Full Paper

Design of Benzothiazole-1,3,4-thiadiazole Conjugates: Synthesis and Anticonvulsant Evaluation

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Various 2-[(6-substituted-1,3-benzothiazol-2-yl)amino]-*N*-[5-substituted-phenyl-1,3,4-thiadiazol-2-yl]acetamides were synthesized with a prospective exploration of "lead hopping", using pharmacophoric elements for *in vivo* anticonvulsant activity. This yielded three potent candidates (**5i**, **5t**, and **5u**) in the preliminary screening employing the maximal electroshock seizure (MES) and the subcutaneous pentylenetetrazole (scPTZ) test, showing minimal neurotoxicity. Their quantitative study indicated an increase of nearly 2–10 times for the MES test and 7- to 67-fold for the scPTZ test in the protective index, the keystone in drug discovery for anticonvulsant activity.

Keywords: Anticonvulsants / Benzothiazole / Neurotoxicity / Phenytoin / Thiadiazole

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Introduction

Epilepsy is one of the common chronic diseases affecting 0.5–1% of the population worldwide. It ranks as the third most frequent neurological disorder, after cerebrovascular disease and dementia [1]. According to the International League against Epilepsy and the International Bureau of Epilepsy, epilepsy is the disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of the condition [2]. Current antiepileptic drugs are effective in controlling seizures in about 70% of the patients, but their use is often limited by side effects [3]. About one-third of the patients do not respond well to the currently available treatments, even if multiple drugs with complementary activities are used [4].

Over the last decades, the optimum therapy for seizures manifests complete control of seizures, absence of bothersome side effects, and an emphasis on maximizing quality of life. In spite of the concerted attempts by the physicians to utilize the available pharmacotherapies to their full advantage, the prospect of freedom from seizures and adverse effects remains elusive for a considerable number of epileptic patients. Approximately 25–30% of patients continue to suffer from seizures despite state-of-the-art treatment [5].

One example of a rationally designed anticonvulsant agent having an aminoacetamide linkage is the phase III drug brivaracetam, chemically 2-(2-oxo-4-propyl-pyrrolidin-1-yl)-butamide. It is an agonist of the synaptic vesicle protein SV2A and an alkylation hybrid of valproic acid and levetiracetam, an anticonvulsant drug made by UCB Pharma (Fig. 1). Moreover, the presynaptic *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist rilutek (riluzole), a 2-aminobenzothiazole analog generated by Rhone-Poulenc Rover, Inc. Collegeville (PA, USA) and marketed by Sanofi-Aventis (chemically, 6-(trifluoromethoxy)benzo[*d*]thiazol-2-amine), contributed to the designing of the synthesized compounds. The Food and Drug Administration (FDA) granted brivaracetam orphan drug designation for the treatment of symptomatic myoclonus in November 2005. The European Medicines Agency (EMA) granted orphan drug designation to brivaracetam for the treatment of progressive myoclonic epilepsies in August 2005. In December 2010, a phase III trial was initiated for partial-onset seizures [6]. A chemical drawing of the new targeted compounds, as rationally designed through brivaracetam along with riluzole, is represented in Fig. 1.

For over a century, despite the refined and efficient methods accrued for the synthesis of thiadiazole, a five-membered aromatic system having three heteroatoms at symmetrical positions has been studied extensively, owing to

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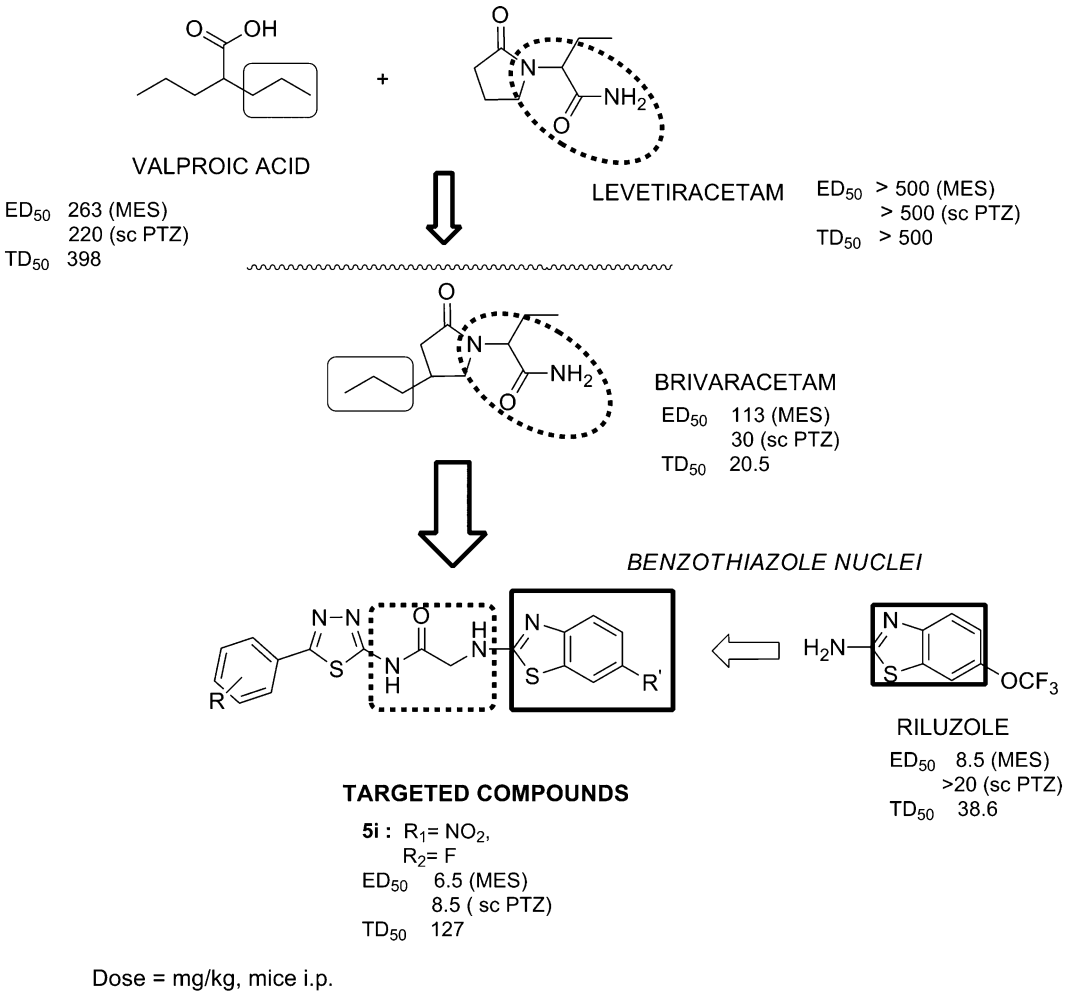


Figure 1. Rationally designed template for the targeted compounds as anticonvulsant agents.

its interesting pharmacological activities [7–12]. Based on our prior research on benzothiazole derivatives as anticonvulsant nuclei [13], it was found that these potential agents lacked the pharmacophoric element of a hydrophobic domain. To gain more insight, the benzothiazole and thiadiazole moieties coalesced through an aminoacetamide linkage were envisioned as postulates with a view to produce promising anticonvulsant agents.

Hence, the following approaches were utilized for the design of these derivatives: (a) hybridization of the thiadiazole and benzothiazole bioactive nuclei to elucidate their synergistic activity [14], (b) the synthesis of molecular scaffolds possessing essential pharmacophoric elements that are present in well-established anticonvulsant drugs such as phenytoin, carbamazepine, gabapentin, zonisamide, etc. [15], (c) a conceivable strategic approach for the discovery of efficacious drugs devoid of toxicity alerts, and (d) the derivatization of a lead to yield compounds with enhanced anticonvulsant activity.

Results and discussion

Chemistry

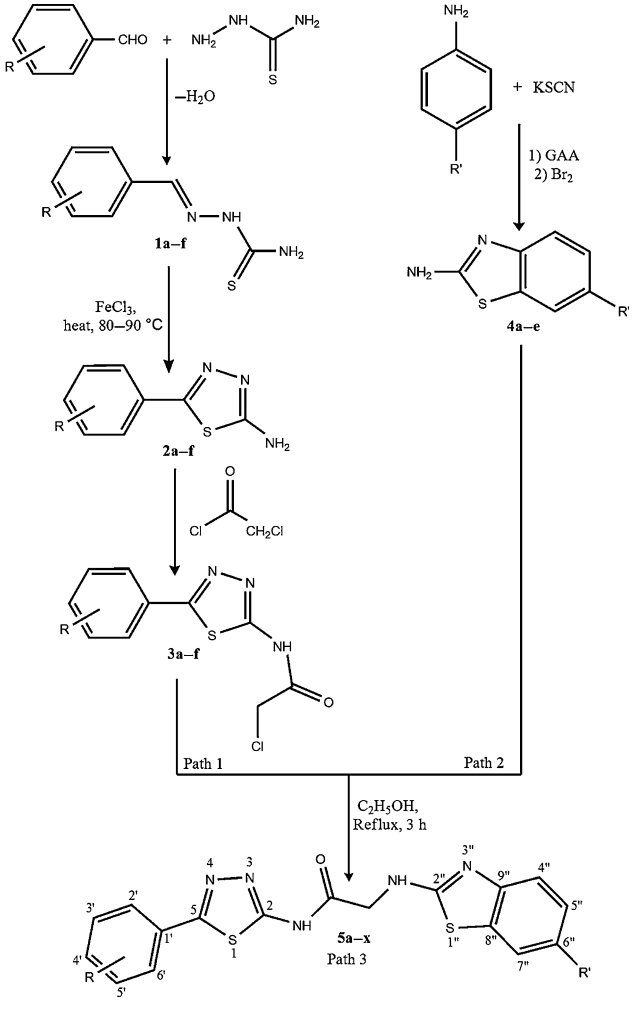
A pragmatic approach to synthesize a series of 2-(1,3-benzothiazol-2-ylamino-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamides **5a–x** is delineated here. Initially, substituted benzaldehyde reacts with thiosemicarbazide to yield substituted (2*E*)-2-benzylidenehydrazinecarbothioamides **1a–f** by extrusion of a water molecule. The Schiff bases undergo intramolecular oxidative cyclization by the coordination of ferric chloride into 5-phenyl-1,3,4-thiadiazole-2-amines **2a–f**. However, mild citric acid in sodium citrate treatment was required to facilitate the cyclization. Further, chloroacetyl chloride selectively condenses with the amine group in the

presence of dimethyl formamide to afford 2-chloro-*N*-(5-phenyl-substituted-1,3,4-thiadiazol-2-yl)acetamides **3a–f** (path 1 in Scheme 1).

