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Original article

Newer GABA derivatives for the treatment of epilepsy including febrile seizures: A bioisosteric approach

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Abstract

The present study aims at design and synthesis of newer γ-aminobutyric acid (GABA) derivatives with the combination of thiosemicarbazone and GABA pharmacophores in order to develop newer anticonvulsants. The reported compounds were designed as bioisosteric analogues of GABA semicarbazones. The structures of the synthesized compounds were confirmed by the use of their spectral data besides elemental analysis. Initial anticonvulsant screening was performed using intraperitoneal (i.p.) maximal electroshock-induced seizure (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine (scSTY), and subcutaneous picrotoxin (scPIC)-induced seizure threshold tests. A model involving 22-day old rat pups was also employed to further screen the effects of the test compounds against hyperthermia-induced febrile seizures. Only compounds **1** and **11** were found to be active in the MES test. Most of the compounds were found to be effective in the scPIC and febrile seizure models and very few compounds showed protection in scPTZ and scSTY models. This is the first report on these new GABA derivatives effective in the treatment of febrile seizures.
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Keywords: Thiosemicarbazones; Anticonvulsants; GABA; Pentylenetetrazole; Picrotoxin; Strychnine; Febrile seizures

1. Introduction

Epilepsy is the most common primary neurological disorder known, affecting 0.4–0.8% of the population and up to 50 million people worldwide [1,2]. Epilepsy is the tendency to experience seizures-intermittent, usually unprovoked and stereotyped episodes that result from abnormal, paroxysmal electrical discharge of neurons of the cerebral cortex [3]. 4-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain [4,5]. It is well documented that attenuation of GABAergic neurotransmission is involved in the pathophysiology of several central nervous system (CNS) disorders in humans, namely anxiety, pain, and epilepsy [6–8]. The peripheral administration of GABA cannot be usefully performed since this neurotransmitter is able to cross the blood–brain diffusion barrier (BBB) only when extremely high doses are applied, which produce severe adverse side effects [9]. Hence, over the past few decades, research aimed at achieving successful delivery of GABA into the CNS has resulted in the discovery of various GABA analogues with improved pharmacological activities [10]. Recently, we reported the anticonvulsant properties of variously substituted *N*,*N*-phthaloyl GABA amides and acid hydrazones [11]. One of the approaches to analog-based drug discovery is the concept of bioisosteric replacement (Fig. 1), which continues to play an important role in bioorganic and medicinal chemistry in the design of novel pharmacological tools as well as new therapeutic agents with optimal pharmacological profile and improved pharmacokinetic properties [12]. In the past decade, aryl semicarbazones had been designed that were structurally dissimilar from many common anticonvulsants containing the dicarboximide function (CONRCO), which may contribute to

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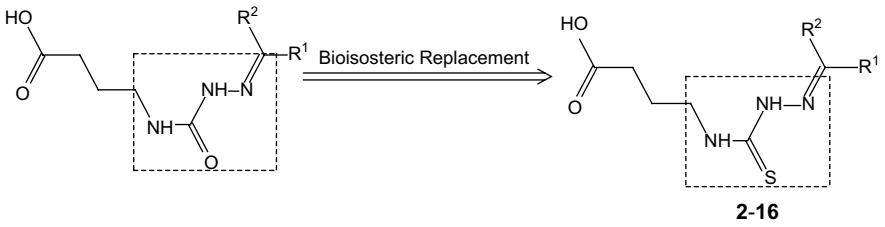
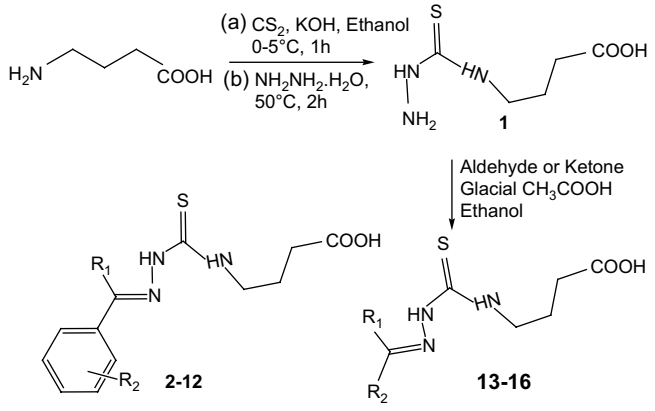


