Short communication

Anticonvulsant and Toxicity Evaluation of Newly Synthesized 1-[2-(3,4-disubstituted phenyl) -3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted -1,3-benzothiazol-2-yl)ureas

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Abstract

A number of new 1-[2-(3,4-disubstituted phenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)urea compounds (**5a-t**) were synthesized and evaluated for their anticonvulsant, hepatotoxic and neurotoxic properties. The titled compounds (**5a-t**) were obtained by cyclization of 3,4-disubstituted benzaldehyde-*N*-(6-substituted-1,3-benzothiazol-2-yl)semicarbazones (**4a-t**) in presence of DMF/triethylamine and chloroacetylchloride. All the newly synthesized compounds were screened for their anticonvulsant activity in i.p. Maximal Electroshock Seizure (MES) model and were compared with the standard drug phenytoin. Interestingly, compounds **5f**, **5n**, and **5p** exhibited 100% protection in the MES test. In the neurotoxicity and hepatotoxicity screening, all the compounds were devoid of toxicity at the dose of 30 mg/kg body weight. The study showed that introduction of F, CH_3 at the 6- position of benzothiazole moiety with H, OCH_3 at the 3-position and OH, OCH_3 at 4-position of the distant phenyl ring led to increased activity. Introduction of F, NO_2, CH_3, OCH_3 substituents at the 6-position of the benzothiazole moiety and unsubstituted distant phenyl ring showed moderate decrease in activity.

Keywords: Azetidine, benzothiazole, maximal electroshock seizure test, anticonvulsant activity, neurotoxicity, hepatotoxicity

1. Introduction

Epilepsy is a brain function disorder characterized by recurrent seizures that has a sudden onset. It was assumed for many years that epilepsy could be treated with just one drug but it is now apparent that is not the case as more than one mechanism may be responsible for various types of seizures. Seizures remain uncontrolled in at least 30% of all epilepsies, even when adequate AED therapy is administered. During recent years, a large number of new AEDs have been marketed worldwide, but the proportion of patients failing to respond to drug treatment has not been changed in a significant extent.

Drugs clinically active against epilepsy include derivatives with common structural characteristics such as nitrogen heterocyclic system with a carbonyl group and an aromatic or heteroaromatic nucleus linked to the heterocyclic system. Benzothiazole derivatives in recent years have acquired conspicuous significance due to their wide spectrum of biological activities. Although they have been known from long ago to be biologically active[1-3] their varied biological features are still of great scientific interest nowadays. In the search of new anticonvulsant agents ha-

ving different substituted benzothiazole nucleus were already reported and all the compounds were found to possess significant activities.[4–6] Since the benzothiazole moiety resembles with the benzisoxazole moiety present in the currently used drug zonisamide, it may have been acting through the inhibition of the sodium channel.

In our previous research we have reported[7–11] several benzfused five membered heterocyclic compounds that have shown considerable anticonvulsant activity.

In the present investigation we have synthesized 1-[2-(3,4-disubstitutedphenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)ureas (Scheme-1). The compounds were evaluated *in vivo* for anticonvulsant activity by MES test and neurotoxicity by rotorod method.

2. Experimental

2. 1. Chemistry

All the solvents were of LR 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). The spots were developed in iodine chamber and visualized with an ultraviolet lamp. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS 135) WIN-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker model DPX 300 FT- NMR spectrometer in (DMSO-d_6) using tetramethylsilane (Me_4Si) TMS as an internal standard. The chemical shifts are recorded in δ ppm scale.

2. 1. 1. Synthesis of 1-[2-(3,4-disubstitutedphenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)ureas (5a-t)

Step I: 6-Substituted-1,3-benzothiazol-2-amines (1a-e)

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 additional 3 h. 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-e**).

Step II: 1-(Substituted -1,3-benzothiazol-2-yl)ureas (2a-e)

To the solution of sodium cyanate (0.01 mol) in minimum quantity of water, glacial acetic acid (5 mL) was added. This solution was heated with 2-amino-6-substituted benzothiazoles (**1a-e**, 0.01 mol) previously dissolved in alcohol, till the contents of mixture become turbid and volume remained half of the original volume. The content was poured on crushed ice. The solid obtained was filtered off and dried.

Step III: *N*-(substituted -1,3-benzothiazol-2-yl)hydrazinecarboxamides (3a-e)

To the warm hydrazine hydrate solution of compounds (**2a-e**) in alcohol, conc. NaOH was added and refluxed for 6 h. Reaction mixture cooled to room temperature and was poured to crushed ice to afford a solid which was filtered and recrystallized from ethanol.

Step IV: 3,4-(Disubstituted benzaldehyde)-*N*-(6-substituted-1,3-benzothiazol-2-yl)semicarbazones (4a-t)

The solution of compounds (**3a-e**, 0.1 mol) in glacial acetic acid (5 mL) and ethanol (10 mL) was heated to boiling and refluxed with appropriate aromatic aldehydes (0.1 mol) for 5 h. The reaction mixture was cooled to room temperature and kept overnight. The solid separated was collected out, washed with methanol, dried and recrystallized from ethanol to get the pure compound.

