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ORIGINAL ARTICLE

Synthesis and biological evaluation of a series of 1,4-disubstituted 1,2,3-triazole derivatives as possible antimicrobial agents

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KEYWORDS

1,3,4-Oxadiazole;
Piperazine;
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Abstract Three series of novel compounds derived from 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid bearing piperazine carboxamides, (5-(substituted phenyl)-1,3,4-oxadiazol-2-yl) and (5-(alkylthio)-1,3,4-oxadiazol-2-yl) substitutions at the 4-position were synthesized. Synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectral analysis and evaluated for their antimicrobial activities. Interestingly, most of the compounds exhibit moderate to good activities against tested Gram-positive, Gram-negative bacterial strains as well as fungal strains.

1. Introduction

1,2,3-Triazole is a well known important heterocycle both in synthetic as well as medicinal chemistry due to its simple synthesis via click chemistry approach and a wide range of biological activities. Simple copper catalyzed 1,3-dipolar cycloadditions of substituted azides and alkynes afford regioselective 1,4-disubstitued 1,2,3-triazoles with high yields. 1,2,3-Triazole with high dipole moment, considerable stability and capability for hydrogen bonding make it a favorable binder of biomolecular targets. 1,2,3-Triazole derivatives were reported to exhibit various biological activities such as antidiabetic [1], antitubercular [2,3], anti-inflammatory [4], antifungal [5–7], antiviral [8,9] and antibacterial [10,11]. Several drugs like carboxyamidotriazole, cefatrizine, and tazobactam bear 1,2,3-triazole in their structure. Moreover, Rufinamide, the amide of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid is an antiepileptic drug, which is used in the treatment of partial seizures and drop attacks associated with the Lennox–Gastaut syndrome [12].

Piperazines are considered as a useful and effective scaffold in drug design as they exhibit a wide range of biological activities such as FAAH and MAGL inhibitors [13], human histamine H_4 antagonist [14], and cannabinoid ligands [15]. Recently, piperazine bearing 1,2,3-triaozles are reported as potential anticancer, antiproliferative agents [16,17]. On the other hand, 1,3,4-oxadiazole is also a significant heterocycle that exhibit activities such as antimicrobial [18–23], anti-inflammatory, analgesic [24,25], antitubercular [26–28], anticonvulsant [29], antidiabetic [30] and anticancer [31]. In view of these findings and in continuation of our earlier attempts for the design and synthesis

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of novel azole containing heterocycles as possible antimicrobial and anti-inflammatory agents [32,33], we report herein the synthesis and antimicrobial evaluation of a series of piperazine carboxamide and 1,3,4-oxadiazole derivatives of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid as possible antimicrobial agents.

2. Experimental

2.1. Materials and measurements

All chemicals used were of laboratory grade and used without further purification. Melting points of compounds were determined in open capillary tubes in a silicon oil bath using a Veego melting point apparatus and are uncorrected. Purity of compounds was monitored by TLC on silica F_{254} coated aluminum plates (Merck) as adsorbent and U.V. light and iodine as visualizing agents. ^{1}H and ^{13}C NMR spectra were recorded on Varian mercury TH-300 operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal standard (Chemical shift in ppm). The High Resolution Mass Spectra were recorded on Waters QT micro-mass analyzer.

2.2. Preparation methods

*2.2.1. Synthesis of 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylic acid (**3**)*

Yield 85%, mp: 165–168 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.72 (s, 2H, Ar-CH_2), 7.14–7.21 (m, 2H, Ar-H), 7.49–7.54 (m, 1H, Ar-H), 8.74 (s, 1H, triazole-H), 13.40 (s, 1H, –COOH).

*2.2.2. General procedure for the synthesis of (1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-substituted piperazin-1-yl)methanone (**4a–k**)*

To a cooled solution of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid (**3**) (1.0 mol) in DMF (10 mL) was added HOBt (1.1 mol) followed by corresponding piperazine (1.1 mol) TEA (2.1 mol) and EDC·HCl (1.1 mol). The reaction mixture was left overnight with stirring. The mixture was then poured onto crushed ice; the product was filtered and washed with water. The crude products (**4a–k**) were purified by column chromatography using hexane:EtOAc (9:1) as eluent.

