Full Paper

Benzothiazole Incorporated Barbituric Acid Derivatives: Synthesis and Anticonvulsant Screening

Nadeem Siddiqui and Waquar Ahsan

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Hamdard Nagar, New Delhi, India

A series of 1-(6-substituted-1,3-benzothiazol-2-yl)-3-(substituted phenyl)hexahydro-2,4,6-pyrimidinetriones **4a–t** were synthesized starting from substituted anilines. These compounds contained two active anticonvulsant pharmacophores, benzothiazole and barbituric acid. Structures of the compounds were confirmed on the basis of different spectroscopic techniques. All the compounds were evaluated for their anticonvulsant activity. Three compounds **4c**, **4d**, and **4s** showed promising anticonvulsant activities in Maximal Electroshock Seizure test (MES) and subcutaneous pentylenetetrazole test (scPTZ). They also displayed a wide safety profile when tested for the minimal motor impairment test.

Keywords: Anticonvulsants / Barbituric acid / Benzothiazole / Pyrimidinetriones

Received: January 7, 2009; accepted: March 19, 2009

DOI 10.1002/ardp.200900002

Introduction

Epilepsy is one of the more common neurological disorders affecting a large number of people. About 20 to 30% of patients have seizures that are resistant to available medical therapies [1]. All currently approved antiepileptic drugs have a dose-related toxicity and idiosyncratic side effects [2]. In response to the premise that major medical breakthroughs in non-pharmacologic therapies for the treatment of epilepsy in the near future seem remote, the search for new antiepileptic drugs with lower toxicities and fewer side effects continues. The realization of such a possibility will necessitate a change in our current antiepileptic drug discovery approach.

Drugs clinically active against epilepsy include derivatives with common structural characteristics such as a nitrogen-heterocyclic system with a carbonyl group and an aromatic or heteroaromatic nucleus linked to the heterocyclic system. Barbituric acid being an essential component of the current antiepileptic drugs phenobarbital and mephobarbital (Fig. 1) has been exploited previously [3, 4] to get newer anticonvulsant agents that are better anticonvulsants in terms of efficacy and safety.

In recent years, benzothiazole derivatives have acquired conspicuous significance due to their wide spectrum of biological activities. Although they have been known from long ago to be biologically active [5–7], their varied biological features are nowadays still of great scientific interest. In search of new anticonvulsant agents, we have already reported different substituted benzothiazole derivatives that possess significant anticonvulsant activities [8–11].

The present series has been synthesized in the light of the features discussed above; effort has been made to produce more potent and safer anticonvulsant agents by adding two heterocyclic nuclei that are known to possess anticonvulsant activity, namely benzothiazole and barbituric acid.

Results and discussion

Chemistry

The synthesis of the target compounds **4a–t** was accomplished as presented in Scheme 1. Appropriate substi-

Correspondence: Nadeem Siddiqui, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Hamdard Nagar, New Delhi 110062, India.
E-mail: nadeems_03@yahoo.co.in / nadeems_03@rediffmail.com
Fax: +91 11 2605-9688 / -5307

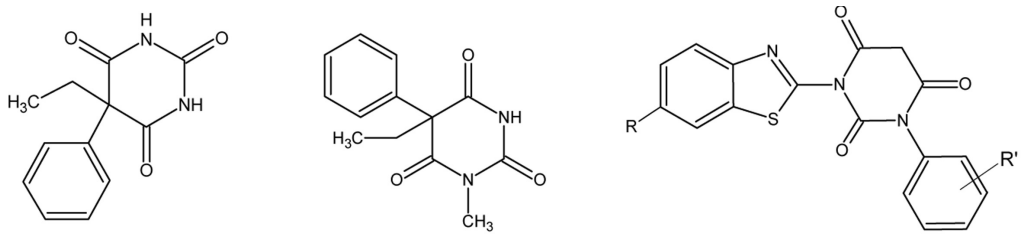
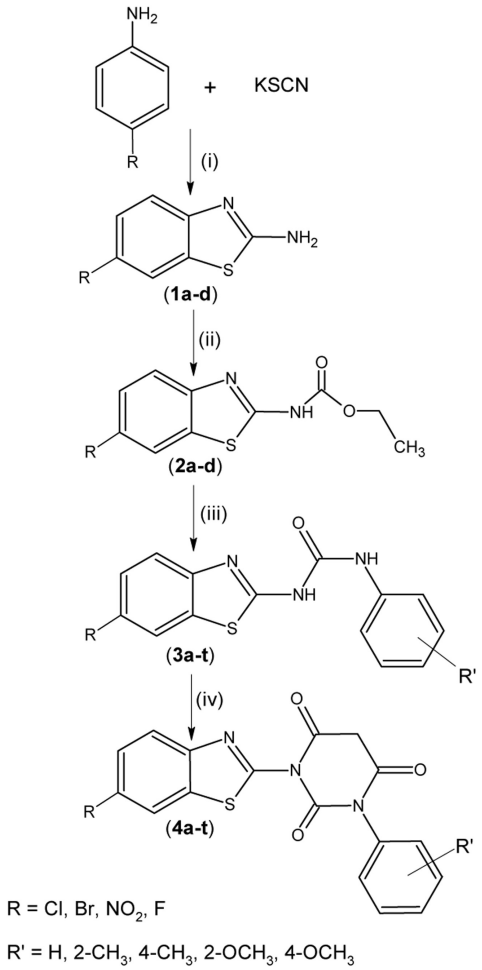


