

**Research article****Synthesis, Characterization and Anticonvulsant Activity on Novel Thiourea Derivatives****Priya Kumar*, Anjana Sharma, S.K.Gupta**

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ABSTRACT

The present research work involves the synthesis of a series of N-Substituted Thiourea derivatives (T1- T3) by refluxing 8-(3-nitrophenoxy) octan-1-amine in THF (Tetrahydrofuran). The synthesized compounds were characterized by elemental analysis, FTIR, ¹H-NMR, mass spectrum. All the synthesized compounds were screened for anti-convulsant activity by using Maximal electroshock seizure (MES) tests. Among the tested compounds, thiourea derivatives of T1 showed most potent activity and were afforded MES tests at 25 mg/kg, respectively.

1. Introduction

The epilepsy is the disordered of the brain function characterized by the periodic and unpredictable occurrence of seizures. Seizures can be "nonepileptic" when evoked in a normal brain or by treatments such as electroshock or chemical convulsants or "epileptic" when occurs without evident provocation.

Anticonvulsants are also increasingly being used in the treatment of bipolar disorder, since many seem to be acts as mood stabilizers, and for the treatment of neuropathic pain. Anticonvulsants suppress the rapid and excessive firing of neurons during seizures. Anticonvulsants also prevent the spread of the seizure within the brain. Some investigators have observed that anticonvulsants themselves may cause reduced IQ in children[1]. However, these adverse effects must be balanced against the significant risk epileptic seizures pose to children and the distinct possibility of death and devastating neurological sequel secondary to seizures. Anticonvulsants are more accurately called antiepileptic drugs (abbreviated "AEDs"), and are often referred to as anti seizure drugs because they provide symptomatic treatment only and have not been demonstrated to alter the course of epilepsy [2]. Next to the voltage-gated sodium channels and components of the GABA system, their targets include GABA_A receptors, the GAT-1 GABA transporter, and GABA transaminase[3].

Additional targets include voltage-gated calcium channels, SV2A, and $\alpha\delta$. By blocking sodium or calcium channels, antiepileptic drugs reduce the release of excitatory glutamate, whose release is considered to be elevated in epilepsy, but also that of GABA. This is probably a side effect

or even the actual mechanism of action for some antiepileptic drugs, since GABA can itself, directly or indirectly, act proconvulsively[4]. The development of epilepsy in an individual at risk, such as after a head injury[5].

Conventional antiepileptic drugs may block sodium channels or enhance γ -amino butyric acid (GABA) function. Several antiepileptic drugs have multiple or uncertain mechanisms of action. The seizure is a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons[6].

During the 5 years, several new drugs have been approved or in the process of being approved, e.g. lamotrigine, gabapentin, tiagabine and milacemide. Although these drugs have been shown to be effective in reducing seizures in a number of patients their efficacy does not appear to be superior to that of the drugs developed earlier.

Available antiepileptic drugs control seizures in about two-thirds of the patients. In 1857, Sir Charles Locock, the attending physician for the birth of Queen Victoria's children, suggested potassium bromide for the treatment of epilepsy. Phenobarbital was introduced in 1912, and later, in 1938, phenytoin was found to be effective in experimental seizures. Significant progress was made both in the development of experimental models and in methods for screening and testing of new epileptic drugs in the period 1935 to 1960. Thirteen new antiepileptic drugs were developed during this period, but in the last 30 years, only a relatively few new epileptic drugs have been developed.

For a long period it was thought that a single drug would be able to treat all forms of epilepsy, but the current consensus is that it is quite unlikely that a wide variety of epilepsies can be managed with one drug. Drugs used in the treatment of two major seizures types, partial and generalized, are quite distinct in their clinical profiles.

The anticonvulsant drug was designed so as to reduce the risk of seizures. After studying a lot many articles related to the convulsant it can be concluded that being a toxic compound thiourea can form a novel series of derivatives which can be used as anti convulsant drug and can be used in reducing the risk of seizures. Thus, the aim of the present study was to prepare a series of thiourea ant to evaluate anticonvulsant properties using MES test in mice[7].