*2.2.2.1. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-Bocpiperazin-1-yl)methanone (**4a**).* Yield 95%, mp: 168–169 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47(s, 9H, –$C(CH_3)_3$), 3.51 (s, 4H, piperazine-H), 3.72 (t, 2H, piperazine-H), 4.27 (t, 2H, piperazine-H), 5.65 (s, 2H, Ar-CH_2), 6.99 (t, 2H, Ar-H), 7.35-7.45 (m, 1H, Ar-H), 8.11 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 41.5, 42.5, 46.4, 80.2, 110.0, 111.7, 112.0, 128.3, 131.8, 144.4, 154.5, 159.7, 162.9.

*2.2.2.2. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(2-methoxyphenyl)piperazin-1-yl) methanone (**4b**).* Yield 89%, mp: 121–122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.11 (s, 4H, piperazine-H), 3.86 (s, 3H, –OCH_3), 3.93 (s, 2H, piperazine-H), 4.45 (s, 2H, piperazine-H), 5.62 (s, 2H, Ar-CH_2), 6.90–7.05 (m, 6H, Ar-H), 7.37–7.43 (m, 1H, Ar-H), 8.15 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.4, 42.8, 46.8, 50.7, 51.2, 55.4, 110.1, 111.2, 111.7, 112.0, 118.4, 121.0, 123.4, 128.2, 131.7, 140.7, 144.6, 152.2, 159.6, 163.0.

*2.2.2.3. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(4-chlorophenyl)piperazin-1-yl)methanone (**4c**).* Yield 90%, mp: 149–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.20 (t, 4H, piperazine-H), 3.90 (t, 2H, piperazine-H), 4.45 (t, 2H, piperazine-H), 5.65 (s, 2H, Ar-CH_2), 6.84 (d, 2H, Ar-H), 6.98 (t, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 7.34-7.43 (m, 1H, Ar-H), 8.11 (s, 1H, triazole-H); LC–MS $[M+H]^+$: 418.1179.

*2.2.2.4. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-p-tolylpiperazin-1-yl)methanone (**4d**).* Yield 95%, mp: 147–149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, –CH_3), 3.20 (s, 4H, piperazine-H), 3.92 (s, 2H, piperazine-H), 4.46 (s, 2H, piperazine-H), 5.66 (s, 2H, Ar-CH_2), 6.88 (s, 2H, Ar-H), 6.98 (t, 2H, Ar-H), 7.09 (d, 2H, Ar-H), 7.34–7.41 (m, 1H, Ar-H), 8.12 (s, 1H, triazole-H).

*2.2.2.5. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (**4e**).* Yield 85%, mp: 170–172 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.52 (t, 4H, piperazine-H), 3.92 (t, 2H, piperazine-H), 4.51 (t, 2H, piperazine-H), 5.66 (s, 2H, Ar-CH_2), 6.83 (d, 2H, Ar-H), 6.99 (t, 2H, Ar-H), 7.34–7.44 (m, 1H, Ar-H), 8.14 (s, 1H, triazole-H), 8.15 (d, 2H, Ar-H); LC–MS $[M+H]^+$: 429.1465.

*2.2.2.6. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)[4-(2-(trifluoromethyl)phenyl)piperazin-1-yl] methanone (**4f**).* Yield 89%, mp: 155–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.29 (t, 4H, piperazine-H), 3.92 (t, 2H, piperazine-H), 4.48 (t, 2H, piperazine-H), 5.65 (s, 2H, Ar-CH_2), 6.98 (t, 2H, Ar-H), 7.06–7.12 (m, 3H, Ar-H), 7.33–7.41 (m, 2H, Ar-H), 8.12 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.5, 42.4, 46.2, 48.9, 49.4, 110.1, 111.7, 112.0, 112.6, 116.5, 119.2, 122.3, 125.3, 128.3, 129.6, 131.7, 131.7, 144.4, 151.1, 159.6, 163.0; LC–MS $[M+H]^+$: 452.1576.

*2.2.2.7. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(3,4-dichlorophenyl)piperazin-1-yl) methanone (**4g**).* Yield 92%, mp: 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (t, 4H, piperazine-H), 3.89 (s, 2H, piperazine-H), 4.46 (s, 2H, piperazine-H), 5.65 (s, 2H, Ar-CH_2), 6.75 (dd, 1H, Ar-H), 6.96–7.01 (m, 2H, Ar-H), 7.27–7.30 (m, 2H, Ar-H), 7.36–7.41 (m, 1H, Ar-H), 8.12 (s, 1H, triazole-H); LC–MS $[M+H]^+$: 452.0840.

