

RESEARCH ARTICLE

Synthesis of 8-substituted-4-(2/4-substituted phenyl)-2H-[1,3,5]triazino[2,1-b][1,3]benzothiazole-2-thiones and their anticonvulsant, anti-nociceptive, and toxicity evaluation in mice

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Abstract

A number of new 8-substituted-4-(2/4-substituted phenyl)-2H-[1,3,5]triazino[2,1-b][1,3]benzothiazole-2-thiones (**4a–t**) were synthesized and evaluated for their anticonvulsant, anti-nociceptive, hepatotoxic, and neurotoxic properties. The titled compounds (**4a–t**) were obtained by cyclization of *N*-[[6-substituted-1,3-benzothiazol-2-yl) amino]carbonothioyl]-2/4-substituted benzamides (**3a–t**) by refluxing in *n*-butanol. All the newly synthesized compounds were screened for their anticonvulsant activity in a mouse seizure model and were compared with the standard drug phenytoin. Compounds **4a**, **4c**, **4f**, and **4l** showed complete protection after time periods of 0.5 h and 4 h. Some of the selected compounds were evaluated for their neurotoxic and hepatotoxic effects, and none of these showed any sign of neurotoxicity or hepatotoxicity. Compounds **4a–t** were also evaluated for their anti-nociceptive activity by a thermal stimulus technique using diclofenac as standard. Compounds **4o**, **4q**, and **4t** displayed highly potent analgesic activity with $p < 0.01$.

Keywords: Benzothiazole; anticonvulsant; analgesic; hepatotoxicity; neurotoxicity

Introduction

Among the different neurological disorders that affect the human condition, epilepsy has been extensively studied during the last century, becoming a dynamic research field in recent years. It describes disorders characterized by recurrent seizure attack due to synchronous neuronal firing. Seizures remain uncontrolled in at least 30% of all epilepsies, even when adequate antiepileptic drug therapy is administered. The classical antiepileptic drugs comprise phenobarbital, available since 1911; phenytoin, marketed in 1939; carbamazepine, used for epilepsy in Europe from the mid-1960s; and valproic acid¹, available in several European countries since the late 1960s. All currently approved antiepileptic drugs have dose-related toxicity and

idiosyncratic side effects². In recent years, benzothiazole derivatives have gained conspicuous significance due to their wide spectrum of biological activities. Although they have been known for a long time to be biologically active^{3–5}, their varied biological features remain of great scientific interest. Newer anticonvulsant agents having a differently substituted benzothiazole nucleus have been reported to possess significant activities^{6–8}.

In our previous work we have reported^{9–13} several benzofused five-membered heterocyclic compounds, including the benzothiazole moiety, which have shown marked anticonvulsant activity. In continuation of our research on the benzothiazole moiety, we have combined the benzothiazole pharmacophore with the active triazine

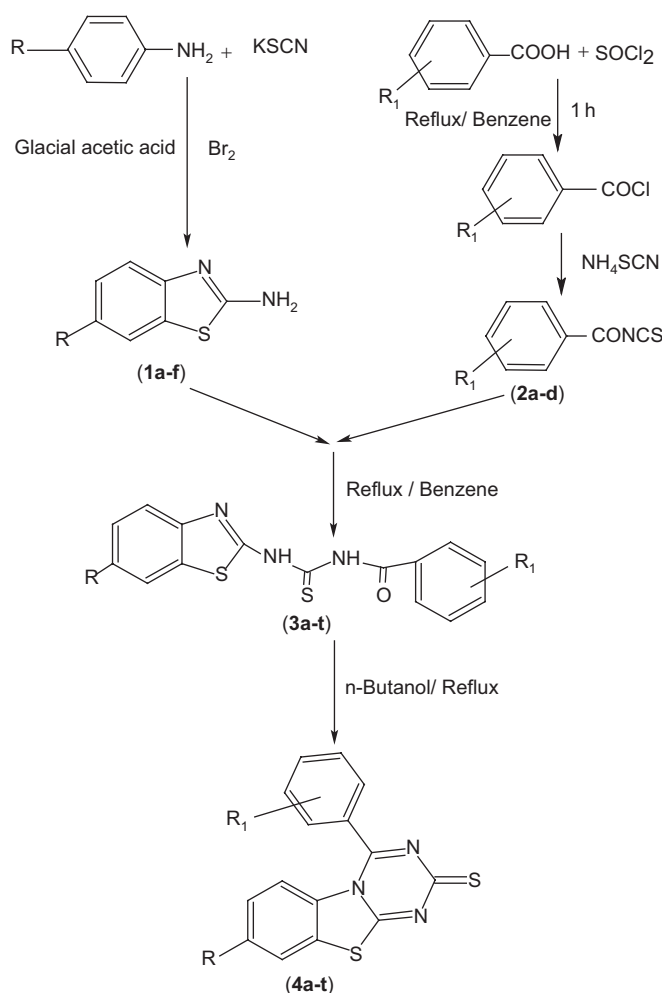
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pharmacophore and studied the effect on anticonvulsant activity thereafter.

