Synthesis and evaluation of 2-(5-(aryl)-1,3,4-oxadiazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamides and N-(4-chloro-3-fluorophenyl)-2-(5-(aryl)-1,3,4-oxadiazol-2-ylthio)acetamides as antimicrobial agents

KALPESH PARIKH and DEEPKUMAR JOSHI*

Chemistry Division, Chemistry Research Laboratory, Sheth MN Science College, Patan 384 265, India
e-mail: deepjoshi359@yahoo.co.in

MS received 9 June 2013; revised 6 January 2014; accepted 11 January 2014

Abstract. A series of 2-mercapto-5-phenyl-1,3,4-oxadiazole derivatives have been condensed with different phenyl acetamide derivatives possessing fluorine atom at *meta* position; resulting in the formation of 2-(5-aryl-1,3,4-oxadiazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamide (**5a–j**) and N-(4-chloro-3-fluorophenyl)-2-(5-aryl-1,3,4-oxadiazol-2-ylthio)acetamide (**5k–t**) derivatives. The antimicrobial properties of the synthesized entities (**5a–t**) measured as their MIC (Minimum Inhibitory Concentration) values were evaluated by using the broth dilution method against Gram-positive bacteria (*S. aureus* and *E. faecalis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*C. albicans* and *A. niger*). The results of antimicrobial activities (in $\mu\text{g/ml}$) revealed the fact that the compounds **5a** and **g** bearing a maximum number of fluorine atoms showed the highest potency among the synthesized compounds against the broad panel of bacterial and fungal strains. The presence of fluorine atom at the *meta* position in the phenyl ring of final derivatives (**5a–t**) brought about an enhancement of their antimicrobial properties to a notable extent.

Keywords. 1,3,4-Oxadiazoles; antifungal activity; antibacterial activity; MIC; SAR study.

1. Introduction

Fluorine containing compounds play a very important role for their applications in medicinal chemistry. Fluorine being the most electronegative atom with small atomic radius helps to enhance the biological activity of newly designed molecules.¹ Fluorinated compounds have been widely used as important antifungal,² anticancer³ and antibacterial drugs.⁴ Some of the fluorinated motifs have been utilized for treating cardiovascular and obesity related diseases.⁵ A new series of potent GPR119 agonists, possessing fluorine atom as a substituent have been reported.⁶ Henceforth, fluorine substitution remains a lucrative aspect in the development of bio-active drug molecules.

Literature study reveals the importance of nitrogen containing heterocyclic compounds as essential antibacterial and antifungal drugs.⁷ Among several different five-membered heterocyclic rings; 1,3,4-oxadiazoles are the class of heterocyclic compounds with a wide range of pharmaceutical and biological action.^{8–10} The different biological activity possessed by 1,3,4-oxadiazoles includes antimicrobial,^{11,12}

antifungal,^{13,14} antiproliferative,¹⁵ anticancer,^{16,17} tyrosinase inhibitor¹⁸ and tiodazosin possessing oxadiazole nucleus is widely used as antihypertensive.¹⁹ Other biological properties associated with 1,3,4-oxadiazole skeletal includes anti-inflammatory^{20,21} as well as analgesic activity.^{22,23} Some derivatives containing 1,3,4-oxadiazole as a core, are utilized as muscle relaxants,²⁴ tranquilizers²⁵ and cathepsin K inhibitors.²⁶ The molecule is also useful as angiogenesis inhibitor and HIV integrase inhibitor.²⁷ In the context of medicinal significance; a number of routes have been developed for synthesizing 1,3,4-oxadiazoles.^{28–32} Several synthetic methods under harsh conditions using different reagents viz. Cu (II)³³ and phosphorous oxychloride³⁴ have been established.

Furthermore, it is observed that the fluorine atom, if substituted with oxadiazole nucleus results in the formation of potent bio-active molecules.^{35,36} Encouraged by the above mentioned findings, it was rationalized to design newer 1,3,4-oxadiazole motifs with fluorine atom substituted as $-\text{CF}_3$ and $-\text{F}$ at the *meta* position (C-15 in the main structure). Strategically placed $-\text{F}$ and $-\text{CF}_3$ at the *meta* position with respect to $-\text{NHCOR}$ -group in the phenyl ring makes them easily removable due to the effect of partial positive charge on *meta* position generated by the presence of partial electronegative

*For correspondence

charge at *ortho* and *para* position in the resonating structure. Thus, easy removal of fluorine substituent exhibits remarkable impact in altering various biological steps; which includes binding with enzymes or receptor and clearance of exogenous substance.³⁷ The action of mechanism of several molecules containing fluoro substituent at *meta* position is explained in a review by Begue and Bonnet-deplon.⁴

2. Experimental

2.1 General

All the chemicals and solvents required for the synthesis were purchased from Merck Ltd., Sdfine Chemicals, LOBA Chemie and HIMEDIA. The majority of the reactions were carried out by standard procedure for the exclusion of moisture. The composition (in %) of elements (CHN) for the synthesized compounds were determined by using Perkin-Elmer 2400 CHN analyzer. Melting points were determined on electro thermal melting point apparatus using the open end capillary method and were reported uncorrected. IR spectra of all compounds were recorded on Bruker *alpha-t* FT-IR spectrophotometer in ATR. The ¹H NMR and ¹³C NMR were obtained using Bruker Spectrophotometer-400 MHz where DMSO-d₆ was used as solvent and TMS as reference. Mass spectral analysis was conducted on Shimadzu mass spectrophotometer LC-MS 2010. TLC plates used for monitoring the completion of the reaction were purchased from Merck (TLC Silica gel 60 F₂₅₄).