*2.2.2.8. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-benzylpiperazin-1-yl)methanone (**4h**).* Yield 87%, mp: 140–142 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47–2.52 (m, 4H, piperazine-H), 3.52 (s, 2H, Ar-CH_2), 3.75 (t, 2H, piperazine-H), 4.27 (t, 2H, piperazine-H), 5.63 (s, 2H, Ar-CH_2), 6.97 (t, 2H, Ar-H), 7.28-7.40 (m, 7H, Ar-H), 8.07 (s, 1H, triazole-H).

*2.2.2.9. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(4-chlorobenzyl)piperazin-1-yl)methanone (**4i**).* Yield 91%, mp: 165–168 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (t, 4H, piperazine-H), 3.74 (s, 2H, piperazine-H), 4.23 (s, 2H, Ar-CH_2), 4.25 (s, 2H, piperazine-H), 5.61 (s, 2H, Ar-CH_2), 6.95 (t, 2H, Ar-H), 7.16–7.41 (m, 5H, Ar-H), 8.05 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.4, 42.7, 46.7, 51.5, 52.3, 75.1, 110.0, 111.8, 127.3, 127.8, 128.0, 128.7, 129.1, 131.7, 132.7, 140.7, 141.5, 144.6, 159.5, 163.0.

*2.2.2.10. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)[4-(bis (4-chlorophenyl)methyl)piperazin-1-yl]methanone (**4j**).* Yield 84%, mp: 170–172 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 4H, piperazine-H), 3.75 (s, 2H, piperazine-H), 4.23 (s, 3H, piperazine-H,-CH), 5.61 (s, 2H, Ar-CH_2), 6.96 (t, 2H, Ar-H), 7.19–7.39 (m, 9H, Ar-H), 8.05 (s, 1H, triazole-H).

*2.2.2.11. (1-(2,6-Difluorobenzyl)-1H-1,2,3-triazol-4-yl)(4-(pyr idin-2-yl)piperazin-1-yl)methanone (**4k**).* Yield 86%, mp: 80–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.64 (s, 4H, piperazine-H), 3.88 (t, 2H, piperazine-H), 4.42 (t, 2H, piperazine-H), 5.66 (s, 2H, Ar-CH_2), 6.64–6.66 (m, 2H, Ar-H), 6.99 (t, 2H, Ar-H), 7.34–7.42 (m, 1H, Ar-H), 7.47–7.53 (m, 1H, Ar-H), 8.15 (s, 1H, triazole-H), 8.19–8.21 (m, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.4, 42.3, 44.8, 45.7, 46.1, 107.0, 110.0, 111.6, 111.9, 113.6, 114.1, 128.2, 130.2, 130.9, 131.6, 132.6, 137.5, 144.4, 147.8, 159.0, 159.7, 162.9; LC–MS $[M+H]^+$: 385.1512.

*2.2.3. Procedure for the synthesis of methyl-1-(2,6-difluoroben zyl)-1H-1,2,3-triazole-4-carboxylate (**5**)*

To a solution of acid **3** in dry MeOH, a catalytic amount of conc. H_2SO_4 was added and the mixture was then refluxed for 7–8 h. After the reaction was complete, excess solvent was removed under vacuum and crushed ice was added to the resulting solid. The solid was filtered and washed with water to obtain the product in 89% yield. mp: 132–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 3H, –OCH_3), 5.69 (s, 2H, Ar-CH_2), 7.00 (t, 2H, Ar-H), 7.36–7.46 (m, 1H, Ar-H), 8.11 (s, 1H, triazole-H).

*2.2.4. Procedure for the synthesis of 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carbohydrazide (**6**)*

The mixture of methyl ester **5** and hydrazine hydrate in ethanol was refluxed for 6 h on a water bath. After the reaction was complete, the reaction mixture was cooled. The solid thus obtained was filtered and washed with water. Yield 85%, mp: 229–231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.45 (s, 2H, –NH_2), 5.72 (s, 2H, Ar-CH_2), 7.20 (t, 2H, Ar-H), 7.48–7.56 (m, 1H, Ar-H), 8.57 (s, 1H, triazole-H), 9.71 (s, 1H, –NH).

*2.2.5. General procedure for the synthesis of 1-(2,6-difluoroben zyl)-4-(5-(substitutedphenyl)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-tr iazoles (**7a–g**)*

The mixture of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbohydrazide **6** and corresponding substituted benzoic acid in $POCl_3$ was refluxed for 8 h. The reaction mixture was then cooled and poured onto the crushed ice. The solid separated was filtered, washed with water. The crude products (**7a–g**) were purified by column chromatography using hexane: EtOAc (8:2) as eluent.