Step V: 1-[2-(3,4-Disubstitutedphenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)ureas (5a-t)

A solution of compound (**4a-t**, 0.1 mol) in DMF (40 mL) and triethylamine (0.1 mol) was stirred well at 0–5 °C. To this solution chloroacetyl chloride (0.2 mol) was added dropwise at the same temperature. The reaction mixture was stirred for 4 h and the separated amine hydrochloride was filtered off. The filtrate was refluxed for 2 h and the separated solid was recrystallized from methanol.

2. 2. Pharmacology

Male albino mice (Swiss, 25–30 g) were used in groups of six each as experimental animals. All the test compounds and standard drug were administered intraperitoneally suspended in polyethylene glycol (PEG). 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 cages for testing. The animals were maintained at room temperature (25 ± 2 °C). All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC). Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-110062. Registration number and date of registration of Animal House Facility is (173/CPCSEA, 28, JAN-2000).

2. 2. 1. Anticonvulsant Activity

Electroshock-induced seizures (MES test)[12, 13]

Each compound was administered as an i.p. injec-

tion at dose level of 30 mg/kg body weight and the anticonvulsant activity was assessed after 0.5 h and 4 h intervals of 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 consists 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 the drug treatment is taken as the end point.

2. 2. 2. Neurotoxic Effects

Rota-rod test. Minimal motor impairment was measured in mice by the rotorod test.[14] The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions/min. The rod diameter was 3.2 cm. Trained animals were given i.p. injection 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.

2. 2. 3. Histopathological Studies

The selected compounds **5f** and **5n** were evaluated for their histopathological study. The Luna's technique[15] was used to assess the liver of mice, which were administered with test compounds at the dose level of 30 mg/kg body weight for 15 days; comparison was done with the control group. Microphotographs of section of liver were taken at the magnification of 100X and 400X. The microphotographs of section of liver of mice administered with compound **5f** and **5n** along with control are presented in Fig. 1-3.

2. 2. 4. Log P Determination

The desired Log P value depends on the nature of the compounds and the testing system. Log P approximately equal to 2.0 is expected to be the best predictor for CNS activity.[16] In this study, we attempted to correlate the anticonvulsant activity with 100% protection against the seizure spread in anti-MES screen, with their calculated log P values (CLOGP). The Log P values were determined for compounds **5f**, **5n** and **5p**. The experimental log P values were determined using the octanol-water method[17] and the CLOGP values were calculated from ACD free ware version 7.1.

2. 2. 5. Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST)

It is mitochondrial enzyme present in large quantities in the liver, heart, skeletal muscles and kidneys, which gets released from the damaged cells when the tissues are destructed. It was estimated using Rietman and Frankel's method.[18–20]

2. 2. 6. Estimation of Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT)

It is cytosolic enzyme present abundantly in liver cells. The serum levels of ALT are elevated in liver diseases. 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 method.[18-20]

2. 2. 7. Estimation of Alkaline Phosphatase

Alkaline phosphatases are enzymes, which catalyze the removal of phosphate group from monophosphate esters under alkaline conditions. This reaction is of considerable importance in several liver diseases. It was determined by the method reported previously.[21]

2. 2. 8. Estimation of total proteins and albumin

Determination of the proteins provides most useful information in chronic liver diseases. It was determined by using Biuret method.[22, 23]

