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Synthesis and anti-bacterial, anti-fungal activity of novel 1,2,4-oxadiazole

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ABSTRACT

*A series of novel 1, 2, 4-oxadiazole derivatives were synthesized and evaluated for in- vitro antibacterial and antifungal activity. All the compounds tested against bacteria showed comparable or less antibacterial activities than the reference drug. Differences in their activity depend on the substitution of different groups. More specifically, best antibacterial activity among synthetic analogues was shown by compound **6** with MIC of 10 µg/ml and compound **14** with MIC of 50 µg/ml against *Bacillus subtilis*. All compounds tested showed significant antifungal activity against all the fungi, compared to the commercial fungicide Miconazole. Among them compounds **4** and **8** showed good antifungal activity against *Fusarium solani* with MIC of 10 and 50 µg/ml.*

Keywords:-Anti-bacterial, Anti-fungal, 1, 2, 4-oxadiazole, MIC value.

INTRODUCTION

During the past decades, the human population affected with life-threatening infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased to an alarming level around the world [1]. Due to this reason, it is imperative to design and develop new antibacterial or antifungal agents with novel chemical structures preferably having different modes of action rather than analogues of the existing ones. 1, 2, 4-Oxadiazoles exhibit diverse biological activities. Oxadiazoles have often been described as bio-isosteres for amides and esters. Due to increased hydrolytic and metabolic stabilities of the oxadiazole ring, improved pharmacokinetic and *in vivo* performance are often observed, which makes these heterocycles an important structural moiety for the pharmaceutical industry. As consequence of these

characteristics, oxadiazoles have often been the target of numerous drug discovery programs as anti-inflammatory agents [2], anti-tumor agents [3], potential anticancer agents [4], Histamine H₃ receptor antagonists [5] as potent inhibitors of MIF biological function [6], and bell-tryptase inhibitors [7], In addition, 1,2,4-oxadiazoles are widely used as hydrolysis resisting amide bioisosteres in the development of peptidomimetics [8]. Also, oxadiazoles exhibit wide range of antibacterial, antifungal and activities against Gram-positive and Gram-negative bacteria [9,10,11].

As a part of research program decide to develop a new antimicrobials, we report the synthesis and evaluation of 1,2,4-oxadiazoles derivative having aryl and alkyl groups at C-5 position and one or more methylene groups as a linker between the aromatic nucleus and carboxylic acid group. Oxadiazoles having aryl, alkyl and one or more methylene groups as a linker exhibit different biological activities such as potent inhibitors of MIF biological function,⁶ interleukin-8 (IL-8) receptor antagonists [12], anticancer activities [13], anti-inflammatory [14], antimicrobial [15], and cytotoxic activity [16], We use electron-withdrawing substituent on starting materials because in literature some reports are available with electron-withdrawing group on different biological activity such as antiinflammatory, antitumor properties [17], and β -amyloid imaging agents [18], Thus it is clear that the 1,2,4-oxadiazole nucleus is quite interesting and we report a simple and straightforward synthesis and also antimicrobial activity of 1,2,4-oxadiazoles.

Based on these reports we embarked on the synthesis of novel 1,2,4-oxadiazoles with introduction of aryl and alkyl moieties at C5 position and also one or more methylene group as a linker between the aromatic nucleus and the carbonyl functionality as a part of our research programme. These compounds are then screened for *in-vitro* antibacterial and antifungal activities.

EXPERIMENTAL SECTION

All reagents were used of analytical grade (Thomas Baker) ¹HNMR spectra were recorded on Cuker Advance spectrometer (30MHz or 500MHz) using tetramethylsilane as internal standard: J values are in Hertz. Chemical shifts are reported in ppm (δ) relative to the solvent peak, Mass spectra were recorded on either GCMS (focus GC with TSQ II mass analyzer and thermoelectro) with autosampler/direct injection (EI/CI) or LCMS (APCI/ESI; Buker daltanoics Micro TOFQ). HPLC purity was checked using Water Alliances or Dionex Ultima 3000 HPLC system. All chromatographic purifications were done on silica gel (100-200 mesh). Ethyl acetate and petroleum ether were used for purification of compounds (Merck Kiesel 60 F254, 0.2mm thickness sheet).