2. Materials and methods

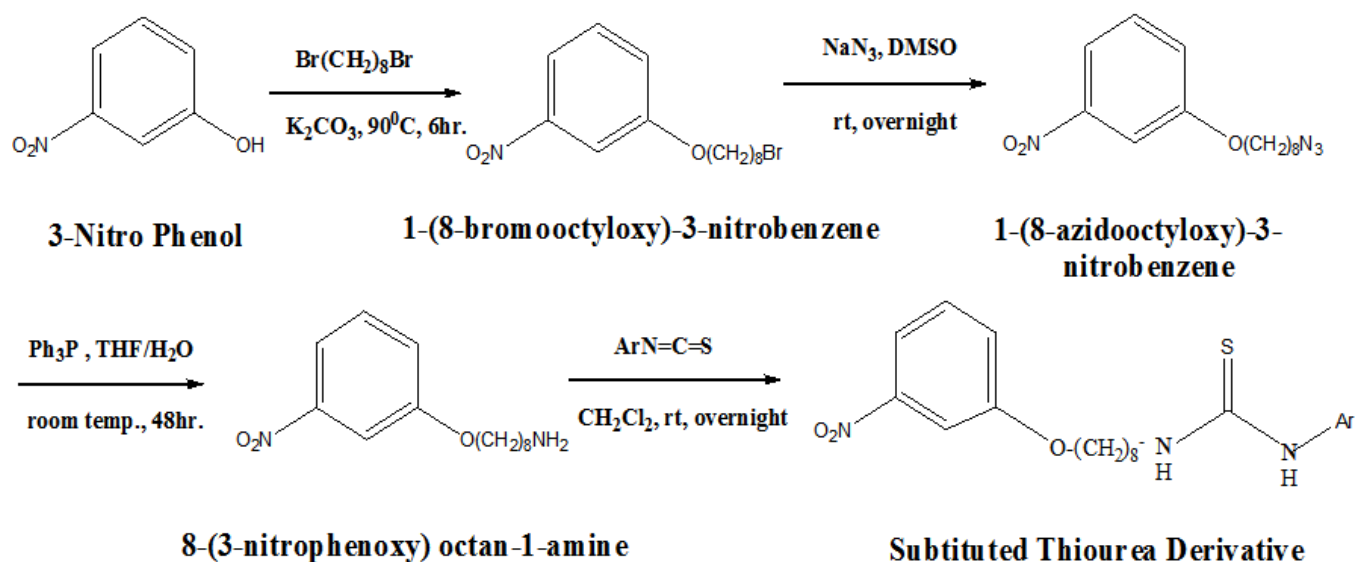
Instrumentation and Chemicals

All the chemicals and reagents were purchased from Sigma-Aldrich (USA), Rankem (Delhi) and CDH (Delhi). The percentage yield was based upon the product obtained from

purification and recrystallization. The solvent used for recrystallization was ethanol. Purity of the compounds was checked on thin layer chromatography (TLC) plates made with silica gel G using solvent system benzene:hexane:methanol, dichloromethane: methanol (2.5:5.0:2.5, 9.7:3.0, 8.0:2.0 v/v). The spots were located by using iodine chamber and UV light. Melting points were determined by digital melting point apparatus. Boiling points were determined by Theil's tube pouring compound in it and were kept in paraffin liquid wax. IR spectra were recorded using KBr on a FTIR Shimadzu 8400S IR spectrophotometer, M.I.E.T, Meerut. All ^1H NMR were recorded on Agilent varion 500 MHz and chemical shift is expressed in TMS as an internal standard and ethanol was used as a solvent. Mass spectra were recorded on max Agilent 6320 ion (ICMS) trap mass spectrometer. Elemental analysis was done by using Chem. Draw Ultra 8.0 software.

2.2 Synthesis

2.2.1 General procedures for the preparation of thiourea derivatives



Scheme 1.1

2.2.1.1 Step I

1-(8-Bromooctyloxy) - 3- nitrobenzene

The 3-nitrophenol (0.5mol) in 1, 8-Dibromooctane (0.25 mol) in 100ml RBF. Then, K_2CO_3 (0.69mg) dissolved in DMSO was added to the mixture. It was refluxed for 6hrs. at 90°C . Then, the product was filtered and dried. **%yield:** 95.238, **M.P:** 70°C , **Solvent system:** Benzene (0.25): Hexane (0.5): Methanol (0.25), **R_f** =0.89, **Visualizing agent:** Iodine chamber and UV light, **Mol. Wt.:** 330.22, **Mol. Form.:** $\text{C}_{14}\text{H}_{20}\text{BrNO}_3$, **Elemental Analysis:** C(50.92), H (6.10), N (4.24), Br (24.20), O (14.54), **I.R (KBr, in cm^{-1}):** N=O : 1327.90-1348.15, C=O : 1050.17-1075.24, Aromatic: 3150-3050, CH_2 (Bending) : 1348.15, Br : 788.83