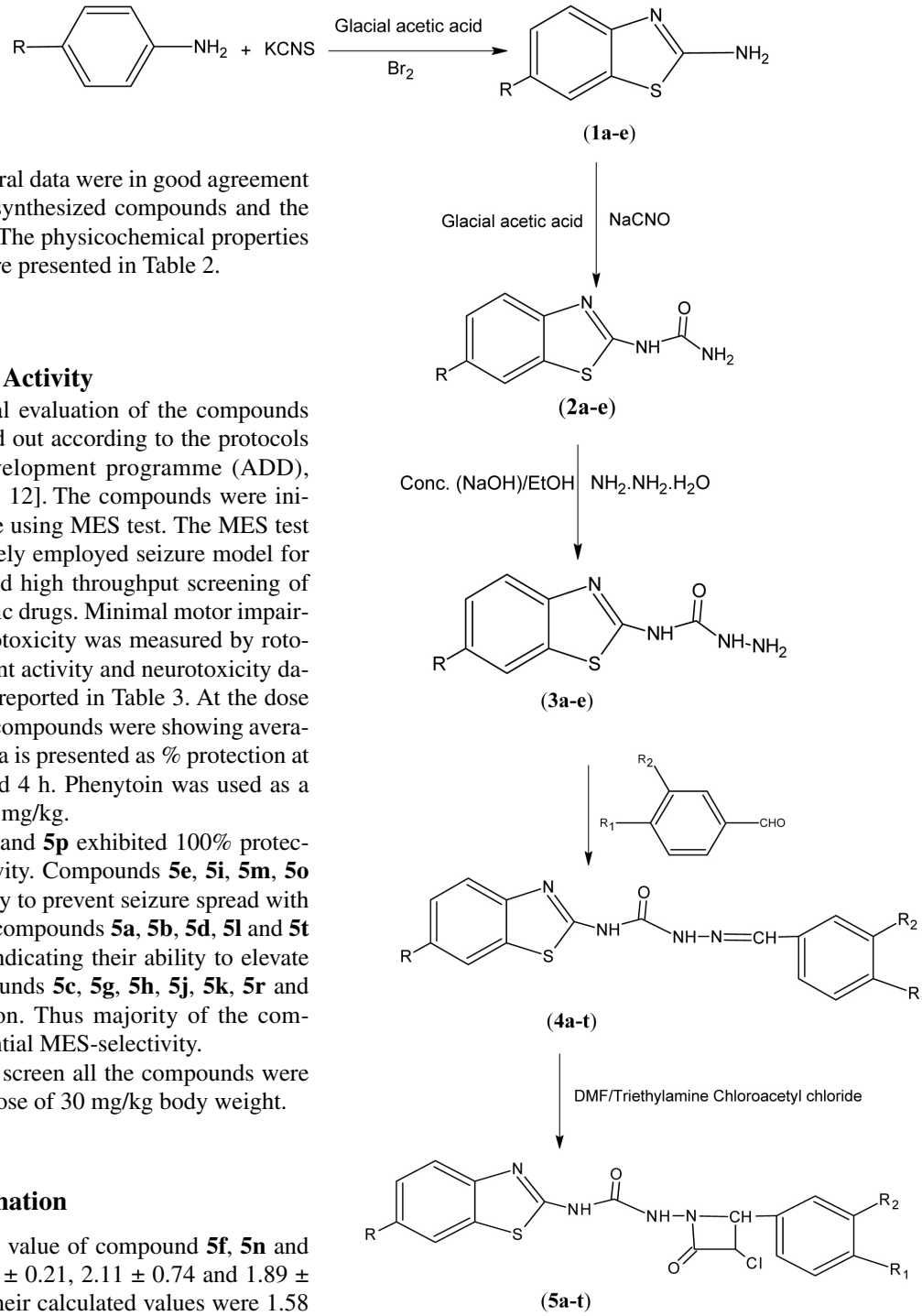
2. 2. 9. Statistical Analysis

All the statistical analyses were carried out using the software SigmaStat 4.0 using ANOVA followed by dunnet's multiple comparison tests and the results are expressed in Mean ± SEM.

3. Results and Discussion

3. 1. Chemistry

The synthesis of 1-[2-(3,4-disubstituted phenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)ureas (**5a-t**) was accomplished as presented in Scheme-1. It involves the cyclization of 3, 4-disubstituted benzaldehyde-*N*-(6-substituted-1,3-benzothiazol-2-yl)semicarbazones (**4a-t**) in the presence of DMF/ triethyl amine and chloroacetyl chloride by stirring and refluxing for 2 h. Synthesized compounds were characterized by elemental analysis, FT-IR, ^{1}H-NMR and mass spectrum. The FT-IR spectrum exhibited characteristic bands for NH, CH-Ar and C=O at 3403-3059, 3091-3010 and 1681-1598 cm$^{-1.}$ The ^{1}H-NMR spectrum showed singlet at δ 4.30–6.87 confirming C*H*Cl, multiplets ranging from δ 6.60, 7.59–7.56, 8.69 confirmed aromatic protons and singlet ranges at δ 8.69–9.02, 11.00–11.57 confirmed the presence of N*H*N– and N*H*C=O respectively which were D_2O exchangeable.



Scheme 1

Analytical and spectral data were in good agreement with the composition of synthesized compounds and the data are given in Table 1. The physicochemical properties of the titled compounds are presented in Table 2.

3. 2. Pharmacology

3. 2. 1. Anticonvulsant Activity

The pharmacological evaluation of the compounds (**5a-t**) was initially carried out according to the protocols of antiepileptic drug development programme (ADD), Epilepsy Branch, NIH [5, 12]. The compounds were initially screened in the mice using MES test. The MES test has become the most widely employed seizure model for the early identification and high throughput screening of investigational antiepileptic drugs. Minimal motor impairment in the form of neurotoxicity was measured by rotorod test. The anticonvulsant activity and neurotoxicity data for the compounds are reported in Table 3. At the dose level of 30 mg/kg, all the compounds were showing average to good protection. Data is presented as % protection at time intervals of 0.5 h and 4 h. Phenytoin was used as a standard at the dose of 30 mg/kg.