Analgesia is a state of reduced awareness to pain, and analgesics are substances that decrease pain sensation by increasing the threshold to painful stimuli. The management of analgesic disorders involves a stepwise approach to the use of therapeutic agents. Relief of pain and reduction of inflammation are major goals in reducing the severity of symptoms¹⁴. A generally accepted stepwise approach to manage painful (nociceptive) disorders includes physical therapy, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), corticosteroids, and immunosuppressive agents. In recent years, a number of benzothiazole derivatives have been synthesized and found to display analgesic and anti-inflammatory activity^{15,16}.

In the present investigation, we have synthesized 8-substituted-4-(2/4-substituted phenyl)-2*H*-[1,3,5]triazino [2,1-*b*][1,3]benzothiazole-2-thiones (Scheme 1). The compounds were evaluated *in vivo* for anticonvulsant activity by the maximal electroshock seizure (MES) test, anti-nociceptive activity by the thermal stimulus technique, and neurotoxicity by the rotarod method.



Scheme 1. Synthetic route of titled compounds 4a-t.

Materials and methods

Animals

Albino mice (Swiss, 25–30 g) were used in groups of six each as experimental animals. All the test compounds and standard drug were suspended in polyethylene glycol (PEG) and administered intraperitoneally. The animals were maintained on an adequate diet and allowed free access to food and water except during the short time they were removed from their cages for testing. The animals were maintained at room temperature (25 ± 2°C). All experimental protocols were carried out with permission of the Institutional Animal Ethics Committee (IAEC). Animals were obtained from the Central Animal House Facility, Jamia Hamdard University, New Delhi (registration number and date of registration of the Animal House Facility: 173/CPCSEA, 28 Jan 2000).

Chemistry

Solvents selected were of LR (laboratory reagent) grade and were obtained from Merck, CDH, and s.d. Fine Chemicals. Melting points were determined in open capillary tubes and are uncorrected. Thin layer chromatography was performed on silica gel G (Merck). Spots were developed in an iodine chamber and visualized under ultraviolet (UV) light. Infrared (IR) spectra were recorded in KBr pellets on a Bio-Rad FTS 135 WIN-IR spectrophotometer. ¹H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker model DPX 300 FT NMR spectrometer in DMSO-*d*₆ using tetramethylsilane (Me₄Si; TMS) as internal standard. The chemical shifts (δ) are recorded in ppm.

General procedure for the synthesis of titled compounds 4a-t (Scheme 1)

6-Substituted-1,3-benzothiazol-2-amines (1a-f) Substituted anilines (0.01 mol) and potassium thiocyanate (0.01 mol) were dissolved in glacial acetic acid, cooled, and stirred for 15 min. Cold bromine solution (0.01 mol, 3 mL in 10 mL acetic acid) was added dropwise. Stirring was continued for an additional 3 h. The separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water, and neutralized with aqueous ammonia solution (25%). The resulting precipitate was filtered off, washed with water, and recrystallized from ethanol to get the desired compounds **1a-f**.

2/4-Substituted benzoylisothiocyanates (2a-d) Substituted benzoic acid (0.1 mol) and thionyl chloride (0.1 mol) were refluxed in benzene (50 mL) (CARE—carcinogenic) for 1 h. The reaction mixture upon filtration yielded substituted benzoyl chloride as a viscous liquid. Ammonium thiocyanate (0.1 mol) was added to the substituted benzoyl chloride (0.1 mol) and refluxed for 30 min in benzene. The resulting mixture was filtered and the substituted benzoyl isothiocyanates **2a-d** were obtained in the form of a liquid with ammonium chloride as a solid residue. Ammonium chloride was removed by filtration as a residue from the mixture.

