Original article

# Synthesis and anticonvulsant activity of some new bishydrazones derived from 3,4-dipropyloxythiophene

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## ABSTRACT

A series of new 3,4-dipropyloxy-$N^2$,$N^5$-bis(substituted)thiophene-2,5-dicarbohydrazides (**4–30**) were synthesized from ethyl thiodiglycolate and diethyloxalate through multistep reactions. Following Dieckmann–Komppa reaction, the required precursor 3,4-dihydroxythiophene-2,5-diester (**1**) was prepared. This was derivatized with propyl bromide and further converted to corresponding hydrazide (**3**), which was finally transformed to targeted hydrazones (**4–30**) by conventional methods. The newly synthesized compounds were characterized using FT-IR, $^1$H and $^{13}$C NMR, EI-MS and elemental analyses. The anticonvulsant activity of all the title compounds was investigated against maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (scMET) models and their neurotoxicity was also evaluated. Some of the selected compounds were subjected to 6 Hz test in order to evaluate their uncover activities. Compound 3,4-dipropyloxy-$N^2$,$N^5$-bis[1-(2-thienyl)ethylidene]thiophene-2,5-dicarbohydrazide (**15**) has emerged as a lead in this series with less neurotoxicity.

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## 1. Introduction

Epilepsy is one of the most common neurological disorders of the brain, due to sudden burst of abnormal electrical discharges. It has been reported that people suffering from epilepsy is increasing day by day in the world [1]. More than 20% of the world epileptic populations are not contented with the marketed antiepileptic drugs (AEDs). However, all currently approved anticonvulsant agents have dose-related toxicity and idiosyncratic side effects [2]. Some of the currently available active drugs have not been directly linked with any specific binding site to receptor within the brain. It is too difficult to identify common pharmacophore responsible for prevention or arrest of seizure activity mainly because of the chemical diversity of organic compounds and their multiple mechanism of action in controlling the seizures [3]. Hence, the search for antiepileptic compounds with more selective activity and minimum toxicity continues to be an area of investigation in medicinal chemistry.

Thiophene derivatives have been known to possess a variety of biological activities such as anti-inflammatory [4], analgesic [5], antidepressant [6], antimicrobial [7] and anticonvulsants [8–10]. Presently available active AEDs like tiagabine [8], etizolam [10] are

containing thiophene moiety in their structure as active pharmacophore. Also, the higher activity of sodium phethenylate [9] is due to the presence of thiophene ring in its structure. On the other hand, many carbazones and semicarbazones have been documented as potent anticonvulsants [11–16]. Their activity is mainly attributed to the presence of aryl binding site with aryl/alkyl hydrophobic group, hydrogen bonding domain and electron donor group, which are the essential requirements [17] for the molecules to show potential activity. Further, introduction of the one more aryl ring in the structure brings about the enhanced van der Waals bonding at the receptor site that leads to increased potency.

The literature survey reveals that very few reports appeared on anticonvulsant activity of different hydrazones [11,12], but none on thiophene carbohydrazides. Against this background, it has been thought of synthesizing novel thiophene derivatives containing bishydrazones at position 2 and 5 and propyloxy group at positions 3 and 4, hoping that the resulting molecules would show enhanced anticonvulsant activity. In the present molecular design, the thiophene ring with propyloxy group acts as electron releasing group and bishydrazones containing aryl/alkyl groups provide effective hydrogen bonding and van der Waals bonding with receptor binding site. Accordingly, Fig. 1 shows the combination of various pharmacophoric features required for an effective anticonvulsant. In continuation of our research program on the synthesis of novel thiophene derivatives exhibiting potential anticonvulsant activity, we herein report the multistep synthesis of hitherto unknown 3,4-dipropyloxy-$N^2$,$N^5$-bis(substituted) thiophene-2,5-dicarbohydrazides (**4–30**) and