Compounds **5f**, **5n**, and **5p** exhibited 100% protection in the anti-MES activity. Compounds **5e**, **5i**, **5m**, **5o** and **5q** showed their ability to prevent seizure spread with 83% protection whereas, compounds **5a**, **5b**, **5d**, **5l** and **5t** showed 66% protection indicating their ability to elevate seizure threshold. Compounds **5c**, **5g**, **5h**, **5j**, **5k**, **5r** and **5s** showed 50% protection. Thus majority of the compounds displayed preferential MES-selectivity.

In the neurotoxicity screen all the compounds were devoid of toxicity at the dose of 30 mg/kg body weight.

3. 2. 2. Log P Determination

Experimental Log P value of compound **5f**, **5n** and **5p** were found to be 2.44 ± 0.21, 2.11 ± 0.74 and 1.89 ± 0.71, respectively while their calculated values were 1.58 ± 0.71, 1.99 ± 0.68 and 2.46 ± 0.69, respectively. Compound **5n** was coinciding to the theoretical value. All the selected compounds were lipophilic in nature.

3. 2. 3. Hepatotoxicity Studies

Liver samples from control group animals and all the experimental groups were within normal histological limits, except the sample **5n** that showed a moderate portal inflammation but these changes are non-specific and insignificant in nature (Fig. 1–3). No hepatocyte necrosis or degeneration was seen in any of the samples.

Enzyme estimation was done for selected compounds (**5f** and **5n**) and was compared to the control and no any significant changes seen (**P < 0.01). Alkaline phosphatase values (± SEM) for compounds **5f**, **5n** and

Table 1: Spectral characterization of synthesized compounds (**5a-t**):

