Med Chem Res (2012) 21:2428–2442
DOI 10.1007/s00044-011-9768-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and anticonvulsant evaluation of some novel 1, 3 benzothiazol-2-yl hydrazones/acetohydrazones

Praveen Kumar · Birendra Shrivastava · Surendra N. Pandeya · Laxmi Tripathi · James P. Stables

Received: 13 December 2010 / Accepted: 27 July 2011 / Published online: 10 August 2011

Abstract A series of 2-[2-(substituted)hydrazinyl]-1,3-benzothiazole and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazide were designed and synthesized keeping in view the structural requirement of pharmacophore and evaluated for anticonvulsant activity and neurotoxicity. The anticonvulsant activity of the titled compounds was assessed using the 6 Hz psychomotor seizure test. The most active compound of the series was 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-bromophenoxy)benzylidene] acetohydrazide **BT 15**, which showed 75% protection (3/4, 1.0 h) and 50% protection (2/4, 0.5 h) at a dose of 100 mg/kg in mice. A computational study was carried out for calculation of pharmacophore pattern and prediction of pharmacokinetic properties. Titled compounds have also exhibited good binding properties with epilepsy molecular targets such as GABA (A) alpha-1, glutamate, GABA (A) delta receptors and Na/H exchanger, in Lamarckian genetic algorithm-based flexible docking studies.

P. Kumar (✉) · B. Shrivastava
School of Pharmaceutical Sciences, Jaipur National University, Jaipur 302025, Rajasthan, India
e-mail: praveensha1977@rediffmail.com

S. N. Pandeya
Saroj Institute of Technology and Management, Lucknow 226 002, Uttar Pradesh, India

L. Tripathi
S D College of Pharmacy & Vocational Studies, Muzaffarnagar 251001, Uttar Pradesh, India

J. P. Stables
Preclinical Pharmacology Section, Epilepsy Branch, National Institute of Health, Bethesda, MD 20892-9020, USA

Keywords 2-[2-(substituted)hydrazinyl]-1,3-benzothiazole · 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazide · 6 Hz psychomotor seizure test · Neurotoxicity · Computational study

Introduction

Epilepsy is a common neurological disorder affecting 0.5–1% of the population, i.e., 45–100 million people worldwide (Bell and Sander, 2002). Current clinically available drugs produce satisfactory seizure control in 60–70% of patients (Perucen, 1996). Several new anticonvulsants like oxacarbazepine, vigabatrin, lamotrigine, gabapentin, topiramate, felbamate, rufinamide, and levetiracetam have been put in clinical practice. Despite familiarity with established antiepileptic drugs and the introduction of these new agents in the past decade, up to one-third of epilepsy patients remain resistant to optimum drug treatment (Sabers and Gram, 2000). Moreover, the current drug therapy is associated with adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, gingival hyperplasia, hirsutism, and megaloblastic anemia (Yogeeshwari *et al.*, 2006). These facts triggered the search for newer more effective and less toxic anticonvulsants.

Earlier two-dimensional (2D) modeling on anticonvulsants has identified that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a spatial arrangement are to be recommended for anticonvulsant activity (Camerman and Camerman, 1980). A pharmacophore model based on some well-known voltage-gated sodium channel blockers including phenytoin and lamotrigine was also proposed (Unverferth *et al.*, 1998). Literature review shows that anticonvulsant activity has been displayed by various hydrazones, acetohydrazones,

semicarbazides, and thiosemicarbazides. Aryl acid hydrazones, similar to semicarbazones, contains large hydrophobic group in close proximity to two electron donor nitrogen atoms, thus fulfilling the structural requirements of maximal electroshock (MES) screen (Jones and Woodbury, 1982). Various benzothiazole derivatives were synthesized and reported as anticonvulsants (Hays *et al.*, 1994; Mizoule *et al.*, 1985; Chopade *et al.*, 2000). 1, 3-benzothiazol-2-yl semicarbazones were synthesized and reported to have significant protection in MES model (Siddiqui *et al.*, 2007). In fact, these evidences suggest that 2-[substituted]-1,3-benzothiazole moiety **1**, possess pharmacophoric character for anticonvulsant activity. In addition, the 4-(aryloxy)phenyl semicarbazone **2** were reported as potential anticonvulsant (Wang *et al.*, 1998).

Continuing our studies on benzfused derivatives that are attractive candidates as anticonvulsant agents, we designed a new series of functionalized 2-[2-(substituted)hydrazinyl]-1,3-benzothiazole **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazide compounds **BT 11–20**, exploring 2-amino benzothiazole and 2-mercapto benzothiazole, respectively, as starting material. The rational design of 2-[2-(substituted)hydrazinyl]-1,3-benzothiazole **BT 1–10**, was planned by molecular hybridization of 2-[substituted]-1,3-benzothiazole **1** and 4-(aryloxy)phenyl semicarbazones **2**. While the rational design of 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazide **BT 11–20**, was planned by molecular hybridization of **1** and **2**, using –S–CH_2–CO– group as bridge (Fig. 1). Their chemical structures were characterized using IR, ^{1}H-NMR, MS, and elemental analysis techniques. All the synthesized titled compounds composed of the essential pharmacophoric elements (Fig. 2) that are necessary for good anticonvulsant activity (Unverferth *et al.*, 1998). In addition, their anticonvulsant activity was evaluated by using 6 Hz psychomotor seizure test in mice. The rotorod assay was performed in mice to evaluate the neurotoxicity of the compounds. Computational study was also carried out to highlight the pharmacophore distance mapping, Log *P* calculation and pharmacokinetic parameters. In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds with six established epilepsy molecular targets and docking affinity & count of probable H-bonds were determined.

Results and discussion

Synthesis and characterization

The reaction sequence leading to the formation of the titled compounds, viz. 2-{2-[substituted]hydrazinyl}-1,3-benzothiazole **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazide **BT 11–20** is shown in Scheme 1. The 4-substituted benzaldehydes **3a–i** were prepared by refluxing various substituted phenols **1a–i** with 4-fluoro benzaldehyde **2** in *N,N*-DMF in the presence of potassium carbonate. To a suspension of 1, 3-benzothiazol-2-amine **4** in ethylene glycol, hydrazine hydrate, and conc. HCl was added with stirring at 5–6°C. This mixture was refluxed to obtain 2-hydrazinyl-1, 3-benzothiazole **5**. A mixture of 1, 3-benzothiazole-2-thiol **6**, chloroethyl acetate and triethyl amine was refluxed in dry methanol to obtain ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate **7**. The **7** was converted to 2-(1, 3-benzothiazol-2-ylsulfanyl) acetohydrazide **8** by reacting with hydrazine hydrate in ethanol. The **5** and **8** were condensed with various 4-substituted benzaldehyde **3a–i**/Isatin **3j** to yield the titled compounds **BT 1–20**. Thin layer chromatography (TLC) was run throughout the reactions to optimize for purity and completion. The physical data for the newly synthesized compounds is presented in Tables 1 and 2.

The structural assignments to new compounds were based on their elemental analysis and spectral (FT-IR, ^{1}H-NMR, and mass) data. The formation of 4-substituted benzaldehydes **3a–i** from 4-substituted phenols **1a–i** was

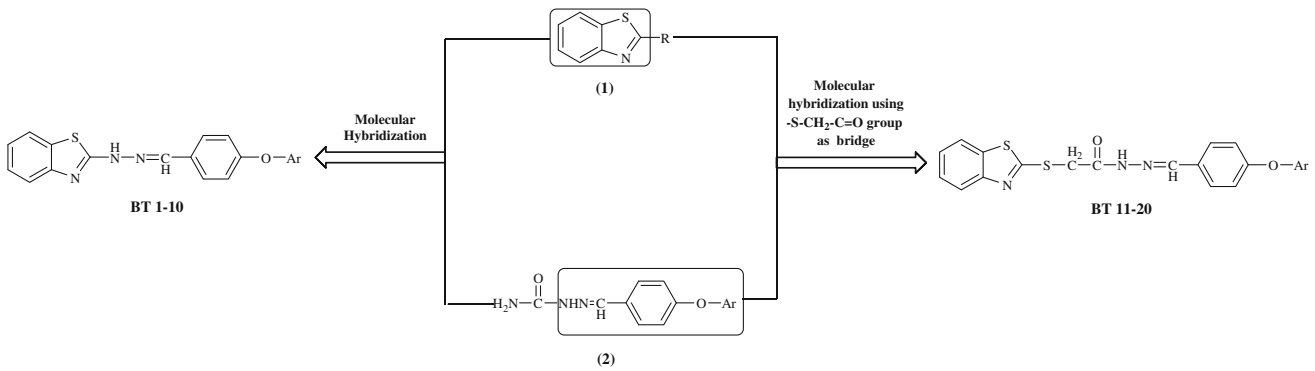


Fig. 1 Rational concept to new 2-{2-[substituted]hydrazinyl}-1,3-benzothiazole **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazide **BT 11–20**