2.2 Synthesis of 5-Aryl-1,3,4-oxadiazole-2-thiol (2a-j)³⁸

Ethanol (40 ml) and potassium hydroxide (0.01 mol, 56.11 g/mol, 0.56 g in 2 ml H₂O) were taken in a dry round bottom flask. Various aryl substituted benzoic acid hydrazide (0.01 mol) were added to it and stirred well to get a clear solution. Carbon disulphide (0.02 mol, 76.14 g/mol, 1.2 ml) was added to the clear solution obtained above and refluxed for 12–15 h. The ethanol was distilled off and then cooled to room temperature. The content was poured into water and acidified with diluted HCl till the precipitates were separated. The separated solid was washed with cold water and dried to get the desired product. The completion of the reaction was monitored on TLC using ethyl acetate: Benzene (6:4) as mobile phase. All the derivatives were obtained using the above stated procedure.

2.2a 5-(4-Fluorophenyl)-1,3,4-oxadiazole-2-thiol (2a): Yield–77%; m.p.: 210° C; IR (ATR, cm⁻¹): 1055, 1253 (-C-O-C- str.), 1423 (-C=C- str. aromatic ring), 1549 (-C=N- str. sec. amine), 2581 (-S-H str.), 3078 (CH str. aromatic ring); ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 7.16–8.15 (4H, m, Ar-*H*), 11.82 (1H, s, -SH); ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 116.2 (C₂), 116.2 (C₄), 122.5 (C₆), 129.4 (C₁), 129.4 (C₅), 163.2 (C₃), 164.8 (C₇), 170.9 (C₈); LCMS (m/z): 197 (M⁺); Anal. calcd. for C₈H₅FN₂OS: C-48.97, H-2.57, N-14.28, S-16.34; Found: C-49.02, H-2.61, N-14.31, S-16.37%.

2.3 Synthesis of 2-chloro-N-phenylacetamide derivatives (4A and B)³⁹

Amines (0.01 mol) were made soluble in appropriate quantity of DMF containing a few drops of TEA (3–4 drops) and stirred well for 10 min. In this well-stirred mixture chloroacetylchloride (0.015 mol, 113 g/mol, 1.19 ml) was added slowly within 15 min maintaining the temperature below 5°C. The mixture thus obtained was stirred for 4 h at room temperature and then the solution containing precipitates was poured onto crushed ice. The solid separated out was well stirred, kept overnight and filtered. The product was crystallized from acetone. The purity of the synthesized derivatives and the extent of completion of reaction were monitored using TLC with mobile phase *n*-hexane: Ethyl acetate (6:4). The process was repeated for differently substituted fluorine derivatives.

2.3a 2-Chloro-N-(3-(trifluoromethyl)phenyl)acetamide (4A): Yield–87%; m.p.: 88° C; IR (ATR, cm⁻¹): 1113 (-C-F, str.), 1667 (C=O str. carbonyl group), 2726 (CH₂ str. methylene), 3313 (NH str. sec. amine); ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 4.06 (2H, s, -CH₂), 7.26–8.06 (4H, m, Ar-*H*), 10.71 (1H, s, -NH); ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 42.4 (C₉), 121.2 (C₁₄), 124.9 (C₁₂), 125.8 (C₁₆), 129.6 (C₁₃), 131.7 (C₁₅), 139.0 (C₁₁), 165.0 (C₁₀); ¹⁹F NMR (376 MHz, DMSO-d₆, δ, ppm): -61.36 (3F, s, -CF₃); LCMS (m/z): 238(M⁺); Anal. calcd. for C₉H₇ClF₃NO: C-45.49, H-2.97, N-5.89 Found: C-45.54, H-3.01, N-5.92%.

2.3b 2-Chloro-N-(4-chloro-3-fluorophenyl)acetamide (4B): Yield–78%; m.p.: 122° C; IR (ATR, cm⁻¹): 1153 (-C-F, str.), 1671 (C=O str. carbonyl group), 2731 (CH₂ str. methylene), 3397 (NH str. sec. amine); ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 4.06 (2H, s, -CH₂), 7.30–7.80 (3H, m, Ar-*H*), 10.51(1H, s, -NH); ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 42.4 (C₉),

111.8 (C₁₆), 116.8 (C₁₄), 124.9 (C₁₂), 129.6 (C₁₃), 139.0 (C₁₁), 163.2 (C₁₅), 165.0 (C₁₀); ¹⁹F NMR (376 MHz, DMSO-d₆, δ, ppm): -115.69 (1F, s, -F); LCMS (m/z): 222(M⁺); Anal. calcd. for C₈H₆Cl₂FNO: C-43.27, H-2.72, N-6.31 Found: C-43.31, H-2.76, N-6.35%.

