of Clubbed [1,2,4]-Oxadiazoles with Fluorobenzimidazoles

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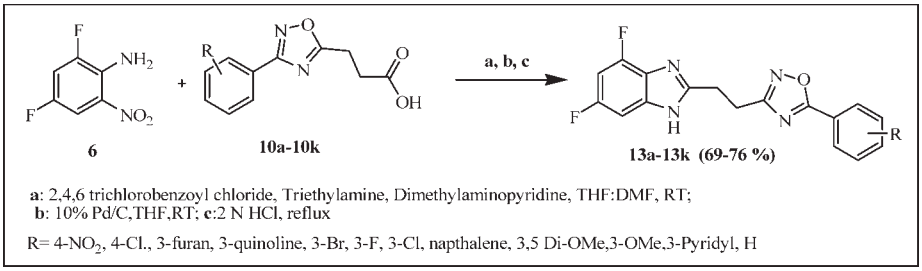

In this study, a novel series of substituted 4,6-difluoro-2-{2-[3-(substituted-phenyl)-[1,2,4]-oxadiazol-5-yl]-ethyl}-1*H*-benzo[d]imidazole derivatives were synthesized by condensation of 2,4-difluoro-6-nitrophenyl amine with 3-(substitutedphenyl-[1,2,4]-oxadiazol-5yl) propionic acid by using 2,4,6-trichlorobenzoyl chloride in the presence of triethyl amine base. The compounds were evaluated for their preliminary *in vitro* antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhosa*. The antibacterial data of the tested compounds indicated that most of the synthesized compounds showed moderate activity with reference standard Gentamycin.

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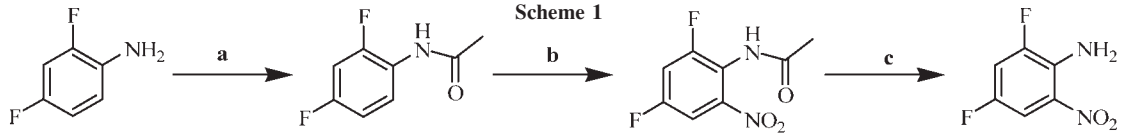
INTRODUCTION

After nitrogen, fluorine occupies the position of second favorite heteroelement in life science-oriented research. Over 10% of newly registered pharmaceutical drugs and some 40% of newly registered agrochemicals contain one or more fluorine atoms [1]. Fluorine containing benzimidazoles, which show promising biological activities, are well documented in the literature. Some of these are like Astemizole (antiallergic, anti-histaminic), Lansoprazole (antiulcerative), Flubendazole (Anthelmintic), and Droperidol (antipsychotic) [2]. So by this idea in view, synthesis of fluorobenzimidazole is the interesting area of research. Interest in benzimidazole containing structure stems not only because of exhibiting broad spectrum of pharmacological activity [3] but also displaying significant activities against several viruses such as casein kinase 2 [4], factor Xa [5], hepatitis C virus [6].

In continuation, heterocyclic species like [1,2,4]-oxadiazole and fluorobenzimidazole derivatives represent a novel emerging major chemical entity as antimicrobial agent. As we know that [1,2,4]-oxadiazoles are an important class of heterocyclic compounds with broad spectrum of pharmacological activity, due to its hydrolytic and metabolic stability of the oxadiazole ring along with improved pharmacokinetics and *in vivo* performance [7]. The biological activities of the compounds containing [1,2,4]-oxadiazoles have been well documented in the literature [8–11]. The oxadiazole moiety is an important structure unit in drugs and chemical materials [12]. Among these oxadiazoles, [1,2,4]-oxadiazoles are gaining interest in the medicinal chemistry [13] and shows numerous biological activities including muscarinic agonists [14], dopamine transporters [15], benzodiazepine receptor partial agonists [16], nematocidal, fungicidal, and microbicides [17], immunosuppressants [18], Fab I inhibitors as antibacterial agents [19], antiplatelet and antithrombotic agents [20], etc. Also, oxadiazoles plays an important role as bioisosteres for amides and esters [21]. Several methods have been reported for the synthesis of [1,2,4]-oxadiazoles in the literature [22–30].

In continuation of our research work [31] and after extensive search, it was observed that enough efforts have not been made till date, to combine these two moieties as a single molecule scaffold. So, we wish to disclose the derivatives of difluorobenzimidazoles clubbed

Scheme 1



a: Acetic anhydride, reflux; **b**: HNO_3, H_2SO_4, RT; **c**: 2N HCl, reflux.

with different substituted [1,2,4]-oxadiazoles and studied their antibacterial activity against different organisms.