A well-established literary procedure incorporating aniline derivatives and potassium thiocyanate via chemoselective bromination and base in cold glacial acetic acid produced annulated 1,3-benzothiazol-2-amines **4a–e** (path 2 in Scheme 1) [16].

Gratifyingly, cyclocondensation of acetamides with benzothiazol-2-amines furnished the quantitative formation of the conjugate addition products **5a–x**, using absolute ethanol as solvent (path 3 in Scheme 1).

The chemical identification studies (^{1}H NMR and IR) of **1a–f** substantiated their formation from aryl aldehyde and thiosemicarbazide. In the IR spectra of the compounds **1a–f**, it was possible to observe the absorption peaks at 1640 and 1440 cm^{-1}, indicating the presence of imine $C{=}N_{str}$ and the characteristic alkene $CH{=}_{bend}$ peak, respectively, formed via the removal of a water molecule through the reaction of the primary substrates. The absence of the exocyclic carbonyl function stretching peak derived from the aryl aldehyde at 1656 cm^{-1} and a broad band at 3338 cm^{-1} for $-NHNH_2$ further supported their formation. The strong absorption peaks at 1510 and 696 cm^{-1} for compounds **2a–f**, attributable to the heteroaromatic ring vibrations and the $C{-}S{-}C_{str}$ band, provided an affirmation for the cyclization of benzylidene hydrazine carbothioamide into the five-membered thiadiazole ring. The $C{=}O_{str}$ peak at 1650 cm^{-1} for the amide bond was assigned to the acetamide formation in compounds **3a–f**.



Scheme 1. Synthetic process for the preparation of compounds **5a–x**.

In path 2, the synthesis of compounds **4a–e** from substituted aniline indicated an IR peak of $C{=}N_{str}$ at 1565 cm^{-1} and a characteristic amidine (N–C=N) peak at 1686 cm^{-1}, evidently from the formation of the aromatic benzothiazole ring. The compounds **5a–x** in path 3 gave a strong $CH_2{-}N$ linkage peak at 2830 cm^{-1}, attributable to the hybrid formation via the amine residue in the benzothiazole ring with the acetamide residue of the thiadiazole ring in the final compounds.

In the ^{1}H NMR spectra of **1a**, a singlet of the imine (CH=N) proton resonated at 8.25 ppm, unlike the aldehydic singlet resonating at 9.08 ppm in the substrate. Two D_2O-exchangeable singlets derived from thiosemicarbazide appeared at 9.26 ($-NH_2$) and 10.80 ppm (=N–NH–CS). The link $NH_2{-}C{=}S$ is considerably more polar and stronger than the fused thiadiazole ring $C{-}S{-}C{-}NH_2$; consequently, the peak is not intense and is deshielded to 4.80 ppm in compounds **2a–f**. The ^{1}H NMR spectra of **3a–f** exhibited the signals belonging to an amide group and a methylene proton at 11.54 and 4.01 ppm, afforded by the acetamide linkage in compounds **4a–e**. The characteristic peak intensity ranging from 4.16 to 4.43 ppm of $-CH_2{-}CO{-}N-$ affirmed the formation of the title compounds **5a–x**.

Pharmacology

The newly synthesized compounds 2-[(6-substituted-1,3-benzothiazol-2-yl)amino]-*N*-[5-(4-substituted-phenyl)-1,3,4-thiadiazol-2-yl]acetamide **5a–x** were subjected to anticonvulsant screening based on the NIH Anticonvulsant Drug Development (ADD) Program protocol (Table 1) [17]. The preliminary activity was established through electrical and chemical induction of convulsions in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) tests, respectively [18, 19]. The neurotoxicity of the active compounds was determined using the minimal motor impairment-Rotarod test [20]. The intraperitoneal injections of the synthesized compounds were administered to the mice at doses of 30, 100, and 300 mg/kg. Phenytoin and carbamazepine were selected as the standard drugs for this purpose [21].

The evaluation was carried out at two time intervals of 0.5 and 4.0 h, respectively.

The MES screen for the evaluation of generalized tonic–clonic seizures identified some clinical drug candidates that prevented seizure spread (Table 2). In this test, compounds **5f**, **5g**, **5i**, **5k**, **5n**, **5r**, **5t**, **5u**, and **5x** were found to be active at 30 mg/kg at 0.5 h of duration. Interestingly, **5g**, **5i**, **5n**, **5t**, and **5u** continued to protect against the seizures at a dose of 30 mg/kg at 4.0 h, indicating rapid onset and longer duration of action at a lower dose, equal to the effects of the standard drug phenytoin. Compounds **5i** and **5t** showed 100% protection, whereas 75% protection was reported for compounds **5g**, **5n**, and **5u**. Compounds **5f**, **5k**, **5r**, and **5x** were also active at 4.0 h, but at a higher dose of 100 mg/kg, suggesting rapid onset and shorter duration of action at a lower dose. The percentage of protection depicted for compounds **5f**, **5k**, and **5x** was less than or equal to 50% at both the administered doses, thereby rendering them less efficient as compared to the standard drug carbamazepine. Compound **5r** had an activity nearly equal to that of carbamazepine.

The synthesized compounds **5b**, **5d**, **5j**, **5l**, **5p**, **5v**, and **5w** had shown activity at a dose of 100 mg/kg at both the reported intervals, except for **5b**, **5j**, **5p**, and **5v**, which exhibited activity at 300 mg/kg after 4.0 h of duration. The compounds **5a**, **5c**, **5e**, **5h**, **5m**, **5o**, **5q**, and **5s** showed nearly 50% protection at the maximum dose of 300 mg/kg at 0.5 and 4.0 h of duration, with the exception of **5e** and **5m**, which exhibited 25% protection at the 0.5-h and no protection at the 4.0-h interval, indicating rapid absorption but cessation of activity at longer duration.

The compounds exhibiting a better anticonvulsant profile against electroshock-induced convulsions were further evaluated for chemically induced convulsions. In the scPTZ screen, the compounds **5i**, **5n**, **5r**, **5t**, and **5u** showed anti-scPTZ activity at the minimum dose of 100 mg/kg at the two chosen time intervals, except for **5n** and **5r**, which showed protection at a higher dose at 4.0 h duration, indicating quick onset and prolonged protection against absence seizures, but at a higher dose. Interestingly, compound **5i** exhibited 100% protection at both the selected time intervals, making it a good anticonvulsant lead compared to carbamazepine. Compound **5g**

Table 1. Physicochemical constants of the synthesized compounds **5a–x** and Lipinski evaluation of active compounds.

						Lipinski rule (for active compounds)[c]				
Compd.	**R**	**R′**	**Molecular formula[a]**	**% Yield**	**M.p. (°C)[b]**	**MW**	**MR**	**No. of atom**	**tPSA**	**Log *P***	**HBD/HBA**
5a	−H	−H	$C_{17}H_{13}N_5OS_2$	71.2	165.5	367.45					
5b	−H	−Br	$C_{17}H_{12}BrN_5OS_2$	74.6	162.6	446.35					
5c	−H	−Cl	$C_{17}H_{12}ClN_5OS_2$	73.9	163.4	401.89					
5d	−H	−F	$C_{17}H_{12}FN_5OS_2$	80.4	160.8	385.44					
5e	−H	$-CH_3$	$C_{18}H_{15}N_5OS_2$	76.8	149.2	381.48					
5f	$-NO_2$	−H	$C_{17}H_{12}N_6O_3S_2$	70.9	167.4	412.45					
5g	$-NO_2$	−Br	$C_{17}H_{11}BrN_6O_3S_2$	75.3	159.8	491.34	117.41	40	130.02	4.90	2/9
5h	$-NO_2$	−Cl	$C_{17}H_{11}ClN_6O_3S_2$	72.7	186.8	446.89					
5i	$-NO_2$	−F	$C_{17}H_{11}FN_6O_3S_2$	78.4	156.2	430.44	109.72	40	130.02	4.26	2/9
5j	$-NO_2$	$-CH_3$	$C_{18}H_{14}N_6O_3S_2$	78.1	150.6	426.47					
5k	−OH	−H	$C_{17}H_{13}N_5O_2S_2$	79.6	190.2	383.45					
5l	−OH	−Br	$C_{17}H_{12}BrN_5O_2S_2$	83.1	175.5	462.34					
5m	−OH	−Cl	$C_{17}H_{12}ClN_5O_2S_2$	78.6	188.1	417.89					
5n	−OH	−F	$C_{17}H_{12}FN_5O_2S_2$	81.1	177.2	401.44	105.05	39	98.44	3.82	3/7
5o	−OH	$-CH_3$	$C_{18}H_{15}N_5O_2S_2$	80.5	160.1	397.47					
5p	$-OCH_3$	−H	$C_{18}H_{15}N_5O_2S_2$	76.2	230.4	397.47					
5q	$-OCH_3$	−Br	$C_{18}H_{14}BrN_5O_2S_2$	77.4	155.2	476.37					
5r	$-OCH_3$	−Cl	$C_{18}H_{14}ClN_5O_2S_2$	76.5	180.2	431.92	114.75	42	87.44	4.86	2/7
5s	$-OCH_3$	−F	$C_{18}H_{14}FN_5O_2S_2$	78.7	172.6	415.46					
5t	$-OCH_3$	$-CH_3$	$C_{19}H_{17}N_5O_2S_2$	79	168.4	411.5	114.68	45	87.44	4.64	2/7
5u	−Cl	−Cl	$C_{17}H_{11}Cl_2N_5OS_2$	77	184.7	436.34	111.37	38	78.21	4.86	2/6
5v	−Cl	−Br	$C_{17}H_{11}BrClN_5OS_2$	78.5	169.3	480.79					
5w	−Br	−Cl	$C_{17}H_{11}BrClN_5OS_2$	71.2	174.3	480.79					
5x	−Br	−Br	$C_{17}H_{11}Br_2N_5OS_2$	74.3	166.7	525.24					

MW, molecular weight; MR, molar refractivity; tPSA, total polar surface area; HBD/HBA, hydrogen bond donor/acceptor.
[a] Solvent used for crystallization of compounds: ethanol.
[b] Melting point of compounds at their decomposition.
[c] Data evaluation based on [32].

