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Synthesis, Characterization and Biological evaluation of novel substituted acid amides containing piperazine 1,2,4-Oxadiazole nucleus

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ABSTRACT

Some new (substituted)(4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazin-1-yl)methanone has been synthesized using piperazine containing 1,2,4-oxadiazole as core moiety with substituted acids. The newly synthesized compounds were characterized by spectroscopic evidences such as IR, ^{1}H NMR, ^{13}C NMR and CHN elemental analysis. All the synthesized compounds were screened for their in vitro antibacterial activity, compounds 8a, 8c, 8d, 8f, 8g and 8i were shown good activity.

Keywords: Cyanogen bromide,1,1-carbonyldiimidazole, trifluoromethoxy benzoicacid , 3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, hydroxybenzotriazole, 1,2,4-oxadiazole and Antibacterial activity.

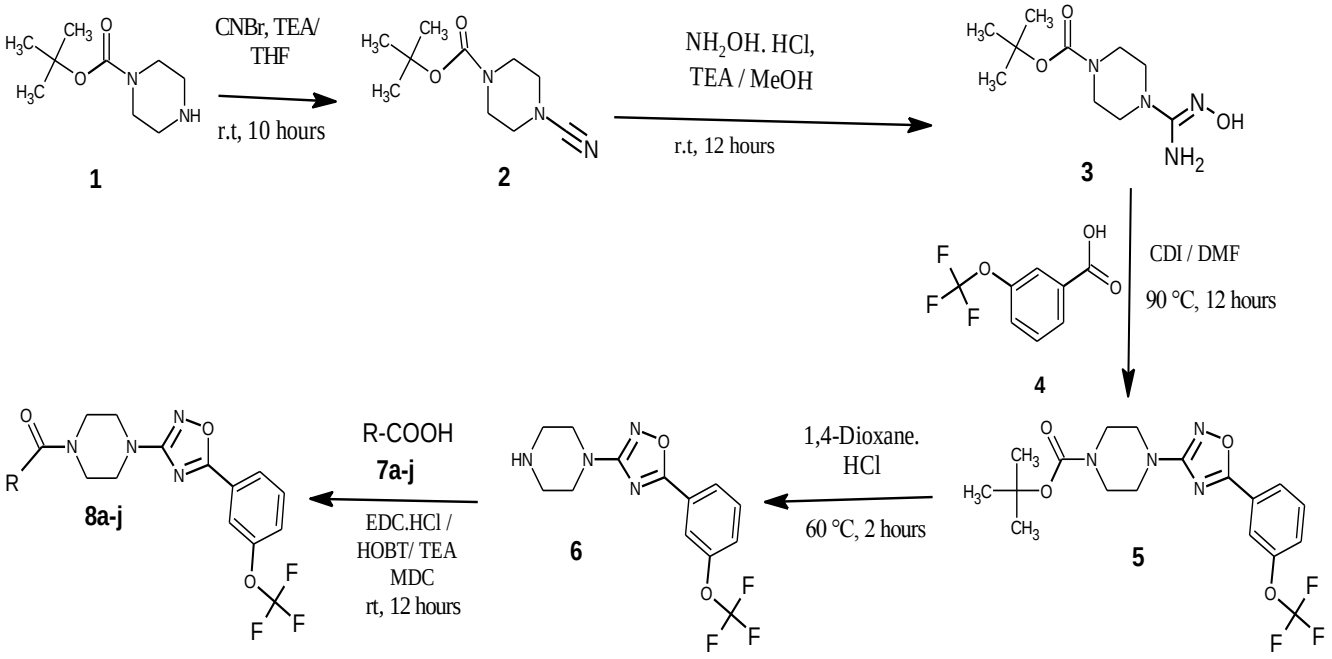
INTRODUCTION

Acid amides having aryl or alkyl groups exhibits different biological activities such as anti-bacterial [1], potent and selective ZAP-70 SH2 inhibitors [2], anti-malarial [3], orally bioavailable cannabinoid receptor 2 (CB_2) agonists [4], dual vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 inhibitors [5], DNA gyrase inhibitor [6] and orally active nonpeptidic inhibitors of human neutrophil elastase [7]. Aromatic acid amides having electron-withdrawing group shows good biological activity such as anti-bacterial, antifungal and 5-HT_3R binding affinities [8].

