Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (4):722-729

(International Peer Reviewed Journal)

Synthesis and *invitro* antibacterial activity of some novel Sulfonamide derivatives bearing 1,4-disbstituted-1,2,4-oxadiazole Moiety

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Received on 29th May and finalized on 15th June 2013.

ABSTRACT

*A strategic synthesis of 1-[(2,5-dimethoxyphenyl)sulfonyl]-4-{5-[substituted]-1,2,4-oxadiazol-3-yl}piperazine, involves construction of 1,2,4-oxadiazole ring by intermolecular condensation of tert-butyl-4-(N-hydroxycarbamimidoyl)piperazine-1-carboxylate with 3-(trifluromethoxy)benzoic acid. Structures of the newly synthesized compounds were established by IR, ^{1}H NMR, ^{13}C NMR, LC-MS and CHNS spectroscopic evidences. All the newly synthesized compounds were tested for their invitro antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus. Tested compounds showed good antibacterial activities when compared to the reference ciprofloxacin. Compounds **10e, 10f, 10g, 10h** and **10j** showed good activity against tested organisms.*

Keywords: Sulfonamide, 1,2,4-oxadiazole, *invitro* antibacterial activity, propylphosphonic anhydride.

INTRODUCTION

Among the chemotherapeutic agents known, sulfonamide drugs were the first to be used for the cure and prevention of bacterial infection in human beings [1]. Sulfonamides play a very important role as key constituent in number of biologically active molecules. Sulfonamides have been exhibiting a wide variety of biological activities such as antibacterial [2], insecticidal [3], antifungal [4], antihepatitis [5], antiinflamatory [6], antitumor [7], anticancer [8], anti-HIV [9] and antitubercular activities [10] from past decades to the present day. These varied biological activities of sulfonamides is due to the presence of 5 or 6 member heterocyclic ring system attached to the sulfonamide group. In recent years extensive research studies have been carried out on the synthesis and evaluation of pharmacological activities of sulfonamide containing heterocycles for different activities, and have been proven to be important pharmacophores. Various substituted sulfonamide derivatives containing oxadiazole ring are of important class of bioactive compounds that have a wide spectrum of pharmacological activities, they also exhibit antimicrobial [11], anti-HIV [11], antbacterial [12], antifungal [13], insecticidal [14], anticancer [15], antiviral [16] and antioxidant activity [17]. Prompted by these observations, we have synthesized a novel series of sulfonamide derivatives bearing 1,4-disubstituted-1,2,4-oxadiazole moiety as shown in Scheme 1.

Synthesized compounds were tested for their *invitro* antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria *Escherichia coli*, and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Chemicals were purchased from Sigma Aldrich, Merck India, SD Fine chemicals and were used without further purifications. Purification and characterization of the newly synthesized sulfonamide derivatives containing oxadiazole ring was done by Column chromatography and silica gel 60-120 mesh size using solvent system Pet ether / Ethyl acetate (7:3). Reactions were monitored by Thin-layer chromatography (TLC) on precoated silica gel plates. Visualization was done with UV light (254 nm) or iodine chamber. Melting point reported was determined in open capillary and was uncorrected. FT-IR Spectra was recorded on Jasco FT-IR Spectrometer, ^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$ at 400 MHz and 100 MHz respectively. LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micromass LCT mass detector. Elemental analysis (C, H, N and S) was performed on an Elementar vario Micro cube analyzer.

Synthesis of tert-butyl piperazine-1-carboxylate (3) : To a stirring solution of anhydrous piperazine **(1)** (0.3 moles, 25 gms) in 300 ml of methylene dichloride at 0^0C, di-tert-butyl dicarbonate **(2)** (0.15 moles, 32.7 gms) in 50 ml MDC was added drop wise under nitrogen atmosphere. A side product di-tert-butyl piperazine-1,4-dicarboxylate formed during the course of the reaction was filtered, washed with methylene chloride and was kept aside. MDC was removed under reduced pressure followed by addition of 300 ml of water; the precipitate obtained was filtered off. Resulting aqueous solution was saturated with solid sodium bicarbonate and was extracted with ethyl acetate. Organic layer was dried and concentrated to get pure tert-butyl piperazine-1-carboxylate **(3)** as white crystalline solid with melting point 46-48 ^{0}C (Lit. mp 45-47^{0}C)**.** [18]