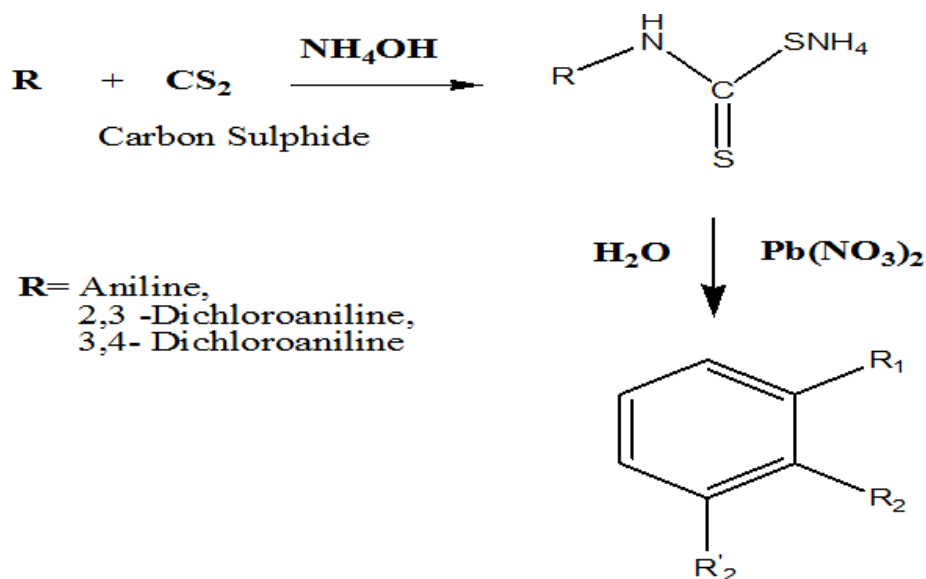
2.2.1.2 Step II

1-(8-azidoctyloxy)-3-nitrobenzene

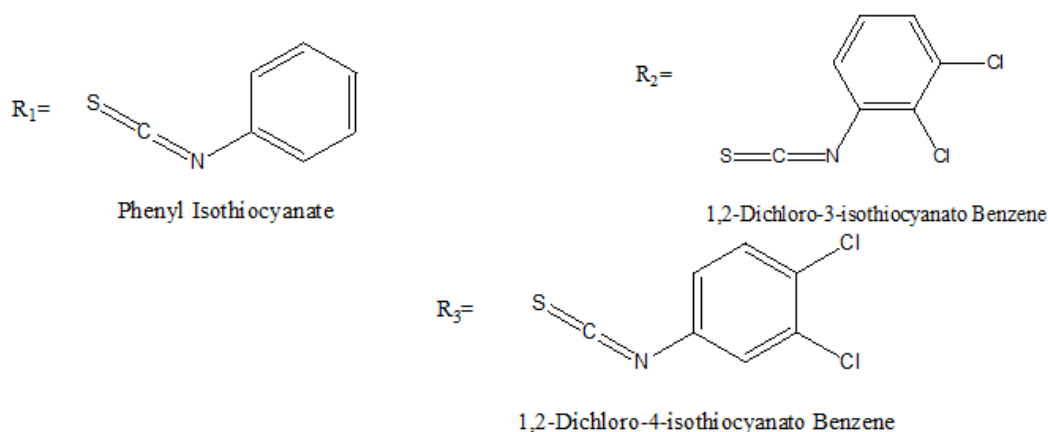
1-(8-bromooctyloxy)-3-nitrobenzene (0.5 mol) was added to the solution of sodium azide (0.25 mol) & in the presence of DMSO. It was refluxed for 24 hrs. at room temperature. Then, the product was filtered and dried. **% yield:** 73.423, **M.P:** 68°C , **Solvent system:** Dichloromethane (0.97): Methanol (0.03), **R_f** =0.90, **Visualizing agent:** Iodine chamber, **Mol. Wt.:**292.33, **Mol. Form.:** $\text{C}_{14}\text{H}_{20}\text{N}_4\text{O}_3$, **Elemental Analysis:** C(57.52), H (6.90), N (19.17), O (16.42), **I.R (KBr, in cm^{-1}):** CH_2 (Bending): 2999.10, N_3 : 2027.05, Aromatic: 1639.38, N=O: 1314.40, C-O: 1022.20