2.4 Synthesis of final compounds (5a-j)

In a round bottom flask (moisture-free) containing 2-chloro-N-(3-(trifluoromethyl)phenyl)acetamide (0.01 mol, 237 g/mol, 2.37 g) in acetone, various aryl substituted 1,3,4-oxadiazole-2-thiols (0.01 mol) were added. K₂CO₃ (0.02 mol, 138 g/mol, 2.76 g) was added to neutralize the liberated hydrochloric acid during the reaction. This mixture was allowed to stir for 4 h at room temperature. The resulting material was poured onto crushed ice and stirred well for 30 min. The solid separated out was filtered and washed with cold water. The product obtained was dried and recrystallized from alcohol. All the targeted derivatives were obtained by the above stated procedure. The completion of the reaction was confirmed by using TLC with mobile phase *n*-hexane: Ethyl acetate (8:2) and (7:3) (as per the resolution).

2.4a 2-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamide (5a): Yield-68%; m.p.: 166° C; IR (ATR, cm⁻¹): 1055, 1253 (-C-O-C- str.), 1113 (-C-F, str.), 1349 (-C-N- str. sec. amine), 1401 (-C=C- str. aromatic ring), 1667 (C=O str. carbonyl group), 2726 (CH₂ str. methylene), 3096 (CH str. aromatic ring), 3313 (NH str. sec. amine); ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 4.32 (2H, s, -CH₂), 7.26–8.06 (8H, m, Ar-H), 10.71 (1H, s, -NH); ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 39.0 (C₉), 116.2 (C₂), 116.2 (C₄), 120.9 (C₁₄), 122.5 (C₆), 124.9 (C₁₂), 125.9 (C₁₆), 129.4 (C₁), 129.4 (C₅), 129.6 (C₁₃), 131.7 (C₁₅), 139.0 (C₁₁), 163.2 (C₃), 164.8 (C₇), 168.4 (C₁₀), 171.1 (C₈); ¹⁹F NMR (376 MHz, DMSO-d₆, δ, ppm): -61.36 (3F, s, -CF₃), -108.56 (1F, s, 4-F); LCMS (m/z): 398(M⁺); Anal. calcd. for C₁₇H₁₁F₄N₃O₂S: C-51.39, H-2.79, N-10.58, S-8.07; Found: C-51.43, H-2.83, N-10.63, S-8.10%.

2.5 Synthesis of final compounds (5k-t)

In a round bottom flask (moisture free); 2-chloro-N-(4-chloro-3-fluorophenyl)acetamide (0.01 mol, 222 g/mol, 2.22 g) was made soluble in appropriate amount of acetone. To this solution, various aryl substituted 1,3,4-oxadiazole-2-thiols (0.01 mol) were added. K₂CO₃ (0.02 mol, 138 g/mol, 2.76 g) was added to neutralize

the liberated hydrochloric acid during the reaction. This mixture was allowed to stir for 4 h at room temperature. The resulting material was poured onto crushed ice and stirred well for 30 min. The solid separated out was filtered and washed with cold water. The product obtained was dried and recrystallized from alcohol. The completion of the reaction was confirmed by using TLC with mobile phase *n*-hexane: Ethyl acetate (6:4). All the entitled derivatives were obtained by the above stated procedure.

2.5a N-(4-Chloro-3-fluorophenyl)-2-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (5k): Yield-69%; m.p.: 186° C; IR (ATR, cm⁻¹): 765 (-C-Cl str.), 1035, 1243 (-C-O-C- str.), 1169 (-C-F, str.), 1341 (-C-N- str. sec. amine), 1452 (-C=C- str. aromatic ring), 1683 (C=O str. carbonyl group), 2725 (CH₂ str. methylene), 3035 (CH str. aromatic ring), 3407 (NH str. sec. amine); ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 4.24 (2H, s, -CH₂), 7.07–8.02 (7H, m, Ar-H), 10.46 (1H, s, -NH); ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 38.9 (C₉), 111.8 (C₁₆), 116.2 (C₄), 116.2 (C₂), 116.8 (C₁₄), 122.5 (C₆), 124.9 (C₁₂), 129.4 (C₅), 129.4 (C₁), 129.6 (C₁₃), 139.0 (C₁₁), 163.2 (C₃), 163.2 (C₁₅), 164.8 (C₇), 168.4 (C₁₀), 171.1 (C₈); ¹⁹F NMR (376 MHz, DMSO-d₆, δ, ppm): -115.69 (1F, s, -F_(c-15)), -108.64 (1F, s, p-F); LCMS (m/z): 382 (M⁺); Anal. calcd. for C₁₆H₁₀ClF₂N₃O₂S: C-50.34, H-2.64, N-11.01, S-8.40 Found: C-50.38, H-2.66, N-11.04, S-8.42%.

2.6 Antimicrobial assay

The newly synthesized entities (5a-t) were screened for their antimicrobial assay against a broad panel of Gram-positive bacteria *S. aureus* (ATCC No. 25923), *E. faecalis* (ATCC No. 29212), Gram-negative bacteria *E. coli* (ATCC No. 25922), *P. aeruginosa* (ATCC No. 27853) and fungi *C. albicans* (ATCC No. 10231), *A. niger* (ATCC No. 1015). The samples for antifungal as well as antibacterial evaluation were tested by standard protocols like Micro dilution/Broth titer method. The stock solution (1000 μg/ml) for each compound was prepared and screening for antimicrobial activity was carried out by diluting the stock solution and preparing the sets consecutively from 1000, 500, 250, 125, 62.5, 31.25, 15.62 up to 7.8 μg/ml. The tubes along with the control were then kept for incubation at 37°C for 24 h. Suspensions were further inoculated on an appropriate media and growth was noted after 48 h. The obtained results (MIC) in μg/ml by observing the highest dilutions (low turbidity) were recorded and compared with the MIC value of standard drugs ciprofloxacin and fluconazole.

