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Preliminary communication

N-{[(6-Substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-substituted benzamides: Synthesis and pharmacological evaluation

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Abstract

A series of 1,3-benzothiazol-2-yl benzamides (**11–30**) were prepared in satisfactory yield and evaluated for their anticonvulsant, neurotoxicity, CNS depressant study and other toxicity studies. All the synthesized compounds were in good agreement with elemental and spectral data. Majority of the compounds were active in MES and scPTZ screen and showed the decrease in the immobility time. None of the compounds had shown neurotoxicity or liver toxicity.

Keywords: Benzamides; Benzothiazole; Anticonvulsant; Neurotoxicity; CNS depressant activity

1. Introduction

The term epilepsy refers to a disorder of the brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsies are common and frequently devastating and affect around 1–2% of the world population. The convulsions of approximately 25% of epileptics are inadequately controlled by the standard drug therapy [1,2]. The number of drugs useful for the treatment of epilepsy is remarkably small. Fewer than 20 drugs are currently marketed in the United States and of these only five or six are widely used [2].

The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry. A rational drug design process of a new anticonvulsant could be achieved in several ways [3,4]. The first strategy is the identification of new targets through better understanding of molecular mechanisms of epilepsy. Another way is to modify already existing drugs and formulations. The long established anticonvulsants control seizures in 50% of patients developing partial seizures and 60–70% of those developing generalized seizures [5–7]. Hence there is an urgent need to develop new anticonvulsants [8].

The benzamides are a class of compounds presenting a wide range of biological applications and anticonvulsant activity [9–12]. A variety of benzamides reported to possess anticonvulsant activity in a number of animal models used to predict potential anticonvulsant activity in man. Recently pharmacophore model was proposed based on the semicarbazones [13] according to which essential features for the anticonvulsant activity are (a) a hydrophobic aryl ring, (b) a hydrogen bonding domain, (c) an electron donor acceptor system and (d) an another hydrophobic aryl ring responsible for metabolism, the size of ring may differ Fig. 1.

In our study *N*-{[(6-substituted-1,3-benzothiazole-2-yl)-amino]carbonothioyl}-2/4-substituted benzamides were synthesized and evaluated for pharmacological activity to test this hypothesis. Benzothiazole, a versatile heterocyclic

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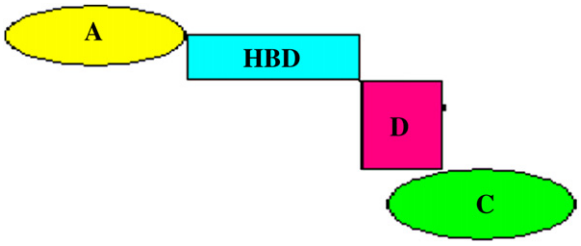


Fig. 1. Suggested pharmacophore model for anticonvulsant activity. A: hydrophobic domain, B: hydrogen bonding site, C: distal hydrophobic domain, D: electron donor moiety.

hydrophobic molecule possessing preliminary anticonvulsant properties [14,15] has been selected for the hydrophobic binding site. Benzothiazole were condensed with the benzamides with the hope to potentiate the biological activities with lesser or limited amount of toxicities.

2. Chemistry

The synthesis of *N*-{[(6-substituted-1,3-benzothiazole-2-yl)-amino]carbonothioyl}-2/4-substituted benzamides **11–30** was accomplished as presented in Scheme 1. The 6-substituted-1,3-benzothiazole-2-amines **1–6** were synthesized by reacting aryl amines with potassium thiocyanates in a satisfactory yield by a known preparation method [16]. In FTIR spectrum bands at 3490–3210 cm^{-1} and 1580–1520 cm^{-1} confirms presence of NH and C=N stretching vibrations, respectively. The ^1H NMR spectrum showed a singlet at δ 5.33–7.21 ppm due to NH_2 protons (D_2O exchangeable). 2/4-Substituted benzoylisothiocyanates **7–10** were obtained by reacting substituted benzoic acid and thionyl chloride, substituted benzoyl chloride was obtained which was further treated with ammonium thiocyanate to get **1–6**. The FTIR bands showed bands for C=O and N–C–S at 1670–1628 cm^{-1} and 2180–2100 cm^{-1}, respectively. The ^1H NMR spectrum showed multiplet at δ 7.20, 7.79–7.48, 8.01 for aromatic protons.

Final compounds *N*-{[(6-substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-substituted benzamides **11–30** were synthesized by refluxing 6-substituted-1,3-benzothiazol-2-amines **1–6** and 2/4-substituted benzoylisothiocyanates **7–10**. The synthesized compounds were characterized by elemental analysis, FTIR, ^1H NMR and mass spectrum. FTIR spectrum revealed three bands for NH, C=O and C=S at 3448–3110 cm^{-1}, 1790–1628 cm^{-1} and 1150–1045 cm^{-1}, respectively. The ^1H NMR of **11–30** confirmed presence of NHC=O and NHC=S by showing singlet at δ 7.78–9.81 and broad singlet at δ 9.88–13.27, respectively, exchangeable with D_2O. The physical and chemical data for newly synthesized compounds are presented in Table 1.

3. Pharmacology

The synthesized compounds (**11–30**) obtained from the reactional sequence, were injected intraperitoneally into mice and in the maximal electroshock (MES) [17,18], subcutaneous pentylenetetrazole (scPTZ) [19] and neurotoxicity screen [20], using doses of 30, 100, 300 mg/kg and observations carried out at two different time intervals. These data are presented in Table 2. These compounds were then evaluated for their CNS behavioral activity in mice using porsolt's swim pool test [21] in rats, results are presented in Table 2. The selected compounds were evaluated for liver toxicity by enzyme estimation [22–24] and histopathological studies [25]. Data are presented in Table 3 and Fig. 2 and 3.