***N*-{[(6-substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-benzamides (3a-t)** The compounds

3a-t were obtained when 6-substituted benzothiazoles (**1a-f**, 0.02 mol) and substituted benzoyl isothiocyanates (**2a-d**, 0.02 mol) were refluxed in benzene for 5 h. The solid material obtained was filtered and recrystallized from benzene.

8-Substituted 4-(2/4-substituted phenyl)-2H-[1,3,5]triazino [2,1-b][1,3]benzothiazole-2-thiones (**4a-t**) A solution of compounds **3a-t** (0.01 mol) in *n*-butanol (30 mL) was refluxed for 5 h to obtain compounds **4a-t**, which were crystallized from *n*-butanol. The spectral data and physicochemical properties of compounds **4a-t** are given in Tables 1 and 2.

Pharmacology

Anticonvulsant activity

Maximal electroshock seizure test (MES) Initial anticonvulsant evaluation of the test compounds was undertaken by following the anticonvulsant drug development (ADD) program protocol^{17,18} devised by the Epilepsy Section of the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), USA. Each compound was administered as an intraperitoneal (i.p.)

injection at a dose level of 30 mg/kg body weight, and the anticonvulsant activity was assessed 0.5 h and 4 h after administration. Maximal electroshock seizures were elicited in mice by delivering 60 Hz, 50 mA electrical stimuli for 0.2 s via ear-clip electrodes. The maximal seizure typically consisted of a short period of tonic extension of the hind limbs and a final clonic episode. Blockade of the hind limbs' tonic extensor component due to drug treatment was taken as the end point.

Anti-nociceptive activity

Thermal stimulus technique The anti-nociceptive activity was evaluated using a previously described procedure¹⁹. The test compounds were suspended in a methylcellulose-water (0.5%) mixture. Each compound was administered orally at a dose of 20 mg/kg. The anti-nociceptive activity was assessed 4 h after administration. The tail of each mouse was gently immersed in thermostatically controlled water at 55°C. The parameter measured in test samples was the time that elapsed between immersion and the attempt to withdraw the tail from hot water, for control as well as treated groups of animals.

Table 1. Spectral characterization of compounds **4a-t**.

