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Original article

Synthesis of novel 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives and evaluation of their *in vivo* anticonvulsant activity

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ABSTRACT

A series of novel 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**) were synthesized and characterized by elemental analyses, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The newly synthesized compounds were screened for their anticonvulsant activity against maximal electroshock seizure (MES) model in male wistar rats and compared with the standard drug phenytoin. The neurotoxic effects were determined by rotorod test by using mice. Compounds **8d**, **8e**, **8f** and **8h** were found to be most potent of this series. The same compounds showed no neurotoxicity at the maximum dose administered (100 mg/kg). The efforts were also made to establish the structure activity relationships among synthesized compounds. The pharmacophore model was used to validate the anticonvulsant activity of the synthesized molecules.

1. Introduction

Epilepsy is a major neurological disorder affecting a large section of people both male and female throughout the world. Currently available drugs for the treatment of epilepsy are symptomatically effective in only 60–70% of patients. Epilepsy also poses a considerable economic burden on society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to treatment. The anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. Anticonvulsants are also increasingly being used in the treatment of bipolar disorder, since many seem to act as mood stabilizers. The goal of anticonvulsants is to suppress the rapid and excessive firing of neurons that start a seizure. An effective anticonvulsant would prevent the spread of the seizure within the brain and offer protection against possible excitotoxic effects that may result in brain damage. Some studies suggest that anticonvulsants themselves are linked to decreased performance of children at school [1]. Anticonvulsants are also called as antiepileptic (AEDs) and antiseizure drugs. The new AEDs and anticonvulsant agents have been reviewed during last few years [2]. The chemical diversity and various mechanisms of action of anticonvulsants make it difficult to find a common way of identifying new drugs. Novel anticonvulsant agents are discovered through conventional screening and/or structure modification rather than a mechanism driven design. Therefore, drug identification is usually conducted via *in vivo* screening tests on the basis of seizure type rather than etiology.

Five membered heterocyclic compounds show various types of biological activities. Among them, 1,3,4-oxadiazoles are important class of heterocyclic compounds with broad spectrum anticonvulsant activity [3,4]. The therapeutic importance of these compounds prompted us to develop selective molecules in which a substituent could be arranged in a pharmacophore pattern to display higher pharmacological activities. 2,5-Disubstituted-1,3,4-oxadiazole derivatives are known for anticonvulsant activity [5,6]. The choice of 1,3,4-oxadiazoles is due to its multi-applicability in the field of medicine. In the present study, some new 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**) have been synthesized and their anticonvulsant effects have been determined through maximal electroshock seizure (MES) test.

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2. Results and discussion

2.1. Chemistry

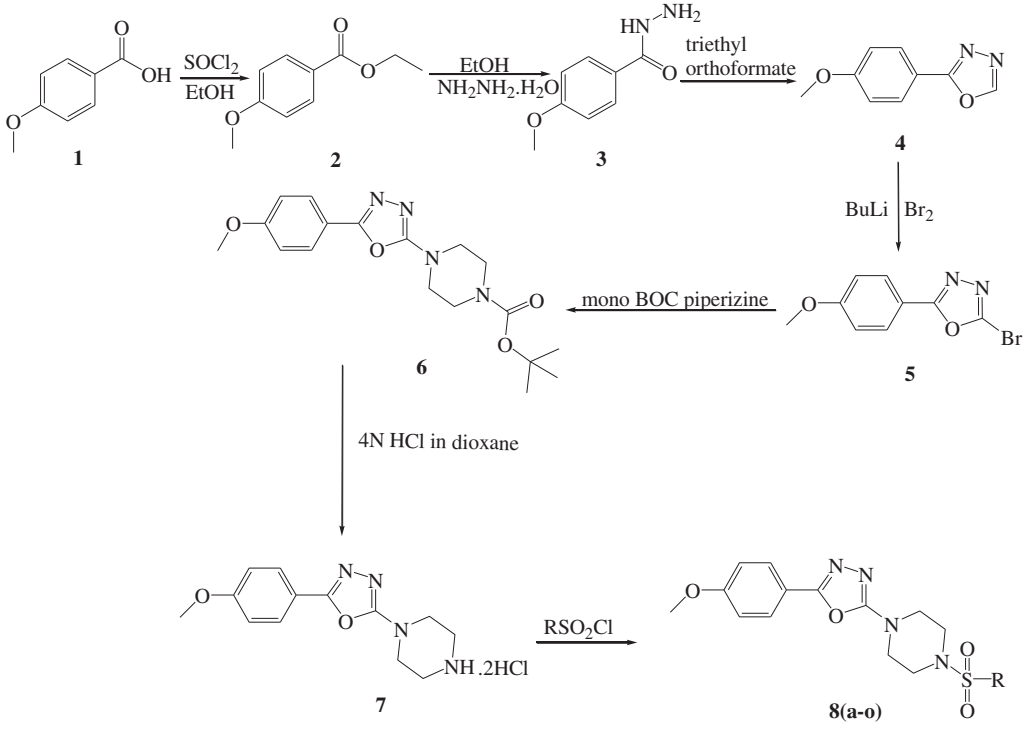
We herein report a versatile method for the synthesis of new 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**) (Scheme 1). This synthetic approach represents the most efficient route to a diverse array of 2,5-disubstituted heterocyclics reported in the literature [7–12]. The synthesis employs readily available starting materials and simple procedures making this method very attractive and convenient for the synthesis of various oxadiazoles. The formation of products was confirmed by recording their elemental analyses, ^{1}H NMR, ^{13}C NMR and mass spectra. The ^{1}H NMR spectra of **8d** and **8e** showed multiplet (piperazine ring) in the region of δ, 3.41–3.60 and 3.06–3.63, respectively. The ^{13}C NMR spectra present the correct number of carbon atoms at the appropriate chemical shift values. The mass spectra of **8d** showed molecular ion peak at *m*/*z* 514.4 which is in agreement with the molecular formula, $C_{20}H_{18}F_3N_5O_6S$. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ±0.4%. The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1.

