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Synthesis and anticonvulsant activity of sulfonamide derivatives-hydrophobic domain

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Abstract—A series of sulphonamide derivatives (**1**–**11**) were synthesized in good yield and evaluated for their possible anticonvulsant activity and neurotoxic study. The structures of the synthesized compounds were confirmed on the basis of their spectral data and elemental analysis. Majority of the compounds were active in MES and scPTZ tests. All the compounds were less toxic than the standard drug phenytoin.

Epilepsy has been recognized as a neurological disorder, affecting a large section of people both male and female across the world. Every year approximately 2,50,000 new cases are added to this figure. Many patients have seizures that are resistant to the available medical therapies. Newer drugs such as flupirtine,[1] topiramate,[2] zonisamide,[3] and vigabatrin[4] have emerged as promising anticonvulsants. Despite introduction of these new drugs women of child bearing age and chronic patients face specific problems of neurotoxicity, symptoms of depression, and CNS related ailments. Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy.

Quite recently, semicarbazones are an emerging new class of potent anticonvulsants.[5,6] More than 300 compounds have been prepared and tested in animal models. Representative semicarbazones, like 4-bromobenzaldehyde semicarbazone, have been shown to have activity comparable with or exceeding that of phenytoin (Dilantin) in the maximal electroshock (MES)-induced seizure test in mice. The MES test is a model, for example, generalized tonic–clonic seizures and identifies compounds that prevent seizure spread and phenytoin is considered as the prototypical in this model. Zonisamide is a sulfonamide derivative, which is indicated as an adjunct for partial seizures in patients over 12 years where seizures are not controlled by first line drugs. During the study of semicarbazones as potential anticonvulsant agents, Pandeya[7] has proposed the identifiable features for anticonvulsant activity like (i) hydrophobic aryl ring, (ii) a hydrogen bonding domain, (iii) an electron-donor group, and (iv) another distal hydrophobic site. To test this hypothesis the size of the hydrophobic aryl ring has been varied to include indole nucleus with active compounds.

In the present study, we describe the synthesis of phenyl and benzothiazoles as potential hydrophobic aryl rings and inclusion of sulfamido functionality resembling the structure of zonisamide. The thioureido moiety was introduced in the structures to increase the lipophilicity of the molecules.

Synthesis of *N*-(6-substituted-1,3-benzothiazol-2-yl)-4-{[(substituted amino) carbonothioyl] amino} benzene sulfonamides (**1**–**6**) involves condensation of the 2-amino-6-substituted benzothiazoles with *p*-acetamidobenzenesulfonyl chloride to get condensed product *N*-(4-{[(6-fluoro-1,3-benzothiazol-2-yl) amino] sulfonyl} phenyl) acetamide. 2-Amino-6-substituted benzothiazoles were synthesized according to the known protocol.[8] The acetamido group was hydrolyzed with acetic acid to give free amino group. Further the free amino group was condensed with different isothiocyanates to get the

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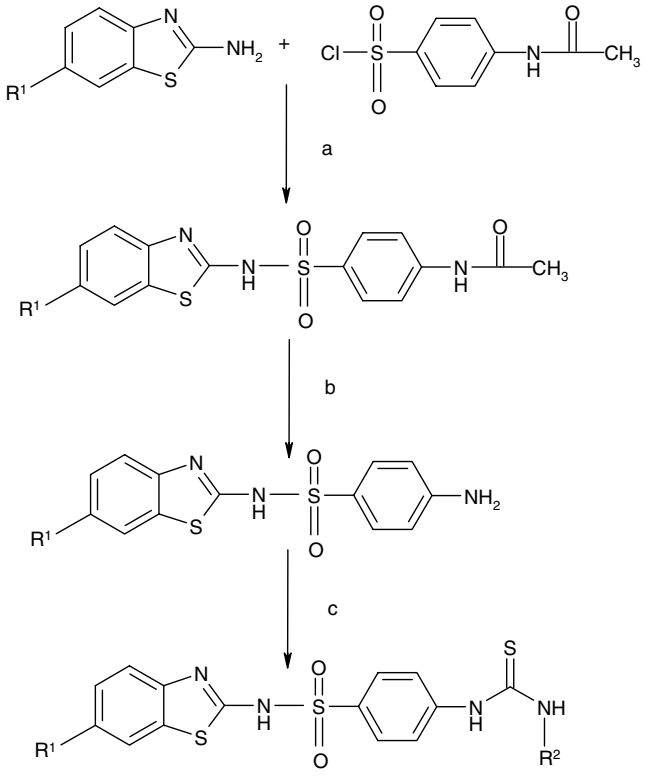
desired compounds[9] (**1**–**6**) (Scheme 1). Similarly *N*-(3-substituted phenyl)-4-{['substituted' amino carbonothionyl] amino} benzene sulfonamides[10] (**7**–**11**) were prepared from *p*-substituted anilines according to Scheme 2. All the synthesized compounds were well characterized by elemental analysis and spectroscopic data. The physical data of all the synthesized compounds are given in (Table 1).