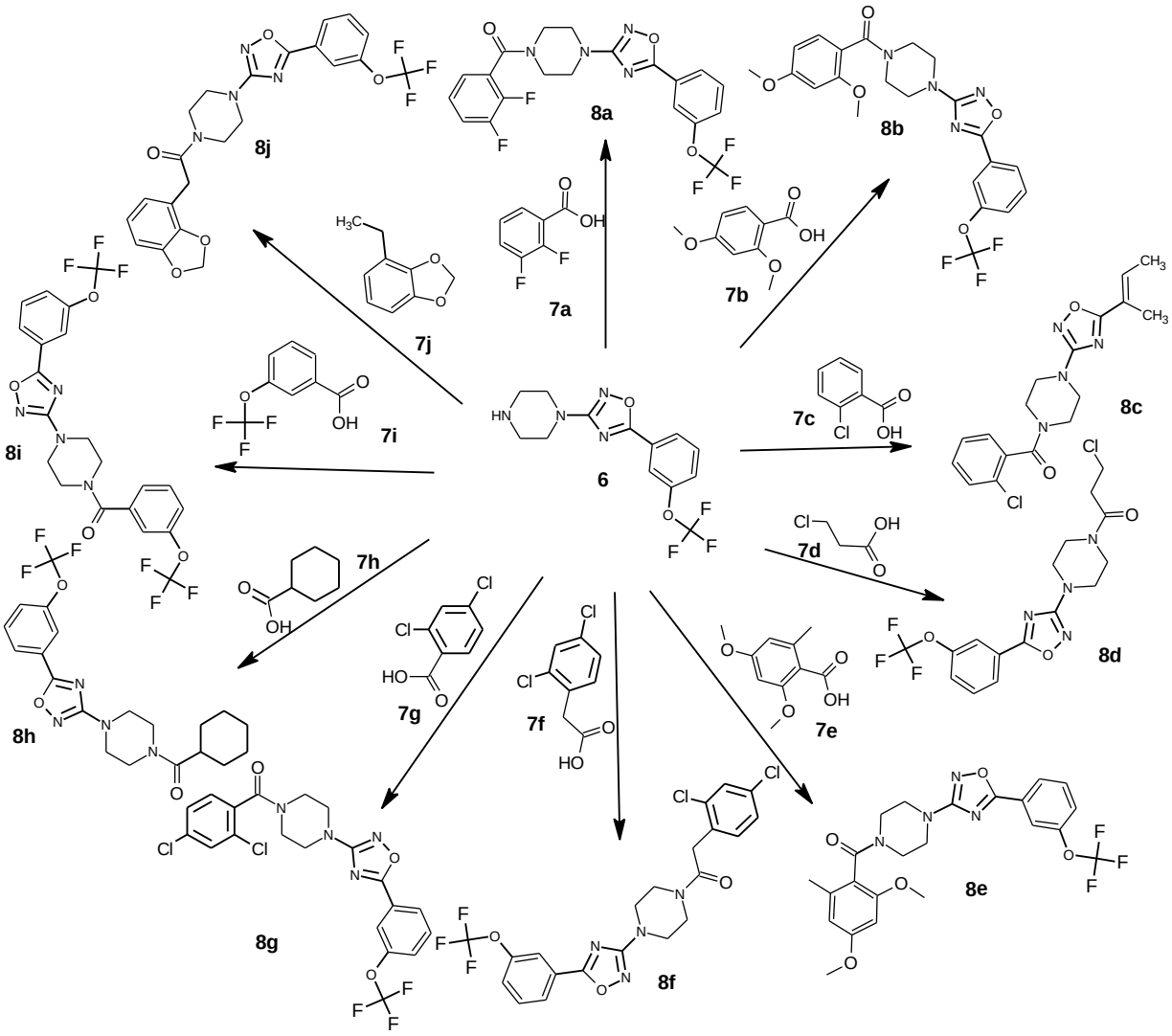
It has been reported that 1,2,4-oxadiazoles were synthesized by treating amidoxime with carboxylicacid derivatives. Amidoxime is obtained from the nitrile by addition of hydroxylamine hydrochloride, these amidoximes are O-acylated by different carboxylic acid derivatives [9-15]. Acid amide containing oxadiazole nucleus and their derivatives were evaluated for a variety of pharmacological activities such as antitubercular [16], antiallergic [17], anti-inflammatory[18], central nervous system depressant activity[19] and ulcergenic activity [20]. Prompted by the above data we planned to synthesize new series of acid amides bearing 1,2,4-oxadiazole nucleus and to evaluate their antimicrobial potential.

