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Synthesis and Anticonvulsant Activity of 3-(6-Substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones

A new series of 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 a**–**j**) has been synthesised using an appropriate synthetic route (Scheme 1) and characterised by elemental analyses and spectral (IR, ^{1}H NMR, ^{13}C NMR, and EI MS) data. The anticonvulsant activity of all the title compounds (**4 a**–**j**) was evaluated against Maximal Electroshock (MES) induced seizures and furthermore the most potent compounds were evaluated against subcutaneous pentylenetetrazole (sc PTZ) induced seizures model in mice. The neurotoxicity was assessed using the rotorod procedure. All the test compounds were administered intraperitoneally at various dose levels ranging from 30–200 mg/kg body wt and the median effective dose (ED_{50}), median toxic dose (TD_{50}), and protection index (PI) values were determined (Table 2). Among the compounds tested, the 3-(6-dimethylaminobenzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 j**) was found to be the most potent (ED_{50}: 9.85 and 14.8 in MES model and 12 and 17 in scPTZ model at t = 0.5 h and 4 h, respectively, and TD_{50} 42.8 and 44 at t = 0.5 h and 4 h, respectively, which has been found to be significant at $p < 0.01$ with respect to reference standard phenytoin) with protection index (PI) 4.85.

Keywords: Anticonvulsant; Synthesis; 3-(Benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones; Maximal Electroshock Seizures (MES); Subcutaneous pentylenetetrazole (sc PTZ); Neurotoxicity

Received: February 28, 2002 [FP677]

Introduction

Epilepsy is a complex and multigenic disease affecting about 30–40 million people worldwide [1–3]. It is mainly due to an electrical hyperexcitability in the central nervous system [4]. Although there are a number of antiepileptic drugs currently available, uncontrolled seizures and medication toxicity are still major problems of antiepileptic drug treatment [5]. The development of novel agents, particularly compounds effective against complex seizures, remains a major focus of antiepileptic drug research [6]. In recent years much effort has been devoted to the development of novel approaches by elucidating the cellular and molecular mechanisms of the hyperexcitability to provide specific targets for novel therapies and as a result several new drugs such as vigabatrin, lamotrigine, gabapentin, tiagabine, felbamate, topiramate, fosphenytoin, and levetiracetam have appeared on the market [5]. Literature survey reveals that 2-aminobenzothiazole derivatives possessed potent anticonvulsant activity [7]. In 1985, Riluzole, (6-(trifluromethoxy)-2-benzothiazolamine) was reported to be a potent anticonvulsant agent that functions by action on voltage-dependent sodium channels [8–10]. Modulation of sodium channels is a mechanism shared by other clinically useful anticonvulsants like phenytoin and carbamazepine [11]. Similarly substituted-[1,3]-oxazine-2-thiones exhibit potential anticonvulsant activity [12]. Previously we reported some 2-aminobenzothiazoles derivatives as potentially active antimicrobial and anti-inflammatory agents [13–15]. In continuation of our work on substituted 2-aminobenzothiazoles and to explore the potential of this nucleus as useful anticonvulsant agent, we report herein the synthesis and anticonvulsant activity of a new series of 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 a**–**j**). Compounds **4a-j** have been designed by incorporating aminobenzothiazoles and oxazinethiones together to get potent anticonvulsant activity.

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Results and discussion

In the present investigation, ten different derivatives of 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones **(4 a**–**j**) were synthesised and evaluat-

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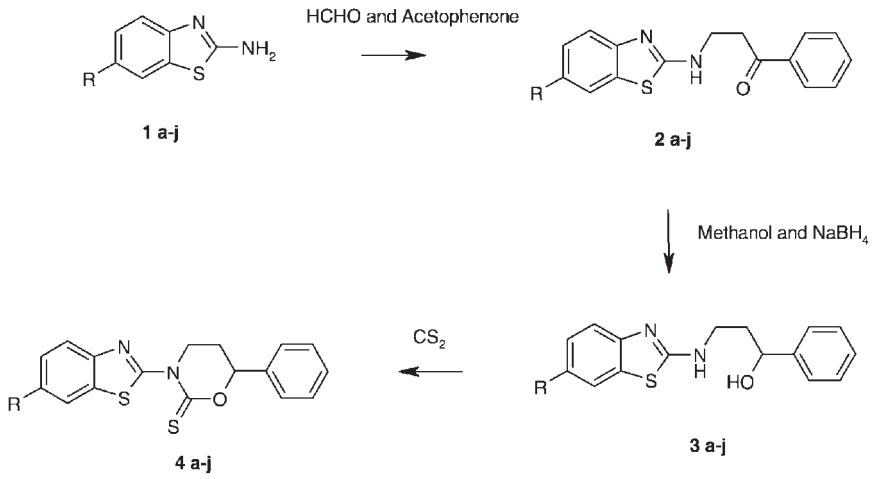