2.2.1.3 Step III**8-(3-nitrophenoxy) octan-1-amine**

8-(3-nitrophenoxy) octan-1-amine (0.00483mol) dissolved in water and filtered to remove black particles then triphenyl phosphine (0.005mol) and THF was added to it and then refluxed for 48 hours at room temperature. Then, the product was filtered and dried. **% yield:** 37.079, **M.P:**120°C, **Solvent system:** Dichloromethane (0.8): Methanol (0.2), **R_f** = 0.77, **Visualizing agent:** Iodine chamber, **Mol. Wt.:**266.34, **Mol. Form.:** C₁₄H₂₂N₂O₃, **Elemental Analysis:** C(63.13), H (8.33), N (10.52), O (18.02), **I.R (KBr, in cm⁻¹):** N=O: 1346.22, Aromatic: 3205.86, C=O: 1012.56, CH₂ (Bending): 1437.83, N=H (Bending): 1971.11

2.2.2 Synthesis of intermediate Isothiocyanate compounds

Scheme 1.2



The salt is dissolved in 200 cc. of water and transferred to round-bottomed flask. To the solution is added with constant stirring a solution of 50 g. of lead nitrate in 100 cc. of water. Lead sulfide separates as a heavy brown precipitate which soon turns black. The mixture is then distilled with steam into a receiver containing 5 cc. of 1 N sulfuric acid as long as any oil comes over. About 2–3 l. of distillate is collected. The product (**1.72**, **1.73** and **1.73**) was separated from the water. The oil was dried over a little calcium chloride and distilled under reduced pressure[8].

2.2.2.1 General procedure for the preparation of N-substituted Isothiocyanates:

In a 250-cc. round-bottomed flask, fitted with a mechanical stirrer and surrounded by an ice-salt cooling bath, are placed 10.75ml of carbon disulfide and 22.5ml of concentrated aqueous ammonia. The stirrer was started, and 14ml of aniline and N- substituted aniline was run into the mixture from a separatory funnel at such a rate that the addition is complete in about twenty minutes. The stirring was continued for thirty minutes after all the aniline has been added, and then the reaction mixture was allowed to stand for another thirty minutes. During this time a heavy precipitate of ammonium phenyldithiocarbamate separates and may even stop the stirrer[8].

Preparation of 1-isothiocyanatobenzene

% yield: 12.48, **B.P:**120°C, **Solvent system:** dichloromethane: Methanol (8:2), **R_f** = 0.80, **Visualizing agent:** Iodine chamber, **Mol. Wt.:** 135.19, **Mol. Form.:** C₇H₅NS, **Elemental analysis:** C (62.19), H (3.73), N (10.36), S (23.72)

Preparation of 1, 2-Dichloro-3-isothiocyanatobenzene

% yield: 81, **B.P:** 130-134°C, **Solvent system:** Dichloromethane: Methanol (8:2), **R_f** = 0.80, **Visualizing agent:** Iodine chamber, **Mol. Wt.:** 2042.94, **Mol. Form.:** C₇H₃Cl₂NS, **Elemental analysis:** C (41.20), H (1.48), Cl (34.74), N (6.86), S(15.71)

Preparation of 1, 2- Dichloro-4-isothiocyanato Benzene

% yield: 76, **B.P:**134-136°C, **Solvent system:** dichloromethane: Methanol (8:2), **R_f**= 0.79, **Visualizing agent:** Iodine chamber, **Mol. Wt.:** 204.08, **Mol. Form.:** C₇H₅Cl₂NS, **Elemental analysis:** C (41.20), H (1.48), Cl (34.74), N (6.86), S (15.71)