MATERIALS AND METHODS

Melting points reported were determined in open capillary and are uncorrected. The structures of the newly synthesized compound were established using IR, ^{1}H NMR, ^{13}C NMR and LC-MS data. FT-IR Spectra was recorded on Jasco FT-IR Spectrometer, ^{1}H NMR and ^{13}C NMR were recorded in DMSO-d_6 at 399.65 MHz and 100.50 MHz respectively. All the chemical shifts were reported in parts per million (ppm). LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micromass LCT mass detector. Elemental analysis (C,H and N) was performed on a Elementar vario MICRO cube. The purity of the compound was confirmed using TLC on precoated silica gel plate and further purification was done using column chromatography.



Scheme 1



Structures of the final compounds 8a-j

Procedure for the preparation of tert-butyl-4-cyanopiperazine-1-carboxylate (2): A mixture of tert-butyl piperazine-1-carboxylate **(1)** (0.13 moles, 25g), cyanogen bromide (0.13 moles, 14.11g) and TEA (0.39 moles, 39.39g) in 250 ml of THF were stirred for 10 hours at room temperature. The THF was removed by vacuum, the residue was dissolved in MDC, washed with water then organic layer was separated, further the organic layer washed with brine, dried over Na_2SO_4 and concentrated. The crude compound was obtained by triturating the concentrated mass with petroleum ether and diethyl ether. It was filtered and dried to get title compound as a white solid. The structure of the compound was confirmed by IR & NMR data as given below. IR: ν_{max} /cm^{-1}: 2228.34 (CN), 1698.2 (CO). ^{1}H-NMR ($CDCl_3$) δ ppm : 3.51-3.48 (t, 4H, H_2CNCH_2), 2.97-2.94 (t, 4H, H_2CNCH_2), 1.40 (s, 9H, $3CH_3$). MP: 107-108^0 C. Yield : 85%

Procedure for the preparation of tert-butyl-4-(N'-hydroxycarbamimidoyl)piperazine-1-carboxylate (3) : Compound **(2)** (0.11 moles, 25g) was dissolved in methanol, TEA (0.33 mloes, 33.33g) and $NH_2OH.HCl$ (0.29 moles, 20.58g) were added the reaction mixture was stirred for 10 hours at room temperature. The solvent was removed under reduced pressure, the residue was dissolved in MDC, washed with water. the organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to

obtained title compound **(3).** Compound **(3)** was crystallized using methanol. ^{1}H-NMR ($CDCl_3$) δ ppm : 9.0 (s, 1H, OH), 6.39 (s, 2H, NH_2) 1.92-1.81 (t, 4H, H_2CNCH_2), 1.75-1.70 (t, 4H, H_2CNCH_2), 1.39 (s, 9H, $3CH_3$). MP: 190-191^0 C. Yield: 88%

Procedure for the preparation of tert-butyl-4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine-1-carboxylate (5) : Trifluoromethoxybenzoic acid **(4)** (0.08 moles, 16.8g) and 1,1-carbonyldiimidazole (CDI) (0.128 moles, 20.75g) were taken in 200 ml DMF. The mixture was stirred for half an hour at 50^0 C in 500 ml round bottomed flask, consumption of acid was confirmed by TLC. To the reaction mixture compound (3) (0.08 moles, 20g) was added and the reaction mixture was heated at 110^0 C for 10 hours. DMF was distilled off and the residue was poured into cold water, the solid **(5)** thus obtained was collected by filtration. The crude product was purified by column chromatography using petroleum ether / ethyl acetate as eluent (7:3). ^{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, $(H_2C\text{-}N\text{-}CH_2)_2$), 1.49 (s, 9H, $3CH_3$). LCMS: 415.12 (M+1). MP: 211-213^0 C .Yield 42 %.