3. Results and discussion

3.1 Chemistry

Synthesis of the desired compounds and intermediates was accomplished according to the steps depicted in scheme 1. The use of two very simple but most important fluorine substituted anilines played an important role in the enhancement of biological results of the synthesized compounds (**5a–t**). The compounds 3-(trifluoromethyl)aniline (**3A**) and 4-chloro-3-fluoroaniline (**3B**) were used as a starting material for the synthesis of the targeted compounds. These two fluoro substituted aniline were treated with chloroacetyl chloride (CAC), triethylamine (TEA) and dimethylformamide (DMF) resulting in the formation of useful intermediates; 2-chloro-N-(3-(trifluoromethyl)phenyl)acetamide (**4A**), and 2-chloro-N-(4-chloro-3-fluorophenyl)acetamide (**4B**), respectively. On the other hand, differently substituted benzo-hydrazide derivatives (**1a–j**) were treated with CS₂ and KOH in an alcoholic medium to undergo cyclization resulting in the formation of potent 1,3,4-oxadiazole derivatives (**2a–j**). The oxadiazole containing fluoro substituent derivatives (**5a–t**) were synthesized under mild conditions using acetone as a solvent and K₂CO₃

as a neutralizing agent by condensing **2a–j** with **4A** and **4B** respectively.

3.2 Characterization

3.2a IR data: IR spectral data obtained here lead to the confirmation about the formation of targeted compounds. The broad stretching band appearing at 3313 cm⁻¹ and 3407 cm⁻¹ indicated the presence of secondary amine in compounds **5a** and **5k**, respectively. The presence of –C–O–C– bond in 1,3,4-oxadiazole ring was confirmed by two stretching bands at 1055 and 1253 cm⁻¹ in compound **5a**. Also the presence of stretching vibration bands at 1035 and 1243 cm⁻¹ in compound **5k** confirmed the presence of –C–O–C– bond in form of oxadiazole nucleus. A weak absorption band at 2726 (**5a**) and 2725 cm⁻¹ (**5k**) helped to conclude the presence of ‘–CH₂’ methylene functional group in the targeted compounds. The presence of –C=O group in vicinity to –NH linkage can be predicted by the stretching frequency of 1667 and 1683 cm⁻¹ in compounds **5a** and **k**, respectively. The presence of –C=C– in aromatic ring was confirmed due to the visibility of a band at 1401 and 1452 cm⁻¹ in **5a** and **k**, respectively. –C–N– linkage in the structure can be proved by a stretching

Scheme 1. Synthetic route for the preparation of compounds **5a–t**.

frequency obtained at 1349 cm^{-1} in molecule **5a** and at 1341 cm^{-1} in compound **5k**.

3.2b ^1H NMR data: The formation of the targeted compounds can be easily confirmed by interpreting the obtained absorption peaks in the proton spectral data. If we take into consideration compound 2-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamide (**5a**); a sharp peak observed at $\delta = 4.32$ confirmed the presence of two protons of the methylene group in the targeted molecule. Absorption peak at $\delta = 10.71$ executed the presence of a proton of secondary amine in the structure. The protons present on the aromatic nucleus showed the absorption details between $\delta = 7.26$ and 8.06 ppm . The compound N-(4-chloro-3-fluorophenyl)-2-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (**5k**) with the 3-fluoro substituent when screened for ^1H NMR spectra, the following absorption details were observed. The absorption spectra at $\delta = 4.24$ exhibited the presence of protons of methylene group. The presence of a proton of secondary amine proved its presence by showing an absorption peak at $\delta = 10.46$. The presence of seven protons on two phenyl rings were confirmed by $\delta = 7.07$ to 8.02 .

3.2c ^{13}C NMR data: The ^{13}C NMR added more confirmation to the formation of the targeted motifs. The δ values were seen to vary between 38.9 and 168.4 . The chemical shifts were assigned by taking into consideration the spectral information produced in an article by Aranda *et al.*⁴⁰ The carbon atom '10' in contact with electronegative atom oxygen corresponding to a carbonyl group showed a maximum downfield shift in the spectrum at $\delta = 168.4$. The ^{13}C NMR signal obtained at 38.9 ppm can be assigned to the carbon '9' of the methylene group present in between the sulphur and the carbonyl group. The carbon '7' present in between the nitrogen and the oxygen atom of the oxadiazole ring and in contact to the phenyl ring with different substitution exhibited a shift at 164.8 ppm . The chemical shift details of the carbon in the phenyl ring with the different substituent was seen to vary between 104.8 and 163.2 ppm , where the $\delta = 104.8$ corresponded to the C-1 and C-5 carbon of the compounds **5e** and **o** due to the presence of a methoxy group at C-2 and C-4. The ^{13}C NMR displaying a signal at 163.2 ppm in **5a** and **k** was due to the presence of fluorine atom to the carbon C-3 by a single bond. The ^{13}C NMR spectra for the compounds **5e** and **o** exhibited sharp peak at 153.5 indicating the presence of $-\text{OCH}_3$ group in direct contact with the carbon C-2 and C-4. The signal of C-3

carbon had a field shift to 139.8 ppm , inspite of having a direct contact to $-\text{OCH}_3$ due to the presence of two methoxy groups each at C-4 and C-2. Similarly, the carbon C-2 (**5j**) and C-3 (**5h**) having a $-\text{NO}_2$ substitution exerted a signal at 149.0 and 148.2 , respectively. The carbon numbering to the structure is given in figure 1.

