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Design and Syntheses of Some New 5–[Benzenesulphonamido]–1,3,4–thiadiazol–2–sulphonamide as Potent Antiepileptic Agent

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*A QSAR study was performed on sulphonamide-1,3,4-thiadiazole derivatives using modified version of Allinger MM_2 force field in Chem 3D Ultra structural descriptors. The relationship between antiepileptic activity and various descriptors was established by stepwise multiple regression analysis. The analyses have produced well predictive and statistically significant QSAR models which were further cross validated. This study helps to design and to synthesize some expectedly potent compounds. The compounds, 5-[(4-acetamido)benzenesulphonamido]-1,3,4-thiadiazol-2-(N-benzoyl) sulphonamide (**8a**), 5-[(4-amino)benzenesulphonamido]-1,3,4-thiadiazol-2-(N-benzoyl) sulphonamide (**9a**) and 5-(4-amino) benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide (**10a**) were synthesized from acetazolamide by modified Schotten-Bouman synthesis method and tested for antiepileptic activity by maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (scPTZ) induced seizure models in mice and have shown significant activity. These compounds were further subjected to diuretic studies, showing up to 86 % reduction in diuresis compared to acetazolamide.*

Keywords: QSAR, antiepileptic activity, diuretic activity, 1,3,4-thiadiazole, acetazolamide.

Разработка и синтез новых 5–[бензилсульфонамидо]–1,3,4–тиадиазол–2–сульфонамидов как противоэпилептических препаратов

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*QSAR исследование проводили на производных сульфонамид-1,3,4-тиадиазола с использованием структурных дескрипторов модифицированной версии силового поля MM_2 Allinger в Chem 3D Ultra. Взаимосвязь противоэпилептической активности и различных дескрипторов была установлена с помощью ступенчатого множественного регрессионного анализа. Получены хорошие предсказательные и статистически значимые QSAR модели, которые в дальнейшем были перекрестно подтверждены. По результатам этого исследования были предложены и синтезированы из ацетазолоамида с использованием модифицированного метода Шоттена-Боумана потенциально перспективные соединения - 5-[(4-ацетамидо)бензолсульфонамидо]-1,3,4-тиадиазол-2-(N-бензоил) сульфонамид (**8a**), 5-[(4-амино)бензолсульфонамидо]-1,3,4-тиадиазол-2-(N-бензоил) сульфонамид (**9a**) и 5-(4-амино)бензолсульфонамидо-1,3,4-тиадиазол-2-сульфонамид (**10a**). Полученные соединения обладают противоэпилептической активностью в опытах на мышах, а также проявляют себя как эффективное противодиуретическое средство.*

Ключевые слова: QSAR модели, противоэпилептическая активность, диуретическая активность, 1,3,4-тиадиазол, ацетазоламид.

Introduction

About 0.5-1% of the world's population is affected by epilepsy, one of the most common neurological disorders, and characterized by recurrent seizure attacks. The drugs that are widely used for treating epileptic seizures have failed to adequately control seizures and have unfavourable adverse effects such as ataxia, hepatotoxicity, gingival hyperplasia and megaloblastic anaemia. The restrictive treatments of epileptic seizures in patients have led to researches to discover new agents with more effectiveness and lesser toxicity.[1-2]

Acetazolamide, a heterocyclic sulphonamide; 1,3,4-thiadiazole derivative, has carbonic anhydrase inhibitor activity and clinically used as diuretic agent and also used in treatment of absence seizures. It is also useful as an adjunct in the treatment of tonic-clonic, myoclonic, and atonic seizures, particularly in women whose seizures occur or are exacerbated at specific times in the menstrual cycle. However, its usefulness is transient often because of rapid development of tolerance.[3] The problem with acetazolamide therapy as an antiepileptic agent is that, it produce diuresis and electrolyte imbalance as an adverse effect.

At least 14 different carbonic anhydrase (CA) isoforms were isolated in higher vertebrates. Several important physiological and physico-pathological functions are played by many CA isozymes, which are strongly inhibited by aromatic and heterocyclic sulphonamides. hCA I and hCA II inhibitors involved in the anticonvulsant activity shown by many sulphonamide drugs with CA inhibitory properties.[4-7]

Among the few reports in the literature our attention was drawn to the earlier discovery by Robin Jr. R.O. and co-workers[8-9] reported that, the only sulphonamides, unsubstituted on the sulphonamide nitrogen in acetazolamide are highly active as carbonic anhydrase inhibitors. The 1,3,4-thiadiazole and its derivatives possess wide variety of activities.[10-25] Furthermore, 1,3,4-thiadiazole nucleus itself exhibit anticonvulsant activity.[23] This increases our interest to design compounds from QSAR study and synthesize the compounds accordingly, having better therapeutic index for epilepsy treatment and very low diuretic activity.

In hope of getting anticonvulsant response of 1,3,4-thiadiazole nucleus itself, substitution of sulphonamide nitrogen at second position and chemically modifying fifth position acetyl group in to aromatic substituted sulphonamide group, that modification of sulphonamido group by some other chemical moiety or any substitution on sulphonamide nitrogen atom yielded structural analogues with loss of diuretic activity and will produce significant anticonvulsant activity.

We report herein designing, synthesis, antiepileptic activity and reduction in diuretic activity of acetazolamide derivatives; 5-[(4-acetamido)benzenesulphonamido]-1,3,4-thiadiazol-2-(*N*-benzoyl) sulphonamide (**8a**), 5-[(4-amino) benzenesulphonamido]-1,3,4-thiadiazol-2-(*N*-benzoyl)-sulphonamide (**9a**) and 5-(4-amino)-benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide (**10a**).

QSAR Study

Several 1,3,4-thiadiazole and sulphonamide derivatives were selected[26] for the study (Figure 1 and Table 1) with the hope to obtain better antiepileptic agents with low dieresis. All physicochemical parameters of each compound from the

Table 1. CA-IV inhibition data for sulphonamides and 1,3,4-thiadiazole derivatives used in this study.

Compd	R	[a]IC_{50} (μM)	[b]pIC_{50}
(**Ia**)	$-NH_2$	300	-2.47712
(**Ib**)	$-CH_2NH_2$	170	-2.23045
(**Ic**)	$-(CH_2)_2NH_2$	160	-2.20412
(**Id**)	$-NHCOCH_3$	246	-2.39094
(**Ie**)	$-NHCOCF_3$	133	-2.12385
(**If**)	$-NHCOCH_2CH_3$	232	-2.36549
(**Ig**)	$-NHCO(CH_2)_2CH_3$	227	-2.35603
(**Ih**)	$-NHCOCH(CH_3)_2$	258	-2.41162
(**Ii**)	$-NHCO(CH_2)_3CH_3$	214	-2.33041
(**Ij**)	$-NHCOCH(CH_3)_3$	230	-2.36173
(**Ik**)	$-NHCO(CH_2)_4CH_3$	63	-1.79934
(**Il**)	$-NHCO(CH_2)_7CH_3$	66	-1.81954
(**Im**)	$-NHCOC_6H_5$	37	-1.5682
(**In**)	$-NHCONHC_6H_5$	240	-2.38021
(**Io**)	$-CH_2NHCONHC_6H_5$	105	-2.02119
(**Ip**)	$-(CH_2)_2NHCONHC_6H_5$	75	-1.87506
(**Iq**)	$-NH(SO)_2C_6H_5$	49	-1.6902
(**IIa**)	$-COCH_3$	12	-1.07918
(**IIb**)	$-COCH(CH_3)_3$	10	-1
(**IIc**)	$-COC_6H_5$	15	-1.17609
(**IId**)	$-COC_6F_5$	2	-0.30103
(**IIe**)	$4\text{-}NHCOCH_3C_6H_4SO_2$	3	-1.44716
(**IIf**)	$3\text{-}F,4\text{-}NHCOCH_3C_6H_4SO_2$	1	0
(**IIg**)	$3\text{-}Cl,4\text{-}NHCOCH_3C_6H_4SO_2$	1	0
(**IIh**)	$3\text{-}Br,4\text{-}NHCOCH_3C_6H_4SO_2$	2	-0.30103
(**IIi**)	$4\text{-}NH_2C_6H_4SO_2$	14	-1.14613
(**IIIa**)	$-COCH_3$	1	0
(**IIIb**)	-CO-2-furyl	6	-0.77895
(**IIIc**)	$-COC_6H_5$	17	-1.23045
(**IIId**)	$-COC_6F_5$	1.5	-0.17609

[a]IC50 valus were determined by CA-IV enzyme inhibition.
[b]negative logarithmic value of IC50 (in moles) [pIC50 = -log10 IC50].