Figure 1. Barbituric acid derivatives as anticonvulsants.



Reagents and conditions: (i) Br_2, AcOH; (ii) ClCOOEt, triethylamine, reflux; (iii) R'$PhNH_2$, reflux; (iv) $CH_2(CO_2H)_2$, CH_3COCl, 40°C, 4 h.

Scheme 1. Synthesis of the titled compounds **4a**–**t**.

tuted anilines were treated with potassium thiocyanate and bromine in acetic acid to yield substituted 2-amino benzothiazole derivatives **1a**–**d**. On refluxing with ethyl-chloroformate and triethylamine, these derivatives afforded the carbamate derivatives of benzothiazoles **2a**–**d**. These carbamates when condensed with different substituted anilines gave the phenylurea derivatives **3a**–**t**. Finally, the phenylurea derivatives were condensed with malonic acid in the presence of acetyl chloride to yield the title compounds **4a**–**t**. The physico-chemical parameters of the synthesized compounds are presented in Table 1. The structures and purity of the final compounds were confirmed on the basis of spectral and elemental analyses and the data were within ± 0.4% of the theoretical values.

Table 1. Physicochemical parameters of the titled compounds **4a**–**t**.

Compound	R	R′	Mol. Formula[a]	CLog P[b]	R_f[c] value
4a	Cl	2-CH_3	$C_{18}H_{12}ClN_3O_3S$	3.17 ± 0.74	0.65
4b	Cl	4-CH_3	$C_{18}H_{12}ClN_3O_3S$	3.17 ± 0.74	0.74
4c	Cl	2-OCH_3	$C_{18}H_{12}ClN_3O_4S$	2.01 ± 0.76	0.57
4d	Cl	4-OCH_3	$C_{18}H_{12}ClN_3O_4S$	1.80 ± 0.76	0.62
4e	Cl	H	$C_{17}H_{10}ClN_3O_3S$	2.71 ± 0.74	0.70
4f	Br	2-CH_3	$C_{18}H_{12}BrN_3O_3S$	3.34 ± 0.78	0.77
4g	Br	4-CH_3	$C_{18}H_{12}BrN_3O_3S$	3.34 ± 0.78	0.82
4h	Br	2-OCH_3	$C_{18}H_{12}BrN_3O_4S$	2.18 ± 0.80	0.69
4i	Br	4-OCH_3	$C_{18}H_{12}BrN_3O_4S$	1.97 ± 0.80	0.71
4j	Br	H	$C_{17}H_{10}BrN_3O_3S$	2.88 ± 0.78	0.74
4k	F	2-CH_3	$C_{18}H_{12}FN_3O_3S$	2.62 ± 0.78	0.54
4l	F	4-CH_3	$C_{18}H_{12}FN_3O_3S$	2.62 ± 0.78	0.63
4m	F	2-OCH_3	$C_{18}H_{12}FN_3O_4S$	1.46 ± 0.80	0.68
4n	F	4-OCH_3	$C_{18}H_{12}FN_3O_4S$	1.25 ± 0.80	0.72
4o	F	H	$C_{17}H_{10}FN_3O_3S$	2.16 ± 0.78	0.66
4p	NO_2	2-CH_3	$C_{18}H_{12}N_4O_5S$	2.30 ± 1.02	0.48
4q	NO_2	4-CH_3	$C_{18}H_{12}N_4O_5S$	2.30 ± 1.02	0.52
4r	NO_2	2-OCH_3	$C_{18}H_{12}N_4O_6S$	1.14 ± 1.03	0.55
4s	NO_2	4-OCH_3	$C_{18}H_{12}N_4O_6S$	0.93 ± 1.03	0.61
4t	NO_2	H	$C_{17}H_{10}N_4O_5S$	1.84 ± 1.02	0.56

[a] Solvent of crystallization: ethanol.
[b] CLog P was calculated using software ACD Labs 8.0 version.
[c] Solvent system: toluene / ethyl acetate / formic acid (5 : 4 : 1).