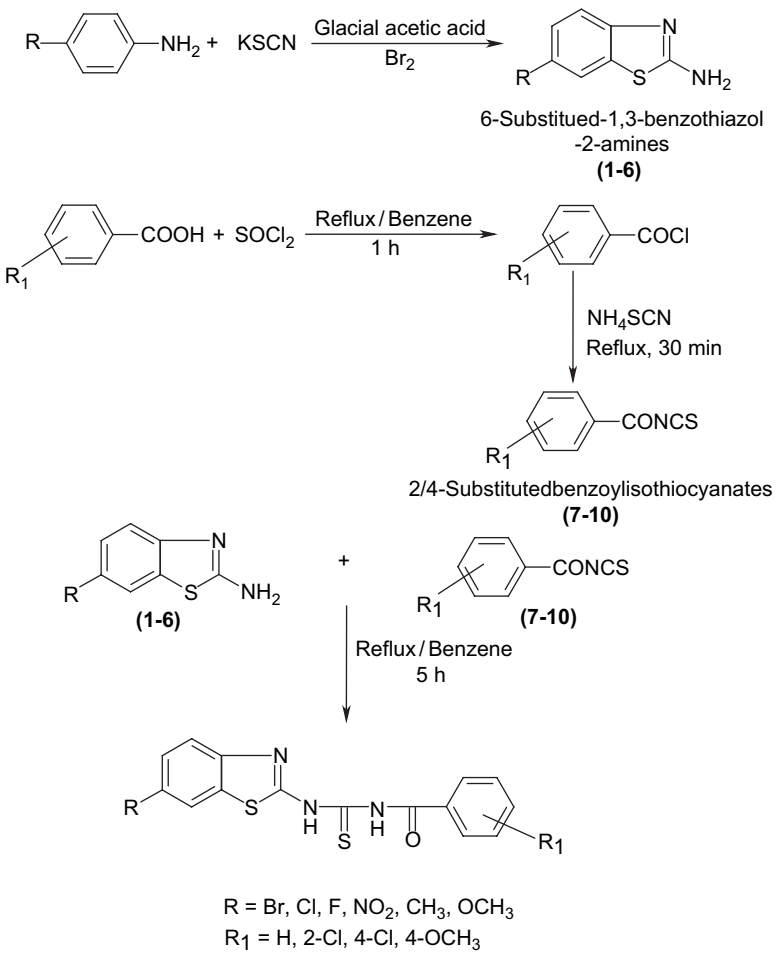
4. Results and discussions

The preliminary evaluation of the synthesized *N*-{[(6-substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-substituted benzamides **11–30** were evaluated for anticonvulsant activity by the use of predictable animal models and the neurotoxicity was checked by rotarod test method. Of these, the MES and scPTZ seizure models represent the two animal seizure models, most widely used in the search for new anticonvulsants. Data is presented in Table 2 after the 0.5 and 4 h time intervals at the dose level of 30, 100 and 300 mg/kg. Phenytoin and carbamazepine were used as the standard drugs for the comparison.

Maximal electroshock seizure (MES) test is a proven method to check the generalized tonic–clonic seizure and identifies clinical candidates that prevent seizure spread. The synthesized compounds **12**, **18**, **19**, **20**, **22**, **24** and **26** had shown activity at the dose level of 30 mg/kg against the seizure spread at both the time intervals except compounds **18**, **20** and **22** which had shown protection at the dose level of 100 mg/kg after 4 h indicating rapid onset but shorter duration of action. Whereas compounds **15**, **21** and **23** had shown protection at both the time intervals 0.5 and 4 h after the dose level of 100 mg/kg, compound **23** had shown activity at 300 mg/kg after the 4 h interval. Compounds **11**, **13**, **14**, **16**, **17**, **25**, **29** and **30** had shown protection at the dose level of 300 mg/kg at both the time intervals. In the scPTZ screen compounds **12**, **19** and **26** had shown activity at 30 mg/kg dose level after both the time intervals except **12**, **26** which had shown activity at the dose of 100 mg/kg at both the time intervals but compounds **11**, **18**, **22** had shown activity at the dose level of 300 mg/kg. Rest of the compounds had activity at the dose level of 300 mg/kg at both the time intervals except compounds **23**, **27–29** which had inactivity. Selected compounds which had shown activity at 30 mg/kg dose level were further tested for their neurotoxicity. None of the compounds showed any sign of neurotoxicity.

Selected compounds which were showing protection against seizures at the dose level of 30 mg/kg in MES and scPTZ screen were further tested for their neurotoxicity. None of the compounds showed any sign of neurotoxicity.

Basic structure of the compounds fulfilled all the pharmacophoric structural requirements like presence of benzothiazole as hydrophobic aryl ring, N as electron donor acceptor system and another hydrophobic aryl ring responsible for metabolism proposed by Pandeya et al. [26] except the presence of hydrophobic domain Fig. 4.



Scheme 1. *N*-{[(6-Substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-substituted benzamides (**11**–**30**).

In general compounds bearing the groups like F, CH_3, OCH_3 at the 6-position of benzothiazole ring with H, 2-Cl, 4-Cl substituted distant phenyl ring showed highly potent activity in MES and scPTZ tests. Whereas replacement with lesser electronegative Br group at the 6-position of benzothiazole ring with 4-OCH_3 substituent at the distant phenyl ring resulted in compounds with decrease in activity to a lesser extent. Presence of substituent like NO_2 at the 6-position of benzothiazole ring, with the unsubstituted distant phenyl ring in compound, showed significant activity whereas substitution with 4-OCH_3 at the distant phenyl ring renders the compound inactive.