4a R=H; **4b** R=CH_3; **4c** R= C_2H_5; **4d** R= Cl;
4e R= Br; **4f** R=OCH_3; **4g** R= OC_2H_5; **4h** R=NO_2
4i R=OH; **4j** R= $N(CH_3)_2$

Scheme 1

ed for their anticonvulsant activity. Synthesis of title compounds **4 a–j** has been carried out as depicted in Scheme 1. Various derivatives of 6-substituted-2-aminobenzothiazoles (**1 a–j**) were prepared by a reported procedure [16]. The compounds **1 a–j** were converted into various derivatives of 3-(6-substituted-benzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 a–j**) by treating with formaldehyde and acetophenone according to the Mannich reaction. The Mannich reaction or α-aminomethylation involves the condensation of the active methyl group in acetophenone with formaldehyde and 2-aminobenzothiazole (in the form of its hydrochloride). The probable mechanism of the reaction involves the intermediate formation of the hydroxy-methyl-aminobenzothiazole, which eliminates water to form the carbocation. This reactive species condenses with the α-carbon atom of acetophenone to yield compound **2 a–j**. Further the compounds **2 a–j** were reduced to 3-(6-substituted-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 a–j**) using sodium borohydride as a mild reducing agent. In this step nucleophilic addition of the hydride ion to the electrophilic carbon atom of the carbonyl group results in the reduction of ketone to secondary alcohol. Finally compounds **3 a–j** were cyclised to give title compounds 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 a–j**) using carbon disulphide.

All of the synthesised compounds were characterised by their physical, analytical, and spectral data (Table 1). The IR spectra of **1 a–j** showed the presence of characteristic absorption peaks at 1600–1650 cm^{-1} (N–H deformation) and 3460–3500 cm^{-1} (N–H stretching). Compounds **2 a–j** showed absorption bands at 1550–1630 cm^{-1} (N–H deformation), 1700–1720 cm^{-1} (C=O stretching), and ca. 3450 cm^{-1} (N–H stretching), due to conversion of primary amine into secondary amine. Compounds **3 a–j** showed strong bands at ca. 3450 cm^{-1} (N–H stretching) and 3590–3650 cm^{-1} (O–H stretching) and the absence of a band at 1700–1720 cm^{-1} (C=O), due to reduction of carbonyl group to hydroxyl group. While compounds **4 a–j** showed disappearance of bands at ca. 3450 cm^{-1} (N–H stretching) and 3590–3650 cm^{-1} (O–H stretching) but shows a prominent band at 1200–1270 cm^{-1} (C=S stretching). Halogen containing compounds (**2 d**, **2 e**, **3 d**, **3 e**, **4 d**, and **4 e**) showed the absorption band at 590–760 cm^{-1} (C–X stretching) and nitro compounds (**2 h**, **3 h**, and **4 h**) showed band at 1490–1550 cm^{-1} (NO_2 stretching). The EI MS ^{1}H NMR and ^{13}C NMR spectral data of all the synthesised compounds were in conformity with the structure assigned.

In the EI-MS spectra, molecular ion (M^+) peaks, which appeared at different intensities, confirmed the molecular weights of the examined compounds (**2 a–j**, **3 a–j**, and **4 a–j**). Molecular ion peaks were the base peak for the compound **2 b**, **2 c**, **2 h**, **2 j**, **3 a**, **3 b**, **3 f**, **3 h**, **4 a**, **4 b**, **4 c**, **4 h**, and **4 i**. Appearance of an isotope peak ($M^+ + 2$) as intense as the molecular ion peak confirmed the presence of halogen atom in compounds **2 d**, **2 e**, **3 d**, **3 e**, **4 d**, and **4 e**. The corresponding ^{1}H NMR, ^{13}C NMR, and mass spectral data are presented in the experimental section.

Table 1. Physical and analytical data of compounds **2 a**–**j**, **3 a**–**j**, and **4 a**–**j**.