Pharmacology

Anticonvulsant activity

The pharmacological testing of all the final compounds was carried out according to the protocol of the Antiepileptic Drug Development (ADD) program, Epilepsy Branch, National Institute of Health (NIH), Rockville, MD, USA.

Table 2. Anticonvulsant activity and minimal motor impairment of the synthesized compounds **4a**–**t**.

Compound	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicity screen	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
4a	300	–[b]	–	–	300	–
4b	100	300	–	300	–	300
4c	30	100	300	–	–	–
4d	30	30	100	–	–	–
4e	–	300	–	–	–	–
4f	100	300	300	300	300	300
4g	100	100	100	300	–	100
4h	300	–	–	–	–	300
4i	100	300	300	–	–	–
4j	–	–	–	–	300	300
4k	100	300	100	–	300	–
4l	100	100	100	300	–	–
4m	100	–	300	–	–	300
4n	100	100	100	300	–	–
4o	–	300	–	–	300	300
4p	300	–	–	–	300	300
4q	–	100	300	–	100	100
4r	100	100	300	–	–	300
4s	30	100	100	300	–	–
4t	100	300	–	300	300	–
Phenytoin[c]	30	30	–	–	100	100
Ethosuximide[d]	–	–	100	300	–	–
Phenobarbital[e]	100	30	–	–	100	300

[a] Number of animals used: 6; solvent used: polyethylene glycol. Dose of 30, 100, and 300 mg/kg were administered i.p. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 h and 4 h after injections were administered.

[b] The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg).

[c] Data from reference [2].

[d] Data from reference [12].

[e] Data from reference [13].

The anticonvulsant activity was assessed by using the electroshock (MES) and chemically-induced seizures (scPTZ) methods. Maximal electroshock seizure (MES) test is the standard model for tonic clonic type of seizures and the subcutaneous pentylenetetrazole(scPTZ) test is the standard model for the absence seizures. All the synthesized compounds were administered intraperitoneally (i.p.) into mice using doses of 30, 100 and 300 mg/kg and the observations were taken at two different time intervals (0.5 h and 4.0 h). Neurological impairment was evaluated by the rotorod method. The data are presented in Table 2.

In the preliminary (Phase I), screening all the compounds except **4e**, **4j**, **4o**, and **4q** showed protection against MES test which indicates the ability of these compounds to prevent the seizure spread. Compounds that showed protection from seizures in the MES model at the 30 mg/kg dose include **4c**, **4d**, and **4s** which were active at 0.5 h of the administration. Interestingly, **4d** continued to protect from the seizures at the same dose after 4.0 h also. It indicates that compound **4d** had a rapid onset of action as well as long duration at the lower dose. Compounds **4c** and **4s** were also active at 4.0 h but at the higher dose 100 mg/kg.

Compounds that were active at 100 mg/kg after 0.5 h in MES tests include **4b**, **4f**, **4g**, **4i**, **4k**, **4l**, **4m**, **4n**, **4r**, and **4t** indicative of their good ability to protect from seizure spread. Among these compounds, **4g**, **4l**, **4n**, and **4r** were also active at the same dose after 4.0 h. This showed that these have quick onset and long duration of action at relatively higher dose. Compounds **4b**, **4f**, **4i**, **4k**, and **4t** were active after 4.0 h at 300 mg/kg. Compounds **4a**, **4h**, and **4p** were active after 0.5 h only at 300 mg/kg which showed the rapid onset and shorter duration of action of these compounds. Compounds **4e** and **4o** showed protection from seizures after 4.0 h at 300 mg/kg.

In the scPTZ screening, compounds that showed protection after 0.5 h were **4c**, **4d**, **4f**, **4g**, **4i**, **4k**, **4l**, **4m**, **4n**, **4q**, **4r**, and **4s**. Among these compounds, **4d**, **4g**, **4k**, **4l**, **4n**, and **4s** were active at a dose of 100 mg/kg which showed the promising nature of the compounds against the model. Interestingly, compounds **4g**, **4l**, **4n,** and **4s** were also active after 4.0 h at 300 mg/kg indicative of the long duration of action of these compounds.