Fig. 1. Anticonvulsant compounds designed by bioisosteric replacement.

toxic side effects [13]. Consistent advances in the design of novel anticonvulsant agents have been obtained through the works of Dimmock and his colleagues [14], which included various aryl semicarbazones and (aryloxy) aryl semicarbazones. Recently, we reported the anticonvulsant and antinociceptive activities of GABA semicarbazones designed as pharmacophoric hybrids [15]. Moreover, various aryl thiosemicarbazides and thiosemicarbazones have also been reported by our group to exhibit anticonvulsant activity in maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) tests. These agents were also found to block the expression of fully kindled seizures [16]. Hence, given the promising biological profile of GABA derivatives, aryl semicarbazones/thiosemicarbazones, we initiated a drug discovery program focusing on the design and synthesis of newer GABA thiosemicarbazones as bioisosteric analogues of GABA semicarbazones. These GABA thiosemicarbazones were found to exhibit anticonvulsant activity in various animal models of seizure including the febrile seizure model with lesser neurotoxicity.

2. Synthesis

The synthesis of GABA thiosemicarbazones was accomplished as per earlier reported procedure [17,18] and presented in Scheme 1. The 4-(hydrazine carbothioamido)butanoic acid (**1**) was synthesized from 4-aminobutanoic acid via a one-pot procedure. 4-Aminobutanoic acid on treatment with carbon disulphide in the presence of potassium hydroxide in ethanol gave the potassium salt of the corresponding 4-dithiocarbamate derivative, which on reaction with hydrazine hydrate yielded 85% of **1**. Finally the required thiosemicarbazones (**2**–**16**) were prepared by the reaction between appropriate aryl/alkyl aldehydes or ketones and 4-(hydrazine carbothioamido)butanoic acid in the presence of glacial acetic acid in ethanol. The yields ranged from 45% to 78%. The purity was assessed by TLC; and the assignments of the structures were based on elemental and spectroscopic methods. The physical properties of the synthesized compounds are presented in Table 1. The chemical shifts obtained from ^{1}H NMR spectra supported the proposed structures. The ^{1}H NMR spectrum revealed that the hydrazino proton (=N–NH) showed a singlet at $\delta = 11.27$–11.68 ppm and the alkyl NH at 1.99–2.03 ppm both of which were D_2O exchangeable. All of the compounds (**2**–**16**) showed a characteristic D_2O exchangeable singlet due to OH proton of the acid function at $\delta = 12.3$–12.36 ppm. The aryl ring protons resonated at $\delta = 6.9$–8.41 ppm. The singlet due to 1H of carbimino proton was observed at $\delta = 8.0$–8.2 ppm and the singlet due to 3H of carbimino CH_3 was observed at $\delta = 0.91$ ppm.



Scheme 1. Synthetic route to GABA thiosemicarbazones.

3. Results and discussion

The synthesized compounds (**1**–**16**) were evaluated at dose levels of 30, 100 and 300 mg/kg intraperitoneally in mice for anticonvulsant activity. In our anticonvulsant drug development program, our approach was to test the new compounds preliminarily in two standard models MES and scPTZ (at NIH) for their ability to reduce seizure spread and to elevate seizure threshold, respectively. Further to understand the mechanistic aspects, we undertook testing in the other two models scSTY and scPIC in our laboratory. From our initial screening results we found that some compounds showed broad spectrum anticonvulsant activity, which prompted us to evaluate their efficacy in a stress-induced febrile seizure model. Table 2 lists the results obtained from the initial anticonvulsant evaluation of the synthesized compounds compared to the clinically proven antiepileptics such as phenytoin and ethosuximide. The acute neurological toxicity was determined by the rotorod test.