Compound no.	FT-IR (KBr): V_{max} (cm ⁻¹)	¹ H-NMR (DMSO-d ₆): δ (ppm)	Mass (EI): m/z
4a	3068 (CH-Ar), 1675 (C=N), 1095 (C=S), 765 (C-Cl), 699 (C-S-C).	7.04-7.74 (m, 8H, Ar-H)	—
4b	3058 (CH-Ar), 1670 (C=N), 1090 (C=S), 690 (C-S-C)	7.17-7.78 (m, 8H, Ar-H)	—
4c	3060 (CH-Ar), 1677 (C=N), 1536 (C-NO ₂), 1129 (C=S), 682 (C-S-C)	7.59-8.71 (m, 8H, Ar-H)	—
4d	3056 (CH-Ar), 2928 (CH-aliph.), 1660 (C=N), 1094 (C=S), 698 (C-S-C)	2.27 (s, 3H, CH ₃), 7.31-7.59 (m, 8H, Ar-H)	—
4e	3062 (CH-Ar), 2924 (CH-aliph.), 1631 (C=N), 1073 (C=S), 704 (C-S-C)	3.77 (s, 3H, OCH ₃), 6.91-7.40 (m, 8H, Ar-H)	325 (M ⁺)
4f	3061 (CH-Ar), 1673 (C=N), 1050 (C=S), 745 (C-Cl), 647 (C-S-C), 552 (C-Br)	7.32-7.93 (m, 7H, Ar-H)	408 (M ⁺)
4g	3066 (CH-Ar), 1664 (C=N), 1054 (C=S), 747 (C-Cl), 652 (C-S-C)	7.41-7.59 (m, 7H, Ar-H)	364 (M ⁺)
4h	3072 (CH-Ar), 1683 (C=N), 1055 (C=S), 1299 (C-F), 747 (C-Cl), 665 (C-S-C)	7.30-7.97 (m, 7H, Ar-H)	—
4i	3052 (CH-Ar), 2910 (CH-aliph.), 1668 (C=N), 1098 (C=S), 768 (C-Cl), 692 (C-S-C)	1.17 (s, 3H, CH ₃), 7.09-7.91 (m, 7H, Ar-H)	343 (M ⁺)
4j	3048 (CH-Ar), 2918 (CH-aliph.), 1662 (C=N), 1084 (C=S), 760 (C-Cl), 688 (C-S-C)	3.74 (s, 3H, OCH ₃), 6.91-8.03 (m, 7H, Ar-H)	—
4k	3042 (CH-Ar), 1656 (C=N), 1078 (C=S), 682 (C-S-C), 560 (C-Br)	7.06-8.01 (m, 7H, Ar-H)	—
4l	3052 (CH-Ar), 1683 (C=N), 1092 (C=S), 761 (C-Cl), 674 (C-S-C)	7.03-7.95 (m, 7H, Ar-H)	—
4m	3069 (CH-Ar), 1674 (C=N), 1323 (C-F), 1094 (C=S), 761 (C-Cl), 674 (C-S-C)	6.96-8.15 (m, 7H, Ar-H)	—
4n	3064 (CH-Ar), 2923 (CH-aliph.), 1674 (C=N), 1085 (C=S), 754 (C-Cl), 695 (C-S-C)	2.43 (s, 3H, CH ₃), 7.30-8.51 (m, 7H, Ar-H)	—
4o	3041 (CH-Ar), 2921 (CH-aliph.), 1686 (C=N), 1092 (C=S), 761 (C-Cl), 674 (C-S-C)	3.82 (s, 3H, OCH ₃), 7.05-8.15 (m, 7H, Ar)	—
4p	3093 (CH-Ar), 2925 (CH-aliph.), 1632 (C=N), 1109 (C=S), 763 (C-Cl), 700 (C-S-C)	3.63 (s, 3H, OCH ₃), 7.20-7.77 (m, 7H, Ar-H)	—
4q	3042 (CH-Ar), 2910 (CH-aliph.), 1648 (C=N), 1301 (C-F), 1074 (C=S)	3.73 (s, 3H, OCH ₃), 6.79-7.60 (m, 7H, Ar-H)	—
4r	3031 (CH-Ar), 2941 (CH-aliph.), 1654 (C=N), 1530 (C-NO ₂), 1122 (C=S), 752 (C-S-C)	3.58 (s, 4H, OCH ₃), 7.40-8.698 (m, 7H, Ar-H)	370 (M ⁺)
4s	3063 (CH-Ar), 2922 (CH-aliph.), 1656 (C=N), 1050 (C=S), 640 (C-S-C)	2.30 (s, 3H, CH ₃), 3.56 (s, 4H, OCH ₃), 6.99-7.43 (m, 7H, Ar-H)	—
4t	3061 (CH-Ar), 2920 (CH-aliph.), 1662 (C=N), 1046 (C=S), 658 (C-S-C)	3.60 (s, 3H, OCH ₃), 3.80 (s, 3H, OCH ₃), 6.88-7.93 (m, 7H, Ar-H)	—

Table 2. Physicochemical properties of compounds **4a-t**.