In the neurotoxicity screen, compounds that did not exhibit any neurotoxicity at the highest dose include **4c**, **4d**, **4e**, **4l**, and **4n**. These compounds did not cause any motor impairment at the three doses after the two time periods. Rest of the compounds showed neurotoxicity but at higher doses. Majority of the compounds were found to be less neurotoxic than phenytoin.

Compounds were designed in such a way that the benzothiazole moiety was substituted with different electron-withdrawing groups and the phenyl ring attached to the pyrimidine ring was substituted with electron-releasing groups at different positions. If we study the structure-activity relationship of these compounds it was found that the methoxy derivatives were more active than the methyl and the unsubstituted derivatives. Compounds with a methoxy group at the fourth position were more active than those that bear the methoxy group at the second position. On the other hand, the chloro substituents were more potent and less neurotoxic than the bromo, fluoro, and nitro derivatives. Introduction of nitro and bromo groups resulted in compounds possessing more neurotoxicity than the chloro- and fluoro-derived compounds.

Table 3. Phase II quantitative anticonvulsant evaluation in mice.

Compound	ED_{50}[a)]		TD_{50}[b)]	PI[c)]	
	MES	scPTZ		MES	scPTZ
4c	22.8 (19.4 – 24.1)[d)]	204.6 (171.6 – 240.4)	432.3 (391.8 – 480.4)	18.9	2.1
4d	13.9 (12.2 – 15.4)	65.2 (56.3 – 73.6)	540.4 (453.8 – 621.1)	38.8	8.2
4s	25.4 (22.8 – 27.3)	74.8 (52.4 – 93.7)	88.6 (64.5 – 101.3)	3.4	1.1
Phenytoin[e)]	9.5 (8.1 – 10.4)	>300	65.5 (52.5 – 72.9)	6.9	<0.22
Carbamazepine[e)]	8.8 (5.5 – 14.1)	>100	71.6 (45.9 – 135)	8.1	<0.22
Phenobarbital[e)]	21.8 (21.8 – 25.5)	13.2 (5.8 – 15.9)	69 (62.8 – 72.9)	3.2	5.2
Valproate[e)]	272 (247 – 338)	149 (123 – 177)	426 (369 – 450)	1.6	2.9

Number of animals used: 10; solvent used: polyethylene glycol (0.1 mL, i.p.).

a) Dose in milligrams per kilogram body mass.

b) Minimal toxicity which was determined by rotorod test 30 min after the test drug was administered.

c) PI = Protective index (TD_{50}/ED_{50}).

d) Data in parentheses are the 95% confidence limits.

e) Data from references [14].

In the Phase-II anticonvulsant screening, the three most active compounds **4c**, **4d**, and **4s** were quantitatively evaluated for their anticonvulsant activity (ED_{50}) and neurotoxicity (TD_{50}). The results are shown in Table 3. All three compounds displayed encouraging activities, which are comparable to some standard drugs. Compounds **4c** and **4d** showed a higher protective index than all the standard drugs.

Lipophilicity determination

The calculated LogP (ClogP) values were generated by using the software ACD Labs 8.0 version. It was found that almost all the compounds have the ClogP value near 2.0, which is considered to be the optimum lipophilicity for the congeners that act on the central nervous system [15]. This may be the reason behind the rapid onset and long duration of action of most of the compounds.

Conclusion

The present series were designed by combining two active pharmacophores showing anticonvulsant activity. Interestingly, all the synthesized compounds showed some degree of protection against the seizures induced by both electroshock and chemically induced seizure methods. Three compounds were found to be very promising as far as efficacy and safety is concerned. They also showed optimum lipophilicity and lesser neurotoxicity than some standard drugs. They may act as lead molecules for future investigations.

One of the authors (W. A.) is thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for the Senior Research Fellowship (SRF) as financial assistance. We are also thankful to Shrenik Pharma Ltd., Mumbai for providing pentylenetetrazole as gift sample.

The authors have declared no conflict of interest.