RESULTS AND DISCUSSION

Synthesis of 2,4-difluoro-6-nitrophenyl amine [32]. In neat reaction condition, acetic anhydride was added slowly in 2,4-difluorophenyl amine **1** at 0°C, a solid material precipitates. The reaction mixture was quenched in ice water and stirred continuously for 30 min. An off violet colored solid compound separated out, which was filtered and suck dried to obtain 2, 4 difluoro acetanilide **2** as a free solid (Scheme 1).

In the second stage, 2,4-difluoro acetanilide **2** was dissolved in nitric acid and conc. sulfuric acid was added slowly at 5–10°C. The reaction mixture was stirred continuously for 3 h at room temperature. Reaction progress was monitored by TLC system (EA: Hexane 2:8). After completion of reaction, the reaction mixture was quenched in ice water, a pale yellow color solid separated, which was filtered through Büchner funnel and suck dried to obtain 2,4-difluoro-6-nitro acetanilide **3** as a free solid.

In the final stage, to a reaction mixture of **3** in 2*N* HCl, a catalytic amount of sulfuric acid was added slowly. Reaction mixture was heated to reflux for 3–4 h. After completion of reaction, the reaction mixture was cooled slowly at 0–5°C, a yellow color needle type crystal separated, which was filtered and suck dried to obtain as 2,4-difluoro-6-nitro aniline **4.**

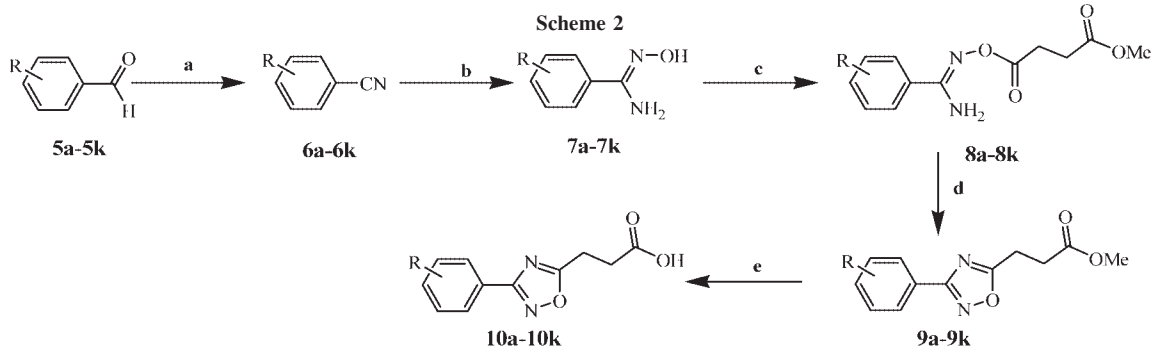
Synthesis of 3-(substitutedphenyl-[1,2,4]-oxadiazol-5-yl)-propionic acid. By using different substituted aromatic aldehydes **5a-5k** in the presence of iodine and aqueous NH_3 in tetrahydrofuran at room temperature, gave substituted benzonitrile **6a-6k.** The compounds were characterized by IR showing cyano functional group at 2210–2245 cm^{-1}. Then followed by amidoxime formation by using hydroxylamine hydrochloride and sodium bicarbonate in methanol at reflux temperature for 8–10 h gives substituted N-hydroxy benzamidine **7a-7k** (Scheme 2).

In the next reaction, condensation of **7a-7k** with monomethyl succinate was carried out by using coupling reagent dicyclohexylcarbodiimide (DCC) and N-hydroxy benzotriazole, and dimethylaminopyridine (DMAP) as a base in dichloromethane at room temperature to obtain **8a-8k**.

In the penultimate step, dehydration followed by cyclization of **8a-8k** was carried out in toluene at reflux temperature in the presence of molecular sieves as a dehydrating agent. It gives 3-(substituted phenyl-[1,2,4]-oxadiazol-5-yl)-propionic acid ethyl ester **9a-9k** as a free solid. In the final step, hydrolysis of **9a-9k** by lithium hydroxide in THF: EtOH: H_2O (7:2:1) at room temperature gives 3-(substituted phenyl-[1,2,4] oxadiazol-5-yl)-propionic acid **10a-10k**.

