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Synthesis of benzothiazole semicarbazones as novel anticonvulsants—The role of hydrophobic domain

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Abstract—A series of 1,3-benzothiazol-2-yl semicarbazones (**1**–**15**) were prepared in satisfactory yield and evaluated for their anticonvulsant, neurotoxicity and other toxicity studies. All the synthesized compounds were in good agreement with elemental and spectral data. Majority of the compounds were active in MES screen. Selected compounds were checked for their lipophilic character.

Epilepsy is not a disease, but a syndrome of different cerebral disorders of central nervous system (CNS), and it is characterized by paroxysmal, excessive and hypersynchronous discharges of large numbers of neurons.[1] Epilepsy is a common neurological condition, affecting 0.5–1% of the population worldwide (45–100 million people).[2] Studies have reported that in India the prevalence rate of epilepsy varies from 1710 to 9780 cases per million population.[3] Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control and others experience the seizure control only at the expense of significant toxic effects.[4] The limitations with conventional AEDs highlighted the need for developing newer agents for epilepsies.

From the study of structures of clinically established drugs, it can be concluded that the anticonvulsant properties have been displayed by various hydrazones (=N—NH—), amides (—$CONH_2$) and carbamides (—NHCO—NH—). The prime need was to search for a molecule that could complement all the above structures in one and semicarbazido group was considered one of them. The events leading to the development of the semicarbazones as promising lead are indicated in Figure 1. In recent years, aryl and heteroaryl semicarbazones[5,6] have emerged as structurally novel anticonvulsants.

In terms of interaction at the binding site, as proposed previously,[7,8] the pharmacophoric elements were thought to be a lipophilic aryl ring and hydrogen-bonding domain. The attachment of a second aryl ring, designated as the distal ring to the proximal aryl ring to increase the van der waal's bonding at the binding site and to increase potency, has also been reported. Substitutions in the aryl ring by halogens have been found to increase potency in the MES screen.[9,10] To test this hypothesis some modifications were made in the structure of semicarbazones. The lipophilic aryl ring was replaced with a versatile heterocyclic molecule benzothiazole, which possesses preliminary anticonvulsant properties.[11] In view of these general requirements for

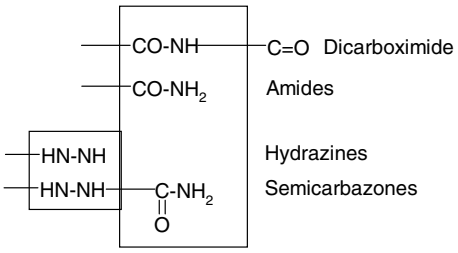

Figure 1. Development of semicarbazones as template for future lead compound.

Keywords: Benzothiazoles; Semicarbazones; Anticonvulsant activity; Hydrophobic domain.
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activity, we have designed aryl-substituted semicarbazones with benzothiazole moiety to be active in convulsant stimuli. These aryl semicarbazones do not possess the dicarboximide group as found in conventional drugs like barbiturates, hydantoins, oxazolidiones, etc., which may be associated with toxicity and side effects. The present work focused on synthesis and pharmacological evaluation of 1-(4-substitutedphenyl) ethan-1-one-*N*-(6-substituted-1,3-benzothiazol-2-yl) semicarbazones and compared its potential with those of established drugs. The lipophilicity character of the compounds was also determined.

1-(4-Substitutedphenyl) ethan-1-one-*N*-(6-substituted-1,3-benzothiazol-2-yl) semicarbazones (**1**–**15**) were synthesized by method outlined in Scheme 1. One of the initial attempts for the synthesis of 6-substituted-1,3-benzothiazole-2-amines was made by treating arylamines with potassium thiocyanate in a satisfactory yield by the known protocol.[12] The benzothiazole-2-yl ureas were prepared by treating required benzothiazole with sodium cyanate in the presence of glacial acetic acid. The benzothiazole-2-yl-ureas were then refluxed with hydrazine hydrate to yield hydrazine carboxamides. The final compounds were synthesized by the reaction of hydrazine carboxamides with appropriate ketones.[13] Analytical and spectral data of all the synthesized compounds were in good agreement with the composition of synthesized compounds. The data of physico-chemical properties of all the compounds are given in Table 1.