Two compounds (**1** and **11**) showed activity in the MES screen at 300 mg/kg with a shorter duration of action (0.5 h) indicative of their ability to prevent seizure spread. In the scPTZ screen, a test used to identify compounds that elevate seizure threshold, six compounds (**2**, **4**, **5**, **7**, **15**, and **16**) showed protection. Compound **16** was the most effective in this model exhibiting protection at 30 mg/kg for a shorter

Table 1
Physical data of 4-(alkylidene/arylidene hydrazine carbothioamido)butanoic acids

Compound	R_1	R_2	Yield (%)	M.P. (°C)[a]	$C \log P$[b]	R_f[c]
1	–	–	85	202–204	−0.58	0.42
2	H	2-OH	54	214–216	1.18	0.63
3	H	4-NO_2	65	213–215	0.73	0.61
4	H	4-Cl	68	206–208	2.76	0.65
5	H	3-NO_2	55	184–187	1.33	0.72
6	H	4-$N(CH_3)_2$	70	256–258	1.74	0.59
7	CH_3	H	54	121–123	1.91	0.62
8	CH_3	4-CH_3	45	116–119	2.18	0.67
9	CH_3	3-NH_2	62	258–260	0.96	0.71
10	CH_3	4-NO_2	54	134–137	0.40	0.65
11	C_6H_5	H	62	52–55	3.17	0.62
12	C_6H_5	4-Br	75	74–76	4.05	0.61
13	CH_2–C_6H_5	CH_2–C_6H_5	56	84–87	2.87	0.66
14	Cyclohexylene		54	127–129	1.84	0.59
15	Cyclopentylene		52	124–127	1.35	0.62
16	Isatinyl		78	117–120	1.78	0.69

[a] Elemental analyses for C, H, N were within ±0.4% of the theoretical values.
[b] $C \log P$ was calculated using www.logp.com.
[c] Mobile phase $CHCl_3$–CH_3OH (9:1).

duration. Compounds **2**, **4**, and **15** exhibited activities at 100 mg/kg and compounds **5** and **7** showed activity at 300 mg/kg at 4 h and 0.5 h, respectively. Five compounds (**2**, **4**–**6**, and **12**) showed protection in the subcutaneous strychnine-induced seizure model. Compounds **2** and **12** showed activity at 100 mg/kg and other compounds showed activity at 300 mg/kg, in which only compounds **4** and **6** showed activity till 4 h period of observation. Therefore, these compounds may be useful in treating not only generalized tonic–clonic and complex partial seizures, but also absence seizures. In the scPIC-induced seizure threshold test, most of the compounds exhibited activity indicative of the possible involvement of GABA-mediation in the anticonvulsant action. All of the compounds except **1**, **8**, **10** and **15** showed protection

Table 2
Anticonvulsant activity of the synthesized compounds

Compound	MES[a]	scPTZ[a]		scSTY[a]		scPIC[a]		Hyperthermia-induced seizures[b]		Neurotoxicity[a]	
	0.5 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	Duration of seizures (s)	% Protection	0.5 h	4 h
1	300	–	–	–	–	–	–	215 ± 3.2^{NS}	27	300	300
2	–	100	–	100	–	100	100	25 ± 1.2	92	–	–
3	–	–	–	–	–	300	300	45 ± 1.5	85	–	–
4	–	100	–	300	300	300	300	150 ± 1.9	49	100	–
5	–	–	300	300	–	100	100	145 ± 2.2	51	–	–
6	–	–	–	300	300	300	300	35 ± 1.7	88	–	–
7	–	300	–	–	–	100	100	30 ± 1.2	90	–	–
8	–	–	–	–	–	–	–	60 ± 2.2	80	–	–
9	–	–	–	–	–	300	300	215 ± 2.8^{NS}	27	–	–
10	–	–	–	–	–	–	–	160 ± 4.5^{NS}	46	100	100
11	300	–	–	–	–	300	300	50 ± 2.6	83	–	–
12	–	–	–	100	–	100	100	240 ± 1.2^{NS}	19	300	–
13	–	–	–	–	–	300	–	80 ± 2.5	73	–	300
14	–	–	–	–	–	300	300	65 ± 1.5	78	–	–
15	–	100	–	–	–	–	–	85 ± 2.8	71	–	–
16	–	30	–	–	–	300	300	25 ± 1.5	92	–	–
Phenytoin	30	–	–	–	–	–	–	–	–	100	100
Ethosuximide	–	300	–	–	–	–	–	–	–	–	–
Lamotrigine	30	30	–	–	–	–	–	145 ± 2.2	51	100	100
Hyperthermic control	–	–	–	–	–	–	–	330 ± 10.0	–	–	–
Vehicle-control	–	–	–	–	–	–	–	295 ± 7.0	–	–	–

