Synthesis, Characterization and Biological evaluation of novel Sulphanamides containing 1,2,4-Oxadiazole Nucleus

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Abstract *Some new amides containing oxadiazole derivatives have been synthesized taking N-Boc piperazine as starting material. These were converted to N-cyano-4-Boc piperazine by nucleophilic substitution reaction. The substituted product was treated with hydroxyl amine hydrochloride in basic condition to give hydroxycarbamimide and cyclized with 3-fluorobenzoic acid to yield N-protected piperazine 1,2,4-oxadiazole. Deprotection of secondary amine was done using trifluoro acetic acid, free secondary amine obtained was condensed with different substituted sulphonyl chlorides in the presence of triethyl amine to yield sulphanamides of piperazine containing 1,2,4-oxadiazole nucleus. The newly synthesized compounds were characterized by spectroscopic studies such as IR, ^{1}H NMR, ^{13}C NMR, LCMS and CHN elemental analysis. All the synthesized compounds were screened for their in vitro antibacterial activity, anthelmintic activity. Some of the compounds showed good biological activity.*

Keywords *Cyanogen bromide, 1,1-carbonyldiimidazole, 3-fluorobenzoicacid, 1,2,4-oxadiazole and antibacterial activity, anthelmintic activity*

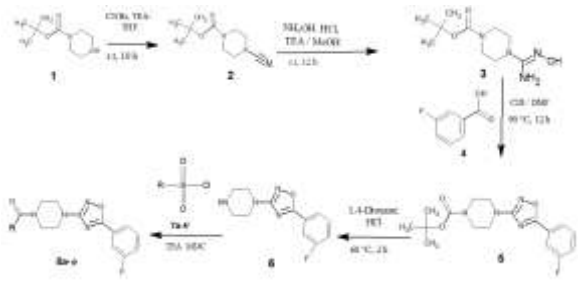
I. INTRODUCTION

Amides having aryl or alkyl groups exhibit different pharmacological properties such as antibacterial [1], antifungal, antiviral [2], anti-malarial and anthelmintic activities [3]. Some of the condensed amides are Potent and selective ZAP-70 SH2 inhibitors [4] and also showed activities as bioavailable cannabinoid receptor 2 (CB_2) agonists [5]. It has been observed that acid amides act as both vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 inhibitors [6], DNA gyrase inhibitor [7]. Aromatic acid amides attached to electron-withdrawing group show good biological activities such as antibacterial, antifungal and 5-HT_3R binding affinities [8].

It has been reported that 1,2,4-oxadiazoles were synthesized by treating amidoxime with carboxylic acid derivatives. Amidoxime is obtained from nitrile by the addition of hydroxylamine hydrochloride and 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]. In view of these pharmacological positive results in literature survey, we planned to synthesize new series of amides bearing 1,2,4-oxadiazole nucleus and evaluated their antimicrobial and anthelmintic potential.

II. MATERIALS AND METHODS

Melting points reported were determined in open capillary and are uncorrected. The structures of the newly synthesized compounds 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 or cdcl$_3$ depends on solubility at 399.65 mhz and 100.50 mhz respectively on Bruker model avance II. 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 by TLC on precoated silica gel plate and further purification was done using column chromatography.



Scheme - 1

Scaffold 6 was synthesized by well known procedure reported by Pushpa Iyengar et al[21]

A. Procedure for the preparation of tert-butyl-4-cyanopiperazine-1-carboxylate (2):

A mixture of tert-butyl piperazine-1-carboxylate (1) (0.13 mol, 25.00 g), cyanogen bromide (0.13 mol, 14.11 g) and TEA (0.39 mol, 39.39 g) were taken in 250 mL THF, contents were stirred for 10 h at room temperature under nitrogen atmosphere. Reaction was monitored by TLC, then THF was removed under vacuum, the residue was dissolved in MDC, washed with water and 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. Structure of the compound was confirmed by IR & ^{1}H-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 (t, 4H, H_2CNCH_2), 2.97 (t, 4H, H_2CNCH_2), 1.40 (s, 9H, $3CH_3$). MP: 107-108 ^{o} C. Yield: 81%