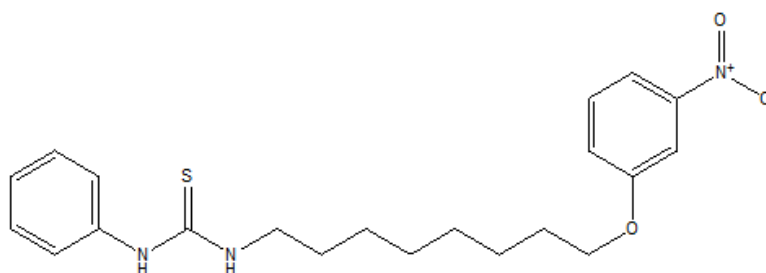
2.2.1.4 Step IV

Synthesis of N-substituted thiourea derivatives

2.2.1.4.1 General procedure for the preparation of N-Substituted Thiourea Derivatives

Compound (0.12gm) was dissolved in a substituted compound (1-isothiocyanatobenzene/ 1, 2-Dichloro-3-Isothiocyanato Benzene/1, 2-Dichloro-4-Isothiocyanato Benzene) then dichloromethane was added into it in 250ml RBF and further it was kept refluxing for overnight at room temperature. At the end, the N-substituted product was filtered and dried to get crude substituted derivative product.

T1: m- [8-(3-Phenylthioureido) octyloxy] nitrobenzene

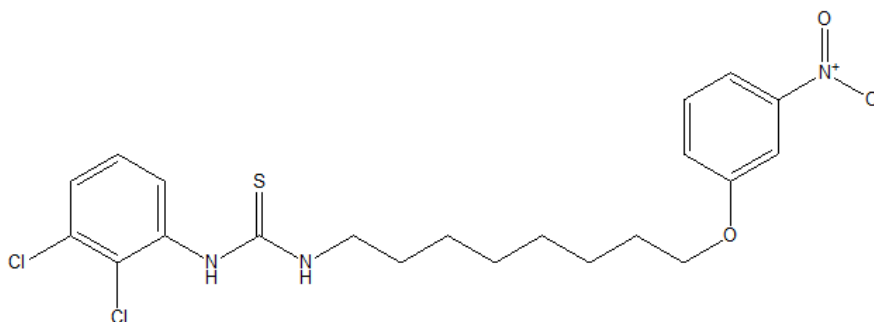


m-[8-(3-Phenylthioureido) octyloxy] nitrobenzene

% yield: 35.35, **B.P:**225°C, **Solvent system:** dichloromethane: Methanol (8:2), **R_f**= 0.85, **Visualizing agent:** Iodine chamber, **Mol. Wt.:**401.52, **Mol. Form.:** C₂₁H₂₇N₃O₃S, **Elemental Analysis:** C (62.82), H (6.78), N (10.47), O (11.95), S (7.99), **IR (KBr, in cm⁻¹):** N=O: 1385.76, CH₂ (Bending): 2186, C-O: 1014.49-1241.11, N-H: 3292.26-

3471.63, C-H (Stretching): 2855.42-2919.06, C=S (Stretching): 828.37, C-H (Aromatic) : 3047.32-3077.21, **¹H NMR, δ ppm:** 7.51 (s, 5H, Aromatic), 1.8 (s, 2H, NH), 2.43 (s, 4H, N-CH₂), 7.61 (s, 4H, Aromatic), **Mass (EI) m/z:** 401.17 Da (M⁺)

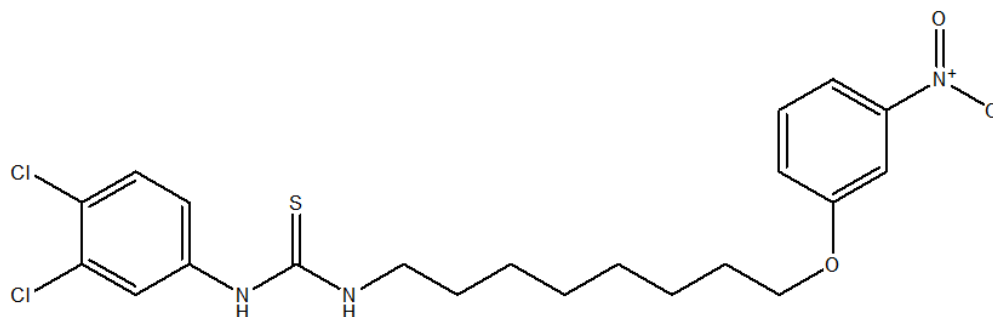
T2: 1-(8-(3-nitrophenoxy) octyl)-3-(2, 3-dichlorophenyl) thiourea