3.2d ^{19}F NMR: The chemical shifts values derived from the ^{19}F NMR spectra clearly confirmed the presence of fluorine atoms in the synthesized molecules. In molecule **5a**, the δ value obtained at -61.36 ppm confirmed the presence of $-\text{CF}_3$ as a substituent. Similarly, a shift at δ value -115.69 ppm helped to prove the presence of $-\text{F}$ (attached to C-15) in the structure **5k**. Furthermore, the presence of fluoro substituents in **5a** and **k** at the para position (C-3) was proved by obtaining δ value at -108.56 and -108.64 ppm , respectively. Thus, fluorine spectral details added more support to the formation of titled compounds.

3.3 Antimicrobial activity

Broad panel of Gram-positive bacteria (*S. aureus* and *E. faecalis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungal strains (*C. albicans* and *A. niger*) were utilized for testing the antimicrobial properties of the targeted compounds (**5a-t**). The results of the antibacterial and antifungal activities in MIC ($\mu\text{g/ml}$) have been reported in tables 1 and 2, respectively. It was observed that most of the compounds exhibited excellent activity as compared to the standards. A very few of them exhibited moderate or poor activity. The standard drug used against antibacterial strains was ciprofloxacin and fluconazole was employed as a standard against fungal strains. The MIC value of ciprofloxacin was recorded to be $62.5\text{ }\mu\text{g/ml}$ against Gram-positive bacterial strain *S. aureus*, where as the same standard responded to MIC $125\text{ }\mu\text{g/ml}$; when used against *E. faecalis*, *E. coli* and *P. aeruginosa*. Fluconazole, when used as a standard drug against fungal strains *C. albicans* and *A. niger*, MIC value was

Figure 1. Carbon numbering of the final derivatives **5a-t**.

Table 1. Antimicrobial screening of the compounds **5a–j**.

Compounds	Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231	<i>A. niger</i> ATCC 1015
5a (R:p-F, X:CF ₃ , Y:H)	62.5	62.5	31.25	62.5	62.5	62.5
5b (R:o-Cl, X:CF ₃ , Y:H)	62.5	62.5	15.63	62.5	62.5	62.5
5c (R:p-CH ₃ , X:CF ₃ , Y:H)	62.5	62.5	31.25	62.5	62.5	62.5
5d (R:o-Br, X:CF ₃ , Y:H)	62.5	62.5	62.5	31.25	125	125
5e (R:tri-OCH ₃ , X:CF ₃ , Y:H)	62.5	31.25	62.5	31.25	125	125
5f (R:o-CH ₃ , X:CF ₃ , Y:H)	31.25	31.25	62.5	31.25	62.5	125
5g (R:H, X:CF ₃ , Y:H)	31.25	31.25	31.25	31.25	62.5	62.5
5h (R:p-NO ₂ , X:CF ₃ , Y:H)	31.25	31.25	31.25	15.63	125	62.5
5i (R:p-Cl, X:CF ₃ , Y:H)	31.25	62.5	31.25	31.25	125	62.5
5j (R:m-NO ₂ , X:CF ₃ , Y:H)	31.25	62.5	15.63	31.25	31.25	62.5
Fluconazole	–	–	–	–	125	62.5
Ciprofloxacin	62.5	125	125	125	–	–

Std.: Standard drug fluconazole for antifungal and ciprofloxacin for antibacterial tests

observed at 125 and 62.5 $\mu\text{g/ml}$, respectively. In general, it was observed that most of the compounds have resulted to be potent antibacterial, and a very few of them were found to exhibit good antifungal activity.

3.4 Antibacterial activity

The targeted compounds were screened for their antibacterial activity and their MIC ($\mu\text{g/ml}$) values were recorded. It was observed that compounds **5f** (o-CH₃), **5g** (H), **5h** (p-NO₂), **5i** (p-Cl) and **5j** (m-NO₂) showed excellent activity (MIC = 31.25 $\mu\text{g/ml}$), when tested against Gram-positive bacteria *S. aureus* (ATCC

No. 25923). Other compounds viz. **5a** (p-F), **5b** (o-Cl), **5c** (p-CH₃), **5d** (o-Br), **5e** (3,4,5-tri-OCH₃), **5k** (p-F), **5l** (o-Cl), **5n** (o-Br), **5o** (3,4,5-tri-OCH₃) and **5r** (p-NO₂) were observed to exhibit MIC value equal to that of the standard drug (MIC 125 $\mu\text{g/ml}$). Only two compounds **5p** (o-CH₃) and **5s** (p-Cl) were exhibiting poor activity (MIC 250 $\mu\text{g/ml}$). A very few exhibited excellent activity (MIC 31.25 $\mu\text{g/ml}$) against *E. faecalis* such as **5e** (3,4,5-tri-OCH₃), **5f** (o-CH₃), **5g** (H) and **5h** (p-NO₂). The other bio-chemical motifs **5a** (p-F), **5b** (o-Cl), **5c** (p-CH₃), **5d** (o-Br), **5i** (p-Cl), **5j** (m-NO₂) and **5l** (o-Cl) showed good activity. It was observed that the targeted molecules **5s** (p-Cl), **5r** (p-NO₂), **5p** (o-CH₃), **5o** (3,4,5-tri-OCH₃) and **5k** (p-F) exhibited activity

Table 2. Antimicrobial screening of the compounds **5k–t**.