B. Procedure for the preparation of tert-butyl-4-(N'-hydroxycarbamimidoyl)piperazine-1-carboxylate (3):

Compound (2) (0.11 mol, 25 g) was dissolved in methanol. To this TEA (0.33 mol, 33.33 g) and $NH_2OH.HCl$ (0.29 mol, 20.58 g) were added, the reaction mixture was stirred for 12 h at room temperature under nitrogen atmosphere. Completion of reaction was confirmed by TLC, solvent was removed under reduced pressure, residue was dissolved in MDC and 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 (t, 4H, H_2CNCH_2), 1.75 (t, 4H, H_2CNCH_2), 1.39 (s, 9H, $3CH_3$). MP: 190-191^0 C. Yield: 86%

C. Procedure for the preparation of tert-butyl 4-[5-(3-fluorophenyl)-1,2,4-oxadiazol-3-yl]piperazine-1-carboxylate (5) :

3-Fluorobenzoic acid (4) (0.08 mol, 16.8 g) and 1,1-carbonyldiimidazole (CDI) (0.128 mol, 20.75 g) were taken in 200 mL DMF. The reaction mixture was stirred for half an hour at 50 ^{o}C in 500 mL round bottomed flask, consumption of acid was confirmed by TLC. To this mixture, compound (3) (0.08 mol, 20 g) was added and the reaction mixture was stirred at 90 ^{o}C for 12 h. Reaction was monitored by TLC, DMF was distilled off and the residue was poured into vigorously stirred ice-cold water, the solid (5) thus obtained was collected by filtration. 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 (d, 1H, Ar-H), 7.78 (s, 1H, Ar-H) 7.51 (t, 1H, Ar-H), 7.29 (d, 1H, Ar-H) 3.57 (t, 8H, $(H_2C\text{-}N\text{-}CH_2)_2$), 1.49 (s, 9H, $3CH_3$). LCMS: 349.3 (M+1). MP: 198-199^{o}C .Yield 32%.

D. Procedure for the preparation of 1-[5-(3-fluorophenyl)-1,2,4-oxadiazol-3-yl]piperazine (6):

Boc group deprotection of was carried out 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) (7.21 g) was taken in excess of 1,4-dioxane.HCl (100 mL) and heated at 50^{o}C for 2h. Solvent was distilled off, to give the title compound (6), formation of title compound was confirmed 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 (d, 1H, Ar-H), 7.92 (s, 1H, Ar-H) 7.59 (t, 1H, Ar-H), 7.46 (d, 1H, Ar-H) 3.86 (t, 4H, H_2CNCH_2), 3.30 (t, 4H, H_2CNCH_2). LCMS: 249.21 (M+1). MP: 182-183 ^{o}C. Yield 82%.

E. General procedure for the preparation of final compounds (8a-e):

Equimolar quantities of 1-[5-(3-fluorophenyl)-1,2,4-oxadiazol-3-yl] piperazine (6) (0.5 g, 0.001 mol), different substituted acids (7a-e) (0.001 mol), 3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) (0.57 g, 0.003 mol), hydroxybenzotriazole (HOBT) (0.010 g, 0.0005 mol) and triethylamine (0.3 g, 0.003 mol) were stirred in dry MDC (6 mL) under nitrogen atmosphere at room temperature for 12 h. Reaction mixture was washed with 10% $NaHCO_3$, organic phase was washed with water and brine, then dried over Na_2SO_4, and solvent was evaporated. Residue was purified by neutral alumina column chromatography using MDC/MeOH as eluent (9:1) to give novel amides containing piperazine oxadiazole nucleus (8a-e) in good yield. Physical data of all the final compounds are represented in Table 1 and IR, ^{1}H-NMR and ^{13}C NMR of few final compounds are tabulated in Table 2.

III. Results and Discussion

In the first step tert-butyl-4-cyanopiperazine-1-carboxylate (2) was prepared from tert-butyl piperazine-1-carboxylate (1) by nucleophilic substitution reaction in presence of triethylamine and cyanogen bromide.Compound (2) was treated with hydroxylamine hydrochloride to give tert-butyl 4-(*N*'-hydroxycarbamimidoyl) piperazine-1-carboxylate (3) which was further reacted with benzoic acid (4) to yield N-protected 1,2,4-oxadiazole (5). Further, deprotection of Boc group was carried out to give the 1-[5-(3-fluorophenyl)-1,2,4-oxadiazol-3-yl]piperazine(6). The unprotected secondary amino group of (6) was treated with different substituted sulphonyl chlorides (7a-e) in presence of triethylamine to get new sulphanamides containing oxadiazole nucleus (8a-e) in good yield.

The newly synthesized compounds were tested for antimicrobial and anthelmintic potential.