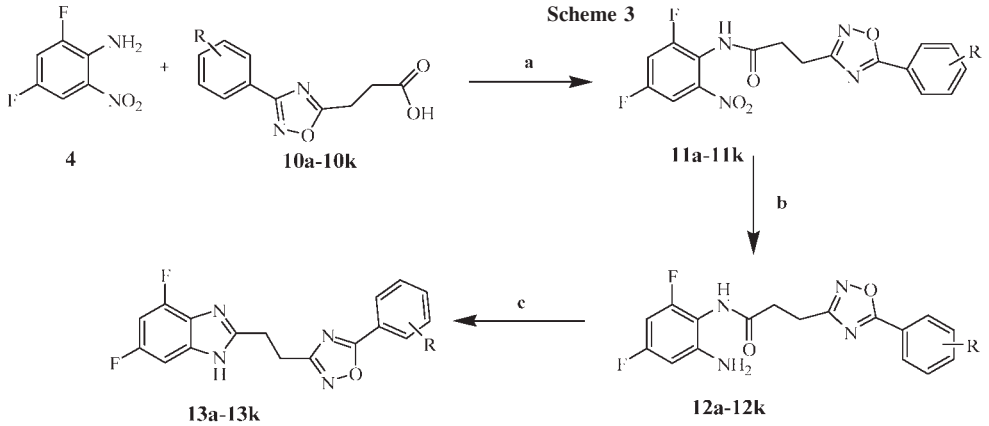
Synthesis of 4,6-difluoro-2-[2-(5-substituted-phenyl-[1,2,4]-oxadiazol-3-yl)-ethyl]-1*H*-benzo[*d*]imidazole. Condensation of 2, 4 difluoro 6-nitro phenyl amine **4** and 3-(substituted phenyl-[1,2,4]oxadiazol-5yl)-propionic acid **10a-10k** in 2,4,6 trichlorobenzoyl chloride [33] as a coupling reagent, in the presence of triethylamine and DMAP in THF: DMF (7:3) solvent at room temperature **11a-11k**. After workup, catalytic reduction of **11a-11k** by using 10% Pd/C in tetrahydrofuran was carried out

Scheme 2



a: I_2, NH_3, THF, RT; **b**: $NH_2OH.HCl$, $NaHCO_3$, methanol, reflux; **c**: monomethyl succinate, dicyclohexylcarbodiimide, dimethylaminopyridine, N-hydroxy benzotriazole, DCM; **d**: toluene, reflux; **e**: LiOH, THF-ETOH-H_2O, RT

Scheme 3



a: 2, 4,6 trichlorobenzoyl chloride, triethylamine, dimethylaminopyridine,THF:DMF, RT; **b**: 10% Pd/C, THF, 25-30 °C; **c**: 2 N HCl , reflux

to obtain **12a-12k**. Then **12a-12k** undergoes cyclization reaction in 2*N* HCl at 100°C to furnish with 4, 6 difluoro-2-{2-[3-(substituted-phenyl)-[1,2,4]-oxadiazol-5-yl]-ethyl}-*1H*-benzo[*d*] imidazole derivatives **13a-13k.** The details of the reaction condition were explained in experimental section (Scheme 3).

ANTIMICROBIAL ACTIVITY

The *in vitro* antibacterial screening of **13a-13k** was assessed against two gram positive and two gram negative bacteria *viz*. *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), and *Salmonella typhosa* (ATCC 14028). From the antibacterial screening, it was observed that all the compounds exhibited activity against different organisms employed. In this, we studied different electron withdrawing or electron donating moieties. Some of the compounds showed better activity against gram positive organisms compared to gram negative organisms. Looking at the structure-activity relationship, marked inhibition in bacteria was observed in the number of compounds **13a**, **13f**, **13g**, and **13i** have shown moderate activity and others **13b**, **13c**, **13d**, **13e**, **13h**, **13j**, and **13k** showed least activity.

EXPERIMENTAL

The melting points were estimated by Veggo programmable (microprocessor based) melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) are reported in ppm Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet), and br s (broad singlet). IR spectra were recorded on (KBr disc) using a FTIR bruker Vector 22 Spectrophotometer. Elemental analyses were determined on Elementor Vario instrument. EIMS spectra recorded on micromass-quatro –II. The purity of the compounds was checked on Merck precoated silica gel 60 F-254.

General experimental procedure for the preparation of 2,4-difluoro-6-nitro phenyl amine (4). In the first stage, under neat reaction condition, acetic anhydride (7.9 g, 77.5 mmol) was added slowly in 2,4-difluoro phenyl amine **1** (10 g, 77.5 mmol) at 0°C, a solid compound precipitates out after 30–45 min. After completion of reaction, the reaction mixture was quenched in ice water with continues stirring for 30 min. The solid was filtered through Büchner funnel and suck dried to obtain 2,4-difluoro acetanilide **2** as a off violet color solid (12.0 g, 92% yield). *m/z:* 172 (M^+).