Table 2. Preliminary evaluation of synthesized compounds **5a–x** for anticonvulsant activity using maximal electroshock test.

	MES screen			
	Dosage (mg/kg)[a)]		Protection[b)]	
Compd. no.	0.5 h	4 h	0.5h	4h
5a	300	300	2/4	2/4
5b	100	300	2/4	2/4
5c	300	300	2/4	2/4
5d	100	100	2/4	1/4
5e	300	–	1/4	0/4
5f	30	100	2/4	1/4
5g	30	30	3/4	3/4
5h	300	300	2/4	2/4
5i	30	30	4/4	4/4
5j	100	100	2/4	1/4
5k	30	100	1/4	1/4
5l	100	300	2/4	3/4
5m	300	–	1/4	0/4
5n	30	30	3/4	3/4
5o	300	300	2/4	2/4
5p	100	300	2/4	1/4
5q	300	300	2/4	2/4
5r	30	100	4/4	3/4
5s	300	300	2/4	2/4
5t	30	30	4/4	4/4
5u	30	30	3/4	3/4
5v	100	300	1/4	1/4
5w	100	100	2/4	1/4
5x	30	100	1/4	1/4
PHY[c)]	30	30	4/4	4/4
CBZ[c)]	30	100	4/4	4/4

(–) Indicates devoid of activity at the maximum dose administered (300 mg/kg).
[a)] All the compounds were administered intraperitoneally.
[b)] Number of animals protected/number of animals tested, the number of mice is four.
[c)] Data taken from [21] and dose in mg/kg.

showed anticonvulsant activity at a maximum dose of 300 mg/kg at both durations of time.

Determination of the minimal motor impairment was performed using the Rotarod test with the compounds exhibiting protection against MES- and scPTZ-induced convulsions. Compounds **5i**, **5t**, and **5u** were found to be devoid of any neurotoxicity, even at the maximum dose, thereby rendering them much safer than the standard drug phenytoin. Compounds **5g** and **5n** gave equivalent neurotoxicity profiles compared to carbamazepine at 0.5 h, but the percentage of toxicity increased after 4.0 h of duration from 25% to 75%, suggesting it to be more toxic than carbamazepine after prolonged absorption. Compound **5r**, although having a minimal motor impairment dose equivalent to that of the standard drug phenytoin, was found to be toxic in nearly 100% of the mice after 4.0 h of duration (Table 3).

The outcomes of the preliminary screening encouraged further studies of the active compounds for quantitative screening [22]. In the quantitative study, MES attainments depicted compound **5i** as having a protective index much higher than the standard drugs phenytoin, carbamazepine, and riluzole [23]. However, compounds **5t** and **5u** showed a much wider protective window than the standard drugs. Likewise the scPTZ quantitative study indicated a remarkable increase in the protective indices for compounds **5i** and **5t**, whereas a comparable value was found for **5u** (Table 4).

A instill into the Antiepileptic Drug Development (ADD) Program disposition compounds **5i**, **5t**, and **5u** were also speculated for the determination of their anticonvulsant activity in the psychomotor 6-Hz seizure test [24–26]. The 6-Hz screen has been validated recently as a model of therapy-resistant epilepsy. It has not been widely used because it lacks clinical validity, since the hydantoins such as phenytoin failed to show protective activity. However, the clinically effective antiepileptic drug levetiracetam, which is not active in the conventional MES and scPTZ tests, does display protective activity in the 6-Hz model. This suggested that the 6-Hz model may be capable of identifying antiseizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action. The results upon intraperitoneal administration in mice are summarized in Table 5 and show **5i** as the most active compound, with maximal (100%) protection at the time points 0.5, 1, and 2 h. The other molecules were less active and protected up to 75% of the mice. Interestingly, the quantitative studies for 2-[(6-fluoro-1,3-benzothiazol-2-yl)amino]-*N*-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**5i**) identified the compound as the most promising candidate, having an ED_{50} comparable to that of levetiracetam [25] used as model anticonvulsant (Table 6).

To infer the effects of the synthesized compounds on the gamma amino butyric acid (GABA) receptor, the highly active compounds **5i**, **5t**, and **5u** were selected and subjected to neurochemical investigation, to estimate the level of GABA in the different regions of the rat brain [27]. The statistical data (Table 7) showed that the concentration of GABA increased significantly in the olfactory lobe and midbrain of the rat brain after administration of the compounds **5i** and **5u** ($p < 0.01$) compared to **5t** ($p < 0.05$). Non-significant results were obtained for **5t** and **5u** in the medulla oblongata and the cerebellum. In the midbrain area, the cerebellum, the olfactory lobe, and the medulla oblongata, compound **5i** significantly elevated the GABA concentration with respect to the control.

Hepatotoxicity and total protein imbalance are the major limitations of the currently marketed antiepileptic drugs. Hence, the results regarding toxicity were established through the concentrations of alkaline phosphatase, serum glutamate oxaloacetate transaminase (SGOT), serum

Table 3. Preliminary evaluation of selected compounds for anticonvulsant activity using subcutaneous pentylenetetrazole test and neurotoxicity.

	scPTZ screen				Minimal motor impairment test			
	Dosage (mg/kg)[a)]		Protection[b)]		Dosage (mg/kg)[a)]		Toxicity[c)]	
Compd. no.	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
5g	300	300	3/4	2/4	100	300	1/4	3/4
5i	100	100	4/4	4/4	300	300	0/4	0/4
5n	100	300	2/4	2/4	100	300	1/4	3/4
5r	100	300	2/4	2/4	100	100	3/4	4/4
5t	100	100	4/4	3/4	300	300	0/4	1/4
5u	100	100	3/4	3/4	300	300	1/4	1/4
PHY[d)]	x	x	x	x	100	100	1/4	0/4
CBZ[d)]	100	300	4/4	4/4	100	300	1/4	0/4

[a)] All the compounds were administered intraperitoneally.
[b)] Number of animals protected/number of animals tested; the number of mice is four.
[c)] Number of animals toxic/number of animals tested; the number of mice is four.
[d)] Data taken from [21] and dose in mg/kg. (x) Indicates not tested.

glutamate pyruvate transaminase (SGPT), and total body protein determined in serum, and the values were represented as mean ± SEM [28–30]. Compounds **5i** and **5u** showed moderate but significant changes in the levels of the mentioned liver enzymes as compared to the control ($p < 0.01$), except for **5u**, which exhibited no significant difference regarding SGPT. Compound **5t** was also found to alter the SGOT level, although the change was not very significant ($p < 0.05$). **5i** showed a significant change in the total protein level ($p < 0.01$) compared to the other selected compounds with $p < 0.05$ significance. All the other values were not significant, indicating the nontoxic nature of the tested compounds. Thus, considering the biochemical parameters estimated to establish the liver function test with selected compounds, it was clearly indicated that none of the compounds showed any malfunctioning or toxicity of the liver as compared to the control (Table 8).

Structure–activity relationship

Dimmock et al. [15] proposed a pharmacophore model for anticonvulsants that consisted of (a) a hydrophobic domain, (b) a hydrogen bonding domain (HBD), (c) a distal hydrophobic domain, and (d) an electron donor moiety, based on which the compounds were synthesized (Fig. 1). The procedure, as depicted in Scheme 1, utilizes constrained pharmacophore characteristics of thiadiazole chemistry, i.e., a "hydrogen binding domain" and a "two-electron donor system" [31]. With the identification of suitable reaction conditions, a survey of the various substituents was executed. The insights into the structure–activity relationship (SAR) of our active compounds

Table 4. Quantification studies of the selected compounds after intraperitoneal administration in mice.

	ED_{50}[a)]			PI[c)]	
Compd. no.	MES	scPTZ	TD_{50}[b)]	MES	scPTZ
5i	6.5	8.5	127.0	19.54	14.94
5t	7.4	10.8	109.5	14.80	10.14
5u	7.8	30	75.0	9.62	2.5
PHY[d)]	9.2	>300	65.6	7.13	<0.22
CBZ[d)]	8.8	>100	71.6	8.14	<0.72
RILZ[d)]	8.5	>20	38.6	4.54	<1.93

All the administered doses are in mg/kg ($n = 6$).
[a)] ED_{50} – median effective dose eliciting anticonvulsant protection in 50% animals.
[b)] TD_{50} – median toxic dose eliciting minimal neurological toxicity in 50% animals.
[c)] PI (protective index) = TD_{50}/ED_{50}.
[d)] Data taken from [23].

Table 5. Anticonvulsant activity – psychomotor seizure test (6-Hz, current 32 mA).

	Intraperitoneal injection into mice[a)]				
Compd. no.	**0.25 h**	**0.5 h**	**1 h**	**2 h**	**4 h**
5i	1	4	4	4	3
5t	0	2	3	3	2
5u	0	0	2	2	1

[a)] Dose of 100 mg/kg was administered. The data indicate the number of mice that were protected as four.

Table 6. Quantification data – psychomotor seizure test (6 Hz, current 32 mA) after intraperitoneal injection into mice.