2.2. Anticonvulsant activity

All the synthesized oxadiazole analogues were screened for their anticonvulsant potential through MES model using male wistar rats in the dose of 100 mg/kg. Antiepileptic drug research has for several decades focused on identifying new potential drugs based on their anticonvulsant activity against a single acute seizure induced by various stimulators, usually in mice and rats. All established antiepileptic drugs have anticonvulsant activity in at least MES model [13]. In the present study, the anticonvulsant activity of the fifteen newly synthesized 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**) was evaluated by MES induced seizure in rats at the dose of 100 mg/kg and the results are summarized in Table 2. Compounds **8d**, **8e**, **8f** and **8h** demonstrated significant protective effect on MES induced seizure, and the effect was similar to that of standard (phenytoin). Similarly, compounds **8a**, **8c**, **8j**, **8k**, **8n** and **8o** showed moderate protective effect and a significant difference in protectiveness were observed when compared to standard group. Compounds **8g**, **8b**, **8i**, **8m** and **8l** have relatively lower anticonvulsant potencies. All the compounds were examined for their neurotoxicity on mice using rotorod method given in the dose of 100 mg/kg. Except compounds **8g**, **8l** and **8m**, none of the compounds showed neurotoxicity. These compounds showed 25% toxicity compared to standard once at 2 h of oral administration (Table 3).

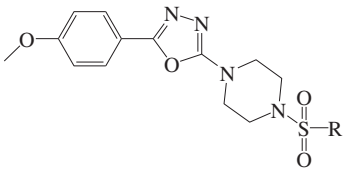
The initial structure activity relationship (SAR) can be drawn for the compounds **8**(**a**–**o**). As proposed by Pandeya and Raja [14], the active compound of the series meets all the requirements vital for anticonvulsant activity. On correlating the structures of the synthesized compounds with their anticonvulsant activity, it was observed that compounds bearing groups like nitro, phenoxy and halogens on phenyl ring possess high potency in MES. The SAR study of these compounds indicate that the introduction of a piperazine group at position 5 of 1,3,4-oxadiazole ring-; trifluoromethyl and nitro substituents at the para and ortho positions of the benzenesulfonyl moiety showed the best anticonvulsant activity in **8d**. Compound **8f** possessing a dichloro thiophene group in the sulfonyl group had good anticonvulsant activity in the MES model. Both compounds did not exhibit neurotoxicity at the highest administered dose. The 4-phenoxy benzene ring in **8e** resulted in increased anticonvulsant activity. The presence of constituents like OC_6H_5 at phenyl ring especially at para position showed higher potent activity. A similar study [15] showed good anticonvulsant property. The presence of isoxazole group in **8h** shows good anticonvulsant activity.

The presence of nitro group in **8j** and halogen groups in **8k**, **8a**, **8n** and **8o** showed moderate anticonvulsant activity. The presence of cyano group in **8c** at aryl ring has moderate activity, and



Scheme 1. Synthetic route of 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives.

Table 1
Chemical structure and melting range of 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**).