Experimental

Chemistry

The melting points were determined in open capillary tubes in a Hicon melting point apparatus (Hicon, India) and are uncorrected. The homogen elemental analyses (C, H, N) of all compounds were performed on the Vario EL III CHNS Elementar (Analysensysteme GmbH, Hanau, Germany). All the Fourier transform infrared (FTIR) spectra were recorded in KBr pellets on a Jasco FT/IR 410 spectrometer (Jasco, Tokyo, Japan). The ^{1}H-NMR spectra were taken on a Bruker 400 Ultra shield™ NMR spectrometer (400 MHz; Bruker Bioscience, USA). Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. The homogeneity of the compounds was checked by thin layer chromatography (TLC) on silica gel G-coated plates (Merck, Germany) by using toluene / ethyl acetate / formic acid (5 : 4 : 1) as solvent system. Iodine chamber and UV lamp were used for the visualization of TLC spots.

*General procedure for the synthesis of 6-substituted-1,3-benzothiazol-2-amines **1a – d***

A mixture of substituted aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid was cooled and stirred. To this solution bromine (0.01 mol) was added dropwise at such a rate to keep the reaction temperature below 10°C throughout the addition. Stirring was continued for additional 3 h and the separated hydrochloride salt was filtered, washed with acetic acid, and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25%). The precipitate obtained

was filtered, washed with water, dried, and recrystallized to afford the 6-substituted-1,3-benzothiazol-2-amine **1a–d**.

*General procedure for the synthesis of ethyl (6-substituted-1,3-benzothiazol-2-yl)carbamates **2a–d***

To the substituted bezothiazol-2-amines (**1a–d**, 0.01 mol) in benzene were added ethylchloroformate (0.011 mol) and triethylamine (2.5 mL), and the reaction mixture was refluxed for 3 h. After cooling, the reaction mixture was poured into cold dil. HCl (50%) and the carbamate thus formed was recrystallized from benzene.

*General procedure for the synthesis of 1-(6-substituted-1,3-benzothiazol-2-yl)-3-(substituted phenyl)ureas **3a–t***

An equimolar mixture of carbamates **2a–d** and substituted anilines in ethanol was refluxed for 18–20 h. After the completion, ethanol was removed and residue obtained was washed with water, dried, and recrystallized from methanol.

*General procedure for the synthesis of 1-(6-substituted-1,3-benzothiazol-2-yl)-3-(substituted phenyl)hexahydro-2,4,6-pyrimidinetriones **4a–t***

An equimolar mixture of phenyl ureas **3a–t** and malonic acid was refluxed in acetyl chloride (10 mL) for 4 h at 40°C. The contents were cooled, poured over crushed ice, and neutralized with dilute sodium bicarbonate solution. The solid thus obtained was filtered, washed with water, dried, and recrystallized from ethanol to get the title compounds **4a–t**.

*1-(6-Chloro-1,3-benzothiazol-2-yl)-3-(2-methylphenyl)hexahydro-2,4,6-pyrimidinetrione **4a***

Yield: 70%; m.p.: 165°C; IR (KBr) ν_{max} [cm^{-1}]: 3071 (CH-Ar), 1681 (C=O), 1520 (C=N), 1104 (C=S), 815 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 2.31 (s, 3H, CH_3), 3.83 (s, 2H, CH_2), 7.05–8.61 (m, 3H, Benzoth.), 7.34–7.81 (m, 4H, ArH).

*1-(6-Chloro-1,3-benzothiazol-2-yl)-3-(4-methylphenyl)hexahydro-2,4,6-pyrimidinetrione **4b***

Yield: 65%; m.p.: 178°C; IR (KBr) ν_{max} [cm^{-1}]: 3111 (CH-Ar), 1632 (C=O), 1507 (C=N), 1135 (C=S), 807 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 1.91 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 6.95–8.25 (m, 3H, Benzoth.), 7.14–7.61 (m, 4H, ArH).

*1-(6-Chloro-1,3-benzothiazol-2-yl)-3-(2-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione **4c***

Yield: 74%; m.p.: 134°C; IR (KBr) ν_{max} [cm^{-1}]: 3072 (CH-Ar), 1671 (C=O), 1504 (C=N), 1460 (OCH_3), 1141 (C=S), 809 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 3.42 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2), 6.91–8.21 (m, 3H, Benzoth.), 7.23–7.68 (m, 4H, ArH).

*1-(6-Chloro-1,3-benzothiazol-2-yl)-3-(4-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione **4d***

Yield: 66%; m.p.: 156°C; IR (KBr) ν_{max} [cm^{-1}]: 3112 (CH-Ar), 1692 (C=O), 1515 (C=N), 1475 (OCH_3), 1171 (C=S), 824 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 3.61 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 6.99–8.37 (m, 3H, Benzoth.), 7.11–7.53 (m, 4H, ArH).