[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 at 0.5 h and 4 h. (−) Indicates an absence of effect at the maximum dose tested.
[b] Compounds were administered intraperitoneally at a single dose of 100 mg/kg; each value represents the mean ± SEM of four rat pups significantly different from the vehicle-control at $P < 0.05$ and NS denotes not significant at $P < 0.05$ (Student's t-test).

in the scPIC test. Compounds that showed activity at 100 mg/kg till the 4 h period included **2**, **5**, **7**, and **12**. All other active compounds except **13** showed activity till 4 h period at 300 mg/kg. Overall it appears that three compounds (**2**, **4**, and **5**) were effective in three animal models of seizure, five (**6**, **7**, **11**, **12**, and **16**) were effective in at least two models and six compounds (**1**, **3**, **9**, and **13**–**15**) showed anticonvulsant activity in one model. Compounds **8** and **10** were completely devoid of anticonvulsant activity.

Due to the promising anticonvulsant activity profile of these derivatives, the effect of newer GABA analogues against febrile seizures was studied in a rat immature model (22-day old rat pups) of hyperthermia (Table 2). Lamotrigine is one of the first-line drugs prescribed for febrile seizures and hence employed as the standard drug. Since the compounds were screened for the first time in an excitotoxic model of febrile seizure, to avoid death of the animals, we started with the single dose (100 mg/kg). Of a total of 16 compounds screened, 11 compounds (**2**, **3**, **5**–**8**, **11**, **13**–**16**) demonstrated activity with 50% or more percentage protection and also shortened the duration of seizures significantly ($P < 0.05$) at a single dose of 100 mg/kg. Compounds **2**, **7**, and **16** showed percentage protections more than or equal to 90%. This is the first report on such derivatives with significant anticonvulsant activity against febrile seizures. Promising compounds could be carried on for lower doses evaluation in the quantitative studies in the future.

In the acute neurological toxicity screen, the compounds **2**, **3**, **5**–**7**, **9**, **11**, **12**, and **14**–**16** emerged as promising anticonvulsants with less or no neurotoxicity. There was no separation between the anticonvulsant dose and the neurotoxic dose for compounds **1**, **4**, and **13**. Compound **10**, which was ineffective in the seizure model showed neurotoxicity at 100 mg/kg.

4. Conclusions

The present study reports the synthesis of GABA thiosemicarbazones designed as bioisosteric analogues of GABA semicarbazones. These thiosemicarbazono derivatives of GABA were found to exhibit anticonvulsant activity in various animal models of seizure including the febrile seizure model. Overall, the synthesized compounds emerged as more active and less neurotoxic when compared to their semicarbazono counter parts.

5. Experimental protocols

5.1. Chemistry

Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Elemental analyses (C, H and N) were undertaken with a Perkin–Elmer model 240C analyzer and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silicagel-G (Merck) coated aluminium plates, visualized by iodine vapor and UV light. Developing solvents were chloroform–methanol (9:1).