Procedure for the preparation of 1-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine (6): Deprotection of Boc group was achieved by using 1,3-Dioxane.HCl. A mixture of tert-butyl 4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine-1-carboxylate **(5)** (7.2 g) was taken in excess of 1,4-Dioxane.HCl (100 ml) and refluxed for 5 hours. 1,4-Dioxane.HCl was distilled off, to obtain the title compound **(6)**, this compound was characterized by ^{1}H NMR and LCMS. Further compound (6) was used in the next step 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_2CNCH_2), 3.30-3.29 (t, 4H, H_2CNCH_2). LCMS: 315.12 (M+1). MP: 191-193^0 C. Yield 88 %

General Procedure **for the preparation of substituted (4-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazin-1-yl)methanone (8a-j) :**
Equimolar quantities of 1-{5-[3-(trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine **(6)** (0.5g, 0.001 moles), different substituted acid **(7a-j)** (0.001 moles)**,** 3-(3-dimethylaminopropyl)carbodiimide hydrochloride **(**EDAC.HCl) (0.003 moles, 0.57g), hydroxybenzotriazole (HOBT) (0.00005 mmol) and triethlamine (0.3g, 0.003 mole) were stirred in dry MDC (6 ml) under nitrogen at room teperature for 12 hours. The reaction mixture was washed with 10% $NaHCO_3$, the organic phase was washed with water and brine, then dried over Na_2SO_4, and evaporated. Residue was purified by neutral alumina column chromatography using MDC / MeOH as a eluent (9:1) to get acid amides containing piperazine oxadiazole nucleus **(8a-j)** in good yield. Physical data of all the final compounds are entered in table 1**.**

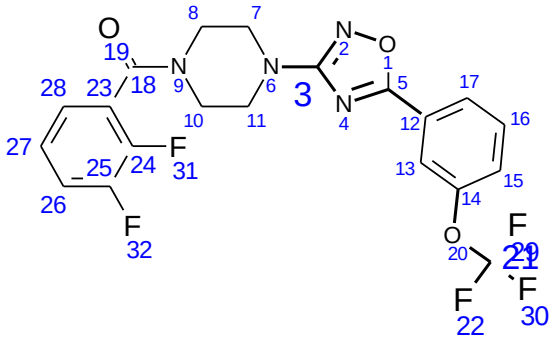
RESULTS AND DISCUSSION

Initially tert-butyl-4-cyanopiperazine-1-carboxylate **(2)** was prepared from tert-butyl piperazine-1-carboxylate **(1)** in the presence of triethylamine and cyanogen bromide. This compound **(2)** was treated with hydroxylamine hydrochloride to afford tert-butyl 4-(*N*'-hydroxycarbamimidoyl)piperazine-1-carboxylate **(3)** which was further reacted with trifluoromethoxybenzoic acid (4) to yield oxadiazole **(5)**. Further deprotection of Boc group gave the 1-{5-[3-trifluoromethoxy)phenyl]-1,2,4-oxadiazol-3-yl}piperazine **(6)**. The unprotected secondary amino group of (**6)** was made to react with different substituted carboxylic aids **(7a-j)** in the presence of triethylamine, EDAC.HCl and HOBT to get new acid amides containing oxadiazole nucleus **(8a-j)** in good yield. The newly synthesized compounds were tested for their antimicrobial activity.

The IR spectrum of a representative compound **8** shows C=O absorption and CF_3 vibration stretching in the range 1650 cm^{-1} and 1301-1165 cm^{-1} respectively. Aromatic C-H band displays at 3077-3018 cm^{-1}. A weak band at 1576-1555 represents C=C bond of the aromatic ring.

In the ^{1}H NMR spectrum of a representative compound **8a** seven protons of the difluorobenzene and trifluoromethoxybenzene appears in the range 8.02-7.42 ppm. Aromatic 3H atoms on difluorobenzene appears as a multiplet in the range 7.30-7.15 ppm, other aromatic 4H atoms on trifluoromethoxybenzene appears at 8.02-8.00 (d, 1H), 7.92 (s, 1H), 7.59-7.58 (t, 1H), 7.44-7.42 (d, 1H),. Eight protons of piperazine methylene groups (CH_2) appears in the range 3.76-3.68 (t, 4H) and 3.56-3.54 (t, 4H). These evidences confirms the formation of the compounds.