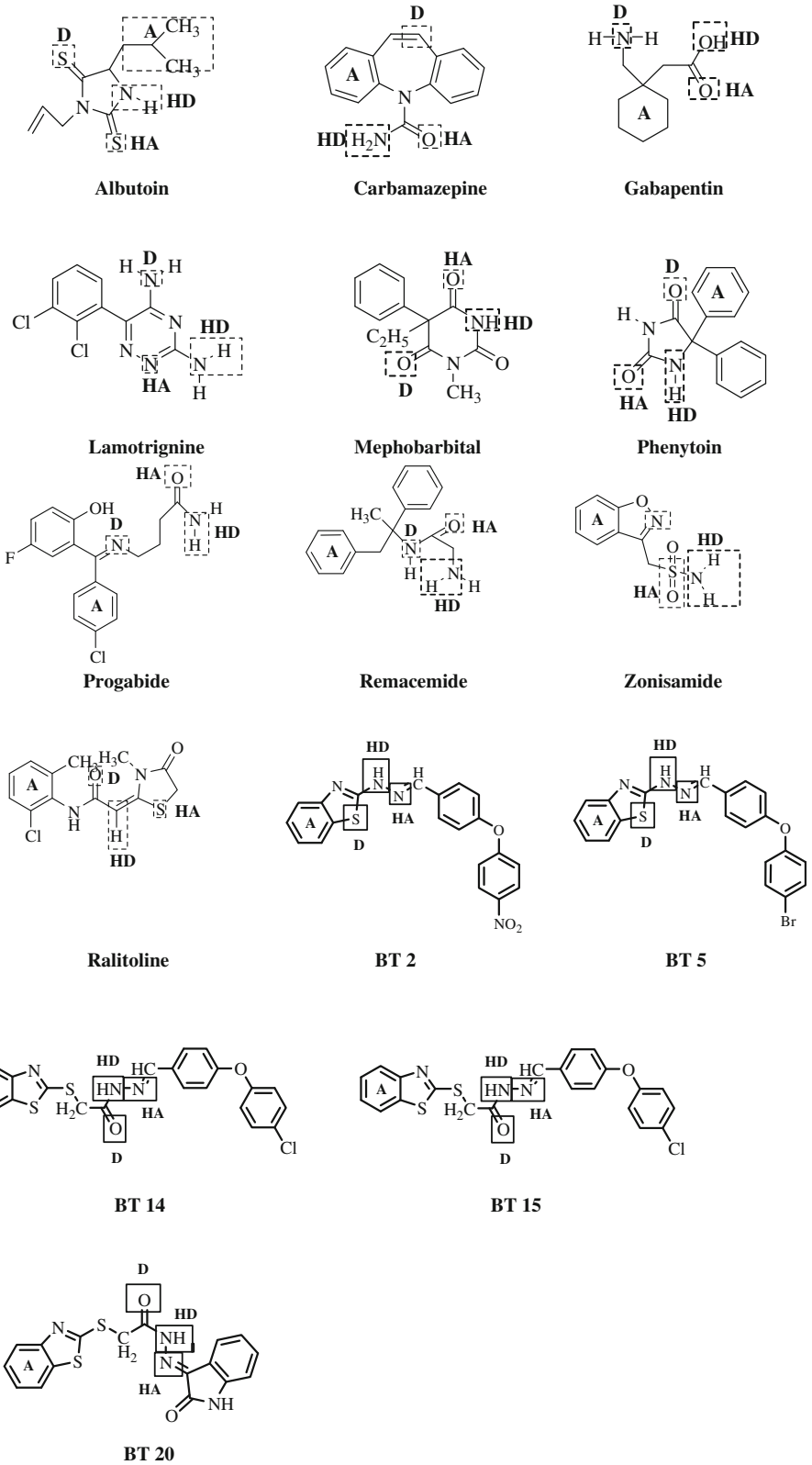
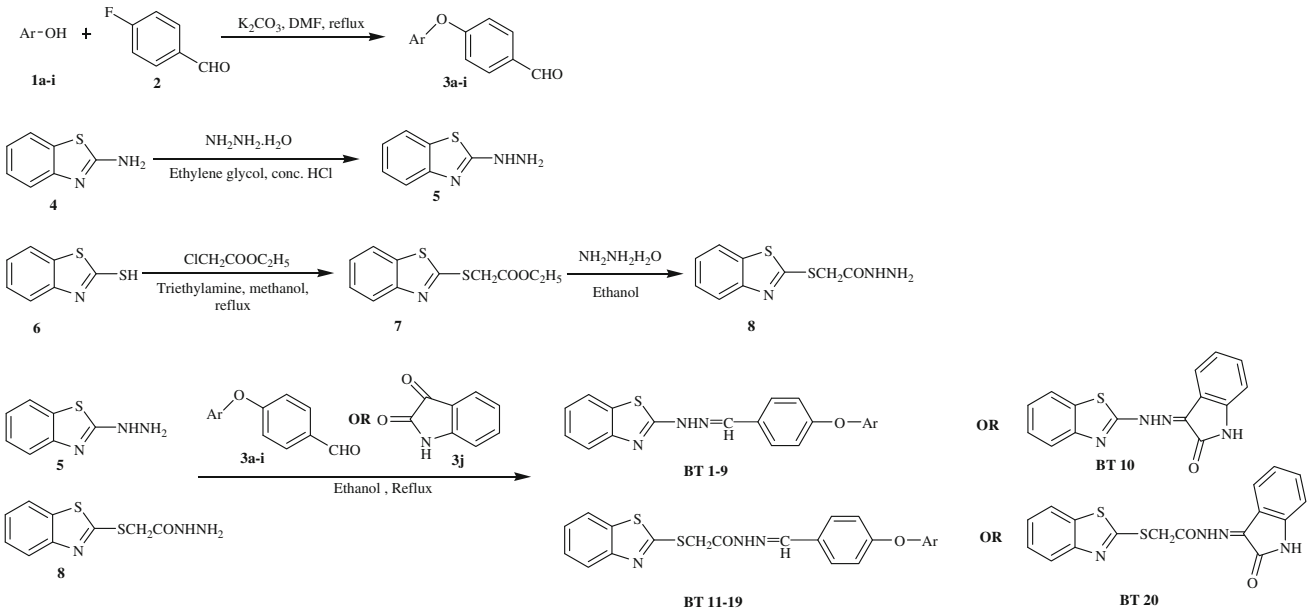


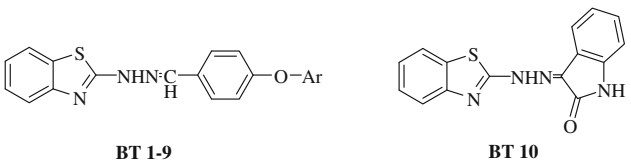
Fig. 2 Pharmacophoric pattern of well-known anticonvulsants and 2-{2-[substituted]hydrazinyl}-1,3-benzothiazole and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazide (**BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20**)

confirmed by its IR and ^{1}H-NMR spectral studies. The IR spectrum of 4-(4-bromophenoxy) benzaldehyde **3e** showed bands at 1662, 3030, and 1241 cm^{-1} indicating the presence of C=O$_{str}$, C–H$_{str}$, and diaryl ether linkage (–O–). The absence of broad band of phenolic –OH at 2900–3000 cm^{-1} confirms the conversion of 4-bromo phenol **1e**



Scheme 1 Synthesis of 2-{2-[substituted]hydrazinyl}-1,3-benzothiazole **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazide **BT 11–20**

Table 1 Physical data of 2-{2-[substituted]hydrazinyl}-1,3-benzothiazoles **BT 1–10**



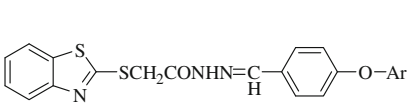
BT 1-9 **BT 10**

Compounds	Ar	Yield (%)	Melting point (°C)	Mol. formula[a] (mol. wt.)	R_f
BT 1	Phenyl	76	174	$C_{20}H_{15}N_3OS$ (345.42)	0.58
BT 2	4-NO_2-Phenyl	81	172	$C_{20}H_{14}N_4O_3S$ (390.42)	0.52
BT 3	4-CH_3-Phenyl	78	182	$C_{21}H_{17}N_3OS$ (359.44)	0.55
BT 4	4-Cl-Phenyl	72	156	$C_{20}H_{14}ClN_3OS$ (379.86)	0.50
BT 5	4-Br-Phenyl	68	240	$C_{20}H_{14}BrN_3OS$ (424.31)	0.44
BT 6	4-F-Phenyl	74	191	$C_{20}H_{14}FN_3OS$ (363.41)	0.45
BT 7	3-Methyl-4-chloro phenyl	70	195	$C_{21}H_{16}ClN_3OS$ (393.89)	0.51
BT 8	Naphthalene-2-yl	76	198	$C_{24}H_{17}N_3OS$ (395.48)	0.55
BT 9	1,3-benzodioxol-5-yl	67	147	$C_{21}H_{15}N_3O_3S$ (389.43)	0.49
BT 10	–	82	308	$C_{15}H_{10}N_4OS$ (294.33)	0.57