*2.2.5.1. 1-(2,6-Difluorobenzyl)-4-(5-phenyl-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**7a**).* Yield 79%, mp: 165–166 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (s, 2H, Ar-CH_2), 7.03 (t, 2H, Ar-H), 7.38–7.46 (m, 1H, Ar-H), 7.49–7.57 (m, 3H, Ar-H), 8.17–8.20 (dd, 2H, Ar-H), 8.32 (s, 1H, triazole-H).

*2.2.5.2. 1-(2,6-Difluorobenzyl)-4-(5-(2-methylphenyl)-1,3,4-oxa diazol-2-yl)-1H-1,2,3-triazole (**7b**).* Yield 84%, mp: 181-184 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.74 (s, 3H, -CH_3), 5.75 (s, 2H, Ar-CH_2), 7.00 (t, 2H, Ar-H), 7.30-7.43 (m, 4H, Ar-H), 8.06 (d, 1H, Ar-H), 8.30 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.0, 41.8, 109.9, 111.9, 122.5, 124.4, 126.1, 129.2, 131.3, 131.7, 131.9, 134.2, 138.6, 157.5, 159.6, 162.9, 164.9.

*2.2.5.3. 1-(2,6-Difluorobenzyl)-4-(5-(3-chlorophenyl)-1,3,4-oxad iazol-2-yl)-1H-1,2,3-triazole (**7c**).* Yield 75%, mp: 190–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.82 (s, 2H, Ar-CH_2), 7.21 (t, 2H, Ar-H), 7.49–7.57 (m, 1H, Ar-H), 7.63–7.74 (m, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 8.06 (d, 1H, Ar-H), 9.15 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.0, 110.0, 111.7, 124.9, 125.3, 126.0, 126.8, 131.4, 131.8, 132.0, 132.4, 133.9, 158.0, 159.1, 162.5; LC–MS $[M+H]^+$: 374.0573.

*2.2.5.4. 1-(2,6-Difluorobenzyl)-4-(5-(4-chlorophenyl)-1,3,4-oxad iazol-2-yl)-1H-1,2,3-triazole (**7d**).* Yield 78%, mp: > 225 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.83 (s, 2H, Ar-CH_2), 7.22 (t, 2H, Ar-H), 7.52-7.58 (m, 1H, Ar-H), 7.71 (d, J = 8.7 Hz, 2H, Ar-H), 8.09 (d, J = 8.7 Hz, 2H, Ar-H), 9.14 (s, 1H, triazole-H).

*2.2.5.5. 1-(2,6-Difluorobenzyl)-4-(5-(4-fluorophenyl)-1,3,4-oxa diazol-2-yl)-1H-1,2,3-triazole (**7e**).* Yield 84%, mp: 184–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (s, 2H, Ar-CH_2), 7.03 (t, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 7.41–7.46 (m, 1H, Ar-H), 8.17–8.21 (m, 2H, Ar-H), 8.32 (s, 1H, triazole-H).

*2.2.5.6. 1-(2,6-Difluorobenzyl)-4-(5-(2,4-dichlorophenyl)-1,3,4 -oxadiazol-2-yl)-1H-1,2,3-triazole (**7f**).* Yield 77%, mp: 190–191 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (s, 2H, Ar-CH_2), 7.03 (t, 2H, Ar-H), 7.38–7.45 (m, 3H, Ar-H), 7.60 (d, 1H, Ar-H), 8.04 (d, 1H, Ar-H), 8.34 (s, 1H, triazole-H); LC–MS $[M+H]^+$: 408.0205.

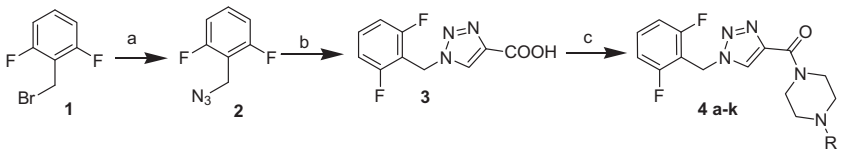
*2.2.5.7. 1-(2,6-Difluorobenzyl)-4-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**7g**).* Yield 74%, mp: 201–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.97 (s, 3H, –OCH_3), 3.99 (s, 3H, –OCH_3), 5.77 (s, 2H, Ar-CH_2), 6.97–7.06 (m, 3H, Ar-H), 7.38–7.46 (m, 1H, Ar-H), 7.66 (d, J = 1.9 Hz, 1H, Ar-H), 7.77 (dd, J_1 = 1.9 Hz, J_2 = 8.5 Hz, 1H, Ar-H), 8.32 (s, 1H, triazole-H); LC–MS $[M+H]^+$: 400.1136.