Compd. no.	**TPE[a)] (h)**	**ED_{50}[b)] (mg/kg)**	**TD_{50}[c)] (mg/kg)**	**PI[d)] (TD_{50}/ED_{50})**
5i	2	25.9 (19.4–26.4)	ND	ND
Levetiracetam[e)]	1	19.4 (9.90–36.0)	>500	>26

ND, not determined.
[a)] Time to peak effect.
[b)] ED_{50} – median effective dose required to assure anticonvulsant protection in 50% animals.
[c)] TD_{50} – median toxic dose eliciting minimal neurological toxicity in 50% animals.
[d)] PI – protective index (TD_{50}/ED_{50}).
[e)] Data taken from Ref. [25].

(**5g**, **5i**, **5n**, **5r**, **5t**, and **5u**) envisaged the thiadiazole–benzothiazole hybrid pharmacophore as a lead towards efficacious anticonvulsant drugs. While the parent unsubstituted hybrid showed moderate activity, substitution of electron-donating groups in compounds **5g**, **5i**, and **5t** or an electron-withdrawing group in **5u** afforded stronger drugs. Furthermore, the combination of an electron-donating substitution on the distal aryl ring attached to the thiadiazole nuclei and an electron-withdrawing substitution on the 6th position of the hydrophobic domain of the benzothiazole nuclei improved the activity (**5n**, **5r**). This can be attributed to the fact that substituents such as –OH and $-OCH_3$ (**5n**, **5r**, and **5t**) on the distal aryl ring, which are capable of forming intermolecular hydrogen bonds with the receptor sites, favored by electronegative substituents or a lipophilic alkyl chain (**5t**), possibly increase the substituent effect of the synthesized compounds. The introduction of hydrophobic substituents in the *para* position of the distal aryl and the 6th position of the benzothiazole ring is proposed to be a significant factor for the difference in anticonvulsant potential of our hybrid compounds. Unsubstituted derivatives of the phenyl ring attached to the thiadiazole nuclei resulted in a substantial loss of activity.

The corporate compound inventory entailed zero Lipinski violations, a polar surface area $\leq$120 and no more than 10 rotatable bonds [32]. The refined hits of the MES, scPTZ, and neurotoxicity analysis (**5i**, **5t**, and **5u**) subjected to quantitative screening rendered a comparable increment of nearly 2–10 times for MES and 7- to 67-fold for scPTZ (Table 4) in the protective index, the mainstay in the drug discovery. Furthermore, these achievements were corroborated with the aid of the psychomotor seizure test (Tables 5 and 6), liver enzyme estimation, total protein estimation of the body, and determination of the GABA concentration in the rat brain. The results confirm levels of significance of $^{**}p < 0.01$ and $^{*}p < 0.05$ (Tables 7 and 8).

Conclusion

In conclusion, the unprecedented study describes a facile synthesis of new promising anticonvulsant thiadiazole derivatives and also probed the importance of joining this nucleus to the benzothiazole hydrophobic domain through aminoacetamide linkage, rendering new effective hybrid molecules. The biological evaluation of the synthesized compounds displayed two most active candidates, **5i** and **5t**, likewise consistent with the toxicity studies. Stimulated by the outcomes of this viable schematic strategy, experiments

Table 7. GABA estimation of the selected compounds.

	GABA conc. (g/100 mg tissue) ± SEM			
Compd. no.[a)]	**Olfactory lobe**	**Mid brain**	**Medulla oblongata**	**Cerebellum**
Control	12.74 ± 0.812	44.35 ± 1.044	38.57 ± 0.665	23.72 ± 1.404
5i	$16.02 \pm 0.221^{**}$	$60.65 \pm 1.881^{**}$	$40.54 \pm 0.377^{*}$	$30.25 \pm 1.226^{**}$
5t	$14.55 \pm 0.019^{*}$	$51.47 \pm 1.108^{*}$	39.65 ± 0.115^{ns}	25.62 ± 1.005^{ns}
5u	$15.65 \pm 0.112^{**}$	$58.02 \pm 1.652^{**}$	40.01 ± 0.169^{ns}	27.69 ± 1.011^{ns}

ns = not significant. The mean level was calculated using ANOVA followed by Dunnett's multiple comparison test ($n = 6$).
[a)] The compounds were tested at a dose of 100 mg/kg intraperitoneally.
$^{*}p < 0.05$.
$^{**}p < 0.01$.

Table 8. Liver enzyme and total protein estimation of the selected compounds.

Compd. no.[a)]	Alkaline phosphatase ± SEM	SGOT ± SEM	SGPT ± SEM	Total protein (g/100 mL) ± SEM
Control	15.51 ± 0.421	151.06 ± 0.958	25.18 ± 0.663	1.93 ± 0.015
5i	11.76 ± 0.201**	125.23 ± 0.855**	20.09 ± 0.990**	2.28 ± 0.025**
5t	14.80 ± 0.200ns	147.46 ± 0.455*	22.37 ± 0.011*	2.03 ± 0.018*
5u	11.20 ± 0.450**	123.67 ± 0.560**	24.18 ± 0.020ns	2.04 ± 0.033*

ns = not significant. The mean level of SGOT/SGPT ± SEM was calculated using ANOVA followed by Dunnett's multiple comparison test ($n = 6$).
a) A dose of 25 mg/kg/day of all the compounds was administered by oral route.
*$p < 0.05$.
**$p < 0.01$.

for elucidating further benefits of these compounds are underway and will be reported in due course.

Experimental

Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} TLC aluminum sheets (Merck KGaA, Darmstadt, Germany) using toluene/ethyl acetate/formic acid (5:4:1) as eluent. Ashless Whatmann No. 1 filter paper was used for vacuum filtration. Melting points were determined by using open capillary tubes in a Hicon melting point apparatus (Hicon, India) and are uncorrected. Elemental data of C, H, and N were within ±0.4% and 0.3%, respectively, of the theoretical value as determined by Vario EL III CHNS Elementar (Analysensysteme GmbH, Hanau, Germany). The infrared spectra of the compounds were recorded on a Bio-Rad FTS-135 spectrophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra of DMSO-d_6/$CDCl_3$ solutions were recorded at 400 and 100 MHz, respectively, with a Bruker 400 Ultra shield TM NMR spectrometer (400 MHz; Bruker Bioscience, USA), using TMS [$(CH_3)_4Si$] as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shifts (δ) values are given in parts per million (ppm). J values are given in Hz. The mass spectra were recorded on a Waters Micro-mass ZQ 2000 spectrophotometer (Jamia Hamdard, New Delhi, India).

*Synthesis of the substituted (2E)-2-benzylidenehydrazine-carbothioamides (**1a–f**)*

Aryl aldehydes (0.05 mol) in warm alcohol (300 mL) and a solution of thiosemicarbazide (0.05 mol) in 300 mL hot water were mixed slowly with continuous stirring. The product (**1a–f**), which was separated, was filtered off after cooling.

*Synthesis of the 5-phenyl substituted-1,3,4-thiadiazol-2-amines (**2a–f**)*

Compounds **1a–f** (0.03 mol) were suspended in 300 mL of distilled water and, after adding a mixture of citric acid (0.06 mol) and sodium citrate (0.03 mol), were heated in the presence of ferric chloride solution (0.15 mol) and stirred for 45 min. After cooling, the solution mixture was neutralized by aqueous ammonia (10%), rendering the solution to precipitate. The precipitate so obtained was filtered off, washed with distilled water, and allowed to dry. The final crystallization was effected by ethanol to afford **2a–f**.

*Synthesis of the 2-chloro-N-(5-phenyl-substituted-1,3,4-thiadiazol-2-yl)acetamides (**3a–f**)*

The amines **2a–f** (1 mol) were reacted with chloroacetylchloride (2 mol) in *N*,*N*-dimethylformamide at room temperature for 2 h to give compounds **3a–f**.

*Synthesis of the 6-substituted-1,3-benzothiazol-2-amines (**4a–e**)*

Aryl amines (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid (10%) were cooled and stirred. During stirring, cold bromine (0.01 mol, 3 mL in 10 mL) was added dropwise. Stirring was continued for an additional 3 h. The separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water, neutralized with aqueous ammonia solution (25%), and filtered off. Finally, the products **4a–e** were washed with cold water, dried, and recrystallized from benzene.

*Synthesis of the 2-(6-substituted-1,3-benzothiazol-2-ylamino-N-(5-phenyl-substituted-1,3,4-thiadiazol-2-yl)-acetamides (**5a–x**)*

2-Chloro-*N*-(5-phenyl-substituted-1,3,4-thiadiazol-2-yl)acetamides **3a–f** (0.01 mol) and 1,3-benzothiazol-2-amines **4a–e** (0.01 mol) were dissolved in ethanol and stirred for 4 h and filtered. The filtrate so collected was left for crystallization overnight, to yield the target products **5a–x**.

2-(1,3-Benzothiazol-2-ylamino)-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (**5a**): Light-brown shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 55.65; H, 3.52; N, 19.01; calcd. C, 55.57; H, 3.57; N, 19.06. IR (KBr) ν (cm^{-1}) 3418.51 (NH str), 3072.08 (C–H str aromatic), 1704.16 (C=C str), 1624.60 (C=O str), 1599.54 (C=N str), 689.74 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 11.76 (s, 1H, NH–CO), 8.68 (s, 1H, NH), 8.10 (d, 2H, 2′,6′H_{Ph}, $J = 7.2$ Hz), 8.05 (m, 3H, 3′,4′,5′H_{Ph}), 8.02 (d, 1H, 4″H_{BT}, $J = 7.9$ Hz), 7.97 (d, 1H, 7″H_{BT}, $J = 7.9$ Hz), 7.56 (m, 1H, 5″H_{BT}), 7.47 (m, 1H, 6″H_{BT}), 4.02 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.2 (NH–CO), 165.4 (C-2), 160.8 (C-2″), 155.5 (C-5), 148.7 (C-8″), 132.2 (C-1′), 130.6 (C-9″), 128.5 (C-4″), 127.3 (C-7″), 124.9 (C-5″), 124.8 (C-6″), 120.7 (C-2′ and C-6′), 120.5 (C-3′ and C-5′), 118.5 (C-4′), 40.5 (CH_2–NH); MS (70 eV): $m/z = 368.31$ (M+1).