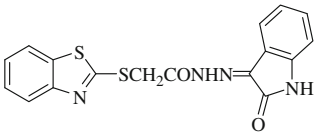
[a] Solvent used for recrystallization—ethanol:water (9:1)

to 4-(4-bromophenoxy) benzaldehyde **3e**. In its ^{1}H-NMR spectrum a singlet at δ 9.81 ppm indicating the presence of –CHO group, where as an absence of a singlet around δ 9.28 ppm for phenolic –OH confirms the conversion of **1e** to **3e**. The IR spectrum of the titled compound **BT 15** showed N–H_{str} at 3178 and 3076 cm^{-1}, C=O_{str} at 1664 cm^{-1}, CH=N_{str} at 1611 cm^{-1}, and diaryl ether linkage (–O–) at 1240 cm^{-1}. Its ^{1}H-NMR spectrum showed a singlet of imine (CH=N) proton at δ 8.00 ppm. The NH proton resonated as a broad singlet at δ 11.15 ppm that was D_2O exchangeable. Aromatic and benzothiazole protons appeared as a set of signals in the region δ 6.88–7.94 ppm. The S-CH_2 protons appeared as singlet at 4.06 ppm. The presence of CH=N_{str} at 1611 cm^{-1} in IR spectrum and a singlet for imine (CH=N) proton at δ 8.00 ppm in ^{1}H-NMR spectrum, confirms the formation of **BT 15**. Further mass spectrum confirmed their purity and molecular weight.

Table 2 Physical data of 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazides **BT 11–20**



BT 11-19



BT 20

Compounds	Ar	Yield (%)	Melting point (°C)	Mol. formula[a] (mol. wt.)	R_f
BT 11	Phenyl	76	184	$C_{22}H_{17}N_3O_2S_2$ (419.52)	0.54
BT 12	4-NO_2-Phenyl	78	112	$C_{22}H_{16}N_4O_4S_2$ (464.52)	0.43
BT 13	4-CH_3-Phenyl	70	178	$C_{23}H_{19}N_3O_2S_2$ (433.55)	0.46
BT 14	4-Cl-Phenyl	64	174	$C_{22}H_{16}ClN_3O_2S_2$ (453.96)	0.41
BT 15	4-Br-Phenyl	62	148	$C_{22}H_{16}BrN_3O_2S_2$ (498.42)	0.38
BT 16	4-F-Phenyl	70	162	$C_{22}H_{16}FN_3O_2S_2$ (437.51)	0.46
BT 17	3-Methyl-4-chloro phenyl	77	168	$C_{23}H_{18}ClN_3O_2S_2$ (467.99)	0.43
BT 18	Naphthalene-2-yl	72	150	$C_{26}H_{19}N_3O_2S_2$ (469.58)	0.48
BT 19	1,3-benzodioxol-5-yl	66	130	$C_{23}H_{17}N_3O_4S_2$ (463.53)	0.42
BT 20	–	80	182	$C_{17}H_{12}N_4O_2S_2$ (368.43)	0.47

[a] Solvent used for recrystallization—ethanol:water (9:1)

Anticonvulsant and neurotoxicity evaluation

The newly synthesized 2-{2-[substituted]hydrazinyl}-1,3-benzothiazoles **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazides **BT 11–20** were subjected to anticonvulsant screening by 6 Hz psychomotor seizure or minimal clonic seizure test to identify their anticonvulsant activity at five different time points, i.e., 0.25, 0.5, 1.0, 2.0, and 4.0 h after i.p. administration in mice at a dose of 100 mg/kg. Neurotoxicity was observed by minimal motor impairment (MMI) which was measured by the rotorod test. The 6 Hz psychomotor seizure test is used to assess a compound's efficacy against electrically induced seizures but uses a lower frequency (6 Hz) and longer duration of stimulation (3 s). It is used to identify clinically useful AEDs that are ineffective in the standard MES and scMET tests but still have anticonvulsant activities in vivo. The results of screening at five different points are summarized in Table 3. As observed from the results of various tested titled compounds, the most active compound of the series was **BT 15**, which showed 75% protection (3/4, 1.0 h) and 50% protection (2/4, 0.5 h) at a dose of 100 mg/kg in mice. At a dose of 100 mg/kg, compounds **BT 14** and **BT 20** showed 75% protection (3/4) at a time point of 1.0 and 0.5 h, respectively. Compounds **BT 2**, **BT 5**, and **BT 11** showed 50% protection at two time point, i.e. (2/4, 0.25 h; 2/4, 1.0 h), (2/4, 0.5 h; 2/4, 2.0 h), and (2/4, 0.25 h; 2/4, 1.0 h), respectively. At a dose of 100 mg/kg, compounds **BT 1**, **BT 4**, **BT 8**, and **BT 16** showed 50% protection (2/4) at a time point of 2.0, 0.5, 2.0, and 0.25 h, respectively. **BT 6**, **BT 13**, and **BT 19** were found inactive. The other compounds displayed mild to moderate protection. None of the compounds showed neurotoxicity in the highest administered dose (300 mg/kg).

Computational study

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.0 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. A computational study of all compounds was performed for prediction of ADME properties such as absorption (% ABS), polar surface area (TPSA), miLog *P*, number of rotatable bonds, and violations of Lipinski's rule of five by using Molinspiration online property calculation toolkit. Calculated miLog *P* for synthesized compounds were then compared with the experimental Log *P* data of these compounds. Docking study of titled compounds was performed with six established epilepsy molecular targets, namely GABA (A) alpha-1 receptor, GABA (A) delta receptor, glutamate receptor, Na/H exchanger, Na channel receptor, and T-type calcium channel receptor by using AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking and their docking affinity (Kcal/mol) and count of probable hydrogen bonds were determined.

Distance mapping

This study involves the correlation of the structural requirement of well-known and structurally different

Table 3 6 Hz anticonvulsant activity and neurotoxicity of 2-{2-[substituted]hydrazinyl}-1,3-benzothiazole **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazide **BT 11–20**

Compound	Test	Dose (mg/kg)	Time (h) to peak effect (N/F)[a]				
			0.25	0.5	1.0	2.0	4.0
BT 1	6 Hz	100	0/4	1/4	0/4	2/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 2	6 Hz	100	2/4	1/4	2/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 3	6 Hz	100	1/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 4	6 Hz	100	0/4	2/4	0/4	1/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 5	6 Hz	100	0/4	2/4	1/4	2/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 7	6 Hz	100	1/4	0/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 8	6 Hz	100	1/4	0/4	1/4	2/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 9	6 Hz	100	0/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 10	6 Hz	100	0/4	0/4	1/4	1/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 11	6 Hz	100	2/4	1/4	2/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 12	6 Hz	100	0/4	0/4	1/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 14	6 Hz	100	0/4	0/4	3/4	1/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 15	6 Hz	100	0/4	2/4	3/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 16	6 Hz	100	2/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 17	6 Hz	100	0/4	1/4	1/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 18	6 Hz	100	0/4	1/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4
BT 20	6 Hz	100	0/4	3/4	0/4	0/4	0/4
	TOX	100	0/4	0/4	0/4	0/4	0/4

[a] N/F = number of animals active or toxic over the number tested at a dose of 100 mg/kg

anticonvulsant compounds with the titled compounds. The 2D modeling on anticonvulsants has identified that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a special spatial arrangement is recommended for anticonvulsant activity. In this study, the 10 well-known and structurally different compounds with anticonvulsant activity—albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, and zonisamide (Fig. 2) with different mechanism of action, were selected so as to propose a generalized pharmacophore model. The pharmacophore group's distance estimation was done by molecular mechanics calculation with the force fields based on both CHARMm force fields and MM3 parameterization. In this study, energy minimization was performed on above mentioned 10 well-known anticonvulsants and the titled compounds using Argus Lab 4.0. Distance between the various structural components essential for activity was determined by ACD/3D viewer. The crucial structural components that were included in the four point pharmacophore model (Fig. 3) were the aryl ring center or the lipophilic group (A), an electron donor atom (D), a hydrogen bond acceptor (HA), and a hydrogen bond donor (HD). An average distance range for every point was obtained and compared to that of 2-{2-[substituted]hydrazinyl}-1,3-benzothiazoles **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazides **BT 11–20**. Now it may be interesting to examine whether **BT 1–20** reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that the titled compounds **BT 1–20** did fulfill the essential demands of the pharmacophore when compared to the average distance requirement (Table 4).

Prediction of ADME properties

A computational study for prediction of ADME properties of titled compounds was performed. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestine and blood–brain barrier (BBB) crossing (Ertl *et al.*, 2000). The percentage of absorption (% ABS) was calculated using TPSA. From all these parameters, it can be observed that all titled compounds exhibited a great % ABS ranging from 71.2 to 92.9% (Table 5). Furthermore, compounds **BT 10** and **BT 20** did not show any violation in Lipinski's parameters, making them potentially promising agents for epilepsy therapy. Other compounds are showing only one violation in Lipinski's parameters.