*2.2.6. Procedure for the synthesis of 5-(1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl)-1,3,4-oxadia-zole-2-thiol (**8**)*

To a solution of carbohydrazide **6** (1.0 mol) in ethanol, an aqueous solution of potassium hydroxide (3.0 mol) was added. To this solution carbon disulfide (1.5 mol) was added and the resulting solution was refluxed in a water bath for 6 h. The reaction mass was cooled, acidified with acetic acid. The solid obtained was filtered, washed with water and dried to afford the target compound in 81% yield. mp: 203–205 °C. ^{1}H NMR (DMSO-d_6): δ 5.79 (s, 2H, Ar-CH_2), 7.21 (t, 2H, Ar-H), 7.49–7.59 (m, 1H, Ar-H), 9.05 (s, 1H, triazole-H), 14.82 (bs, 1H, –SH); LC–MS $[M+H]^+$: 296.0354.

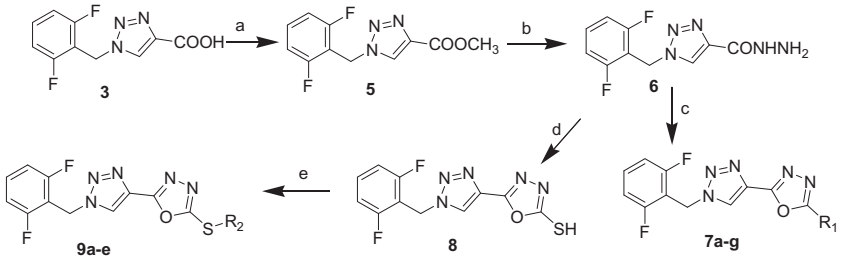
*2.2.7. General procedure for the synthesis of 1-(2,6-difluorobenzyl)-4-(5-(alkylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9a–e**)*

To a solution of compound **8** (1.0 mol) suspended in DMF, alkyl halide (1.2 mol) and a catalytic amount of triethylamine



Compd	R	Compd	R
4a	Boc	**4g**	3,4-Dichlorophenyl
4b	2-Methoxyphenyl	**4h**	Benzyl
4c	4-Chlorophenyl	**4i**	4-Chlorobenzyl
4d	4-Methylphenyl	**4j**	bis(4-Chlorophenyl)methyl
4e	4-Nitrophenyl	**4k**	Pyridin-2-yl
4f	2-(Trifluoromethyl)phenyl		

Scheme 1 Synthetic scheme for the target compounds **4a–k**. *Reagent and reaction conditions:* (a) NaN_3, TBAB, toluene, rt; (b) Propiolic acid, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, H_2O–^{t}BuOH (1:1), rt; (c) HOBt, sub. piperazines, TEA, EDC·HCl, DMF, rt.



Compd	R_2	Compd	R_1
9a	Ethyl	**7a**	Phenyl
9b	iso-Propyl	**7b**	o-Tolyl
9c	n-Butyl	**7c**	3-Chlorophenyl
9d	iso-Pentyl	**7d**	4-Chlorophenyl
9e	Benzyl	**7e**	4-Fluorophenyl
		7f	2,4-Dichlorophenyl
		7g	3,4-Dimethoxyphenyl

Scheme 2 Synthetic scheme for the target compounds **7a–g** and **9a–e**. *Reagent and reaction conditions:* (a) Dry MeOH, cat. H_2SO_4 reflux; (b) $N_2H_4{\cdot}H_2O$, ethanol, reflux; (c) sub. benzoic acids, $POCl_3$, reflux; (d) CS_2, aq. KOH, ethanol, reflux; (e) alkyl halides, TEA, DMF, 50 °C.

was added. The reaction mixture was then warmed in a water bath at 50 °C for 3–4 h. After the reaction was complete as monitored on TLC, the mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water. The crude products (**9a–e**) were purified by column chromatography using hexane-ethyl acetate (8:2) as eluant.

*2.2.7.1. 1-(2,6-Difluorobenzyl)-4-(5-(ethylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9a**)*. Yield 90%, mp: 102–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, 3H, –CH_3), 3.28–3.35 (q, 2H, -SCH_2), 5.75 (s, 2H, Ar-CH_2), 7.01 (t, 2H, Ar-H), 7.38–7.48 (m, 1H, Ar-H), 8.24 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5, 26.9, 41.8, 109.8, 111.9, 124.1, 131.9, 133.8, 158.8, 159.5, 162.9, 164.7; LC–MS $[M+H]^+$: 324.0704.