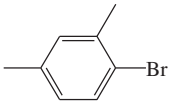
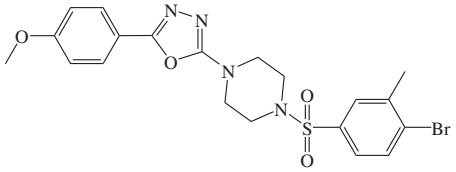
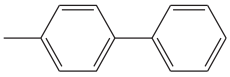
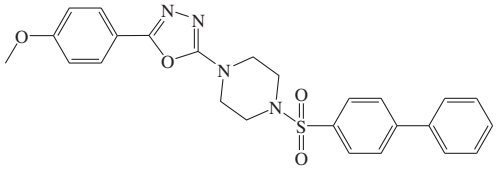
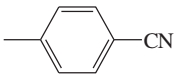
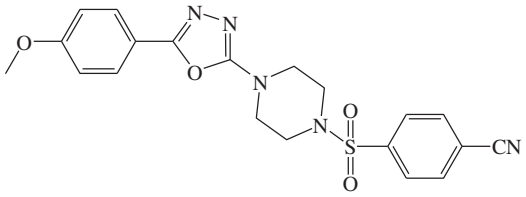
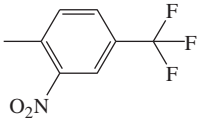
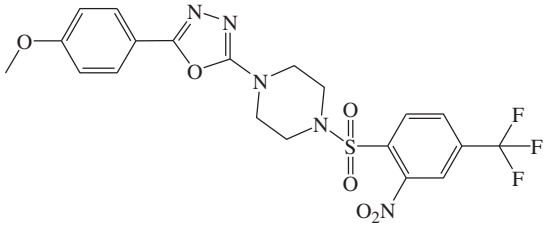
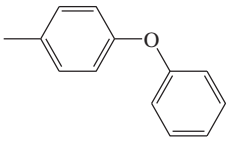
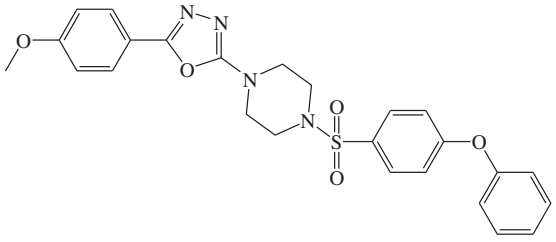
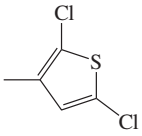
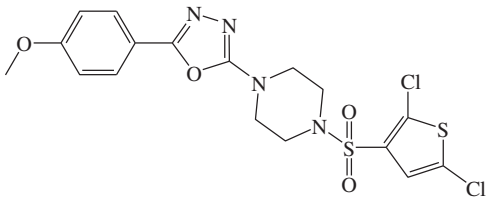
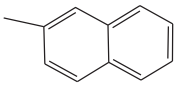
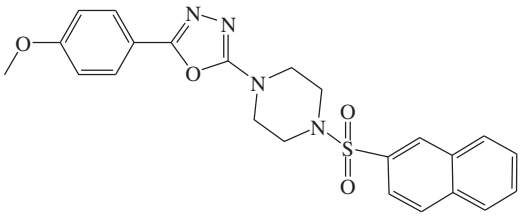
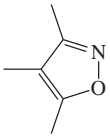
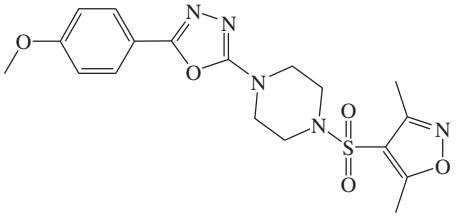
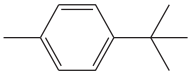
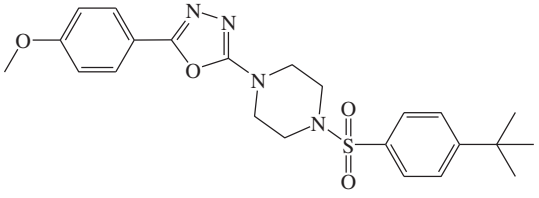
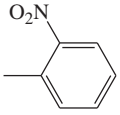
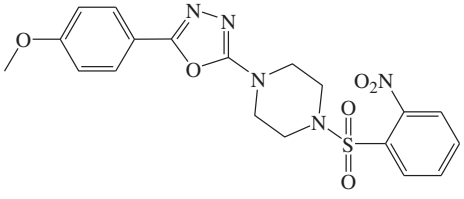
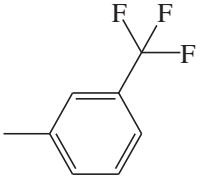
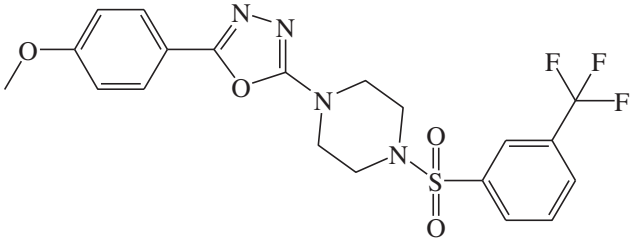
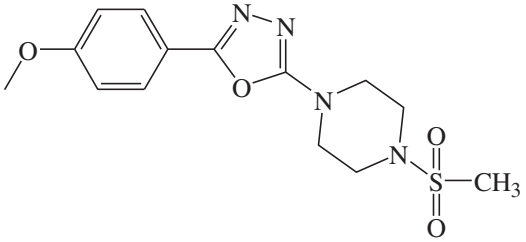
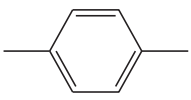
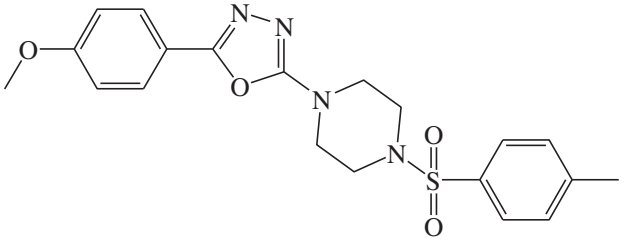
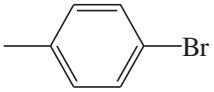
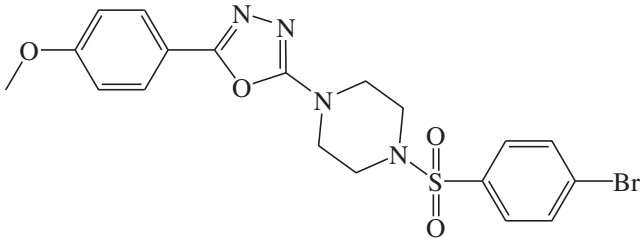
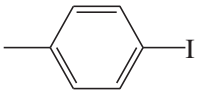
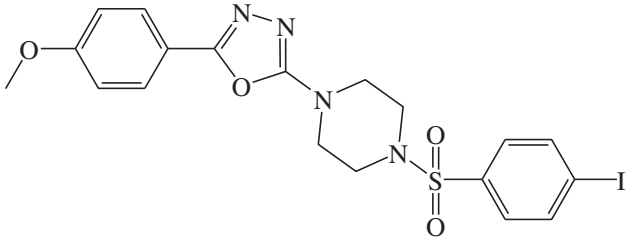
Compound	R	Structure
8a		
8b		
8c		
8d		
8e		
8f		

Table 1 (*continued*)

Compound	R	Structure
8g		
8h		
8i		
8j		
8k		
8l	—CH_3	
8m		

(*continued on next page*)

Table 1 (*continued*)

Compound	R	Structure
8n		
8o		

naphthalene group in **8g**, biphenyl in **8b** and tert-butyl in **8i** were weakly active in the MES test. Anticonvulsant activity has increased considerably when methyl group in **8l** was replaced by tolyl variation **8m**. The presence of electron releasing methyl group in benzene ring in **8m** has more anticonvulsant activity as compared to methyl group alone in **8l**. The presence of sulfonyl linker is important for MES activity, and it is common in all derivatives. These compounds contribute to the 25% neurotoxicity at 2 h. Among the synthesized compounds **8**(**a**–**o**), all the compounds showed activity in the range of 10.6–77.6% in comparison to phenytoin which completely inhibited the convulsions produced by electro-convulsometer, but compound **8d** having electron withdrawing groups showed excellent anticonvulsant activity. It has already been established that there are at least a few parameters for anticonvulsant drugs such as lipophilic domain (L), hydrophobic unit (R) and electron donor (D) system [15]. Thus the proposed pharmacophore model for **8d** includes all the above factors important for bioactivity (Fig. 1).