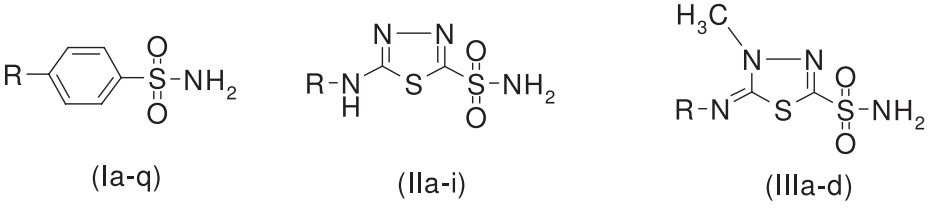


Figure 1. 1,3,4-Thiadiazole and sulphonamide derivatives selected for the study.

series were calculated and subjected to stepwise, multiple and sequential regression analysis with respect to biological activity (Table 2). Correlation of each parameter was generated with biological activity and is summarised in Table 3. The number of developed models was high, so further analysis was bases on statistically significant parameters, namely correlation coefficient (R), it's square (R^2), variance ratio (F), cross-validation method (Q^2) standard deviation based on predicted residual sum of squares (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}). The parameters used in the model were almost independent, which can be seen from the Pearson correlation matrix (Table 4, Figure 2).

Chemistry

5-Amino-1,3,4-thiadiazol-2-[*N*-(substituted benzoyl)] sulphonamides (**4a-d**) was prepared by hydrolysis of the benzoylated acetazolamides (**3a-d**), which was prepared from the acetazolamide (**1**) by benzoylation with substituted benzoyl chlorides (**2a-d**). Substituted 4-acetamidobenzenesulphonyl chlorides (**7a-c**) were prepared according to the literature method[27] from the substituted acetanilides (**6a-c**) by sulphonation with chlorosulphonic acid. The crude product was used in the next step immediately. Modified Schotten-Boumann synthesis method[26-27] was used to synthesized

Table 2. Descriptors Calculated for the QSAR Study.

Sr. No.	Descriptor	Abbreviations	Unit	Type
1	Heat of formation	HF	kcal/mol	Thermodynamic
2	Boiling Point	BP	Kelvin	Thermodynamic
3	Critical Pressure	CP	Bar	Thermodynamic
4	Critical Volume	CV	cm^2/mol	Thermodynamic
5	Critical Temperature	CT		Thermodynamic
6	Henry's law constant	HLC		Thermodynamic
7	Ideal gas thermal capacity	IGTC	J/mol K	Thermodynamic
8	Log p	LP		Thermodynamic
9	Melting Point	MP	Kelvin	Thermodynamic
10	Molar Refractivity	MR	cm^3/mol	Thermodynamic
11	Standard Gibbs free energy	GBS	kJ/mol	Thermodynamic
12	Connolly accessible area	CAA	Angstrom S2	Steric
13	Connolly molecular surface area	MS	Angstrom S2	Steric
14	Connolly solvent excluded volume	CSEV	Angstrom S2	Steric
15	Ovality			Steric
16	Principal Moment of Inertia-X	PMI- X		Steric
17	Principal Moment of Inertia-Y	PMI- Y		Steric
18	Principal Moment of Inertia-Z	PMI- Z		Steric
19	Dipole Moment	D	Debye	Electronic
20	Dipole Moment – X axis	DX	Debye	Electronic
21	Dipole Moment – Y axis	DY	Debye	Electronic
22	Dipole Moment – Z axis	DZ	Debye	Electronic
23	Electronic energy	EE	eV at 0 ^{0}C	Electronic
24	Total energy	TE	eV	Electronic
25	HOMO energy	HOMO	eV	Electronic
26	LUMO energy	LUMO	eV	Electronic
27	Repulsion energy	RE	eV	Electronic
28	Bending energy	E_b	kcal/mol	Thermodynamic
29	Charge-charge energy	Ec	kcal/mol	Thermodynamic
30	Charge-dipole energy	Ed	kcal/mol	Thermodynamic
31	Dipole-dipole energy	DDE	kcal/mol	Thermodynamic
32	Non-1,4-*Van der Waals* energy	NVDWE	kcal/mol	Thermodynamic
33	Stretch energy	SE		Thermodynamic
34	Stretch bend energy	SBE	kcal/mol	Thermodynamic
35	Torsion energy	TOE	kcal/mol	Thermodynamic
36	Total energy	TE	kcal/mol	Thermodynamic
37	*Van der Waals* energy	VDE	kcal/mol	Thermodynamic

Table 3. Descriptors, observed, calculated and predicted CA-IV inhibition activity data of compounds of training set.

Compd	Descriptors			pIC		
	HLC	LUMO	VDWE	[a]Obs.	[b]Cal.	LOO
(**Ia**)	2.4950	-1.4370	8.2817	7.6353	7.61418	3.228892
(**Ib**)	2.3305	-0.3276	3.1643	6.6177	6.60267	2.656621
(**Ic**)	1.7620	-0.6241	4.4457	6.8029	6.80039	2.734382
(**Id**)	1.7620	-0.6029	6.0813	6.7930	6.79324	2.756201
(**Ie**)	2.3305	-0.8487	1.3968	6.9469	6.92539	1.930475
(**If**)	2.3305	-0.8488	1.3991	6.9285	6.92513	1.737577
(**Ig**)	2.3305	-0.8377	2.7005	6.9248	6.90509	1.952210
(**Ih**)	2.3305	-0.8317	3.7304	6.8950	6.87545	1.953405
(**Ii**)	2.3305	-0.8289	3.8343	6.9654	6.94487	1.951018
(**Ij**)	2.3305	-0.5802	6.5330	6.6646	6.66516	1.588046
(**Ik**)	2.3305	-0.5625	7.1177	6.6904	6.68507	1.610167
(**Il**)	2.3305	-0.8294	5.4088	6.9343	6.94855	1.440459
(**Im**)	2.3305	-1.2295	8.0392	6.4709	cro	cro
(**In**)	2.3305	-0.7771	4.3643	7.0677	7.09076	1.652787
(**Io**)	2.3305	-1.8565	4.7188	8.0725	7.98791	1.762105
(**Ip**)	2.3305	-0.9266	3.1096	7.3222	7.27296	0.889326
(**Iq**)	1.7620	-0.9547	4.8646	7.3505	7.29577	1.256420
(**IIa**)	1.7620	-0.8601	6.5776	6.9636	6.97175	1.278754
(**IIb**)	1.7620	-0.9373	8.5701	7.0206	7.06207	2.440922
(**IIc**)	2.3305	-1.0746	8.1744	7.2723	7.27390	1.662270
(**IId**)	1.7620	-0.9003	10.0460	7.1909	7.21692	1.771029
(**IIe**)	1.7620	-0.8147	11.3092	7.1254	7.19688	1.963489
(**IIf**)	2.3305	-1.3112	7.8988	7.4437	7.48955	2.344829
(**IIg**)	2.3305	-2.0134	9.1096	8.1297	8.08186	2.253290
(**IIh**)	1.7620	-0.7769	10.8721	6.9047	6.91702	2.134695
(**IIi**)	7.3867	-1.4747	1.1745	8.5932	8.57619	2.476107
(**IIIa**)	7.3196	-1.6986	1.0305	8.8500	8.88264	2.698101
(**IIIb**)	7.5169	-1.6800	2.1094	8.8937	8.91858	2.698101
(**IIIc**)	7.7864	-1.7131	2.3540	9.1877	9.05720	2.298853
(**IIId**)	4.8975	-1.2736	3.0051	8.0309	8.09029	2.476107

[a]Observed value
[b]Calulated (Cal) and predicted (LOO) values of pIC from Model II
[cro]Compound removed as outlier

Table 4. Pearson Correlation Matrix for descriptors influencing inhibition of CA-IV.