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HB - Hydrogen Bonding Domain;   E - Electron Donor;   A - Aryl binding site
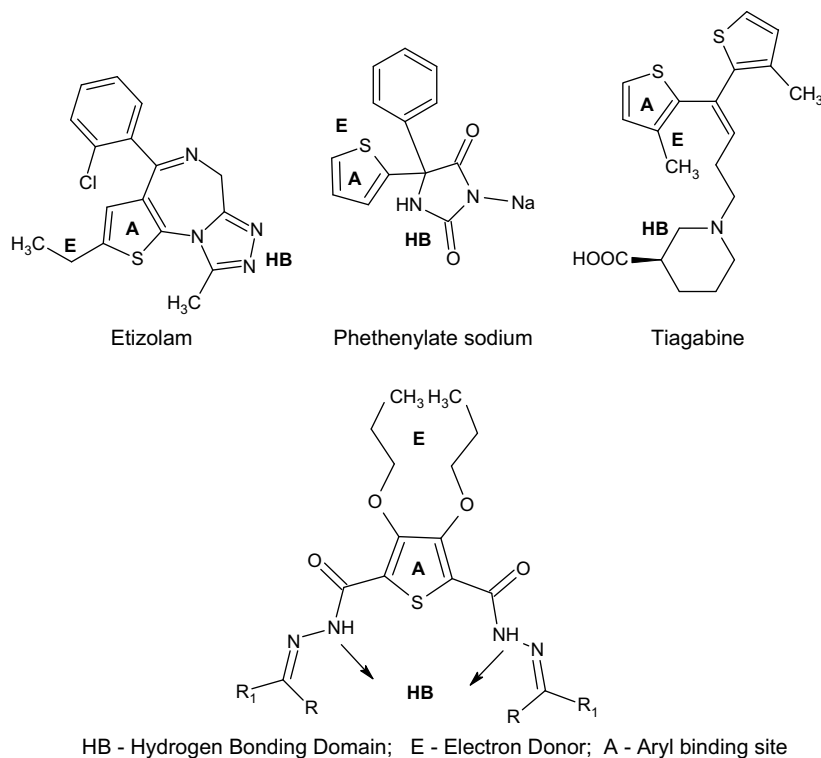
**Fig. 1.** Molecular design of new thiophene based antiepileptic agents by pharmacophore combination.

screening of their anticonvulsant activity by MES, scMET, and 6 Hz models and neurotoxicity.

## 2. Chemistry

The reaction sequences employed for synthesis of title compounds, viz. 3,4-dipropyloxy-$N^2$,$N^5$-bis(substituted)thiophene-2,5-dicarbohydrazides (**4**–**30**) are shown in Scheme 1.

The starting material, thiodiglycolic ester was prepared by refluxing the corresponding acid and absolute alcohol in the presence of sulfuric acid. This ester was condensed with diethyloxalate in the presence of sodium ethoxide to obtain compound (**1**). This compound was substituted with propyl bromide in the presence of anhydrous potassium carbonate to get compound (**2**). The key intermediate, 3,4-dipropoxythiophene-2,5-dicarbohydrazide (**3**) was synthesized by condensing corresponding ester (**2**) with hydrazine hydrate in methanol. It was then converted to hydrazones by condensing it with appropriate aryl/alkyl aldehydes or ketones in the presence of Conc. HCl as catalyst. The physiochemical properties and characterization data of compounds **4**–**30** are summarized in Tables 1 and 2.

The structural assignments to new compounds were based on their elemental analysis and spectral (FT-IR, $^1$H NMR, $^{13}$C NMR and Mass) data. The formation of hydrazide **3** from the corresponding ester **2** was confirmed by its IR and $^1$H NMR spectral data. Its IR spectrum showed bands at 3286, 3191 and 1641 cm$^{-1}$ indicating the presence of –NH$_2$, >NH and >C=O groups respectively. In its $^1$H NMR spectrum it displayed two triplet peaks at $\delta$ 0.9 (–CH$_3$), 4.1 (–OCH$_2$–) and multiplet at 1.8 (–CH$_2$–). Further it showed broad peaks at 5.0 (–NH$_2$) and singlet at 8.3 (–NH–) conforming the formation of hydrazide group in the molecule.

The condensation of carbonyl compounds with hydrazide **3** to form hydrazone **4** was evidenced from its spectral data. Its IR spectrum showed absorption bands at 3211, 2969, 1642 and 1601 cm$^{-1}$ indicating the presence of >NH, propyl, >C=O and >C=N groups respectively. The absence of absorption bands due to –NH$_2$ group at

3286 cm$^{-1}$ and –NH– group at 3191 cm$^{-1}$ of hydrazide (**3**) conformed the product formation. The compounds **5**–**30** behave similarly. $^1$H NMR spectrum of it showed two triplets at $\delta$ 0.9 (–CH$_3$), 4.2 (–OCH$_2$–) and multiplet (–CH$_2$–) at 1.8 confirming the presence of propyloxy group. The azomethine proton resonated at $\delta$ 8.4 (–CH=N–) as singlet and the aromatic protons appeared as multiplet at $\delta$ 7.4–7.7. The lone >CONH– proton resonated as sharp singlet at $\delta$ 11.2, while the absence of broad peak at $\delta$ 5.0 (–NH$_2$) and sharp singlet at $\delta$ 8.3 (–NH–) (which correspond to the peaks of bishydrazide) further confirmed the formation of condensation product **4**. The spectral data of other hydrazones are given in the Experimental section.