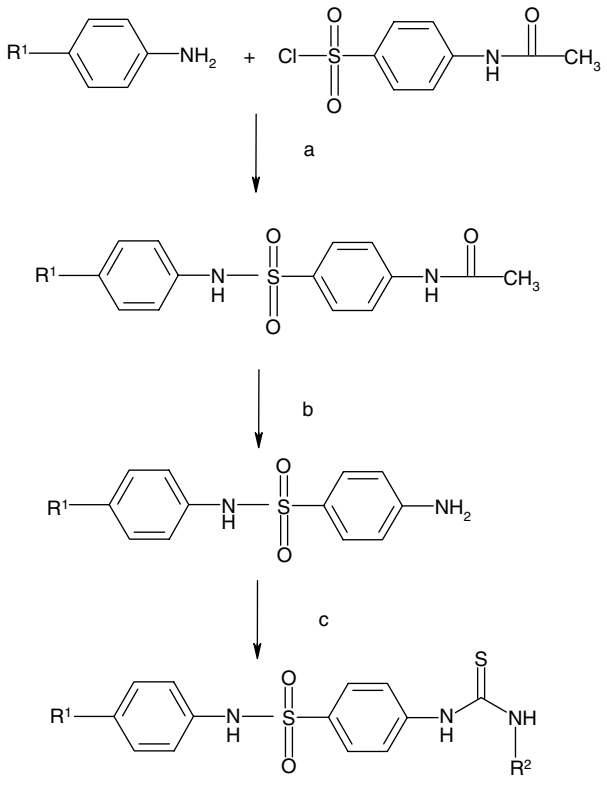
The anticonvulsant evaluation was undertaken using reported procedures.[11,12] Male albino mice (CF-1 strain or Swiss, 18-258) and rats (Spraque–Dawley or Wistar, 100–150 g) were used as experimental animals. The tested compounds were suspended in 0.5% methyl cellulose–water mixture or in polyethylene glycol (PEG).

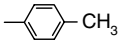
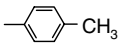
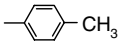
MES—maximal electroshock seizure test: maximal electroshock seizures were elicited with a 60 cycle altering current of 50 mA intensity (5–7 times that necessary to elicit minimal electroshock seizures) delivered for



	R^1	R^2
1	F	CH_3
2	F	C_2H_5
3	Cl	CH_3
4	Cl	C_2H_5
5	OCH_3	CH_3
6	OCH_3	C_2H_5

Scheme 1. Reagents and conditions: (a) pyridine/Ac_2O; (b) CH_3COOH (80%) boiling 6 h; (c) R^2 NCS, EtOH/reflux, 2 h.



	R^1	R^2
7	Cl	CH_3
8	Cl	
9	Br	CH_3
10	Br	
11	CH_3	

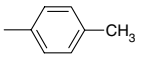
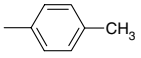
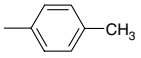
Scheme 2. Reagents and conditions: (a) pyridine/Ac_2O; (b) CH_3COOH (80%) boiling 6 h; (c) R^2 NCS, EtOH/reflux, 2 h.

0.25 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extensor component of the seizure is defined as protection, and results are expressed as number of animal protected/number of animals tested.

NT—neurotoxicity: the rotorod test was used to evaluate neurotoxicity. The animal was placed on a 3.2 cm diameter Knurled rod rotating at 6 rpm. Normal mice can remain on a rod rotating at this speed indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min. and is expressed as number of animals exhibiting toxicity/number of animals tested.