	HLC	LUMO	VDWE	pIC_{50}
HLC	1.000			
LUMO	-0.600	1.000		
VDWE	-0.560	0.113	1.000	
pIC_{50}	0.787	-0.792	-0.130	1.000

5-[(4-acetamido) substituted benzenesulphonamido]-1,3,4-thiadiazol-2-[*N*-(substituted benzoyl)]sulphonamides (**8a-g**), where the nucleophilic substitution reaction occurs. In this reaction acetone:water mixture (1:1) was used instead of pyridine, sodium hydroxide or any other strong base. Compounds (**8a-g**) were prepared from the 5-amino-1,3,4-thiadiazol-2-[*N*-(substituted benzoyl)]sulphonamides (**4a-d**) (Scheme 1) and substituted benzene sulphonyl chlorides (**7a-c**) (Scheme 2). After purification, further, subjected to hydrolysis with aqueous sodium hydroxide (20 %) solution to obtain the compounds (**9a-g**) while, 5-(4-amino) benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamides

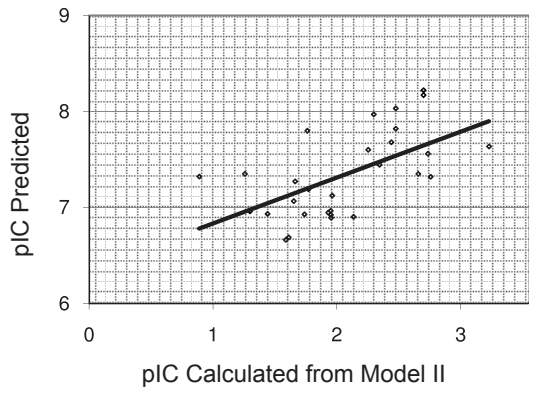


Figure 2. Predictive performance of the model II built on datasets for 29 compounds. The plots show the correlations between predicted (in cross validation) versus experimental log IC values.

(**10a-b**) were obtained by hydrolysis of (**9**a and **9**g) using 70 % sulphuric acid (Scheme 3).

The FT-IR spectra show S=O stretching at 1070 –1030, sulphonamide stretching at1177-1125 cm^{-1}, sulphonamide N-H stretching at 3385-3265 cm^{-1}, C-S stretching at 712-662 cm^{-1}, S=O stretching at 1128-1026 cm^{-1}, but C = O stretching peak at 1687-1513 cm^{-1} is absent in compounds (**10a-b**). The ^{1}H-NMR spectra of all compounds indicated expected peaks in the region of 1.249-1.254 δ ppm singlet of Ar-SO_2NH, while multiplets of aromatic ring are in the range of 6.6-8.2 δ ppm. Thin layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion.

Pharmacology

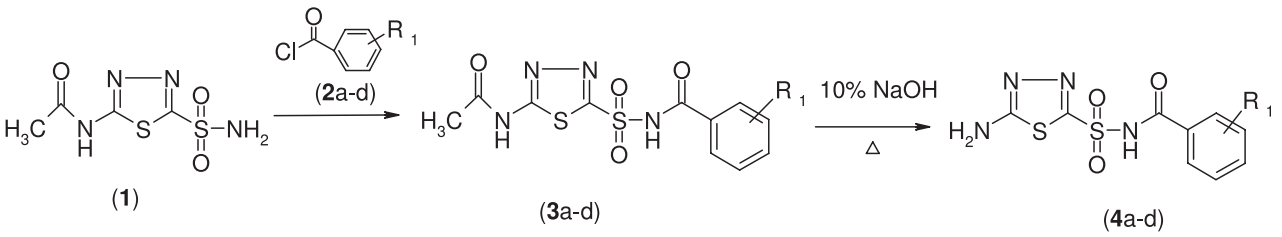
The synthesized derivatives obtained from the reaction sequence were administered orally into mice and evaluated for their antiepileptic activity in maximal electroshock (MES) [28-29] and subcutaneous pentylenetetrazole (scPTZ)[30] using a dose of 100 mg/kg. The same derivatives were also studied for their diuretic activity in conscious rats, by collecting urine for four hrs. The results of antiepileptic and diuretic activity are shown in Table 5 and Table 6 respectively.

Results and Discussion

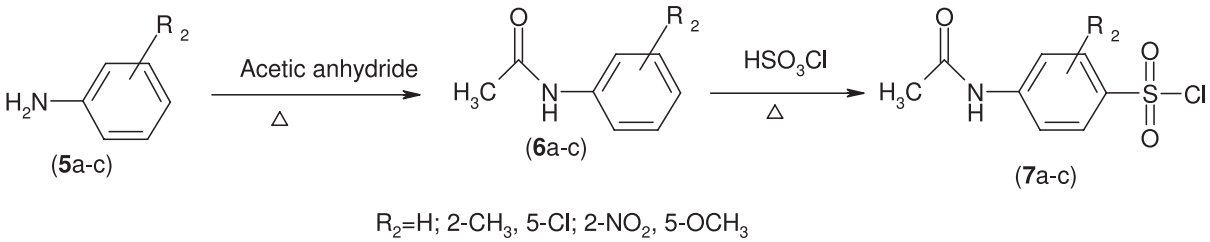
The descriptors of 1,3,4-thiadiazole were found to have good correlation with biological activity for designing antiepileptic agent which are summarised in Table 3. Herein the result of QSAR study for antiepileptic activity of the mentioned series are reported.

The Model II tested for 29 compounds as a test set. The predicted activity shows linear relationship with observed activity in the test set (R=0.9310) showing the robustness of the model. Henry's law constant (HLC), lowest unoccupied molecular orbital energy (LUMO) and *Van der Waals* energy (VDWE) have better correlation with biological activity and have low value of standard deviation. The data show (Model II) overall significant level greater than a 99.9 as it exceeded the tabulated F value and Q^2 value found to be greater than 0.5. The equation was validated by leave one out cross validation method and bootstrapping method as an internal validation, which gives statistically significant values.