Comp	R	Mol. formula[b] (Mol. wt)	Mp [°C]	Yield[a] [%]	Mass (M^+)
2 a	H	$C_{16}H_{14}ON_2S$ (282)	188–90	76	282
2 b	CH_3	$C_{17}H_{16}ON_2S$ (296)	206–08	58	296
2 c	C_2H_5	$C_{18}H_{18}ON_2S$ (310)	208–09	55	310
2 d	Cl	$C_{16}H_{13}ON_2SCl$ (316)	210–12	64	318[d]
2 e	Br	$C_{16}H_{13}ON_2SBr$ (361)	221–14	69	363[d]
2 f	OCH_3	$C_{17}H_{16}O_2N_2S$(312)	240–42	70	312
2 g	OC_2H_5	$C_{18}H_{18}O_2N_2S$ (324)	148–50	55	324
2 h	NO_2	$C_{16}H_{15}O_3N_3S$ (337)	252–54	68	337
2 i	OH	$C_{16}H_{14}O_2N_2S$ (298)	242–03	58	298
2 j	$N(CH_3)_2$	$C_{18}H_{19}ON_3S$ (325)	266–07	61	325
3 a	H	$C_{16}H_{16}ON_2S$ (284)	196–98	72	284
3 b	CH_3	$C_{17}H_{18}ON_2S$ (298)	212–14	68	298
3 c	C_2H_5	$C_{18}H_{20}ON_2S$ (312)	216–17	63	312
3 d	Cl	$C_{16}H_{15}ON_2SCl$ (318)	218–20	67	320[d]
3 e	Br	$C_{16}H_{10}ON_2SBr$ (363)	220–22	69	365[d]
3 f	OCH_3	$C_{17}H_{18}O_2N_2S$ (314)	248–50	80	314
3 g	OC_2H_5	$C_{18}H_{20}O_2N_2S$ (326)	154–56	78	326
3 h	NO_2	$C_{16}H_{15}O_3N_3S$ (339)	260–62	74	339
3 i	OH	$C_{16}H_{16}O_2N_2S$ (300)	242–43	68	300
3 j	$N(CH_3)_2$	$C_{18}H_{21}ON_3S$ (327)	236–37	66	327
4 a	H	$C_{17}H_{14}ON_2S_2$ (326)[c]	207–209	77	326
4 b	CH_3	$C_{18}H_{18}ON_2S_2$ (342)	172–74	72	342
4 c	C_2H_5	$C_{19}H_{20}ON_2S_2$ (356)	178–79	69	356
4 d	Cl	$C_{17}H_{14}ON_2S_2Cl$ (360)	184–86	67	362[d]
4 e	Br	$C_{17}H_{14}ON_2S_2Br$ (405)	188–90	70	407[d]
4 f	OCH_3	$C_{18}H_{16}O_2N_2S_2$ (356)	228–30	60	356
4 g	OC_2H_5	$C_{19}H_{18}O_2N_2S_2$ (370)	132–34	72	370
4 h	NO_2	$C_{17}H_{15}O_3N_3S_2$ (381)	240–41	69	381
4 i	OH	$C_{17}H_{14}O_2N_2S_2$ (342)	166–67	66	342
4 j	$N(CH_3)_2$	$C_{19}H_{19}ON_3S_2$ (369)	192–93	71	369

[a] Compounds **2 a**–**j** and **3 a**–**j** were recrystallised from methanol, and compounds **4 a**–**j** from benzene-petroleum ether 60–40 °C (6 : 4).
[b] CHN analyses were found to be within the limit of ± 0.4 %.
[c] Lit mp: 208–210 °C, Ref.[19].
[d] values represent (M^+ + 2) due to appearance of an isotopic peak.

The anticonvulsant activity of all the title compounds (**4 a**–**j**) was evaluated against Maximal Electroshock (MES) induced seizures and furthermore the most potent compounds were evaluated against subcutaneous pentylenetetrazole (sc PTZ) induced seizures model in mice [17]. Using the rotorod procedure the neurotoxicity was assessed [18]. All the test compounds were administered intraperitoneally at various dose level ranging from 30 to 200 mg/kg body wt and the median effective dose (ED_{50}), median toxic dose (TD_{50}), and protection index (PI) values were determined. Suspensions of the test compounds in propylene glycol were administered to mice at half and hour or 4 hours before evaluation of their activity. The results of anticonvulsant activity and neurotoxicity are presented in Table 2.

The anti MES activity (ED_{50} values in Table 2) indicated significant anticonvulsant activity for all of the test compounds **4 a**–**j**. However, they were found to be less potent when compared with the reference standard phenytoin

Table 2. Anticonvulsant and neurotoxicity of compounds **4 a–j** in mice[a].

Comp	MES		scPTZ		Toxicity		PI
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h	
4 a	53 (46–61)	65.5 (60–71)	ND	ND	88.5 (79–98)	110 (109–111)	1.66
4 b*	16.9 (14.2–19.3)	22 (20–24)	20 (19–21)	29.5 (28–31)	53.5 (47–60)	70 (68–72)	3.13
4 c*	14.8 (12.9–16.6)	20 (19–21)	18 (15.6–20.4)	27.6 (25.2–30)	53.5 (45–62)	71 (70–72)	3.58
4 d	86 (83–88)	95 (89–101)	ND	ND	110 (106–114)	130 (125–135)	1.27
4 e	88 (79–98)	99 (81–121)	ND	ND	123 (120–126)	150 (146–154)	1.39
4 f	70 (59–83)	82 (79–85)	ND	ND	112 (110–114)	140 (135–145)	1.6
4 g	77 (68–88)	86 (80–92)	ND	ND	118 (110–126)	135 (130–140)	1.53
4 h	99 (81–121)	110 (109–111)	ND	ND	140 (139–141)	175 (174–176)	1.41
4 i	95 (89–101)	107 (99–115)	ND	ND	131 (126–136)	156 (140–172)	1.37
4 j**	9.85 (8.77–10.7)	14.8 (13–16)	12 (11.4–12.6)	17 (15.6–18.4)	47.8 (39.2–59.2)	60 (56–64)	4.85
Std	6.48	7.1	7.5	8.2	42.8	44	6.60
(PTN)	(5.65–7.24)	(5.9–8.3)	(6.4–8.6)	(7.8–8.6)	(36.4–47.5)	(37–51)	