In the second stage, to a solution of 2,4-difluoro acetanilide **2** (12 g) in nitric acid (25 mL), Conc. sulfuric acid (25 mL) was added slowly at 5–10°C. The reaction mixture was stirred continuously for 3 h at room temperature. The progress of the reaction was monitored on TLC system (Ethyl acetate: Hexane, 2:8). After completion of reaction, the reaction mass was quenched slowly in ice water and a pale yellow color solid was observed, which was filtered through Büchner funnel and suck dried to obtain 2,4-difluoro-6-nitro acetanilide **3** as a free solid (12.0 g, 80% yield). The compound was characterized by mass and NMR. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.23 (3H, —CH_3, s), 7.26 (1H, ArH, m), 7.62 (1H, ArH, m), 8.01 (1H, —NH, s). *m/z:* 215 (M^-).

In the final stage, to a suspension of 2, 4 difluoro 6-nitro acetanilide **3** (12 g, 69.0 mmol) in 2*N* HCl (25 mL), a catalytic amount of sulfuric acid (1.2 mL) was added slowly and the reaction mixture was heated to reflux for 3–4 h. After completion of reaction, the reaction mixture was cooled gradually at 10°C, a yellow color needle type crystal separated out. The solid compound was filtered and suck dried to obtain 2,4-difluoro-6-nitro aniline **4** as a free solid (7.0 g, 72.9% yield). The compound was characterized by mass and NMR. ^{1}H NMR ($CDCl_3$) δ (ppm): 6.05 (2H, —NH_2, s), 7.12 (1H, ArH, m), 7.73 (1H, ArH, s). *m/z:* 173 (M^-).

General experimental procedure for the preparation of 3-{3-substituted-phenyl-[1,2,4]-oxadiazole-5-yl)-propionic acid (10a). To a solution of 4-nitrobenzaldehyde **5a** (6.0 g, 39.73 mmol) in tetrahydrofuran (30 mL), aqueous NH_3 (120 mL) was added and then followed by iodine (10.59 g, 41.69 mmol). The reaction mixture was stirred further for 2 h. After

completion of reaction, color of the reaction mixture changed from brownish to colorless. The reaction mixture was extracted with ethyl acetate (50 mL × 2) twice, and the organic layer was dried over sodium sulfate and evaporated under vaccum till dryness. The crude compound was triturated with diethyl ether to obtain a free solid of 4-nitro benzonitrile **6a** (5.1 g, 84% yield). The compound was characterized by IR showing cyano functional group at around 2238 cm^{-1} in this example.

To a solution of **6a** (5.0 g, 33.78 mmol) in methanol (50 mL), hydroxylamine hydrochloride (2.56 g, 37.16 mmol), sodium bicarbonate (4.35 g, 50.67 mmol) were added and the reaction mixture was heated to reflux for 8–10 h. Progress of reaction was monitored on TLC. After completion of reaction, methanol was distilled completely under vaccum. A pale yellow solid compound precipitates after charging water (100 mL), which was filtered through funnel and suck dried to obtain N-hydroxy-4-nitrobenzamidine (4.9 g, 80% yield) **7a** as a free solid.

In the second stage, monomethyl succinate (5.14 g, 38.94 mmol) was dissolved in THF: DMF (7:3 ratio) (25 mL) at 25–30°C. Then DCC (11.15 g, 54.14mmol) a coupling reagent, N-hydroxybenzo triazole (1.09 g, 8.12 mmol) was added at 25–30°C and stirred continuously for 30 min. Mixed anhydride formation, charged 4- nitrobenzamidine, **7a** (4.8 g, 27.07 mmol), and DMAP (0.99 g, 8.12 mmol) with continuous stirring for 60 min. Progress of the reaction was monitored on TLC (ethyl acetate: hexane 3:7). After completion of reaction, the reaction mixture was filtered through Büchner funnel with bed wash of ethyl acetate (25 mL) twice. Water wash given to the organic layer (100 mL × 2) twice separated the organic solution, which was dried over sodium sulphate and distilled out as organic layer under vaccum. The crude compound was triturated with diethyl ether to obtain **8a** (6.2 g, 74% yield) as a free off white solid.

In the penultimate stage, **8a** compound (6.2 g, 20.06 mmol) was dissolved in toluene (60 mL) and molecular sieves were added as dehydrating agent. The reaction mass was stirred at reflux temperature for 2–3 h. After completion of reaction, the reaction mixture was filtered through Büchner funnel with bed wash of toluene (10 mL). The filtrate was concentrated under vaccum and recrystallized with diethyl ether to obtain 3-(3-4-nitrophenyl-[1,2,4]-oxadiazol-5-yl)-propionic acid ethyl ester **9a** (4.5 g, 77% yield) as a pure compound.