Table 2
Effect of the tested compounds in the maximal electroshock seizure test.

Treatment	E/F	% Protection
8a	6.29	52.42
8b	7.11	23.34
8c	6.34	42.40
8d	1.91	77.64
8e	1.96	74.31
8f	2.11	73.47
8g	6.96	31.54
8h	2.34	71.25
8i	7.64	17.32
8j	5.49	59.64
8k	6.11	53.01
8l	8.15	10.63
8m	7.68	17.03
8n	6.51	50.79
8o	6.57	50.43
Standard	1.98	75.88
Control (Vehicle)	8.21	–

Values are expressed as mean, $n = 6$ animals in each group.
E/F = Extension/Flexion [Decrease in ratio of extension phase (in seconds)/flexion phase (in seconds)].
% Protection = (control-Test)/(Control)*100.

3. Conclusion

In conclusion, a series of novel 1-[5-(4-methoxy-phenyl)-[1,3,4] oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**) were synthesized in good yield, characterized by different spectral studies and their anticonvulsant activity have been evaluated. Various substituted sulfonamides showed potent anticonvulsant activity. Compounds with electron withdrawing groups and hetero aryl derivatives like **8d**, **8e**, **8f** and **8h** showed excellent anticonvulsant activity on MES model. Therefore, the nature of groups in sulfonyl moiety is very important for anticonvulsant activity in MES model.

4. Experimental

4.1. Chemistry

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. Elemental analyses were recorded on VarioMICRO superuser V1.3.2 Elementar. ^{1}H NMR and ^{13}C NMR

Table 3
Neurotoxicity screening of the compounds **8**(**a**–**o**).

Compound	Neurotoxicity screen			
	0.5 h	1 h	2 h	4 h
8a	0/4	0/4	0/4	0/4
8b	0/4	0/4	0/4	0/4
8c	0/4	0/4	0/4	0/4
8e	0/4	0/4	0/4	0/4
8f	0/4	0/4	0/4	0/4
8g	0/4	0/4	1/4	1/4
8h	0/4	0/4	0/4	0/4
8i	0/4	0/4	0/4	0/4
8j	0/4	0/4	0/4	0/4
8k	0/4	0/4	0/4	0/4
8l	0/4	0/4	1/4	1/4
8m	0/4	0/4	1/4	1/4
8n	0/4	0/4	0/4	0/4
8o	0/4	0/4	0/4	0/4
Standard	0/4	0/4	0/4	0/4

The data in the table represent ratio between the numbers of the animals that exhibited neurotoxicity against the number of tested animals.

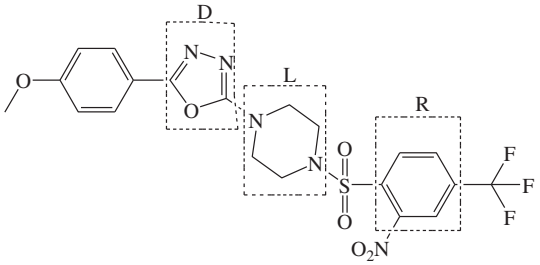


Fig. 1. The pharmacophore model of **8d** for anticonvulsant activity.

spectra were recorded on Bruker DRX -500 spectrometer at 400 MHz using DMSO-d_6/chloroform as solvent and TMS as an internal standard. Mass spectral data were obtained by LC/MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.

*4.1.1. Preparation of ethyl 4-methoxybenzoate (**2**)*

To a stirred solution of 4-methoxy benzoic acid (25.0 g, 0.1643 mol) in ethanol (250 mL) at 0–5 °C, thionyl chloride (0.197 mol) was added. Reaction mass was heated to reflux for 5 h. Reaction completion was monitored by TLC. It was concentrated to syrup and diluted with ethyl acetate (250 mL). Ethyl acetate layer was washed with saturated sodium bicarbonate solution followed by water and brine. Combined organic layer was dried over sodium sulphate and concentrated to get the title compound as pale yellow liquid. (Yield: 26.7 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (t, J = 7.22 Hz, 3H, C–CH_3), 3.72 (s, 3H, O–CH_3), 4.33 (q, J = 7.9 Hz, 2H, O–CH_3), 7.08 (dd, J = 2.08, 6.88 Hz, 2H, Ar–H), 7.80 (dd, J = 2.12, 6.84 Hz, 2H, Ar–H). MS (ESI) m/z: 181.0.

*4.1.2. Preparation of 4-methoxybenzohydrazide (**3**)*

To a mixture of **2** (25.0 g, 0.1387 mol) in ethanol (200 mL) at 0–5 °C, hydrazine hydrate (13.87 mL, 0.2774 mol) was added. The reaction mass was heated to reflux for 2.0 h. The reaction completion was monitored by TLC. The reaction mixture was cooled to 0–5 °C stirred for 60 min. The solid formed was filtered and dried to get compound **3** as white solid (Yield: 18.4 g, 80%). ^{1}H NMR (400 MHz, DMSO- d_6): δ 3.72 (s, 3H, O–CH_3), 4.54 (s, br, 2H, NH_2), 7.10 (dd, J = 2.18, 6.89 Hz, 2H, Ar–H), 7.80 (dd, J = 2.22, 6.94 Hz, 2H, Ar–H), 9.82 (s, br, 1H, NH). MS (ESI) m/z: 167.1.

*4.1.3. Preparation of 2-(4-methoxyphenyl)-1,3,4-oxadiazole (**4**)*

To a mixture of **3** (18.0 g, 0.1083 mol) and triethyl orthoformate (80.3 g, 0.541 mol) was heated to 100–110 °C and maintained for 12 h. The reaction completion was monitored by TLC. The reaction mixture was cooled to room temperature and concentrated to syrup. Traces triethyl orthoformate was removed by azeotropic distillation with toluene. The residue was purified by column chromatography on silica gel using eluent 0–5% ethyl acetate in petroleum ether. The title compound was yielded as pale yellow liquid (Yield: 17.55 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 3H, O–CH_3), 7.09 (dd, J = 2.10, 6.90 Hz, 2H, Ar–H), 7.82 (dd, J = 2.12, 6.92 Hz, 2H, Ar–H), 9.83 (s, 1H, Het–CH). MS (ESI) m/z: 177.2.