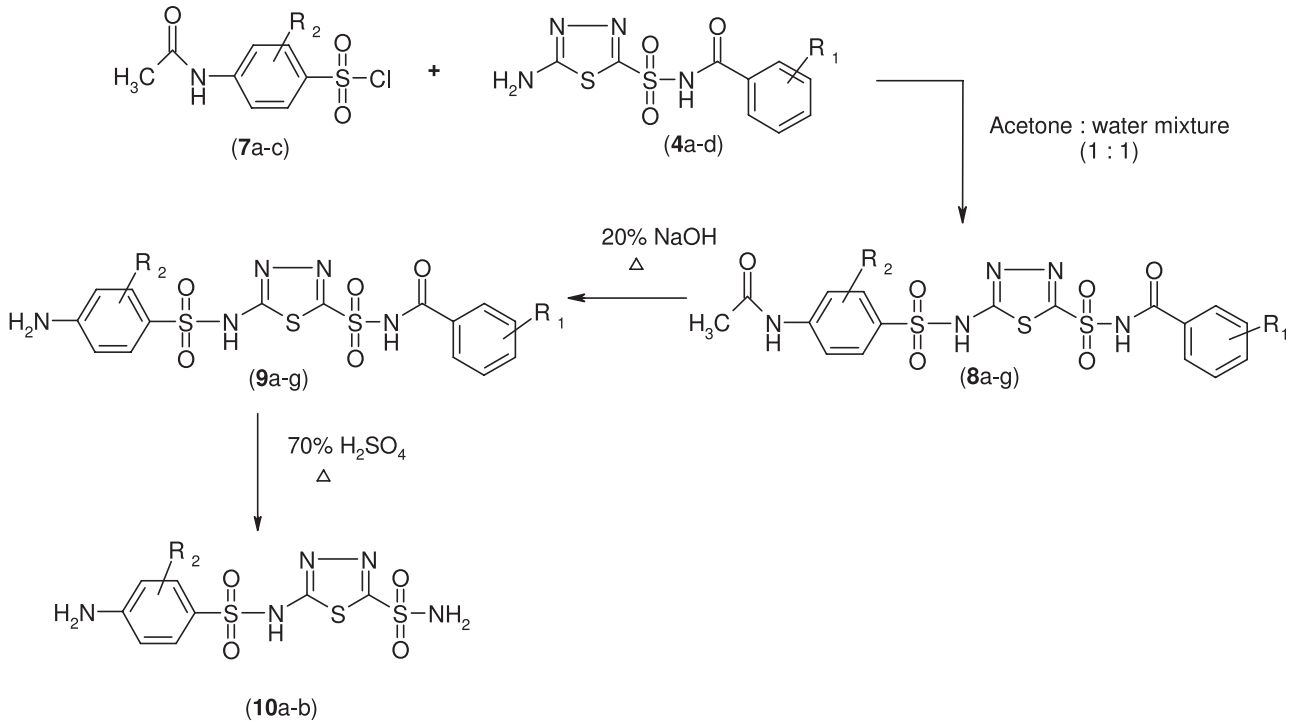
Epileptic mediators are membrane based and increasing the lipophillic nature of antiepileptic drug molecule may improve its pharmacokinetic and pharmacodynamic properties. Increase in lipophilicity and minimum electronic interference increases antiepileptic activity suggested by positive value of HLC, VDWE and negative value of LUMO, as former two are molecular properties describing thermodynamic and later one is molecular property describing electronic parameters of the moiety. Normally both of parameters are referred to certain groups, thus substitution of bulkier, lipophillic, aromatic group will contribute to



Scheme 1.



Scheme 2.



Compd. No.	R_1	R_2	Compd. No.	R_1	R_2
8a	H	H	**9b**	4-Cl	H
8b	4-Cl	H	**9c**	4-NO_2	H
8c	4-NO_2	H	**9d**	3-NO_2	H
8d	3-NO_2	H	**9e**	H	2-CH_3, 5-Cl
8e	H	2-CH_3, 5-Cl	**9f**	4-Cl	2-CH_3, 5-Cl
8f	4-Cl	2-CH_3, 5-Cl	**9g**	H	5-OCH_3, 2-NO_2
8g	H	5-OCH_3, 2-NO_2	**10a**	--	H
9a	H	H	**10b**	--	5-OCH_3, 2-NO_2

Scheme 3.

HLC and VDWE. Furthermore, reduced electron density of $–NH_2$ (amine and sulphonamide) increases the electrostatic attraction for receptor. The LUMO descriptor indicates the strength and orientation behaviour of molecule in an electrostatic field. It is also important in determining the behaviour of the molecule in vicinity of molecule. Based on the above results, some new derivatives of 1,3,4-thiadiazole were designed and synthesized, accordingly as depicted in Scheme 1, 2 and 3.

In this series, all the analogues show more potent anticonvulsant activity while diuretic activity is absent. In the earlier reports it was highlighted that the only sulphonamides, unsubstituted on the sulphonamide nitrogen in acetazolamide[8-9] are highly active as carbonic anhydrase inhibitors. It is also reported that presence of electron rich atom/group attached at the para position of the aryl ring showed increased potency in the MES screen.[31-32]

All the tested compounds were found to exhibit anticonvulsant activity in MES screening; however, compound **8c** is more potent while compound **10b** shows potency similar to standard drug (Phenytion). All the synthesized compounds were active in MES screen and showed recovery without any mortality. Compound **8c** displayed activity in the MES screen with recovery of animals in 130.30 sec while compound **8g** showed 100% incidence of recovery from mortality in the scPTZ test since animals does not produce any clonic convulsions. This compound exhibited rapid onset of action and long duration of activity. The most active compound in the scPTZ test, a test used to identify compound that elevates seizure threshold, were **8d** and **8g**. Experimental results indicated that our compound exhibited better anticonvulsant activity as compared to diuretics so could be better drugs compared to acetazolamide in treatment of convulsions (Table 5). All the compounds were screened for diuretic activity. In the diuretic study, compounds showed decrease in urine output up to 80% when compared to acetazolamide as reported in Table 6. Generally compounds possessing higher log p value showed higher decrease in diuretic activity. Bulkier compounds are more lipophillic and can cross blood brain barrier to exert their

Table 5. Effect of the synthesized compounds on MES induced and scPTZ induced convulsions in mice.

Treatment	Dose (mg/kg)	MES Test Time Duration (in Sec)					scPTZ Test Time Duration (in Sec)	
		flexion	extension	Clonus	stupor	Recovery	Onset of clonic convulsions in sec	Incidence of protection against mortality (%)
Solvent Control		5.87 ±0.56	54.15±5.61	15.75±1.02	147.2±6.05	204.4	142.4±12.9	00
Phenytoin	100	3.23±0.49***	10.20±0.67***	9.23±1.78*	60.03±3.88***	126.2	A	100
Acetazolamide	100	4.03±0.78*	12.46±1.62*	11.29±1.55*	85.19±4.02***	182.67	487.17±29.08	16.6
8a	100	3.83±0.86*	10.45±0.79***	8.83±0.51***	62.86±3.80***	143.72	664.4 ± 74.1*	66.6
8b	100	3.97±0.93*	10.56±1.19*	10.76±0.64***	58.85±3.76***	137.43	NT	–
8c	100	3.05±0.77*	11.35±1.54*	9.80±1.54*	63.50±5.29***	113.30	369.16±23.64	16.6
8d	100	4.15±0.96*	10.48±0.77***	11.00±1.02*	70.96±4.83***	130.30	779.9 ± 52.7*	83.3
8e	100	2.92±0.67*	11.49±0.88***	11.11±0.81***	65.30±4.00***	129.51	570.16±39.64***	66.66
8f	100	3.50±0.93*	11.04±1.01*	12.24±1.53*	71.71±3.66***	158.64	503.16±27.30*	50
8g	100	2.80±0.59*	10.88±1.05*	11.22±0.71***	68.15±4.23***	132.67	A	100
9a	100	4.07±0.97*	11.55±1.43*	11.61±1.32*	67.47±3.01***	172.10	440.16±40.97	16.6
9b	100	3.02±0.62*	10.90±1.00*	9.65±1.88*	62.02±6.53***	155.33	NT	–
9c	100	3.57±0.66*	11.29±0.94***	10.12±0.85***	64.85±3.08***	163.98	396.84±41.87	33.33
9d	100	3.42±0.81*	12.47±1.61*	10.46±1.16*	59.06±4.70***	164.40	NT	–
9e	100	3.52±0.64*	11.09±0.76***	9.95±1.27*	63.81±3.76***	157.67	NT	–
9f	100	3.62±0.86*	11.45±0.81***	11.46±1.63*	67.85±5.99***	144.71	780.0±12.4	66.6
9g	100	3.52±0.55*	12.60±1.53*	10.97±1.41*	67.82±6.46*	176.20	NT	–
10a	100	4.13±0.71*	13.13±1.12***	11.69±1.18*	66.64±4.33***	134.87	629.73±32.7	66.6
10b	100	4.07±0.79*	11.44±1.37*	10.39±1.22*	55.96±3.09***	127.29	667.6±95.0	50

Values are expressed as mean±SEM, n=6,
P<0.05* and <0.001*** as compared to control group,
A: Absence of convulsions, NT: Not tested

Table 6. Diuretic activity of the synthesized compounds.