Hepatotoxic reactions have contributed to the decline of many therapies. Table 3 shows the liver function tests with reference to the control. Studies reported earlier have shown that phenytoin is a possible cause of acetaminophen hepatotoxicity [27] and anticonvlsants such as carbamazepine [28] and valporic acid [29] were also found to enhance or show heptotoxic side effects. In the present study some selective compounds showing protection at 30 mg/kg in both the tests were administered chronically to animals for 15 days and the biochemical parameters were estimated. The values of alkaline phosphatase, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and total protein suggested that none of the compounds had shown any significant increase or decrease. Thus considering the biochemical parameters estimated to establish liver function test with selected compounds, it was clearly indicated that none of the compounds showed any malfunctioning or toxicity of the liver as compared to control. Further histopathological study of liver confirmed that there is no liver toxicity by showing normal hepatic parenchyma with portal triad, central vein and heptocytes in comparison to control (Figs. 2 and 3).

The forced swim test is a well validated and extensively used screen for compounds with antidepressant activity. Compounds **11**–**30** were also evaluated for their antidepressant activity with the standard fluoxetine at the dose level of 30 mg/kg. Antidepressant activity was assessed as mean average immobility time in seconds and data is presented as mean ± SEM (Table 3). Data was analyzed by using students'*t*' test against the individual untreated controls with the treated compounds. Compounds **13**, **15**–**17**, **20**, **22**, **24** and **30** showed decrease in the immobility time and were highly potent ($p < 0.001$) equivalent to the standard fluoxetine ($p < 0.001$). Compounds **11**, **21**, **23**, **25**, **29** had shown highly significant activity with ($p < 0.01$). Compounds **18** and **19** had shown statistically significant

Table 1
Physico-chemical properties of compounds **11–30**

Compd. no.	R	R_1	Mol. formula (MW)[a]	Yield (%)	[b]Mp. (°C)	R_f[c] (R_m)[d]	Found (calcd.)%		
							C	H	N
11	Cl	H	$C_{15}H_{10}ClN_3OS_2$ (347.84)	80	252	0.71 (−0.38)	51.87 (51.79)	2.96 (2.90)	10.21 (10.19)
12	F	H	$C_{15}H_{10}FN_3OS_2$ (331.38)	82	217	0.88 (−0.86)	54.32 (54.37)	3.06 (3.04)	12.86 (12.68)
13	NO_2	H	$C_{15}H_{10}N_4O_3S_2$ (358.39)	69	295	0.69 (−0.34)	50.47 (50.27)	2.84 (2.81)	15.60 (15.63)
14	CH_3	H	$C_{16}H_{13}N_3OS_2$ (327.42)	68	198	0.81 (−0.62)	58.88 (58.69)	3.97 (4.00)	12.72 (12.83)
15	CH_3O	H	$C_{16}H_{13}N_3O_2S_2$ (343.42)	75	212	0.82 (−0.65)	56.00 (55.96)	3.79 (3.82)	12.20 (12.24)
16	Br	2-Cl	$C_{15}H_9ClBrN_3OS_2$ (426.73)	67	180	0.94 (−1.19)	42.42 (42.22)	2.16 (2.10)	9.81 (9.85)
17	F	2-Cl	$C_{15}H_9Cl_2N_3OS_2$ (382.28)	88	217	0.73 (−0.43)	47.34 (47.13)	2.30 (2.37)	10.95 (10.99)
18	NO_2	2-Cl	$C_{15}H_9ClFN_3OS_2$ (365.83)	75	170	0.87 (−0.82)	49.46 (49.25)	2.58 (2.48)	11.44 (11.49)
19	CH_3	2-Cl	$C_{16}H_{12}ClN_3OS_2$ (361.86)	79	215	0.86 (−0.78)	53.32 (53.11)	3.30 (3.34)	11.56 (11.61)
20	CH_3O	2-Cl	$C_{16}H_{12}ClN_3O_2S_2$ (377.86)	80	175	0.81 (−0.62)	51.01 (50.86)	2.98 (3.20)	11.07 (11.12)
21	Br	4-Cl	$C_{15}H_9ClBrN_3OS_2$ (426.73)	65	187	0.92 (−1.06)	44.20 (42.22)	2.16 (2.13)	10.81 (9.85)
22	F	4-Cl	$C_{15}H_9Cl_2N_3OS_2$ (382.28)	85	195	0.77 (−0.52)	47.34 (47.13)	2.34 (2.37)	11.03 (10.99)
23	NO_2	4-Cl	$C_{15}H_9ClFN_3OS_2$ (365.83)	83	173	0.89 (−0.90)	50.14 (49.25)	2.45 (2.48)	11.79 (11.49)
24	CH_3	4-Cl	$C_{16}H_{12}ClN_3OS_2$ (361.86)	80	213	0.88 (−0.86)	53.17 (53.11)	3.37 (3.34)	11.66 (11. 61)
25	CH_3O	4-Cl	$C_{16}H_{12}Cl\ N_3O_2S_2$ (377.86)	78	178	0.85 (−0.75)	51.08 (50.86)	3.15 (3.20)	11.17 (11.12)
26	Br	CH_3O	$C_{16}H_{12}ClN_3O_2S_2$ (377.86)	75	203	0.69 (−0.34)	51.07 (50.80)	3.19 (3.20)	11.08 (11.12)
27	F	CH_3O	$C_{16}H_{12}FN_3O_2S_2$ (361.41)	73	157	0.91 (−1.00)	53.27 (53.17)	3.85 (3.35)	11.02 (11.63)
28	NO_2	CH_3O	$C_{16}H_{12}N_4O_4S_2$ (388.42)	61	257	0.90 (−0.95)	49.69 (49.47)	3.15 (3.11)	14.38 (14.42)
29	CH_3	CH_3O	$C_{17}H_{15}N_3O_2S_2$ (357.44)	70	214	0.83 (−0.68)	57.32 (57.12)	4.13 (4.23)	11.70 (11.76)
30	CH_3O	CH_3O	$C_{17}H_{15}N_3O_3S_2$ (373.44)	71	162	0.93 (−1.12)	54.88 (54.67)	4.06 (4.05)	11.20 (11.25)