Compd.	FT-IR (KBr, V_{max} cm^{-1})	^{1}H-NMR (DMSO-d_6) δ ppm	Mass (EI) m/z
5a	3403 (NH), 3059 (CH–Ar), 1598 (C=O), 1462 (C=N), 1254 (C–N), 1094 (N–N), 812 (C–Cl), 719 (C–S–C) .	4.48 (s, 1H, CHCl) , 6.28 (s, 1H,CH), 6.60–7.86 (m, 8H, Ar–H), 8.99 (s, 1H, NHN–, D_2O exchangeable), 11.26 (bs, IH, NHC=O, D_2O exchangeable	407 (M^+)
5b	3490 (OH), 3310, 3291 (NH), 3063 (CH–Ar), 1603 (C=O), 1520 (C=N), 1270 (C–N), 1073 (N–N), 823 (C–Cl), 690 (C–S–C).	4.46 (s, 1H, CHCl), 6.12 (bs, 1H, D_2O exchangeable), 6.87 (s, 1H, CH), 7.22–8.01 (m, 7H, Ar–H), 8.89 (s, 1H, NHN–, D_2O exchangeable), 11.01 (bs, 1H, NHC=O, D_2O exchangeable)	–
5c	3430 (OH), 3315(NH), 3090 (CH–Ar), 2918, 2849 (CH-Aliph), 1600 (C=O), 1462 (C=N), 1271 (C–N), 1124 (N–N), 814 (C–Cl), 719 (C–S–C).	3.76 (s, 3H, OCH_3), 4.41 (d, 1H, CHCl), 5.84 (bs, 1H, OH, D_2O exchangeable), 6.25 (d, 1H, CH), 7.20–7.96 (m, 6H, Ar–H), 8.86 (bs, 1H, NHN–, D_2O exchangeable), 11.00 (bs, 1H, NHC=O, D_2O exchangeable).	–
5d	3179 (NH), 3056 (CH–Ar), 2921, 2851 (CH–Aliph.), 1681 (C=O), 1476 (C=N), 1272 (C–N), 1102 (N–N), 808 (C–Cl), 664 (C–S–C).	3.70 (s, 6H, 2–OCH_3), 4.30 (d, 1H, CHCl), 6.68 (d, 1H, CH), 7.17–7.59 (m, 6H, Ar–H), 8.69 (bs, 1H, NHN–, D_2O exchangeable), 11.57 (bs, 1H, NHC=O, D_2O exchangeable).	467 (M^+)
5e	3230 (NH), 3070 (CH–Ar), 1610 (C=O), 1458 (C=N), 1253(C–N), 1171 (C–F), 1102 (N–N), 840 (C–Cl), 693 (C–S–C).	4.45 (d, 1H, CHCl), 6.58 (d, 1H, CH), 6.74 – 7.76 (m, 8H, Ar–H), 8.90 (s, 1H, NHN–, D_2O exchangeable), 11.16 (bs, 1H, NHC=O, D_2O exchangeable).	–
5f	3495 (OH), 3250 (NH), 3056 (CH–Ar), 1610 (C=O), 1460 (C=N), 1253 (C–N), 1173 (C–F), 1115 (N–N), 815 (C–Cl), 670 (C–S–C).	4.58 (d, 1H, CHCl), 6.10 (s, 1H, OH), 6.54 (d, 1H, CH), 7.21–7.64 (m, 7H, Ar–H), 8.94 (s, 1H, NHN–,D_2O exchangeable), 11.12 (bs, 1H, NHC=O, D_2O exchangeable).	–
5g	3394 (OH), 3270 (NH), 3058 (CH–Ar), 2923 (CH– Aliph.), 1609 (C=O), 1459 (C=N), 1250 (C–N), 1179(C–F), 1103 (N–N), 815 (C–Cl).	3.81 (s, 3H, OCH_3), 4.46 (d, 1H, CHCl), 5.75 (s, 1H, OH), 6.42 (d, 1H, CH), 6.91–7.62 (m, 6H, Ar–H), 8.87 (bs, 1H, NHN–, D_2O exchangeable), 11.03 (bs, 1H, NHC=O, D_2O exchangeable).	–
5h	3297(NH), 3090 (CH–Ar), 2922 (CH–Aliph.), 1632 (C=O), 1463 (C=N), 1258 (C–N), 1165(C–F), 1080 (N–N), 797 (C–Cl).	3,80 (s, 6H, 2–OCH_3), 4.36(d, 1H, CHCl), 6.62 (d, 1H, CH), 6.95–7.41 (m, 6H, Ar–H), 8.72 (s, 1H, NHN–, D_2O exchangeable), 11.54 (bs, 1H,NHC=O, D_2O exchangeable).	–
5i	3396 (NH), 3060 (CH–Ar), 1650 (C=O), 1463 (C=N), 1405 (C–NO_2), 1251(C–N), 1131 (N–N), 819 (C–Cl), 719 (C–S–C).	4.44 (d, 1H, CHCl), 6.55 (d, 1H, CH), 7.38–8.43 (m, 8H, Ar–H), 8.96 (bs, 1H, NHN– D_2O exchangeable), 11.22 (bs, 1H, NHC=O, D_2O exchangeable).	–
5j	3493(OH), 3270, 3167 (NH), 3077 (CH–Ar), 1603 (C=O), 1464 (C=N), 1420 (C–NO_2), 1248 (C–N), 1099 (N–N), 814 (C–Cl), 697 (C–S–C).	4.46 (d, 1H, CHCl), 5.79(bs, 1H, OH), 6.45 (d, 1H, CH), 7.41–8.05 (m, 7H, Ar–H), 8.97 (bs, 1H, NHN–, D_2O exchangeable), 11.01 (bs, 1H, NHC=O, D_2O exchangeable).	
5k	3409 (OH), 3301 (NH), 3066 (CH–Ar), 2923 (CH–Aliph.), 1610 (C=O), 1465 (C=N), 1430 (C–NO_2), 1238 (C–N), 1097 (N–N), 819 (C–Cl), 701(C–S–C).	3.86 (s, 3H, OCH_3), 4.44 (d, 1H, CHCl), 5.38 (bs, 1H, OH), 6.40 (d, 1H, CH), 6.89–8.69 (m, 6H, Ar–H), 8.90 (s, 1H, NHN–, D_2O exchangeable), 11.06 (bs, 1H, NHC=O, D_2O exchangeable).	463 (M^+)
5l	3310 (NH), 3067 (CH–Ar), 2920 (CH–Aliph.), 1630 (C=O), 1439 (C=N), 1382 (C–NO_2), 1243 (C–N), 1044 (N–N), 853 (C–Cl), 697 (C–S–C).	3.75 (s, 6H, 2–OCH_3), 4.87 (d, 1H, CHCl), 6.58 (d, 1H, CH), 7.22–8.45 (m, 6H, Ar–H), 9.02 (bs, 1H, NHN–, D_2O exchangeable), 11.19 (bs, 1H, NHC=O, D_2O exchangeable).	477 (M^+)