*2-[(6-Bromo-1,3-benzothiazol-2-yl)amino]-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (**5b**):* Yellowish-brown shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 45.79; H, 2.69; N, 15.65; calcd. C, 45.75; H, 2.71; N, 15.69. IR (KBr) ν (cm^{-1}) 3401.53 (NH str), 3084.70 (C–H str aromatic), 1699.22 (C=C str), 1652.86 (C=O str), 1607.14 (C=N str), 883.78 (C–Br str), 692.45 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 11.96 (s, 1H, NH–CO), 8.35 (s, 1H, NH), 8.04 (d, 2H, 2′,6′H_{Ph}, J = 7.0 Hz), 7.94 (m, 3H, 3′,4′,5′H_{Ph}), 7.92 (s, 1H, 7″H_{BT}), 7.20 (d, 1H, 5″H_{BT}, J = 7.8 Hz), 6.89 (d, 1H, 4″H_{BT}, J = 8.0 Hz), 4.12 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 169.3 (NH–CO), 165.6 (C-2), 161.4 (C-2″), 155.8 (C-5), 148.2 (C-8″), 132.5 (C-1′), 130.1 (C-9″), 126.3 (C-6″), 125.5 (C-4″), 124.7 (C-7″), 122.9 (C-5″), 120.5 (C-2′ and C-6′), 120.4 (C-3′ and C-5′), 118.8 (C-4′), 40.8 (CH_2–NH); MS (70 eV): m/z = 447.38 (M+1), 448.19 (M+2).

*2-[(6-Chloro-1,3-benzothiazol-2-yl)amino]-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (**5c**):* Light-yellow shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 50.88; H, 2.98; N, 17.38; calcd. C, 50.81; H, 3.01; N, 17.43. IR (KBr) ν (cm^{-1}) 3416.11 (NH str), 3083.75 (C–H str aromatic), 1703.05 (C=C str), 1657.39 (C=O str), 1609.13 (C=N str), 865.95 (C–Cl str), 691.02 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.08 (s, 1H, NH–CO), 9.22 (s, 1H, NH), 8.07 (d, 2H, 2′,6′H_{Ph}, J = 6.9 Hz), 8.03 (m, 3H, 3′,4′,5′H_{Ph}), 7.98 (s, 1H, 7″H_{BT}), 7.25 (d, 1H, 5″H_{BT}, J = 7.7 Hz), 7.01 (d, 1H, 4″H_{BT}, J = 8.0 Hz), 4.34 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.8 (NH–CO), 165.2 (C-2), 161.8 (C-2″), 155.6 (C-5), 148.9 (C-8″), 132.7 (C-1′), 129.8 (C-9″), 128.4 (C-4″), 125.7 (C-7″), 124.8 (C-5″), 124.1 (C-6″), 120.6 (C-2′ and C-6′), 120.3 (C-3′ and C-5′), 118.9 (C-4′), 40.7 (CH_2–NH); MS (70 eV): m/z = 402.74 (M+1), 403.63 (M+2).

*2-[(6-Fluoro-1,3-benzothiazol-2-yl)amino]-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (**5d**):* Yellow crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 53.03; H, 3.09; N, 18.11; calcd. C, 52.97; H, 3.14; N, 18.17. IR (KBr) ν (cm^{-1}) 3441.52 (NH str), 3091.20 (C–H str aromatic), 1708.25 (C=C str), 1646.28 (C=O str), 1608.89 (C=N str), 874.81 (C–F str), 693.23 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.01 (s, 1H, NH–CO), 9.10 (s, 1H, NH), 8.06 (d, 2H, 2′,6′H_{Ph}, J = 7.3 Hz), 8.04 (s, 1H, 7″H_{BT}), 7.99 (m, 3H, 3′,4′,5′H_{Ph}), 7.98 (d, 1H, 5″H_{BT}, J = 7.7 Hz), 7.56 (d, 1H, 4″H_{BT}, J = 7.9 Hz), 4.18 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.6 (NH–CO), 165.4 (C-2), 160.9 (C-2″), 156.1 (C-5), 155.4 (C-6″), 148.1 (C-8″), 132.9 (C-1′), 129.3 (C-9″), 120.8 (C-5″), 120.5 (C-2′ and C-6′), 120.2 (C-3′ and C-5′), 118.7 (C-4′), 118.2 (C-4″), 114.5 (C-7″), 40.8 (CH_2–NH); MS (70 eV): m/z = 386.35 (M+1).

*2-[(6-Methyl-1,3-benzothiazol-2-yl)amino]-N-(5-phenyl-1,3,4-thiadiazol-2-yl)acetamide (**5e**):* Brown powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 56.69; H, 3.92; N, 18.31; calcd. C, 56.67; H, 3.96; N, 18.36. IR (KBr) ν (cm^{-1}) 3400.93 (NH str), 3073.76 (C–H str aromatic), 1701.67 (C=C str), 1653.77 (C=O str), 1604.28 (C=N str), 685.41 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 11.82 (s, 1H, NH–CO), 8.87 (s, 1H, NH), 8.03 (d, 2H, 2′,6′H_{Ph}, J = 7.0 Hz), 7.97 (m, 3H, 3′,4′,5′H_{Ph}), 6.89 (d, 1H, 4″H_{BT}, J = 7.8 Hz), 6.55 (d, 1H, 5″H_{BT}, J = 8.0 Hz), 6.47 (m, 1H, 7″H_{BT}), 4.28 (s, 2H, CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 167.9 (NH–CO), 165.5 (C-2), 160.7 (C-2″), 155.8 (C-5), 148.2 (C-8″), 131.9 (C-1′), 130.5 (C-9″), 130.2 (C-6″), 125.4 (C-4″), 124.7 (C-7″), 120.9 (C-5″), 120.5 (C-2′ and C-6′), 120.1 (C-3′ and C-5′), 118.3 (C-4′), 40.6 (CH_2–NH), 22.4 (–CH_3); MS (70 eV): m/z = 382.11 (M+1).

*2-(1,3-Benzothiazol-2-ylamino)-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**5f**):* Dark-brown crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 49.55; H, 2.90; N, 20.32; calcd. C, 49.51; H, 2.93; N, 20.38. IR (KBr) ν (cm^{-1}) 3416.06 (NH str), 3078.83 (C–H str aromatic), 1709.02 (C=C str), 1647.26 (C=O str), 1624.74 (C=N str), 1596.39 (N=O), 692.75 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.60 (s, 1H, NH–CO), 9.54 (s, 1H, NH), 8.90 (d, 2H, 3′,5′H_{Ph}, J = 7.5 Hz), 8.50 (d, 2H, 2′,6′H_{Ph}, J = 7.2 Hz), 7.88 (d, 1H, 4″H_{BT}, J = 7.9 Hz), 7.54 (d, 1H, 7″H_{BT}, J = 7.9 Hz), 7.25 (m, 1H, 5″H_{BT}), 7.13 (m, 1H, 6″H_{BT}), 4.13 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 165.6 (C-2), 160.5 (C-2″), 155.2 (C-5), 146.9 (C-8″), 132.5 (C-1′), 130.8 (C-9″), 130.1 (C-4′), 128.3 (C-4″), 127.9 (C-7″), 125.7 (C-3′ and C-5′), 124.8 (C-5″), 124.7 (C-6″), 124.4 (C-2′ and C-6′), 40.7 (CH_2–NH); MS (70 eV): m/z = 413.36 (M+1).

*2-[(6-Bromo-1,3-benzothiazol-2-yl)amino]-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**5g**):* Creamy-white shiny needle-shaped crystals; solubility: chloroform, ethanol, methanol. Elemental analysis (%): Found C, 41.37; H, 2.19; N, 17.16; calcd. C, 41.56; H, 2.26; N, 17.10. IR (KBr) ν (cm^{-1}): 3472.20 (NH str), 3190.65 (C–H str aromatic), 1598.70 (C=C str), 1585.20 (C=O str), 1552.42 (C=N str), 1509.99 (N=O), 879.38 (C–Br str), 663.39 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.67 (s, 1H, NH–CO), 9.58 (s, 1H, NH), 8.83 (d, 2H, 3′,5′H_{Ph}, J = 7.4 Hz), 8.41 (d, 2H, 2′,6′H_{Ph}, J = 6.8 Hz), 7.80 (s, 1H, 7″H_{BT}), 7.09 (d, 1H, 5″H_{BT}, J = 7.6 Hz), 6.97 (d, 1H, 4″H_{BT}, J = 7.9 Hz), 4.16 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.7 (NH–CO), 165.3 (C-2), 160.8 (C-2″), 156.2 (C-5), 146.7 (C-8″), 132.6 (C-1′), 130.4 (C-9″), 130.2 (C-4′), 126.4 (C-6″), 125.5 (C-3′ and C-5′), 125.1 (C-4″), 124.8 (C-7″), 124.3 (C-2′ and C-6′), 122.8 (C-5″), 40.7 (CH_2–NH); MS (70 eV): m/z = 492.22 (M+1), 493.45 (M+2).

*2-[(6-Chloro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**5h**):* Light-yellow shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 45.74; H, 2.46; N, 18.78; calcd. C, 45.69; H, 2.48; N, 18.81. IR (KBr) ν (cm^{-1}): 3456.75 (NH str), 3090.30 (C–H str aromatic), 1707.62 (C=C str), 1633.41 (C=O str), 1534.11 (C=N str), 1445.36 (N=O), 859.19 (C–Cl str), 689.43 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.68 (s, 1H, NH–CO), 9.62 (s, 1H, NH), 8.80 (d, 2H, 3′,5′H_{Ph}, J = 7.2 Hz), 8.60 (d, 2H, 2′,6′H_{Ph}, J = 7.0 Hz), 7.67 (s, 1H, 7″H_{BT}), 7.42 (d, 1H, 5″H_{BT}, J = 7.7 Hz), 7.08 (d, 1H, 4″H_{BT}, J = 8.1 Hz), 4.19 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.5 (NH–CO), 165.2 (C-2), 160.9 (C-2″), 156.1 (C-5), 148.3 (C-8″), 132.4 (C-1′), 130.4 (C-4′), 128.6 (C-9″), 128.4 (C-4″), 126.5 (C-7″), 125.4 (C-3′ and C-5′), 124.9 (C-2′ and C-6′), 124.7 (C-5″), 124.3 (C-6″), 40.9 (CH_2–NH); MS (70 eV): m/z = 447.56 (M+1), 448.40 (M+2).