5.1.1. Synthesis of 4-(hydrazine carbothioamido)butanoic acid (*1*)

4-Aminobutanoic acid (0.1 mol, 10.32 g) was dissolved in 30 mL of ethanol and was stirred at 0–5 °C vigorously. To the stirred solution, an equimolar amount of potassium hydroxide (0.1 mol, 5.60 g) and double the molar amount of carbon disulphide (0.2 mol, 15.20 mL) were added and stirred for the next 1 h. The reaction mixture was brought back to room temperature, after which hydrazine hydrate (99%) (0.1 mol, 4.85 mL) was added. Stirring was continued for 2 h, while simultaneously heating the reaction mixture to 50 °C. The resulting precipitate was filtered, dried and recrystallized using ethanol, M.P. 202–204 °C, IR (KBr) ν_{max} 3300, 1740, 1640, 1550, 1270, 835 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 1.82 (m, 2H, CH_2 β to COOH), 2.03 (s, 2H, NHCSNH, D_2O exchangeable), 2.23 (t, 2H, CH_2 α to COOH), 3.36 (t, 2H, CH_2 γ to COOH), 4.34 (s, 2H, NH_2, D_2O exchangeable), 12.24 (s, 1H, OH of COOH, D_2O exchangeable).

5.1.2. General procedure for the synthesis of 4-(alkylidene/arylidene hydrazine carbothioamido)butanoic acids (*2*–*16*)

To a solution of 4-(hydrazinecarbothioamido)butanoic acid (0.005 mol, 0.89 g) in ethanol (20 mL), an equimolar amount of appropriate alkyl/aryl aldehydes or ketones (including isatin) was added and refluxed in the presence of glacial acetic acid for 1–3 h until the completion of the reaction. The solvent was then removed *in vacuo* and the residue obtained was dried and recrystallized from ethanol. The physical data of the compounds are presented in Table 1. The IR spectra of the compounds were identical in the following aspects: 3300, 1640, 1590, 1170, 1015 cm^{-1}; ^{1}H NMR (DMSO) δ (ppm) spectra of some representative compounds are as follows.

5.1.2.1. 4-(2-(2-Hydroxybenzylidene)hydrazine carbothioamido)butanoic acid (2). 1.80 (m, 2H, CH_2 β to COOH), 2.01 (s, 1H, NHCS, D_2O exchangeable), 2.20 (t, 2H, CH_2 α to COOH), 3.40 (t, 2H, CH_2 γ to COOH), 6.9–7.4 (m, 4H, Ar–H), 8.0 (s, 1H, carbimino H), 9.9 (s, 1H, Ar–OH, D_2O exchangeable), 11.56 (s, 1H, NHN=, D_2O exchangeable), 12.3 (s, 1H, OH of COOH, D_2O exchangeable).

5.1.2.2. 4-(2-(4-Nitrobenzylidene)hydrazine carbothioamido)-butanoic acid (3). 1.81 (m, 2H, CH_2 β to COOH), 2.03 (s, 1H, NHCS, D_2O exchangeable), 2.21 (t, 2H, CH_2 α to COOH), 3.43 (t, 2H, CH_2 γ to COOH), 7.93–8.41 (m, 4H, Ar–H), 8.2 (s, 1H, carbimino H), 11.62 (s, 1H, NHN=, D_2O exchangeable), 12.32 (s, 1H, OH of COOH, D_2O exchangeable).

5.1.2.3. 4-(2-(4-Chlorobenzylidene)hydrazine carbothioamido)butanoic acid (4). 1.79 (m, 2H, CH_2 β to COOH), 2.02