Compd.	FT-IR (KBr,V_{max} cm^{-1})	^{1}H-NMR (DMSO-d_6) δ ppm	Mass (EI) m/z
5m	3279 (NH), 3091 (CH–Ar), 2926 (CH–Aliph.), 1607 (C=O), 1467 (C=N), 1238 (C–N), 1103 (N–N), 819 (C–Cl), 690 (C–S–C).	2.43 (s, 3H, CH_3), 4.42 (d, 1H,.CHCl), 6.53 (d, 1H, CH), 7.25–7.75 (m, 8H, Ar–H), 8.91 (bs, 1H, NHN–, D_2O exchangeable), 11.09 (bs, 1H, NHC=O, D_2O exchangeable).	–
5n	3399 (OH), 3278, 3109 (NH), 3058 (CH–Ar), 1615 (C=O), 1464 (C=N), 1253 (C–N), 1107 (N–N), 817 (C–Cl), 691 (C–S–C).	2.46 (s, 3H, CH_3), 4.49 (d, 1H, CHCl), 5.60 (bs, 1H, OH), 6.49 (d, 1H, CH), 6.70–7.65 (m, 7H, Ar–H), 8.92 s, 1H, NHN–, D_2O exchangeable), 11.12 (bs, 1H, NHC=O, D_2O exchangeable).	–
5o	3388 (OH), 3107 (NH), 3059 (CH–Ar), 2923 (CH–Aliph.), 1630 (C=O), 1452 (C=N), 1267 (C–N), 1114 (N–N), 814 (C–Cl), 691 (C–S–C).	2.48 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.52 (d, 1H, CHCl), 5.50 (bs, 1H, OH), 6.44 (d, 1H, CH), 7.32–7.67 (m, 6H, Ar–H), 8.92 (s, 1H, NHN–, D_2O exchangeable), 11.08 (bs, 1H, NHC=O, D_2O exchangeable).	–
5p	3310 (NH), 3058 (CH–Ar), 2923 (CH–Aliph.), 1632 (C=O), 1486 (C=N), 1265 (C–N), 111O (N–N), 813 (C–Cl), 691 (C–S–C).	2.17 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3),4.52 (d, 1H, CHCl), 6.38 (d, 1H, CH), 6.79–7.86 (m, 7H, Ar–H), 9.02 (bs, 1H, NHN–, D_2O exchangeable, 11.00 (bs, 1H, NHC=O, D_2O exchangeable)	–
5q	3241 (NH), 3056 (CH–Ar), 2919 (CH–Aliph.), 1604 (C=O), 1466 (C=N), 1242 (C–N), 1111 (N–N), 815 (C–Cl), 701 (C–S–C).	3.79 (s, 3H, OCH_3), 4.61 (d, 1H, CHCl), 6.61 (d, 1H, CH), 7.19–7.51 (m, 8H, Ar–H), 8.90 (s, 1H, NHN–, D_2O exchangeable, 11.11 (bs, 1H, NHC=O, D_2O exchangeable).	–
5r	3490 (OH), 3287, 3167 (NH), 3088 (CH–Ar), 2933 (CH–Aliph.), 1610 (C=O), 1490 (C=N), 1247 (C–N), 1097 (N–N), 819 (C–Cl), 692 (C–S–C).	3.75 (s, 3H, OCH_3), 4.48 (d, 1H, CHCl), 6.09 (bs, 1H, OH), 6.68 (d, 1H, CH), 7.56–7.99 (m, 7H, Ar–H), 9.01 (s, 1H, NHN–, D_2O exchangeable), 11.17 (bs, 1H, NHC=O, D_2O exchangeable).	–
v5s	3488 (OH), 3323 (NH), 3058 (CH–Ar), 2923 (CH–Aliph.), 1598 (C=O), 1462 (C=N), 1238 (C–N), 1104 (N–N), 813 (C–Cl), 691 (C–S–C).	3.69 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.75 (d, 1H, CHCl), 5.57 (bs, 1H, OH), 6.23 (d, 1H, CH), 7.08–7.51 (m, 6H, Ar–H), 8.93 (s, 1H, NHN–, D_2O exchangeable), 11.13 (bs, 1H, NHC=O, D_2O exchangeable).	448 (M^+)
5t	3251 (NH), 3010 (CH–Ar), 2918 (CH–Aliph.), 1604 (C=O), 1479 (C=N), 1253 (C–N), 1103 (N–N), 813 (C–Cl), 703 (C–S–C).	3.77 (s, 9H, 3–OCH_3), 4.51 (d, 1H, CHCl), 6.22 (d, 1H, CH), 7.37–7.44 (m, 6H, Ar–H), 8.88 (bs, 1H, NHN–, D_2O exchangeable), 11.08 (bs, 1H, NHC=O, D_2O exchangeable).	462 (M^+)

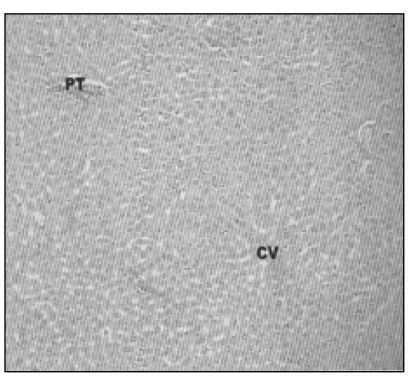


Fig: 1 (100X)

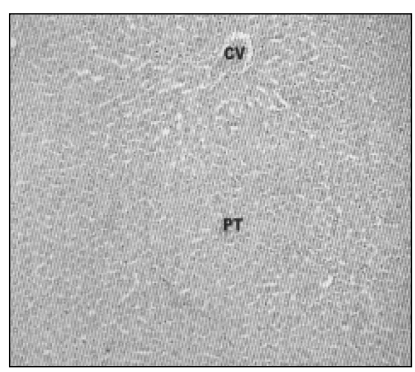


Fig: 2 (100X)