The IR spectrum of representative compound 8a shows C=O absorption and C-F vibration stretching in the range 1638 cm^{-1} and 1356-1284 cm^{-1} respectively. Aromatic C-H band displays at 2863 cm^{-1}. A weak band at 1442-1402 cm^{-1} 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 fluorobenzene appears in the range 7.87-7.15 ppm. Aromatic 2H appears as a doublet, remaining 5H appears as multiplet due to the fluorine interaction. Eight protons of piperazine methylene groups (CH_2) appear in the range 3.95-3.47 as triplet. These evidences confirm the formation of the compound.

The ^{13}C NMR spectrum of a representative compound 8a is discussed below. Aromatic carbons of difluorobenzene and fluorobenzene appear in the range of 118-130 ppm and four ipso carbons appear 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 appears at 41-46 ppm. All these data confirm the assigned structure of the compound.

TABLE – I
PHYSICAL DATA OF FINAL MOLECULES 8a-e

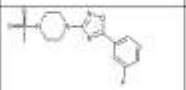
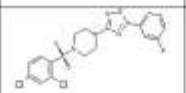
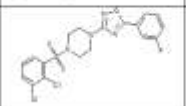
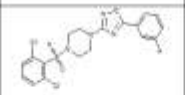
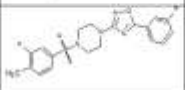
Compound	MP (°C)	Yield (%)	Structure	LCMS (M+1)	Cal. (found) %		
					C	H	N
8a	142	65		327.08	47.84 (47.81)	4.65 (4.62)	17.17 (17.15)
8b	188	52		458.51	47.28 (47.25)	3.31 (3.30)	12.25 (12.23)
8c	217	41		458.21	47.28 (47.22)	3.31 (3.29)	12.25 (12.22)
8d	209	68		458.27	47.28 (47.24)	3.31 (3.28)	12.25 (12.23)
8e	218	52		487.1	54.28 (54.27)	4.32 (4.30)	13.33 (13.32)

TABLE II
IR, ^{1}H NMR, ^{13}C NMR OF THE FINAL COMPOUNDS 8a-h

Comp	IR	^{1}H NMR	^{13}C NMR
8a	IR (KBr,cm-1) : 1640 (C=O), 1442 (C=C), 1358-1281 (C-F stretching)	^{1}H NMR ; $CDCl_3$ (ppm): 7.87 (d, 1H, Ar-H); 7.77 (d, 1H, Ar-H); 7.52-7.46 (m, 1H, Ar-H); 7.30-7.23 (m, 2H, Ar-H); 7.20-7.15 (m, 2H, Ar-H); 3.66 (t, 4H, H_2CNCH_2); 3.55 (t, 4H, H_2CNCH_2); 2.51(s, 3H, CH_3).	^{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, 21.39.
8b	IR (KBr,cm-1) : 1629 (C=O), 1441 (C=O), 1259-1202 (C-F stretching)	^{1}H NMR ; $CDCl_3$ (ppm): 7.79 (s, 1H, Ar-H); 7.66 (dd, 1H, Ar-H); 7.52 (t, 2H, Ar-H); 7.42 (d, 1H, Ar-H); 7.25 (d, 2H, Ar-H); 3.83 (t, 4H, H_2CNCH_2); 3.65 (t, 4H, H_2CNCH_2).	^{13}C NMR; CDCl3 (ppm): 173.46, 170.15, 167.72, 161.54, 158.58, 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.
8c	IR (KBr,cm-1) : 1613 (C=O), 1571 (C=O), 1297-1200 (C-F stretching)	^{1}H NMR ; $CDCl_3$ (ppm): 7.79 (s, 1H, Ar-H); 7.66 (dd, 1H, Ar-H); 7.52 (t, 2H, Ar-H); 7.42 (d, 1H, Ar-H); 7.25 (d, 2H, Ar-H); 3.83 (t, 4H, H_2CNCH_2); 3.65 (t, 4H, H_2CNCH_2).	^{13}C NMR; CDCl3 (ppm): 173.46, 170.15, 167.72, 161.54, 158.58, 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.
8d	IR (KBr,cm-1) : 1614 (C=O), 1574 (C=O), 1279-1251 (C-F stretching)	^{1}H NMR ; $CDCl_3$ (ppm): 7.79 (s, 1H, Ar-H); 7.66 (dd, 1H, Ar-H); 7.52 (t, 2H, Ar-H); 7.42 (d, 1H, Ar-H); 7.25 (d, 2H, Ar-H); 3.83 (t, 4H, H_2CNCH_2); 3.65 (t, 4H, H_2CNCH_2).	^{13}C NMR; CDCl3 (ppm): 173.46, 170.15, 167.72, 161.54, 158.58, 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
8e	IR (KBr,cm-1) : 1675 (C=O), 1574 (C=O), 1276-1231 (C-F stretching)	------	------

