DESIGN, SYNTHESIS AND EVALUATION OF ANTICONVULSANT ACTIVITY OF SOME THIOPHENE ANALOGUES

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ABSTRACT

The present research work was carried out with main aim to synthesize novel series of thiophene derivatives and evaluate their anticonvulsant activity. In this work, 20 molecular structures of thiophene have been docked using GABAa protein structure and score was obtained. All synthesized derivatives were subjected for physicochemical studies such as melting point, TLC, solubility and the spectral analysis using IR, 1HNMR, 13C NMR and MS. Compounds were screened for anticonvulsant activity by Isoniazid (INH) and Pentylenetetrazole (PTZ) induced convulsions in mice. All the derivatives were given satisfactory reaction yields that represent the efficiency of the employed synthetic route. In INH and PTZ induced convulsion model; T10, T12, T13 and T15 delayed the onset of convulsion significantly when compared to an induction control group. The compound with electron releasing group such CH_3, NH_2, OH etc. as a substitution showed activity in both the model while substitution of electron releasing group and electron withdrawing group showed selective action towards isoniazide induced convulsion.

Keywords: *Epilepsy, Thiophene, Isoniazid (INH), Pentylenetetrazole (PTZ), GABAa*

1. INTRODUCTION

Convulsion is one of the important CNS disorders growing with alarming speed [1]. The unattended and poorly managed convulsion cases may lead to complications which many times are beyond management [2]. A large number of anticonvulsant agents in the modern medicine are being used clinically but dose and duration dependent side effect including tolerance is one of the major limitations [3]. Thus there is an ongoing need to develop more antiepileptic drugs that are effective and endowed with improved safety profile [4-7].

Thiophene is class of heterocyclic compound containing a 5 membered ring that contains one sulphur as heteroatom with the molecular formula C_4H_4S. Thiophene and its derivatives are obtained from petroleum or coal. The word Thiophene is discovered from the Greek word Theion for sulphur, and other Greek word Phaino which is a synonym of shinning. Thiophene molecules got from certain natural resources and are also included in several pharmacologically active compounds.

According to medicinal chemistry, thiophene and its derivatives have been well known for their therapeutic properties. The simple thiophenes are stable liquid compounds that are similar to the corresponding benzene compounds by comparing their physicochemical parameters such as boiling point, odor and also, they found in coal tar distillates. Thiophene and its derivatives are structurally analogous to pyrrole and act as an extremely reactive benzene derivative attributed to their π electron cloud [8]. On this background current study titled synthesis and evaluation of anticonvulsant activity of some thiophene analogues was undertaken.

2. MATERIAL AND METHODS

2.1 Chemistry

All the compounds were characterized by IR, 1H and 13C NMR spectra recorded with Bruker WM- 300 in deuterated DMSO at 400 and 100 MHz, respectively using tetramethyl sylane (TMS) as the internal standard. All chemical shifts are reported on δ scale. Thin layer chromatography (TLC) was carried out using Merck silica gel 60 F-254 plates (layer thickness: 0.25 mm) and UV lamp; all solvents were distilled before using.

2.2 Synthesis:

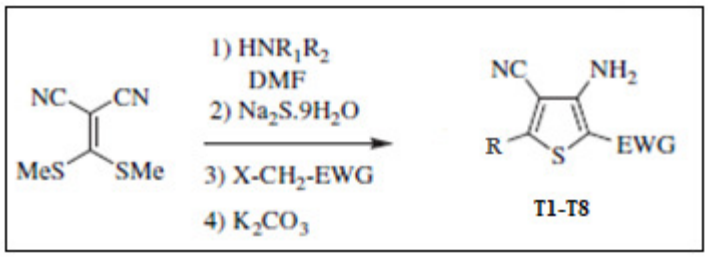


Figure 1: Synthesis of Thiophene derivatives (Scheme 1)

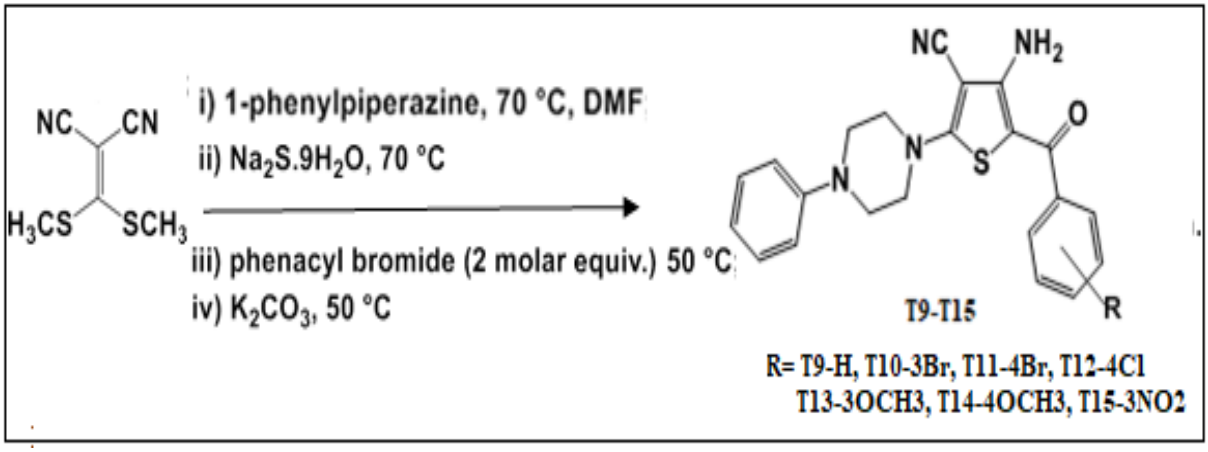


Figure 2: Synthesis of Thiophene derivatives (Scheme 2)