Treatment[a]	Body weight of five rats	Normal saline intake (mL)	Urine output (mL)[b]	% Output	T/C	T/S	% Diuretic activity[c]	% Reduction in Diuretic Activity[d]
Control	730	36.5	7.2	19.73	1.0			
Acetazolamide	660	33	20.7	62.73	3.18	1.0	100	0
8a	690	34.5	3.8	11.01	0.56	0.184	18.4	81.6
8f	542	27.1	2.9	10.7	0.54	0.14	14	86
9e	769	38.5	5.3	13.77	0.697	0.256	25.6	74.4
9h	680	34	4.1	12.06	0.569	0.198	19.8	80.2
10a	687	34.4	12.4	36.047	1.72	0.599	59.9	40.1

[a] The animals of each groups received normal saline (5 mL/100 g p.o.). Except the control group, the animals of each group received the dose of 45 mg/kg. n=6,
[b] Urine volume collected in 4 hr,
[c] Diuretic activity = (T/S)×100,
[d] Compared to standard
T/C = ratio of test to control,
T/S = ratio of test to standard

effect at CNS. Present study explored that substitution of 1,3,4-thiadiazoles at third position and sulphonamido moiety at fifth position leads to the development of new chemical entities with potent anticonvulsant activity as compared to diuretic activity.

Seizures are caused by abnormal stimulation of nerves in the brain by other nerves. Generally, anticonvulsants reduce the excitability of the neurons (nerve cells) of the brain. When neuron excitability is decreased, seizures are theoretically reduced in intensity and frequency of occurrence or, in some instances, are virtually eliminated.[1] CA isozymes, which are strongly inhibited by aromatic and heterocyclic sulphonamides. hCA I and hCA II isoenzyme inhibitors involved in the anticonvulsant activity, shown by many sulphonamide drugs with CA inhibitory properties.[4-7]

The synthesized compounds showed antiepileptic effect, may be due to their inhibitory effect on brain carbonic anhydrase; specifically inhibition of hCA I and hCA II isoenzyme, leads to an increased transneuronal chloride gradient, increased chloride current, and increased inhibition.

Experimental Protocols

QSAR Study

a) The dataset and parameters. The CA inhibition data of sulphonamides and 1,3,4-thiadiazole data have been reported in terms of inhibitory concentration of 50% of enzyme inhibition (IC_{50} in micromoles). The inhibition data were converted to negative logarithmic values (concentration in moles). These values were used for subsequent QSAR analyses as response variable. The models for CA-IV inhibition were constructed based on the training set and the generated models were then validated: internally (using the leave one out technique) and externally (predicting the activities of the test set).[28-30,33] Molecular structure were generated and optimised with CS Chem Draw Ultra 6.0 and Chem 3D Ultra (Cambridge Soft.) respectively,[34] first by molecular mechanics (MM2) and re-optimised by MOPAC-AM1 until the root mean square (RMS) gradient value becomes smaller than 0.0001 kcal/mol·Å.

b) Statistical computation. The relationship between response variable (as a dependent variable) and various physicochemical as well as structural descriptors (as independent variables), were established by step-wise linear multiple regression analysis using SYSTAT 10.2 and VALSTAT running on a Pentium 4 processor (CPU 3.0 GHz HT).[35-36] Significant descriptors were chosen on the basis of statistical data of analysis.[37-38]

Model I

pIC=[5.3164(±0.4683)]+HLC[0.3005(±0.1034)]+LUMO[-0.7018(±0.3847)]+VDWE [0.0835(±0.0522)]

N=30, *R*=0.9186, R^2=0.8439, *variance*=0.1040, *SD*=0.3225, *F*=46.8575, Q^2=0.7669, S_{PRESS}=0.3941, S_{DEP}=0.3669

This model has an outlier (compound Im) because their residual values exceeded twice the standard error of estimate. When this outlier was removed from the dataset, a significant Model II has been found which is able to explain 0.0829 of variance of CA-IV inhibition. This model has a high internal predictivity as shown by the good Q^2 value of 0.8066.

Model II

pIC=[5.3104(±0.4189)]+HLC[0.2875(±0.0930)]+LUMO[-0.7033(±0.3441)]+VDWE [0.0864(±0.0467)]

N=29, R=0.9310, R^2=0.8668, variance=0.0829, std=0.2879, F=54.219, Q^2=0.8066, S_{PRESS}=0.3633, S_{DEP}=0.3374

The parameters used in the model were almost independent, which can be seen from the Pearson correlation matrix (Table 4, Figure 2). Two best models were selected from series, out of which Model II is selected as the best. Since, this model has better statistically significant value, minimum standard deviation, low variance, and statistically significant F value.

Synthetic Study

Melting point was determined in one end open capillary tubes on a Thermonik Precision melting point apparatus (C-PMP-2, Mumbai, India) and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Shimadzu FT-IR 8400s spectrophotometer in KBr. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Varian EM-390 apparatus. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis (C, H, N and S) were undertaken with Elemental Vario EL III Carlo Erba 1106 analyzer. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates and a solvent system of benzene: ethanol (8:2). The spots were developed in iodine chamber and visualized under ultra violet lamp. The log p values were determined using ChemDraw software

General procedure. 5-(4-Amino)benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide was synthesized in following sequence:

*5-[(4-Acetamido)benzenesulphonamido]-1,3,4-thiadiazol-2-(N-benzoyl)-sulphonamide (**8a**).* [5-Amino-1,3,4-thiadiazol-2-[*N*-benzoylsulphonamide] (**4a**) was suspended in a 1:1 mixture of acetone-water, and the stoichometric amount of 4-acetamidobenzenesulphonyl chloride (**7a**) and base was added concomitantly. The reaction mixture was magnetically stirred for several hours, the solvent was evaporated, then the p*H* was adjusted to 2 with 5N hydrochloric acid and the recrystallised from aqueous ethanol.

*5-[(4-Amino)benzenesulphonamido]-1,3,4-thiadiazol-2-(N-benzoyl)sulphonamide (**9a**).* The synthesized compound, (**8a**) (2.0 g, 0.0041 mol) was dissolved in 20 % aqueous sodium hydroxide and refluxed for 45 minutes, allowed to cool and acidified with dilute hydrochloric acid, to precipitate out the hydrolyzed product (**9a**), which was separated by filtration at vacuum, and recrystallised.

*5-(4-Amino)benzenesulphonamido-1,3,4-thiadiazol-2-sulphonamide (**10a**).* The compound (**8a**) (2.0 g, 0.0042 mol) was refluxed with 10-15 mL of 70 per cent sulphuric acid (3:4 by volume) for 30 minutes, allowed to cool, filtered off and rendered the filtrate alkaline with 10-20 per cent sodium hydroxide solution. The precipitate thus formed was filtered at vacuum and recrystallised to obtained compound (**10a**).

Adopting the above procedures, other compounds (**8b-g**), (**9b-g**) and (**10b**) were synthesized and characterized. The characterized data of these compounds are as follows:

*N-[(5-amino-1,3,4-thiadiazol-2-yl)sulfonyl]benzamide (**4a**).* Yield 76 %, m.p. 234^{0}C, R_f 0.61. *m/z* (GC-MS): 268 (100%, Base peak, M^+). Found: C 38.04, H 2.82, N 19.72, S 22.57 %. $C_9H_8N_4O_3S_2$ (284.31) requires C 38.02, H 2.84, N 19.71, S 22.56 %. FT-IR (KBr) ν_{max} cm^{-1}: 3446-3368 (N-H), 3193-3002 (Ar C-H), 1685 (C=O), 683-588 (C–S), 1072 –1026 (S=O), 1326, 1178 (sulphonamide), 3070-3002 (heteroaromatic C-H), 1685-1629 (heteroaromatic C=N). ^{1}H NMR (DMSO-d_6) δ_H ppm: 3.320 (s, 1H, CONH), 7.018-7.853 (m, 5H, Ar-H), 8.025 (s, 1H, thiadiazole, C-H), 8.611 (s, 1H, acetamido-H).