Synthesis of 4-(N-Hydroxycarbamimidoyl)-benzoic acid methyl ester (2): Hydroxylamine hydrochloride (321 mg, 4.6 mmol) and sodium carbonate (326 mg, 3.1 mmol) were added to a solution of 4-Cyano-benzoic acid methyl ester (**1**), (500 mg, 3.1 mmol) in ethanol. The resultant mixture was stirred at room temperature, following which water was added. The reaction mixture was stirred at room temperature for 6 h. then reaction mixture was poured in to ice cold water and filtered to obtain the crude. This was purified by precipitation using ethyl acetate and hexane to obtain a title compound as white solid. Yield: 68%; ¹HNMR (DMSO, 300MHz): δ 8.16 (d, $J = 9$ Hz, 2H), 7.89 (d, $J = 9$ Hz, 2H), 6.16 (bs, 2H), 3.98 (s, 3H); MS (APCI); m/z 195 [M+1]⁺

Synthesis of 4-(5-Methyl-[1,2,4]oxadiazol-3-yl)-benzoic acid methyl ester (3): 4-(N-Hydroxycarbamimidoyl)-benzoic acid methyl ester (**2**), (100 mg, 0.5 mmol) in pyridine was added dropwise to a solution of acticanhydride (26 mg, 0.25 mmol) in dry pyridine. The resultant mixture was stirred at 100-120C for 8 h. following the completion of reaction time, ice

cold water was added and extracted with ethyl acetate, organic layer wash with 1N HCl followed by brine to obtain the crude compound which was purified by silica gel column chromatography to obtain the title compound as white solid. Yield 52%; $^1\text{H NMR}$ (CDCl_3 , 300MHz): δ 8.06 (d, $J = 9$ Hz, 2H), 7.78 (d, $J = 9$ Hz, 2H), 3.93 (s, 3H); 2.86 (s, 3H); MS (APCI); m/z 219 $[\text{M}+1]^+$; HPLC: 93.32%.

Synthesis of 4-(5-Trifluoromethyl-[1,2,4]oxadiazol-3-yl)-benzoic acid methyl ester (4): The compound was prepared from (2) using trifluoroacetic anhydride and pyridine. The synthetic method followed the same as for 3. White solid. Yield 56%; $^1\text{H NMR}$ (DMSO, 300MHz): δ 8.18 (d, $J = 9$ Hz, 2H), 7.85 (d, $J = 9$ Hz, 2H), 3.95 (s, 3H); MS (APCI); m/z 273 $[\text{M}+1]^+$; HPLC: 96.31%.

Synthesis of 4-{5-[2-(Carboxy-methyl-amino)-phenyl]-[1,2,4]oxadiazol-3-yl}-benzoic acid methyl ester (5): The compound was prepared from (2) using N-methylisatoic anhydride and pyridine. The synthetic method followed the same as for (3). White solid. Yield 64%; $^1\text{H NMR}$ (DMSO, 300MHz): δ 11.13 (bs, 1H), 8.17 (d, $J = 6$ Hz, 1H), 8.04 (d, $J = 9$ Hz, 2H), 7.91 (d, $J = 9$ Hz, 2H), 7.60 (d, $J = 6$ Hz, 1H), 7.44 (t, 1H), 6.61 (t, 1H), 3.89 (s, 3H), 2.85 (s, 3H); MS (APCI); m/z 354 $[\text{M}+1]^+$; HPLC: 96.04%.

Synthesis of 4-[5-(2-Carboxy-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (6): The compound was prepared from (2) using succinic anhydride and pyridine. The synthetic method followed the same as for (3). White solid. Yield 61%; $^1\text{H NMR}$ (CDCl_3 , 300MHz): δ 11.02 (bs, 1H), 8.07 (d, $J = 9$ Hz, 2H), 7.76 (d, $J = 9$ Hz, 2H), 3.95 (s, 3H), 3.29 (t, 2H), 2.98 (t, 2H); MS (APCI); m/z 277 $[\text{M}+1]^+$; HPLC: 94.38%.

Synthesis of 4-[5-(2-Methoxycarbonyl-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (7): In a solution of compound 4-[5-(2-Carboxy-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester 6 (100 mg, 0.3 mmol) in methanol, SOCl_2 (106 mg, 0.9 mmol) was added dropwise at 0°C and stirred the reaction mixture at room temperature for 10-12 h. following the completion of reaction time, SOCl_2 was removed from the reaction mixture using distillation. ice cold water was added and extracted with ethyl acetate, organic layer wash with brine to obtain the crude compound which was purified by precipitation using ethyl acetate and hexane to obtain a white solid. Yield 52%; $^1\text{H NMR}$ (CDCl_3 , 300MHz): δ 8.14 (d, $J = 9$ Hz, 2H), 7.91 (d, $J = 9$ Hz, 2H), 3.95 (s, 3H), 3.73 (s, 3H), 3.30 (t, 2H), 2.98 (t, 2H); MS (APCI); m/z 291 $[\text{M}+1]^+$; HPLC: 92.75%.