*1-(6-Chloro-1,3-benzothiazol-2-yl)-3-phenylhexahydro-2,4,6-pyrimidinetrione **4e***

Yield: 83%; m.p.: 155°C; IR (KBr) ν_{max} [cm^{-1}]: 3056 (CH-Ar), 1702 (C=O), 1524 (C=N), 1165 (C=S), 803 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 3.73 (s, 2H, CH_2), 6.84–8.12 (m, 3H, Benzoth.), 7.10–7.67 (m, 5H, ArH).

*1-(6-Bromo-1,3-benzothiazol-2-yl)-3-(2-methylphenyl)hexahydro-2,4,6-pyrimidinetrione **4f***

Yield: 71%; m.p.: 179°C; IR (KBr) ν_{max} [cm^{-1}]: 3072 (CH-Ar), 1679 (C=O), 1478 (C=N), 1126 (C=S), 563 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 2.41 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 7.12–8.16 (m, 3H, Benzoth.), 7.21–7.59 (m, 4H, ArH).

4.1.4.7 1-(6-Bromo-1,3-benzothiazol-2-yl)-3-(4-methylphenyl)hexahydro-2,4,6-pyrimidinetrione 4g

Yield: 84%; m.p.: 196°C; IR (KBr) ν_{max} [cm^{-1}]: 3056 (CH-Ar), 1684 (C=O), 1474 (C=N), 1118 (C=S), 574 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 1.76 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 7.08–8.06 (m, 3H, Benzoth.), 7.16–7.43 (m, 4H, ArH).

*1-(6-Bromo-1,3-benzothiazol-2-yl)-3-(2-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione **4h***

Yield: 61%; m.p.: 144°C; IR (KBr) ν_{max} [cm^{-1}]: 3066 (CH-Ar), 1686 (C=O), 1512 (C=N), 1448 (OCH_3), 1074 (C=S), 612 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 3.51 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2), 6.87–8.13 (m, 3H, Benzoth.), 7.21–7.69 (m, 4H, ArH).

*1-(6-Bromo-1,3-benzothiazol-2-yl)-3-(4-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione **4i***

Yield: 73%; m.p.: 186°C; IR (KBr) ν_{max} [cm^{-1}]: 3046 (CH-Ar), 1654 (C=O), 1504 (C=N), 1467 (OCH_3), 1026 (C=S), 603 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 3.71 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 6.93–8.24 (m, 3H, Benzoth.), 7.11–7.58 (m, 4H, ArH).

*1-(6-Bromo-1,3-benzothiazol-2-yl)-3-phenylhexahydro-2,4,6-pyrimidinetrione **4j***

Yield: 61%; m.p.: 155°C; IR (KBr) ν_{max} [cm^{-1}]: 3116 (CH-Ar), 1712 (C=O), 1504 (C=N), 1161 (C=S), 631 (C-Br); ^{1}H-NMR ($CDCl_3$) δ: 3.94 (s, 2H, CH_2), 6.92–7.96 (m, 3H, Benzoth.), 7.21–7.57 (m, 5H, ArH).

*1-(6-Fluoro-1,3-benzothiazol-2-yl)-3-(2-methylphenyl)hexahydro-2,4,6-pyrimidinetrione **4k***

Yield: 56%; m.p.: 178°C; IR (KBr) ν_{max} [cm^{-1}]: 3024 (CH-Ar), 1667 (C=O), 1472 (C=N), 1323 (C-F), 1153 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.34 (s, 3H, CH_3), 3.67 (s, 2H, CH_2), 7.01–8.34 (m, 3H, Benzoth.), 7.34–7.68 (m, 4H, ArH).

*1-(6-Fluoro-1,3-benzothiazol-2-yl)-3-(4-methylphenyl)hexahydro-2,4,6-pyrimidinetrione **4l***

Yield: 69%; m.p.: 193°C; IR (KBr) ν_{max} [cm^{-1}]: 3056 (CH-Ar), 1673 (C=O), 1452 (C=N), 1334 (C-F), 1201 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.59 (s, 3H, CH_3), 3.77 (s, 2H, CH_2), 6.87–8.14 (m, 3H, Benzoth.), 7.23–7.56 (m, 4H, ArH).

1-(6-Fluoro-1,3-benzothiazol-2-yl)-3-(2-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione ***4m***

Yield: 86%; m.p.: 193°C; IR (KBr) ν_{max} [cm^{-1}]: 3019 (CH-Ar), 1679 (C=O), 1508 (C=N), 1467 (OCH_3), 1356 (C-F), 1034 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.54 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 6.84 – 8.10 (m, 3H, Benzoth.), 7.23 – 7.64 (m, 4H, ArH).