The ^{13}C NMR spectrum of a representative compound **8a** is discussed bellow.



Aromatic carbons of difluorobenzene and trifluoeomethoxybenzene appears in the range of 118-130 ppm and ipso carbons C_5, C_3, C_{14} & C_{25} appears at 170.33, 151.58, 149.48 and 145.23 ppm respectively. The carbonyl (C=O) carbon appears at 164.01 ppm and aliphatic carbon of piperazine ring C_7, C_8, C_{10} and C_{11} appears at 41-46 ppm. All these evidences confirms the assigned structure for the compound.

Table 1; Physical data of final molecules 8a-j

Compound	MP (^{0}C)	Yield (%)	Molecular formula	Cal. (found) %		
				C	H	N
8a	145	55	$C_{20}H_{15}F_5N_4O_3$	52.87 (52.82)	3.33 (3.30)	12.33 (12.31)
8b	138	62	$C_{22}H_{21}F_3N_4O_5$	55.23 (55.19)	4.42 (4.43)	11.71 (11.70)
8c	187	61	$C_{20}H_{16}ClF_3N_4O_3$	53.05 (53.00)	3.56 (3.50)	12.37 (12.30)
8d	197	76	$C_{16}H_{16}ClF_3N_4O_3$	47.48 (47.46)	3.98 (3.91)	13.84 (13.79)
8e	178	52	$C_{23}H_{23}F_3N_4O_5$	56.10 (59.98)	4.71 (4.62)	11.38 (11.30)
8f	158	68	$C_{21}H_{17}Cl_2F_3N_4O_3$	50.32 (50.01)	3.42 (3.32)	11.38 (11.29)
8g	132	71	$C_{20}H_{15}Cl_2F_3N_4O_3$	49.30 (48.90)	3.10 (2.91)	11.50 (11.40)
8h	156	58	$C_{20}H_{23}F_3N_4O_3$	56.6 (56.1)	5.46 (5.44)	13.20 (13.00)
8i	143	57	$C_{21}H_{16}F_6N_4O_4$	50.21 (50.10)	3.21 (3.16)	11.15 (11.06)
8j	167	64	$C_{22}H_{19}F_3N_4O_5$	55.46 (55.39)	4.02 (4.00)	11.76 (11.66)

Antibacterial activity: The newly synthesized compounds **8a-j** was screened for their antibacterial activity. Different concentrations of test compounds were prepared using DMSO and were tested against *S. aureus, S. citreus, B. polymyx and B. cereus* bacterial stains by disc diffusion method [21] using ciprofloxacin as standard. The discs with 6.0 mm in diameter were prepared using filter paper. Discs were kept in screw capped bottle and sterilized at 140 ^{0}C for 1 h. Discs for the experiment were prepared by taking twice the amount of test compounds solution required for each disc was added to the bottle

containing discs. Discs with different concentration of test compound were placed on the nutrient agar media in two sets on fresh bacteria seeded on agar media and incubated for 12 h at 35 ^{o}C .