Compound no.	R	R ₁	Mol. formula ^a	Mol. weight	Yield (%)	MP ^b (°C)	R _i value ^c	Experimental log P ^d
4a	Cl	H	C ₁₅ H ₈ ClN ₃ S ₂	329.5	53	160	0.89	2.80
4b	F	H	C ₁₅ H ₈ FN ₃ S ₂	313	51	118	0.87	—
4c	NO ₂	H	C ₁₅ H ₈ N ₄ O ₂ S ₂	340	46	240	0.86	2.78
4d	CH ₃	H	C ₁₆ H ₁₁ N ₃ S ₂	309	48	140	0.90	—
4e	OCH ₃	H	C ₁₆ H ₁₁ N ₃ O ₂ S ₂	325	52	286	0.92	—
4f	Br	2-Cl	C ₁₅ H ₇ BrClN ₃ S ₂	407.5	52	120	0.79	3.10
4g	Cl	2-Cl	C ₁₆ H ₇ Cl ₂ N ₃ S ₂	376	56	280	0.77	3.12
4h	F	2-Cl	C ₁₅ H ₇ ClFN ₃ S ₂	347.5	50	174	0.82	—
4i	CH ₃	2-Cl	C ₁₆ H ₁₀ ClN ₃ S ₂	343.5	40	212	0.80	—
4j	OCH ₃	2-Cl	C ₁₆ H ₁₀ ClN ₃ O ₂	311.5	54	170	0.94	—
4k	Br	4-Cl	C ₁₅ H ₇ BrClN ₃ S ₂	407.5	60	138	0.78	—
4l	Cl	4-Cl	C ₁₆ H ₇ Cl ₂ N ₃ S ₂	376	65	216	0.91	3.16
4m	F	4-Cl	C ₁₅ H ₇ ClFN ₃ S ₂	328.5	48	180	0.83	3.17
4n	CH ₃	4-Cl	C ₁₆ H ₁₀ ClN ₃ S ₂	343.5	58	218	0.94	—
4o	OCH ₃	4-Cl	C ₁₆ H ₁₀ ClN ₃ O ₂	359.5	50	162	0.84	—
4p	Cl	4-OCH ₃	C ₁₆ H ₁₀ ClN ₃ O ₂	359.5	56	154	0.76	—
4q	F	4-OCH ₃	C ₁₆ H ₁₀ FN ₃ S ₂	327	52	130	0.83	—
4r	NO ₂	4-OCH ₃	C ₁₆ H ₁₀ N ₄ O ₃ S ₂	370	58	220	0.88	—
4s	CH ₃	4-OCH ₃	C ₁₇ H ₁₃ N ₃ O ₂ S ₂	329	55	100	0.85	—
4t	4-OCH ₃	4-OCH ₃	C ₁₇ H ₁₃ N ₃ O ₂ S ₂	355	50	160	0.81	—

^aSolvent of crystallization: ethanol.^bMelting point of compounds at their decomposition.^cSolvent system benzene:acetone (8:2); elemental analysis for C, H, and N was in good agreement ($\pm 0.4\%$) with the theoretical value.^dExperimental log *P* values of the most active compounds were calculated using octanol-water system (— denotes not determined).

Neurotoxic effects

Rotarod test Minimal motor impairment was measured in mice by the rotarod test²⁰. The mice were trained to stay on an accelerating rotarod that rotated at 10 rev/min. The rod diameter was 3.2 cm. Trained animals were given i.p. injections of the test compounds at a dose of 30 mg/kg. Unimpaired mice can easily remain on a rod rotating at this speed. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three concurrent trials.

Histopathological studies

The cellular effects of selected compounds **4a** and **4o** were evaluated by histopathology. Luna's technique²¹ was used to assess the livers of the mice, which had received the test compounds at the dose of 30 mg/kg body weight for 15 days. Comparison was made with the control group receiving the dosing vehicle. Microphotographs of liver sections were taken at magnifications of $\times 100$ and $\times 400$.

Log *P* determination

The desired log *P* value depends on the nature of the compounds and the testing system. A log *P* of approximately 2.0 is considered to be the best predictor for penetration of the blood-brain barrier and therefore of central nervous system (CNS) activity²². In this study, we attempted to correlate the anticonvulsant activity with 100% protection against seizure spread in the anti-MES screen with calculated log *P* values (CLOGP). Log *P* values were determined for compounds **4a**, **4c**, **4f**, **4g**, **4l**, and **4m**. Experimental log *P* values were determined using the octanol-water method²³ and CLOGP values were calculated using ACD freeware version 7.1.

Estimation of liver enzymes and proteins

Serum glutamate oxaloacetate transaminase (SGOT) This is a mitochondrial enzyme present in large quantities in the liver, heart, skeletal muscles, and kidneys. It is released from the cells when the tissues are damaged. It was estimated using Rietman and Frankel's method²⁴⁻²⁶.

Serum glutamate pyruvate transaminase (SGPT) This cytosolic enzyme is present abundantly in liver cells. Thus, the serum levels of SGPT are elevated by liver disease. This is considered one of the most sensitive indications of liver damage, particularly in viral hepatic necrosis, e.g. viral hepatitis or toxin-induced liver injury. It was determined using Rietman and Frankel's method²⁴⁻²⁶.