Synthesis of 4-[5-(2-Cyclopropylcarbamoyl-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (8): Thionyl chloride (106 mg, 0.9 mmol) was added dropwise to a solution of 4-[5-(2-Carboxy-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (6) (100 mg, 0.3 mmol) at 0°C . The reaction mixture was stirred at $40-45^\circ\text{C}$ for 2 h. subsequently; SOCl_2 was removed from the reaction mixture using distillation. Following this, added Cyclopropylamine (30 mg, 0.5 mmol) was added. This was followed by addition of triethylamine (Et_3N) (43 mg, 0.4 mmol). The reaction mixture was stirred for 15-16 h at room temperature. Following the completion of reaction, the mixture was poured in to ice-cold water and extracted with ethyl acetate to obtain crude compound which was purified by silica gel column chromatography to obtain the title compound as white solid. Yield 58%; $^1\text{H NMR}$ (CDCl_3 , 300MHz): δ 8.05 (bs, 1H), 8.03 (d, $J = 9$ Hz, 2H), 7.76 (d, $J = 9$ Hz, 2H), 3.95 (s, 3H), 3.16 (t, 2H), 2.85 (t, 2H), 2.32 (m, 1H), 0.56 (m, 4H); MS (APCI); m/z 316 $[\text{M}+1]^+$; HPLC: 90.00%.

Synthesis of 4-[5-(3-Carboxy-propyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (9): The compound was prepared from (2) using Glutaric anhydride and pyridine. The synthetic

method followed the same as for (3). White solid. Yield 68%; ¹HNMR (DMSO, 300MHz): δ 12.03 (bs, 1H), 8.13 (d, *J* = 9Hz, 2H), 7.89 (d, *J* = 9Hz, 2H), 3.88 (s, 3H), 3.07 (t, 2H), 2.41 (t, 2H), 2.02 (m, 2H); MS (APCI); *m/z* 291[M+1]⁺; HPLC: 86.59%.

Synthesis of 4-[5-(3-Methoxycarbonyl-propyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (10): The compound was prepared following same synthetic method similar to that for (7). White solid. Yield 56%; ¹HNMR (DMSO, 300MHz): δ 8.14 (d, *J* = 9Hz, 2H), 7.93 (d, *J* = 9Hz, 2H), 3.95 (s, 3H), 3.69 (s, 3H), 3.07 (t, 2H), 2.54 (t, 2H), 2.27 (m, 2H); MS (APCI); *m/z* 307[M+1]⁺; HPLC: 93.22%.

Synthesis of 4-[5-(3-Cyclopropylcarbamoyl-propyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (11): The compound was prepared following same synthetic method similar to that for 8. White solid. Yield 50%; ¹HNMR (DMSO, 300MHz): δ 8.13 (s, 1H), 8.04 (d, *J* = 9Hz, 2H), 7.76 (d, *J* = 9Hz, 2H), 3.95 (s, 3H), 3.29 (t, 2H), 2.58 (t, 2H), 2.12 (m, 1H), 1.57 (m, 2H), 0.54 (m, 4H); MS (APCI); *m/z* 330[M+1]⁺; HPLC: 82.38%.

Synthesis of 4-Bromo-N-hydroxy-benzamidine (13): Hydroxylamine hydrochloride (284 mg, 4.1 mmol) and sodium carbonate (288 mg, 2.7 mmol) were added to a solution of 4-Bromo-benzonitrile (12), (500 mg, 2.7 mmol) in ethanol. The resultant mixture was stirred at room temperature, following which water was added. The reaction mixture was stirred at room temperature for 6 h. then reaction mixture was poured in to ice cold water and filtered to obtain the crude. This was purified by precipitation using ethyl acetate and hexane to obtain a title compound as white solid. Yield: 71%; ¹HNMR (DMSO, 300MHz): δ 7.79 (d, *J* = 6Hz, 2H), 7.62 (d, *J* = 6Hz, 2H), 5.81 (bs, 2H); MS (APCI); *m/z* 216[M+1]⁺