The structure of hydrazone **4** was further confirmed by its Mass spectrum. It displayed the molecular ion peak at m/z 492 (100%), which is in agreement with the molecular formula C$_{26}$H$_{28}$N$_4$O$_4$S. The major peaks at m/z 389, 372, 347 and 289 were due to the fragmentation of molecular ion leading to formation of important species with complex structures, as shown in Scheme 2. It has been also seen that similar fragmentation patterns were observed for other hydrazone derivatives.

## 3. Pharmacology

The newly synthesized compounds were evaluated for anticonvulsant activity by the maximal electroshock (MES), subcutaneous metrazol (MET) and neurotoxicity screening by Rotorod technique. Some of the selected compounds were evaluated in 6 Hz model at five time intervals. The results of anticonvulsant activity of newly synthesized compounds **4**–**30** are presented in Tables 3 and 4. The detailed test procedures are given the Experimental section.

## 4. Results and discussion

The results of MES test reveal that compounds **14**, **15**, **20**, **28**, **29** and **30** showed activity at a dose of 300 mg/kg (1/1 of the animals protected at 4 h). The presence of coumarin, phenyl and thiophene
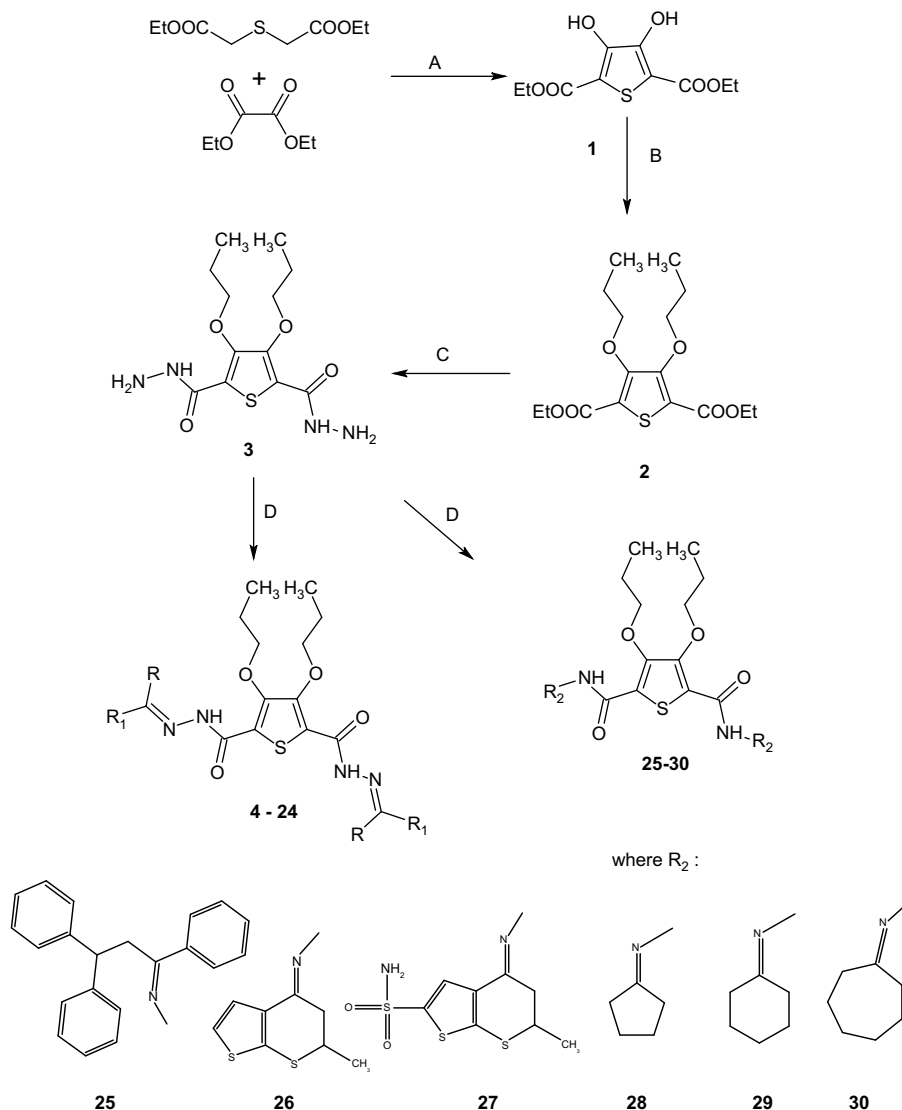
**Scheme 1.** Synthesis of substituted bishydrazones. A: NaOEt, H$^+$; B: CH$_3$CH$_2$CH$_2$Br, K$_2$CO$_3$, DMF; C: NH$_2$NH$_2$ H$_2$O, EtOH; D: aldehyde/ketone, EtOH, H$^+$.