*N-{[5-({[4-(acetylamino)phenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl]sulfonyl}benzamide (**8a**).* Yield 32.54 %, m.p. 216^{0}C, R_f 0.54, LogP 2.159. Found: C 42.44, H 3.12, N 14.55, S 19.87 %. $C_{17}H_{15}N_5O_6S_3$ (481.52) requies C 42.40, H 3.14, N 14.54, S 19.98 %. *m/z* (GC-MS): 458 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3520-3400 (N-H), 3110-3012 (Ar C-H), 1700-1680 (amide C=O), 700-600 (C–S), 1070-1030 (S=O), 1370-1335, 1170-1155 (sulphonamide), 3265 (sulphonamide N-H), 3077-3003

(heteroaromatic C-H), 3500-3220 (heteroaromatic N-H), 1640 (heteroaromatic C=N). λ_{max} (chloroform) (lgε)/nm 235. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.103 (s, 1H, Ar-SO_2H), 2.378 (s, 3H, CH_3), 3.320 (s, 1H, CONH), 4.740 (s, 1H, N-H), 7.281- 7.785 (m, 6H, Ar-H), 8.025 (s, 1H, thiadiazole, C-H), 8.611 (s, 1H, acetamido-H). ^{13}C NMR δ_c ppm: 169.7, 168.9, 141.6, 135.5, 134.3, 128.9, 128.7, 128.7, 127.5, 127.5, 127.4, 122.0, 121.9, 121.8, 23.1.

*N-{[5-({[4-(acetylamino)phenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-chlorobenzamide (**8b**)*. Yield 40.12 %, m.p. 224^{0}C, R_f 0.62, LogP 2.717. Found: C 39.56, H 2.74, N 13.57, S 18.63 %. $C_{17}H_{14}N_5O_6S_3Cl$ (515.97) requires C 39.57, H 2.73, N 13.57, S 18.64 %. *m/z* (GC-MS): 462 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3567-3385 (N-H), 3108-3045 (Ar C-H), 1661-1525 (amide C = O), 760-636 (C–S), 1097-1026 (S=O), 1383, 1330, 1177, 1141 (sulphonamide), 3385 (sulphonamide N-H), 2920 (CH_3-O), 1531-1482 (Ar C-NO_2), 852 (Ar C-N) 543 (C-Cl). λ_{max} (chloroform) (lgε)/nm 238. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.217 (s, 1H, Ar-SO_2H), 2.332 (s, 3H, CH_3), 3.496 (s, 1H, CONH), 4.740 (s, 1H, N-H), 7.242- 7.453 (m, 3H, Ar-H), 8.009 (s, 1H, thiadiazole, C-H), 8.036 (s, 1H, Acetamido-H). ^{13}C NMR δ_c ppm: 170.1, 169.1, 141.0, 137.3, 135.4, 132.2, 129.5, 129.1, 128.8, 128.7, 128.2, 127.6, 122.0, 121.8, 22.5.

*N-{[5-({[4-(acetylamino)phenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl]sulfonyl}-4-nitrobenzamide (**8c**)*. Yield 28.13 %, m.p. 242^{0}C, R_f 0.58, LogP 2.156. Found: C 38.79, H 2.69, N 15.94, S 18.27 %. $C_{18}H_{17}N_6O_8S_3$ (526.52) requires C 38.78, H 2.68, N 15.96, S 18.27 %. *m/z* (GC-MS): 531 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3367-3110 (N-H), 3110-3024 (Ar C-H), 1445 (amide C=O), 711-655 (C–S), 1128-1080 (S=O), 1348, 1313, 1184 (sulphonamide), 3238 (sulphonamide N-H), 3077-3003 (heteroaromatic C-H), 2360-2341 (heteroaromatic N-H), 1720 (heteroaromatic C=N), 1515-1452 (Ar C-NO_2), 896-852 (Ar C-N). λ_{max} (chloroform) (lgε)/nm 248. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.232 (s, 3H, CH_3), 3.541 (s, 1H, CON(-H) Ar), 7.241- 7.483 (m, 4H, Ar-H), 8.017-8.259 (m, 4H, Ar-H), 8.110 (s, 1H, acetamido N-H), 8.129 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 170.1, 168.9, 141.7, 140.3, 135.2, 128.7, 128.5, 127.4, 121.8, 121.7, 127.5, 121.3, 23.0.

N-{[5-({[4-(acetylamino)phenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl]sulfonyl}-3-nitrobenzamide (***8d***). Yield 21.87 %, m.p. 237^{0}C, R_f 0.61, LogP 1.481. Found: C 38.76, H 2.69, N 15.96, S 18.29 %. $C_{18}H_{17}N_6O_8S_3$ (526.52) requires C 38.78, H 2.68, N 15.96, S 18.27 %. *m/z* (GC-MS): 534 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3527-3415 (N-H), 3100-3000 (Ar C-H), 1531 (amide C=O), 713-619 (C–S), 1087 (S=O), 1352, 1311, 1176 (sulphonamide), 3265 (sulphonamide N-H), 3077-3003 (heteroaromatic C-H), 2341-2331 (heteroaromatic N-H), 1720 (heteroaromatic C=N), 1456-1531 (Ar C-NO_2), 827 (Ar C-N). λ_{max} (chloroform) (lgε)/nm 252. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.214 (s, 3H, CH_3), 3.452 (s, 1H, CON(-H) Ar), 7.447- 7.881 (m, 2H, Ar-H), 8.219 (s, 1H, acetamido N-H), 8.437 (s, 1H, Sulphonamido N-H), 7.904-8.724 (m, 4H, Ar-H). ^{13}C NMR δ_c ppm: 169.9, 169.1, 148.3, 141.3, 135.3, 135.1, 133.2, 129.6, 127.4, 127.2, 124.5, 122.4, 121.4, 121.4, 22.8.

N-{[5-({[4-(acetylamino)-5-chloro-2-methylphenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl] sulfonyl} benzamide (***8e***). Yield 26.60 %, m.p. 249^{0}C, R_f 0.64, LogP 3.204. Found: C 40.79, H 3.05, N 13.20, S 18.17 %. $C_{18}H_{16}N_5O_6S_3Cl$ (529.99) requires C 40.79, H 3.04, N 13.21, S 18.15 %. *m/z* (GC-MS): 519 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3398-3137-(N-H), 3107-3010-(Ar C-H), 1708-1685 (amide C=O), 692-621 (C–S), 1081-1054 (S=O), 1365, 1311, 1187 (sulphonamide), 3319 (sulphonamide N-H), 2360-2341 (heteroaromatic N-H), 1708 (heteroaromatic C=N), 592-532 (C-Cl), 972-846 (C-N), 2894 (C-H). λ_{max} (chloroform) (lgε)/nm 252. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.054 (s, 1H, C(=O)CH_3), 2.452 (s, 1H, Ar C-CH_3), 3.365 (s, 1H, CON(-H) Ar), 7.404-7.753 (m, 3H, Ar-H), 7.647- 7.818 (m, 2H, Ar-H), 8.124 (s, 1H, acetamido N-H), 8.548 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 170.1, 168.8, 140.3, 136.2, 134.2, 134.2, 132.4, 128.9, 128.8, 127.7, 127.6, 127.3, 126.3, 123.0, 22.6, 16.2.

*N-{[5-({[4-(acetylamino)-5-chloro-2-methylphenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl] sulfonyl}-4-chlorobenzamide (**8f**)*. Yield 39.44 %, m.p. 257^{0}C, R_f 0.61, LogP 3.763. Found: C 38.29, H 2.65, N 12.42, S 17.07 %. $C_{18}H_{15}N_5O_6S_3Cl_2$ (564.44) requires C 38.30, H 2.68, N 12.41, S 17.04 %. *m/z* (GC-MS): 581 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3359-3338 (N-H), 3082-3045 (Ar C-H), 1691-1629 (amide C=O), 678-669 (C–S), 1035 (S=O), 1342, 1319, 1157 (sulphonamide), 3338 (sulphonamide N-H), 2381-2341 (heteroaromatic N-H), 1785-1735 (heteroaromatic C=N), 559 (C-Cl), 960-827 (C-N), 2943 (C-H). λ_{max} (chloroform) (lgε)/nm 248. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.062 (s, 1H, C(=O)CH_3), 2.564 (s, 1H, Ar C-CH_3), 3.447 (s, 1H, CON(-H) Ar), 7.173-7.392 (m, 2H, Ar-H), 7.423- 7.588 (m, 2H, Ar-H), 7.973 (s, 1H, acetamido N-H), 8.213 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 169.8, 169.0, 140.2, 137.4, 135.9, 135.1, 132.4, 129.1, 129.1, 128.9, 128.8, 127.7, 126.4, 122.9, 23.0, 16.5.