Synthesis of tert-butyl-4-cyanopiperazine-1-carboxylate (4) : A mixture of tert-butyl piperazine-1-carboxylate **(3)** (0.13 moles, 25 gms), cyanogen bromide (0.13 moles, 14.13 gms) and potassium carbonate (0.39 moles, 18.5 gms) in dry acetonitrile (250 ml) was stirred at room temperature for 10 hours and reaction mixture was concentrated under reduced pressure. The crude mass obtained was dissolved in MDC, washed with water and brine. Organic layer was separated, dried using sodium sulfate and concentrated to get pale yellow solid. Solid obtained was triturated in pet-ether, filtered to obtain compound **(4)** as a white solid. Further recrystallization was carried out using pet-ether/MDC (8:2) to get pure tert-butyl-4-cyanopiperazine-1-carboxylate **(4)** as colorless crystals with melting point 107-108 ^{0}C. IR: ν_{max}/cm^{-1}: 2228.34 (CN), 1698.2 (CO). ^{1}H-NMR (CDCl3) δ ppm: 3.51-3.48 (t, 4H, H_2C-N-CH_2), 2.97-2.94 (t, 4H, H_2C-N-CH_2), 1.40 (s, 9H, $3CH_3$).

Synthesis of tert-butyl-4-(N'-hydroxycarbamimidoyl) piperazine-1-carboxylate (5) : A mixture of *tert*-butyl-4-cyanopiperazine-1-carboxylate **(4)** (0.11 moles, 25 gms), hydroxylamine chloride (0.29 moles, 20.58 gms) and triethylamine (Et_3N) (0.33 moles, 33.33 gms) in methanol (250 ml) was stirred for 12 hours at room temperature. Reaction mixture was then concentrated and crude solid obtained was dissolved in methylene dichloride, washed with water, organic layer was separated, dried and concentrated to get crude white solid which was recrystallized using methanol to get pure *tert*-butyl-4-(*N*'-hydroxycarbamimidoyl)piperazine-1-carboxylate **(5)** as colorless solid with melting point 190-191 ^{0}C. ^{1}H-NMR ($CDCl_3$) δ ppm: 9.01 (s, 1H, OH), 6.39 (s, 2H, NH_2) 1.92-1.81 (t, 4H, H_2C-N-CH_2), 1.75-1.70 (t, 4H, H_2C-N-CH_2), 1.39 (s, 9H, $3CH_3$).

Synthesis of tert-butyl-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine-1-carboxylate (7) : A equimolar mixture of *tert*-butyl-4-(*N*'-hydroxycarbamimidoyl)piperazine-1-carboxylate **(5)** (0.08 moles, 20 gms) and 3-(trifluoromethoxy)benzoic acid **(6)** (0.08 moles, 16.8 gms)