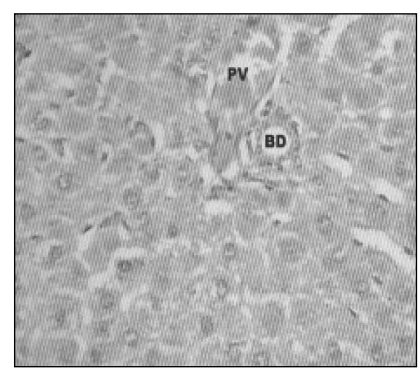


Fig: 3 (400X)

Fig. 1–3. Low power (HE x 100x) and high power (HE x 400x) photomicrographs of liver from control (Fig. 1) and compound **5f** (Fig. 2) showing a normal hepatic parenchyma with Portal Triad (PT), Central Vein (CV) and the hepatocytes. While in case of compound **5n** (Fig. 3), liver showing normal Portal triad and Bile duct (BD) structures except here is mild but insignificant periportal inflammatory cell infiltration.

Table 2: Physicochemical properties of compounds (**5a-t**)

Compd No.	R	R_1	R_2	[a]Mol. Formula	Yield (%)	[b]M.P. (^oC)	[c]Elemental analysis (% N) Calcd.(Found)	[d]R_f (R_m) [e]
5a	Cl	H	H	$C_{17}H_{12}Cl_2N_4O_2S$	50	286	13.70 (13.76)	0.96 (–1.38)
5b	Cl	OH	H	$C_{17}H_{12}Cl_2N_4O_3S$	49	240	13.28 (13.24)	0.98 (–1.69)
5c	Cl	OH	OCH_3	$C_{18}H_{14}Cl_2N_4O_4S$	55	270	13.40 (12.36)	0.85 (–0.75)
5d	Cl	OCH_3	OCH_3	$C_{19}H_{16}Cl_2N_4O_4S$	52	296	12.03 (11.99)	0.99 (–1.99)
5e	F	H	H	$C_{17}H_{12}ClFN_4O_2S$	54	230	14.40 (14.34)	0.84 (–0.72)
5f	F	OH	H	$C_{17}H_{12}ClFN_4O_3S$	53	242	13.81 (13.77)	0.90 (–0.95)
5g	F	OH	OCH_3	$C_{18}H_{14}ClFN_4O_4S$	50	260	12.80 (12.83)	0.92 (–1.06)
5h	F	OCH_3	OCH_3	$C_{19}H_{16}ClFN_4O_4S$	51	188	12.47 (12.43)	0.87 (–0.82)
5i	NO_2	H	H	$C_{17}H_{12}ClN_5O_4S$	54	265	16.70 (16.76)	0.83 (–0.68)
5j	NO_2	OH	H	$C_{17}H_{12}ClN_5O_5S$	56	290	16.18 (16.14)	0.91 (–1.00)
5k	NO_2	OH	OCH_3	$C_{18}H_{14}ClN_5O_6S$	45	280	15.14 (15.10)	0.89 (–0.90)
5l	NO_2	OCH_3	OCH_3	$C_{19}H_{16}ClN_5O_6S$	53	238	14.70 (14.65)	0.82 (–0.65)
5m	CH_3	H	H	$C_{18}H_{15}ClN_4O_2S$	53	268	14.44 (14.48)	0.87 (–0.82)
5n	CH_3	OH	H	$C_{18}H_{15}ClN_4O_3S$	52	250	13.96 (13.91)	0.93 (–1.12)
5o	CH_3	OH	OCH_3	$C_{19}H_{17}ClN_4O_4S$	50	300	13.00 (12.94)	0.88 (–1.18)
5p	CH_3	OCH_3	OCH_3	$C_{20}H_{19}ClN_4O_4S$	55	250	12.56 (12.54)	0.94 (–1.19)
5q	OCH_3	H	H	$C_{18}H_{15}ClN_4O_3S$	56	220	13.95 (13.91)	0.86 (–1.18)
5r	OCH_3	OH	H	$C_{18}H_{15}ClN_4O_4S$	54	254	13.42 (13.38)	0.97 (–1.50)
5s	OCH_3	OH	OCH_3	$C_{19}H_{17}ClN_4O_5S$	58	288	12.54 (12.48)	0.95 (–1.27)
5t	OCH_3	OCH_3	OCH_3	$C_{20}H_{19}ClN_4O_5S$	54	270	12.16 (12.10)	0.81 (–0.62)

[a]Solvent of crystallization : Ethanol; [b]Melting point of the compounds at their decomposition; [c]The elemental analysis data were in agreement to the calculated values (in the range of ± 0.4%) [d]Solvent system Benzene: Acetone (8:2); [e]A logarithmic function of R_f value was also calculated ; $R_m = \log (1–1/R_f)$.