Synthesis of 3-(4-Bromo-phenyl)-5-methyl-[1,2,4]oxadiazole (14): 4-Bromo-N-hydroxy-benzamidine (100 mg, 0.4 mmol) (13) in pyridine was added dropwise to a solution of acetic anhydride (23 mg, 0.2 mmol) in dry pyridine. The resultant mixture was stirred at 100-120C for 8 h. following the completion of reaction time, ice cold water was added and extracted with ethyl acetate, organic layer wash with 1N HCl followed by brine to obtain the crude compound which was purified by silica gel column chromatography to obtain the title compound as white solid. Yield 61%; ¹HNMR (CDCl₃, 300MHz): δ 7.69 (d, *J* = 9Hz, 2H), 6.98 (d, *J* = 9Hz, 2H), 2.58 (s, 3H); MS (APCI); *m/z* 240[M+1]⁺; HPLC: 82.51%.

Synthesis of 3-(4-Bromo-phenyl)-5-trifluoromethyl-[1,2,4]oxadiazole (15): The compound was prepared from (13) using trifluoroacetic anhydride and pyridine. The synthetic method followed the same as for (14). White solid. Yield 56%; ¹HNMR (CDCl₃, 300MHz): δ 7.74 (d, *J* = 9Hz, 2H), 6.98 (d, *J* = 9Hz, 2H), MS (APCI); *m/z* 294[M+1]⁺; HPLC: 90.76%.

Synthesis of {2-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-phenyl}-methyl-carbamic acid (16): The compound was prepared from (13) using N-methylisatoic anhydride and pyridine. The synthetic method followed the same as for (14). White solid. Yield 67%; ¹HNMR (DMSO, 300MHz): δ 12.18 (bs, 1H), 7.81 (d, *J* = 9Hz, 2H), 7.60 (d, *J* = 9Hz, 2H), 7.43 (t, 1H), 6.92 (d, *J* = 6Hz, 1H), 6.74 (d, *J* = 6Hz, 1H), 6.61 (t, 1H), 2.83 (s, 3H); MS (APCI); *m/z* 375[M+1]⁺; HPLC: 91.29%

Synthesis of 3-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-propionic acid (17): The compound was prepared from (13) using succinic anhydride and pyridine. The synthetic method followed the same as for (14). White solid. Yield 68%; ¹HNMR (CDCl₃, 300MHz): δ 11.09 (bs, 1H), 7.92 (d, *J* = 9Hz, 2H), 7.77 (d, *J* = 9Hz, 2H), 3.20 (t, 2H), 2.84 (t, 2H); MS (APCI); *m/z* 298[M+1]⁺; HPLC: 87.82%.

Synthesis of 3-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-propionic acid methyl ester (18): In a solution of compound 3-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-propionic acid (**17**), (100 mg, 0.3 mmol) in methanol, SOCl₂ (99 mg, 0.8 mmol) was added dropwise at 0 °C and stirred the reaction mixture at room temperature for 10-12 h. following the completion of reaction time, SOCl₂ was removed from the reaction mixture using distillation. ice cold water was added and extracted with ethyl acetate, organic layer wash with brine to obtain the crude compound which was purified by precipitation using ethyl acetate and hexane to obtain a white solid. Yield 58%; ¹HNMR (CDCl₃, 300MHz): δ 7.88 (d, *J* = 9Hz, 2H), 7.76 (d, *J* = 9Hz, 2H), 3.57 (s, 3H), 3.22 (t, 2H), 2.90 (t, 2H); MS (APCI); *m/z* 312[M+1]⁺; HPLC; 85.83%.

Synthesis of 3-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-N-cyclopropyl-propionamide (19): Thionyl chloride (99 mg, 0.8 mmol) was added dropwise to a solution of 3-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-propionic acid (**17**), (100 mg, 0.3 mmol) at 0°C. The reaction mixture was stirred at 40-45 °C for 2 h. subsequently; SOCl₂ was removed from the reaction mixture using distillation. Following this, added Cyclopropylamine (28 mg, 0.5 mmol) was added. This was followed by addition of triethylamine (Et₃N) (47 mg, 0.4 mmol). The reaction mixture was stirred for 15-16 h at room temperature. Following the completion of reaction, the mixture was poured in to ice-cold water and extracted with ethyl acetate to obtain crude compound which was purified by silica gel column chromatography to obtain the title compound as white solid. Yield 51%; ¹HNMR (DMSO, 300MHz): δ 8.05 (s, 1H), 7.88 (d, *J* = 9Hz, 2H), 7.74 (d, *J* = 9Hz, 2H), 3.14 (t, 2H), 2.60 (t, 2H), 2.20 (m 1H), 0.52 (m, 4H); MS (APCI); *m/z* 337[M+1]⁺; HPLC; 96.23%.