*2.2.7.2. 1-(2,6-Difluorobenzyl)-4-(5-(isopropylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9b**)*. Yield: 92%, mp: 150–152 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (d, 6H, 2 × –CH_3), 3.91–4.00 (m, 1H, -CH), 5.73 (s, 2H, Ar-CH_2), 7.00 (t, 2H, Ar-H), 7.36–7.46 (m, 1H, Ar-H), 8.22 (s, 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.2, 39.0, 41.8, 109.8, 111.9, 124.1, 131.9, 133.9, 158.8, 159.6, 162.9, 164.4; LC–MS $[M+H]^+$: 338.0885.

*2.2.7.3. 1-(2,6-Difluorobenzyl)-4-(5-(butylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9c**)*. Yield 89%, mp: 66–67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, –CH_3), 1.42–1.55 (m, 2H,-CH_2CH_3), 1.75–1.85 (m, 2H, –CH_2), 3.27–3.32 (t, 2H, –SCH_2), 5.73 (s, 2H, Ar-CH_2), 7.01 (t, 2H, Ar-H), 7.37–7.47 (m, 1H, Ar-H), 8.22 (s, 1H, triazole-H).

*2.2.7.4. 1-(2,6-Difluorobenzyl)-4-(5-(isopentylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9d**)*. Yield 85%, mp: 154–156 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (d, 6H, 2 × –CH_3), 1.40–1.48 (m, 1H, -CH), 1.57–1.68 (m, 2H, –CH_2), 2.77 (t, 2H, –SCH_2), 5.73 (s, 2H, Ar-CH_2), 6.99 (t, 2H, Ar-H), 7.35–7.45 (m, 1H, Ar-H), 8.12 (s, 1H, triazole-H).

*2.2.7.5. 1-(2,6-Difluorobenzyl)-4-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)-1H-1,2,3-triazole (**9e**)*. Yield 91%, mp: 159–161 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (s, 2H, –SCH_2), 5.71 (s, 2H, Ar-CH_2), 6.92–7.01 (m, 3H, Ar-H), 7.37–7.40 (m, 5H, Ar-H), 8.20 (s, 1H, triazole-H); LC–MS $[M+H]^+$: 386.0885.

Table 1 Antimicrobial activity of the title compounds; diameter of inhibition zone in mm.

Compd	Microorganisms*										
	A	B	C	D	E	F	G	H	I	J	K
4a	–†	–†	17.66	19.52	18.09	18.24	18.69	–†	16.71	16.66	18.21
4b	–†	17.33	19.01	18.89	18.00	16.50	17.75	18.08	–†	17.68	19.03
4c	17.82	19.23	18.23	18.64	19.26	18.24	19.04	17.56	–†	18.22	19.01
4d	18.86	17.69	–†	–†	–†	–†	–†	–†	18.71	16.96	17.64
4e	18.82	19.00	16.94	18.72	19.35	18.92	17.86	19.87	18.87	18.00	19.03
4f	–†	–†	17.24	–†	18.96	18.21	19.01	18.77	–†	–†	–†
4g	18.71	18.72	17.92	20.11	19.23	17.99	18.09	20.22	18.96	19.21	18.87
4h	–†	17.66	–†	19.97	20.01	–†	17.09	19.83	16.97	20.07	17.56
4i	17.98	–†	17.39	20.07	18.07	18.22	15.69	20.09	–†	18.66	19.08
4j	–†	19.04	18.77	–†	–†	–†	–†	18.87	16.55	–†	–†
4k	19.00	–†	15.69	–†	20.34	20.01	19.04	–†	–†	19.24	16.54
7a	19.02	–†	18.22	17.83	19.00	17.82	17.23	–†	18.88	16.64	17.01
7b	17.98	16.96	18.27	16.86	–†	18.84	17.52	15.69	17.66	15.96	19.00
7c	19.11	18.71	–†	–†	17.84	–†	–†	18.00	18.08	19.23	18.87
7d	18.92	–†	17.62	19.06	18.88	17.96	19.01	18.09	17.86	19.22	17.62
7e	18.74	19.07	–†	–†	18.24	–†	17.55	16.66	–†	18.21	17.86
7f	16.66	18.71	–†	19.00	17.52	–†	18.27	–†	19.24	17.28	16.90
7g	–†	18.00	17.92	17.91	–†	19.54	–†	–†	18.77	19.08	17.81
9a	16.69	–†	18.36	18.00	–†	17.66	16.96	17.23	18.06	17.58	16.46
9b	17.66	–†	17.02	–†	–†	–†	–†	16.86	17.86	18.01	18.23
9c	18.03	18.22	–†	17.65	17.76	16.91	17.53	16.88	15.62	19.03	–†
9d	–†	19.06	18.22	16.64	18.82	17.22	18.07	–†	–†	–†	17.84
9e	–†	17.42	18.21	–†	18.91	18.62	–†	–†	–†	–†	16.69
Chloramphenicol	22.26	22.64	21.26	24.18	23.56	22.67	22.50	NA‡	NA‡	NA‡	NA‡
Nystatin	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	23.09	20.12	23.11	22.01