[a] Solvent of crystallization: benzene.
[b] Melting point of the compounds at their decomposition.
[c] Solvent system T:E:F (5:4:1).
[d] A logarithmic function of R_f value was also calculated; $R_m = \log (1 - 1/R_f)$.

results ($p < 0.05$), whereas remaining compounds were devoid of activity. Compounds with substitution with Br, Cl, F, NO_2, CH_3 and OCH_3 at the 6-position of benzothiazole and distant phenyl ring substituted with H, 2-Cl, 4-Cl, 4-OCH_3 were highly potent.

From the present study it was concluded that all the pharmacophoric elements were present in the titled compounds; although the HBD is not in accordance of the proposed pharmacophore model (Fig. 4). All the compounds except **23**, **27–29** showed protection in the two animal models of seizures. Compounds **12**, **19**, **26** emerged as anticonvulsants with no neurotoxicity and can be claimed to detect compounds possessing against generalized tonic–clonic (grand mal) and generalized absence (petit mal) seizures, respectively. Different substitutions at the distal aryl ring with halogens and alkyl groups resulted in variations like increase and decrease in the activity. In the toxicity studies none of the compounds showed neurotoxicity or liver toxicity. In CNS depressant study, most of the compounds showed the decrease in the immobility time. Dose used for anticonvulsant and CNS depressant activity are 30 mg/kg, but we cannot suggest the mechanism of action involved for both the activities effect. However, to confirm this fact, further studies are required to be carried out.

5. Experimental protocols

5.1. Chemistry

The melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. The homogen elemental analyses (C, H, N) of all compounds were performed on the CHNS Elimentar (Analysen systime, GmbH) Germany Vario EL III. All the Fourier transform infra red (FTIR) spectra were recorded in KBr pellets on a Jasco FT/IR 410 spectrometer. The ^{1}H NMR spectra were taken on a Bruker model dpx 300 (300 MHz) NMR spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethyl silane (TMS) as an internal standard. Mass spectra were measured on a Brooker Ion trap (Esquire 3000) mass spectrometer from Regional Research Laboratory (RRL), Jammu, India.

The homogeneity of the compounds was checked by thin layer chromatography (TLC) on silica gel G (Merck) coated plates 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.

5.1.1. 6-Substituted-1,3-benzothiazol-2-amine (*1–6*)

A mixture of aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid (10%) was cooled and stirred. To this solution bromine (0.01 mol) was added dropwise at such a rate to keep the temperature below 10 °C throughout the addition. Stirring was continued for an 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%), filtered, washed with water and dried, recrystallized with benzene to obtain 6-substituted-1,3-benzothiazol-2-amine.

*5.1.1.1. 6-Chloro-1,3-benzothiazol-2-amine (**1**).* Yield 74%; IR: ν_{max} (cm^{-1}) 3490 (NH), 1580 (C=N); ^{1}H NMR

Table 2
Anticonvulsant, neurotoxicity screening and CNS depression study of compounds **11–30**

Compd. no	Intraperitoneal injection in mice[a]						Mean average immobility time (s)[c]	
	MES screen		scPTZ		NT[b]		Mean ± SEM	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	Untreated	Treated
11	300	300	100	300	X	X	42.0 ± 1.8	29.0 ± 1.9**
12	30	30	30	100	(−)	(−)	34.4 ± 1.4	24.0 ± 1.6
13	300	300	300	300	X	X	34.0 ± 1.6	14.8 ± 1.5***
14	300	300	300	300	X	X	35.8 ± 1.6	29.0 ± 1.6
15	100	100	300	300	X	X	41.0 ± 2.1	19.8 ± 1.8***
16	300	300	300	300	X	X	42.0 ± 1.2	22.6 ± 1.0***
17	300	300	300	300	X	X	49.0 ± 1.2	32.6 ± 1.5***
18	30	100	100	300	(−)	(−)	34.6 ± 1.6	25.0 ± 1.4*
19	30	30	30	30	(−)	(−)	58.2 ± 1.8	40.4 ± 1.5*
20	30	100	100	100	(−)	(−)	50.6 ± 1.3	42.6 ± 2.4***
21	100	100	300	300	X	X	48.2 ± 2.5	38.0 ± 2.3**
22	30	100	100	300	(−)	(−)	39.8 ± 1.3	20.2 ± 1.5***
23	100	300	–	–	X	X	39.8 ± 1.3	24.6 ± 1.4**
24	30	100	100	100	(−)	(−)	47.0 ± 1.0	30.0 ± 1.5***
25	300	300	300	300	X	X	55.0 ± 1.9	43.0 ± 1.7**
26	30	30	30	100	(−)	(−)	40.8 ± 2.5	34.2 ± 2.6
27	–	–	–	–	X	X	31.4 ± 1.3	31.2 ± 1.2
28	–	–	–	–	X	X	28.8 ± 0.9	27.8 ± 0.7
29	300	300	–	–	X	X	26.2 ± 1.7	10.2 ± 1.1**
30	300	300	300	300	X	X	23.2 ± 0.8	16.8 ± 0.9***
Phenytoin	30	30	–	–	(−)	(−)	–	–
Carbamazepine	30	100	100	100	(−)	(−)	–	–
Fluoxetine	29.6 ± 0.6	14.2 ± 0.9***	–	–	–	–	–	–

$n = 6$.
[a] Doses of 30, 100 and 300 mg/kg were administered. The data indicates the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4 h.
[b] NT indicates neurotoxicity screening using rotorod test. A dash (−) indicates an absence of activity. X denotes not tested.
[c] Dose = 30 mg/kg (p.o); $p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$. Data was analyzed by unpaired students '*t*' test.