Compounds	Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231	<i>A. niger</i> ATCC 1015
5k (R:p-F, X:F, Y:Cl)	62.5	125	62.5	125	250	250
5l (R:o-Cl, X: F, Y:Cl)	62.5	62.5	31.25	125	250	125
5m (R:p-CH ₃ , X:F, Y:Cl)	–	–	–	–	–	–
5n (R:o-Br, X:F, Y:Cl)	62.5	125	62.5	125	125	125
5o (R:tri-OCH ₃ , X:F, Y:Cl)	62.5	125	62.5	62.5	250	250
5p (R:o-CH ₃ , X:F, Y:Cl)	125	125	31.25	62.5	125	125
5q (R:H, X:F, Y:Cl)	62.5	125	62.5	125	125	250
5r (R:p-NO ₂ , X:F, Y:Cl)	62.5	125	62.5	62.5	125	125
5s (R:p-Cl, X:F, Y:Cl)	125	125	62.5	62.5	125	125
5t (R:m-NO ₂ , X:F, Y:Cl)	31.25	125	125	62.5	125	125
Fluconazole	–	–	–	–	125	62.5
Ciprofloxacin	62.5	125	125	125	–	–

Std.: Standard drug fluconazole for antifungal and ciprofloxacin for antibacterial tests

of similar MIC value to that of a standard (MIC 125 $\mu\text{g/ml}$). The compound **5m** (p-CH₃) was observed to show zero activity against both *S. aureus* and *E. faecalis*.

The targeted series of compounds (**5a–t**); when introduced against Gram-negative bacteria *E. coli*; other than **5m** (p-CH₃) and **5t** (m-NO₂), all compounds exhibited excellent to good activity as compared to the standard drug. The compounds **5b** (o-Cl) and **5j** (p-NO₂) have shown good activity and far better than the standard drug with the MIC value of 15.63 $\mu\text{g/ml}$. The compounds **5a** (p-F), **5c** (p-CH₃), **5g** (H), **5h** (p-NO₂), **5i** (p-Cl), **5l** (o-Cl) and **5p** (o-CH₃) showed excellent activity (MIC 31.25 $\mu\text{g/ml}$). The MIC values of the compounds **5d** (o-Br), **5e** (3,4,5-tri-OCH₃), **5f** (o-CH₃), **5k** (p-F), **5n** (o-Br), **5o** (3,4,5-tri-OCH₃), **5q** (H), **5r** (p-NO₂) and **5s** (p-Cl) were good as compared to standard with a MIC value of 62.5 $\mu\text{g/ml}$. Thus, all the compounds were showing better activity than the standard ciprofloxacin (MIC 125 $\mu\text{g/ml}$), when treated against Gram-negative bacteria *E. coli*. Another Gram-negative bacteria *P. aeruginosa* was employed to check the activity of the series of compounds synthesized. Compound **5h** (p-NO₂) exhibited best MIC value (15.63 $\mu\text{g/ml}$) among all the synthesized compounds. Several compounds such as **5d** (o-Br), **5e** (3,4,5-tri-OCH₃), **5f** (o-CH₃), **5g** (H), **5k** (p-F) and **5l** (o-Cl) also showed excellent activity (MIC 31.25 $\mu\text{g/ml}$) compared to the standards against *P. aeruginosa*. Chemical entities **5a** (p-F), **5b** (o-Cl), **5c** (p-CH₃), **5o** (3,4,5-tri-OCH₃), **5r** (p-NO₂), **5s** (p-Cl) and **5t** (m-NO₂) responded to Gram-negative bacteria *P. aeruginosa* up to 62.5 $\mu\text{g/ml}$. Compounds **5k** (p-F), **5l** (o-Cl), **5n** (o-Br) and **5q** (H) exhibited activity similar to that of the standard drug ciprofloxacin (MIC 125 $\mu\text{g/ml}$) against *P. aeruginosa*.

Overall, amongst the targeted molecules, only compound **5m** did not respond to any of the bacterial strain, where as compounds **5f** (o-CH₃), **5g** (H), **5h** (p-NO₂), **5j** (m-NO₂) and **5b** (o-Cl) have exhibited excellent activity against all the bacterial strains utilized for conducting the MIC value tests.