*2-[(6-Fluoro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**5i**):* Light-yellow needle shaped crystal; solubility: ethanol, chloroform. Elemental analysis (%): Found C, 47.28; H, 2.52; N, 19.57; calcd. C, 47.44; H, 2.58; N, 19.52. IR (KBr) ν (cm^{-1}) 3386.39 (NH str), 3071.08 (C–H str

aromatic), 1707.66 (C=C str), 1640.16 (C=O str), 1606.41 (C=N str), 1576.52 (N=O), 852.38 (C–F str), 689.43 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.66 (s, 1H, NH–CO), 9.58 (s, 1H, NH), 8.80 (d, 2H, 3′,5′H_{Ph}, $J = 7.0$ Hz), 8.41 (d, 2H, 2′,6′H_{Ph}, $J = 7.3$ Hz), 8.06 (s, 1H, 7″H_{BT}), 7.99 (d, 1H, 5″H_{BT}, $J = 7.8$ Hz), 7.62 (d, 1H, 4″H_{BT}, $J = 8.2$ Hz), 4.18 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 167.8 (NH–CO), 164.9 (C-2), 161.2 (C-2″), 156.2 (C-5), 155.5 (C-6″), 147.9 (C-8″), 132.7 (C-1′), 130.4 (C-4′), 128.7 (C-9″), 126.3 (C-3′ and C-5′), 124.2 (C-2′ and C-6′), 120.8 (C-5″), 118.1 (C-4″), 114.6 (C-7″), 41.2 (CH_2–NH)); MS (70 eV): $m/z = 431.34$ (M+1).

2-[(6-Methyl-1,3-benzothiazol-2-yl)amino]-N-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide (5j): Light-brown shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 50.75; H, 3.27; N, 19.67; calcd. C, 50.69; H, 3.31; N, 19.71. IR (KBr) ν (cm^{-1}) 3401.16 (NH str), 3081.33 (C–H str aromatic), 1696.17 (C=C str), 1652.02 (C=O str), 1620.91 (C=N str), 1584.77 (N=O), 694.98 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.64 (s, 1H, NH–CO), 9.57 (s, 1H, NH), 8.90 (d, 2H, 3′,5′H_{Ph}, $J = 7.3$ Hz), 8.30 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 6.81 (d, 1H, 4″H_{BT}, $J = 7.9$ Hz), 6.57 (d, 1H, 5″H_{BT}, $J = 7.6$ Hz), 6.50 (m, 1H, 7″H_{BT}), 4.16 (s, 2H, CH_2), 2.19 (s, 3H, CH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 164.8 (C-2), 160.5 (C-2″), 155.7 (C-5), 148.1 (C-8″), 131.8 (C-1′), 130.5 (C-4′), 130.3 (C-9″), 130.1 (C-6″), 126.0 (C-3′ and C-5′), 125.4 (C-4″), 124.9 (C-7″), 124.8 (C-2′ and C-6′), 120.6 (C-5″), 40.6 (CH_2–NH), 22.6 (–CH_3); MS (70 eV): $m/z = 427.30$ (M+1).

2-(1,3-Benzothiazol-2-ylamino)-N-[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5k): Cream crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 53.30; H, 3.39; N, 18.22; calcd. C, 53.25; H, 3.42; N, 18.26. IR (KBr) ν (cm^{-1}): 3446.27 (NH str), 3263.11 (OH str), 3080.00 (C–H str aromatic), 1636.24 (C=C str), 1530.12 (C=O str), 1510.29 (C=N str), 700.08 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.72 (s, 1H, NH–CO), 12.60 (s, 1H, OH), 9.73 (s, 1H, NH), 7.99 (d, 1H, 4″H_{BT}, $J = 7.8$ Hz), 7.97 (d, 2H, 2′,6′H_{Ph}, $J = 6.8$ Hz), 7.84 (d, 1H, 7″H_{BT}, $J = 7.9$ Hz), 7.58 (m, 1H, 5″H_{BT}), 7.54 (d, 2H, 3′,5′H_{Ph}, $J = 7.2$ Hz), 7.49 (m, 1H, 6″H_{BT}), 4.18 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.1 (NH–CO), 165.3 (C-2), 160.6 (C-2″), 155.4 (C-5), 148.6 (C-8″), 136.8 (C-4′), 132.2 (C-1′), 130.7 (C-9″), 128.4 (C-4″), 127.9 (C-7″), 127.5 (C-3′ and C-5′), 124.5 (C-5″), 124.2 (C-6″), 123.6 (C-2′ and C-6′), 40.5 (CH_2–NH); MS (70 eV): $m/z = 384.22$ (M+1).

2-[(6-Bromo-1,3-benzothiazol-2-yl)amino]-N-[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5l): Light-yellow shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 44.22; H, 2.59; N, 15.09; calcd. C, 44.16; H, 2.62; N, 15.15. IR (KBr) ν (cm^{-1}): 3451.94 (NH str), 3269.75 (OH str), 3086.55 (C–H str aromatic), 1631.49 (C=C str), 1527.32 (C=O str), 1443.40 (C=N str), 862.01 (C–Br str), 695.28 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.78 (s, 1H, NH–CO), 12.12 (s, 1H, OH), 9.76 (s, 1H, NH), 8.01 (d, 2H, 2′,6′H_{Ph}, $J = 7.1$ Hz), 7.89 (s, 1H, 7″H_{BT}), 7.59 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 7.15 (d, 1H, 5″H_{BT}, $J = 7.7$ Hz), 6.91 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.23 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.2 (NH–CO), 165.6 (C-2), 160.9 (C-2″), 155.8 (C-5), 148.1 (C-8″), 137.1 (C-4′), 132.5 (C-1′), 130.2 (C-9″), 127.5 (C-3′ and C-5′), 126.3 (C-6″), 125.3 (C-4″), 124.7 (C-7″), 123.3 (C-2′ and C-6′), 122.8 (C-5″), 40.7 (CH_2–NH); MS (70 eV): $m/z = 463.12$ (M+1), 464.00 (M+2).

2-[(6-Chloro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5m): Light-brown crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 48.92; H, 2.84; N, 16.72; calcd. C, 48.86; H, 2.89; N, 16.76. IR (KBr) ν (cm^{-1}) 3450.04 (NH str), 3394.14 (OH str), 3080.77 (C–H str aromatic), 1693.13 (C=C str), 1633.47 (C=O str), 1609.31 (C=N str), 837.90 (C–Cl str), 709.62 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.79 (s, 1H, NH–CO), 12.14 (s, 1H, OH), 9.81 (s, 1H, NH), 8.02 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 7.93 (s, 1H, 7″H_{BT}), 7.68 (d, 2H, 3′,5′H_{Ph}, $J = 7.4$ Hz), 7.62 (d, 1H, 5″H_{BT}, $J = 7.8$ Hz), 7.44 (d, 1H, 4″H_{BT}, $J = 8.1$ Hz), 4.22 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 165.1 (C-2), 161.4 (C-2″), 155.5 (C-5), 148.6 (C-8″), 138.3 (C-4′), 132.2 (C-1′), 129.2 (C-9″), 127.8 (C-4″), 127.1 (C-3′ and C-5′), 124.8 (C-7″), 124.5 (C-5″), 124.3 (C-6″), 123.6 (C-2′ and C-6′), 40.8 (CH_2–NH); MS (70 eV): $m/z = 418.71$ (M+1), 419.54 (M+2).

2-[(6-Fluoro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5n): White crystalline powder; solubility: DMF. Elemental analysis: Found C, 50.70; H, 2.95; N, 17.54; calcd. C, 50.86; H, 3.01; N, 17.45. IR (KBr) ν (cm^{-1}) 3390.24 (NH str), 2924.52 (OH str), 2853.17 (C–H str aromatic), 1692.23 (C=C str), 1639.20 (C=O str), 1608.34 (C=N str), 838.88 (C–F str), 708.71 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.77 (s, 1H, NH–CO), 12.12 (s, 1H, OH), 9.70 (s, 1H, NH), 8.04 (s, 1H, 7″H_{BT}), 8.01 (d, 2H, 2′,6′H_{Ph}, $J = 6.8$ Hz), 7.91 (d, 1H, 5″H_{BT}, $J = 7.8$ Hz), 7.74 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 7.71 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 4.25 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 165.5 (C-2), 161.5 (C-2″), 155.8 (C-5), 155.3 (C-6″), 148.4 (C-8″), 137.9 (C-4′), 132.4 (C-1′), 129.3 (C-9″), 127.3 (C-3′ and C-5′), 123.1 (C-2′ and C-6′), 120.4 (C-5″), 118.2 (C-4″), 114.6 (C-7″), 40.8 (CH_2–NH)); MS (70 eV): $m/z = 402.50$ (M+1).

N-[5-(4-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl]-2-[(6-methyl-1,3-benzothiazol-2-yl)amino]acetamide (5o): Creamish-white powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 54.45; H, 3.75; N, 17.58; calcd. C, 54.39; H, 3.80; N, 17.62. IR (KBr) ν (cm^{-1}) 3395.04 (NH str), 3052.72 (OH str), 2920.61 (C–H str aromatic), 1693.11 (C=C str), 1636.03 (C=O str), 1608.34 (C=N str), 705.58 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.76 (s, 1H, NH–CO), 12.01 (s, 1H, OH), 9.68 (s, 1H, NH), 7.98 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 7.66 (d, 2H, 3′,5′H_{Ph}, $J = 7.4$ Hz), 6.79 (d, 1H, 4″H_{BT}, $J = 7.9$ Hz), 6.56 (d, 1H, 5″H_{BT}, $J = 7.5$ Hz), 6.48 (m, 1H, 7″H_{BT}), 4.23 (s, 2H, CH_2), 2.27 (s, 3H, CH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.4 (NH–CO), 165.4 (C-2), 161.2 (C-2″), 155.4 (C-5), 148.3 (C-8″), 137.9 (C-4′), 130.8 (C-1′), 130.4 (C-9″), 130.1 (C-6″), 127.2 (C-3′ and C-5′), 125.1 (C-4″), 124.8 (C-7″), 123.6 (C-2′ and C-6′), 120.2 (C-5″), 40.8 (CH_2–NH), 22.5 (–CH_3); MS (70 eV): $m/z = 398.09$ (M+1).