[a] All compounds were administered by ip injection at doses spanning the range 30–200 mg kg^{-1}, 30 min and 4 h before evaluation of activity and at least 6 animals were used to calculate each ED_{50} and TD_{50} values. In scPTZ induced seizures test, 200 µL/kg body wt. of 10 µM solution of PTZ was administered by subcutaneous route 15 min after the ip injection of test compounds; the anticonvulsant activity was recorded at t = 0.5 and 4 h and represented in terms of the ED_{50}, i.e., dose of test compounds required to assure anticonvulsant protection in 50 % of animals from hind limb tonic extension (tonic phase); the TD_{50}, dose eliciting minimal neurological toxicity in 50 % of animals as assessed by the rotorod test (locomotor deficit); the PI, protection index ($PI = TD_{50}/ED_{50}$) from MES induced seizures after 0.5 h; ED_{50} and TD_{50} values are expected as mg kg^{-1}; All data were calculated according to the method of Litchfield and Wilcoxon [20], and significant differences between ED_{50} values of the test compounds with respect to std PTN (phenytoin) are denoted as $^*p < 0.05$ and ** $p < 0.01$; ND: activity not determined.

(ED_{50}: 6.48 and 7.1 at t = 0.5 and 4 h in MES model). The different substituents on the aromatic ring exert a significant influence on the biological activity by modulating the lipophilicity and thereby facilitating penetration across the blood-brain barrier. The presence of electron-withdrawing groups (halogen, alkoxy, hydroxy, and nitro substituents) on the aromatic ring in general decreases the potency of test compounds compared to compounds having electron-donating groups (alkyl and dialkylamino substituents). This is because of decreased lipophilicity, which in turn inhibits permeability across biological membranes. Further, it has been found that the ED_{50} and TD_{50} values of test compounds increase significantly at t = 4 h, compared to t = 0.5 h, in contrast to the refer-

ence standard, indicating that the test compounds were metabolised with time in the biological environment. This trend was found to be more pronounced in compounds **4 d**, **4 e**, **4 f**, **4 g**, **4 h**, and **4 i** (having electron-withdrawing groups) compare to compounds **4 b**, **4 c**, and **4 j** (having electron-donating groups). In order to confirm this fact *in vitro*, the most potent compound **4 j** was incubated in phosphate buffer solution (pH 7.4) at 37 °C and after every hour 10 µL of aliquot was analysed by analytical RP-HPLC (Reverse Phase High Performance Liquid Chromatography) over a period of 6 h. Further the individual peaks were collected from HPLC, freeze-dried, and characterised by Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI TOF MS). The results of this study (Figure 1) show that after 2 h compound **4 j** (parent peak, retention time: 36 min) has been hydrolysed (appearance of second peak at 30 min). The hydrolysis product (metabolite of compound **4 j**) was found to be less lipophilic compared to the parent compound as it elutes before the parent peak from a C_{18} column in RP HPLC. As shown in Scheme 2, at physiological pH, the test compound **4 j** undergo hydrolysis across the oxazinane-2-thione ring (as the observed mass, Figure 2 was found to be in agreement with the hydrolysis product of compound **4 j**). As in the case of **4 j**, all the test compounds may possibly undergo hydrolysis and therefore the ED_{50} and TD_{50} values increase with time. In particular, electron-withdrawing substituents favour this hydrolysis compares to electron-donating group and a significant increase in the

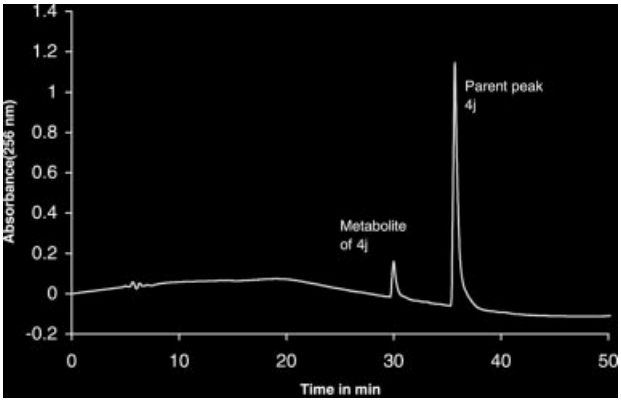
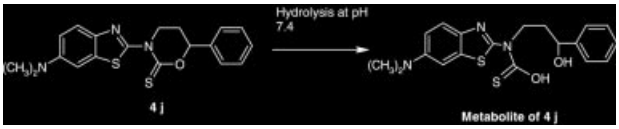