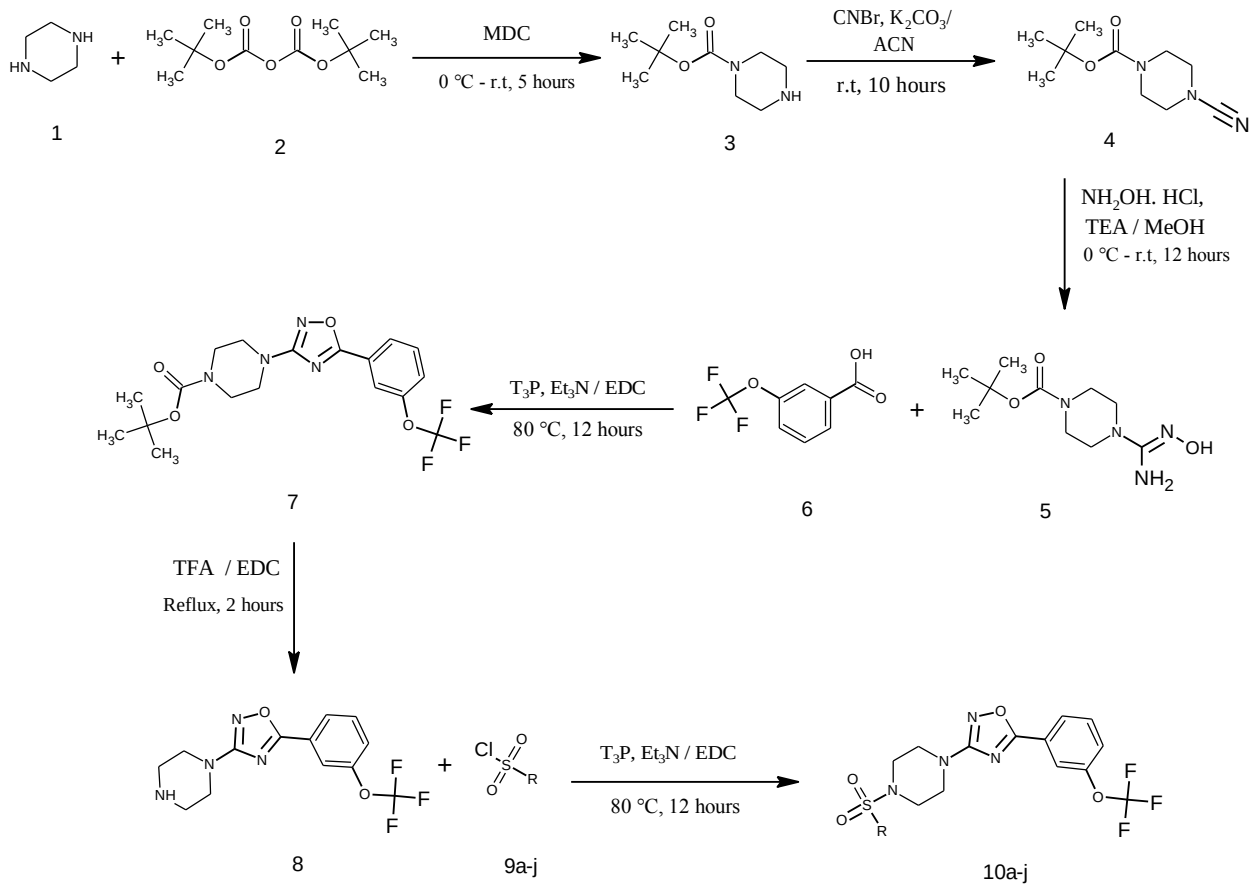
along with propylphosphonic anhydride (T_3P) (0.24 moles, 76.32 gms) and triethylamine (Et_3N) (0.24 moles, 24.24 gms) in ethylene dichloride (200 ml) was refluxed under nitrogen atmosphere for 12 hours. Reaction mixture was washed with water, organic layer was separated, dried and concentrated to obtain crude compound **(7)** which was further purified by Column chromatography using (7:3) pet-ether/ethyl acetate as eluent to obtain pure *tert*-butyl-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine-1-carboxylate **(7)** as colorless solid with melting point 211-213 ^{0}C. ^{1}H-NMR ($CDCl_3$) δ ppm : 7.87-7.86 (d, 1H, Ar-H), 7.78 (s, 1H, Ar-H) 7.51-7.46 (t, 1H, Ar-H), 7.29-7.28 (d, 1H, Ar-H) 3.57-3.55 (t, 8H, Piperazine methylenes), 1.49 (s, 9H, $3CH_3$). LC-MS: 415.12 (M+1).

Synthesis of 1-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine (8) : A mixture of *tert*-butyl-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine-1-carboxylate **(7)** (6 gms) along with trifluroacetic acid (6 ml) in ethylene dichloride (60 ml) was refluxed for about 2 hours. Reaction mixture was cooled to room temperature and concentrated to get deprotected crude 1-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine **(8)** as pale yellow solid with melting point 191-193 ^{0}C, which was taken for next step directly without purification. ^{1}H-NMR ($CDCl_3$) δ ppm: 8.01-7.99 (d, 1H, Ar-H), 7.92 (s, 1H, Ar-H) 7.59-7.57 (t, 1H, Ar-H), 7.46-7.44 (d, 1H, Ar-H) 3.86-3.84 (t, 4H, H_2C-N-CH_2), 3.30-3.29 (t, 4H, H_2C-N-CH_2). LC-MS: 315.12 (M+1).

General procedure for the synthesis of 1-(substitutedsulfonyl)-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl} piperazine (10a-j) : A equimolar mixture of 1-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine **(8)** (0.001 moles, 0.5 gms), with substituted sulfonyl chlorides **(9a-j)** (0.001 moles) along with triethylamine (Et_3N) (0.003 moles, 0.3 gms) in ethylene dichloride (5 ml) was reflux for 12 hours. Reaction mixture was then cooled to room temperature, washed with water; organic layer was separated, dried using sodium sulfate and concentrated to obtain crude pale yellow solids **(10a-j)**. Crude compound obtained was further purified by Column chromatography using pet-ether: ethyl acetate (7:3) as eluent to get the pure 1-(substitutedsulfonyl)-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl} piperazine **(10a-j)** in good yield. Physical data and spectroscopic evidences of some of the synthesized sulfonamide derivatives bearing 1,2,4-oxadiaole moiety are given in the Table 1 and Table 2 respectively.