moieties along with methyl group attached to azomethine carbon of hydrazones showed marginal activity at higher concentration. However, at lower concentration no activity was observed probably due to lack of hydrogen bonding with receptor site. The marginal activity of compounds **28**, **29** and **30** may be due to the presence of alicyclic substituents. The size of the alicyclic substituent did not affect the activity. The results showed that compounds **19** and **22** displayed protection at 100 mg/kg dose (1/7 animals protected at 0.5 h). But this effect was not found to be linear at higher dose. The observed activity in MES screening may be due to the presence of electron releasing substituents in hydrazones. In scMET model, compounds **4**, containing phenyl group and **15**, containing thiophene and methyl groups showed marginal efficacy at 300 mg/kg dose (1/5 of the animals protected at 4 h) and compound **22** showed protection at a dose 30 mg/kg (1/5 of the animals protected at 4 h).

As seen from the results of neurotoxicity screening, compounds **6** and **26** showed toxicity at a dose 30 mg/kg. It has been observed that compounds **5**, **9**, **10**, **11**, **12**, **20**, **23** and **27** exhibited toxicity at

a dose level of 100 mg/kg, whereas compounds **8**, **21** and **25** showed toxicity at 300 mg/kg. The remaining compounds did not show neurotoxicity at the maximum administered dose level of 300 mg/kg. These results clearly indicated that toxicity is not attributed to the basic moiety, i.e., 3,4-dipropyloxy thiophene, but it is due to the presence of other substituents such as pyridine, carbazole, furan, indole, coumarin and certain alkyl groups attached to it. The observed toxicity in the above compounds may be due to the active involvements of these substituents in the metabolism.

Some of the compounds were selected for 6 Hz model to identify their activity at five different time points, i.e., 0.25, 0.5, 1.0, 2.0 and 4 h. As observed from the results, amongst various tested hydrazones, compounds **4**, **7** and **15** showed good response at a dose 100 mg/kg and hence they emerged as lead compounds. These compounds have been selected for second phase of screening in order to explore their efficacy in detail. It is interesting to note that presence of methyl and thiophene moieties as substituents attached to azomethine group of title compound (**15**) has increased