The sulfonamido benzothiazoles were initially screened at 30, 100, and 300 mg/kg intraperitoneally in mice for anticonvulsant activity (Table 2). All

Table 1. Physical data of compounds (**1**–**11**)

Compound	R^1	R^2	Molecular formula	Found (Cal.) %			Yield%	Mp °C	R_f[a]
				C	H	N			
1	F	CH_3	$C_{15}H_{13}FN_4O_2S_3$	45.41 (45.44)	3.36 (3.30)	14.20 (14.13)	50	220	0.92
2	F	C_2H_5	$C_{16}H_{15}FN_4O_2S_3$	46.43 (46.83)	3.25 (3.65)	14.04 (13.64)	70	226	0.90
3	Cl	CH_3	$C_{15}H_{13}ClN_4O_2S_3$	44.04 (43.64)	3.54 (3.14)	13.16 (13.56)	58	232	0.86
4	Cl	C_2H_5	$C_{16}H_{15}ClN_4O_2S_3$	45.42 (45.02)	3.11 (3.51)	13.42 (13.12)	54	230	0.90
5	OCH_3	CH_3	$C_{16}H_{16}N_4O_3S_3$	46.66 (47.06)	3.51 (3.91)	13.41 (13.71)	55	235	0.79
6	OCH_3	C_2H_5	$C_{17}H_{18}N_4O_3S_3$	48.04 (48.34)	4.46 (4.26)	13.35 (13.25)	52	215	0.94
7	Cl	CH_3	$C_{14}H_{14}ClN_3O_2S_2$	49.07 (49.49)	4.50 (4.12)	12.76 (12.37)	68	150	0.70
8	Cl		$C_{20}H_{18}ClN_3O_2S_2$	57.35 (57.76)	4.74 (4.33)	10.52 (10.10)	70	160	0.75
9	Br	CH_3	$C_{14}H_{14}BrN_3O_2S_3$	44.19 (43.76)	3.26 (3.64)	10.51 (10.94)	60	140	0.80
10	Br		$C_{20}H_{18}BrN_3O_2S_2$	52.57 (52.18)	3.54 (3.91)	9.57 (9.13)	65	130	0.87
11	CH_3		$C_{21}H_{21}N_3O_2S_2$	63.37 (63.79)	5.73 (5.31)	10.22 (10.63)	60	119	0.80

[a] Solvent system: toluene/ethylacetoacetate/formic acid (5:4:1).

Table 2. Anticonvulsant activity and minimal motor impairment of sulfonamido derivatives

Compound	Intraperitoneal injection in mice[a]				Neurotoxicity screen	
	MES		scPTZ			
	0.5 h	4.0 h	0.5 h	4.0 h		
1	300	—	—	—	—	—
2	300	—	300	—	—	—
3	—	100	—	300	—	300
4	—	30	—	300	—	300
5	300	—	—	—	—	—
6	—	—	—	—	—	—
7	300	300	300	[b]	300	300
8	100	300	300	[c]	300	300
9	300	[d]	300	—	300	—
10	100	300	300	—	300	300
11	300	300	300	—	300	—
Phenytoin	30	30	—	—	100	100
Ethosuximide	—	—	300	—	—	—
Carbamazepine	30	—	100	—	100	300
Valporic acid	—	—	300	—	—	—

[a] Doses of 30, 100, and 300 mg/kg were administered, the figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4.0 h after injection was made. The dash (—) indicates an absence of activity at maximum dose administered (300 mg kg).

[b] Exhibited activity (2/5 after 4.25 and 5.0 h).

[c] Exhibited activity (2/5 and 1/5 after 4.25 and 5.0 h, respectively).