1-(8-(3-nitrophenoxy)octyl)-3-(2,3-dichlorophenyl)thiourea

% yield: 56.25, **B.P:** 240°C, **Solvent system:** dichloromethane: Methanol (8:2), **R_f**= 0.91, **Visualizing agent:** Iodine chamber, **Mol. Wt.:** 469.10, **Mol. Form.:** C₂₁H₂₅Cl₂N₃O₃S, **Elemental Analysis:** C (53.62), H (5.36), Cl (15.07), N (8.93), O (10.20), S (6.82), **IR (KBr, in cm⁻¹):**

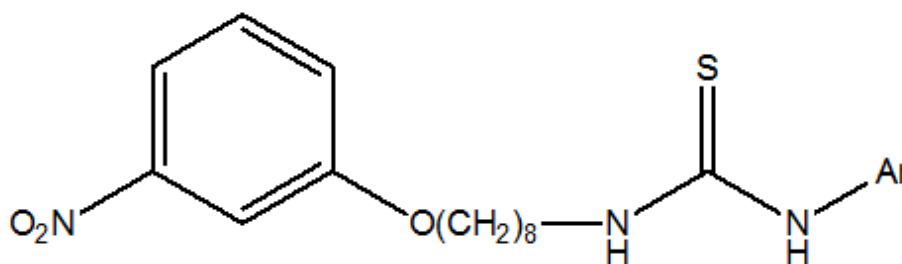
N=O : 1385.76, C-O : 1031.85, CH (Stretching) : 28122.98, N-H : 3304.80, C=S : 3304.80, C=S (Stretch) : 855.37-871.76, C-Cl : 574.75, C-H (Aromatic) : 3180.26, **¹H NMR, δ ppm:** 6.92 (s, 3H, Aromatic), 1.8 (s, 2H, NH), 2.43 (s, 8H, N-CH₂), 7.61 (s, 4H, Aromatic), **Mass (EI) m/z:** 469.09 Da (M⁺)



1-(8-(3-nitrophenoxy)octyl)-3-(3,4-dichlorophenyl)thiourea

% yield: 57, **B.P:** 230°C, **Solvent system:** dichloromethane: Methanol (8:2), **R_f** = 0.88, **Visualizing agent:** Iodine chamber, **Mol. Wt.:** 470.41, **Mol. Form.:** C₂₁H₂₅Cl₂N₃O₃S, **Elemental Analysis:** C (53.62), H (5.36), Cl (15.07), N (8.93), O (10.20),

S (6.82), **I.R (KBr, in cm⁻¹)** : N=O : 1458.08, C-O : 1014.50, C-H (Aromatic) : 3197.76, C-H (Stretching): 2811.06, C-Cl : 787.87, N-H: 3382.91, **¹H NMR, δ ppm:** 6.92 (s, 3H, Aromatic), 9.57 (s, 8H, CH₂), 7.26 (s, 3H, Aromatic), 1.8 (s, 2H, NH), **Mass (EI) m/z:** 469.09 Da (M⁺)



2.3 Structure determination

Substitution of isothiocyanates helps in enhancing the activity. Substitution of 1- Isothiocyanatobenzene at position 10, showed most potent anticonvulsant activity. Substitution of 1, 2-dichloro-3-isocyanatobenzene at position 10, shows good anticonvulsant activity but is found to be most potent anti-inflammatory compound. Substitution of 1, 2- dichloro-4-isothiocyanatobenzene at position 10, shows good anti-inflammatory activity.

3. Results

3.1 Anti convulsant activity

Male and female adult Swiss albino mice weighing 20–40 g were used. The animals were housed in colony cages, under standard laboratory conditions, with free access to food and tap water. After the adaption period of 10 days, experimental groups were chosen randomly. Each mouse was used only once.

3.1.1 By MES method

All compounds were administered 25 mg/kg intraperitoneally and injected 30 min before MES. Each mouse was used only once. The electroshocks were evoked through a current transmitter producing 50 Hz square waves. Flow duration of current and the duration of each square wave were fixed at 0.2 s, and at 0.4 m, respectively. During the shock, electrodes were attached to each animal's ears and the animals lay on their backs, their tails being fixed. Thus, observation of the tonic and clonic convulsions that appeared during the seizure was ensured. In mice, maximal electroshock seizures consist of a latency period lasting 1.6 s. Followed by a short initial flexion period, then 13.2 s of tonic hind limb extension and 7.6 s of terminal clonus. Total duration of seizure was 22.3 s [9].