Synthesis of 4-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-butyric acid (20): The compound was prepared from (**13**) using Glutaric anhydride and pyridine. The synthetic method followed the same as for (**14**). White solid. Yield 65%; ¹HNMR (DMSO, 300MHz): δ 11.83 (s, 1H), 7.93 (d, *J* = 9Hz, 2H), 7.77 (d, *J* = 9Hz, 2H), 2.70 (t, 2H), 2.37 (t, 2H), 1.82 (m, 2H), MS (APCI); *m/z* 312[M+1]⁺; HPLC; 91.90%.

Synthesis of 4-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-butyric acid methyl ester (21): The compound was prepared following same synthetic method similar to that for (**18**). White solid. Yield 50%; ¹HNMR (DMSO, 300MHz): δ 7.89 (d, *J* = 9Hz, 2H), 7.68 (d, *J* = 9Hz, 2H), 3.69 (s, 3H), 3.02 (t, 2H), 2.49 (t, 2H), 1.93 (m, 2H); MS (APCI); *m/z* 326[M+1]⁺; HPLC; 83.23%.

Synthesis of 4-[3-(4-Bromo-phenyl)-[1,2,4]oxadiazol-5-yl]-N-cyclopropyl-butyramide (22): The compound was prepared following same synthetic method similar to that for (**19**). White solid. Yield 61%; ¹HNMR (DMSO, 300MHz): δ 8.03 (s, 1H), 7.78 (d, *J* = 9Hz, 2H), 7.56 (d, *J* = 9Hz, 2H), 2.57 (t, 2H), 2.43 (m, 1H), 2.20 (t, 2H) 1.67 (m, 2H), 0.52 (m, 4H); MS (APCI); *m/z* 351[M+1]⁺; HPLC; 85.65%.