In the final stage, 3-(3-4 nitrophenyl-[1,2,4] oxadiazol-5-yl)-propionic acid ethyl ester **9a** (4.5 g, 15.46 mmol) was dissolved in THF: EtOH: H_2O (7:2:1) with 25 mL volume. $LiOH.H_2O$ was added (0.630 g, 18.55 mmol) at room temperature and the reaction mixture was stirred for 2 h at 25–30°C. After completion of reaction, the mixture was acidified by 2*N* HCl, a solid material separated, which was filtered through Büchner funnel and suck dried to obtain 3-(3-4-nitrophenyl-[1,2,4]-oxadiazol-5-yl) propionic acid **10a** (2.9 g) as a pale yellow solid.

All the intermediates **10b-10k** were synthesized by the aforementioned procedure using different substituted aldehydes **5b-5k**, respectively, and were characterized by ^{1}H NMR and ms. The details of the intermediates were given in Table 1.

General procedure for the preparation of 4,6-difluoro-2-[2-(5-substituted-phenyl-[1,2,4]-oxadiazol-3-yl)-ethyl]-1H-benzimidazole: (13a-13k). In the reaction, 3-(3-4-nitrophenyl-[1,2,4] oxadiazol-5-yl)-propionic acid **10a** (1.5 g, 5.74 mmol) was dissolved in THF: DMF (7:3) 25 mL volume. Then 2,4,6-trichlorobenzoyl chloride (1.47 g, 6.03 mmol) followed by triethyl amine (0.696 g, 6.89 mmol) was added for the mixed anhydride formation. The reaction mass was stirred for 60 min at 25–30°C. DMAP (0.701 g, 5.74mmol) and **4** (1.0 g, 5.74 mmol) were added and stirred continuously for 2 h. Progress of the reaction was monitored on TLC system (ethyl acetate: hexane 3:7). After completion of reaction, the reaction mixture was filtered through Büchner funnel and bed wash of ethyl acetate. Water wash given to the organic solution, dried over sodium sulphate and concentrated under vacuum completely. Finally, the crude compound was triturated with diethyl ether to obtain as a off white color solid **11a** (1.6 g, 66% yield).

Similarly, by using the respective 3-{3-substituted-phenyl-[1,2,4]-oxadiazol-5-yl)-propionic acid **10b-10k,** we have synthesized **11b-11k** for the next stage.

In the second stage. **11a** (1.5 g, 3.58 mmol) was dissolved in THF (50 mL), and 10% Pd/C was added (0.3 g, 20% w/w) at room temperature under nitrogen. Reaction mass was stirred for 2 h at 25–30°C. Progress of the reaction was monitored on TLC system (ethyl acetate: hexane 3:7). After completion of the reaction the mixture was filtered through celite with bed wash of tetrahydrofuran (25 mL). The filtrate was concentrated under vacuum to obtain **12a-12k.** Without isolation of **12a-12k**, next cyclization reaction in 2*N* HCl (15 mL) at reflux temperature for 4–5 h was proceeded. After completion of reaction, aqueous $NaHCO_3$ solution (50 mL) was added to the reaction mixture slowly at 5–10°C. A solid product was separated out, which was filtered through Büchner funnel and suck dried to obtain 4-6-difluoro-2-{2-[3-(4-nitrophenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-4-6-difluoro-*1H*-benzo[*d*]imidazole **13a** as pale yellow colored solid.

Similarly, by using respective substituted [1,2,4]-oxadiazoles **10a-10k**, we synthesized the respective **13b-13k** by using aforementioned procedure. ^{1}H NMR, ms, IR, and elemental analysis characterized all the synthesized compounds. The details of the intermediates were given in Table 2.