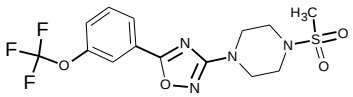
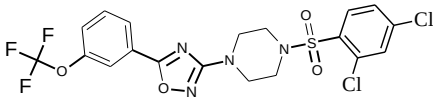
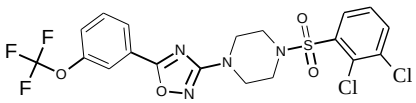
***Invitro* ANITBACTERIAL STUDY :** The antibacterial activity was evaluated against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion method using Ciprofloxacin as standard. Out of all the synthesized compounds **(10a-j)** some of the compounds showed potent antibacterial activities.

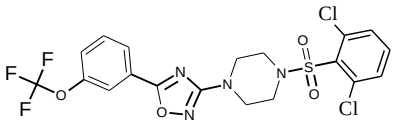
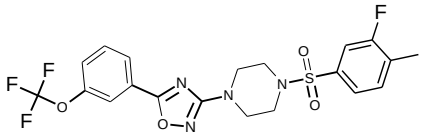
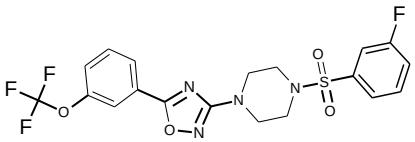
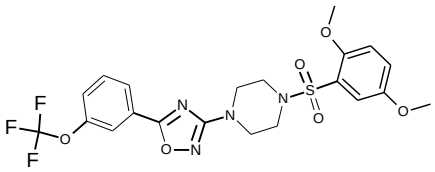
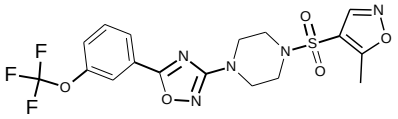
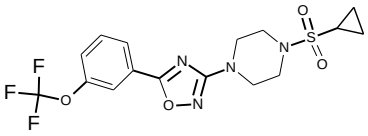
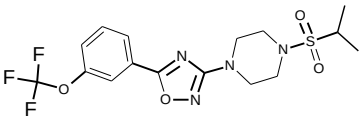
The newly synthesized compounds **10a-j** was screened for their antibacterial activity against bacterial strains by disc diffusion method [19]. The discs with 6.25 mm in diameter were punched from Whatman No. 1 filter paper. 10 disc batches were dispensed to each screw-capped bottle and sterilized by subjecting to dry heat at 140 °C for 60 minutes. The test compounds were prepared with varied concentrations using DMSO. Discs were prepared by taking 10 times the amount of chemical required for each disc was added to each bottle containing 10 discs. These discs of different concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria and incubated at 37.8°C for 24 hours. Ciprofloxacin was used as a standard drug. Solvent and growth pedals were kept. The zone of inhibition and minimum inhibitory concentrations [MIC] was noted.



Scheme 1

Table 1: Physical data of 1-(substitutedsulfonyl)-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl} piperazine derivatives **(10a-j)**.

Comp	Molecular Formula	Structural formula	Elemental Analysis: Calculated % (Found %)				MP ^{0}C
			C %	H %	N %	S %	
10a	$C_{14}H_{15}F_3N_4O_4S$		43.46 (43.35)	3.08 (3.12)	11.42 (11.50)	6.54 (6.57)	185
10b	$C_{19}H_{15}Cl_2F_3N_4O_4S$		43.61 (43.58)	2.89 (2.91)	10.71 (10.75)	6.13 (6.18)	216
10c	$C_{19}H_{15}Cl_2F_3N_4O_4S$		43.61 (43.59)	2.89 (2.90)	10.71 (10.76)	6.13 (6.14)	217