Figure 1. Analytical reverse phase high performance liquid chromatogram of compound **4 j** and its metabolite: RP-HPLC Conditions: Beckman Gold Nouveau System equipped with a 168 photodiode-array detector. Column; Vydac 218TP53 (C_{18}, 300 Å, 5 mm, 3.2 mm i.d. × 250 mm). 0.5 mL min^{-1}, Buffer A = 0.1 % TFA in water. Buffer B = 90 % ACN containing 10 % buffer A. Linear gradient from 0 % B to 90 % B over 30 min, total run time 50 min. Retention time (*Rt*) for parent peak 36 Min, metabolite *Rt* = 30 min.



Scheme 2

ED_{50} and TD_{50} values of compounds **4 d**, **4 e**, **4 f**, **4 g**, **4 h**, and **4 i** was therefore found at t = 4 h. These results also reflect that the oxazinane-2-thione ring is necessary for anticonvulsant activity; in turn, it may be essential to maintain the lipophilicity of the test compounds. Similar results were found when some of the most potent compounds were evaluated for anticonvulsant activity against the sc PTZ model. Further, to confirm this phenomenon *in vivo*, a kinetic study should be carried out in an animal model. Based upon the results it will also be necessary to optimise the lead compound by substituting a series of electron-donating groups on aromatic ring and selectively modifying the oxazinane-2-thione ring itself. The protection index (PI) values are found to be more significant for determining the relation between lipophilicity and toxicity. Table 2 shows that PI values > 3 were found for more potent compounds in contrast to less lipophilic compounds. Thus, as the lipophilicity increases so does the toxicity and therefore also the protection index (PI). In the series, 3-(6-dimethylaminoben-

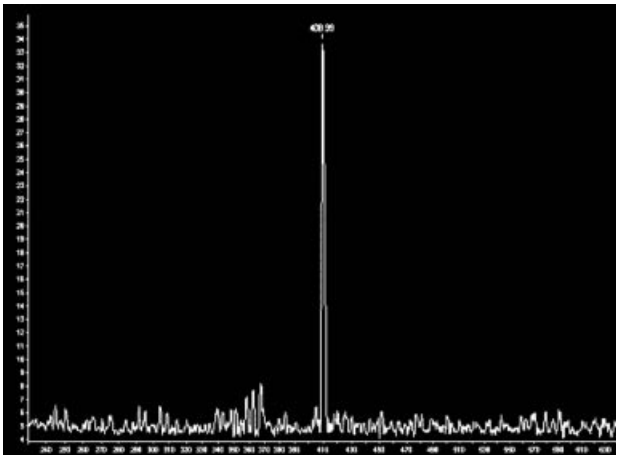


Figure 2. Matrix assisted laser desorption ionisation time of flight mass spectrum of **4 j** metabolite: MALDI TOF MS Conditions; Lasermat 2000 (Thermo Bioanalysis Ltd., UK). The test material (ca. 0.2 mg mL^{-1}) dissolved in a mixture of water and methanol (9 : 1) and 2 µL was applied to the sample plate and allowed to dry at room temperature prior to analysis. The spectrum was acquired in positive mode without matrix at laser power 20 and typically 10–12 shots were obtained per spectrum. Expected mass: 387 (M^+); Observed: 409 ($M + Na^+$).

zothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 j**) was found to be the most potent (ED_{50}: 9.85 and 14.8 in MES model and 12 and 17 in the scPTZ model at t = 0.5 h and 4 h, respectively, and TD_{50} 42.8 and 44 at t = 0.5 h and 4 h, respectively, which have been found to be significant at $p < 0.01$ with respect to reference standard phenytoin) with a protection index (PI) of 4.85. Further studies are in progress to optimise this lead compound and fully characterise the mode of action.

Acknowledgments

The authors wish to thank the Regional Sophisticated Instrumentation Center (Central Drug Research Institute), Lucknow for recording spectra, the Principals of the Department of Pharmaceutical Sciences, Nagpur University Nagpur (India), Nagpur College of Pharmacy, for providing laboratory facilities, and the University Grant Commission for financial assistance.

Experimental

Melting points were determined in open capillaries using a Thermonik precision melting point cum boiling point apparatus, Model C-PMB-2 (Mumbai, India) and are uncorrected. Purity of the compounds was checked by precoated TLC plates (E. Merck Kieselgel 60 F_{254}, Mumbai, India). IR spectra were recorded using KBr pellets on a Perkin-Elmer 337 Spectrophotometer from Perkin Elmer International Incorporation, Rorkreuz, Switzerland (ν_{max} in cm^{-1}). ^1H NMR and ^{13}C NMR spectra on a Bruker W.M. 400 Spectrometer (Bruker AG, Fallanden, Switzerland) at 360 MHz using TMS as internal standard (chemical shifts in δ ppm) and mass spectra (EI-MS) were recorded at 70 eV on a Jeol D-300 spectrometer (Jeol Ltd, Tokyo, Japan). Elemental analyses were carried out using Heraeus Carlo Erba 1180 CHN analyser (from Heraeus Instrument GmbH, Hanau, Germany). All the chemicals were purchased from Aldrich Company Ltd, Dorset (UK).