A. Antibacterial activity

The newly synthesized compounds **8a-e** were screened for their antibacterial activity. Different concentrations of test compounds were prepared using DMSO and tested against *S. aureus*, *S. citreus*, *B. polymyx and B. cereus* bacterial stains by disc diffusion method [22,23] using ciprofloxacin (5 µg/50µL) 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 1h. Disc for the experiment was prepared by taking twice the amount of test compounds solution required for each disc and added to bottle containing discs. Discs with different concentration of test compounds were placed on the nutrient agar media in two sets on fresh bacteria seeded on agar media and incubated for 12h at 35^{o}C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug which resulted in inhibition of bacterial growth. Out of all the synthesized compounds, some showed good antibacterial property. Results are tabulated in Table 3.

B. Anthelmintic Activity

Anthelmintic activity of 8a-e compounds was done by using *P. posthuma* (Indian Earthworm).Worms were maintained under normal vermicomposting medium with adequate supply of nourishment and water for about three weeks. Adult earthworms of approximately 4 cm in length and 0.2

- 0.3cm in width were chosen for the experiment. Different concentrations of 50 and 100 mg samples were evaluated as per the standard method reported [24]. Five groups of each with six earth worms were taken. Each *P. posthuma* was washed separately with normal saline before the initiation of experimental procedure and placed into a 20mL of normal saline. Group I earthworms were placed in 20mL saline in a clean petri plate and Group II earthworms were placed in 20mL saline containing standard drug piperazine citrate (50mg/mL). Similarly, Group III to X earthworms was placed in 20mL saline containing 50 and 100mg/mL of test samples respectively. Observation was done keeping time taken for paralysis and the time taken for death as objective and was documented in minutes. Paralysis time was analyzed based on behaviour of the worms with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body color and the results are illustrated in Table 4.

IV. Applications

In the present study, the derivatives which have been synthesized were screened for their antibacterial and anthelmintic activity, also promising as active pharmacophores.

V. Conclusions

In the present research, we synthesized some novel acid amides containing piperazine 1,2,4-oxadiazole nucleus compounds and are screened for their antimicrobial and anthelmintic activities. Compounds 8a, 8b and 8e shown good activity against the tested bacteria which may be due to the presence of halogens particularly fluorine present in the acid amides with 1, 2, 4-oxadiazole ring system.

TABLE-III
ANTIBACTERIAL ACTIVITIES OF AMIDES CONTAINING OXADIAZOLE DERIVATIVES (8a-e)

Compound	*S.aureus*	*S.citreus*	*B. polymyxa*	*B. cereus*
8a	03	22	21	22
8b	04	09	17	13
8c	05	13	12	15
8d	04	11	10	13
8e	10	24	24	22
CIPX	28	27	24	24

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

TABLE-IV
ANTHELIMINTIC ACTIVITY OF 8a-e AGAINST PHERETIMA POSTHUMA

Test Samples	*Concentration (mg/mL)*	*Time taken for paralysis(min)*	*Time taken for death(min)*
Control	–	142.33±0.49	167.17±0.87
Piperazine citrate	50	39.17±0.48**	57.00±0.58**
8a	100	37.00±1.59	57.50±0.76
	50	61.00±1.46**	106.00±0.86**
8b	100	45.00±0.82**	54.17±0.60**
	50	69.33±1.23**	96.17±0.60**
8c	100	39.50±0.76	49.67±0.71**
	50	72.83±0.95**	92.50±1.26**
8d	100	58.33±1.23**	88.00±0.58**
	50	88.17±0.60**	112.67±0.95**
8e	100	31.17±0.48	43.50±0.89
	50	62.17±0.48**	86.33±0.67**

Values are the mean±S.E.M of three earthworms. Symbols represent statistical significance *p<0.05, **p<0.01, ns: not significant as compared to control group.

Acknowledgment

The authors would like to thank Sapala organics Hyderabad & Siddaganga College of Pharmacy, Tumkur for spectral analysis and biological experiments, Department of Chemistry, Tumkur University, Tumkur for their valuable suggestions and constant encouragement.

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