2-(1,3-Benzothiazol-2-ylamino)-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5p): Yellowish-brown shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 54.44; H, 3.76; N, 17.59; calcd. C, 54.39; H, 3.80; N, 17.62. IR (KBr) ν (cm^{-1}) 3399.03 (NH str), 3053.28 (C–H str aromatic), 1698.51 (C=C str), 1642.36 (C=O str), 1605.79 (C=N str), 1268.27 (O–CH_3), 681.02 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.66 (s, 1H, NH–CO), 9.54 (s, 1H, NH), 7.96 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 7.86 (d, 2H, 2′,6′H_{Ph}, $J = 6.7$ Hz), 7.75 (d, 1H, 7″H_{BT}, $J = 7.7$ Hz), 7.59 (m, 1H, 5″H_{BT}), 7.58 (d, 2H, 3′,5′H_{Ph}, $J = 7.2$ Hz), 7.51 (m, 1H, 6″H_{BT}), 4.28 (s, 2H, CH_2), 4.19 (s, 3H, OCH_3); ^{13}C NMR δ

(ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 165.3 (C-2), 160.5 (C-2″), 158.5 (C-4′), 155.6 (C-5), 148.1 (C-8″), 132.2 (C-1′), 130.2 (C-9″), 128.7 (C-4″), 127.4 (C-7″), 124.9 (C-5″), 124.4 (C-6″), 123.7 (C-2′ and C-6′), 116.6 (C-3′ and C-5′), 45.6 (O–CH_3), 40.9 (CH_2–NH); MS (70 eV): $m/z = 398.14$ (M+1).

2-[(6-Bromo-1,3-benzothiazol-2-yl)amino]-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5q): Yellowish-white shiny powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 45.42; H, 2.92; N, 14.67; calcd. C, 45.38; H, 2.96; N, 14.70. IR (KBr) ν (cm^{-1}): 3450.70 (NH str), 3080.37 (C–H str aromatic), 1693.01 (C=C str), 1633.84 (C=O str), 1609.23 (C=N str), 1275.66 (O–CH_3), 837.59 (C–Br str), 709.86 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.68 (s, 1H, NH–CO), 9.57 (s, 1H, NH), 7.98 (d, 2H, 2′,6′H_{Ph}, $J = 6.8$ Hz), 7.88 (s, 1H, 7″H_{BT}), 7.59 (d, 2H, 3′,5′H_{Ph}, $J = 7.1$ Hz), 7.25 (d, 1H, 5″H_{BT}, $J = 7.7$ Hz), 6.99 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.32 (s, 2H, CH_2), 4.20 (s, 3H, OCH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.7 (NH–CO), 165.2 (C-2), 160.9 (C-2″), 158.2 (C-4′), 155.5 (C-5), 148.3 (C-8″), 132.3 (C-1′), 130.4 (C-9″), 126.2 (C-6″), 125.7 (C-4″), 124.7 (C-7″), 123.1 (C-2′ and C-6′), 122.6 (C-5″), 116.2 (C-3′ and C-5′), 45.4 (O–CH_3), 40.8 (CH_2–NH); MS (70 eV): $m/z = 477.06$ (M+1), 478.02 (M+2).

2-[(6-Chloro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5r): Creamy white amorphous flakes; solubility: DMF. Elemental analysis: Found C, 49.88; H, 3.22; N, 16.29; calcd. C, 50.05; H, 3.27; N, 16.21. IR (KBr) ν (cm^{-1}) 3456.78 (NH str), 3081.69 (C–H str aromatic), 1693.19 (C=C str), 1634.38 (C=O str), 1609.31 (C=N str), 1275.68 (O–CH_3), 837.92 (C–Cl str), 700.03 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.71 (s, 1H, NH–CO), 9.61 (s, 1H, NH), 7.91 (s, 1H, 7″H_{BT}), 7.86 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 7.71 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 7.30 (d, 1H, 5″H_{BT}, $J = 7.6$ Hz), 7.01 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.39 (s, 2H, CH_2), 4.26 (s, 3H, OCH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.5 (NH–CO), 165.4 (C-2), 160.3 (C-2″), 158.3 (C-4′), 155.5 (C-5), 148.2 (C-8″), 132.7 (C-1′), 129.1 (C-9″), 127.1 (C-4″), 125.3 (C-7″), 124.7 (C-5″), 124.4 (C-6″), 123.2 (C-2′ and C-6′), 116.6 (C-3′ and C-5′), 45.6 (O–CH_3), 40.8 (CH_2–NH)); MS (70 eV): $m/z = 433.10$ (M+1), 434.40 (M+2).

2-[(6-Fluoro-1,3-benzothiazol-2-yl)amino]-N-[5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (5s): Yellow shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis (%): Found C, 52.10; H, 3.36; N, 16.82; calcd. C, 52.04; H, 3.40; N, 16.86. IR (KBr) ν (cm^{-1}) 3392.13 (NH str), 3055.60 (C–H str aromatic), 1693.14 (C=C str), 1639.22 (C=O str), 1608.73 (C=N str), 1275.86 (O–CH_3), 837.19 (C–F str), 708.70 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.70 (s, 1H, NH–CO), 9.59 (s, 1H, NH), 8.08 (s, 1H, 7″H_{BT}), 8.01 (d, 1H, 5″H_{BT}, $J = 7.9$ Hz), 7.89 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 7.84 (d, 2H, 2′,6′H_{Ph}, $J = 7.2$ Hz), 7.69 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 4.38 (s, 2H, CH_2), 4.24 (s, 3H, OCH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.4 (NH–CO), 165.3 (C-2), 160.8 (C-2″), 158.3 (C-4′), 155.5 (C-5), 155.1 (C-6″), 148.1 (C-8″), 132.4 (C-1′), 129.3 (C-9″), 123.7 (C-2′ and C-6′), 120.4 (C-5″), 118.4 (C-4″), 116.3 (C-3′ and C-5′), 114.5 (C-7″), 45.5 (O–CH_3), 40.9 (CH_2–NH); MS (70 eV): $m/z = 416.18$ (M+1).

N-[5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl]-2-[(6-methyl-1,3-benzothiazol-2-yl)amino]acetamide (5t): Yellowish-brown shiny crystalline powder; solubility: chloroform, methanol. Elemental analysis: Found C, 55.50; H, 4.22; N, 17.09; calcd. C, 55.46; H, 4.16; N, 17.02. IR (KBr) ν (cm^{-1}) 3408.02 (NH str), 3082.79 (C–H str aromatic), 1701.08 (C=C str), 1684.96 (C=O str), 1617.18 (C=N str), 1274.54 (O–CH_3), 697.32 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, $CDCl_3$): 12.70 (s, 1H, NH–CO), 9.70 (s, 1H, NH), 7.96 (d, 2H, 2′,6′H_{Ph}, $J = 6.7$ Hz), 7.68 (d, 2H, 3′,5′H_{Ph}, $J = 7.0$ Hz), 6.82 (d, 1H, 4″H_{BT}, $J = 8.1$ Hz), 6.60 (d, 1H, 5″H_{BT}, $J = 7.9$ Hz), 6.44 (m, 1H, 7″H_{BT}), 4.21 (s, 2H, CH_2), 3.58 (s, 3H, OCH_3), 2.27 (s, 3H, CH_3); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.3 (NH–CO), 165.5 (C-2), 160.3 (C-2″), 158.3 (C-4′), 155.6 (C-5), 148.2 (C-8″), 131.1 (C-1′), 130.8 (C-9″), 130.4 (C-6″), 125.4 (C-4″), 124.6 (C-7″), 123.6 (C-2′ and C-6′), 120.5 (C-5″), 116.4 (C-3′ and C-5′), 45.6 (O–CH_3), 40.8 (CH_2–NH), 22.6 (–CH_3); MS (70 eV): $m/z = 412.31$ (M+1).

2-(6-Chlorobenzo[d]thiazol-2-ylamino-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide (5u): Creamy-white shiny crystals; solubility: chloroform, ethanol. Elemental analysis: Found C, 46.71; H, 2.46; N, 15.99; calcd. C, 46.79; H, 2.54; N, 16.05. IR (KBr) ν (cm^{-1}) 3418.54 (NH str), 3072.84 (C–H str aromatic), 1704.67 (C=C str), 1624.09 (C=O str), 1599.56 (C=N str), 836.98 (C–Cl str), 689.71 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.68 (s, 1H, NH–CO), 9.58 (s, 1H, NH), 7.91 (d, 2H, 3′,5′H_{Ph}, $J = 7.4$ Hz), 7.88 (s, 1H, 7″H_{BT}), 7.82 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 7.32 (d, 1H, 5″H_{BT}, $J = 7.8$ Hz), 7.04 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.43 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.4 (NH–CO), 165.4 (C-2), 160.3 (C-2″), 155.5 (C-5), 148.2 (C-8″), 134.3 (C-4′), 132.7 (C-1′), 129.1 (C-9″), 128.6 (C-3′ and C-5′), 127.1 (C-4″), 125.3 (C-7″), 124.7 (C-5″), 124.4 (C-6″), 124.2 (C-2′ and C-6′), 40.8 (CH_2–NH); MS (70 eV): $m/z = 437.35$ (M+1), 438.54 (M+2), 440.85 (M+4).