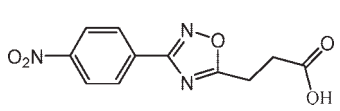
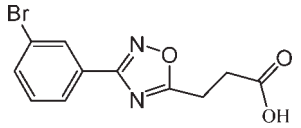
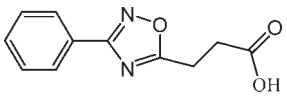
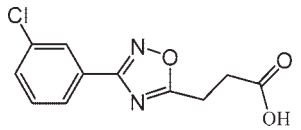
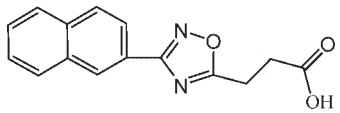
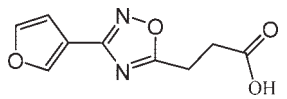
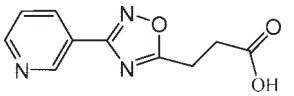
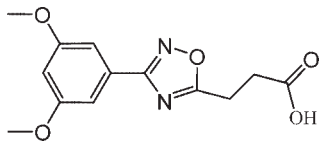
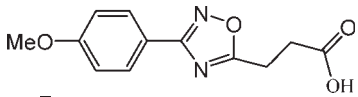
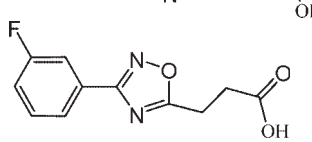
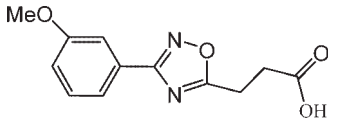
Synthesis of 4,6-difluoro-2-{2-[3-(4-nitrophenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-4,6-difluoro-1*H*-benzo[*d*]-imidazole (13a). The compound was obtained using 3-(3-(4-nitrophenyl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a pale yellow solid (diethyl ether); IR (KBr): 3242, 2768, 2547, 2362, 1719, 1578, 1516, 1438, 1340, 1219, 914, 869, 719 cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.87 (2H, t), 3.19 (2H, t), 7.12 (1H, m), 7.29 (1H, d), 8.22 (2H, d), 8.39 (2H, d), 12.4 (1H, s —NH).

Synthesis of 2-(2-(3-(3-bromophenyl)-[1,2,4]-oxadizol-5-yl)-4,6-difluoro-1*H*-benzo[*d*]imidazole (13b). The compound was obtained using 3-[3-(3-bromo-phenyl)-[1,2,4]-oxadiazol-5-yl) propionic acid as a pale yellow solid (diethyl ether); IR (KBr): 3433, 2714, 2608, 2361, 1701, 1566, 1535, 1443, 1358, 846, 744, cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.84 (2H, t), 3.19 (2H, t), 7.18(1H, m), 7.52 (2H, m), 7.78–7.81 (1H, m), 7.98 (1H, m), 8.08 (1H, m), 12.14 (1H, s, —NH).

Synthesis of 4,6-difluoro-2-[2-(3-phenyl-[1,2,4]-oxadizol-5-yl)-ethyl]-1*H*-benzo[*d*]imidazole (13c). The compound was obtained using 3-(3-phenyl-[1,2,4]-oxadiazol-5-yl) propionic acid as a off white solid (diethyl ether); IR (KBr): 3133, 2933 2714, 2608, 2361, 1701, 1632, 1590, 1443, 1358, 1231, 912, 846, 718 cm^{-1} ;^{1}H NMR (400 MHz, DMSO d_6): δ 2.8 (2H, t), 3.15 (2H, t), 7.08(1H, m), 7.12 (1H, d), 7.55 (3H, m), 7.95 (2H, m), 12.21 (1H, s, —NH).

Table 1

Characterization of intermediates **10a–10k**.

No.	Intermediates	ms (*m*/*z*)	^{1}H NMR (δ ppm)
10a		264.0	2.9 (2H, t), 3.3 (2H, t), 8.2 (2H,d), 8.4 (2H,d), 12.0 (1H,s)
10b		298.1	2.85 (2H,t), 3.25 (2H,t), 7.4 (1H,t), 7.8 (1H,d), 8.0 (1H,d) 8.1(1H,s), 12.16 (—COOH, 1H,s)
10c		219.2	2.75 (2H,t), 3.05 (2H,m), 7.4 (2H,t), 7.8 (2H,m), 8.0(1H,dd),12.2 (—COOH,1H,s)
10d		253.2	2.9(2H,t), 3.2 (2H,t), 7.4–7.8 (2H, m), 8.0 (2H, m).12.22 (—COOH,1H,s)
10e		269.3	2.8 (2H,t), 3.2 (2H,t), 7.45–7.55 (2H,m), 8.0–8.3 (2H,m) 8.8 (1H,d) 12.22 (—COOH, 1H, s)
10f		209.0	2.8 (2H, t), 3.3 (2H, t), 6.7(1H, d), 7.2(1H, d), 8.0 (1H, s), 12.2(1H, s)
10g		220.1	2.8 (2H, t), 3.2 (2H, t), 7.6(1H, m) 7.7(1H, m), 8.0 (1H, m), 8.8 (1H, s), 12.2(1H, s).
10h		279.2	2.8 (2H,t),3.2 (2H,t),3.9 (3H,s), 6.7 (1H,t), 7.1 (2H,d), 12.18 (—COOH,1H,s).
10i		249.1	2.8 (2H,t), 3.2(2H,t), 3.85(3H,s), 7.1(2H,d),7.9(2H, d), 12.4 (1H, s)
10j		237.0	2.9 (2H, t), 3.2 (2H, t), 7.4 (1H, t), 7.6 (1H,t), 7.7 (1H, d), 7.8 (1H, d), 12.2(1H, s)
10k		249.2	2.78 (2H, t), 3.25 (2H, t), 7.15 (2H, d), 8.01 (2H, d), 12.29 (1H, s)