10d	$C_{19}H_{15}Cl_2F_3N_4O_4S$		43.61 (43.60)	2.89 (2.91)	10.71 (10.78)	6.13 (6.19)	215
10e	$C_{20}H_{18}F_4N_4O_4S$		49.38 (49.35)	3.73 (3.72)	11.52 (11.50)	6.59 (6.57)	240
10f	$C_{19}H_{16}F_4N_4O_4S$		48.31 (48.33)	3.41 (3.44)	11.86 (11.88)	6.79 (6.80)	248
10g	$C_{21}H_{21}F_3N_4O_6S$		49.03 (49.07)	4.11 (4.15)	10.89 (10.91)	6.23 (6.26)	256
10h	$C_{17}H_{16}F_3N_5O_5S$		44.45 (44.49)	3.51 (3.55)	15.24 (15.26)	6.98 (6.95)	239
10i	$C_{16}H_{17}F_3N_4O_4S$		45.93 (45.95)	4.10 (4.13)	13.39 (13.42)	7.66 (7.69)	219
10j	$C_{16}H_{19}F_3N_4O_4S$		45.71 (45.75)	4.56 (4.59)	13.33 (13.36)	7.63 (7.67)	214

RESULTS AND DISCUSSION

Characterization of synthesized compounds was done by IR, ^{1}H NMR and ^{13}C NMR spectral data's. IR, ^{1}H NMR and ^{13}C NMR of compounds **10d, 10f, 10i** and **10g** are given in the Table 2.

In compound **10d,** IR stretching for carbonyl group was observed at 1576.7 cm^{-1} and two kinds of stretchings were observed for sulfonamide group at 1278.88 cm^{-1} and 1168.65 cm^{-1} corresponding to strong asymmetric and symmetric stretchings respectively.

In compound **10d**, the four protons of trifluromethoxy benzene resonates at 7.99-7.98(d, 1H, Ar-H); 7.90(s, 1H, Ar-H); 7.57-7.55(t, 1H, Ar-H) and 7.43-7.41 (d, 1H, Ar-H) ppm. Eight protons in Piperazine nucleus appear at 3.75-3.63(t, 4H, H_2C-N-CH_2) and 3.56-3.53 (t, 4H, H_2C-N-CH_2) ppm. The three proton

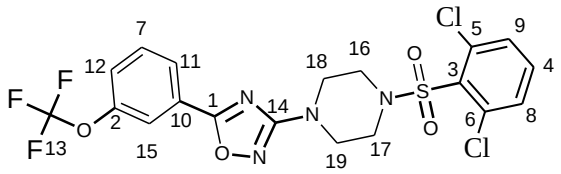
on 2,6-dichloro benzene attached to the sulfonyl group appears at 7.49-7.47 (d, 2H, Ar-H) and 7.36-7.32 (t, 1H, Ar-H) ppm. ^{1}H NMR splitting patron confirms the proton count and the predicted structure for the compound.

^{13}C NMR spectrum of the compound **10d** accounts for the five ipso carbon atoms C_1, C_2, C_3, C_{10} and C_{14} signals at 173.31, 170.23, 149.50, 130.66 and 120.36 ppm respectively. Nine aromatic carbon atoms C_4, C_5, C_6, C_7, C_8, C_9, C_{11}, C_{12} and C_{15} appears at 135.69, 134.61, 134.61, 132.68, 131.75, 131.75, 126.19, 124.89 and 119.01 ppm respectively. C_{13} Carbon atom present in the trifluromethoxy group appears at 121.61 ppm and four carbon atoms C_{16}, C_{17}, C_{18} and C_{19} of the piperazine nucleus appears at 45.99, 45.61, 44.89 and 42.58 respectively, confirming the predicted carbon skeleton.