(DMSO-d_6): (δ, ppm) 6.12 (s, 2H, NH_2, D_2O exchangeable), 6.79–7.13 (m, 3H, Ar–H).

*5.1.1.2. 6-Fluoro-1,3-benzothiazol-2-amine (**2**).* Yield 81%; IR: ν_{max} (cm^{-1}) 3318 (NH), 1568 (C=N); ^1H NMR (DMSO-d_6): (δ, ppm) 6.73–7.06 (m, 2H, Ar–H), 7.21 (s, 2H, NH_2, D_2O exchangeable), 7.30 (m, 1H, Ar–H).

*5.1.1.3. 6-Nitro-1,3-benzothiazol-2-amine (**3**).* Yield 77%; IR: ν_{max} (cm^{-1}) 3318 (NH), 1568 (C=N); ^1H NMR (DMSO-d_6): (δ, ppm) 6.30 (s, 2H, NH_2, D_2O exchangeable), 7.17–8.21 (m, 3H, Ar–H).

*5.1.1.4. 6-Methyl-1,3-benzothiazol-2-amine (**4**).* Yield 80%; IR: ν_{max} (cm^{-1}) 3240 (NH), 1520 (C=N); ^1H NMR (DMSO-d_6): (δ, ppm) 2.05 (s, 3H, CH_3), 5.21 (s, 2H, NH_2, D_2O exchangeable), 6.63–6.94 (m, 3H, Ar–H).

*5.1.1.5. 6-Methoxy-1,3-benzothiazol-2-amine (**5**).* Yield 78%; IR: ν_{max} (cm^{-1}) 3300 (NH), 1583 (C=N); ^1H NMR

Table 3
Enzyme estimation of the selected compounds

Treatment	Alkaline phosphatase ± SEM[a, ns]	SGOT ± SEM[b, ns]	SGPT ± SEM[c, ns]	Total Protein (g/100 ml) ± SEM[d, ns]
Control	13.06 ± 0.25	148.6 ± 1.50	27.67 ± 0.84	1.80 ± 0.01
12	13.64 ± 0.06	149.3 ± 1.23	27.89 ± 0.71	1.83 ± 0.03
18	13.30 ± 0.03	148.0 ± 0.45	29.67 ± 0.60	1.71 ± 0.03
19	13.96 ± 0.07	147.9 ± 0.02	29.33 ± 0.61	1.98 ± 0.75
20	13.04 ± 0.09	148.6 ± 0.08	27.98 ± 0.34	1.90 ± 0.32
22	12.98 ± 1.00	147.3 ± 0.01	27.81 ± 0.62	1.88 ± 0.23
24	13.48 ± 0.20	149.1 ± 0.02	27.48 ± 0.45	1.79 ± 0.08
26	13.53 ± 0.38	146.8 ± 0.40	28.49 ± 0.08	1.81 ± 0.30

ns denotes non-significant. Results given as mean ± SEM were calculated using ANOVA followed by Dunnett's comparison test.
[a] $P = 0.6382$.
[b] $P = 0.2133$.
[c] $P = 0.0847$.
[d] $P = 0.9995$.

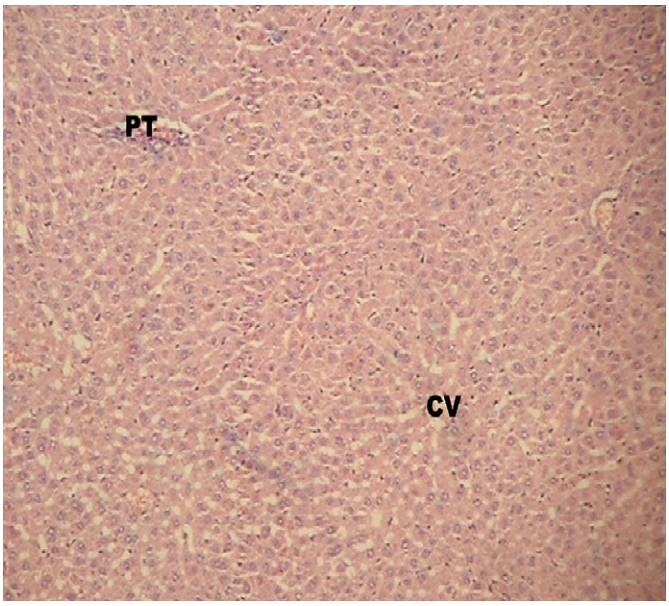


Fig. 2. Section of liver. Group: control, interference: normal hepatic parenchyma with portal triad (PT), central vein (CV). Magnification: 100×.

(DMSO-d_6): (δ, ppm) 3.81 (s, 3H, OCH_3), 5.33 (s, 2H, NH_2, D_2O exchangeable), 6.66–7.07 (m, 3H, Ar–H).

*5.1.1.6. 6-Bromo-1,3-benzothiazol-2-amine (**6**).* Yield 82%; IR: ν_{max} (cm^{-1}) 3210 (NH), 1560 (C=N); ^1H NMR (DMSO-d_6): (δ, ppm) 6.03 (s, 2H, NH_2, D_2O exchangeable), 6.99–7.33 (m, 3H, Ar–H).

*5.1.2. 2/4-Substituted benzoylisothiocyanates (**7–10**)*

Substituted benzoic acid (0.1 mol) and thionyl chloride (0.1 mol) were refluxed in benzene (50 ml) for one hour.