Synthesis of 3-(6-substituted-benzothiazol-2-ylamino)-1-phenylpropan-1-one **2 a**–**j**; *General procedure*

To a solution of 2-aminobenzothiazole (0.01 mole) in methanol (150 mL), acetophenone (0.01 mole), formaldehyde (0.01 mole) and a few drops of concentrated hydrochloric acid were added dropwise with constant stirring. The reaction mixture was heated under reflux for 2 hours on a water bath. The reaction mixture was filtered and cooled. The separated solid was filtered off, dried, and recrystallised from methanol. Using the above procedure, ten such compounds **2 a**–**j** were synthesised and characterised and their physical data are listed in Table 1.

Some representative spectral data for compounds **2**:

3-(6-Methylbenzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 b**): White crystals; R_f: 0.56 (chloroform); IR (KBr): 1560 (N–H deformation), 1720 (C=O stretching), 3450 (N–H stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.28 (t, 3 H, *CH_3*–C_6H_5–), 2.2–2.3 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.2 (s,1 H, –*NH*–CH_2–CH_2–CO–), 7.2–8.1 (m, 8 H, Aromatic) ppm; EI MS (*m*/*z*, %): 296 (M^+, 100 %).

3-(6-Ethyl-benzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 c**): White crystals; R_f: 0.57 (chloroform); IR (KBr): 1568 (N–H deformation), 1710 (C=O stretching), 3440 (N–H stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.28 (t, 3 H, *CH_3*–CH_2–C_6H_5–), 1.9–1.98 (d, 2 H, CH_3–*CH_2*–C_6H_5–), 2.2–2.3 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.21 (s, 1 H, –*NH*–CH_2–CH_2–CO–), 7.2–8.0 (m, 8 H, Aromatic) ppm; EI MS (*m*/*z*, %): 310 (M^+, 100 %).

3-(6-Bromo-benzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 e**): White crystals; R_f: 0.66 (chloroform); IR (KBr): 700 (Ar–Br stretching),1580 (N–H deformation), 1700 (C=O stretching), 3450 (N–H stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.23–2.35 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.21 (s, 1 H, –*NH*–CH_2–CH_2–CO–), 7.1–8.0 (m, 8 H, Aromatic) ppm; EI MS (*m*/*z*, %): 363 ([M^+ + 2], 100 %).

3-(6-Nitro-benzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 h**): White crystals; R_f: 0.61 (chloroform); IR (KBr): 1490 (NO_2 stretching), 1560 (N–H deformation), 1720 (C=O stretching), 3450 (N–H stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.22–2.34 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.20 (s, 1 H, –*NH*–CH_2–CH_2–CO–), 7.1–8.0 (m, 8 H, Aromatic) ppm; EI MS (*m*/*z*, %): 337 (M^+, 100 %).

3-(6-Dimethylaminobenzothiazol-2-ylamino)-1-phenylpropan-1-one (**2 j**): White crystals; R_f: 0.63 (chloroform); IR (KBr): 1580 (N–H deformation), 1710 (C=O stretching), 3450 (N–H stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.30 (t, 6 H, *$(CH_3)_2N$*–C_6H_5–), 2.2–2.3 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.1 (s, 1 H, –*NH*–CH_2–CH_2–CO–), 7.1–8.1 (m, 8 H, Aromatic) ppm; EI MS (*m*/*z*, %): 325 (M^+, 100 %).

Synthesis of 3-(6-substituted-benzothiazol-2-ylamino)-1-phenylpropan-1-ol **3 a**–**j**

To a solution of 3-(6-substituted benzothiazol-2-ylamino)-1-phenylpropan-1-one (0.01 mole) in methanol (100 mL), sodium borohydride (0.01 mole) was added in fraction with constant stirring over the period of 3 hours. After stirring for 3 hours, the reaction mixture was allowed to stand overnight. The separated solid was filtered off, dried, and recrystallised from methanol. Using the above procedure ten such compounds **3 a**–**j** were synthesised and characterised and their physical data have been listed in Table 1.

Some representative spectral data for compounds **3**:

3-(Benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 a**): Pale white crystals; R_f: 0.66 (chloroform); IR (KBr): 3450 (N–H stretching), 3590 (O–H stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.8–1.83 (m, 1 H, Ar–*CH*(OH)–), 2.2–2.3 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.2 (s, 1 H, –*NH*–CH_2–CH_2(OH)–), 7.2–8.1 (m, 9 H, Aromatic) and 8.9 (bs, 1 H, Ar–CH(*OH*)–) ppm; EI MS (*m*/*z*, %): 284 (M^+, 100 %).