[d] Exhibited activity (1/3) after 4.25 and 5.0 h.

the compounds, except **6**, exhibit anticonvulsant activity. Majority of the compounds, except **1** and **5**, were active in both MES and scPTZ tests making them useful for broad spectrum of seizure types. In the MES test compounds **8** and **10** with chloro- and bromo substituents showed activity at 100 mg/kg after 0.5 h. On the other hand, compounds **1**, **2**, **5**, **7**, **9**, and **11** showed protection in mice at the dose level of 300 mg/kg after 0.5 h. Some compounds like **7**, **8**, **10** and **11** were also active after 4.0 h extended period of activity. Compounds **3** and **4** with chloro substitution in the benzothiazole molecules were active at lower doses of 100 and 30 mg/kg, respectively, after 4.0 h. Thus, compound **4** showing activity at a lower dose of 30 mg/kg seems to be very potent in anticonvulsant MES screening. scPTZ screen is useful for petitmal seizures. Majority of the compounds also showed activity in scPTZ screen. Compounds **2**, **7**, **8**, **9**, **10**, and **11** showed activity at 300 mg/kg after 0.5 h. However, compounds **3** and **4** showed activity at 300 mg/kg after 4 h. Compounds showing activity in scPTZ test were comparable to standard drug ethosuximide (300 mg/kg). Some of the selected compounds **3** and **4** were further tested in oral MES screen using rats at 30 mg/kg (Table 3) in which compound **3** exhibited bioactivity throughout the time period up to 4.0 h similar to phenytoin. Compound **4** also showed some activity after 4.0 h.

Table 3. Evaluation of some components in the MES test after oral administration (30 mg/kg) to rats

Compound	Time (h) Oral administration to rats[a]				
	0.25	0.5	1.0	2.0	4.0
3	1	1	1	2	3
4	0	0	0	1	1
Phenytoin	1	4	3	3	3

[a] The figures indicate the number of rats out of four which were protected. Both compounds **3** and **4** exhibited no toxicity at dose of 30 mg/kg up to 4.0 h.

In the rotorod neurotoxicity screening compounds **1**, **2**, **5**, and **6** did not show any toxicity at the dose 300 mg/kg. Compounds **7**, **8**, and **10** were toxic at 0.5 and 4.0 h. Whereas compounds **9** and **10** showed toxicity only after 0.5 h and did not show toxicity after 4.0 h. Two compounds **3** and **4** showed delayed toxicity that is toxicity only after 4.0 h, which is comparable with that of carbamazepine (300 mg/kg). However, all the compounds were less toxic than phenytoin (100 mg/kg).

The bioactivity in MES test exhibited by chloro-substituted derivative **8** demonstrated that of the hydrophobic center could be expanded to greater size than the phenyl ring. The compound **3** has shown good oral activity for a longer duration of action. It has also shown activity in the scPTZ screen. Thus, it has shown broad-spectrum of activity. On the other hand, compound **4** had shown activity at a lower dose of 30 mg/kg in the MES test again with longer duration of activity. The only difference being the substitution in the thioureido moiety, while **3** has methyl group, **4** has more lipophilic ethyl group. Again the compound **4** has also exhibited activity in the scPTZ test. Both these compounds were not toxic at a dose level of 300 mg/kg. The fluoro- and methoxy-containing compounds were inactive. Compound **8** has shown activity in MES test at 100 mg/kg immediately after 0.5 h. Compound **8** also exhibited activity at 100 mg/kg in scPTZ test. The rapid onset of action is believed to be due to the substitution of more lipophilic tolyl group in the distal thioureido moiety. Evidently, this distal hydrophobic centre alters the bioavailability of the compounds. These findings confirm to our hypothesis of action of anticonvulsant compounds.

In conclusion from this study, it is quite apparent that there are at least four parameters for the activity of anticonvulsant drugs, that is, (i) a lipophilic domain, (ii) a distal hydrophobic center whose size affects the pharmacokinetic property, (iii) SO_2NH-group acts as a hydrogen donor, and (iv) a two-electron donor (–C═N–) system is also present. Hydrophobic size appears to govern the MES or scPTZ activity. If there is larger hydrophobic moiety as in chloro versus bromo, then MES activity is favored. Compound **9** with a bromo group shows anticonvulsant activity in MES test after 4.25 and 5.0 h as compared with compound **7** with chloro substituents. These results further confirm our observation and proposals[5] for a pharmacophore model and modifying the size of the hydrophobic domain (A) (Fig. 1). The structure of benzothiazole derivatives can be superimposed on the structure of zonisamide, a potent clinically effective drug to see the geometrical requirements of pharmacophore molecules for anticonvulsant drugs hitherto unknown (Fig. 2).

The synthesized compounds confirmed the pharmacophore model requirements for activity such as A, hydrophobic domain; C, distal hydrophobic domain in some compounds like **8**, **10**, and **11** of the series **6**–**11**, and D, electron donor moiety (NH) in all the synthesized compounds.