Alkaline phosphatase Alkaline phosphatases are enzymes that catalyze removal of the phosphate group from monophosphate esters under alkaline conditions. This reaction is of considerable importance in several liver diseases²⁷.

Estimation of proteins Determination of the proteins provides most useful information in chronic liver diseases. They were determined using the Biuret method^{28,29}.

Statistical analyses

All statistical analyses were carried out using SigmaStat 4.0 software by means of analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, and the results are expressed as mean \pm SEM.

Results and discussion

Anticonvulsant activity

The anticonvulsant activity and neurotoxicity data for the compounds are reported in Table 3. At the dose level of

Table 3. Anticonvulsant, anti-nociceptive, and neurotoxicity data of compounds **4a-t**.

Compound no.	MES screen ^a (% protection)		Neurotoxicity screen ^a		Mean average reaction time ^b (s) (mean ± SEM)		Anti-nociceptive (%)
	0.5 h	4 h	0.5 h	4 h	Control	Treated	
4a	100	100	—	—	1.2±0.985	2.1±0.242**	175
4b	66	66	×	×	0.7±0.583	1.0±0.057**	142
4c	100	100	—	—	0.9±0.532	1.9±0.453**	211
4d	66	66	×	×	0.9±0.049	2.2±0.317**	244
4e	66	66	×	×	0.9±0.140	1.7±0.349*	188
4f	100	100	—	—	1.0±0.259	1.7±0.407	170
4g	100	83	—	—	1.0±0.140	1.5±0.259	150
4h	83	66	—	—	1.7±0.239	3.0±0.239**	176
4i	66	66	×	×	0.8±0.021	1.7±0.303*	212
4j	66	50	×	×	0.8±0.047	1.3±0.160**	162
4k	66	66	×	×	1.1±0.383	1.3±0.414	118
4l	100	100	—	—	0.9±0.095	2.1±0.388*	233
4m	100	83	—	—	0.7±0.054	1.3±0.341	185
4n	83	66	—	—	1.2±0.373	1.8±0.558	150
4o	83	66	—	—	1.8±0.109	2.3±0.179***	127
4p	66	66	×	×	0.9±0.657	1.4±0.197*	155
4q	66	66	×	×	0.8±0.037	1.8±0.111***	225
4r	66	50	×	×	1.4±0.287	2.1±0.322	150
4s	50	50	×	×	1.5±0.171	2.1±0.060**	140
4t	83	66	—	—	0.9±0.540	0.2±0.176***	222
Phenytoin	100	100	—	—			
Diclofenac					1.8±0.200	19.6±0.89***	1088

Note. $n=6$; * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Data were analyzed by unpaired Student's t test.

^aIntraperitoneal dose of 30 mg/kg was administered and the animals were examined 0.5 and 4 h after administration. — indicates an absence of activity; × denotes not tested.

^bDose=20 mg/kg (p.o).

30 mg/kg, all compounds exhibited average to good protection. Results are presented as percent protection at time intervals of 0.5 h and 4 h. The anticonvulsant drug phenytoin was used as the standard at a dose of 30 mg/kg.

Compounds **4a**, **4c**, **4f**, **4g**, **4l**, and **4m** showed 100% protection after the 0.5 h time period and the compounds **4g** and **4m** showed 83% protection after 4 h. All the compounds, with the exception of compound **4s** (which showed 50% protection), showed 66–83% protection at both time intervals.

Neurotoxicity screening of compounds is also presented in Table 3. In the neurotoxicity screen all the selected compounds were devoid of neurotoxicity at the dose of 30 mg/kg body weight.

Anti-nociceptive activity

8-Substituted-4-(2/4-substitutedphenyl)-2H-[1,3,5]triazino [2,1-b][1,3]benzothiazole-2-thiones (**4a-t**) were evaluated for their anti-nociceptive activity by the thermal stimulus technique. Results of the screening are presented in Table 3. Results are reported as mean average reaction time in seconds for untreated control and treatment compounds at the dose level of 20 mg/kg. Diclofenac was used as the standard for comparison. The level of significance was determined by Student's t test. Compounds **4o**, **4q**, and **4t** showed highly potent activity with $p<0.001$, whereas compounds **4a**, **4b**, **4c**, **4d**, **4h**, **4j** and **4s** showed significant anti-nociceptive activity with $p<0.01$. Compounds **4e**, **4i**, **4l** and **4p** also displayed the activity with $p<0.05$, however

compounds **4f**, **4g**, **4k**, **4m**, **4n**, and **4r** were devoid of anti-nociceptive activity at the tested dose.