3-(6-Methyl-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 b**): Pale white crystals; R_f: 0.68 (chloroform); IR (KBr): 3450 (N–H stretching), 3600 (O–H stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.27 (t, 3 H, *CH_3*–C_6H_5–), 1.81–1.84 (m, 1 H, Ar–*CH*(OH)–), 2.1–2.3 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.2 (s, 1 H, –*NH*–CH_2–CH_2(OH)–), 7.2–8.0 (m, 8 H, Aromatic) and 8.88 (bs, 1 H, Ar–CH(*OH*)–) ppm; EI MS (*m*/*z*,%): 298 (M^+, 100 %).

3-(6-Chloro-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 d**): Pale white crystals; R_f: 0.62 (chloroform); IR (KBr): 710 (Ar–Cl stretching), 3452 (N–H stretching), 3590 (O–H stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.81–1.83 (m, 1 H, Ar–*CH*(OH)–), 2.1–2.33 (d, 4 H, –NH–*CH_2*–*CH_2*–CO–), 4.22 (s, 1 H, –*NH*–CH_2–CH_2(OH)–), 7.2–8.0 (m, 8 H, Aromatic) and 9.0 (bs, 1 H, Ar-CH(*OH*)–) ppm; EI MS (*m*/*z*, %): 320 ([M^+ + 2], 100 %).

3-(6-Methoxy-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 f**): Pale white crystals; R_f: 0.68 (chloroform); IR (KBr): 3450 (N–H stretching), 3605 (O–H stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.28 (t, 3 H, *CH_3O*–C_6H_5–), 1.81–1.83 (m, 1 H, Ar–*CH*(OH)–), 2.1–2.3 (d, 4 H, –NH–*CH_2–CH_2*–CO–), 4.2 (s, 1 H, –*NH*–CH_2–CH_2(OH)–), 7.2–8.0 (m, 8 H, Aromatic) and 8.88 (bs, 1 H, Ar–CH(*OH*)–) ppm; EI MS (*m*/*z*, %): 314 (M^+, 100 %).

3-(6-Nitro-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (**3 h**): Pale white crystals; R_f: 0.69 (chloroform); IR (KBr): 1496 (NO_2 stretching), 3450 (N–H stretching), 3590 (O–H stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.81–1.83 (m, 1 H, Ar–*CH*(OH)–), 2.1–2.33 (d, 4 H, –NH–*CH_2–CH_2*–CO–), 4.22 (s, 1 H, –*NH*–CH_2–CH_2(OH)–), 7.2–8.0 (m, 8 H, Aromatic) and 9.0 (bs, 1 H, Ar–CH(*OH*)–) ppm; EI MS (*m*/*z*, %): 339 (M^+, 100 %).

Synthesis of 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones **4 a**–**j**

To a solution of 3-(6-substituted-benzothiazol-2-ylamino)-1-phenylpropan-1-ol (0.01 mole) in dry chloroform (50 mL), carbon disulphide (0.01 mole) was added dropwise. The reaction mixture was heated under reflux for 7 hours on a water bath. Excess of the solvent was distilled-off *in vacuo*. The residue so obtained was recrystallised from a mixture of benzene-petroleum ether (60–80 °C; 6 : 4). Using the above procedure ten such compounds **4 a**–**j** were synthesised and characterised and their physical data have been listed in Table 2.

Some representative spectral data for compounds **4**:

3-(Benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 a**): White crystals; R_f: 0.36 (chloroform); IR (KBr): 1200 (C=S stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.89–1.90 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*-CH-Ar), 7.2–8.1 (m, 9 H, Aromatic) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 122 (*C*H, Aromatic) and 182 (*C*=S). EI MS (*m*/*z*, %): 326 (M^+, 100 %), 210 (86 %), 150 (25 %), 134 (33 %), 78 (56 %).

3-(6-Methyl-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 b**): White crystals; R_f: 0.38 (chloroform); IR (KBr): 1250 (C=S stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.27 (t, 3 H, *CH_3*–C_6H_5–),1.89–1.90 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*–CH–Ar), 7.2–8.1 (m, 8 H, Aromatic) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 19.6 (*C*H_3, benzothiazole), 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 122–124 (*C*H, Aromatic) and 182 (*C*=S); EI MS (*m*/*z*, %): 342 (M^+, 100 %), 226 (80 %), 166 (22 %), 150 (32 %), 77 (52 %).

3-(6-Ethyl-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 c**): White crystals; R_f: 0.38 (chloroform); IR (KBr): 1270 (C=S stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1–1.27 (t, 3 H, *CH_3*–CH_2–C_6H_5–), 1.87–1.89 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 1.9–1.96 (d, 2 H, CH_3–*CH_2*–C_6H_5–), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*–CH–Ar), 7.2–8.1 (m, 8 H, Aromatic) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 19.6 (*C*H_3–CH_2–, benzothiazole), 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 35.0 (CH_3–*C*H_2–, benzothiazole), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 122–125 (*C*H, Aromatic) and 182 (*C*=S); EI MS (*m*/*z*, %): 356 (M^+, 100 %), 240 (80 %), 180 (22 %), 164 (32 %), 77 (50 %).