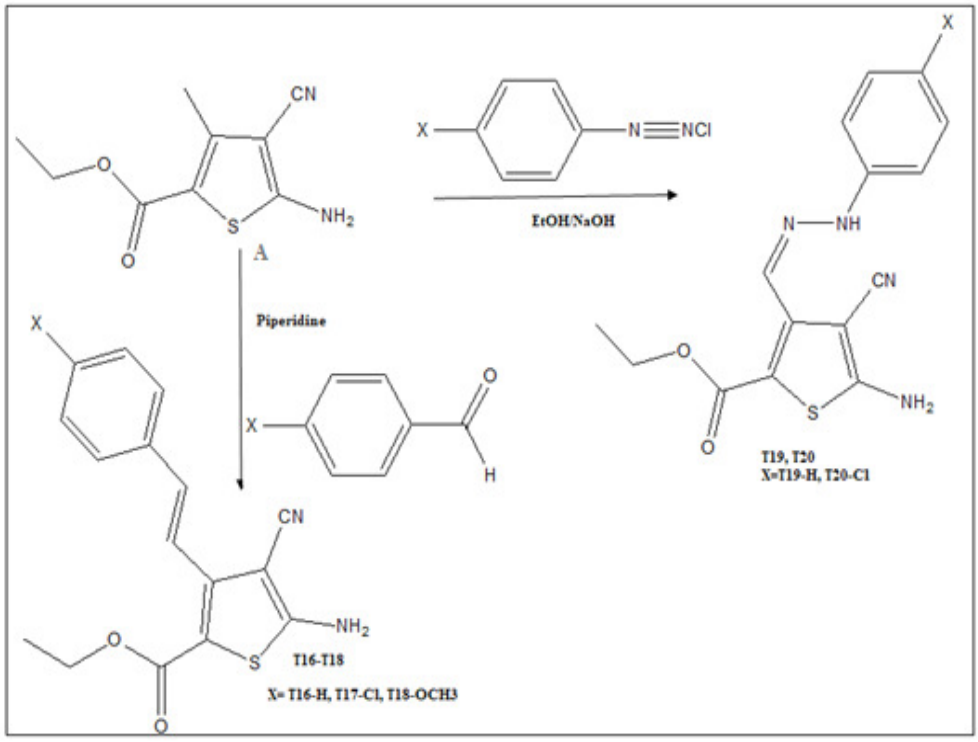


Figure 3: Synthesis of Thiophene derivatives (Scheme 3)

i) Synthesis of T1-T8:

2-[Bis (methylsulfanyl) methylene) malononitrile (0.01 mol) was dissolved in 15 ml DMF. The respective secondary amine (0.01 mol) was added and the mixture was heated at 70ºC for 75 min. Then, (0.01 mol) $Na_2S.9H_2O$ was added and heated for 2 h at 70ºC. The activated halide (0.02 mol) was added dropwise at 70ºC. The mixture was heated at 70 ºC for 2 h and the potassium carbonate (0.02 mol) was added. The reaction was stirred at 70ºC for 90 min more. The mixture was poured onto 100 ml water with good stirring. When a precipitate looked, it was filtered, washed with water, and dried at RT until constant weight. When a viscous mixture get, 25 ml of ether was added and the precipitate was filtered, washed with water, and dried at RT until constant weight. The isolated solid was purified by recrystallization in ethanol [9].

ii) Synthesis of T9-T15:

A mixture of 0.51 g 2-[Bis (methylsulfanyl) methylene] malononitrile and 0.47ml 1-phenylpiperazine in 5 ml DMF was stirred at 70°C for 75 min. Then, 0.72 g $Na_2S \cdot 9H_2O$ was added, and the stirring was continued at 70°C for additional 2 h. The corresponding phenacyl bromide (6 mmol) was added portion wise to the reaction mixture, and the reaction mixture was stirred for 2 h at 70°C. Lastly, the reaction was basified with 0.41 g K_2CO_3 and stirring was continued at 70°C for 90 min. After cooling, the reaction mixture was poured onto 50 ml ice water and stirred for 1 h. The precipitate formed was filtered, washed twice with 20 ml ethanol, then with 10 ml diethyl ether, dried and crystallized with methanol [10].

iii) Synthesis of T16-18:

To a solution of **A** (2.10 g) containing a catalytic amount of 0.5 ml piperidine, either 1.06 g benzaldehyde/1.40 g 4-chlorobenzaldehyde/1.36 g 4-methoxybenzaldehyde/ 1.22 g salicyaldehyde was added and heated in an oil bath at 120ºC for about 2 hours, then boiled in 20 ml ethanol for a few minutes. The solid products obtained upon pouring into an acidified ice/water mixture were crystallized from ethanol [11].

iv) Synthesis of T19-T20:

To a cold solution (0-5ºC) of A (2.10 g) in 20 ml ethanol (98%) containing 5 ml sodium hydroxide (10 %), an equimolar amount of either diazotized aniline (0.93 g)/ diazotized 4-chloroaniline (1.27g) /diazotized 4-methoxyaniline (1.23g)/diazotized *p*-toluidene (1.07 g) was gradually added under stirring. The solid products formed upon cooling in an ice bath were collected by filtration, washed with water and crystallized from ethanol [11].

2.3 Anticonvulsant activity

2.3.1. Isoniazid (INH) induced convulsions in mice

In present study, 48 mice (18-30g) divided into eight groups each consisting of six (n=6) animals. Test compounds were suspended in Carboxy Methyl Cellulose (CMC). Diazepam 10 mg/kg i.p. used as a reference standard. After 60 minute of dose administration, isoniazid (200 mg/kg, i.p.) was administered to all mice to induce convulsions. Immediately after INH injection, each mice was kept in a separate cage and observed for the convulsions. Onset of convulsions and percentage protection (i.e. number of mice survived after 60 minutes of INH induced convulsion) were recorded in each group and compared against control group. All results statistically analysed by one way ANOVA followed by Turkey-Kramer multiple comparison test [12].

2.3.2 Pentylenetetrazole (PTZ) induced convulsions in mice

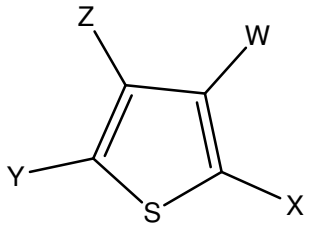
In PTZ induced convulsion model, 48 mice (18-30g) divided into eight groups each consisting of six (n=6) animals. Test compounds were suspended in Carboxy Methyl Cellulose (CMC). Diazepam 10 mg/kg i.p. used as a reference standard. After 60 minute of dose administration, pentylenetetrazole (80 mg/kg) was administered to all mice to induce convulsions. Immediately after PTZ injection, each mouse was kept in a separate cage and observed for the convulsions. Onset of convulsions and percentage protection (i.e. number of mice survived after 60 minutes of PTZ induced convulsion) were recorded in each group and compared against control group. All results statistically analysed by one way ANOVA followed by Turkey-Kramer multiple comparison test [13, 14].

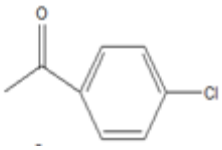
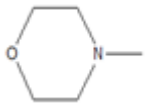
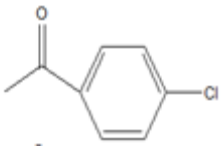
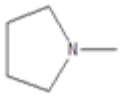
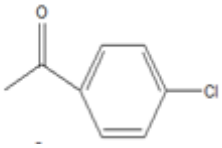
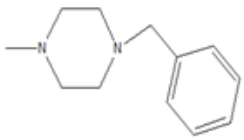
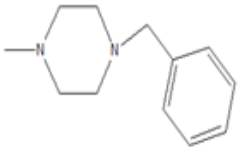
3. RESULTS AND DISCUSSION

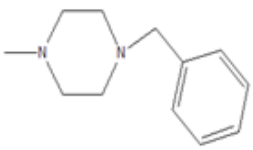
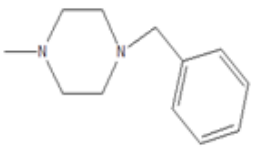
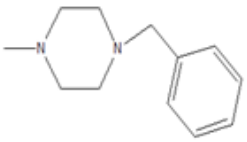
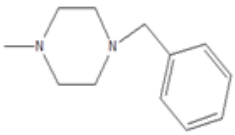
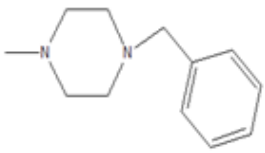
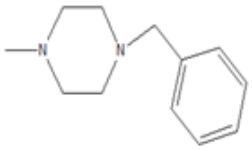
3.1 Physicochemical Characterization of Thiophene Derivatives

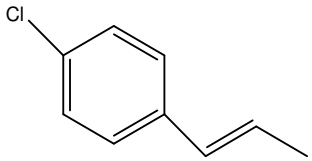
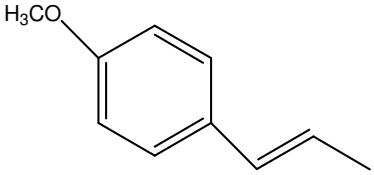
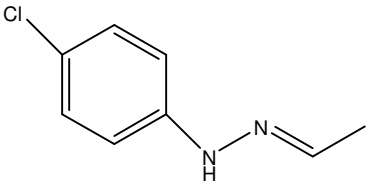
The table 1 shows physicochemical characterization of the compounds estimated in this study. All the compounds were given satisfactory reaction yields and properly separated from the reaction mixture, that representing the efficiency of the employed synthetic route.