**Table 1**
Physical characterization data of new compounds, **4**–**24**.

| Cd No | R | R$_1$ | Mol. for/Mol. mass | Recry. Sol | Elemental analysis (%): Found (cal.) | | | | Mp (°C)/yield % |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | S | |
| 4 | H | C$_6$H$_5$ | C$_{26}$H$_{28}$N$_4$O$_4$S/492 | MDC | 63.52 (63.40) | 5.67 (5.73) | 11.43 (11.37) | 6.42 (6.51) | 229–230/45 |
| 5 | H | 3-Indolyl | C$_{30}$H$_{30}$N$_6$O$_4$S/570 | DMF | 62.58 (63.14) | 5.19 (5.30) | 14.88 (14.73) | 5.69 (5.62) | 294–295/55 |
| 6 | H | 5-Nitro-2-furyl | C$_{22}$H$_{22}$N$_6$O$_{10}$S/562 | DMF | 46.62 (46.97) | 3.85 (3.94) | 15.01 (14.94) | 5.82 (5.70) | 280–281/51 |
| 7 | H | 3,4,5-Meth-oxyphenyl | C$_{32}$H$_{40}$N$_4$ O$_{10}$S/672 | DMF | 57.01 (57.13) | 5.85 (5.99) | 8.45 (8.33) | 4.62 (4.77) | 297–298/57 |
| 8 | H | 2-Fluorenyl | C$_{40}$H$_{36}$N$_4$O$_4$S/668 | DMF | 71.42 (71.83) | 5.31 (5.43) | 8.23 (8.12) | 4.89 (4.79) | 304–205/45 |
| 9 | H | 2-Thienyl | C$_{22}$H$_{24}$N$_4$O$_4$S$_3$/504 | DMF | 52.30 (52.36) | 4.68 (4.79) | 11.32 (11.10) | 19.11 (19.06) | 259–260/57 |
| 10 | H | 1-Butyl | C$_{20}$H$_{32}$N$_4$O$_4$S/424 | MDC | 56.41 (56.58) | 7.52 (7.60) | 13.39 (13.20) | 7.48 (7.55) | 192–193/74 |
| 11 | H | N-Ethyl-3-carbazolyl | C$_{42}$H$_{42}$N$_6$O$_4$S/726 | DMF | 69.10 (69.40) | 5.72 (5.82) | 11.61 (11.56) | 4.31 (4.41) | 278–279/76 |
| 12 | H | 4-Biphenyl | C$_{38}$H$_{36}$N$_4$O$_4$S/644 | DMF | 70.29 (70.78) | 5.60 (5.63) | 8.75 (8.69) | 5.02 (4.97) | 268–269/65 |
| 13 | H | 6-Methoxy-2-phenyl | C$_{36}$H$_{36}$N$_4$O$_6$S/652 | DMF | 66.01 (66.24) | 5.48 (5.56) | 8.68 (8.58) | 5.00 (4.91) | 291–292/72 |
| 14 | CH$_3$ | Phenyl | C$_{28}$H$_{32}$N$_4$O$_4$S/520 | MDC | 64.28 (64.59) | 6.28 (6.20) | 10.71 (10.76) | 6.25 (6.16) | 229–230/79 |
| 15 | CH$_3$ | 2-Thienyl | C$_{24}$H$_{28}$N$_4$O$_4$S$_3$/532 | MDC | 54.01 (54.11) | 5.22 (5.30) | 10.62 (10.52) | 18.09 (18.06) | 253–254/71 |
| 16 | CH$_3$ | 2-Pyrrolyl | C$_{24}$H$_{30}$N$_6$O$_4$S/498 | CHCl$_3$ | 57.42 (57.71) | 5.99 (6.06) | 16.90 (16.86) | 6.72 (6.63) | 232–233/51 |
| 17 | CH$_3$ | 2-Pyridyl | C$_{26}$H$_{30}$N$_6$O$_4$S/522 | CHCl$_3$ | 60.03 (59.75) | 5.78 (5.79) | 16.21 (16.08) | 6.15 (6.14) | 242–243/54 |
| 18 | CH$_3$ | 3-Indolyl | C$_{32}$H$_{34}$N$_6$O$_4$S/598 | DMF | 64.05 (64.19) | 5.62 (5.72) | 14.11 (14.04) | 5.43 (5.36) | 293–294/47 |
| 19 | CH$_3$ | 2-Thiazolyl | C$_{22}$H$_{26}$N$_6$O$_4$S$_3$/534 | DMF | 49.32 (49.42) | 4.81 (4.90) | 15.80 (15.72) | 18.03 (17.99) | 288–289/59 |
| 20 | CH$_3$ | 3-Couma-rinyl | C$_{34}$H$_{32}$N$_4$O$_8$S/656 | DMF | 62.37 (62.18) | 4.93 (4.91) | 8.65 (8.53) | 4.89 (4.88) | 251–252/72 |
| 21 | CH$_3$ | CH$_3$ | C$_{18}$H$_{28}$N$_4$O$_4$S/396 | CHCl$_3$ | 54.32 54.52) | 6.98 (7.12) | 14.18 (14.13) | 8.20 (8.09) | 219–220/48 |
| 22 | C$_6$H$_5$ | 4-Pyridyl | C$_{36}$H$_{34}$N$_6$O$_4$S/646 | DMF | 66.65 (66.85) | 5.20 (5.30) | 13.08 (12.99) | 4.86 (4.96) | 255 d*/50 |
| 23 | C$_6$H$_5$ | 5-Br-2-thienyl | C$_{34}$H$_{30}$Br$_2$N$_4$O$_4$S$_3$/814 | DMF | 50.03 (50.13) | 3.71 (3.75) | 6.81 (6.88) | 11.89 (11.81) | 297–298/54 |
| 24 | C$_6$H$_5$ | C$_6$H$_5$ | C$_{38}$H$_{36}$N$_4$O$_4$S/644 | CHCl$_3$ | 70.58 (70.78) | 5.53 (5.63) | 8.79 (8.69) | 5.05 (4.97) | 254–255/79 |

d*: decomposed.