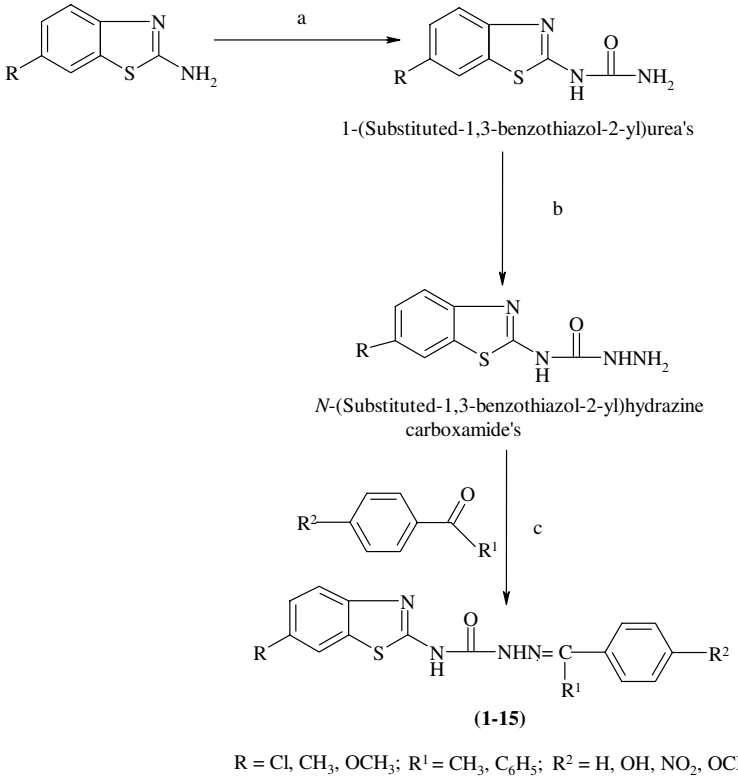
The anticonvulsant evaluation was undertaken using reported procedures.[14–16] Swiss albino mice (25–30 g) of either sex were used as experimental animals. The test compounds and standard drug were administered intraperitoneally which were suspended in Tween 80 (1%) or in 0.5% methyl cellulose–water mixture.

MES—maximal electroshock test: Maximal electroshock seizure was elicited with a 60 cycle altering current of 50 mA intensity delivered for 0.25 s via ear clip electrodes. The maximal seizure typically consists of a short period of tonic extension of the hind limbs and a final clonic episode. Abolition of the hind limb tonic extensor component of the seizure is defined as protection, and results are expressed as % protection.

NT—neurotoxicity: The rotarod 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. Results are expressed as number of animals exhibiting toxicity/number of animals tested.

Assessment of liver function: The biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method[17] and alkaline phosphate was measured by using King method.[18] The total protein and total albumin were also measured according to reported methods.[19,20]

Histopathological study of liver: The histopathological studies were carried out by a reported method.[21] The



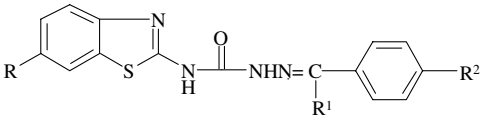
Scheme 1. Reagents and conditions: (a) glacial acetic acid/NaOCN; (b) NaOH/EtOH, $NH_2NH_2{\cdot}H_2O$, reflux 6 h; (c) EtOH/reflux, 5 h.

Table 1. Physical data of compounds (**1**–**15**)