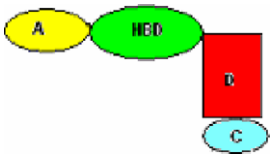


Figure 1. Suggested pharmacophore model for anticonvulsant activity. (A) Hydrophobic domain, (B) hydrogen bonding site, (C) distal hydrophobic domain, and (D) electron donor moiety.

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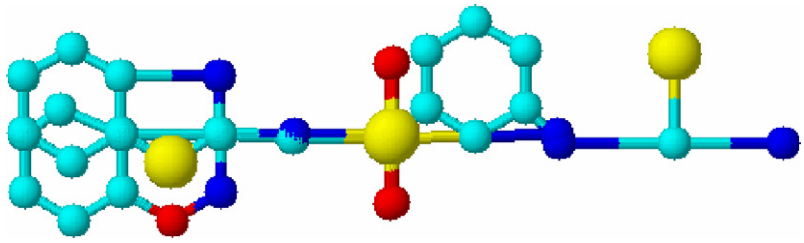

Figure 2. Superimposed structures of zonisamide on benzothiazole derivatives.

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9. Typical procedure: the respective benzothiazole (1.7 g, 0.01 mol) was taken in a mixture of pyridine (4 ml) and acetic anhydride (20 ml). To this mixture *p*-acetamidobenzenesulphonyl chloride (2.5 g, 0.01) was added and the mixture was heated for 2 h on a water bath. The reaction mixture was poured onto 30 ml of water; the solid product obtained was filtered and crystallized from ethanol (80%). Prepared *N*-(4-{[(6-fluoro-1,3-benzothiazol-2-yl) amino] sulfonyl} phenyl) acetamide was hydrolyzed by boiling it in (50 ml) of 80% acetic acid for 6 h to obtain the product. Further 4-amino-*N*-(6-fluoro-1,3-benzothiazol-2-yl)benzene sulfonamide hydrolyzed product was refluxed in ethanol (25 ml) with ethyl isothiocyanate for 2 h. The final product *N*-(6-fluoro-1,3-benzothiazol-2-yl)-4-{[(substituted amino) carbonothioyl] amino} benzene sulfonamide **2** so obtained was filtered and recrystallized from chloroform and DMSO. Yield: 70%; mp °C: 220; FT-IR (KBr) cm^{-1}: 3300–3250 (NH); 1300(SO_2NH_2); 1200(C═S); ^1H NMR (DMSO-d_6) δ ppm: 2.19 (q, 2H, CH_2): 3.44(t, 3H, CH_3): 7.20–7.84 (m, 7H, Ar–H); 12.31 (s, 3H, NH).
10. Typical procedure: *p*-chloroaniline (1.27 g, 0.01 mol) was taken in a mixture of pyridine (4 ml) and acetic anhydride (20 ml). To this mixture *p*-acetamidobenzene sulfonyl chloride (2.33 g, 0.01 mol) was added and the mixture was heated for 2 h on a water bath. The reaction mixture was filtered and crystallized from ethanol (80%). Prepared *N*-(4-{[(3-chlorophenyl)amino]sulfonyl}phenyl)acetamide was hydrolyzed by boiling it in (50 ml) of 80% acetic acid for 6 h to obtain the product. The final product *N*-(3-chlorophenyl)-4-{[(methylamino) carbonothionyl] amino} benzene sulfonamide **7** was obtained by refluxing hydrolyzed product 4-amino-*N*-(3-chlorophenyl) benzene sulfonamide (1.69 g, 0.006 mol) in ethanol (25 ml) with methyl isothiocynate (0.438 g, 0.006 mol) for 2 h. The solid product was filtered and recrystallized from ethanol. Yield: 68%; mp °C: 150; FT-IR (KBr) cm^{-1}: 3313–3204 (NH); 1172 (C═S), 1310 ($NHSO_2$); ^1H NMR (DMSO-d_6) δ ppm: 7.58–7.61 (d, 4H, $J = 14$ Hz, Ar–H); 7.32–7.35 (d, 4H, $J = 14$ Hz, Ar–H); 2.04 (s, 3H, CH_3); 10.07(s, 3H, NH, NHC═S).
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