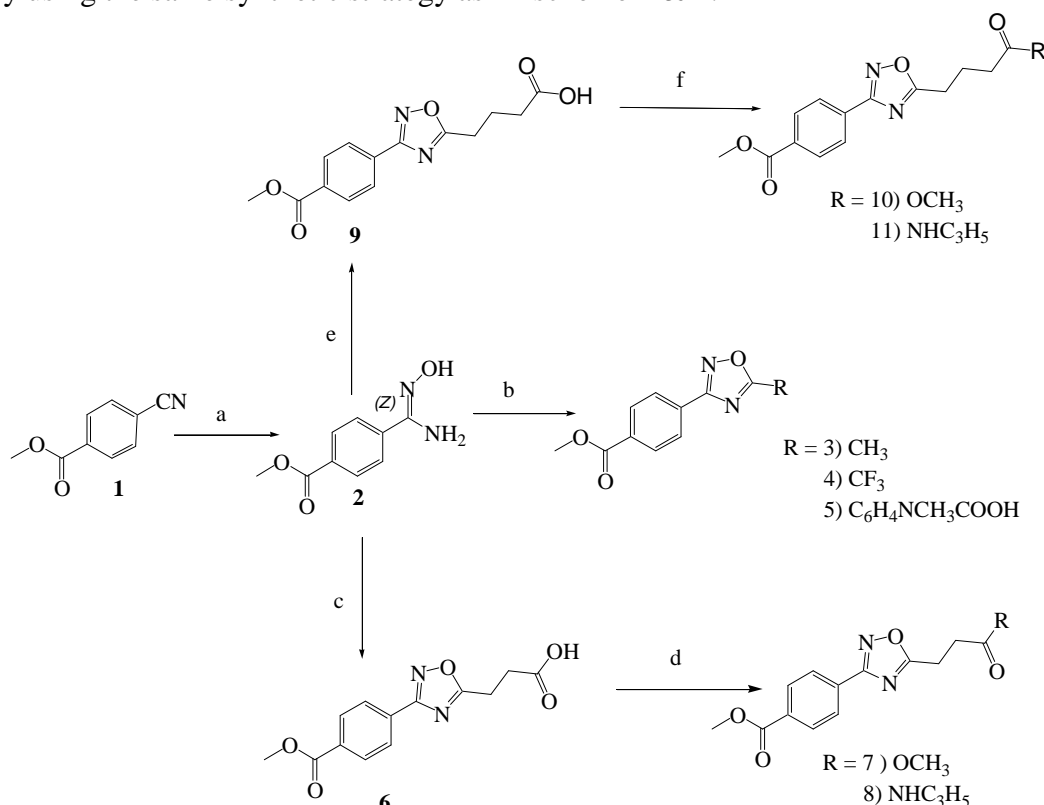
RESULTS AND DISCUSSION

Chemistry

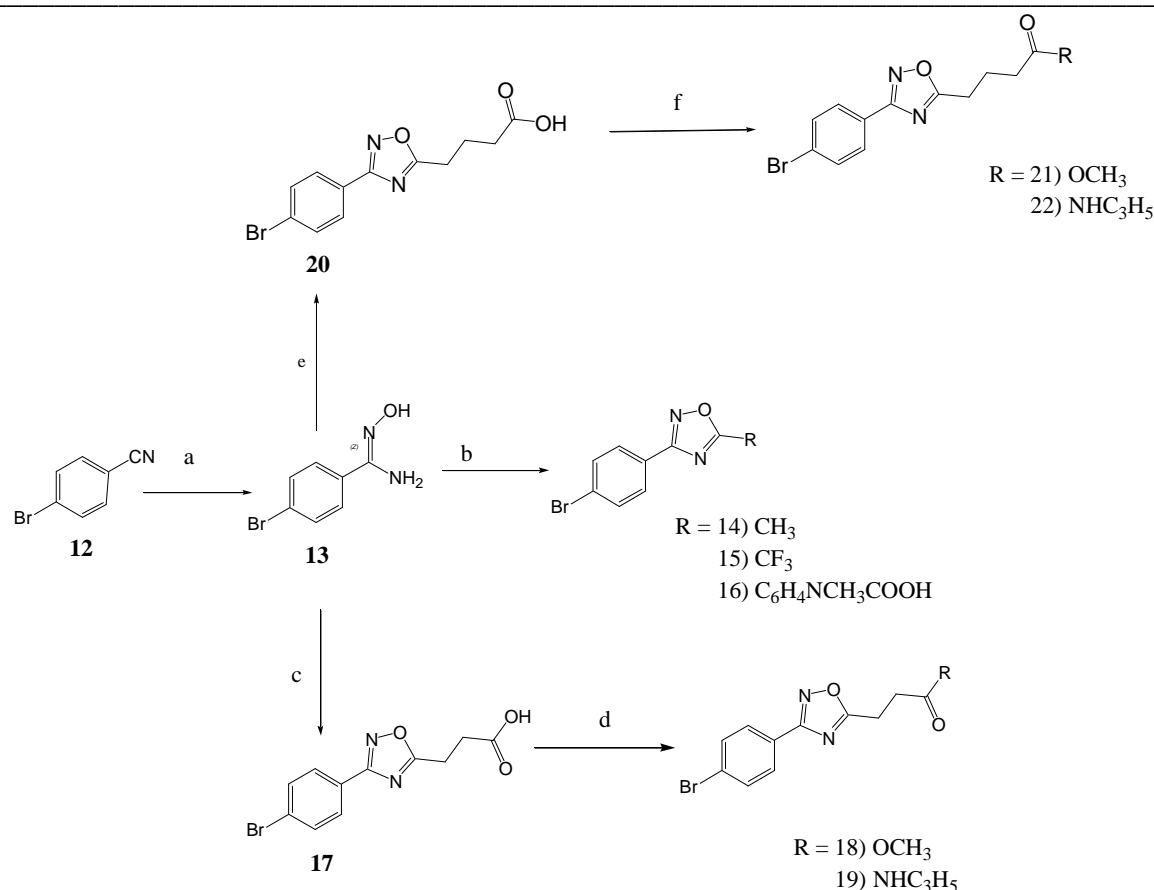
Numerous synthetic methodologies are known in the literature for synthesis of 1, 2, 4-oxadiazoles. These include the condensation of amidoximes with different substituted carboxylic acids in presence of different coupling reagents such as 1,1-carbonyldiimidazole (CDI), dicyclohexylcarbodiimide (DCC) to give O-acylamidoximes which are cyclized to 1,2,4-oxadiazoles [19,20,21], green synthesis from beta-keto esters and amidoximes under solvent free conditions [22], microwave-assisted efficient synthesis [23,24], and solid supported protocols [25].