Compound	R	R^1	R^2	Molecular formula	Found (Calcd)%			Yield (%)	Mp[a] (°C)	R_f[b](R_m)[c]
					C	H	N			
1	Cl	CH_3	H	$C_{16}H_{13}ClN_4OS$	55.77(55.73)	3.84(3.80)	16.30(16.25)	55	185	0.76(−0.50)
2	Cl	CH_3	OH	$C_{16}H_{13}ClN_4O_2S$	53.32(53.26)	3.60(3.63)	15.50(15.53)	60	180	0.79(−0.57)
3	Cl	CH_3	NO_2	$C_{16}H_{12}ClN_5O_3S$	49.34(49.30)	3.14(3.10)	18.00(17.97)	58	170	0.82(−0.65)
4	Cl	CH_3	OCH_3	$C_{17}H_{15}ClN_4O_2S$	54.52(54.47)	4.07(4.03)	14.90(14.95)	55	164	0.84(−0.72)
5	Cl	C_6H_5	H	$C_{21}H_{15}ClN_4OS$	62.01(61.99)	3.76(3.72)	13.72(13.77)	65	168	0.86(−0.78)
6	CH_3	CH_3	H	$C_{17}H_{16}N_4OS$	62.90(62.94)	5.00(4.97)	17.29(17.27)	60	150	0.72(−0.41)
7	CH_3	CH_3	OH	$C_{17}H_{16}N_4O_2S$	60.02(59.98)	4.70(4.74)	16.40(16.46)	62	142	0.70(−0.36)
8	CH_3	CH_3	NO_2	$C_{17}H_{15}N_5O_3S$	55.29(55.27)	4.05(4.09)	18.90(18.96)	60	200	0.80(−0.60)
9	CH_3	CH_3	OCH_3	$C_{18}H_{18}N_4O_2S$	61.04(61.00)	5.16(5.12)	15.84(15.81)	45	80	0.85(0.75)
10	CH_3	C_6H_5	H	$C_{22}H_{18}N_4OS$	68.42(68.37)	4.62(4.69)	14.54(14.50)	50	140	0.73(−0.43)
11	CH_3O	CH_3	H	$C_{17}H_{16}N_4O_2S$	59.84(59.88)	4.78(4.74)	17.00(16.46)	55	138	0.75(−0.47)
12	CH_3O	CH_3	OH	$C_{17}H_{16}N_4O_3S$	57.25(57.29)	4.56(4.52)	15.76(15.72)	60	120	0.83(−0.68)
13	CH_3O	CH_3	NO_2	$C_{17}H_{15}N_5O_4S$	52.21(52.98)	3.98(3.92)	18.21(18.17)	65	70	0.88(−0.86)
14	CH_3O	CH_3	OCH_3	$C_{18}H_{18}N_4O_3S$	58.30(58.36)	4.94(4.90)	15.16(15.12)	55	90	0.90(−0.95)
15	CH_3O	C_6H_5	H	$C_{22}H_{18}N_4O_2S$	65.60(65.65)	4.54(4.51)	13.96(13.92)	40	100	0.68(−0.32)

[a] Melting point of the compounds at their decomposition.
[b] Solvent system benzene/acetone (9:1).
[c] A logarithmic function of R_f value was also calculated; $R_m = \log(1-1/R_f)$.

Table 2. Anticonvulsant and neurotoxicity screening of compounds **1**–**15**



Compound	R	R^1	R^2	Intraperitoneal injection in mice[a]			
				MES screen (% protection)		Neurotoxicity screen	
				0.5 h	4 h	0.5 h	4 h
1	Cl	CH_3	H	100	83.3	(−)	(−)
2	Cl	CH_3	OH	50	50	X	X
3	Cl	CH_3	NO_2	33.3	33.3	X	X
4	Cl	CH_3	OCH_3	66.6	66.6	X	X
5	Cl	C_6H_5	H	50.0	33.3	X	X
6	CH_3	CH_3	H	33.3	33.3	X	X
7	CH_3	CH_3	OH	66.6	66.6	X	X
8	CH_3	CH_3	NO_2	100	100	(−)	(−)
9	CH_3	CH_3	OCH_3	83.3	83.3	(−)	(−)
10	CH_3	C_6H_5	H	33.3	33.3	X	X
11	CH_3O	CH_3	H	83.3	66.6	(−)	(−)
12	CH_3O	CH_3	OH	66.6	50.0	X	X
13	CH_3O	CH_3	NO_2	100	100	(−)	(−)
14	CH_3O	CH_3	OCH_3	83.3	83.3	(−)	(−)
15	CH_3O	C_6H_5	H	100	100	(−)	(−)
Phenytoin	—	—	—	100	100	(−)	(−)

[a] Dose of 30 mg/kg was administered. The animals were examined 0.5 and 4 h after administration. The dash (−) indicates an absence of activity. X denotes not tested.

rats were scarified under light ether anaesthesia after 24 h of last dosage; the livers were removed and washed with normal saline. Small pieces of liver tissue were processed and embedded in paraffin wax. Sections of 5–6 μm in thickness were cut, stained with haematoxylin and eosin and then studied under an electron microscope.

Candidate anticonvulsants are often evaluated initially in the MES screens. Compounds affording protection

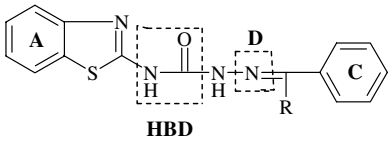


Figure 2. Proposed elements for anticonvulsant activity in basic structure of compounds. (A) Aryl ring system, (HBD) hydrophobic domain, (D) electron donor moiety and (C) distal aryl ring.