1-(6-Fluoro-1,3-benzothiazol-2-yl)-3-(4-methoxyphenyl)hexahydro-2,4,6-pyrimidinetrione ***4n***

Yield: 79%; m.p.: 212°C; IR (KBr) ν_{max} [cm^{-1}]: 3051 (CH-Ar), 1693 (C=O), 1526 (C=N), 1434 (OCH_3), 1361 (C-F), 1027 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.61 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 6.89 – 8.24 (m, 3H, Benzoth.), 7.21 – 7.67 (m, 4H, ArH).

1-(6-Fluoro-1,3-benzothiazol-2-yl)-3-phenylhexahydro-2,4,6-pyrimidinetrione ***4o***

Yield: 74%; m.p.: 136°C; IR (KBr) ν_{max} [cm^{-1}]: 3116 (CH-Ar), 1698 (C=O), 1523 (C=N), 1349 (C-F), 1154 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.84 (s, 2H, CH_2), 6.97 – 8.16 (m, 3H, Benzoth.), 7.31 – 7.66 (m, 5H, ArH).

1-(2-Methylphenyl)-3-(6-nitro-1,3-benzothiazol-2-yl)hexahydro-2,4,6-pyrimidinetrione ***4p***

Yield: 82%; m.p.: 184°C; IR (KBr) ν_{max} [cm^{-1}]: 3023 (CH-Ar), 1676 (C=O), 1454 (C=N), 1339 (NO_2), 1189 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.54 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 7.13 – 8.21 (m, 3H, Benzoth.), 7.31 – 7.64 (m, 4H, ArH).

1-(4-Methylphenyl)-3-(6-nitro-1,3-benzothiazol-2-yl)hexahydro-2,4,6-pyrimidinetrione ***4q***

Yield: 78%; m.p.: 216°C; IR (KBr) ν_{max} [cm^{-1}]: 3056 (CH-Ar), 1664 (C=O), 1459 (C=N), 1345 (NO_2), 1176 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 2.64 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 7.10 – 8.24 (m, 3H, Benzoth.), 7.21 – 7.56 (m, 4H, ArH).

1-(2-Methoxyphenyl)-3-(6-nitro-1,3-benzothiazol-2-yl)hexahydro-2,4,6-pyrimidinetrione ***4r***

Yield: 71%; m.p.: 171°C; IR (KBr) ν_{max} [cm^{-1}]: 3034 (CH-Ar), 1665 (C=O), 1531 (C=N), 1447 (OCH_3), 1356 (NO_2), 1064 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.34 (s, 3H, OCH_3), 3.67 (s, 2H, CH_2), 6.94 – 8.11 (m, 3H, Benzoth.), 7.24 – 7.71 (m, 4H, ArH).

1-(4-Methoxyphenyl)-3-(6-nitro-1,3-benzothiazol-2-yl)hexahydro-2,4,6-pyrimidinetrione ***4s***

Yield: 56%; m.p.: 198°C; IR (KBr) ν_{max} [cm^{-1}]: 3059 (CH-Ar), 1651 (C=O), 1550 (C=N), 1489 (OCH_3), 1368 (NO_2), 1021 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.41 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 7.14 – 8.21 (m, 3H, Benzoth.), 7.29 – 7.64 (m, 4H, ArH).

1-(6-Nitro-1,3-benzothiazol-2-yl)-3-phenylhexahydro-2,4,6-pyrimidinetrione ***4t***

Yield: 61%; m.p.: 116°C; IR (KBr) ν_{max} [cm^{-1}]: 3112 (CH-Ar), 1667 (C=O), 1513 (C=N), 1343 (NO_2), 1135 (C=S); ^{1}H-NMR ($CDCl_3$) δ: 3.81 (s, 2H, CH_2), 6.98 – 8.19 (m, 3H, Benzoth.), 7.21 – 7.55 (m, 5H, ArH).

Pharmacology

The pharmacological testing of all the final compounds was performed according to the standard protocol given by the epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program [16].

The investigations were conducted with albino mice of either sex (25 to 30 g; Animals were obtained from Central Animal House Facility., Hamdard University, New Delhi-62. Registration no. and date of registration is 173/CPCSEA, 28 Jan., 2000.). The albino mice were kept under standard conditions at an ambient temperature of 25 ± 2°C and allowed free access to food and water except at the time they were brought out of the cage. All the experimental protocols were carried out with permission from Institutional Animal Ethics committee (IAEC), form no. 416.