Our synthetic strategy for 1,2,4-oxadiazole derivatives is illustrated in scheme 1 and 2. 4-(N-Hydroxycarbamimidoyl)-benzoic acid methyl ester (**2**), a key intermediate for the proposed synthesis was synthesized from 4-cyano-benzoic acid methyl ester (**1**) using hydroxylamine hydrochloride (68% yield). The reaction of (**2**) with Acetic anhydride in pyridine afforded 4-(5-methyl-[1,2,4]oxadiazol-3-yl)-benzoic acid methyl ester (**3**) (52% yield). Similarly 4-(5-Trifluoromethyl-[1,2,4]oxadiazol-3-yl)-benzoic acid methyl ester (**4**) in (56% yield), 4-[5-[2-(Carboxy-methyl-amino)-phenyl]-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**5**) (64% yield), 4-[5-(2-Carboxy-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**6**) (61% yield), and 4-[5-(3-Carboxy-propyl)-4,5-dihydro-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**9**) (68% yield) were prepared using different substituted anhydride.

Esterification of compound (**6**) and (**9**) using (SOCl₂)/methanol afforded 4-[5-(3-Methoxycarbonyl-propyl)-4,5-dihydro-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**10**) (56% yield) and 4-[5-(2-Methoxycarbonyl-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**7**) (52% yield) respectively. 4-[5-(2-Cyclopropylcarbamoyl-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**8**) (58% yield) and 4-[5-(3-Cyclopropylcarbamoyl-propyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (**11**) (50% yield) were obtained by treating the compound (**6**) and (**9**) with SOCl₂ to prepare 4-[5-(2-Chlorocarbonyl-ethyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester and 4-[5-(3-Chlorocarbonyl-propyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (not isolated) which is in-situ treated with triethylamine (Et₃N)/methylcyclopropane to give the title compounds. All the compounds (13–22) were prepared in good yields by using the same synthetic strategy as in scheme 1 & 2.



Scheme 1. Reagents and conditions (a) NH₂OH.HCl, Na₂CO₃/EtOH, H₂O, r.t, 6h, 68%; (b) (3) acetic anhydride, pyridine, 100–120^oC, 8 h, 52%, (4) trifluoroacetic anhydride/pyridine, 100–120^oC, 8 h, 56%, (5) N-methylisatoic anhydride/pyridine, 100–120^oC, 8 h, 64% (c) succinic anhydride/pyridine, 100–120^oC, 8 h, 61%, (d) (7) SOCl₂/MeOH, r.t, 12-14h, 52% (8) SOCl₂, 40^oC, 2h, Et₃N/cyclopropylamine, r.t.12-14h, 58% (e) Glutaric anhydride/Pyridine, 80^oC, 7-8h, 68% (f) (10) SOCl₂/MeOH, r.t, 12-14h, 56% (11) SOCl₂, 40^oC, 2h, Et₃N/cyclopropylamine, r.t.12-14h, 50%



Scheme 2. Reagent and conditions (a) $\text{NH}_2\text{OH}\cdot\text{HCl}$, $\text{Na}_2\text{CO}_3/\text{EtOH}$, H_2O , r.t, 6h, 71%; (b) (14) acetic anhydride, pyridine, $100\text{--}120^\circ\text{C}$, 8 h, 61%, (15) trifluoroacetic anhydride/pyridine, $100\text{--}120^\circ\text{C}$, 8 h, 56%, (16) *N*-methylisatoic anhydride/pyridine, $100\text{--}120^\circ\text{C}$, 8h,67% (c) succinic anhydride/pyridine, $100\text{--}120^\circ\text{C}$, 8 h, 68%, (d) (18) $\text{SOCl}_2/\text{MeOH}$, r.t, 12-14h, 58% (19) SOCl_2 , 40°C , 2h, $\text{Et}_3\text{N}/\text{cyclopropylamine}$, r.t.12-14h, 51% (e) Glutaric anhydride/Pydrine, 80°C , 7-8h, 65% (f) (21) $\text{SOCl}_2/\text{MeOH}$, r.t, 12-14h, 50% (22) SOCl_2 , 40°C , 2h, $\text{Et}_3\text{N}/\text{cyclopropylamine}$, r.t.12-14h.