Table 3: Anticonvulsant and neurotoxicity data of the titled compounds (**5a-t**)

Compd. No.	MES screen[a] (% Protection)		Neurotoxicity screen[a]	
	0.5 h	4 h	0.5 h	4 h
5a	66	66	×	×
5b	66	66	×	×
5c	50	50	×	×
5d	66	66	×	×
5e	83	83	(–)	(–)
5f	100	100	(–)	(–)
5g	50	50	×	×
5h	50	33	×	×
5i	83	66	(–)	(–)
5j	50	50	×	×
5k	50	50	×	×
5l	66	66	×	×
5m	83	83	(–)	(–)
5n	100	100	(–)	(–)
5o	83	83	(–)	(–)
5p	100	100	(–)	(–)
5q	83	83	(–)	(–)
5r	50	50	×	×
5s	50	50	×	×
5t	66	66	×	×
Phenytoin	100	100	(–)	(–)

[a]Intraperitoneal dose of 30 mg/kg was administered and the animals were examined 0.5 and 4 h after administration. The dash (-) indicates an absence of activity. x denotes not tested.

control were found to be 19.46 ± 0.80, 23.70 ± 0.30 and 13.06 ± 0.25, respectively. SGOT ± SEM values for compounds **5f**, **5n** and control were found to be 150.0 ± 1.50, 176.1 ± 1.96 and 148.67 ± 1.50, respectively. SGPT ± SEM values for compounds **5f**, **5n** and control were found to be 19.50 ± 0.85, 39.17 ± 1.83 and 27.67 ± 0.84, respectively.

Protein estimation was also done for the compounds (**5f** and **5n**) and was compared to the control and no any significant changes seen (**P < 0.01). Albumin (g/100 mL) ± SEM values for compounds **5f**, **5n** and control were found to be 1.70 ± 0.27, 1.98 ± 0.03 and 1.67 ± 0.009, respectively. Globulin (g/100 mL) ± SEM values for compounds **5f**, **5n** and control were found to be 0.74 ± 0.02, 0.12 ± 0.007 and 0.13 ± 0.01 respectively. Total Protein (g/100 mL) ± SEM values for compounds **5f**, **5n** and control were found to be 2.45 ± 0.02, 2.11 ± 0.02 and 1.80 ± 0.01, respectively. Albumin / Globulin (g/100 mL) ± SEM values for compounds **5f**, **5n** and control were found to be 2.30 ± 0.09, 16.00 ± 1.16 and 13.84 ± 1.57, respectively.

4. Conclusions

In this study it was concluded that the basic structure of the compounds have all the pharmacophoric elements necessary for the anticonvulsant activity. In general, compounds bearing F, CH_3 at the 6-position of benzot-

hiazole moiety with H, OCH_3 at the 3-position and OH, OCH_3 at 4-position of the distant phenyl ring showed a highly potent activity. Whereas, replacements with Cl, NO_2, OCH_3 at the 6-position of benzothiazole ring with H, OCH_3 at the 3-position and OH at 4-position of distant phenyl ring resulted in 50% decrease in potency. Compounds with F, NO_2, CH_3, OCH_3 substituents at the 6-position of the benzothiazole moiety and unsubstituted distant phenyl ring showed moderate decrease in activity. A compound substituted with OCH_3 at the 6-position of the benzothiazole moiety and 3, 4-positions of distant phenyl ring displayed significant activity.

5. Acknowledgement

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Povzetek

Sintetizirali smo številni nove spojine 1-[2-(3,4-disubstituirani fenil)-3-kloro-4-oksoazetidin-1-il]-3-(6-substituirani-1,3-benzotiazol-2-il) uree poimenovane (**5a-t**), ovrednotili smo njihovo antikonvulzivno delovanje, hepatotoksičnost in nevrotoksičnost. Spojine (**5a-t**) smo pridobili s ciklizacijo 3,4-disubstituirani benzaldehid-*N*-(6-substituirani-1,3-benzotiazol-2-il) semikarbazonov (**4a-t**) v prisotnosti DMF/trietilamina in kloracetilklorida. Vse novo sintetizirane spojine smo testirali za antikonvulzivno delovanje s testom z elektrošokom izzvanega epileptičnega napada (model MES i.p.) in jih primerjali z delovanjem standardnega zdravila fenitoina. Zanimivo, spojine **5f**, **5n** in **5p** so 100 % zaščitile pred epileptičnimi napadi. Pri odmerku 30 mg/kg telesne teže nobena izmed substanc ni imela hepatotoksičnih ali nevrotoksičnih učinkov. Naša študija je pokazala, da uvedba F, zamenjava CH_3 na položaju 6 benzotiazolne strukture z H, OCH_3 na položaju 3 in OH, OCH_3 na položaju 4 oddaljenega fenilnega obroča povzroči povečano aktivnost. Uvedba F, NO_2, CH_3, OCH_3 na položaju 6 benzotiazola in nesbstituiranega oddaljenega fenilnega obroča povzroči zmerno zmanjšanje aktivnosti.