Table 1: Physicochemical characteristics of Thiophene derivatives



Deri-vative	X	Y	Z	W	Yield (%)	Melting Point (°C)	Rf *
T-6			-CN	-NH_2	61	236-238	0.77
T-7			-CN	-NH_2	81	232-234	0.75
T-8			-CN	-NH_2	70	186-188	0.58
T-9		-COC_6H_5	-CN	-NH_2	74	204-206	0.67

T-10		-COC_6H_4(3-Br)	-CN	-NH_2	70	231-233	0.73
T-11		-COC_6H_4(4-Br)	-CN	-NH_2	71	226-228	0.68
T-12		-COC_6H_4(4-Cl)	-CN	-NH_2	74	217-219	0.64
T-13		-COC_6H_4(3-OCH_3)	-CN	-NH_2	68	208-210	0.56
T-14		-COC_6H_4(4-OCH_3)	-CN	-NH_2	78	221-223	0.81
T-15		-COC_6H_4(3-NO_2)	-CN	-NH_2	85	243-245	0.76

T-17	$-NH_2$	$-CO_2Et$		-CN	86	91-93	0.62
T-18	$-NH_2$	$-CO_2Et$		-CN	90	93-95	0.84
T-20	$-NH_2$	$-CO_2Et$		-CN	58	118-120	0.60

Rf* Solvent system used for TLC was Chloroform: Methanol (40:60)

The Characterization of synthesized derivatives was carried out by using Infrared spectroscopy (IR), Proton NMR (H1 NMR) and Carbon NMR (C13 NMR) for structure elucidation (Table 2).

Table 2: Characterization of synthesized derivatives using IR, H1 NMR and C13 NMR

T6	IR	1328 (C-N), 2258 (C≡N), 1694 (C=O), 647(C-S-C), 2936(C-H stretching)
	H1 NMR	δ 3.05 (ddd, 4H, *J* = 2.5, 6.7,15.8Hz), 3.71 (ddd,4H, *J* = 2.5, 6.7, 15.5 Hz), 7.52 (ddd,2H, *J* = 0.5, 1.7, 8.7Hz), 7.88 (ddd,2H, *J* = 0.5, 1.8, 8.7Hz)
	C13 NMR	81.27, 95.92, 150.35, 157.78 (4C; Thiophene CH), 183.72 (Carbonyl;1C), 133.27, 138.22 (Benzene C;2C), 129.04, 131.15 (Benzene CH;4C), 46.52, 66.48 (Cyclohexane CH_2;4C), 115.35 (Nitrile;1C)
	Mass	Calculated: 347.82 g/mol, Found: 347.60 g/mol (M+H)

T7	IR	3427 (Ar-NH_2), 3269 (Ar), 2196 (C≡N), 1705 (C=O),832(C-Cl)
	H1 NMR	δ 2.04 (dtdd, 4H, *J* = 3.5,3.9,7.4,11.3Hz), 3.45 (ddd, 4H, *J* = 4.0, 7.3, 15.2Hz), 7.58 (ddd, 2H, *J* = 0.5, 1.7, 8.7 Hz), 7.95 (ddd, 2H, *J* = 0.5, 1.8, 8.7Hz)
	C13 NMR	81.26, 95.92, 150.35, 157.71 (4C; Thiophene CH), 183.76 (Carbonyl;1C), 133.28, 138.23 (Benzene C;2C), 129.0, 131.15 (Benzene CH;4C), 52.27, 25.56 (Pyrrolidine CH_2;4C), 115.33 (Nitrile;1C)
	Mass	Calculated: 331.82 g/mol, Found: 331.75 g/mol (M+H)
T8	IR	3279 (Aromatic C-H), 2202 (C≡N), 1684 (C=O), 695 (C-Cl)
	H1 NMR	δ 2.71 (ddd, 4H, *J* = 2.5, 6.7,17.7Hz), 3.05 (ddd, 4H, *J* = 2.5, 6.7, 13.9 Hz), 3.68 (s, 2H), 7.17-7.34 (5H, 7.23 (dddd, *J* = 0.5, 0.9, 1.4,7.8 Hz), 7.27 (tdd, *J* = 0.5, 1.8, 7.7 Hz), 7.34 (tt, *J* = 1.4,7.7 Hz)), 7.55 (ddd, 2H, *J* =0.5, 1.7, 8.7Hz), 7.96 (ddd, 2H, *J* = 0.5, 1.8, 8.7Hz)
	C13 NMR	81.25, 95.92, 150.36, 157.74 (Thiophene CH;4C), 183.75 (Carbonyl;1C), 133.29, 138.16, 135.67 (Benzene C; 3C), 129.02, 131.16, 128.93, 128.57, 127.32 (Benzene CH; 9C), 52.27, 25.52 (Cyclohexane CH_2;4C), 60.16 (Aliphatic CH;1C), 115.35 (Nitrile;1C)
	Mass	Calculated: 436.96g/mol, Found:436.82 g/mol (M+H)