* (A) *E. coli*; (B) *P. aeruginosa*; (C) *B. subtilis*; (D) *S. pyogenes*; (E) *K. pneumonia*; (F) *S. aureus*; (G) *K. terrigena*; (H) *C. albicans*; (I) *T. viride*; (J) *A. flavus*; (K) *A.* brasiliensis.
† (–) Inactive.
‡ (NA) Not Applicable.

3. Results and discussion

3.1. Chemistry

Methodologies adopted for the synthesis of the title compounds **4a–k**, **7a–g** and **9a–e** are shown in Schemes 1 and 2, respectively. Starting from commercially available 2,6-difluorobenzyl bromide **1**, the corresponding 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid **3** was obtained by reported procedure [34]. ^{1}H NMR data were in accordance with the expected structure. Emergence of signals at δ 8.74 and δ 12.20 for triazole ring proton and acid proton respectively confirmed the formation of acid **3**. The ^{1}H NMR analysis of corresponding carboxamide derivatives **4a–k** revealed three sets of protons in the aliphatic region (between δ 3–5) for piperazine. Further, esterification of **3** was achieved by refluxing the acid in dry MeOH, and a catalytic amount of conc. H_2SO_4 for 7–8 h. The formation of methyl ester **5** was confirmed by ^{1}H NMR in which the methoxy protons appeared at δ 3.93. Thereafter, the Methyl-1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylate **5** was converted to its carbohydrazide derivative **6** by refluxing it with hydrazine hydrate in ethanol for 6 h. ^{1}H NMR of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbohydrazide **6** showed signals at δ 4.45 for $-NH_2$ protons and at δ 9.71 for –NH proton. Synthesis of the title compounds 1-(2,6-difluorobenzyl)-4-(5-(substitutedphenyl)-1,3,4-oxadiazol-2-yl)-1*H*-1,2,3-triazoles **7a–g** was accomplished by refluxing carbohydrazide **6** with different substituted benzoic acids in $POCl_3$ for 8 h. 5-(1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)-1,3,4-oxadiazole-2-thiol **8** was obtained by refluxing carbohydrazide **6** with carbon disulfide and potassium hydroxide in ethanol. Thiol **8** was alkylated using different alkyl halides in DMF containing a catalytic amount of triethylamine to obtain final compounds 1-(2,6-difluorobenzyl)-4-(5-(alkylthio)-1,3,4-oxadiazol-2-yl)-1*H*-1,2,3-triazoles **9a–e**.

3.2. Antimicrobial activity

The newly synthesized compounds were evaluated for their antibacterial activity against seven different bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Streptococcus aureus*, and *Klebsiella terrigena*. Four fungal strains, *Candida albicans*, *Trichoderma viride*, *Aspergillus flavus*, and *Aspergillus* brasiliensis were used for the antifungal screening of the compounds. Agar disc diffusion assay method was used for testing. Discs with 6 mm diameter were used for sample loading. The test compounds with concentration 100 µg/mL were prepared using DMSO. Chloramphenicol and Nystatin were used as standard antibacterial and antifungal drugs for the comparison of activities. Inhibition zone in mm of diameter and minimum inhibitory concentrations (MIC) in µg/mL for the tested compounds are summarized in Tables 1 and 2 respectively. The investigation of antibacterial and antifungal screening results revealed that most of the test compounds exhibited moderate to good activities as compared to the standard drugs.

Table 2 Antimicrobial activity of the title compounds; minimum inhibitory concentrations (MIC) in μg/mL.