*4.1.4. Preparation of 2-bromo-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**5**)*

To a 500 mL four necked flask was charged tetra hydro furan (170 mL) and **4** (17.0 g, 0.096 mol). The reaction mass was cooled to −65 to −70 °C under nitrogen atmosphere with stirring and added *n*-butyl lithium (50.3 mL, 2.3 M in *n*-hexane, 0.1157 mol) over a period of 1 h, further reaction mass was stirred at same temperature for 1.0 h. Bromine (9.2 g, 0.1157 mol) was added slowly to reaction mass by maintaining the reaction temperature −65 to −70 °C. Further stirred the reaction mass for 1.0 h. Reaction completion was monitored by TLC. Reaction mass was warmed to around −10 °C and quenched with 1.0 N HCl solution (100 mL). Reaction mass was warmed to room temperature and the compound was extracted with ethyl acetate (2 × 100 mL). Combined organic layer was washed with water (1 × 100 mL) followed by brine (100 mL), dried over anhydrous sodium sulphate and concentrated to one volume stage. Compound was crystallized using petroleum ether to yield the title compound as white solid. (Yield: 22.64 g, 93%). ^{1}H NMR (400 MHz, DMSO- d_6): δ 3.72 (s, 3H, O–CH_3), 7.12 (dd, J = 2.11, 6.91 Hz, 2H, Ar–H), 7.78 (dd, J = 2.12, 6.93 Hz, 2H, Ar–H). MS (ESI) m/z: 256.0.

*4.1.5. Preparation of tert-butyl-4-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]piperazine-1-carboxylate (**6**)*

To a stirred solution of **5** (20.0 g, 0.078 mol), potassium carbonate (32.511 g, 0.2352 mol) in dimethyl formamide (240 mL), mano boc piperazine (17.52 g, 0.0940 mol) were added and stirred the reaction mass for 10 h at room temperature. Reaction completion was monitored by TLC. Reaction mass was quenched into ice water (2.0 L), stirred at room temperature for 1 h. The solid formed was filtered and dried to yield the above compound as white solid (Yield: 23.73 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.44 (s, 9H, t-butyl-H), 3.49 (s, 8H, 2(–CH_2–N–CH_2–)), 3.72 (s, 3H, O–CH_3), 7.10 (dd, J = 2.10, 6.82 Hz, 2H, Ar–H), 7.78 (dd, J = 2.12, 6.83 Hz, 2H, Ar–H). MS (ESI) m/z: 361.4.

*4.1.6. Preparation of 1-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl] piperazine hydrochloride (**7**)*

To a stirred solution of **6** (23 g, 0.088 mol) in 1,4 dioxane at 0–50 °C, 4 N HCl in dioxane (40.0 mL) was added. Reaction was stirred at room temperature for 10 h, filtered the solid and it was washed with diethyl ether, then dried under nitrogen atmosphere and packed in air tight container. The title compound was obtained as white hygroscopic solid (Yield: 24.75 g, 90%). ^{1}H NMR (400 MHz, DMSO- d_6): δ 3.25 (s, 4H, –CH_2–N–CH_2–), 3.70 (s, 3H, O–CH_3), 3.80 (t, J = 4.00 Hz, 4H, –CH_2–N–CH_2–), 7.13 (dd, J = 2.13, 6.92 Hz, 2H, Ar–H), 7.79 (dd, J = 2.2, 6.89 Hz, 2H, Ar–H), 9.42 (s, br, 1H, NH), 9.81 (s, br, 2H, HCl). MS (ESI) m/z: 261.3.

*4.1.7. General procedure for the synthesis of 1-[5-(4-methoxyphenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **8**(**a**–**o**)*

To a mixture of **7** (1.0 eq) and triethyl amine (3.5 eq) in dichloromethane (10 volume), substituted sulphonyl chloride (1.2 eq) was added at 5–10 °C and stirred overnight at room temperature. The completion of the reaction was confirmed by TLC. The reaction mixture was poured into separating funnel washed with water followed by brine solution, dried over anhydrous sodium sulphate and concentrated to syrup. Crude syrup was purified by crystallization using dichloromethane and petroleum ether to yield the oxadiazole substituted sulfonamide as white to white solid.

*4.1.7.1. 1-(4-Bromo-3-methyl-benzenesulfonyl)-4-[5-(4-methoxyphenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8a**).* Yield: 82% (White solid); m.p.: 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (s, 3H, Ar–CH_3), δ 3.10 (t, J = 4.32 Hz, 4H, –CH_2–N–CH_2–), 3.63 (t, J = 4.20 Hz, 4H, –CH_2–N–CH_2–),3.82 (s, 3H, O–CH_3), 7.10 (dd, J = 2.14, 6.90 Hz, 2H, Ar–H), 7.69 (dd, J = 2.18, 6.79 Hz, 2H, Ar–H),7.82–7.87 (m, 3H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.4 (Ar–C), 44.9, 45.1 (–CH_2–N–CH_2–), 55.4 (O–CH_3), 123.7, 125.4, 126.8, 129.3, 129.5, 130.1, 130.8, 133.3, 134.4 (Ar–C), 153.4 (het–C), 158.6 (Ar–C), 163.6 (het–C). MS (ESI) m/z: 494.04. Anal. Calcd. for $C_{20}H_{21}BrN_4O_4S$: C, 48.69; H, 4.29; N, 11.36; Found: C, 48.71; H, 4.32; N, 11.45%.