Log *P* determination

Titled compounds showed dependence of biological activity on lipophilic character in a congeneric series. In particular, for drugs acting on central nervous system to be potent, they have to cross BBB, thus potency has been correlated with optimum lipophilicity (Log *P*) near 2. In this study, we attempted to correlate the anticonvulsant activity of congeners with their calculated Log *P* value. The experimental Log *P* values were determined using the octanol-phosphate buffer method. The data is presented in

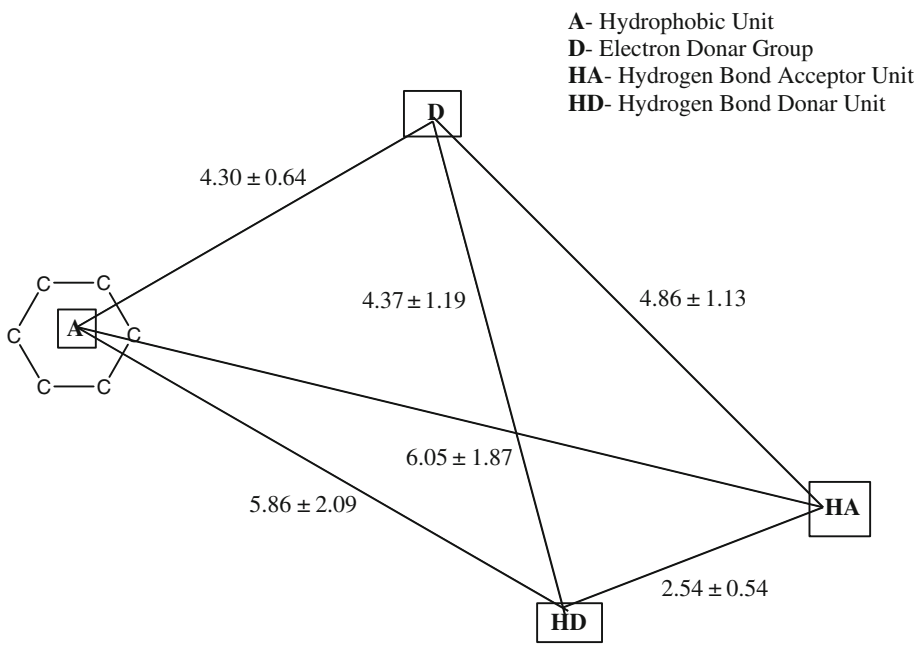


Fig. 3 Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 and CHARMm parametrization (Argus Lab 4.0 and ACD/3D viewer)

Table 4 Distance range between the essential structural elements A, D, and HA–HD

Compounds	A–HA	A–HD	A–D	HA–HD	HD–D	HA–D
Albutoin	5.37	2.72	4.51	2.72	4.03	5.40
Carbamazepine	4.28	4.28	4.25	2.33	5.75	5.67
Gabapentin	4.26	4.93	3.83	2.23	3.57	4.50
Lamotrigine	5.30	7.42	4.54	2.42	4.94	4.25
Mephobarbital	3.78	5.50	4.81	2.34	4.63	5.23
Phenytoin	6.20	4.01	4.35	2.63	3.88	5.17
Progabide	9.51	9.23	3.79	2.41	6.72	6.79
Ralitoline	8.30	5.55	4.56	2.75	2.52	4.85
Remacemide	7.51	8.75	5.38	3.87	3.96	2.43
Zonisamide	6.02	6.22	3.01	1.78	3.71	4.31
Mean ± SD	6.05 ± 1.87	5.86 ± 2.09	4.30 ± 0.64	2.54 ± 0.54	4.37 ± 1.19	4.86 ± 1.13
BT 2	5.60	4.69	2.92	1.41	2.68	3.07
BT 5	5.47	4.70	2.92	1.42	2.67	3.04
BT 14	9.16	7.95	8.09	1.42	2.24	2.67
BT 15	9.16	7.95	8.09	1.42	2.24	2.67
BT 20	8.45	8.47	7.70	1.42	2.24	3.52

Distances calculated for 3D optimized structures using MM3 and CHARMm parameterization (Argus Lab 4.0 and ACD/3D viewer)

Table 6. As observed some of the experimental values were in good agreement with the theoretical values. All the titled compounds showed lipophilic character.

Docking study

In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds **BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20** with six established epilepsy molecular targets namely GABA (A) alpha-1 receptor, GABA (A) delta receptor, glutamate receptor, Na/H exchanger, Na channel receptor, T-type calcium channel receptor. The description of receptors is provided in Table 7. The docking affinity (Kcal/mol) and count of probable hydrogen bonds was evaluated. **BT 2** exhibited good binding properties with GABA (A) delta receptor (affinity value −6.2 kcal/mol and 1 H-bond), GABA (A) alpha-1 receptor (affinity value −5.8 kcal/mol and 0 H-bond), glutamate receptor (affinity value −6.4 kcal/mol and 0 H-bond), and Na/H exchanger (affinity value

Table 5 Pharmacokinetic parameters important for good oral bioavailability of compounds **BT 1–20**

Compound	% ABS	TPSA (A^2)	n-ROTB	MW	MV	n-OHNH donors	n-ON acceptors	Lipinski's violations
Rule	–	–	–	<500	–	<5	<10	≤1
BT 1	92.9	46.51	5	345.43	302.05	1	4	1
BT 2	77.1	92.34	6	390.42	325.38	1	7	1
BT 3	92.9	46.51	5	359.45	318.61	1	4	1
BT 4	92.9	46.51	5	379.87	315.59	1	4	1
BT 5	92.9	46.51	5	424.32	319.94	1	4	1
BT 6	92.9	46.51	5	363.41	306.98	1	4	1
BT 7	92.9	46.51	5	393.89	332.15	1	4	1
BT 8	92.9	46.51	5	395.48	346.04	1	4	1
BT 9	86.5	64.98	5	389.43	325.98	1	6	1
BT 10	84.8	70.14	2	294.33	242.20	2	5	0
BT 11	87.0	63.58	7	419.53	355.96	1	5	1
BT 12	71.2	109.41	8	464.52	379.30	1	8	1
BT 13	87.0	63.58	7	433.55	372.53	1	5	1
BT 14	87.0	63.58	7	453.97	369.50	1	5	1
BT 15	87.0	63.58	7	498.42	373.85	1	5	1
BT 16	87.0	63.58	7	437.52	360.90	1	5	1
BT 17	87.0	63.58	7	468.00	386.06	1	5	1
BT 18	87.0	63.58	7	469.59	399.96	1	5	1
BT 19	80.6	82.05	7	463.54	379.89	1	7	1
BT 20	78.9	87.21	4	368.44	296.12	2	6	0

−5.5 kcal/mol and 0 H-bond). **BT 5** exhibited good binding properties with glutamate receptor (affinity value −6.3 kcal/mol and 2 H-bonds), Na/H exchanger (affinity value −5.7 kcal/mol and 2 H-bonds), GABA (A) alpha-1 receptor (affinity value −6.4 kcal/mol and 1 H-bond), and GABA (A) delta receptor (affinity value −6.4 kcal/mol and 0 H-bond). **BT 14** exhibited good binding properties with glutamate receptor (affinity value −7.7 kcal/mol and 3 H-bonds), Na/H exchanger (affinity value −5.5 kcal/mol and 1 H-bond), GABA (A) alpha-1 receptor (affinity value −5.2 kcal/mol and 1 H-bond), and GABA (A) delta receptor (affinity value −5.9 kcal/mol and 0 H-bond). **BT 15** exhibited good binding properties with GABA (A) alpha-1 receptor (affinity value −6.6 kcal/mol and 2 H-bonds), glutamate receptor (affinity value −6.6 kcal/mol and 1 H-bond), GABA (A) delta receptor (affinity value −6.2 kcal/mol and 0 H-bond), and Na/H exchanger (affinity value −5.2 kcal/mol and 0 H-bond). **BT 20** exhibited good binding properties with GABA (A) alpha-1 receptor (affinity value −5.9 kcal/mol and 3 H-bonds), glutamate receptor (affinity value −6.8 kcal/mol and 2 H-bonds), GABA (A) delta receptor (affinity value −6.2 kcal/mol and 1 H-bond), and Na/H exchanger (affinity value −5.2 kcal/mol and 0 H-bond). The above-mentioned compounds did not show affinity and H-bonding with Na channel and T-type calcium channel receptors. The docking study results of **BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20** with these six receptors are shown in Table 8. The docking images are shown in Fig. 4. Docking study results shows that the titled compounds exhibited good binding properties with glutamate, GABA (A) alpha-1, GABA (A) delta receptor, and Na/H exchanger.