Hepatotoxicity studies

Microphotographs of sections of the livers of mice receiving compounds **4a** and **4o** along with control are presented in Figure 1. Liver samples from control group animals and all experimental groups were within normal histological limits, except sample **4a** which showed moderate portal inflammation. These changes were non-specific in nature. No hepatocyte necrosis or degeneration was seen in any of the samples.

Log P determination

Experimental log P values of compounds **4a**, **4c**, **4f**, **4g**, **4l**, and **4m** were found to be $2.80±0.24$, $2.78±0.35$, $3.10±0.47$, $3.12±0.21$, $3.16±0.54$, and $3.17±0.13$, respectively, while their calculated values were $3.46±0.86$, $1.52±1.08$, $4.44±0.90$, $4.26±0.87$, $4.23±0.87$, and $3.69±0.90$, respectively. All the selected compounds were lipophilic in nature.

Estimation of liver enzymes and proteins

Enzyme estimation was performed for the most active compounds (**4a** and **4o**) and the data are presented in Table 4. All enzymes estimated were compared to the control. Alkaline phosphatase and SGPT values were found to be increased slightly by both compounds ($p<0.01$). Compound **4a** also increased the SGOT level in blood slightly ($p<0.01$).

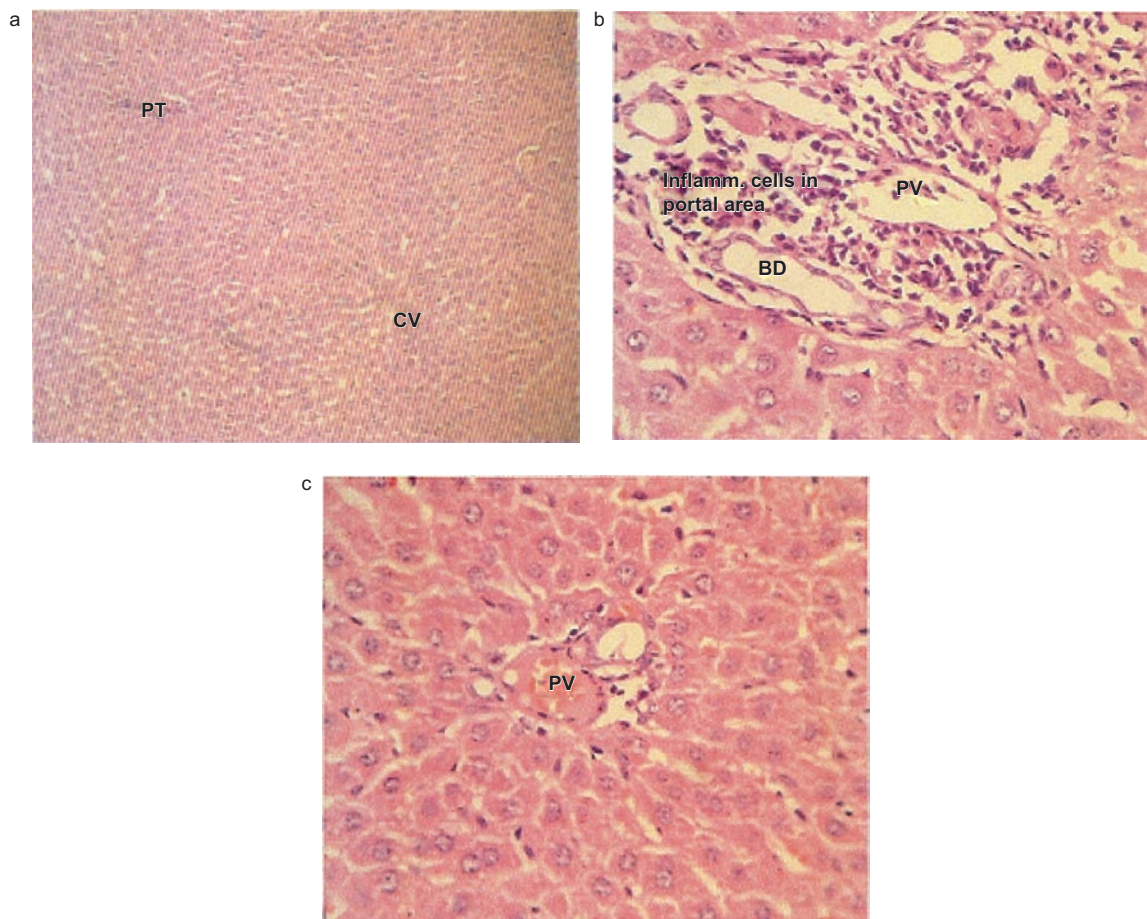


Figure 1. (a) Low power (HE, $\times 100$) and (b, c) high power (HE, $\times 400$) photomicrographs of liver. Sample from control (a) shows normal hepatic parenchyma with portal triad (PT), central vein (CV), and hepatocytes. In the case of compound **4a** (b), liver shows hepatic parenchyma with inflammatory cells infiltrating the portal triad (PT). The central vein and hepatocytes do not show any inflammatory cells. BD, Biliary duct. In the case of compound **4o** (c), liver shows normal portal triad structures.

Table 4. Effect of selected compounds on enzyme levels.

Treatment	Alkaline phosphatase (\pm SEM)	SGOT (\pm SEM)	SGPT (\pm SEM)
Control	13.06 \pm 0.25	148.67 \pm 1.50	27.67 \pm 0.840
4a	17.30 \pm 0.16**	183.00 \pm 1.00**	45.67 \pm 1.310**
4o	20.29 \pm 0.18**	142.83 \pm 1.40*	32.67 \pm 0.760**

Note. * $p < 0.05$, ** $p < 0.01$. The mean level of SGOT/SGPT \pm SEM was calculated using ANOVA followed by Dunnett's multiple comparison test.