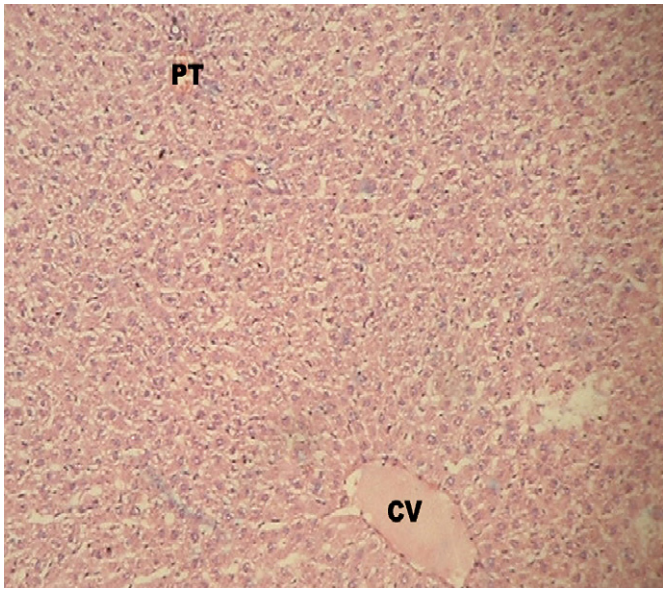


Fig. 3. Section of liver. Group: 2, interference: normal hepatic parenchyma with portal triad (PT), central vein (CV). Magnification: 100×.

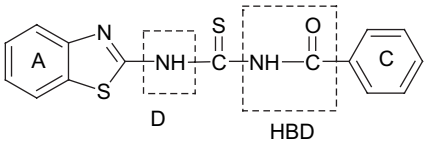


Fig. 4. Showing A: hydrophobic aryl ring, C: distal hydrophobic domain, D: electron donor domain, HBD: Hydrogen bond acceptor/donor moiety in basic structure of titled compounds **11–30**.

The reaction mixture upon filtration yielded substituted benzoyl chloride as a plummy liquid.

Ammonium thiocyanate (0.1 mol) was added to substituted benzoyl chloride (0.1 mol) and refluxed for 30 min in benzene. The resultant mixture was filtered and substituted benzoyl isothiocyanates (**7–10**) were obtained in the form of liquid and ammonium chloride as a residue. Ammonium chloride was removed by filtration as a residue from the mixture.

*5.1.2.1. Benzoyl isothiocyanate (**7**).* Yield 68%; IR: ν_{max} (cm^{-1}) 2154 (N–C–S), 1628 (C=O); ^1H NMR (DMSO-d_6): (δ, ppm) 7.48–8.01 (dd, 5H, Ar–H).

*5.1.2.2. 2-Chlorobenzoyl isothiocyanate (**8**).* Yield 62%; IR: ν_{max} (cm^{-1}) 2100 (N–C–S), 1650 (C=O), 840 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.30–7.89 (dd, 4H, Ar–H).

*5.1.2.3. 4-Chlorobenzoyl isothiocyanate (**9**).* Yield 60%; IR: ν_{max} (cm^{-1}) 2250 (N–C–S), 1670 (C=O), 836 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.38–7.95 (dd, 4H, Ar–H).

*5.1.2.4. 4-Methoxybenzoyl isothiocyanate (**10**).* Yield 66%; IR: ν_{max} (cm^{-1}) 2180 (N–C–S), 1634 (C=O); ^1H NMR (DMSO-d_6): (δ, ppm) 3.63 (s, 3H, OCH_3), 7.20–7.79 (dd, 4H, Ar–H).

*5.1.3. N-{[(6-Substituted-1,3-benzothiazol-2-yl)amino]-carbonothioyl}-2/4-substituted benzamides (**11–30**)*

The final compounds **11–30** were obtained when 6-substituted benzothiazoles **1–6** (0.02 mol) and substituted benzoyl isothiocyanate **7–10** (0.02 mol) were refluxed in benzene for 5 h, solid material obtained was filtered and recrystallized from the benzene. The spectral data of **11–30** are given below. The physico-chemical properties compounds are presented in Table 1.

*5.1.3.1. N-{[(6-Chloro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**11**).* IR: ν_{max} (cm^{-1}) 3379, 3180 (NH), 3072 (CH–Ar), 1673 (C=O), 1551 (C=N), 1090 (C=S), 803 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.47–7.95 (m, 8H, Ar–H), 8.17 (s, 1H, NHC=O, D_2O exchangeable), 12.98 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.2. N-{[(6-Fluoro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**12**).* IR: ν_{max} (cm^{-1}) 3290, 3185 (NH), 3070 (CH–Ar), 1674 (C=O), 1562 (C=N), 1321 (C–F) 1092 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 7.32–7.96 (m,

8H, Ar–H), 8.14 (s, 1H, NHC=O, D_2O exchangeable), 12.80 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 331 (M^+).

*5.1.3.3. N-{[(6-Nitro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**13**).* IR: ν_{max} (cm^{-1}) 3223, 3174 (NH), 3083 (CH–Ar), 1684 (C=O), 1556 (C=N), 1349 (C–NO_2), 1128 (C=S), 806 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.36–8.33 (m, 8H, Ar–H), 9.09 (s, 1H, NHC=O, D_2O exchangeable), 13.27 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 358 (M^+).

*5.1.3.4. N-{[(6-Methyl-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**14**).* IR: ν_{max} (cm^{-1}) 3357, 3185 (NH), 3056 (CH–Ar), 2921 (CH–Aliph), 1676 (C=O), 1509 (C=N), 1466 (CH_3), 1090 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 2.43 (s, 3H, CH_3), 7.14–7.96 (m, 8H, Ar–H), 9.84 (s, 1H, NHC=O, D_2O exchangeable),12.81 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 327 (M^+).