Table 2 : IR, ^{1}H NMR, ^{13}C NMR of the final compounds 8a,8e,8i and 8j

Comp	IR	^{1}H NMR	^{13}C NMR
8a	IR (KBr,cm-1) : 1650 (C=O), 1301-1165 (CF_3 streching).	^{1}H NMR ; $CDCl_3$ (ppm): 8.02-8.00 (d, 1H, Ar-H); 7.92 (s, 1H, Ar-H); 7.59-7.58 (t, 1H, Ar-H); 7.44-7.42 (d, 1H, Ar-H); 7.30-7.15 (m, 3H, Ar-H); 3.76-3.68 (t, 4H, H_2CNCH_2); 3.56-3.54 (t, 4H, H_2CNCH_2).	^{13}C NMR; $CDCl_3$ (ppm): 173.33, 170.33, 164.01, 151.58, 149.48, 147.72, 145.23, 130.66, 126.19, 125.80, 124.92, 123.81, 121.60, 119.03, 118.64, 46.27, 45.88, 42.57, 41.39
8e	IR (KBr,cm-1) : 1680 (C=O), 1293-1264 (CF_3 streching).	^{1}H NMR ; $CDCl_3$ (ppm): 8.02-8.00 (d, 1H, Ar-H); 7.93 (s, 1H, Ar-H); 7.59-7.56 (t, 1H, Ar-H); 7.45-7.43 (d, 1H, Ar-H); 6.32(s,6H, OCH_3); 3.73-3.76 (t, 4H, H_2CNCH_2); 3.65-3.64 (t, 4H, H_2CNCH_2); 2.32 (s, 3H, CH3).	^{13}C NMR; CDCl3 (ppm): 173.46, 170.15, 167.72, 161.54, 158.38, 155.83, 149.48, 138.52, 130.72, 126.11, 125.00, 120.37, 117.71, 115.63, 106.68, 96.14, 55.83, 55.29, 46.06, 45.06, 42.56, 41.53, 20.03.
8i	IR (KBr,cm-1) : 1634 (C=O), 1252-1166 (CF_3 streching).	^{1}H NMR ; $CDCl_3$ (ppm): 8.02-8.00 (d, 1H, Ar-H); 7.92 (s, 1H, Ar-H); 7.58-7.30 (m, 6H, Ar-H); 3.93-3.88 (t, 4H, H_2CNCH_2); 3.55-3.50 (t, 4H, H_2CNCH_2).	^{13}C NMR; $CDCl_3$ (ppm): 173.33, 170.35, 168.86, 149.48, 149.20, 137.11, 130.66, 130.44, 126.18, 126.12, 125.40, 124.93, 122.42, 121.61, 120.37, 119.78, 119.04, 46.90, 46.90, 41.58, 41.49.
8j	IR (KBr,cm-1) : 1635 (C=O), 1280-1160 (CF_3 streching).	^{1}H NMR ; $CDCl_3$ (ppm): 8.02-7.99 (d, 1H, Ar-H); 7.91 (s, 1H, Ar-H); 7.57-7.55 (t, 1H, Ar-H); 7.43-7.41 (d, 1H, Ar-H); 6.77-6.75 (d, 2H, Ar-H); 6.70-6.68 (t, 1H, Ar-H); 5.95 (s, 2H, O-CH_2-O); 3.78-3.77 (t, 4H, H_2CNCH_2); 3.69 (s, 2H, CH_2); 3.59-3.51 (t, 4H, H_2CNCH_2).	^{13}C NMR; $CDCl_3$ (ppm): 173.19, 170.34, 169.68, 149.48, 147.97, 146.54, 130.62, 128.23, 126.19, 126.09, 124.85, 121.51, 120.33, 119.01, 108.92, 108.44, 101.03, 45.91, 45.79, 45.25, 40.99, 40.65.

The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug which resulted in inhibition of bacterial growth. Out all the synthesised compounds some of the compounds showed good antibacterial activities.

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antibacterial activity, which are promising as active pharmacophore. Further studies are undergoing.

CONCLUSIONS

In the present research we synthesized some novel acid amides containing piperazine 1,2,4-oxadiazole nucleus compounds and they are initially screened for their antimicrobial activities. compounds **8a, 8c, 8d, 8f, 8g** and **8i** shown good activity against the tested bacteria which may be due to the presence of halogens in the acid amides with 1,2,4-oxadiazole ring system.

Table-3. Antibacterial activities of acid amides containing oxadiazzole derivatives (8a-j)

Compound	*S. aureus*	*S. citreus*	*B. polymyxa*	*B. cereus*
8a	27	24	25	20
8b	08	04	06	10
8c	26	27	24	24
8d	26	27	24	14
8e	02	02	02	02
8f	24	26	24	23
8g	26	24	23	20
8h	08	04	08	04
8i	27	25	23	24
8j	11	12	02	04
CIPX	28	27	24	24

CIPX = Ciprofloxacin is used as a positive control, and the zone of inhibition is expressed in mm.

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