*N-{[5-({[4-(acetylamino)-5-methoxy-2-nitrophenyl]sulfonyl}amino)-1,3,4-thiadiazol-2-yl]sulfonyl}benzamide (**8g**)*. Yield 25.30 %, m.p. 264^{0}C, R_f 0.52 LogP 0.694.. Found: C 38.88, H 2.88, N 15.12, S,, 17.27 %. $C_{18}H_{15}N_6O_9S_3$ (590.99) requires C 38.85, H 2.90, N 15.10, S 17.28 %. *m/z* (GC-MS): 541 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3537-3427 (N-H), 3124-3028 (Ar C-H), 1691-1528 (amide C=O), 717-609 (C–S), 1022-1078 (S=O), 1367, 1333, 1181, 1157 (sulphonamide), 3265 (sulphonamide N-H), 2847 (CH_3-O), 1528-1487 (Ar C-NO_2), 823 (Ar C-N stretching). λ_{max} (chloroform) (lgε)/nm 261. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.054 (s, 1H, C(=O)CH_3), 3.714 (s, 1H, CON(-H) Ar), 4.245 (s, 1H, Ar O-CH_3), 7.204-7.938 (m, 3H, Ar-H), 8.12- 8.417 (m, 2H, Ar-H), 8. 241 (s, 1H, acetamido N-H), 8.548 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 169.7, 169.1, 159.3, 138.2, 137.7, 134.5, 132.3, 131.1, 131.1, 130.8, 129.1, 128.9, 127.7, 127.6, 116.9, 116.5, 112.4, 55.9, 22.8.

*N-[(5-{[(4-aminophenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl) sulfonyl]benzamide (**9a**)*. Yield 60.27 %, m.p. 259^{0}C, R_f 0.57, LogP 2.448. Found: C 40.98, H 2.97, N 15.95, S 21.89 %. $C_{15}H_{13}N_5O_5S_3$ (439.49) requires C 40.99, H 2.98, N 15.94, S 21.89 %. *m/z* (GC-MS): 429 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3573-3295 (N-H), 3075 (Ar C-H), 1687-1513 (amide C=O), 712-662 (C–S), 1128-1026 (S=O), 1330, 1177, 1125 (sulphonamide), 3385 (sulphonamide N-H). λ_{max} (chloroform) (lgε)/nm 238. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.249 (s, 1H, Ar-SO_2NH), 3.526 (s, 2H, Ar-NH_2), 6.895-6.923 (s, 2H, Ar-unsymmetrical pattern), 7.236-8.392 (m, 3H, Ar-H), 8.143 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 170.2, 152.0, 134.4, 132.3, 129.8, 128.8, 128.7, 128.2, 128.1, 127.6, 127.6, 116.5, 116.3.

*N-[(5-{[(4-aminophenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl)sulfonyl]-4-chloro benzamide (**9b**)*. Yield 70.64 %, m.p. 267^{0}C, R_f 0.52, LogP 3.007. Found: C 38.01, H 2.57, N 14.75, S 20.29 %. $C_{15}H_{12}N_5O_5S_3Cl$ (473.93) requires C 38.01, H 2.55, N 14.78, S 20.30 %. *m/z* (GC-MS): 481 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3561-3372 (N-H), 3097-3015 (Ar C-H), 1698 (amide C=O), 763-553 (C–S), 1087-1056 (S=O), 1341, 1330, 1170, 1139 (sulphonamide), 3324-3197 (sulphonamide N-H), 782 (C-Cl). λ_{max} (chloroform) (lgε)/nm 242. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.329 (s, 1H, Ar-SO_2NH), 3.225 (s, 2H, Ar-NH_2), 6.985-7.014 (s, 2H, Ar-unsymmetrical pattern), 7.623-8.432 (m, 2H, Ar-H), 8. 344 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 170.1, 151.5, 137.5, 132.2, 130.1, 129.2, 129.1, 129.0, 128.9, 128.2, 128.1, 116.3, 116.2.

N-[(5-{[(4-aminophenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl)sulfonyl]-4-nitro benzamide (***9c***). Yield 52.06 %, m.p. 283^{0}C, R_f 0.44, LogP 1.263. Found: C 37.20, H 2.51, N 17.34, S 19.86 %. $C_{15}H_{12}N_6O_7S_3$ (484.49) requires C 37.19, H 2.50, N 17.35, S 19.86 %. *m/z* (GC-MS): 512 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3407-3126 (N-H), 3107-3010 (Ar C-H), 1708-1685 (amide C=O), 692-621 (C–S), 1515-1452 (Ar C-NO_2), 1054-1081 (S=O), 1365, 1311, 1187 (sulphonamide), 3319 (sulphonamide N-H),

2360-2341 (heteroaromatic N-H), 1708 (heteroaromatic C=N), 972-846 (C-N), 2894 (C-H). λ_{max} (chloroform) (lgε)/nm 273. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.421 (s, 1H, Ar-SO_2NH), 4.032 (s, 2H, Ar-NH_2), 6.985-7.014 (s, 2H, Ar-unsymmetrical pattern), 6.634-7.327 (m, 2H, Ar-H), 8.611 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 169.9, 151.9, 151.4, 140.2, 129.2, 128.6, 128.5, 128.4, 128.1, 121.3, 121.3, 116.4, 116.2.

*N-[(5-{[(4-aminophenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl)sulfonyl]-3-nitro benzamide (**9d**)*. Yield 46.41 %, m.p. 274^{0}C, R_f 0.47, LogP 1.151. Found: C 37.19, H 2.51, N 17.36, S 19.87 %. $C_{15}H_{12}N_6O_7S_3$ (484.49) requires C 37.19, H 2.50, N 17.35, S 19.86 %. *m/z* (GC-MS): 519 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3412-3197 (N-H), 3107-2925 (Ar C-H), 1712-1654 (amide C=O), 723-681 (C–S), 1487-1411 (Ar C-NO_2), 1109-1020 (S=O), 1368, 1333, 1173 (sulphonamide), 3320 (sulphonamide N-H), 2378-2331 (heteroaromatic N-H), 1712 (heteroaromatic C=N), 2862 (C-H). λ_{max} (chloroform) (lgε)/nm 265. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1. 495 (s, 1H, Ar-SO_2NH), 3. 503 (s, 2H, Ar-NH_2), 6. 985-7.014 (s, 2H, Ar-unsymmetrical pattern), 6. 436-7. 273 (m, 2H, Ar-H), 7.932 (s, 1H, Sulphonamido N-H). ^{13}C NMR δ_c ppm: 169.8, 151.4, 148.3, 135.2, 133.4, 129.9, 129.6, 128.4, 128.1, 124.3, 122.3, 116.6, 116.3.

*N-[(5-{[(4-amino-5-chloro-2-methylphenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl) sulfonyl] benzamide (**9e**)*. Yield 68.62 %, m.p. 263^{0}C, R_f 0.48, LogP 3.494. Found: C 39.40, H 2.88, N 14.34, S 19.70 %. $C_{16}H_{14}N_5O_5S_3Cl$ (487.96) requires C 39.38, H 2.89, N 14.35, S 19.71 %. *m/z* (GC-MS): 495 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3467-3178 (N-H), 3102-2936 (Ar C-H), 1734-1686 (C=O), 713-690 (C-S), 559-522 (C-Cl), 1134-1047(S=O), 1387, 1324, 1157 (Sulphonamide), 3370 (sulphonamide N-H), 2395 (heteroaromatic N-H), 1784 (heteroaromatic C=N), 2912 (C-H stretching). λ_{max} (chloroform) (lgε)/nm 272. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.249 (s, 1H, Ar-SO_2NH), 3.990 (s, 3H, Ar-CH_3), 3.526 (s, 2H, Ar-NH_2), 6.895-6.923 (s, 2H, Ar-unsymmetrical pattern), 7.236-7.581 (m, 6H, Ar-H), 7.604-7.735 (s, 2H, NH_2), 8.367-8.392 (s, 1H, thiadiazole, C-H). ^{13}C NMR δ_c ppm: 170.1, 147.6, 135.6, 134.3, 132.2, 130.6, 128.5, 128.5, 127.8, 127.7, 127.3, 122.6, 117.9, 16.5.