Table 3. Enzyme estimation of selected compounds

Compound	Alkaline phosphatase ± SEM	SGOT[a] ± SEM[ns]	SGPT[b] ± SEM[ns]	Albumin ± SEM[ns]	Gloublin ± SEM[ns]	Totalprotein ± SEM[ns]
Control	13.06 ± 0.25	148.67 ± 1.50	27.67 ± 0.840	1.67 ± 0.009	0.13 ± 0.01	1.80 ± 0.01
1	13.67 ± 0.56	148.11 ± 0.54	26.78 ± 0.78	1.67 ± 0.100	0.13 ± 0.05	1.82 ± 0.05
8	14.46 ± 0.06*	147.89 ± 0.95	26.17 ± 0.75	1.70 ± 0.028	0.13 ± 0.03	1.79 ± 0.02
13	13.69 ± 0.06	149.00 ± 1.90	27.90 ± 0.48	1.66 ± 0.010	0.14 ± 0.56	1.81 ± 0.04
15	12.78 ± 0.45	148.56 ± 1.34	27.67 ± 0.56	1.68 ± 0.030	0.14 ± 0.38	1.80 ± 0.02

[a] Denotes serum glutamate oxaloacetate transaminase.
[b] Denotes serum glutamate pyruvate transaminase.
* $p < 0.05$, ns denotes not significant. The mean level was calculated using ANOVA followed by Dunnett's test.

in the MES test may prove to be useful in treating generalized tonic–clonic and complex partial seizure. Neurotoxicity in mice is measured by the rotarod procedure, while minimal motor impairments in rats are detected by overt evidence of ataxia and abnormal gait and stance. The 1-(4-substitutedphenyl) ethan-1-one-*N*-(6-substituted-1,3-benzothiazol-2-yl) semicarbazone derivatives were screened at 30 mg/kg intraperitoneally in mice for anticonvulsant activity (Table 2). Phenytoin was used as the standard at the dose level of 30 mg/kg. Compounds **1**, **8**, **13** and **15** had shown 100% protection at both the time intervals, that is, 0.5 and 4 h except the compound **1** whose % protection decreased to 83.3% at the 4 h indicating rapid onset but shorter duration of action. Compounds **9**, **11** and **14** had shown 83.3% protection at both time intervals except **11**, which also had rapid onset but shorter duration of action with 66.6% protection at the 4 h interval. Compounds **4**, **7** and **12** showed 66.6% protection in the MES screen, whereas compounds **2**, **3**, **5**, **6** and **10** had 50–33.3% protection at the dose level of 30 mg/kg compared with standard phenytoin. The selected compounds with 100–83.3% protection were evaluated for the neurotoxicity at the 30-mg/kg dose level, none of the compound had shown the sign of neurotoxicity.

The bioevaluation led to an understanding of the correlation of bioactivity with structure of compounds. The substituents like CH_3, OCH_3 at the aryl ring with NO_2 and unsubstituted distant phenyl ring lead to highly potent compounds having longer duration of activity. Whereas lesser lipophilic substituent Cl at the aryl ring with OH at distal phenyl ring had shown 50% decrease in the potency.

The basic structure of the synthesized compounds has all the proposed pharmacophoric elements necessary for the anticonvulsant activity like (i) benzothiazole as a aryl ring system (A), (ii) NHC═O acted as hydrophobic domain (HBD), (iii) N as electron donor moiety (D) and (iv) distal aryl ring (C) as shown in Figure 2.

The dependence of biological activity inset of congeneric agents on lipophilic character has been shown in many types of drug action. In particular, some reports have indicated that anticonvulsant activity of different types of compounds was correlated with lipophilicity.[22] The experimental log *P* values were determined using the octanol–water method[23] for the compounds having 100% protection against the seizure spread in anti-MES screen. The compound **1** had log *P* 3.12 and **8** had log *P* 3.22. Among the compounds showing anticonvulsant activity, compounds with more lipophilic substitution like CH_3, Cl group in benzothiazole ring are more active.

Enzyme estimation and histopathological studies of the selected compounds with 100% protection were also done to check the magnitude of liver toxicity. Table 3 shows the liver function tests with reference to selected compounds. The estimation revealed that there was no significant increase in alkaline phosphatase, SGOT, SGPT and decrease in protein level in serum as compared to the control level (Table 3). It was clearly indicated that none of the compound showed any malfunctioning or toxicity of the liver as compared to control.