3.5 Antifungal activity

From the antifungal activity results, it can be evaluated that the compound **5j** (m-NO₂) exhibited excellent activity (MIC 31.25 $\mu\text{g/ml}$) from the complete series, when employed against *C. albicans* (ATCC No. 10231) as compared to the standard drug fluconazole (125 $\mu\text{g/ml}$). Other compounds **5a** (p-F), **5b** (o-Cl), **5c** (p-CH₃), **5f** (o-CH₃) and **5g** (H) showed good activity (62.5 $\mu\text{g/ml}$). Compounds **5k** (p-F), **5l** (o-Cl) and **5o** (3,4,5-tri-OCH₃) resulted in poor activity (MIC

250 $\mu\text{g/ml}$) against *C. albicans*. When the synthesized compounds were tested against *A. niger* (ATCC No. 1015); very few compounds **5a** (p-F), **5b** (o-Cl), **5c** (p-CH₃), **5g** (H), **5h** (p-NO₂), **5i** (p-Cl) and **5j** (m-NO₂) exhibited moderate activity (MIC 62.5 $\mu\text{g/ml}$) as compared to the standard drug. Other compounds did not turn up to be good antifungal against *A. niger* as they exhibited poor activity as compared to the fluconazole drug.

3.6 Structure-activity relationship

The study of SAR helped in revealing that the use of different substitution and their effect on various microbial strains. Different electronic environments were made possible on the phenyl ring as to confer the change in activity due to different functional groups. Both electron-withdrawing and electron-donating groups were chosen as substitutions during the designing and synthesis of target molecules as indicated in figure 2. The use of different substitution in the target entities proved to be the most effective parameter in the synthesis of bio-active molecules. From the antimicrobial activity data available in the report, it can be concluded that majority of compounds with electron-withdrawing functional groups like –NO₂, –F, –Cl and –Br exhibited better activity than the compounds with electron-donating groups like –CH₃ and –OCH₃. The compounds **5a** (p-F), **5b** (o-Cl), **5d** (o-Br), **5h** (p-NO₂), **5i** (p-Cl) and **5j** (m-NO₂) possessing electron withdrawing groups showed excellent activity against all the bacterial strains and a moderate activity against fungal

Figure 2. General structures of targeted bioactive compounds **5a–t**.

Table 3. Antimicrobial screening of the compounds **5g**, **5q** and N-phenyl-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetamide (indicating the correlation between fluorine atom and biological activity).

Compounds	Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231	<i>A. niger</i> ATCC 1015
5* (R:H, X: H, Y:H)	125	125	250	250	250	125
5g (R:H, X:CF ₃ , Y:H)	31.25	31.25	31.25	31.25	62.5	62.5
5q (R:H, X:F, Y:Cl)	62.5	125	62.5	125	125	250

5*= N-phenyl-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetamide

strains. It was also observed that the compounds **5c** (p-CH₃), **5e** (3,4,5-tri-OCH₃), **5f** (o-CH₃), **5m** (p-CH₃), **5o** (3,4,5-tri-OCH₃) and **5p** (o-CH₃) possessing electron- donating groups showed moderate activity or even less than the standards used.

The antibacterial as well as antifungal MIC values for compounds **5g** (H) well supports even in the absence of any substitutions the antimicrobial activity which seems to be an excellent quality with respect to some electron-donating groups against each bacterial and fungal strains used. This has helped to confirm that the fluorine substitution on the main structure at position X (attached to C-15) in the form of -CF₃ shoots up the potency of target molecules as an effective antimicrobial agent. The effect of decrease in the potency of target molecule due to reduction of electron-withdrawing functional group 'fluorine', can be proved by comparing the biological activity data between the compounds **5g** (R:H, X:CF₃, Y:H) and **5q** (R:H, X:F, Y:Cl). Also if a compound (**5***) with no fluorine substituent or no electron-withdrawing group is taken, activity is found to be diminishing as shown in table 3. The compound **5g** exerted excellent activity (31.25 $\mu\text{g/ml}$) against both the Gram-positive and Gram-negative bacterial strains, where as the compound **5q** showed comparatively low activity (62.5 $\mu\text{g/ml}$) against the same bacterial strains.

Along with the presence of electron-withdrawing groups, the presence of fluorine substitution at position X in each compound has helped to enhance the antimicrobial potency across the panel of Gram-positive, Gram-negative and fungal strains. Overall, the presence of electron-withdrawing groups like -F, -Cl, -Br and -NO₂ in the chemical entities as substitution and fluorine atom (-CF₃ or -F) in the heterocyclic skeleton have helped to increase the antimicrobial potency. Thus, in the future, the combination of different electron-withdrawing groups will be performed on oxadiazole ring for generating better lead entities as potent antibacterial and antifungal agents.

4. Conclusion

In the present work, more emphasis was given on development of new bio-active heterocyclic derivatives bearing fluorine atoms. The presence of hydrophobic group fluorine at position 15 (in the final structure as shown in figure 1) helped to demonstrate its importance leading to higher activity of the synthesized scaffolds, even higher than the standard drugs used. The SAR study reveals that the electronic effect of the various substituents plays a very important role in identifying the targeted compounds with electron with-drawing groups (NO₂, F, Cl and Br) as potent microorganism growth inhibitors. Also the importance of fluorine atom (quantitatively) is proved to be of much importance in the enhancement of biological properties of the derived compounds as indicated in the SAR study. The results prompted the titled oxadiazole compounds as interesting leads for further physiochemical optimization and are also interesting for future scope, to be used as templates for generating better antibacterial and antifungal lead molecules.

Supplementary information

Supplementary information (spectral data) of compounds, can be seen in the website of Journal of Chemical Sciences (www.ias.ac.in/chemsci).