*N-[(5-{[(4-amino-5-chloro-2-methylphenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl) sulfonyl]-4-chlorobenzamide (**9f**)*. Yield 53.29 %, m.p. 268^{0}C, R_f 0.58, LogP 4.052. Found: C 39.80, H 2.51, N 14.43, S 18.42 %. $C_{16}H_{13}N_5O_5S_3Cl_2$ (522.41) requires C 39.79, H 2.51, N 13.41, S 18.41 %. *m/z* (GC-MS): 516 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3407-3126 (N-H), 3107-3010 (Ar C-H), 1708-1685 (amide C=O), 692-621 (C–S), 1054-1081 (S=O), 1365, 1311, 1187 (sulphonamide), 3319 (sulphonamide N-H), 2360-2341 (heteroaromatic N-H), 1708 (heteroaromatic C=N), 972-846 (C-N), 2894 (C-H), 592-532 (C-Cl). λ_{max} (chloroform) (lgε)/nm 285. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.071 (s, 1H, Ar-SO_2NH), 4.139 (s, 3H, Ar-CH_3), 4. 652 (s, 2H, Ar-NH_2), 6. 539-7.214 (s, 2H, Ar-unsymmetrical pattern), 7.623-7.851 (m, 6H, Ar-H), 7.046-7.973 (s, 2H, NH_2), 8.534-8.743 (s, 1H, thiadiazole, C-H). ^{13}C NMR δ_c ppm: 169.8, 147.5, 137.7, 135.6, 132.2, 130.5, 129.0, 129.0, 128.8, 128.8, 127.7, 122.4, 117.8, 16.9.

*N-[(5-{[(4-amino-5-methoxy-2-nitrophenyl)sulfonyl]amino}-1,3,4-thiadiazol-2-yl) sulfonyl]benzamide (**9g**)*. Yield 21.24 %, m.p. 252^{0}C, R_f 0.47, LogP 4.006. Found: C 37.37, H 2.71, N 16.33, S 18.72 %. $C_{16}H_{13}N_6O_8S_3Cl$ (514.51) requires C 37.35, H 2.74, N 16.33, S 18.70 %. *m/z* (GC-MS): 523 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3359-3338 (N-H), 3082-3045 (Ar C-H), 1691-1629 (amide C=O), 678-669 (C–S), 1035 (S=O), 1342, 1319, 1157 (sulphonamide), 3338 (sulphonamide N-H), 2381-2341 (heteroaromatic N-H), 1515-1452 (Ar C-NO_2), 1785-1735 (heteroaromatic C=N), 559 (C-Cl), 960-827 (C-N), 2943 (C-H). λ_{max} (chloroform) (lgε)/nm 288. ^{1}H NMR (DMSO-d_6) δ_H ppm: 2.146 (s, 1H, Ar-SO_2NH), 3.927 (s, 3H, Ar-OCH_3), 4.201 (s, 2H, Ar-NH_2), 6.539-7.412 (s, 2H, Ar-unsymmetrical pattern), 7.023-7.851 (m, 5H, Ar-H), 6.957-7.077 (s, 2H, NH_2). ^{13}C NMR δ_c ppm: 170.0, 154.1, 140.8, 138.4, 134.3, 132.2, 128.6, 128.4, 127.6, 127.3, 125.3, 115.6, 112.8, 56.1.

*5-{[(4-Aminophenyl)sulfonyl]amino}-1,3,4-thiadiazole-2-sulfonamide (**10a**)*. Yield 42.04 %, m.p. 194^{0}C, R_f 0.60, LogP 0.840. Found: C 28.67, H 2.71, N 20.89, S 28.68 %. $C_8H_9N_5O_4S_3$ (335.38) requires C 28.65, H 2.70, N 20.88, S 28.68 %. *m/z* (GC-MS): 319 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3398-3378 (N-H), 3120-2793 (Ar C-H), 706 (C–S), 1080-1026 (S=O), 1358, 1293, 1080 (sulphonamide), 3320 (sulphonamide N-H), 2378-2351 (heteroaromatic N-H), 1686 (heteroaromatic C=N). λ_{max} (chloroform) (lgε)/nm 260. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1.254 (s, 1H, Ar-SO_2NH), 7.228-8.123 (m, 6H, Ar-H), 8.611 (s, 1H, sulphonamido-H). ^{13}C NMR δ_c ppm: 151.4, 129.9, 128.2, 128.1, 116.4, 116.3.

*5-{[(4-Amino-5-methoxy-2-nitrophenyl)sulfonyl]amino}-1,3,4-thiadiazole-2-sulfonamide (**10b**)*. Yield 34.58 %, m.p. 222^{0}C, R_f 0.65, LogP -1.174. Found: C 26.36, H 2.47, N 20.49, S 23.43 %. $C_9H_{10}N_6O_7S_3$ (410.41) requires C 26.34, H 2.46, N 20.48, S 23.44 %. *m/z* (GC-MS): 396 (100%, Base peak, M^+). FT-IR (KBr) ν_{max} cm^{-1}: 3358-3298 (N-H), 3127-2761 (Ar C-H), 726 (C–S), 1078-1014 (S=O), 1532-1476 (Ar C-NO_2), 1358, 1293, 1080 (sulphonamide), 3320 (sulphonamide N-H), 2398-2371 (heteroaromatic N-H), 1677 (heteroaromatic C=N), 569-527 (C-Cl), 2873 (C-H). λ_{max} (chloroform) (lgε)/nm 278. ^{1}H NMR (DMSO-d_6) δ_H ppm: 1. 425 (s, 1H, Ar-SO_2NH), 3.108(s, 3H, OCH_3), 3.527 (s, 2H, Ar-NH_2), 7.334-8.312 (m, 2H, Ar-H), 8.611 (s, 1H, sulphonamido-H). ^{13}C NMR δ_c ppm: 153.1, 140.2, 138.7, 125.3, 115.2, 112.9, 56.1.

Pharmacology

The anticonvulsant evaluations were undertaken using reported procedure.[28-30,33] Male albino mice (CF-1 strain or swiss, 25-35 g) and rats (Sprague-Dawley or Wistar, 100-150 g) were used as experimental animals. The tested compounds were suspended in polyethylene glycol 400.

Anticonvulsant screening. Initially all the compounds were administered orally in a volume of 100 mg/kg body weight of mice, 60 min prior to the electroshock. The electroshock induced in animal by passing a current of 45 mA for 0.2 sec duration through electroconvulsiometer (Techno India) using corneal electrodes. The following phases in sequence and time in each phase (in seconds) was noted.[39-41]

1. Tonic flexon	:	Contraction of muscle throughout the body and forelimbs.
2. Tonic extension	:	Extension of extremities.
3. Clonus	:	Stage of relaxation after extension.
4. Stupor	:	Stage of unconsciousness before recovery (Generally more than one minute).

While in scPTZ test, all the drugs were administered orally 30 min prior to the administration of pentylenetetrazole (6 mg/kg) by subcutaneous injection. The animals were observed for 1 and 3 hour by placing in a separate cage. The duration of seizures (tonic-clonic convulsions) were recorded.[42] Activity was established using the MES and scPTZ test and these data are presented in Table 5.

Diuretic activity. The Lipschitz method was employed for the assessment of diuretic activity.[43] The rats were deprived of food for 18 h and tap water *ad libitum* was allowed. The urine was collected quantitatively for a total period of four hours and the urine collected of initially of 20 min. was discarded.[44] The effect of the compounds was compared with standard and the control group. The ratio (T/C, T/S) of urine volume of treated and control group was determined.[45-46] The results are shown in Table 6**.**

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