2-(6-Bromobenzo[d]thiazol-2-ylamino)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)acetamide (5v): Light-brown crystals; solubility: chloroform, ethanol. Elemental analysis: Found C, 42.53; H, 2.40; N, 14.52; calcd. C, 42.47; H, 2.31; N, 14.57. IR (KBr) ν (cm^{-1}) 3415.49 (NH str), 3070.44 (C–H str aromatic), 1703.59 (C=C str), 1622.00 (C=O str), 1600.03 (C=N str), 838.94 (C–Br str), 832.09 (C–Cl str), 686.57 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.62 (s, 1H, NH–CO), 9.55 (s, 1H, NH), 7.89 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 7.86 (s, 1H, 7″H_{BT}), 7.79 (d, 2H, 2′,6′H_{Ph}, $J = 7.0$ Hz), 7.14 (d, 1H, 5″H_{BT}, $J = 7.7$ Hz), 7.01 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.39 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.8 (NH–CO), 162.9 (C-2), 160.8 (C-2″), 154.8 (C-5), 147.6 (C-8″), 138.4 (C-4′), 132.9 (C-1′), 130.5 (C-9″), 127.8 (C-3′ and C-5′), 126.1 (C-6″), 125.5 (C-4″), 124.6 (C-7″), 123.9 (C-2′ and C-6′), 122.3 (C-5″), 39.9 (CH_2–NH); MS (70 eV): $m/z = 481.51$ (M+1), 482.19 (M+2), 484.86 (M+4).

N-(5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl)-2-(6-chlorobenzo[d]thiazol-2-ylamino)acetamide (5w): Creamy-yellow shiny crystals; solubility: chloroform, methanol. Elemental analysis: Found C, 42.50; H, 2.39; N, 14.49; calcd. C, 42.47; H, 2.31; N, 14.57. IR (KBr) ν (cm^{-1}) 3417.42 (NH str), 3070.90 (C–H str aromatic), 1702.55 (C=C str), 1625.67 (C=O str), 1596.24 (C=N str), 836.74 (C–Br str), 833.01 (C–Cl str), 687.05 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.63 (s, 1H, NH–CO), 9.56 (s, 1H, NH), 7.90 (s, 1H, 7″H_{BT}), 7.84 (d, 2H, 3′,5′H_{Ph}, $J = 7.5$ Hz), 7.66 (d, 2H, 2′,6′H_{Ph}, $J = 7.2$ Hz), 7.30 (d, 1H, 5″H_{BT}, $J = 7.8$ Hz), 7.04 (d, 1H, 4″H_{BT}, $J = 7.9$ Hz), 4.31 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 167.8 (NH–CO), 163.4 (C-2), 161.2 (C-2″), 155.0 (C-5), 148.3

(C-8″), 137.7 (C-4′), 132.5 (C-1′), 131.4 (C-9″), 126.8 (C-3′ and C-5′), 126.5 (C-6″), 125.7 (C-4″), 124.9 (C-7″), 123.5 (C-2′ and C-6′), 122.8 (C-5″), 40.4 (CH_2–NH); MS (70 eV): $m/z = 481.54$ (M+1), 482.27 (M+2), 484.91 (M+4).

2-(6-Bromobenzo[d]thiazol-2-ylamino)-N-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)acetamide (5x): Dark yellowish crystals; solubility: chloroform, ethanol. Elemental analysis: Found C, 38.79; H, 2.06; N, 13.39; calcd. C, 38.87; H, 2.11; N, 13.33. IR (KBr) ν (cm^{-1}) 3416.07 (NH str), 3073.99 (C–H str aromatic), 1701.65 (C=C str), 1623.75 (C=O str), 1597.98 (C=N str), 837.06 (C–Br str), 688.25 (C–S–C str); ^{1}H NMR δ (ppm) (400 MHz, DMSO-d_6): 12.58 (s, 1H, NH–CO), 9.55 (s, 1H, NH), 7.85 (s, 1H, 7″H_{BT}), 7.81 (d, 2H, 2′,6′H_{Ph}, $J = 6.7$ Hz), 7.64 (d, 2H, 3′,5′H_{Ph}, $J = 7.0$ Hz), 7.17 (d, 1H, 5″H_{BT}, $J = 7.7$ Hz), 7.00 (d, 1H, 4″H_{BT}, $J = 8.0$ Hz), 4.24 (s, 2H, CH_2); ^{13}C NMR δ (ppm) (DMSO-d_6, TMS): 168.9 (NH–CO), 163.1 (C-2), 160.5 (C-2″), 155.2 (C-5), 147.8 (C-8″), 137.5 (C-4′), 132.5 (C-1′), 130.4 (C-9″), 126.7 (C-3′ and C-5′), 126.0 (C-6″), 125.4 (C-4″), 124.8 (C-7″), 123.5 (C-2′ and C-6′), 122.2 (C-5″), 40.1 (CH_2–NH); MS (70 eV): $m/z = 526.09$ (M+1), 527.00 (M+2), 529.79 (M+4).

Pharmacology

The investigations were conducted on Swiss male albino mice (20 ± 2 g) and adult Wistar rats (180 ± 10 g). The animals were housed in wire-mesh cages under laboratory conditions (26 ± 2°C, 12 h/12 h light/dark). They were allowed to acclimatize, with free access to food and water, for a 24-h period before testing. All the experimental protocols were carried out with the permission from the Institutional Animal Ethics Committee (IAEC). Animals were obtained from the Central Animal House Facility (173/CPCSEA, 28 January, 2000), Hamdard University, New Delhi.

Preliminary anticonvulsant screening

The synthesized compounds were assessed for anticonvulsant activity using the MES test and the scPTZ test. The electroconvulsometer (Ugo Basile ECT unit, pulse generator 57800; Comerio VA, Italy) with an ear clip electrode was used for the assessment of the anticonvulsant potential against electroshock-induced seizures. Neurotoxicity was ascertained by the minimal motor impairment test using the Rotarod test (Ugo Basile Srl motor function unit, 47600–Mouse Rota-Rod; Comerio VA, Italy). Four animals (Swiss albino mice) were selected for each treatment and standard group. Phenytoin and carbamazepine were used as standards.

MES test

In the MES test [18], mice were pre-screened for 24 h before delivering the maximal electroshock (50 mA, 60 Hz, 0.2 s duration) by means of corneal electrodes. A drop of 0.9% sodium chloride was instilled in each eye prior to the application of the electrodes, in order to prevent death of the animal. The test compounds were suspended in a 0.5% methyl cellulose/water mixture or in polyethylene glycol (PEG). The test solutions of each compound at three doses (30, 100, and 300 mg/kg body mass) were administered intraperitoneally and the anticonvulsant activity was assessed after 0.5- and 4.0-h intervals, respectively. Abolition of the hind limb tonic extensor component of the seizure in half or more of the animals was defined as protection.

scPTZ test

In the scPTZ test [19], a dose of pentylenetetrazol of 70 mg/kg was utilized. This produced clonic seizures in >95% of the animals, lasting for a period of at least 5 s. The test compounds were administered at three graded doses, viz., 30, 100, and 300 mg/kg intraperitoneally. At the anticipated time, the convulsant was administered as 0.5% solution, subcutaneously, in the posterior midline. Animals were observed over a 30-min period. Absence of clonic spasms in half or more of the animals in the observed time period indicates the ability of a compound to abolish the effect of pentylenetetrazol at the seizure threshold.

Minimal motor impairment test (Rotarod test)

The minimal motor impairment is measured in mice by the Rotarod test [20]. The mice were trained to stay on an accelerating Rotarod of 3.2 cm in diameter, rotating at 10 rpm. The animals were injected intraperitoneally with the test compounds at a dose of 25 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least 1 min in each of the three trials. The dose at which 50% of the animals were unable to balance themselves and fell off the rotating rod was determined.

Quantitative anticonvulsant study

The quantification study of the selected compounds for the determination of the median effective dose (ED_{50}), the median neurotoxic dose (TD_{50}), and the protective index (PI) was done using the literary procedure. For the determination of ED_{50} and TD_{50} values, groups of six mice were given a range of intraperitoneal doses of the test drug, until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program [22].

Psychomotor seizure test

The psychomotor seizure analysis was executed according to the protocol first described by Brown et al. [24] and more recently by Barton et al. [25] and Kaminski et al. [26]. The kernels employed for this alternate electroshock paradigm included a constant-current corneal stimulation (0.2 ms rectangular pulse width) at a low frequency (6 Hz) for a longer duration (3 s) at a dose of 100 mg/kg intraperitoneally in a group of four mice. The mice were manually restrained and instantly released into the observation cage following the stimulation. The stunned posture colligating rearing, forelimb automatic movements, and clonus, twitching of the vibrissae and Straub tail conduced to the seizure. The duration of seizure in untreated animals varied from 60 to 120 s, following which the animals resumed their normal exploratory behavior. Protection is manifested by attainment of exploratory behavior within 10 s from the stimulation.

GABA estimation

The most active compounds found after initial anticonvulsant screening were subjected to neurochemical estimation of the GABA levels in adult Wistar rat brain, to assess the effects of the synthesized compounds on the GABA levels in various regions of the rat brain. Adult Wistar rats weighing 180 ± 10 g were used in this study. The animals were divided into groups of six. The

control group was treated only with the vehicle (30% v/v PEG-400). The animals were sacrificed after 2 h of intraperitoneal drug administration by decapitation, and the brain regions, i.e., midbrain, olfactory lobe, cerebellum, and medulla oblongata, were transferred to separate vessel containing 6–8 mL of ice-cold 80% ethanol and processed further according to the reported procedure [27].

Liver function test

The animals were divided into groups of six, and the control group received a basal diet and vehicle (0.5% methylcellulose). The active drugs were administered orally to each animal at a dose of 25 mg/kg/day (in 0.5% methylcellulose) for 2 weeks. After the stipulated period, each animal was anesthetized by anesthetic ether and blood was collected by cardiac puncture to assess the biochemical parameters such as SGOT, SGPT, alkaline phosphate, and total protein, according to the 2,4-dinitrophenyl hydrazine method using SPAN diagnostic reagent kits [28–30].

Statistical analysis

All the statistical analyses were performed using the software GraphPad InStat version 3.0, and the method used was ANOVA followed by Dunnett's multiple comparison test.

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