T9	IR	3371,3271 (NH2), 2198 (C≡N), 1612 (C=O)
	H1 NMR	δ3.09-3.29 (8H, 3.17 (ddd, *J* = 2.5,6.7,14.4Hz), 3.24 (ddd, *J* = 2.5,6.7,14.3Hz)), 6.65-6.93 (3H, 6.72 (dtd, *J* = 0.5, 1.2, 8.3Hz), 6.86 (tt, *J* = 1.2, 8.1 Hz)), 7.21 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3Hz), 7.38-7.70 (5H, 7.46 (dddd, *J* = 0.4, 1.5,7.6, 8.4 Hz), 7.62 (dddd, *J* = 0.4, 1.5, 1.7, 8.4 Hz), 7.67 (tt, *J* = 1.5,7.6 Hz))
	C13 NMR	81.28, 95.91, 150.36, 157.72 (Thiophene CH;4C), 183.75 (Carbonyl;1C), 135.13, 149.65 (Benzene C;2C), 129.74, 128.98, 132.73, 114.36, 118.35 (Benzene CH;10C), 49.82, 49.69 (Cyclohexane CH_2;4C), 115.35 (Nitrile;1C)
	Mass	Calculated: 388.49g/mol, Found: 388.32 g/mol (M+H)
T10	IR	3394, 3259 (NH2); 2198 (C≡N); 1597 (C=O)
	H1 NMR	δ3.11-3.35 (8H, 3.23 (ddd, *J* = 2.5, 6.7, 14.4 Hz), 3.21 (ddd, *J* = 2.5, 6.7, 14.3 Hz)), 6.67-6.93 (3H, 6.74 (dtd, *J* = 0.5, 1.2, 8.3 Hz), 6.85 (tt, *J* =1.2, 8.1Hz)), 7.25 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3Hz), 7.31-7.56 (2H, 7.42 (ddd, *J* =1.3, 1.7, 7.8 Hz), 7.52 (td, *J* = 0.4,7.8 Hz)), 7.73 (ddd,1H, *J* = 0.4, 1.7, 1.9 Hz), 7.86 (ddd, 1H, *J* = 1.3, 1.9, 7.8Hz)
	C13 NMR	81.25, 95.91, 150.32, 157.77 (Thiophene CH;4C), 183.72 (Carbonyl;1C), 137.32, 123.27, 149.65 (Benzene C;3C), 114.32, 118.31, 128.75, 129.77, 131.18, 133.26, 135.61 (Benzene CH; 9C), 49.84, 49.67 (CyclohexaneCH_2;4C), 115.29 (Nitrile;1C)

	Mass	Calculated: 467.38 g/mol, Found: 467.25 g/mol (M+H)
T11	IR	3383, 3282 (NH2); 2198 (C≡N); 1600 (C=O)
	H1 NMR	δ 3.12-3.33 (8H, 3.17 (ddd, *J* = 2.5, 6.7, 14.4 Hz), 3.24 (ddd, *J* = 2.5, 6.7, 14.3 Hz)), 6.68-6.92 (3H, 6.73 (dtd, *J* = 0.5,1.2,8.3Hz), 6.88 (tt, *J* = 1.2 8.1 Hz)), 7.24 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3 Hz), 7.67 (ddd, 2H, *J* = 0.5, 1.4, 8.6Hz), 7.94 (ddd, 2H, *J* = 0.5, 1.7, 8.6 Hz)
	C13 NMR	81.25, 95.97, 150.32, 157.71 (Thiophene CH; 4C), 183.74 (Carbonyl;1C), 137.31, 123.27, 149.62 (BenzeneC;3C), 128.68, 131.15, 135.67, 133.22, 114.34, 129.78, 118.39 (Benzene CH; 9C), 49.86, 49.61 (Cyclohexane CH_2;4C), 115.28 (Nitrile;1C)
	Mass	Calculated: 467.38 g/mol, Found: 467.32 g/mol (M+H)
T12	IR	3383, 3286 (NH2), 2198 (C≡N), 1600 (C=O)
	H1 NMR	δ3.12-3.33 (8H, 3.19 (ddd, *J* = 2.5, 6.7, 14.4 Hz), 3.24 (ddd, *J* = 2.5,6.7,14.3Hz)), 6.68-6.93 (3H, 6.74 (dtd, *J* = 0.5, 1.2, 8.3Hz), 6.85 (tt, *J* = 1.2, 8.1 Hz)), 7.26 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3Hz), 7.54 (ddd, 2H, *J* = 0.5, 1.7,8.7Hz), 7.95 (ddd, 2H, *J* = 0.5, 1.8, 8.7Hz)
	C13 NMR	81.23, 95.96, 150.35, 157.74 (Thiophene CH; 4C), 183.71 (Carbonyl;1C), 133.27, 138.22, 149.68 (Benzene C;3C), 131.16, 129.04, 114.37, 129.72, 118.39 (Benzene CH; 9C), 49.85, 49.61 (Cyclohexane CH_2; 4C), 115.28 (Nitrile;1C)