(s, 1H, NHCS, D_2O exchangeable), 2.24 (t, 2H, CH_2 α to COOH), 3.42 (t, 2H, CH_2 γ to COOH), 7.47–7.74 (m, 4H, Ar–H), 8.13 (s, 1H, carbimino H), 11.64 (s, 1H, NHN=, D_2O exchangeable), 12.35 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.4. 4-(2-(4-(Dimethylamino)benzylidene)hydrazine carbothioamido)butanoic acid (**6**).* 1.83 (m, 2H, CH_2 β to COOH), 2.01 (s, 1H, NHCS, D_2O exchangeable), 2.22 (t, 2H, CH_2 α to COOH), 3.04 (s, 6H, $N(CH_3)_2$), 3.46 (t, 2H, CH_2 γ to COOH), 6.81–7.48 (m, 4H, Ar–H), 8.11 (s, 1H, carbimino H), 11.68 (s, 1H, NHN=, D_2O exchangeable), 12.33 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.5. 4-(2-(1-Phenylethylidene)hydrazine carbothioamido)-butanoic acid (**7**).* 0.88 (s, 3H, carbimino CH_3), 1.81 (m, 2H, CH_2 β to COOH), 2.02 (s, 1H, NHCS, D_2O exchangeable), 2.24 (t, 2H, CH_2 α to COOH), 3.43 (t, 2H, CH_2 γ to COOH), 7.51–7.62 (m, 5H, Ar–H), 11.27 (s, 1H, NHN=, D_2O exchangeable), 12.35 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.6. 4-(2-(1-p-Tolylethylidene)hydrazine carbothioamido)-butanoic acid (**8**).* 0.91 (s, 3H, carbimino CH_3), 1.83 (m, 2H, CH_2 β to COOH), 2.03 (s, 1H, NHCS, D_2O exchangeable), 2.22 (t, 2H, CH_2 α to COOH), 2.41 (s, 3H, Ar–CH_3), 3.44 (t, 2H, CH_2 γ to COOH), 7.20–7.65 (m, 4H, Ar–H), 11.28 (s, 1H, NHN=, D_2O exchangeable), 12.36 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.7. 4-(2-(1-(3-Aminophenyl)ethylidene)hydrazine carbothioamido)butanoic acid (**9**).* 0.89 (s, 3H, carbimino CH_3), 1.81 (m, 2H, CH_2 β to COOH), 2.02 (s, 1H, NHCS, D_2O exchangeable), 2.24 (t, 2H, CH_2 α to COOH), 3.43 (t, 2H, CH_2 γ to COOH), 5.82 (s, 2H, Ar–NH_2, D_2O exchangeable), 6.52–7.10 (m, 4H, Ar–H), 11.30 (s, 1H, NHN=, D_2O exchangeable), 12.33 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.8. 4-(2-(Diphenylmethylene)hydrazine carbothioamido)-butanoic acid (**11**).* 1.83 (m, 2H, CH_2 β to COOH), 2.03 (s, 1H, NHCS, D_2O exchangeable), 2.22 (t, 2H, CH_2 α to COOH), 3.46 (t, 2H, CH_2 γ to COOH), 7.32–7.82 (m, 10H, Ar–H), 11.27 (s, 1H, NHN=, D_2O exchangeable), 12.32 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.9. 4-(2-(1,3-Diphenylpropan-2-ylidene)hydrazine carbothioamido)butanoic acid (**13**).* 1.84 (m, 2H, CH_2 β to COOH), 2.02 (s, 1H, NHCS, D_2O exchangeable), 2.25 (t, 2H, CH_2 α to COOH), 2.63 (s, 4H, CH_2–Phenyl) 3.43 (t, 2H, CH_2 γ to COOH), 7.05–7.30 (m, 10H, Ar–H), 11.31 (s, 1H, NHN=, D_2O exchangeable), 12.33 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.10. 4-(2-Cyclohexylidene hydrazine carbothioamido)butanoic acid (**14**).* 1.29 (m, 4H, *o*-position of cyclohexane ring), 1.65 (m, 6H, *m*- and *p*-positions of cyclohexane ring), 1.81 (m, 2H, CH_2 β to COOH), 2.03 (s, 1H, NHCS, D_2O exchangeable), 2.24 (t, 2H, CH_2 α to COOH), 3.44 (t, 2H, CH_2 γ to COOH), 11.27 (s, 1H, NHN=, D_2O exchangeable), 12.35 (s, 1H, OH of COOH, D_2O exchangeable).