Anticonvulsant activity

Maximal electroshock test (MES)

The maximal electroshock seizure test was carried out according to the standard protocol [17]. Albino mice were stimulated through corneal electrodes to 50 mA current at a pulse of 60 Hz applied for 0.25 s. Animals were previously administered with the test drug i.p. Abolition of hind-limb tonic extension spasm was recorded as the anticonvulsant activity. The test compounds were suspended in 0.5% methyl cellulose-water mixture or in polyethylene glycol (PEG). In the preliminary screening, each compound was administered as an i.p. injection at three dose levels (30, 100, and 300 mg/kg body mass) and the anticonvulsant activity assessed after 0.5 h and 4.0 h intervals of administration.

Subcutaneous pentylenetetrazole-induced seizure test (scPTZ)

The subcutaneous pentylenetetrazole test was performed according to the known protocol [18]. This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in >95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min., failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

The pharmacological parameters estimated in phase-I screening were quantified in phase-II screening (Table 3). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For the determination of ED_{50} and TD_{50} values, groups of ten mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10 to 90% seizure protection or minimal observed neurotoxicity. From the plot 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 the computer program by Litchfield and Wilcoxon's method [19].

Neurotoxicity screening (NT)

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod (diameter 3.2 cm) that rotates at 10 rpm. Trained animals were injected intraperitoneally 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 [20].

References

[1] S. R. Benbadis., W. O. Tatum, *J. Clin. Neurophysiol.* **2003**, *20*, 42 – 44.

[2] J. R. Dimmock, S. N. Pandey, J. W. Quail, U. Pugazhenthi, *et al.*, *Eur. J. Med. Chem*. **1995**, *30*, 303 – 314.

[3] A. Agarwal, S. Lata, K. K. Saxena, V. K. Srivastava, A. Kumar, *Eur. J. Med. Chem.* **2006**, *41*, 1223 – 1229.

[4] Archana, V. K. Srivastava, A. Kumar, *Bioorg. Med. Chem.* **2004**, *12*, 1257 – 1264.

[5] I. Chulak, V. Sutorius, V. Sekerka, *Chem. Pap.* **1990**, *44*, 131 – 138.

[6] M. Lacova, J. Chavancova, O. Hyblova, S. Varkonda, *Chem. Pap*. **1991**, *45*, 411 – 418.

[7] T. Papenfuhs, *Ger. Offen. De* **1987**, *3*, 528 – 532.

[8] N. Siddiqui, S. N. Pandeya, A. P. Sen, G. S. Singh, *Pharmakeftiki* **1992**, *4*, 121 – 125.

[9] N. Siddiqui, S. N. Pandeya, S. A. Khan, J. P. Stables, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 255 – 259.

[10] N. Siddiqui, A. Rana, S. A. Khan, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4178 – 4182.

[11] A. Rana, N. Siddiqui, S. A .Khan, S. E. Haque, M. A. Bhat, *Eur. J. Med. Chem*. **2008**, *43*, 1114 – 1122.

[12] R. Thirumurugan, D. Sriram, A. Saxena, J. P. Stables, P. Yogeeswari, *Bioorg. Med. Chem.* **2006**, *14*, 3106 – 3112.

[13] P. T. Flaherty, T. D. Greenwood, A. L. Manheim, J. F. Wolfe, *J. Med. Chem.* **1996**, *39*, 1509 – 1513.

[14] U. Huseyin, V. D. Kim, C. Silvia, S. Santi, *et al.*, *J. Med. Chem.* **1998**, *41*, 1138 – 1145.

[15] E. J. Lien, R. C. H. Liuo, H. G. Shinoucla, *J. Pharm. Sci.* **1979**, *68*, 463 – 465.

[16] *Anticonvulsant Screening Project, Antiepileptic Drug Development Program*; National Institute of Health, DHEW Publ (NIH) (US) **1978**; pp. 78 – 1093.

[17] C. Krall, J. K. Penny, B. G. White, H. G. Kupferberg, *Epilepsia* **1978**, *19*, 409 – 428.

[18] G. N. Kelekci, S. Yabanoglu, E. Kupeli, U. Salgin, A. A. Bilgin, *Bioorg. Med. Chem.* **2007**, *15*, 5775 – 5786.

[19] J. T. Litchfield, F. Wilcoxon, *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99 – 113.

[20] S. G. Kucukguzel, S. Rollas, *Il Farmaco* **2005**, *57*, 583 – 587.