Structure–activity relationship

In this study, various 2-{2-[substituted]hydrazinyl}-1,3-benzothiazoles **BT 1–10** and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted)acetohydrazides **BT 11–20** were synthesized by using different substituent, in order to investigate the pharmacophoric substituent, responsible for better activity. In the synthetic design, the various substituents were introduced at positions 2 of 1, 3-benzothiazol-2-yl backbone structure. At position 2, hydrazones/acetohydrazones having substituent viz. phenyl, 4-nitro phenyl, 4-methyl phenyl, 4-chloro phenyl, 4-bromo phenyl, 4-flouro phenyl, 4-chloro-3-methyl phenyl, naphthalene-2-yl, 1, 3-benzodioxol-5-yl at position 19 and 23, respectively, were introduced. Keeping in view, the pharmacophoric character of isatin for anticonvulsant activity, the isatinyl moiety was also attached to position N-11 and N-15 of 1, 3-benzothiazol-2-yl

Table 6 Log *P* value for compounds **BT 1–20**

Compounds	Experimental Log *P*	Theoretical Log *P* (miLog *P*[a])
Rule	–	≤5
BT 1	5.2	5.5
BT 2	5.1	5.5
BT 3	5.4	5.9
BT 4	5.6	6.2
BT 5	5.5	6.3
BT 6	5.0	5.7
BT 7	5.5	6.5
BT 8	5.6	6.7
BT 9	5.0	5.4
BT 10	3.2	3.5
BT 11	5.6	5.9
BT 12	5.3	5.8
BT 13	5.7	6.3
BT 14	5.6	6.5
BT 15	5.7	6.7
BT 16	5.5	6.0
BT 17	5.7	6.9
BT 18	5.9	7.1
BT 19	5.5	5.8
BT 20	3.2	3.4

[a] miLog *P*, logarithm of compound partition coefficient between *n*-octanol and water calculated as per Molinspiration Online Property Toolkit

hydrazones/acetohydrazones. These substituents were chosen based on the literature review and computational study.

The most active compound **BT 15** has 4-bromo phenyl at position 23 of 1, 3-benzothiazol-2-yl acetohydrazones. It has been observed that at the presence of 4-chloro phenyl and phenyl at position 23 of 1, 3-benzothiazol-2-yl acetohydrazones yields active compounds. The presence of isatinyl at position N-15 of 1, 3-benzothiazol-2-yl acetohydrazones also yields the active compound. Among 1, 3-benzothiazol-2-yl hydrazones only 4-nitro phenyl and 4-chloro phenyl substitution at position 19, yields compound with moderate protection. This observation clearly indicates that benzothiazol-2-yl acetohydrazones are more promising anticonvulsant agents than 1, 3-benzothiazol-2-yl hydrazones.

Experimental

Chemistry

All the chemicals and solvents, purchased from Merck (India), Spectrochem (India), Himedia (India) and S. d. Fine were used without further purification. The progress of reaction was monitored by TLC, performed on a silica gel 60 F_{254} coated aluminum sheet. The melting points were determined by using Thomas-Hoover melting point apparatus and are uncorrected. The FT-IR spectra were recorded on Perkin-Elmer Spectrum BX-II Spectrophotometer. The ^{1}H-NMR spectra were recorded on Bruker 300 MHz High Resolution NMR spectrometer using TMS as an internal standard. Chemical shifts were reported in ppm (δ) and signals were described as singlet (s), doublet (d), triplet (t), and multiplet (m). All exchangeable protons were confirmed by addition of D_2O. The mass spectra were recorded on a Waters Micromass ZQ 2000 mass spectrometer. Elemental analysis (C, H, N) was undertaken with Perkin Elmer Model 240C analyzer.

Synthesis of 4-substituted benzaldehyde (**3a–i**)

A mixture of substituted phenol **1a–i** (37.4 mmol), 4-fluorobenzaldehyde **2** (37.4 mmol), and potassium carbonate (38.8 mmol) in *N,N*-dimethylformamide (30 ml) was refluxed for 16–18 h under nitrogen. After cooling, the product was extracted from the reaction mixture and purified by chromatography. (**3e**) Yield: 72%; m.p.: 47°C; IR (KBr, cm^{-1}) υ: 1662 (C=O), 1241 (–O–); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.81 (s, 1H, –CHO), 6.80–7.82 (a set

Table 7 Description of target receptors used in docking study

Receptor	Description
GABA(A) alpha-1	gi|27808653|sp|P14867.3|GBRA1_HUMAN RecName: Full=Gamma-aminobutyric acid receptor subunit alpha-1; AltName: Full=GABA(A) receptor subunit alpha-1; Flags: Precursor
GABA(A) delta	gi|59802571|sp|O14764.2|GBRD_HUMAN RecName: Full=Gamma-aminobutyric acid receptor subunit delta; AltName: Full=GABA(A) receptor subunit delta; Flags: Precursor
Glutamate	gi|4504117|ref|NP_000821.1| glutamate receptor, ionotropic kainate 1 isoform 1 precursor [Homo sapiens]
Na/H exchanger	gi|6919937|sp|Q92581.2|SL9A6_HUMAN RecName: Full=Sodium/hydrogen exchanger 6; AltName: Full=Na(+)/H(+) exchanger 6; Short = NHE-6; AltName: Full = Solute carrier family 9 member 6
Na channel	gi|126362949|ref|NP_008853.3| sodium channel protein type 3 subunit alpha isoform 1 [Homo sapiens]
T-type calcium	gi|21361077|ref|NP_066919.2| voltage-dependent T-type calcium channel subunit alpha-1I isoform a [Homo sapiens]

Table 8 Docking study results of compounds **BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20**

Ligand	Receptor	Affinity (Kcal/mol)	H-bonds	H-binding ligand			H-binding receptor			
				Element	Atom No.	Type	Residue	Element	Atom No.	Type
BT 2	GABA(A) alpha-1	−5.8	00	–	–	–	–	–	–	–
	GABA (A) delta	−6.2	01	N	09	Donor	Ala324	O	452	Acceptor
	Glutamate	−6.4	00	–	–	–	–	–	–	–
	Na/H exchanger	−5.5	00	–	–	–	–	–	–	–
	Na channel	0.0	00	–	–	–	–	–	–	–
	T-type calcium	0.0	00	–	–	–	–	–	–	–
BT 5	GABA(A) alpha-1	−6.4	01	N	09	Donor	Ser326	O	349	Acceptor
	GABA (A) delta	−6.4	00	–	–	–	–	–	–	–
	Glutamate	−6.3	02	N	09	Donor	Val541	O	952	Acceptor
				N	08	Acceptor	His523	N	780	Donor
	Na/H exchanger	−5.7	02	N	09	Donor	Gly254	O	112	Acceptor
				N	09	Donor	Glu255	O	117	Acceptor
	Na channel	0.0	00	–	–	–	–	–	–	–
	T-type calcium	0.0	00	–	–	–	–	–	–	–
BT 14	GABA(A) alpha-1	−5.2	01	N	6	Acceptor	Ser299	O	185	Both
	GABA (A) delta	−5.9	00	–	–	–	–	–	–	–
	Glutamate	−7.7	03	N	6	Acceptor	Thr333	O	865	Both
				O	30	Acceptor	Thr333	O	865	Both
				N	12	Donor	Thr333	O	865	Both
	Na/H exchanger	−5.5	01	N	12	Donor	Val256	O	135	Acceptor
	Na channel	0.0	00	–	–	–	–	–	–	–
	T-type calcium	0.0	00	–	–	–	–	–	–	–
BT 15	GABA(A) alpha-1	−6.6	02	O	30	Acceptor	Ser297	O	168	Both
				O	30	Acceptor	Ser297	N	162	Donor
	GABA (A) delta	−6.2	00	–	–	–	–	–	–	–
	Glutamate	−6.6	01	N	12	Donor	Leu455	O	102	Acceptor
	Na/H exchanger	−5.2	00	–	–	–	–	–	–	–
	Na channel	0.0	00	–	–	–	–	–	–	–
	T-type calcium	0.0	00	–	–	–	–	–	–	–
BT 20	GABA(A) alpha-1	−5.9	03	N	23	Donor	Ser299	O	185	Both
				O	25	Acceptor	Ser299	O	185	Both
				N	06	Acceptor	Ser297	O	168	Both
	GABA (A) delta	−6.2	01	N	13	Donor	Ala323	O	446	Acceptor
	Glutamate	−6.8	02	N	06	Acceptor	Glu456	N	107	Donor
				N	06	Acceptor	Glu457	N	117	Donor
	Na/H exchanger	−5.2	00	–	–	–	–	–	–	–
	Na channel	0.0	00	–	–	–	–	–	–	–
	T-type calcium	0.0	00	–	–	–	–	–	–	–

Affinity and H-bonds calculations were determined by docking study using AutoDock 4.0 software

of signals, 8H, Ar–H); MS (*m*/*z*, %): 276.95 (M^++1 for ^{79}Br, 100.00), 278.93 (M^++1 for ^{81}Br, 96.70).