Compd	Microorganisms*										
	A	B	C	D	E	F	G	H	I	J	K
4a	–†	–†	70	60	80	80	60	–†	80	100	60
4b	–†	70	50	70	80	90	70	60	–†	90	50
4c	80	50	60	70	70	80	50	70	–†	80	50
4d	70	70	–†	–†	–†	–†	–†	–†	70	100	70
4e	70	50	80	70	70	70	70	50	70	80	50
4f	–†	–†	70	–†	80	80	50	60	–†	–†	–†
4g	70	60	70	60	70	90	60	50	70	70	60
4h	–†	70	–†	70	60	–†	70	50	80	60	70
4i	80	–†	70	60	80	80	90	50	–†	80	50
4j	–†	50	60	–†	–†	–†	–†	60	80	–†	–†
4k	60	–†	90	–†	60	60	50	–†	–†	70	70
7a	60	–†	60	80	60	80	70	–†	80	80	70
7b	80	80	60	90	–†	70	70	80	90	90	50
7c	60	60	–†	–†	80	–†	–†	60	80	60	60
7d	70	–†	80	60	70	70	50	60	90	60	70
7e	70	50	–†	–†	70	–†	70	70	–†	70	70
7f	90	60	–†	60	80	–†	60	–†	70	80	70
7g	–†	60	70	80	–†	60	–†	–†	80	60	70
9a	80	–†	60	70	–†	70	70	70	70	80	80
9b	80	–†	70	–†	–†	–†	–†	80	80	70	60
9c	70	60	–†	80	70	80	60	60	90	60	–†
9d	–†	50	60	90	70	70	50	–†	–†	–†	70
9e	–†	70	60	–†	60	60	–†	–†	–†	–†	80
Chloramphenicol	50	40	40	50	50	50	40	NA‡	NA‡	NA‡	NA‡
Nystatin	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	40	60	50	40

* (A) *E. coli*; (B) *P. aeruginosa*; (C) *B. subtilis*; (D) *S. pyogenes*; (E) *K. pneumonia*; (F) *S. aureus*; (G) *K. terrigena*; (H) *C. albicans*; (I) *T. viride*; (J) *A. flavus*; (K) *A.* brasiliensis.
† (–) Inactive.
‡ (NA) Not Applicable.

Compounds **4e** and **4g** were active against all the tested species. Compound **4c** was active against all the species except *T. viride*. Compounds **4b**, **4c**, **4e**, and **4i** were significantly effective against *A.* brasiliensis. Compounds **4e**, **4g**, **4h**, and **4i** were significantly active against *C. albicans*. Compound **4k** was effective against *E. coli*, *K. pneumonia*, *S. aureus*, *K. terrigena* and *A. flavus*. In fact, it was the most active compound against *S. aureus*. Compounds **4c**, **4e**, and **4j** showed significant inhibition of bacteria *P. aeruginosa*. For *E. coli*, **4k** exhibited good activity. The most active compound against the *B. subtilis* was **4b**. Compound **4g** and **4i** were active against *S. pyogenes*. The enhanced activities can be attributed to the presence of biologically active piperazine ring bearing different aryl substitutions at the nitrogen. In case of the 4-(5-(substituted aryl)-1,3,4-oxadiazol-2-yl)1*H*-1,2,3-triazole derivatives, almost all the title compounds showed moderate to good activities. Compound **7b** was found to be most active against all the tested pathogens except *K. pneumonia*. Compound **7d** was significantly active against all the species except *P. aeruginosa*. Compound **7f** was found to be most active and comparable with the standard drug against *T. viride*. Compound **7e** was good against bacteria *P. aeruginosa*. Against *E. coli*, **7a**, and **7c** exhibited good activity. It was noteworthy that almost all the compounds having halo-substituted phenyl rings at the 5-position of the 1,3,4-oxadiazole ring exhibit significant activity against the tested microorganisms. When the aryl substitutions at the 5-position of the 1,3,4-oxadiazole ring were replaced by thioalkyl substitutions, compound **9a**, **9b** and **9c** bearing ethyl, *iso*-propyl and *n*-butyl substitutions respectively, retained the activities. Although compound **9d** and **9e** were least active against the tested fungi, they retained activities against the bacterial strains. The overall results indicate that most of the synthesized compounds showed moderate to good activity against different tested microorganisms.

4. Conclusions

The present work reports the synthesis and in vitro biological evaluation of a new series of 1,4-disubstituted 1*H*-1,2,3-triazole derivatives having carboxamide and 1,3,4-oxadiazolyl substitutions at the 4-position. The antimicrobial study revealed that 1,2,3-triazole clubbed with 5-substituted 1,3,4-oxadiazoles and N-substituted piperazines showed good activities against pathogenic strains. The presence of a piperazine ring, halo-substituted phenyl rings in the structure, enhanced the activities of the synthesized compounds. Further modifications of these new molecules are warranted which could provide more interesting results and new lead molecules for further studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2015.03.003.

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