Acknowledgements

Authors would like to express their sincere thanks to Sheth M. N. Science College, Patan for providing laboratory facilities. We are highly thankful to SAIF Chandigarh and Indian Institute of Science (IISc), Bangalore, for analytical and instrumentation facilities. One of our author DSJ is highly indebted to Department of Science and Technology (DST), India for awarding INSPIRE fellowship (DST-JRF).

References

- Joshi K, Dandia A and Khanna S 1990 *Indian J. Chem.* **29B** 1125
- Xu W, He J, He M, Han F, Chen X, Pan Z, Wang J and Tong M 2011 *Molecules* **16** 9129
- Isanbor C and Hagan D 2006 *J. Fluor. Chem.* **127** 303
- Begue J and Bonnet-Delpon D 2006 *J. Fluor. Chem.* **127** 992
- Kirk K 2006 *J. Fluor. Chem.* **127** 1013
- Sakairi K, Kogami M, Torii M, Makino M, Kataoka D, Okamoto R, Miyazawa T, Inoue M, Takahashi N, Harada S and Watanabe N 2012 *Chem. Pharm. Bull.* **60** 1093
- Parikh K and Joshi D 2013 *Med. Chem. Res.* **22** 3688
- Amir M and Shikha K 2004 *Eur. J. Med. Chem.* **39** 535
- Padmavathi V, Reddy G, Padmaja A, Kondaiah P and Shazia A 2009 *Eur. J. Med. Chem.* **44** 2106
- Weng J, Liu H, Huang H, Tan C and Chen J 2012 *Molecules* **17** 989
- Gaonkar S, Rai K and Prabhuswamy B 2006 *Eur. J. Med. Chem.* **41** 841
- Manjunatha K, Poojary B, Lobo P, Fernandes J and Kumari N 2010 *Eur. J. Med. Chem.* **45** 5225
- Li Y, Liu J, Zhang H, Yang X and Liu Z 2006 *Bioorg. Med. Chem. Lett.* **16** 2278
- Hamada N and Sharshira E 2011 *Molecules* **16** 2304
- Loetchutinat C, Chau F and Mankhetkorn S 2003 *Chem. Pharm. Bull.* **51** 728
- Abadi A, Eissa A and Hassan G 2003 *Chem. Pharm. Bull.* **51** 838
- Kumar D, Sundaree S, Johnson E and Shah K 2009 *Bioorg. Med. Chem. Lett.* **19** 4492
- Khan M, Choudhary M, Khan K, Rani M and Rahman A 2005 *Bioorg. Med. Chem.* **13** 3385
- Vardan S, Smulyan H, Mookherjee S and Eich R 1983 *Clin. Pharmacol. Ther.* **34** 290
- Raman K, Singh K, Salzman S and Parmar S 1993 *J. Pharm. Sci.* **82** 167
- Sahin G, Palaska E, Kelicen P, Demirdamar R and Altinok, G 2001 *Arzneimittel Forschung - Drug Res.* **51** 478
- Angilini I, Angilini L and Sparacc F 1969 British Patent 1:161, 801, 1969; *Chem. Abstr.* **71**: 112936. 1964
- Najer H, Giudicelli R, Moral C and Menin M 1966 *Bull. Soc. Chim. Fr.* **1** 153
- Yale H and Losee K 1966 *J. Med. Chem.* **9** 478
- Piala J and Yale H 1964 US Patent: 3,141, 022. *Chem. Abstr.* **61**, 8317, 1964
- Palmer J, Hirschbein B, Cheung H, McCarter J, Janc J, Yu W and Wesolowski G 2006 *Bioorg. Med. Chem. Lett.* **16** 2909
- Johns B 2004 *PCT Int. Appl. WO* 2004101512
- Baxendale I, Ley S and Martinelli M 2005 *Tetrahedron* **61** 5323
- Coppo F, Evans K, Graybill T and Burton G 2004 *Tetrahedron Lett.* **45** 3257
- Brain C, Paul J, Loong Y and Oakley P 1990 *Tetrahedron Lett.* **40** 3275
- Wang Y, Sauer D and Djuric S 2006 *Tetrahedron Lett.* **47** 105
- Al-Talib M, Tashtoush H and Odeh N 1990 *Syn. Comm.* **20** 1811
- Patel B, Guin S, Ghosh T, Rout S and Banerjee A 2011 *Org. Lett.* **13** 5976
- Theocharis A and Alexandrou N 1990 *J. Hetero. Chem.* **27** 1685
- Zhenga X, Zhong B, Wang Y, Chena W, Huang Q, Liua Q and Songa G 2003 *J. Fluor. Chem.* **123** 163
- Cao S, Qian X, Song G and Huang Q 2002 *J. Fluor. Chem.* **117** 63
- Bégué J and Bonnet-Delpon D 2005 *Chimie bioorganique et Médicinale du Fluor*, EDP Science/CNRS, Paris
- Dash S, Ashok B, Singh J, Maiti B and Maity T 2011 *Med. Chem. Res.* **20** 1206
- Metwally M, Abdel-latif E, Amer F and Kaupp G 2004 *Dyes Pigments* **60** 249
- Aranda G, Dessolin M, Golfier M and Guillerez M-G 1982 *Org. Magn. Res.* **18** 159