Synthesis of 2-hydrazinyl-1, 3-benzothiazole (**5**)

To a suspension of 1, 3-benzothiazol-2-amine **4** (0.01 mol) in ethylene glycol (8 ml), hydrazine hydrate (0.03 mol), and conc. HCl (2 ml) was added at 5–6°C. This mixture was refluxed for 5–6 h to obtain 2-hydrazinyl-1, 3-benzothiazole **5.** Yield: 82%; m.p.: 212–214°C; IR (KBr, cm^{-1}) υ: 3345 ($NHNH_2$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 11.12 (s, 1H, NH, D_2O exchangeable), 7.12–7.96 (m, 4H, Benzothiazole-H), 4.52 (s, 2H, NH_2). MS (*m*/*z*, %): 166.02 (M^++1, 87.43).

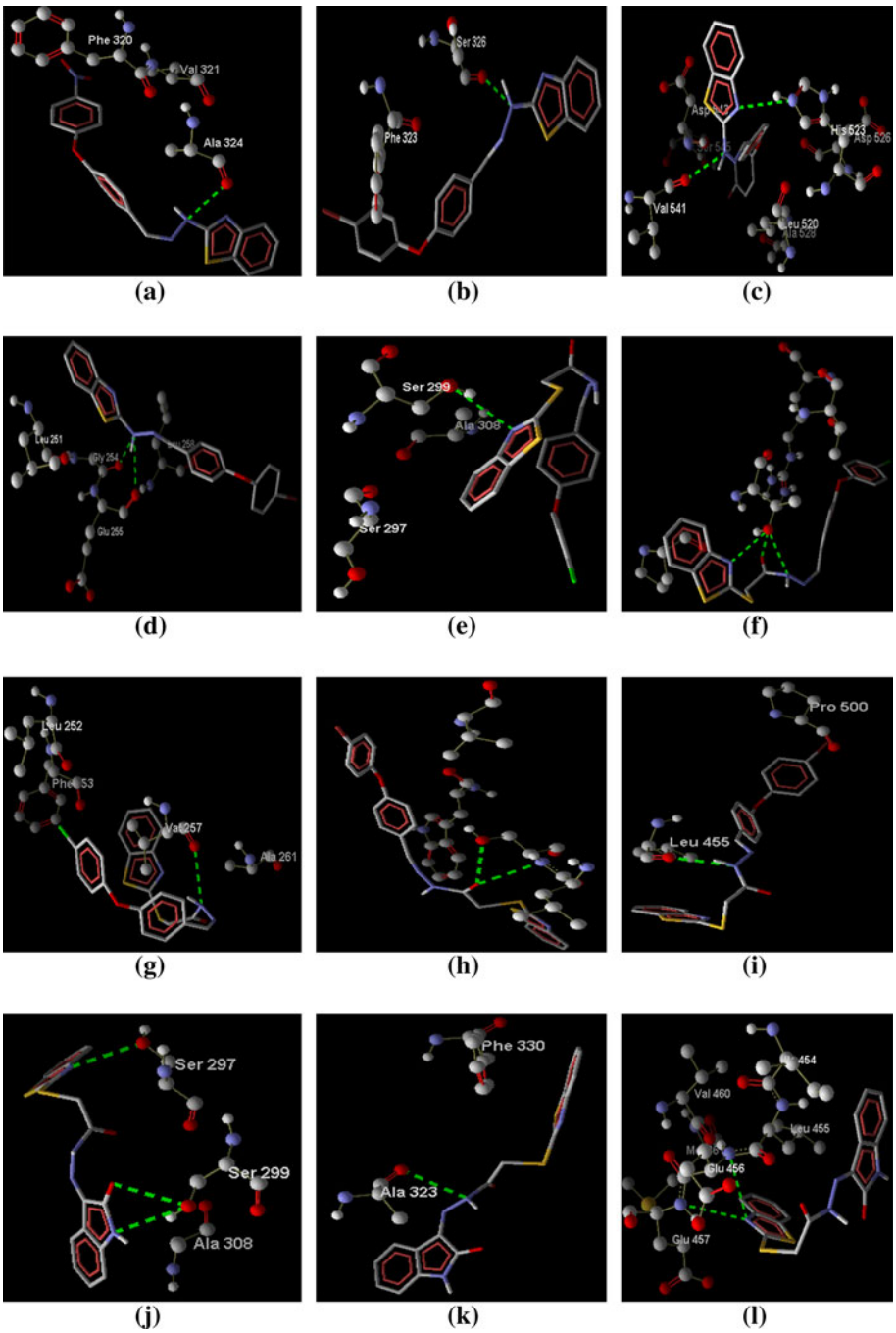


Fig. 4 Docking images. **a BT 2** with GABA (A) delta receptor; **b BT 5** with GABA(A) alpha-1 receptor; **c BT 5** with glutamate receptor, **d BT 5** with Na/H exchanger; **e BT 14** with GABA (A) alpha-1 receptor; **f BT 14** with glutamate receptor, **g BT 14** with Na/H exchanger; **h BT 15** with GABA (A) alpha-1 receptor; **i BT 15** with glutamate receptor; **j BT 20** with GABA (A) alpha-1 receptor; **k BT 20** with GABA (A) delta receptor; and **l BT 20** with glutamate receptor

Synthesis of ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate (**7**)

A mixture of 1, 3-benzothiazole-2-thiol **6** (1 mmol), chloroethyl acetate (1.1 mmol) and triethyl amine (1 mmol) was refluxed in dry methanol for 2–3 h. Ethanol was removed under reduced pressure and the product was recrystallized with ethanol to obtain white crystals of **7**. Yield: 86%; m.p.: 112°C; IR (KBr, cm^{-1}) υ: 1672 (C=O), 721 (C–S–C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.06–7.92 (m, 4H, Benzothiazole-H), 4.33 (q, 2H, O-CH_2), 4.00 (s, 2H, S-CH_2), 1.34 (t, 3H, OCH_2CH_3); MS (*m*/*z*, %): 254.00 (M^++1, 43.26).

Synthesis of 2-(1,3-benzothiazol-2-ylsulfanyl)acetohydrazide (**8**)

Compound **7** (0.1 mol) and hydrazine hydrate (0.3 mol) in ethanol (50 ml) were refluxed for 2 h. The excess of solvent was removed under reduced pressure and recrystallized from chloroform-hexane (3:1) to yield white crystals of compound **8**. Yield: 81%; m.p.: 168–170°C; IR

(KBr, cm^{-1}) υ: 3335 ($NHNH_2$), 1668 (C=O), 726 (C–S–C); ^1H NMR ($CDCl_3$, 300 MHz) δ: 11.02 (s, 1H, NH, D_2O exchangeable), 7.02–7.90 (m, 4H, Benzothiazole-H), 4.48 (s, 2H, NH_2), 4.06 (s, 2H, S-CH_2). MS (*m*/*z*, %): 240.02 (M^++1, 37.43).

Synthesis of 2-{2-[substituted]hydrazinyl}-1,3-benzothiazoles (**BT 1–10**) and 2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(substituted) acetohydrazides (**BT 11–20**)

Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes **3a–i**/isatin **3j** and 2-hydrazinyl-1, 3-benzothiazole **5**/2-(1, 3-benzothiazol-2-ylsulfanyl) acetohydrazide **8** were dissolved in warm ethanol containing 0.5 ml of glacial acetic acid. The reaction mixture was refluxed for 4–6 h and set aside. The resultant solid was washed with ethanol and recrystallized from 90% ethanol. The physical data and elemental analysis data of the titled compounds **BT 1–20** are presented in Tables 1 and 2. The spectral data of titled compounds **BT 1–20** are given below.

2-[2-(4-phenoxybenzylidene)hydrazinyl]-1,3-benzothiazole **(BT 1)**

IR (KBr, cm^{-1}) υ: 3251, 3068 (NH_{str} associated), 1605 ($CH=N_{str}$), 1241 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.72 (s, 1H, NH, D_2O exchangeable), 7.01–8.28 (a set of signals, 13H, Ar–H, and Benzothiazole-H), 8.02 (s, 1H, CH=N). MS (*m*/*z*, %): 346.07 (M^++1, 94.11).

2-{2-[4-(4-nitrophenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole **(BT 2)**

IR (KBr, cm^{-1}) υ: 3245, 3066 (NH_{str} associated), 1607 ($CH=N_{str}$), 1523 (N=O), 1240 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.76 (s, 1H, NH, D_2O exchangeable), 7.04–8.23 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 8.09 (s, 1H, CH=N). MS (*m*/*z*, %): 391.00 (M^++1, 100.00).

2-{2-[4-(4-methylphenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 3**)

IR (KBr, cm^{-1}) υ: 3248, 3064 (NH_{str} associated), 1603 ($CH=N_{str}$), 1239 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.71 (s, 1H, NH, D_2O exchangeable), 7.01–8.22 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 8.05 (s, 1H, CH=N), 2.34 (s, 3H, CH_3). MS (*m*/*z*, %): 360.07 (M^++1, 91.71).