Table 2: Spectral Interpretations of **10d, 10f, 10i** and **10g**

Compounds	IR	^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm	^{13}C NMR (100 MHz, $CDCl_3$) δ/ppm	LC-MS (M+1)
10d	C=O: 1576.47 cm^{-1} S=O: 1278.88 cm^{-1} (Asymmetric), 1168.65 cm-1 (Symmetric)	7.99-7.98 (d, 1H, Ar-H); 7. 90 (s, 1H, Ar-H); 7.57-7.55 (t, 1H, Ar-H); 7.49-7.47 (d, 2H, Ar-H); 7.43-7.41 (d, 1H, Ar-h); 7.36-7.32 (t, 1H, Ar-H); 3.75-3.63 (t, 4H, H_2C-N-CH_2); 3.56-3.53 (t, 4H, H_2C-N-CH_2)	173.31, 170.23, 149.50, 135.69, 134.61, 132.68, 131.75, 130.66, 127.19, 126.19, 124.89, 121.61, 120.36, 119.04, 45.99, 45.61, 44.89, 42.58	524.31
10f	C=O: 1578.45 cm^{-1} S=O: 1300.75 cm^{-1} Asymmetric), 1165.76 cm-1 (Symmetric)	7.97-7.95 (d, 1H, Ar-H); 7.88-7.58 (s, 1H, Ar-H); 7.56-754 (m, 4H, Ar-H); 7.43-7.40 (d, 1H, Ar-H); 7.34-7.29 (t, 1H, Ar-H); 3.68-3.65 (t, 4H, H_2C-N-CH_2); 3.18-3.16 (t, 4H, H_2C-N-CH_2)	173.36, 170.04, 163.76, 161.25, 149.51, 137.72, 131.10, 130.66, 126.11, 124.94, 123.45, 121.62, 120.47, 119.05, 115.15, 45.49, 45.19, 44.89, 42.58	473.41
10i	C=O: 1579.45 cm^{-1} S=O: 1285.32 cm^{-1} (Asymmetric), 1146.47 cm^{-1} (Symmetric)	8.02-8.00 (d, 1H, Ar-H); 7.99-7.92 (s, 1H, Ar-H); 7.58-7.54 (t, 1H, Ar-H); 7.44-7.42 (d, 1H, Ar-H); 3.68-3.66 (t, 4H, H_2C-N-CH_2); 3.44-3.42 (t, 4H, H_2C-N-CH_2); 2.31-2.25 (m, 1H, CH); 1.22-1.20 (m, 2H, CH_2): 1.04-0.99 (m, 2H, CH_2)	173.36, 170.21, 149.53, 130.62, 126.22, 124.93, 121.62, 120.39, 119.06, 45.90, 45.80, 45.31, 45.21, 25.67, 4.37, 4.35	419.32
10j	C=O: 1575.45 cm^{-1} S=O: 1267.00 cm^{-1} (Asymmetric), 1163.83 cm^{-1} (Symmetric)	8.01-7.99 (d, 1H, Ar-H); 7.92 (s, 1H, Ar-H); 7.58-7.54 (t, 1H, Ar-H); 7.44-7.42 (d, 1H, Ar-H); 3.68-3.66 (t, 4H, H_2C-N-CH_2); 3.50-3.43 (t, 4H, H_2C-N-CH_2); 3.27-3.19 (m, 1H, CH); 1.38-1.20 (d, 6H, CH_3)	173.30, 170.35, 149.50, 130.66, 126.25, 126.14, 124.90, 124.81, 120.39, 53.49, 46.52, 45.89, 45.53, 45.31, 16.72, 16.70	421.02

*All the chemical shifts were reported in parts per million (ppm).



Compound 10d

Table 3: Antimicrobial activities of 1-(substitutedsulfonyl)-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl} piperazine derivatives. (MIC values µg/ml)

Compounds	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus aureus	***Bacillus cereus***	***Escherichia coli***	***Pseudomonas aeruginosa***
10a	6	8	9	7
10b	13	12	14	15
10c	15	13	14	12
10d	14	15	12	13
10e	23	19	18	22
10f	20	18	17	20
10g	21	15	13	23
10h	12	14	15	13
10i	9	-	6	-
10j	20	18	18	23
Ciprofloxacin	25	20	20	25

* No activity was observed up to 200µg/ml; dimethylsulfoxide (DMSO) is used as control.

From the Table 3, Compounds **10e, 10f, 10g, 10h** and **10j** were found to have comparable potency against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa* compared to standard drug Ciprofloxacin while some of them have less or no potency against tested strains.

APPLICATIONS

Antibacterial activity results of the sulfonamide derivatives bearing 1,4-disubstituted-1,2,4-oxadiazole indicates that these derivatives possess promising pharmacophore property and can be used against bacterial infections. Antifungal activity studies of these compounds are underway.

CONCLUSIONS

Our present study describes a convenient and efficient synthetic route to synthesize sulfonamide derivatives bearing 1,4-disustituted-1,2,4-oxadiazole moiety and we believe the procedure can be conveniently reproduced. Synthesized sulfonamide derivative bearing 1,4-disustituted-1,2,4-oxadiazolemoiety structures were supported by IR, ^{1}H NMR, ^{13}C NMR and LC-MS spectral data. Synthesized compounds were screened for their antibacterial activity against Gram-positive and Gram-negative bacterial strains. Thus the study was found very useful to identify antibacterial targets among the synthesized sulfonamide derivatives.