3-(6-Chloro-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 d**): White crystals; R_f: 0.32 (chloroform); IR (KBr): 710 (Ar–Cl stretching),1260 (C=S stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.89–1.90 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*–CH–Ar), 7.2–8.0 (m, 8 H, Aromatic) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 122–126 (*C*H, Aromatic), 132 (Ar–Cl), and 182 (*C*=S); EI MS (*m*/*z*, %): 362 ([M^+ + 2], 100 %) 248 (80 %), 188 (20 %), 172 (30 %), 77 (52 %).

3-(6-Nitro-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 h**): White crystals; R_f: 0.35 (chloroform); IR (KBr): 1250 (C=S stretching), 1490 (NO_2 stretching) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.19–1.90 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*–CH–Ar), 7.2–8.1 (m, 8 H, Aromatic) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 124–128 (CH, Aromatic), 142 (Ar–NO_2) and 182 (C=S); EI MS (*m*/*z*, %): 381 (M^+, 100 %) 265 (79 %), 205 (22 %), 189 (32 %), 78 (49 %).

3-(6-Hydroxybenzothiazol-2-yl)-6-phenyl-[1,3]-oxazinane-2-thiones (**4 i**): White crystals; R_f: 0.39 (chloroform); IR (KBr): 1265 (C=S stretching), cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.89–1.90 (t, 1 H, –N–CH_2–CH_2–*CH*–Ar), 2.1–2.33 (q, 4 H, –N–*CH_2–CH_2*–CH–Ar), 7.2–8.1 (m, 8 H, Aromatic), 9.2–9.4 (bs, 1 H, Ar–*OH*) ppm; ^{13}C NMR (360 MHz, $CDCl_3$) δ: 24.5, 25.0 (–*C*H_2–*C*H_2–, oxazinanethione), 42.73 (*C*H, benzothiazole), 52.6 (–*C*H–Ar, oxazinanethione), 124–126 (*C*H, Aromatic),151 (Ar–OH) and 182 (*C*=S); EI MS (*m*/*z*, %): 342 (M^+, 100 %) 226 (80 %), 166 (22 %), 150 (30 %), 77 (42 %).

Pharmacology

Maximal Electroshock Seizures (MES) method was used to check the anticonvulsant activity of all the title compounds **4 a**–**j** [17]. This study was conducted on albino mice of either sex weighing between 20 to 25 g. The animals were divided into two groups (control and standard) and each experimental group consisted of six animals. All the animals were left for 2 days under laboratory conditions for acclimatisation and maintained on a standard pellet diet and water *ad libitum* before the day of the experiment. On the last day food was withdrawn and they were given water only. A 12 hours dark:light cycle was also maintained. All the experimental protocols were approved by the institutional review committee and experiments were conducted in accordance with the standard guidelines. The homogeneous suspension of the test compounds (**4 a**–**j**) and standard drug (phenytoin) were prepared in polyethylene glycol and distilled water (1 : 9/mL). All the test compounds were administered intraperitoneally (ip) at a dose of 30–200 mg/kg body wt., 30 min prior to the start of experiments. The Maximal Electroshock Seizures (MES) were induced by an electroconvulsometer (Techno Instruments, Lucknow), using a technique described earlier [17]. The animals were subjected to electroshock (60 mA/0.2 s) via the transauricular electrodes. Further the most potent compounds **4 b**, **4 c**, and **4 j** were evaluated against the sc PTZ model in mice. The anticonvulsant effect was assessed by recording the Tonic Hind-limb Extension (THE) at various dose level at t = 0.5 and 4 h. Absence of seizure component like hind leg tonic extension with drug treatment was considered to be evidence of protection. Median effective dose (ED_{50}) was calculated for each compound and are presented in Table 2.

Acute neurotoxicity of all the test compounds was assessed in mice by evaluating the Rolling Roller Performance (RRP) according to the method described by Dunham and Miya (1957) [18]. Briefly, group of animals (mice) were trained to balance on a rotating rod (3 cm diameter and 6 rpm speed) and

was allowed three attempts to remain on the rotating rod for 20 s. Such trained animals were treated with the test compounds at various dose level ranging from 30–200 mg/kg body wt., by intraperitoneal administration. Test compounds were considered to be neurotoxic at a particular dose level if the trained animal showed lack of Rolling Roller Performance. Each of the trained animals was tested in this manner at 30 min and 4 h after the drug administration, and the neurotoxic effect was recorded in terms of TD_{50} and based upon these results PI values were calculated as shown in Table 2.

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