Synthesis of 2-{2-[3-(3-chlorophenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-4,6-difluoro-1*H*-benzo[*d*]-imidazole (13d). The compound was obtained using 3-[3-(3-chloro-phenyl)-[1,2,4]-oxadiazol-5-yl) propionic acid as a brownish solid (diethyl ether); IR (KBr): 3233, 2878, 2731, 2624, 2548, 2362, 1696, 1591, 1432, 1337, 1239, 1168, 898,752 cm^{-1} ;^{1}H NMR (400 MHz, DMSO d_6): δ 2.8–3.08 (4H, s), 7.04 (1H, m), 7.21 (1H, d), 7.62 (2H, m), 7.74 (1H, m), 7.92 (1H, m), 12.1 (1H, s, —NH).

Synthesis of 4,6-difluoro-2-[2-(3-pyridin-3-yl-[1,2,4]-oxadizol-5-yl)-ethyl]-1*H*-benzo[*d*]imidazole (13e). The compound was obtained using 3-(3-pyridin-3-yl)-[1,2,4]-oxadiazol-

Table 2

Characterization of the compounds **13a–13k**.

Compound	R	Time (h)	Mp (°C)	Yield %	Molecular Formula	Analysis (%) Calcd./Found C	H	N
13a	4-NO_2	4	120–122	75	$C_{17}H_{11}F_2N_5O_3$	54.99	2.99	18.86
						55.09	3.01	18.88
13b	3-Br	4	129–131	71	$C_{17}H_{11}BrF_2N_4O$	50.39	2.74	13.83
						50.47	2.79	13.85
13c	H	5	112–114	74	$C_{17}H_{12}F_2N_4O$	62.58	3.71	17.17
						62.71	3.67	17.26
13d	3-Cl	5	118–120	69	$C_{17}H_{11}ClF_2N_4O$	56.60	3.07	15.53
						56.74	3.15	15.74
13e	3-Pyridyl	4	162–164 (dec)	69	$C_{16}H_{11}F_2N_5O$	58.72	3.39	21.40
						58.72	3.46	21.52
13f	3-furan	6	134–136	72	$C_{15}H_{10}F_2N_4O_2$	56.97	3.19	17.71
						56.89	3.30	17.70
13g	Naphthalene	5	123–125	73	$C_{21}H_{14}F_2N_4O$	67.02	3.75	14.89
						67.11	3.78	14.99
13h	3,5-Di-OMe	5	129–131	75	$C_{19}H_{16}F_2N_4O_3$	59.07	4.17	14.50
						59.12	4.23	14.53
13i	4-OMe	5	136–138	70	$C_{18}H_{14}F_2N_4O_2$	60.67	3.96	15.72
						60.62	3.97	15.71
13j	3-F	4	141–143	68	$C_{17}H_{11}ClF_2N_4O$	56.60	3.07	15.53
						56.59	3.16	15.52
13k	3-OMe	4	156–158	76	$C_{18}H_{14}F_2N_4O_2$	60.67	3.96	15.72
						60.80	4.06	15.91

5-yl) propionic acid as a off white solid (diethyl ether); IR (KBr): 3331, 2922, 2731, 2624, 2362, 1695, 1570, 1440, 1358, 1244, 910, 844, 738 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.75 (2H, t), 3.18 (2H, t) 7.18 (1H, d), 7.31 (1H, m), 7.89 (2H, m), 8.51 (2H, m), 12.14 (1H, s —NH).

Synthesis of 4,6-difluoro-2-[2-(3-furan-3-yl-[1,2,4]-oxadizol-5-yl)-ethyl]-1*H*-benzo[*d*] imidazole (13f). The compound was obtained using 3-(3-(furan-2-yl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a off white solid (diethyl ether); IR (KBr): 3327, 2928, 2714, 2604, 2361, 1628, 1580, 1437, 1312, 1275, 1127, 853, 743 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.77 (2H, t), 3.12 (2H, t) 7.27 (1H, d), 7.57 (2H, m), 7.69 (2H, m), 12.11(1H, s —NH).