	Mass	Calculated: 422.93 g/mol, Found: 422.85 g/mol (M+H)
T13	IR	3375, 3275 (NH2), 2954, 2927, 2831 (aliphatic C–H), 2198 (C≡N), 1612 (C=O)
	H1 NMR	δ 3.11-3.29 (8H, 3.19 (ddd, *J* = 2.5, 6.7, 14.4 Hz), 3.21 (ddd, *J* = 2.5, 6.7, 14.3Hz)), 3.76 (s, 3H), 6.67-7.05 (4H, 6.72 (dtd, *J* = 0.5, 1.2, 8.3 Hz), 6.89 (tt, *J* = 1.2,8.1 Hz), 6.97 (ddd, *J* = 1.4, 1.9, 8.2Hz)), 7.22 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3Hz), 7.43 (td,1H, *J* = 0.5, 8.2 Hz), 7.64 (ddd, 1H, *J* =0.5, 1.6, 1.9 Hz), 7.88 (ddd,1H, *J* = 1.4, 1.6, 8.1 Hz)
	C13 NMR	81.21, 95.95, 150.37, 157.71 (Thiophene CH; 4C), 183.72 (Carbonyl;1C), 136.18, 149.62, 160.86 (Benzene C;3C), 122.03, 129.94, 118.28, 113.91, 114.34, 129.78, 118.36 (Benzene CH; 9C), 49.82, 49.67 (Cyclohexane CH_2; 4C), 115.32 (Nitrile;1C), 55.92 (Aliphatic CH; 1C)
	Mass	Calculated: 418.51 g/mol, Found: 418.44 g/mol (M+H)
T14	IR	3379,3278 (NH2), 2935, 2835 (aliphatic C–H), 2202 (C≡N), 1604 (C=O)
	H1 NMR	δ 3.12-3.30 (8H, 3.17 (ddd, *J* = 2.5, 6.7, 14.4Hz), 3.24 (ddd, *J* = 2.5, 6.7, 14.3Hz)), 3.83 (s, 3H), 6.67-6.92 (3H, 6.73 (dtd, *J* = 0.5, 1.2, 8.3 Hz), 6.86 (tt, *J* = 1.2, 8.1 Hz)), 7.04 (ddd, 2H, *J* = 0.5, 1.2, 8.3 Hz), 7.21 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3Hz), 7.57 (ddd, 2H, *J* = 0.5, 1.8, 8.3Hz)
	C13 NMR	81.17, 95.92, 150.38, 157.76 (Thiophene CH; 4C),

		183.75 (Carbonyl;1C), 127.42, 149.63, 164.52 (BenzeneC;3C), 130.73, 114.45, 114.39, 129.72, 118.34 (Benzene CH; 8C), 49.82, 49.67 (Cyclohexane CH_2; 4C), 115.27 (Nitrile; 1C), 55.94 (Aliphatic CH;1C)
	Mass	Calculated: 418.51 g/mol, Found: 418.29 g/mol (M+H)
T15	IR	3394, 3263 (NH2), 2198(C≡N), 1600 (C=O), 1527, 1342 (NO2)
	H1 NMR	δ 3.14-3.33 (8H, 3.21 (ddd, *J* = 2.5, 6.7, 14.4 Hz), 3.21 (ddd, *J* = 2.5, 6.7, 14.3 Hz)), 6.67-6.92 (3H, 6.73 (dtd, *J* = 0.5, 1.2, 8.3Hz), 6.85 (tt, *J* = 1.2, 8.1 Hz)), 7.28 (dddd, 2H, *J* = 0.5, 1.4, 8.1, 8.3 Hz), 7.68 (ddd,1H, *J* = 0.4, 7.8, 8.3 Hz), 8.05-8.22 (2H, 8.11 (dt, *J* = 1.8, 7.8 Hz), 8.16 (dt, *J* = 1.8, 8.3 Hz)), 8.74 (ddd,1H, *J* = 0.4, 1.7, 1.9 Hz)
	C13 NMR	81.22, 95.97, 150.33, 157.79 (Thiophene CH; 4C), 183.76 (Carbonyl;1C), 136.02, 148.57, 149.61 (Benzene C;3C), 135.84, 129.76, 125.01, 124.67, 114.39, 129.72, 118.38 (Benzene CH; 9C), 49.87, 49.65 (Cyclohexane CH_2; 4C), 115.39 (Nitrile;1C)
	Mass	Calculated: 433.48 g/mol, Found: 433.36 g/mol (M+H)
T17	IR	3397–3204 (NH2), 2979–2859 (CH2, CH3), 2205 (CN), 1677 (C=O), 1634,1495 (C=C)
	H1 NMR	δ 1.17 (t, 3H, *J* = 7.1 Hz), 4.07 (q, 2H, *J* = 7.1 Hz), 7.19-7.49 (5H, 7.25 (ddd, *J* = 0.5, 1.4, 8.2Hz), 7.35 (ddd, *J* = 0.5, 1.5, 8.2 Hz), 7.46 (d, *J* = 16.2 Hz)), 7.79 (d,1H, *J* = 16.1 Hz)