The Luncas technique was used to access the livers of mice, which were administered with test compounds at the dose level of 30 mg/kg body weight for 15 days and a comparison was done with the control group. Liver samples from control group and all the experimental groups were within normal histological limits (Figs. 3 and 4).

In conclusion, the present results indicate that the compounds possessed marked anticonvulsant activity as indicated by their prevention of the effects of maximal electroshock induced seizures in comparison with standard drug. The results further confirmed the pharmacophoric model elements necessary for the anticonvulsant

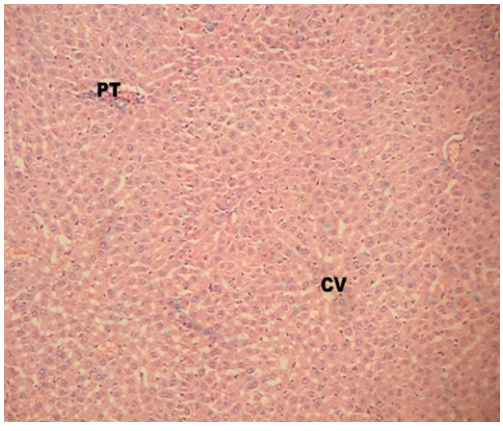


Figure 3. Microphotograph of the section of liver. Group: control, interference: normal hepatic parenchyma with portal triad (PT), central vein (CV) and the hepatocytes. Magnification: 100×.

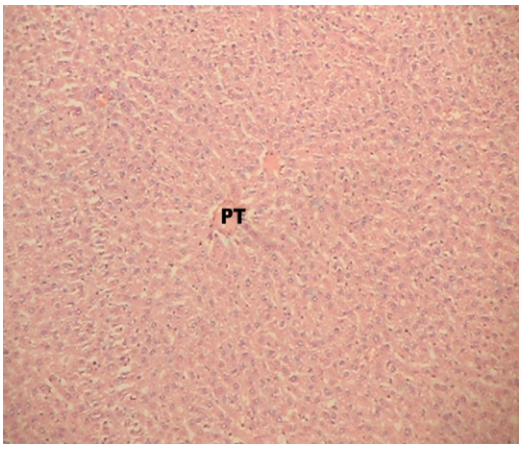


Figure 4. Microphotograph of the section of liver. Group: **8**, interference: showing normal portal triad structures. Magnification: 100×.

activity, which will account for bioactivity of majority of compounds. Our recent results with 4-Cl and 4-alkyl substituted phenyl ring of benzothiazole moiety have given impetus to the present investigation. Substitution with NO_2 at the distal aryl ring showed favoured MES activity as compared to hydroxy (OH) substitutent with the bigger hydrophobic aryl ring. This observation suggests that distal hydrophobic centre alters the bioavailability of the compounds. In the toxicity studies none of the compounds had shown neurotoxicity and liver toxicity.

Acknowledgments

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13. *Typical procedure:* To the solution of cyanate (0.5 g) in minimum quantity of water, glacial acetic acid (5 ml) was added. This solution was heated with respective 2-amino-6-substituted benzothiazole (1.7 g, 0.01 mol) in alcohol, until the contents of mixture became turbid and volume remained half of the original volume. The contents were added to ice cool water. The solid obtained was filtered off and dried. Further, to the warm hydrazine hydrate (5 ml), solution of prepared urea in alcohol, NaOH (.04 g) was added and solid obtained was filtered off and dried. The solution of carboxamide in glacial acetic acid (5 ml) and ethanol (10 ml) were heated to boiling and refluxed with aromatic ketones (1 g, 0.122 mol) for 5 h. Refluxed solution was cooled to room temperature and kept overnight. The solid was collected out, washed with methanol, dried and recrystallized from ethanol to get the compound **1**. FTIR (KBr) cm^{-1}: 3304, 3218 (NH), 3066 (CH—Ar.), 2918 (CH—aliph.), 1662 (C=O), 1577 (C=N), 1088 (C—Cl), 657 (C—S—C); ^1H NMR (DMSO-d_6): (δ, ppm) 2.39 (s, 3H, CH_3), 6.96–7.59 (m, 8H, Ar-H), 9.18 (s, 1H, NHN=, D_2O exchangeable), 9.32 (br s, 1H, NHC=O, D_2O exchangeable).
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