Biology

In this present work a novel series of 1,2,4-oxadiazole compounds were synthesized. All the synthesized compounds were screened for their antibacterial and antifungal activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Aspergillus niger*, and *Fusarium solani*. The minimum inhibitory concentration (MIC) of all compounds were also determined. The antibacterial data (Table-1) revealed that all tested compounds exhibit moderate to good activity against all the tested bacteria. As compared to the standard drug ciprofloxacin which has of MICs 10, 50, 50, 10 $\mu\text{g}/\text{ml}$ against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, and *Escherichia coli*, respectively. Compound **6** showed very promising activity with MIC 10 $\mu\text{g}/\text{ml}$ against *Bacillus subtilis* and also significant activities of 50, 100, 100 $\mu\text{g}/\text{ml}$ against *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*. 3-(4-Bromo-phenyl)-5-methyl-[1,2,4]oxadiazole(**14**) showed comparable activity with MIC of 50 $\mu\text{g}/\text{ml}$ against *Bacillus subtilis* and showed moderate activity against all tested bacteria. The other compounds showed moderate or weak MIC against all tested bacteria.

The screening data of antifungal activity of these series of compounds spans wide range of antifungal activity. It is of interest that compound **4** was found to exhibit the most potent in vitro antifungal activity with MIC 10 $\mu\text{g}/\text{ml}$ against *Fusarium solani* which is even so much more potent than standard drug miconazole with MIC of 50 $\mu\text{g}/\text{ml}$ against *Fusarium solani*. Also compound **8** showed significant activity with MICs of 100, 50 $\mu\text{g}/\text{ml}$ against *Aspergillus niger*

and *Fusarium solani*. The most potent antibacterial activity exhibited by compound **6** might be due to the presence of two methylene group linker between the aromatic nucleus and the carboxylic acid group on the other hand the most potent antifungal activity exhibited by compound **4** might be due to the presence of CF₃ group attached to oxadiazole. It is interesting to note that a minor change in the molecular structure of investigated compounds may have a pronounced effect on antimicrobial screening e.g. compound **9** with three methylene group linker has very weak antimicrobial activity. Compound **17** and **20** showed poor antimicrobial activity when introduced electron-withdrawing group (4-Bromo). When we introduced amide instead of acid compound **8** showed good antifungal activity and introduction of electron-withdrawing group in compound **19** again drops the antifungal activity. Introduction of alkyl moieties as in compound **14** showed comparable antibacterial activity but compounds **3** showed poor antimicrobial activity with having alkyl moieties. By replacing alkyl CH₃ group by CF₃, a significant change in activity was observed as compound **4** showed most potent antifungal activity than compounds **14** and **3**. On the other hand changes in the molecular structure of compounds **5, 7, 10, 11, 16, 18, 19, 21** and **22** did not showed any antimicrobial activity.

Table 1: Antimicrobial activity expressed as MIC (µg/mL)

Compounds	Grampositive		Gram-negative		Fungi	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S.typhimurium</i>	<i>E. coli</i>	<i>A. niger</i>	<i>F. solani</i>
2	200	200	200	100	100	100
3	200	200	100	200	200	200
4	500	200	500	500	200	10
5	100	500	200	200	200	200
6	50	10	100	100	50	200
7	100	200	200	100	500	100
8	100	100	100	100	100	50
9	500	500	500	200	100	200
10	500	200	500	200	500	500
11	200	100	200	200	100	200
13	200	200	100	100	100	100
14	100	50	100	100	200	200
15	500	500	500	500	500	500
16	200	200	200	500	500	500
17	200	200	200	200	500	200
18	500	200	200	200	100	200
19	200	200	200	100	200	100
20	200	500	100	100	500	500
21	200	500	500	500	500	500
22	200	200	100	200	100	200
Ciprofloxan	10	50	50	10	NT	NT
Miconazole	NT	NT	NT	NT	10	50

The screening organisms. Gram-positive bacteria: S. aureus - Staphylococcus aureus, B. subtilis - Bacillus subtilis, Gram-negative bacteria: S.typhimurium - Salmonella typhimurium, E. coli - Escherichia coli, Fungi: A. niger - Aspergillus niger and F. solani - Fusarium Solani

CONCLUSION

In summary we have synthesized a novel series of the 1,2,4-oxadiazole compounds and evaluated them using a antimicrobial screen. The antimicrobial activity of the synthesized compounds may

be due to the presence of two methylene group linker and alkyl group. The data revealed that compound **6** having two methylene linker between aromatic nucleues and carboxylic acid showed antibacterial activity, compound **14** with two methylene linker between aromatic nucleus and amide is also responsible for antifungal activity which mean methylene group linker is tolerated for activity. Also compounds **4 & 8** showed anti fungal activity hence alkyl substituent is also responsible for activity.

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