Synthesis of 4,6-difluoro-2-[2-(3-napthalen-2-yl-[1,2,4]-oxadizol-5-yl)-ethyl]-1*H*-benzo[*d*]imidazole (13g). The compound was obtained using 3-(3-naphthalen-2-yl-[1,2,4]-oxadiazol-5-yl)-propionic acid as a buff colored solid (diethyl ether); IR (KBr): 3274, 3054, 2932, 2720, 2362, 1739, 1708, 1584,1499, 1428, 1306, 1157, 898, 749 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.96 (2H, t), 3.21 (2H, t), 7.22 (1H, d),

Table 3

Antibacterial activity of compounds **13a–13k**.

Compound	R	Organisms Sa	Pa	Ec	St
13a	4-NO_2	27	25	20	19
13b	3-Br	23	18	16	14
13c	H	21	23	18	19
13d	3-Cl	21	25	18	14
13e	3-Pyridyl	20	22	23	23
13f	3-Furan	25	23	21	24
13g	Naphthalene	27	26	24	20
13h	3,5 Di-OMe	19	20	15	13
13i	4-OMe	29	26	22	19
13j	3-F	20	22	19	18
13k	3-OMe	22	23	21	20
Gentamycin	–	34	35	30	29

Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, St: *Salmonella typhosa*.

7.52– 7.74 (4H, m), 8.05 (1H, d), 8.2 (2H, m), 8.8 (1H, d), 12.36 (1H, s —NH).

Synthesis of 4,6-difluoro-2-{2-[3-(3,5-dimethoxy phenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-1*H*-benzo[d] imidazole (13h). The compound was obtained using 3-(3-(3,5-dimethoxyphenyl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a buff colored solid (diethyl ether); IR (KBr): 3101, 2714, 2612, 2361, 1701, 1559, 1535, 1443, 1358, 900, 740 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.75–3.1(4H, s), 3.99 (6H, s), 7.11 (2H, m), 7.22 (2H, m), 7.46 (1H, m), 12.14 (1H, s —NH).

Synthesis of 4,6-difluoro-2-{2-[3-(4-methoxy phenyl)–[1,2,4]-oxadizol-5-yl]-ethyl}-1*H*-benzo[*d*]-imidazole (13i). The compound was obtained using 3-(3-(4-methoxyphenyl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a brownish colored solid (diethyl ether); IR (KBr): 2940, 2608, 2362, 1710, 1596, 1482, 1362, 1255, 1029, 843, 749 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.75–3.1(4H, s), 4.02 (3H, s), 7.11 (1H, d), 7.24 (3H, m), 7.46 (2H, m), 12.04 (1H, s —NH).

Synthesis of 4,6-difluoro-2-{2-[3-(3-fluorophenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-1*H*-benzo[*d*]imidazole (13j). The compound was obtained using 3-(3-(3-fluorophenyl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a brownish solid (diethyl ether); IR (KBr): 3233, 2878, 2731, 2624, 2548, 2362, 1696, 1591, 1432, 1337, 1239, 1168, 898,752 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.8–3.08 (4Hs), 7.04 (1H,m), 7.21 (1H,d), 7.62 (2H,m), 7.74 (1H,m), 7.92 (1H,m), 12.1 (1H,s —NH).

Synthesis of 4,6-difluoro-2-{2-[3-(4-methoxy phenyl)-[1,2,4]-oxadizol-5-yl]-ethyl}-1*H*-benzo[*d*]-imidazole (13k). The compound was obtained using 3-(3-(4-methoxyphenyl)-[1,2,4]-oxadiazol-5-yl) propanoic acid as a brownish colored solid (diethyl ether); IR (KBr): 2940, 2608, 2362, 1710, 1596, 1482, 1362, 1255, 1029, 843, 749 cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 2.75–3.1(4H, s), 4.02 (3H, s), 7.11 (1H, d), 7.24 (3H, m), 7.46 (2H, m), 12.04 (1H, s —NH).

PHARMACOLOGICAL ACTIVITY

Antimicrobial activity. The title compounds were screened for the antimicrobial activity against two different gram positive and two gram negative microorganisms *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), and *Salmonella typhosa* (ATCC 14028) under the following conditions. (Table 3).

Method: Well diffusion method [34], Medium: The nutrient agar medium,

Solvent: Chloroform: Concentrations: 50 and 100 μ*M*.

Condition: 24 h at 24–28°C, Standard: The antibiotic Gentamycin.

The nutrient agar medium, 20 mL was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 h sub cultured bacteria was inoculated in the petri plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 and 100 μ*M* using a micropipette. The plates were left over for 24 h at 24–28°C. The antibiotic Gentamycin was used as a standard for comparative study.

The percentage of inhibition was calculated by the formula% Inhibition = Diameter of the inhibition zone × 100.

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