The two proteins found in the liver, albumin and globulin, were also estimated, and the albumin/globulin ratio was also determined. The results are presented in Table 5. All parameters determined were compared to the control. In advanced liver disease, albumin decreases and globulin often increases. It was found that the albumin level in blood increased significantly after administration of the two compounds. There was no significant change in globulin level.

Conclusions

All the compounds were active in the MES test, indicative of their ability to prevent seizure spread. Maximum potency was observed when halogen substituents such as Br, Cl, and F and nitro substituent at the 8-position of the benzothiazole

Table 5. Effect of selected compounds on protein levels.

Treatment	Albumin (g/100 mL)	Globulin (g/100 mL)	Total protein (g/100 mL)	Albumin/ globulin
Control	1.67 \pm 0.009	0.13 \pm 0.01	1.80 \pm 0.01	12.84 \pm 1.57
4a	1.91 \pm 0.03**	0.35 \pm 0.02	2.26 \pm 0.02**	5.45 \pm 0.42**
4o	1.88 \pm 0.03**	0.21 \pm 0.09	2.09 \pm 0.02**	8.95 \pm 0.82**

Note. ** $p < 0.01$. Results are given as mean total protein level \pm SEM and were calculated using ANOVA followed by Dunnett's multiple comparison test.

ring with a 2/4-Cl substituted phenyl ring were present in the compounds. However, substitution with CH_3 at the 8-position of the benzothiazole ring with 4- OCH_3 at the phenyl ring reduced the potency by 50%. Derivatives with OCH_3 at the 8-position of the benzothiazole ring and 4-Cl/ OCH_3 substituent at the phenyl ring exhibited a decrease in activity to a lesser extent.

The anti-nociceptive activity of the compounds was evaluated by a thermal stimulus technique. Substitution with 8-F, 8- OCH_3 at the benzothiazole ring and with 4-Cl, 4- OCH_3 substituents at the distal phenyl ring led to high potency of the compounds. The presence of 8-Cl, 8- NO_2 , 8- CH_3 substituents in the benzothiazole ring and H, 4- OCH_3 substituents in the distal phenyl ring was responsible for marked

anti-nociceptive activity. However, substitution at 8-CH₃ in the benzothiazole ring with 2-Cl substitution in the distal phenyl ring resulted in a significant decrease in activity.

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