REFERENCES

[1] K. Shiva Prasad, L. Shiva Kumara, K.B. Vinaya, S. Chandra Shekarb, B. Jayalakshmic, H.D. Revanasiddappaa, *Int J Chem Res*, **2011**, *2(1)*, 1-6.

[2] N. Subhakara Reddy, A. Srinivas Raoa, M. Adharvana Chari, V. Ravi Kumara, V. Jyothyc, V. Himabindu, *J. Chem. Sci*, **2012**, *124(3)*, 723–730.
[3] Chester M. Himel, Wissam G. Aboul-Saad, Solang Uk, *J. Agr. Food Chem*, **1971**, *19(6)*, 1175-1180.
[4] Ahmed Hanafy, Jun Uno, Hiroki Mitani, Yingqian Kang, Yuzuru Mikami, *Jpn. J. Med. Mycol*, **2007**, *48(1)*,47-50.
[5] Zhao Yan-fang, Feng Run-liang, Liu Ya-jing, Zhang Yi-kun, Gong Ping, *Chem. Res. Chinese Universities*, **2010**, *26(2)*, 272—277.
[6] S. Guniz Kuçukguzel, Inci Coskun, Sevil Aydın, Goknur Aktay, Sule Gursoy, Ozge Cevik, Ozlem Bingol Ozakpınar, Derya Ozsavc, Azize Sener, Neerja Kaushik-Basu, Amartya Basu, Tanaji T. Talele, *Molecules*, **2013**, *18*, 3595-3614.
[7] Mostafa M. Ghorab, Fatma A. Ragab, Helmy I. Heiba, Hebaallah M. Agha, *J. Basic. Appl .Chem*, **2011**, *1(2)*, 8-14.
[8] Mansour S. Al-Said, Mostafa M. Ghorab, Mohammed S. Al-Dosari, Mostafa M. Hamed, *Eur. J. Med. Chem*, **2011,** *46*, 201-207.
[9] Kamlesh Kumar Sahu, Veerasamy Ravichandran, Vishnu Kant Mourya, Ram Kishore Agrawal, *Med. Chem. Res*, **2007**, *15*, 418–430
[10] P.J. Vora, A.G. Mehta, Russ. *IOSR. J. Appl. Chem*, **2012**, *1(4)*, 34-39.
[11] R. Iqbal, M. Zareef, S. Ahmed, J.H. Zaidi, M. Arfan, M. Shafiqueb, N.A. Al-Masoudic, *J. Chin. Chem. Soc.* **2006**, *53*, 689-696
[12] G.C. Ramaprasad[c], B. Kalluraya, B. Sunil Kumar, Sahana Mallya, *Int J Pharm Pharm Sci*, **2012**, *4(4)*, 210-213
[13] Weiming Xu, Jiang He, Ming He, Feifei Han, Xuehai Chen, Zhaoxi Pan, Jian Wang, Maoguo Tong, *Molecules*, **2011**, *16*, 9129-9141.
[14] Wei-Ming Xu, Fei-Fei Han, Ming He, De-Yu Hu, Jiang He, Song Yang, Bao-An Song, *J. Agric.Food Chem.* **2012**, *60*, 1036−1041.
[15] Jesus M. Ontoria, Sergio Altamura, Annalise Di Marco, Federica Ferrigno, Ralph Laufer, Ester Muraglia, Maria Cecilia Palumbi, Michael Rowley, Rita Scarpelli, Carsten Schultz-Fademrecht, Sergio Serafini, Christian Steinkuhler, and Philip Jones, *J. Med. Chem*, **2009**, *52*, 6782–6789.
[16] Vasant B. Jagrut, Prashant D. Netankar, Dhanaji V. Jawale, Ramrao A. Mane, Wamanrao N. Jadhav, *Bull. Korean Chem. Soc*, **2009**, *30(11)*, 2812-2814.
[17] V. Sumangalaa, Boja Poojarya, N. Chidanandaa, T. Arulmoli, Shalini Shenoy, *J. Chem. Pharm. Res*, **2012,** *4(3)*, 1661-1669.
[18] Material Safety Data Sheet, *Sigma Aldrich*, Product number: *343536*.
[19] W. Droge, *Physiol. Rev.* **2002**, *82*, 47–95.