*5.1.3.5. N-{[(6-Methoxy-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**15**).* IR: ν_{max} (cm^{-1}) 3270, 3110 (NH), 3070 (CH–Ar), 2930 (CH–Aliph), 1670 (C=O), 1545 (C=N), 1085 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 3.82 (s, 3H, OCH_3), 7.05–7.96 (m, 8H, Ar–H), 8.13 (s, 1H, NHC=O, D_2O exchangeable), 12.81(bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 343 (M^+).

*5.1.3.6. N-{[(6-Bromo-1,3-benzothiazol-2-yl)amino]carbonothioyl}-2-chlorobenzamide (**16**).* IR: ν_{max} (cm^{-1}) 3290, 3280 (NH), 3062 (CH–Ar), 1690 (C=O), 1583 (C=N), 1048 (C=S), 802 (C–Cl), 545 (C–Br); ^1H NMR ($CDCl_3$): (δ, ppm) 7.32–7.87 (m, 7H, Ar–H), 9.19 (s, 1H, NHC=O, D_2O exchangeable), 11.78 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.7. 2-Chloro-N-{[(6-chloro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**17**).* IR: ν_{max} (cm^{-1}) 3310, 3298 (NH), 3060 (CH–Ar), 1690 (C=O), 1599 (C=N), 1049 (C=S), 808 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.46–7.79 (m, 7H, Ar–H), 8.14 (s, 1H, NHC=O, D_2O exchangeable), 12.92 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.8. 2-Chloro-N-{[(6-fluoro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**18**).* IR: ν_{max} (cm^{-1}) 3249, 3135 (NH), 3100 (CH–Ar), 1638 (C=O), 1548 (C=N), 1045 (C=S), 801 (C–Cl), 1300 (C–F); ^1H NMR (DMSO-d_6): (δ, ppm) 7.30–7.81 (m, 7H, Ar–H), 7.97 (s, 1H, NHC=O, D_2O exchangeable), 13.02 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 365 (M^+).

*5.1.3.9. 2-Chloro-N-{[(6-methyl-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**19**).* IR: ν_{max} (cm^{-1}) 3300, 3170 (NH), 3045 (CH–Ar), 2970 (CH–Aliph), 1650 (C=O), 1556 (C=N), 1433 (CH_3), 1126 (C=S), 815 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 2.45 (s, 3H, CH_3), 7.15–7.68 (m, 7H, Ar–H), 7.80 (s, 1H, NHC=O, D_2O exchangeable), 12.71 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.10. 2-Chloro-N-{[(6-methoxy-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**20**).* IR: ν_{max} (cm^{-1}) 3363, 3190 (NH), 3040 (CH–Ar), 2940 (CH–Aliph), 1700 (C=O), 1460 (OCH_3), 1578 (C=N), 1050 (C=S), 835 (C–Cl); ^1H NMR(DMSO-d_6): (δ, ppm) 3.85 (s, 3H, OCH_3), 7.06–7.69 (m, 7H, Ar–H), 7.78 (s, 1H, NHC=O, D_2O exchangeable), 12.61 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 377 (M^+).

*5.1.3.11. N-{[(6-Bromo-1,3-benzothiazol-2-yl)amino]carbonothioyl}-4-chlorobenzamide (**21**).* IR: ν_{max} (cm^{-1}) 3390, 3210 (NH), 3075 (CH–Ar) 1790 (C=O), 1555 (C=N), 1150 (C=S), 812 (C–Cl), 563 (C–Br); ^1H NMR ($CDCl_3$): (δ, ppm) 7.36–8.16 (m, 7H, Ar–H), 8.42 (s, 1H, NHC=O, D_2O exchangeable), 12.73 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 426 (M^+).

*5.1.3.12. 4-Chloro-N-{[(6-chloro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**22**).* IR: ν_{max} (cm^{-1}) 3239, 3177 (NH), 3077 (CH–Ar), 1673 (C=O), 1562 (C=N), 1055 (C=S), 804 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 7.37–7.99 (m, 7H, Ar–H), 8.17 (s, 1H, NHC=O, D_2O exchangeable), 12.61 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.13. 4-Chloro-N-{[(6-fluoro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**23**).* IR: ν_{max} (cm^{-1}) 3285, 3172 (NH), 3072 (CH–Ar), 1675 (C=O), 1562 (C=N), 1322 (C–F), 1095 (C=S), 802 (C–Cl); ^1H NMR (DMSO-d_6): (δ,ppm) 7.28–8.17 (m, 7H, Ar–H), 8.36 (s, 1H, NHC=O, D_2O exchangeable), 12.81 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.14. 4-Chloro-N-{[(6-methyl-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**24**).* IR: ν_{max} (cm^{-1}) 3390, 3205 (NH), 3081 (CH–Ar), 2950 (CH–Aliph), 1675 (C=O), 1560 (C=N), 1094 (C=S), 806 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 2.27 (s, 3H, CH_3), 7.36–7.80 (m, 7H, Ar–H), 8.19 (s, 1H, NHC=O, D_2O exchangeable), 13.07 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.15. 4-Chloro-N-{[(6-methoxy-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**25**).* IR: ν_{max} (cm^{-1}) 3448, 3174 (NH), 3094 (CH–Ar), 2932 (CH–Aliph) 1675 (C=O), 1574 (C=N), 1091 (C=S), 805 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm)3.82 (s, 3H, OCH_3), 7.36–8.15 (m, 7H, Ar–H), 8.31 (s, 1H, NHC=O, D_2O exchangeable), 12.90 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.16. N-{[(6-Chloro-1,3-benzothiazol-2-yl)amino]carbonothioyl}-4-methoxybenzamide (**26**).* IR: ν_{max} (cm^{-1}) 3271, 3228 (NH), 3093 (CH–Ar), 2932 (CH–Aliph), 1634 (C=O), 1533 (C=N), 1049 (C=S), 812 (C–Cl); ^1H NMR (DMSO-d_6): (δ, ppm) 3.38 (s, 3H, OCH_3), 7.20–7.77 (m, 7H, Ar–H),