*4.1.7.2. 1-[Biphenyl-sulfonyl]-4-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)piperazine (**8b**).* Yield: 80% (White solid); m.p.: 171 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.08 (t, J = 4.14 Hz, 4H, −CH_2−N−CH_2−), 3.60 (t, J = 5.01 Hz, 4H, −CH_2−N−CH_2−), 3.79 (s, 3H, O−CH_3), 7.04 (dd, J = 2.10, 6.91 Hz, 2H, Ar−H), 7.40−7.52 (m, 3H,Ar−H), 7.64−7.87 (m, 6H,Ar−H), 7.93 (d, J = 8.55 Hz, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 44.8, 45.6 (−CH_2−N−CH_2−), 55.3 (O−CH_3), 114.6, 116.3, 125.9, 127.1, 127.6, 128.6, 129.4, 132.6, 133.6, 138.2, 144.8 (Ar−C), 158.5 (het−C), 161.1 (Ar−C), 163.0 (het−C). MS (ESI) m/z: 476.5. Anal. Calcd. for $C_{25}H_{24}N_4O_4S$: C, 63.01; H, 5.08; N, 11.76; Found: C, 63.21; H, 5.12; N, 11.70%.

*4.1.7.3. 4-{4-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine-1-sulfonyl}-benzonitrile (**8c**).* Yield: 80% (White solid); m.p.: 225 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.12 (s, br, 4H, −CH_2−N−CH_2−), 3.58 (s, br, 4H, CH_2−N−CH_2−), 3.79 (s, 3H, O−CH_3), 7.04 (d, J = 8.55 Hz, 2H, Ar−H), 7.75 (d, J = 8.58 Hz, 2H, Ar−H), 7.93 (d, J = 8.16 Hz, 2H, Ar−H), 8.12 (d, J = 8.13 Hz, 2H, Ar−H).

^{13}C NMR (400 MHz, DMSO-d_6): δ 45.1, 45.6 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 115.0 (−CN), 116.2, 116.8, 118.0, 127.6, 128.6, 134.1, 139.8 (Ar−C), 159.0 (het−C), 161.5 (Ar−C), 163.5 (het−C). MS (ESI) m/z: 426.4. Anal. Calcd. for $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46; Found: C, 56.53; H, 4.43; N, 16.65%.

*4.1.7.4. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(2-nitro-4-trifluoromethyl-benzene sulfonyl)-piperazine (**8d**).* Yield: 79% (White solid); m.p.: 193 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.41 (t, J = 4.95 Hz, 4H, −CH_2−N−CH_2−), 3.60 (t, J = 5.22 Hz, 4H, −CH_2−N−CH_2−), 3.80 (s, 3H, O−CH_3), 7.06 (d, J = 8.91 Hz, 2H, Ar−H), 7.79 (d, J = 8.88 Hz, 2H, Ar−H), 8.25 (d, J = 8.82 Hz, 2H, Ar−H), 8.65 (s, 1H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.0, 45.9 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 115.0, 116.8, 122.4 (Ar−C), 124.5 (C−F_3), 127.6, 129.8, 132.4, 134.3, 134.8, 148.4 (Ar−C), 159.1 (het−C), 161.6 (Ar−C), 163.5 (het−C). MS (ESI) m/z: 514.4. Anal. Calcd. for $C_{20}H_{18}F_3N_5O_6S$: C, 46.78; H, 3.53; N, 13.64; Found: C, 46.84; H, 3.63; N, 13.73%.

*4.1.7.5. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(4-phenoxy-benzenesulfonyl) piperazine (**8e**).* Yield: 83% (White solid); m.p.: 151 °C; ^{1}H NMR (400 MHz, DMSO- d_6): δ 3.06 (t, J = 4.84 Hz, 4H, −CH_2−N−CH_2−), 3.63 (t, J = 5.24 Hz, 4H, −CH_2−N−CH_2−), 3.71 (s, 3H, O−CH_3), 7.16 (dd, J = 1.92, 6.80 Hz, 4H, Ar−H), 7.25−7.29 (m, 1H, Ar−H), 7.44−7.49 (m, 2H, Ar−H), 7.51−7.56 (m, 2H, Ar−H), 7.76 (dd, J = 2.04, 6.86 Hz, 2H, Ar−H), 7.84−7.89 (m, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.3, 45.5 (−CH_2−N−CH_2−), 55.3 (O−CH_3), 118.1, 120.7, 124.4, 125.5, 125.8, 129.0, 129.6, 130.6, 130.9, 131.2 (Ar−C), 155.0, 159.0 (het−C), 161.7 (Ar−C), 163.8 (het−C). MS (ESI) m/z: 493.1. Anal. Calcd. for $C_{25}H_{24}N_4O_5S$: C, 60.96; H, 4.91; N, 11.37; Found: C, 60.85; H, 4.78; N, 11.46%.

*4.1.7.6. 1-(2,5-Dichloro-thiophene-3-sulfonyl)-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8f**).* Yield: 80% (White solid); m.p.: 187 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.30 (t, J = 3.42 Hz, 4H, −CH_2−N−CH_2−), 3.59 (t, J = 4.50 Hz, 4H, −CH_2−N−CH_2−), 3.80 (s, 3H, −O−CH_3), 7.06 (d, J = 8.46 Hz, 2H, Ar−H), 7.39 (s, 1H, het−H), 7.78 (d, J = 8.49 Hz, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.1, 45.6 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 115.1, 116.9 (Ar−H), 127.3, 127.5, 127.6 (het−C), 131.0 (Ar−C), 132.8, 159.0 (het−C), 161.6(Ar−C), 163.5 (het−C). MS (ESI) m/z: 476.3. Anal. Calcd. for $C_{17}H_{16}Cl_2N_4O_4S_2$: C, 42.95; H, 3.39; N, 11.79; Found: C, 42.86; H, 3.43; N, 11.83%.