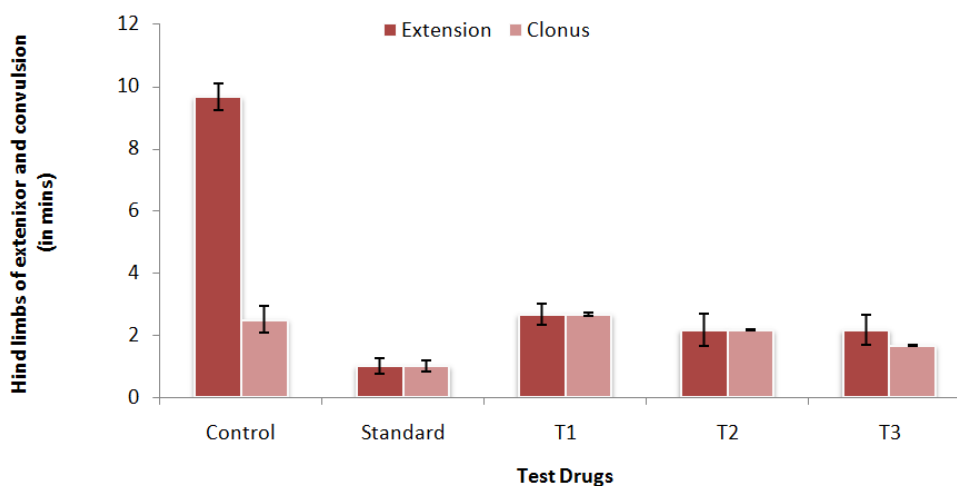
All the three synthesized novel derivatives were used for the evaluation of anti-convulsant activity by Maximal electroshock Method and the results are recorded and shown in **Table 1.1**.

Table 1.1: Anti-convulsant activity of thiourea derivatives

S.No.	Compd. Code	Hind limbs of extensor & convulsion (MEAN±SEM)	
		Extensor	Convulsion
1.	Control	9.7±0.67	2.5±0.42
2.	Standard	1±0.25	1.1±0.16
3.	T1	2.6±0.33***	2.7±0.49
4.	T2	2.1±0.30**	2.1±0.40
5.	T3	2.1±0.47	1.6±0.33*

Anti-convulsant activity of synthesized compounds are determined at the dose (25mg/kg). All values express on MEAN±SEM: * p≤0.05 less significant, ** p≤0.01 significant,

*** p≤0.001 highly significant from standard (phenytoin). Data were analyzed by using one-way ANOVA followed by Tukey's test.

**Figure 1.1:** Graph of anti-convulsant activity of thiourea derivatives by using maximal electroshock method.

Discussion

All the compounds T1, T2 and T3 were screened for anti-convulsant activity taking 1% distilled water as control and phenytoin as standard drug. Significance of the compounds was found out in relation to untreated group and data were analyzed one way ANOVA using Tukey's test. Compound T1 and T2 exhibit a significant activity at both the phases i.e. extensor and convulsion. Compound T1 showed highly significant activity at convulsion phase among all the tested compounds.

Conclusion

The anticonvulsant activity of the new compounds was determined by using MES test. The rodent models are widely used as standard methods for predicting protection against generalized absence seizures in humans.

The thiourea derivatives were administered 25mg/kg intraperitoneally. Firstly, in control group, distilled water was administered at a dose of 25mg/kg in mice. Secondly, in standard group, phenytoin (standard drug) was administered at a dose of 25mg/kg in mice. Then after every thirty minutes synthesized derivative was administered at a dose of 25mg/kg

to the three different groups. Statistical methods was applied on MES test using one way – ANOVA followed by Tukey's test for comparison. The results of anticonvulsant activity were shown in Table 1.1.

The results from MES test shows that out of three different compounds, T1 was found to be the 100% effective and most active compound at a dose of 25mg/kg.

In conclusion, the synthesis of a number of thiourea derivatives as a candidate anticonvulsant was reported in this study. Thiourea derivative T1 displayed noteworthy activity in MES screens. Hence, this compound (T1) in particular serves as prototypic molecule for subsequent molecular modifications in the search of novel anticonvulsant.

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