*5.1.2.11. 4-(2-(2-Oxoindolin-3-ylidene)hydrazine carbothioamido)butanoic acid (**16**).* 1.84 (m, 2H, CH_2 β to COOH), 1.99 (s, 1H, NHCS, D_2O exchangeable), 2.21 (t, 2H, CH_2 α to COOH), 3.47 (t, 2H, CH_2 γ to COOH), 7.1–7.8 (m, 4H, Ar–H), 11.20 (s, 1H, NH of isatinyl, D_2O exchangeable), 11.31 (s, 1H, NHN=, D_2O exchangeable), 12.32 (s, 1H, OH of COOH, D_2O exchangeable).

5.2. Pharmacology

The preliminary anticonvulsant and neurotoxicity evaluations were done using reported procedures [19,20]. Male albino mice (Swiss strain, 18–25 g) were used as experimental animals. All of the test compounds were suspended in 0.5% w/v methyl cellulose in the case of MES and scPTZ screens and 30% v/v PEG 400 for screening in the picrotoxin and strychnine-induced seizure models. The animals were maintained at an ambient temperature of 22 ± 1 °C, in groups of 5 per cage under standard laboratory conditions, receiving standard laboratory chow and water *ad libitum*. A 12 h:12 h light/dark cycle was maintained throughout the experimental studies. All the tests have been performed in accordance with the guidelines laid out by the Institutional Animal Ethics Committee.

5.2.1. Anticonvulsant screening

All of the test compounds were administered intraperitoneally in a volume of 0.01 mL/g for mice at doses of 30, 100 and 300 mg/kg. Anticonvulsant activity was assessed after 30 min and 4 h of drug administration. Activity in the scSTY and scPIC tests was established according to the earlier reported procedures [21,22] and the data are presented in Table 2.

5.2.1.1. Hyperthermia-induced seizures. Wistar rat pups obtained at age 15 days were housed with their mothers under standard laboratory conditions until they were weaned at 21 days. At age 22 days, the pups had a mean weight of 100 g. They were then housed in groups of four with *ad libitum* access to food and water. A daylight cycle of 12 h:12 h was maintained. The majority of our studies began with animals aged 22 days. Exposure to hyperthermia was carried out as reported earlier [23]. Briefly, the rat pups were placed in a glass chromatography tank (30 × 30 × 60 cm), which contained water to a depth that the animal could stand upright supported by the side of the tank with only its head above water level. Exposure to hyperthermia was achieved by maintaining the water in the tank at a temperature of 45 °C by placing it in a temperature-controlled water bath. This temperature (45 °C) does not produce skin damage at exposures <1 h. In human subjects, only a mild ''pricking pain'' is reported at 45 °C, and this sensation disappears in a few seconds. The pups were placed in the water unrestrained for 4 min or until a seizure occurred (whichever was shorter). They were immediately removed

from the water and placed in an observation chamber at the first sign of seizure onset. The seizure duration was measured as the time from seizure onset to the instant when the pup first righted itself and appeared conscious. The state of consciousness was determined by the responsiveness of the pups to one or all of several stimuli (tapping on cage, loud clap, responsiveness to touch, and the movement of a small object before its eyes). At the end of the period of observation, the pup was gently toweled dry, placed beneath a lamp until its fur appeared free of moisture, and then returned to its home cage.

5.2.2. Neurotoxicity screen

Rotarod test has been performed to detect the minimal motor deficit in mice. Animals were divided into groups (4–8) and trained to stay on an accelerating rotarod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals (able to stay on the rotarod for at least two consecutive trials of 90 s each) were given an i.p. injection of the test compounds at doses of 30, 100 and 300 mg/kg. Neurological deficit was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. The dose at which the animal fell off the rod was determined and the data are presented in Table 2.

6. Statistical analysis

The results of the febrile seizure model were expressed as mean ± standard error of mean (SEM) and the data were analyzed using Student's *t*-test. Statistical significance was assigned to a P value of less than 0.05. The statistical software package PRISM (Graphpad Software Inc., San Diego, CA) was used for the analyses.

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