*4.1.7.7. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(naphthalene-1-sulfonyl)-piperazine (**8g**).* Yield: 78% (White solid); m.p.: 167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.26 (t, J = 5.00 Hz, 4H, −CH_2−N−CH_2-), 3.74 (t, J = 5.32 Hz, 4H, −CH_2−N−CH_2−),3.90 (s, 3H, O−CH_3), 7.08 (dd, J = 2.08, 6.88 Hz, 2H, Ar−H), 7.60 (dd, J = 2.12, 6.84 Hz, 2H, Ar−H), 7.77 (dd, J = 1.72, 8.70 Hz, 1H, Ar−H), 7.86 (dd, J = 1.40, 5.26 Hz, 2H, Ar−H), 7.94−7.96 (m, 1H, Ar−H), 8.00−8.02 (m, 2H, Ar−H), 8.38 (s, 1H, Ar−H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 45.1, 45.8 (−CH_2−N−CH_2−), 55.6 (O−CH_3), 122.6, 124.1, 125.8, 128.0, 128.8, 129.1, 129.2, 129.6, 130.7, 132.4 (Ar−C), 155.0 (het−C), 161.7 (Ar−C), 163.4 (het−C). MS (ESI) m/z: 451.5. Anal. Calcd. for $C_{23}H_{22}N_4O_4S$: C, 61.32; H, 4.92; N, 12.44; Found: C, 61.22; H, 4.83; N, 12.52%.

*4.1.7.8. 1-(3,5-Dimethyl-isoxazole-4-sulfonyl)-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8h**).* Yield: 79% (White solid); m.p.: 152 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, het−CH_3), 2.63 (s, 3H, het−CH_3), 3.22 (t, J = 4.96 Hz, 4H, −CH_2−N−CH_2−), 3.63 (t, J = 5.28 Hz, 4H, −CH_2−N−CH_2−), 3.82 (s, 3H, O−CH_3), 7.08 (dd, J = 2.08, 6.88 Hz, 2H, Ar−H), 7.80 (dd, J = 2.12, 6.84 Hz, 2H, Ar−H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 11.4, 13.1 (het−CH_3) 44.6, 46.1 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 112.7, 115.1, 116.9, 127.6 (Ar−C), 158.0, 159.0, 161.6 (het−C), 163.5 (Ar−C), 174.7 (het−C). MS (ESI) m/z: 420.4. Anal. Calcd. for $C_{18}H_{21}N_5O_5S$: C, 51.54; H, 5.05; N, 16.70; Found: C, 51.64; H, 5.12; N, 16.63%.

*4.1.7.9. 1-(4-Tert-butyl-benzenesulfonyl)-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8i**).* Yield: 79% (White solid); m.p.: 187 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (s, 9H, t-butyl H), 3.13 (s, br, 4H, −CH_2−N−CH_2−), 3.58 (s, br, 4H, −CH_2−N−CH_2−), 3.79 (s, 3H, O−CH_3), 7.04 (d, J = 8.55 Hz, 2H, Ar−H), 7.75 (d, J = 8.58 Hz, 2H, Ar−H), 7.93 (d, J = 8.16 Hz, 2H, Ar−H), 8.12 (d, J = 8.13 Hz, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 31.2, 41.3 (t-butyl C), 45.1, 45.6 (−CH_2−N−CH_2−), 55.8(O−CH_3), 115.0, 116.2, 118.0, 127.6, 128.6, 134.1, 139.8 (Ar−C), 158.0 (het−C), 161.5 (Ar−C), 163.5 (het−C). MS (ESI) m/z: 457.5. Anal. Calcd. for $C_{23}H_{28}N_4O_4S$: C, 60.51; H, 6.18; N, 12.27; Found: C, 60.44; H, 6.24; N, 12.31%.

*4.1.7.10. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(2-nitro-benzenesulfonyl)-piperazine (**8j**).* Yield: 78% (Off white solid); m.p.: 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.37 (t, J = 4.72 Hz, 4H, −CH_2−N−CH_2−), 3.61 (t, J = 5.20 Hz, 4H, −CH_2−N−CH_2−), 3.81 (s, 3H, O−CH_3), 7.07 (dd, J = 1.84, 8.42 Hz, 2H, Ar−H), 7.80 (dd, J = 2.00, 8.34 Hz, 2H, Ar−H), 7.85−7.94 (m, 2H, Ar−H), 8.00−8.05 (m, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.1, 45.9 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 115.0, 116.8, 124.7, 127.6, 129.5, 130.8, 132.9, 135.4, 148.3 (Ar−C), 159.1 (het−C), 161.6 (Ar−C), 163.6 (het−C). MS (ESI) m/z: 446.4. Anal. Calcd. for $C_{19}H_{19}N_5O_6S$: C, 51.23; H, 4.30; N, 15.72; Found: C, 51.34; H, 4.23; N, 15.83%.

*4.1.7.11. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(3-trifluoromethyl-benzenesulfonyl)-piperazine (**8k**).* Yield: 79% (Off white solid); m.p.: 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.15 (t, J = 4.40 Hz, 4H, −CH_2−N−CH_2−), 3.60 (t, J = 5.20 Hz, 4H, −CH_2−N−CH_2−), 3.81 (s, 3H, O−CH_3), 7.07 (dd, J = 1.84, 8.42 Hz, 2H, Ar−H), 7.76 (dd, J = 2.00, 8.34 Hz, 2H, Ar−H), 7.90−7.94 (m, 1H, Ar−H), 8.00 (s, 1H, Ar−H), 8.09−8.14 (m, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.2, 46.0 (−CH_2−N−CH_2−), 55.8 (O−CH_3), 115.0, 116.8, 124.2 (Ar−C), 124.3 (Ar−CF_3), 127.6, 130.6, 130.8, 131.7, 132.0, 137.0 (Ar−C), 159.0 (het−C), 161.6 (Ar−C), 163.5 (het−C). MS (ESI) m/z: 469.4. Anal. Calcd. for $C_{20}H_{19}F_3N_4O_4S$: C, 51.28; H, 4.09; N, 11.96; Found: C, 51.11; H, 4.15; N, 11.84%.