	C13 NMR	111.07, 118.24, 144.13, 146.59 (Thiophene CH; 4C), 160.62 (Carbonyl; 1C), 133.52, 133.65 (Benzene C; 2C), 127.82, 128.85 (Benzene CH; 4C), 115.31 (Nitrile;1C), 127.45, 133.38 (Ethylene CH; 2C), 60.94 (Aliphatic CH_2; 1C), 14.2 (Aliphatic CH_3; 2C)
	Mass	Calculated: 332.80 g/mol, Found: 332.72 g/mol (M+H)
T18	IR	3399–3204 (NH2), 2931–2840 (CH2, 2CH3), 2204 (CN), 1677 (C=O), 1632,1502 (C=C)
	H1 NMR	δ 1.22 (t, 3H, *J* = 7.1 Hz), 3.76 (s, 3H), 4.06 (q, 2H, *J* = 7.1 Hz), 7.05 (ddd, 2H, *J* = 0.4, 1.3, 8.8 Hz), 7.24 (ddd, 2H, *J* = 0.4, 1.9, 8.8 Hz), 7.37 (d, 1H, *J* = 15.4 Hz), 7.75 (d, 1H, *J* = 15.3 Hz)
	C13 NMR	111.27, 118.21, 144.39, 146.16 (Thiophene CH; 4C), 160.62 (Carbonyl;1C), 127.52, 159.98 (Benzene C; 2C), 114.26, 127.82 (Benzene CH; 4C), 115.31 (Nitrile; 1C), 127.45, 133.38 (Ethylene CH; 2C), 60.94 (Aliphatic CH_2; 1C), 14.19, 55.94 (Aliphatic CH_3; 2C)
	Mass	Calculated: 328.80 g/mol, Found: 328.78 g/mol (M+H)
T20	IR	3425–3201 (NH, NH2), 3100 (CH aromatic), 2900 (CH2, CH3), 2216(CN), 1672 (C=O), 1600, 1486 (C=C),1520 (=N–NH)
	H1 NMR	δ 1.29 (t, 3H, *J* = 7.1 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 7.47 (ddd, 2H, *J* = 0.5, 1.6, 8.4Hz), 7.65 (ddd, 2H, *J* = 0.5, 1.8, 8.4 Hz), 8.26 (s, 1H)
	C13 NMR	112.38, 123.92,136.27, 145.52 (Thiophene CH; 4C),

		160.61 (Carbonyl; 1C), 124.38,141.21 (Benzene C; 2C), 117.75, 129.68 (Benzene CH; 4C), 115.28 (Nitrile; 1C), 60.94 (Aliphatic CH_2; 1C), 14.16 (Aliphatic CH_3;1C), 143.27 (Imine CH; 1C)
	Mass	Calculated: 348.81 g/mol, Found: 348.74 g/mol (M+H)

3.2 Anticonvulsant activity

3.2.1. Isoniazid (INH) induced convulsions in mice

In the Isoniazid induced convulsions, Isoniazid at 200 mg/kg, i.p., exhibited mean onset of convulsions at 1203 seconds in induction control group animals. In test groups, one hour prior intraperitoneal administration of Thiophene derivatives at 100mg/kg to INH, delayed the onset of convulsion significantly T10, T12, T13 and T15 ($P<0.01$, $P<0.001$, $P<0.01$ and $P<0.001$respectively) when compared to induction control group. Whereas delayed onset of convulsion was non-significant for T6, T7, T8, T9, T11, T14, T17, T18 and T20.

3.2.2 Pentylenetetrazole (PTZ) induced convulsions in mice

The mean onset of convulsions in Pentylenetetrazole (80 mg/kg i.p.) induction control group observed at 335 seconds. In test groups, one hour prior intraperitoneal administration of Thiophene derivatives at 100mg/kg to PTZ delayed the onset of convulsion significantly for T10, T12 and T15 ($P<0.01$) were found to show more significant in increasing the onset of action with % protection ranging from 66.66-100%. Whereas delayed onset of convulsion was non-significant for T8 and T14.