2-{2-[4-(4-chlorophenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 4**)

IR (KBr, cm^{-1}) υ: 3254, 3061 (NH_{str} associated), 1611 ($CH=N_{str}$), 1242 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.76 (s, 1H, NH, D_2O exchangeable), 7.08–8.21 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 8.09 (s, 1H, CH=N). MS (*m*/*z*, %): 380.06 (M^++1 for ^{35}Cl, 100.00), 382.01 (M^++1 for ^{37}Cl, 35.13).

2-{2-[4-(4-bromophenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 5**)

IR (KBr, cm^{-1}) υ: 3251, 3068 (NH_{str} associated), 1606 ($CH=N_{str}$), 1240 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.79 (s, 1H, NH, D_2O exchangeable), 7.01–8.26 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 8.05 (s, 1H, CH=N). MS (*m*/*z*, %): 426.03 (M^++1 for ^{81}Br, 100.00), 424.07 (M^++1 for ^{79}Br, 95.30).

2-{2-[4-(4-fluorophenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 6**)

IR (KBr, cm^{-1}) υ: 3255, 3066 (NH_{str} associated), 1602 ($CH=N_{str}$), 1241 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.78 (s, 1H, NH, D_2O exchangeable), 7.08–8.21 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 8.03 (s, 1H, CH=N). MS (*m*/*z*, %): 364.02 (M^++1, 78.10).

2-{2-[4-(4-chloro-3-methylphenoxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 7**)

IR (KBr, cm^{-1}) υ: 3246, 3061 (NH_{str} associated), 1609 ($CH=N_{str}$), 1240 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.72 (s, 1H, NH, D_2O exchangeable), 7.09–8.25 (a set of signals, 11H, Ar–H, and Benzothiazole-H), 8.06 (s, 1H, CH=N), 2.36 (s, 3H, CH_3). MS (*m*/*z*, %): 394.05 (M^++1 for ^{35}Cl, 100.00), 396.10 (M^++1 for ^{37}Cl, 36.17).

2-{2-[4-(naphthalen-2-yloxy)benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 8**)

IR (KBr, cm^{-1}) υ: 3253, 3065 (NH_{str} associated), 1607 ($CH=N_{str}$), 1240 (–O–), 838, 826 (β-naphthyl). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.74 (s, 1H, NH, D_2O exchangeable), 7.03–8.28 (a set of signals, 15H, Ar–H, naphthyl-H, and Benzothiazole-H), 8.03 (s, 1H, CH=N). MS (*m*/*z*, %): 396.13 (M^++1, 52.13).

2-{2-[4-(1,3-benzodioxol-5-yloxy) benzylidene]hydrazinyl}-1,3-benzothiazole (**BT 9**)

IR (KBr, cm^{-1}) υ: 3251, 3063 (NH_{str} associated), 1608 ($CH=N_{str}$), 1242 (–O–). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.78 (s, 1H, NH, D_2O exchangeable), 7.11–8.21 (a set of signals, 11H, Ar–H, Benzodioxol-H, and

Benzothiazole-H), 8.07 (s, 1H, CH=N). MS (*m/z*, %): 390.07 (M^++1, 91.03).

3-[2-(1,3-benzothiazol-2-yl)hydrazinylidene]-1,3-dihydro-2H-indol-2-one (**BT 10**)

IR (KBr, cm^{-1}) υ: 3257, 3082 (NH_{str} associated), 1673 ($C{=}O_{str}$), 1621 (C=N). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 12.72 (s, 1H, NH of Isatin, D_2O exchangeable), 11.68 (s, 1H, NH, D_2O exchangeable), 7.20–8.18 (a set of signals, 8H, Isatin-H, and Benzothiazole-H). MS (*m/z*, %): 295.05 (M^++1, 92.11).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(4-phenoxybenzylidene)acetohydrazide (**BT 11**)

IR (KBr, cm^{-1}) υ: 3188, 3079 (NH_{str} associated), 1661 ($C{=}O_{str}$), 1607 ($CH{=}N_{str}$), 1242 (–O–), 723 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.09 (s, 1H, NH, D_2O exchangeable), 8.09 (s, 1H, CH=N), 6.80–7.96 (a set of signals, 13H, Ar–H, and Benzothiazole-H), 4.11 (s, 2H, S-CH_2). MS (*m/z*, %): 420.10 (M^++1, 63.06).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-nitrophenoxy)benzylidene]acetohydrazide (**BT 12**)

IR (KBr, cm^{-1}) υ: 3177, 3075 (NH_{str} associated), 1666 ($C{=}O_{str}$), 1604 ($CH{=}N_{str}$), 1523 (N=O), 1240 (–O–), 728 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.13 (s, 1H, NH, D_2O exchangeable), 8.04 (s, 1H, CH=N), 6.83–7.91 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 4.10 (s, 2H, S-CH_2). MS (*m/z*, %): 465.05 (M^++1, 42.17).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-methylphenoxy)benzylidene]acetohydrazide (**BT 13**)

IR (KBr, cm^{-1}) υ: 3182, 3076 (NH_{str} associated), 1667 ($C{=}O_{str}$), 1610 ($CH{=}N_{str}$), 1240 (–O–), 723 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.12 (s, 1H, NH, D_2O exchangeable), 8.07 (s, 1H, CH=N), 6.82–7.94 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 4.07 (s, 2H, S-CH_2), 2.35 (s, 3H, CH_3). MS (*m/z*, %): 434.06 (M^++1, 57.78).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-chlorophenoxy)benzylidene]acetohydrazide (**BT 14**)

IR (KBr, cm^{-1}) υ: 3188, 3079 (NH_{str} associated), 1666 ($C{=}O_{str}$), 1612 ($CH{=}N_{str}$), 1240 (–O–), 723 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.18 (s, 1H, NH, D_2O exchangeable), 8.08 (s, 1H, CH=N), 6.85–7.92 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 4.05 (s, 2H, S-CH_2). MS (*m/z*, %): 454.02 (M^++1 for ^{35}Cl, 77.31), 456.05 (M^++1 for ^{37}Cl, 27.05).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-bromophenoxy)benzylidene]acetohydrazide (**BT 15**)

IR (KBr, cm^{-1}) υ: 3178, 3076 (NH_{str} associated), 1664 ($C{=}O_{str}$), 1611 ($CH{=}N_{str}$), 1240 (–O–), 725 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.15 (s, 1H, NH, D_2O exchangeable), 8.00 (s, 1H, CH=N), 6.88–7.94 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 4.06 (s, 2H, S-CH_2). MS (*m/z*, %): 499.84 (M^++1 for ^{81}Br, 100.00), 497.85 (M^++1 for ^{79}Br, 94.12).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-fluorophenoxy)benzylidene]acetohydrazide (**BT 16**)

IR (KBr, cm^{-1}) υ: 3176, 3077 (NH_{str} associated), 1668 ($C{=}O_{str}$), 1607 ($CH{=}N_{str}$), 1240 (–O–), 725 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.11 (s, 1H, NH, D_2O exchangeable), 8.06 (s, 1H, CH=N), 6.85–7.98 (a set of signals, 12H, Ar–H, and Benzothiazole-H), 4.03 (s, 2H, S-CH_2). MS (*m/z*, %): 438.04 (M^++1, 76.66).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(4-chloro-3-methylphenoxy)benzylidene] acetohydrazide (**BT 17**)

IR (KBr, cm^{-1}) υ: 3183, 3078 (NH_{str} associated), 1672 ($C{=}O_{str}$), 1607 ($CH{=}N_{str}$), 1240 (–O–), 721 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.18 (s, 1H, NH, D_2O exchangeable), 8.01 (s, 1H, CH=N), 6.82–7.91 (a set of signals, 11H, Ar–H, and Benzothiazole-H), 4.05 (s, 2H, S-CH_2), 2.37 (s, 3H, CH_3). MS (*m/z*, %): 468.05 (M^++1 for ^{35}Cl, 100.00), 470.02 (M^++1 for ^{37}Cl, 35.34).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-[4-(naphthalen-2-yloxy)benzylidene]acetohydrazide (**BT 18**)

IR (KBr, cm^{-1}) υ: 3212, 3068 (NH_{str} associated), 1669 ($C{=}O_{str}$), 1611 ($CH{=}N_{str}$), 1238 (–O–), 837, 822 (β-naphthyl), 727 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.24 (s, 1H, NH, D_2O exchangeable), 8.07 (s, 1H, CH=N), 6.72–7.98 (a set of signals, 15H, Ar–H, naphthyl-H, and Benzothiazole-H), 4.12 (s, 2H, S-CH_2). MS (*m/z*, %): 470.05 (M^++1, 41.92).

N′-[4-(1,3-benzodioxol-5-yloxy)benzylidene]-2-(1,3-benzothiazol-2-ylsulfanyl)aceto hydrazide (**BT 19**)

IR (KBr, cm^{-1}) υ: 3208, 3063 (NH_{str} associated), 1667 ($C{=}O_{str}$), 1606 ($CH{=}N_{str}$), 1240 (–O–), 725 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 11.08 (s, 1H, NH, D_2O exchangeable), 8.02 (s, 1H, CH=N), 6.78–7.91 (a set of signals, 11H, Ar–H, Benzodioxol-H, and Benzothiazole-H), 4.09 (s, 2H, S-CH_2). MS (*m/z*, %): 464.02 (M^++1, 62.21).