8.34 (s, 1H, NHC=O, D_2O exchangeable), 12.39 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.17. N-{[(6-Fluoro-1,3-benzothiazol-2-yl)amino]carbonothioyl}-4-methoxybenzamide (**27**).* IR: ν_{max} (cm^{-1}) 3271, 3185 (NH), 3069 (CH–Ar), 2925 (CH–Aliph), 1687 (C=O), 1561 (C=N), 1303 (C–F), 1058 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 3.80 (s, 3H, OCH_3), 7.09–7.58 (m, 7H, Ar–H), 9.65 (s, 1H, NHC=O, D_2O exchangeable), 9.88 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.18. 4-Methoxy-N-{[(6-nitro-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**28**).* IR: ν_{max} (cm^{-1}) 3411, 3287 (NH), 3099 (CH–Ar), 2916 (CH–Aliph), 1674 (C=O), 1561 (C=N), 1325 (NO_2), 1125 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 3.58(s, 3H, OCH_3), 7.40–8.25 (m, 7H, Ar–H), 8.69 (s, 1H, NHC=O, D_2O exchangeable), 12.46 (bs, 1H, NHC=S, D_2O exchangeable).

*5.1.3.19. 4-Methoxy-N-{[(6-methyl-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**29**).* IR: ν_{max} (cm^{-1}) 3400, 3255 (NH), 3126 (CH–Ar), 2943 (CH–Aliph), 1674 (C=O), 1544 (C=N), 1464 (CH_3), 1099 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 2.30 (s, 3H, CH_3), 3.44 (s, 3H, OCH_3), 6.99–7.43 (m, 7H, Ar–H), 9.81 (s, 1H, NHC=O, D_2O exchangeable), 12.79 (bs, 1H, NHC=S, D_2O exchangeable); mass (EI) *m/z*: 357 (M^+).

*5.1.3.20. 4-Methoxy-N-{[(6-methoxy-1,3-benzothiazol-2-yl)amino]carbonothioyl}benzamide (**30**).* IR: ν_{max} (cm^{-1}) 3391, 3298 (NH), 3103 (CH–Ar), 2943 (CH–Aliph), 1641 (C=O), 1550 (C=N), 1054 (C=S); ^1H NMR (DMSO-d_6): (δ, ppm) 3.73 (s, 6H, $2OCH_3$), 6.79–7.61 (m, 7H, Ar–H), 8.46 (s, 1H, NHC=O, D_2O exchangeable), 12.40 (bs, 1H, NHC=S, D_2O exchangeable).

5.2. Pharmacology

The anticonvulsant evaluations were undertaken by the National Institute of Health, using their reported procedures. Male albino mice (CF-1 strain, 25–30 g) and male rats (Sprague-dawley, 100–150 g) were used as experimental animals. The test compounds and standard drug were administered intraperitoneally suspended in Tween 80 (1%) or in 0.5% methyl cellulose–water mixture.

5.2.1. Anticonvulsant screening

Initially all compounds were administered i.p. at doses of 30, 100, 300 mg/kg to one to four mice. Activity was established using the MES, scPTZ tests.

Maximal electroshock test: maximal electroshock seizure were elicited with a 60 cycle altering current of 50 mA intensity delivered for 0.25 s via ear clip electrodes. The maximal seizure typically consists of a short period of tonic extension of the hind limbs and a final clonic episode. Abolition of the hind limb tonic extensor component of the seizure is defined as protection.

Subcutaneous pentylenetetrazole seizure threshold test: scPTZ was conducted by administering PTZ dissolved in 0.9% sodium chloride solution in the posterior midline of the animals. A minimal time of 30 min subsequent to sc administration of PTZ was used for seizure detection. Protection was referred to as the failure to observe an episode of clonic spasms of at least 5 s duration during this time period.

5.2.2. Neurotoxicity screening

The rotorod test was used to evaluate neurotoxicity. The animal was placed on a 3.2 cm diameter knurled rod rotating at 6 rpm. Normal mice can remain on a rod rotating at this speed indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min. Results are expressed as number of animals exhibiting toxicity/number of animals tested.

5.2.3. CNS depressant study

The forced swim pool method described earlier was followed, Wistar rats were placed in a chamber (diameter: 45 cm, height: 20 cm) containing water up to a height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted, an initial attempt of 15 min pre-test, followed by 5 min test session 24 h later. The animals were administered an i.p. injection (30 mg/kg) of the test compounds 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which are necessary to keep its head above the surface of water) during the 5 min test period were measured.

5.2.4. Assessment of liver function

The animals were divided into groups of six, and the control group received a basal diet and vehicle. Other groups were administered the test drug in a dose of 30 mg/kg/day po (in methylcellulose) for two weeks. After the stipulated period, each animal was anesthetized by anesthetic ether, and blood was collected from the liver to assess the biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate, total protein and total albumin were measured according to the reported methods.

5.2.5. Histopathological study of liver

The histopathological studies were carried out by a reported method. The rats were scarified under light ether anesthesia after 24 h of last dosage, the livers were removed and washed with normal saline. Small pieces of liver tissue were processed and embedded in paraffin wax. Sections of 5–6 μm in thickness were cut, stained with haematoxylin and eosin, and then studied under an electron microscope.

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