*4.1.7.12. 1-Methanesulfonyl-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8l**).* Yield: 80% (Off white solid); m.p.: 169 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.84 (s, 3H, S−CH_3), 3.10 (t, J = 4.32 Hz, 4H, −CH_2−N−CH_2−), 3.40 (t, J = 5.10 Hz, 4H, −CH_2−N−CH_2−), 3.71 (s, 3H, O−CH_3), 7.10 (dd, J = 1.92, 8.42 Hz, 2H, Ar−H), 7.86 (dd, J = 2.00, 8.34 Hz, 2H, Ar−H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 39.6 (s-CH_3), 45.4, 46.1 (−CH_2−N−CH_2−), 55.7 (O−CH_3), 117.0,

124.2, 128.1 (Ar–C), 157.0 (het–C), 159.8 (Ar–C), 163.5 (het–C). MS (ESI) *m/z*: 339.4. Anal. Calcd. for $C_{14}H_{18}N_4O_4S$: C, 49.69; H, 5.36; N, 16.56; Found: C, 49.83; H, 5.26; N, 16.72%.

*4.1.7.13. 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-4-(toluene-4-sulfonyl)-piperazine (**8m**).* Yield: 80% (Off white solid); m.p.: 161 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.2 (s, 3H, Ar–CH_3) 3.13 (s, br, 4H, –CH_2–N–CH_2–), 3.60 (s, br, 4H, –CH_2–N–CH_2–), 3.79 (s, 3H, O–CH_3), 7.04 (d, J = 8.55 Hz, 2H, Ar–H), 7.75 (d, J = 8.58 Hz, 2H, Ar–H), 7.93 (d, J = 8.16 Hz, 2H, Ar–H), 8.12 (d, J = 8.13 Hz, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 28.3 (Ar–CH_3), 45.1, 45.6 (–CH_2–N–CH_2–), 55.8, 115.0, 116.2, 118.0, 127.6, 128.6, 134.1, 139.8 (Ar–C), 159.0 (het–C), 161.5 (Ar–C), 163.5 (het–C). MS (ESI) *m/z*: 415.5. Anal. Calcd. for $C_{20}H_{22}N_4O_4S$: C, 57.96; H, 5.35; N, 13.52; Found: C, 58.02; H, 5.57; N, 13.42%.

*4.1.7.14. 1-(4-Bromo-benzenesulfonyl)-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8n**).* Yield: 80% (Off white solid); m.p.: 211 °C; ^{1}H NMR(400 MHz, DMSO-d_6): δ 3.06 (t, J = 3.30 Hz, 4H, –CH_2–N–CH_2–), 3.45 (t, J = 3.90 Hz, 4H, –CH_2–N–CH_2–), 3.80 (s, 3H, O–CH_3), 7.05 (d, J = 8.85 Hz, 2H, Ar–H), 7.68 (d, J = 8.58 Hz, 2H, Ar–H), 7.76 (d, J = 8.79 Hz, 2H, Ar–H), 7.86 (d, J = 8.55 Hz, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.2, 45.6 (–CH_2–N–CH_2–), 55.8 (O–CH_3), 115.1, 116.8, 127.6, 127.9, 129.9, 133.1, 134.8 (Ar–C), 159.0 (het–C), 161.6 (Ar–C), 163.5 (het–C). MS (ESI) *m/z*: 480.3. Anal. Calcd. for $C_{19}H_{19}BrN_4O_4S$: C, 47.61; H, 4.00; N, 11.69; Found: C, 47.79; H, 4.34; N, 11.82%.

*4.1.7.15. 1-(4-Iodo-benzenesulfonyl)-4-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine (**8o**).* Yield: 80% (Off white solid); m.p.: 208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10 (t, J = 3.34 Hz, 4H, –CH_2–N–CH_2–), 3.46 (t, J = 3.92 Hz, 4H, –CH_2–N–CH_2–), 3.80 (s, 3H, O–CH_3), 7.15 (d, J = 8.83 Hz, 2H, Ar–H), 7.69 (d, J = 8.57 Hz, 2H, Ar–H), 7.78 (d, J = 8.75 Hz, 2H, Ar–H), 7.85 (d, J = 8.56 Hz, 2H, Ar–H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 45.1, 45.5 (–CH_2–N–CH_2–), 55.8 (O–CH_3), 115.1, 116.8, 127.7, 127.9, 129.8, 133.2, 134.8 (Ar–C), 158.9 (het–C), 161.6 (Ar–C), 163.4 (het–C). MS (ESI) *m/z*: 527.0. Anal. Calcd. for $C_{19}H_{19}IN_4O_4S$: C, 43.36; H, 3.64; N, 10.64; Found: C, 43.37; H, 3.74; N, 10.43%.

4.2. Anticonvulsant evaluation

Animals: Male wistar rats procured from National Institute of Nutrition, Hyderabad (190–220 g) were used in the present study. The animals were kept in individual cages for one week to acclimatize for the laboratory conditions. They were allowed to free access of water and food.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee, G Pulla Reddy College of Pharmacy, Hyderabad, India.

Maximal Electroshock Seizure Model (*MES*): Maximal electroshock seizure model was used in the present study to evaluate the anticonvulsant activity of the compounds on male wistar rats. Seizures were induced in rats by delivering electroshock of 150 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. The test compounds (100 mg/kg) were administered by oral route in the form of solution (The compounds were dissolved in 1% sodium carboxymethyl cellulose), 30 min before the maximal electroshock seizure test. The animals were observed closely for 2 min. The percentage of inhibition of seizure relative to control was recorded and calculated [16]. Phenytoin (100 mg/kg) was used as a standard drug.

Neurotoxicity screening: The minimal motor impairment was measured in mice by the rotorod test. Acute neurological toxicity in mice was evaluated by rotorod test [16]. The mice were trained to stay on the accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were administered with the test compounds at dose of 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trails. Phenytoin was used as a standard drug.

Statistical analysis: In the present study, data were analyzed by one way analysis of variance (ANOVA) followed by dunnet test to compare difference between the groups.

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