2-(1,3-benzothiazol-2-ylsulfanyl)-*N*′-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)acetohydrazide (**BT 20**)

IR (KBr, cm^{-1}) υ: 3239, 3080 (NH_{str} associated), 1677 ($C{=}O_{str}$), 1621 (C=N), 723 (C–S–C). ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 12.77 (s, 1H, NH of Isatin, D_2O exchangeable), 11.11 (s, 1H, NH, D_2O exchangeable), 6.89–7.93 (a set of signals, 8H, Isatin-H, and Benzothiazole-H), 4.08 (s, 2H, S-CH_2). MS (*m/z*, %): 369.02 (M^++1, 56.83).

Anticonvulsant and neurotoxicity evaluation

The evaluation of anticonvulsant activity and neurotoxicity was carried out by the Epilepsy Branch, National Institute of Neurological Disorder and Stroke, National Institute of Health, Bethesda, USA following the reported procedures.

Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague-Dawley, 100–150 g) were used as experimental animals. The synthesized derivatives were suspended in 0.5% methyl cellulose and the test compound is usually manipulated with a mortar pestle to help preparation of suspension. The titled compounds **BT 1–20** were subjected to anticonvulsant screening by 6 Hz psychomotor seizure test to identify the anticonvulsant activity of the compounds at five different time points, i.e., 0.25, 0.5, 1.0, 2.0, and 4.0 h after i.p. administration in mice at a dose of 100 mg/kg. Neurotoxicity was observed by MMI which was measured by the rotorod (neurotoxicity) test.

6 Hz psychomotor seizure test

6 Hz psychomotor seizure test or minimal clonic seizure (6 Hz) test was used to assess compound's efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3 s). Test compounds were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% animals (32 mA for 3 s). The untreated mice would display seizure characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors, described originally as being similar to the aura of human patients with partial seizure. Animals not displaying this behavior are considered to be protected.

Neurotoxicity—minimal motor impairment

MMI was measured by the rotorod (neurotoxicity) test. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for a long period of time. The compound was considered toxic if the treated animal falls off this rotating rod three times during 1-min period.

Computational study

Distance mapping

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.01 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. In conformational analysis of the 10 clinically effective, well-known and structurally different anticonvulsant drugs such as albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, zonisamide; a molecular model was suggested on the basis of molecular dynamics distance estimations (Yogeeswari *et al.*, 2005).

Calculation of physicochemical parameters

A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA) (Ertl *et al.*, 2000), miLog *P*, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five (Lipinski *et al.*, 2001) were calculated using Molinspiration online property calculation toolkit (Molinspiration Cheminformatics, 2010). Absorption (% ABS) was calculated by: $\%\ ABS = 109 - (0.345 \times TPSA)$ (Zhao *et al.*, 2002).

Log *P* determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature (Farrar *et al.*, 1993). 10 ml of octanol and 10 ml phosphate buffer were taken in a glass stoppered graduated tube and 5 mg of accurately weighed compound was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to dynamic equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy. Theoretical miLog *P* for synthesized compounds was then compared with the experimental Log *P* data.

Docking study

Compounds **BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20** were selected as ligands for docking study with six established epilepsy receptors namely GABA(A) alpha-1, GABA(A) delta, glutamate, Na/H exchanger, Na channel, and T-type

calcium channel receptor. The description of receptors used is shown in Table 7. These receptors are the most important molecular targets in the design and discovery of successful antiepileptic drugs (Landmark, 2007). In this study, AutoDock 4.0 with its Lamarckian genetic algorithm (LGA) was used for automated flexible ligand docking of **BT 2**, **BT 5**, **BT 14**, **BT 15**, and **BT 20** with above-mentioned receptors.

The grid maps were calculated using AutoGrid. In all dockings, a grid map with $60 \times 60 \times 60$ points, a grid spacing of 0.375 Å (roughly a quarter of the length of a carbon–carbon single bond) were used, and the mps were centered on the ligand binding site. For all dockings, 100 independent run with, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2.5×10^6 energy evaluations, maximum number of generations of 27,000, an elitism value of 1 and a number of active torsion of 9 were used. Auto Dock 4.0 was used to generate both grid and docking parameter files (i.e., .gpf and.dpf files) and docking affinity (Kcal/mol) and count of probable H-bonds were determined.

Acknowledgments The authors would like to express their gratitude to IIT, Delhi and CDRI, Lucknow for providing the spectral and elemental data. One of the authors (Praveen Kumar) is thankful to S. D. College of Pharmacy and Vocational Studies, Muzaffarnagar-251001, India for providing research facilities. The authors also appreciate the help and support by Mr. Om Prakash during docking study.

References

Bell GS, Sander JW (2002) The epidemiology of epilepsy: the size of the problem. Seizure 11(Suppl A):306–314

Camerman A, Camerman N (1980) Stereochemical similarities in chemically different antiepileptic drugs. In: Glaser GH, Penry JK, Woodbury JM (eds) Antiepileptic drugs: mechanism of action. Raven Press, New York, pp 223–231

Chopade RS, Bahekar RH, Khedekar PB, Bhusari KP, Rao AR (2000) Synthesis and anticonvulsant activity of 3-(5-substituted-benzothiazol-2-yl)-6-phenyl-[1, 3]-oxazinane-2-thiones. Arch Pharm (Weinheim) 335:881

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43:3714–3717

Farrar VA, Ciechanowicz MR, Grochowski J, Serda P, Pilati T, Filippini G, Hinko CN, El-Assadi E, Moore JA, Edafiogho IO, Andrews CW, Cory M, Nicholson JM, Scott JR (1993) Synthesis and CLOGP correlation of imidooxy anticonvulsants. J Med Chem 36:3517–3525

Hays SJ, Rice MJ, Ortwine DF, Johnson G, Schwarz RD, Boyd DK (1994) Substituted 2-benzothiazolamines as sodium flux inhibitors: quantitative structure activity relationships and anticonvulsant activity. J Pharm Sci 83:1425

Jones GL, Woodbury DM (1982) Principles of drug action: structure activity relationships and mechanisms. In: Woodbury DM, Penry JK, Pippenger CE (eds) Antiepileptic drugs. Raven Press, New York, pp 83–109

Landmark CJ (2007) Targets for antiepileptic drugs in the synapse. Med Sci Monit 13:RA1–RA7

Lipinski CA, Lombardo L, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Mizoule J, Meldrum B, Martine M, Croucher M, Ollat C, Uzar A (1985) 2-amino-6-trifluromethoxy benzothiazole, a possible antagonist of excitatory amino acid neurotransmission-1. Anticonvulsant properties. Neuropharmacology 24:767

Molinspiration Cheminformatics, Bratislava, Slovak Republic, http://www.molinspiration.com/services/properties.html. Accessed September 16, 2010

Perucen E (1996) The new generation of antiepileptic drugs: advantages and disadvantages. Br J Clin Pharmacol 42:531–543

Sabers A, Gram L (2000) Newer anticonvulsants: comparative review of drug interactions and adverse effects. Drugs 60:23–33

Siddiqui N, Rana A, Khan SA, Bhat MA, Haque SE (2007) Synthesis of benzothiazole semicarbazones as novel anticonvulsants–the role of hydrophobic domain. Bioorg Med Chem Lett 17(15): 4178–4182

Unverferth K, Engel J, Hofgen N, Rostock A, Gunther R, Lankau HJ, Menzer M, Rolfs A, Liebscher J, Muller B, Hofmann HJ (1998) Synthesis, anticonvulsant activity, and structure-activity relationships of sodium channel blocking 3-aminopyrroles. J Med Chem 41:63–73

Wang Y, Cai S, Lan N, Keana J, Ilyin V, Weber E (1998) Carbocyclic and heterocyclic substituted semicarbazones and thiosemicarbazones and the use thereof. PCT/US98/08004:1-81

Yogeeshwari P, Sriram D, Thirumurgan R, Jit LR, Ragavendran JV, Kavya R, Rakhra K, Saraswat V (2006) Synthesis of N4-(2, 4-dimethylphenyl) semicarbazones as 4-aminobutyrate aminotransferase inhibitors. Acta Pharm 56:259–272

Yogeeswari P, Sriram D, Thirumurugan R, Raghavendran JV, Sudhan K, Pavana RK, Stables JP (2005) Discovery of N-(2, 6-dimethylphenyl)-substituted semicarbazones as anticonvulsants: hybrid pharmacophore-based design. J Med Chem 48:6202–6211

Zhao Y, Abraham MH, Lee J, Hersey A, Luscombe NC, Beck G, Sherborne B, Cooper I (2002) Rate-limited steps of human oral absorption and QSAR studies. Pharm Res 19:1446–1457