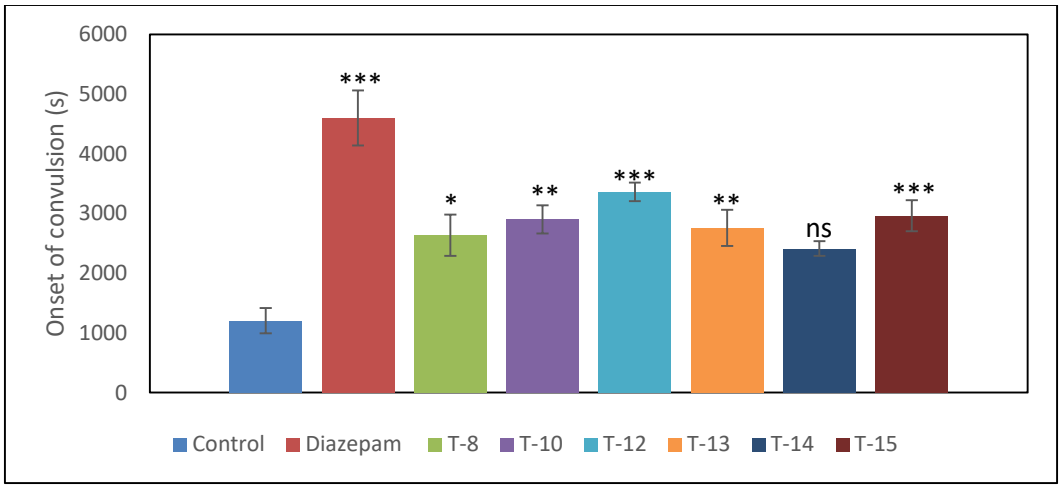


Figure 4. Effect of Thiophene derivatives in Isoniazide induced convulsions in mice

*Test drugs: significant from normal control, * P < 0.05; ** P < 0.001*

Mean ± S.E.M = Mean values ± Standard error of means of six experiments

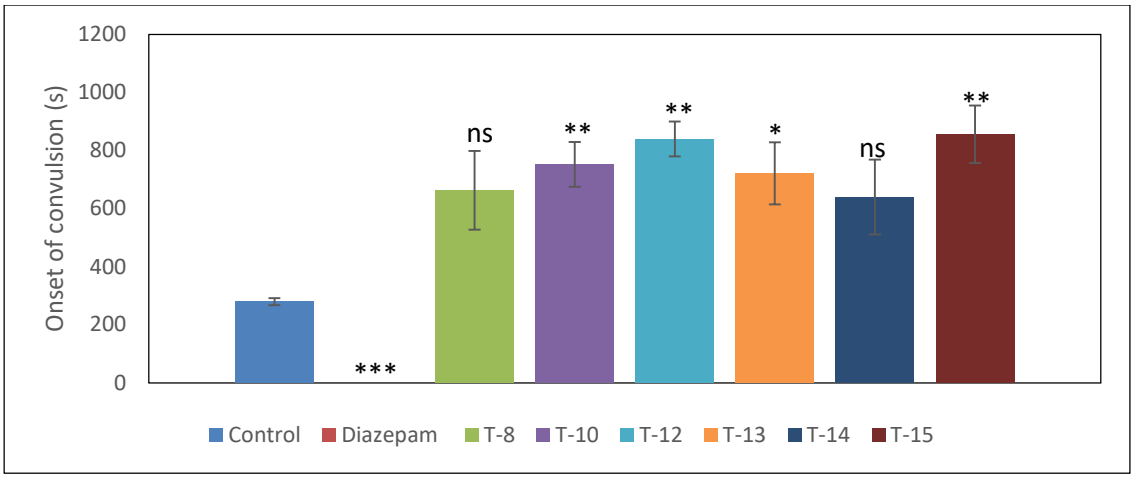


Figure 5. Effect of Thiophene derivatives in PTZ induced convulsions in mice

*Test drugs: significant from normal control, * P < 0.05; ** P < 0.001*

Mean ± S.E.M = Mean values ± Standard error of means of six experiments

4. CONCLUSION

Convulsions being an important symptom of epilepsy precipitates either due to enhanced activity of excitatory neurotransmitters like Glutamate or depletion of inhibitory neurotransmitter like GABA in the CNS. Current pharmacotherapy for convulsions reported limited therapeutic outcome and are associated with many deleterious side effects [15, 16]. This indicates the need

to develop new drugs for the treatment of convulsions with improved therapeutic outcome. Hence, in the present study preclinical trials for different derivatives of Thiophene compounds were carried out for anticonvulsant activity in chemical (Isoniazid and PTZ) induced convulsions in mice.

INH reported to inhibit GABA synthesis and glutamic acid decarboxylase by inhibiting pyridoxine phosphokinase and thereby depleting GABA concentration in the CNS and precipitate the convulsions [17, 18]. In present study Thiophene derivative T-8, T-10, T-12, T-13 and T-15 at 100 mg/kg,i.p, significantly delayed the mean onset of convulsion when compared to induction control group. This indicates the anticonvulsant activity to these derivatives which might be due to antagonizing the INH effect on GABA synthesis.

PTZ induced convulsions is a well-established animal model for absence seizure. PTZ is GABA antagonists leads to CNS stimulation that produces convulsion in mice [19, 20]. Thiophene derivatives T10, T12, T13, T14 and T15 prolonged mean onset of convulsions significantly when compared to induction control group. This indicates the anticonvulsant activity to these derivatives which might be due to potentiating GABA activity in the CNS.

On the basis of result, it can be concluded that Thiophene derivatives exhibited anticonvulsant activity. All synthetic compounds of Thiophene derivatives showed prolonged onset of convulsions and protecting maximum animals suggesting increase level of GABA by its potentiation or prevention its depletion.

CONSENT

The present study did not involve Patients.

COMPETING INTERESTS

Authors have declared that no competing.

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