the activity considerably. Also, introduction of phenyl (**4**) and trimethoxy phenyl (**7**) groups to azomethine carbon caused moderate activity. These active molecules have satisfied the structural requirements such as aryl binding site with a hydrophobic group, hydrogen bonding domain –NHCO– group, electron donor group and another hydrophobic aryl ring for showing the activity.

## 5. Conclusion

In the present work, twenty-seven newly designed bispropyloxyhydrazones were successfully synthesized. Their structures were confirmed by spectral and elemental analyses. Further, their anticonvulsant activity was evaluated by MES, scMET and 6 Hz models. Also, they were screened for neurotoxicity. From the results of antiepileptic activity, it can be concluded that compounds **4**, **7** and **15** displayed good activity with less neurotoxicity. Here the activity is attributed to the presence of favorable structural environment such as aryl binding site with a hydrophobic group, hydrogen bonding domain –NHCO– group, electron donor group and another hydrophobic aryl ring in these hydrazones. It can be also concluded that the 3,4-dipropyloxythiophene acts as an important pharmacophore for showing anticonvulsant activity. As this moiety showed

least contribution towards the neurotoxicity, it can be used as basic moiety in structural designing of new molecules for AEDs.

## 6. Experimental procedure

### 6.1. Chemistry

All the chemicals and the solvents, purchased from Aldrich and Merck were used without further purification. The progress of the reaction was monitored by thin layer chromatography, performed on a Silica gel 60 F$_{254}$ coated Aluminium sheet. Melting points were determined on open capillaries using a Stuart SMP3 (BIBBY STERLIN Ltd. UK) apparatus and they are uncorrected. FT-IR spectra were recorded on Nicolet Avatar 330 FTIR spectrophotometer. The $^1$H NMR and $^{13}$C NMR spectra were recorded on Varian 300 & 400 MHz NMR spectrophotometers using TMS as an internal standard. Chemical shifts were reported in ppm ($\delta$) and signals were described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br) and multiplet (m). The coupling constant (J) values are expressed in Hz. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 spectrophotometer/Data system using Argon/Xenon (6 kV, 10 mA) FAB gas, at 70 eV. Elemental analysis was carried out using FLASH EA

**Table 2**
Physical and characterization data of new compounds, **25**–**30**.

| Cd No | R$_2$ | Mol. for/Mol. mass | Recry. Sol | Elemental analysis (%): Found (cal.) | | | | Mp (°C)/yield(%) |
|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S | |
| 25 | β,β-Diphenyl propiophenyl | C$_{54}$H$_{52}$N$_4$O$_4$S/852 | MDC | 75.91 (76.03) | 6.11 (6.14) | 6.65 (6.57) | 3.71 (3.76) | 234–235/64 |
| 26 | 6-Methyl-5,6-dihydro-4-H-thieno [2,3-b] thiopyran-4-yl | C$_{28}$H$_{32}$N$_4$O$_4$S$_5$/648 | DMF | 51.63 (51.83) | 4.87 (4.97) | 8.53 (8.63) | 24.91 (24.71) | 262–263/63 |
| 27 | 6-Methyl-5,6-di-hydro-4-H-thieno [2,3-b] thiopyran(2-sulfonamide)-4-yl | C$_{28}$H$_{34}$N$_6$O$_8$S$_7$/806 | DMF | 41.50 (41.67) | 4.32 (4.25) | 10.52 (10.41) | 27.65 (27.81) | >300/70 |
| 28 | Cyclopentenyl | C$_{22}$H$_{32}$N$_4$O$_4$S/448 | MDC | 58.61 (58.91) | 7.10 (7.19) | 12.59 (12.49) | 7.21 (7.15) | 203–204/66 |
| 29 | Cyclohexenyl | C$_{24}$H$_{36}$N$_4$O$_4$S/476 | CHCl$_3$ | 60.42 (60.48) | 7.69 (7.61) | 11.82 (11.75) | 6.79 (6.73) | 182–183/66 |
| 30 | Cycloheptenyl | C$_{26}$H$_{40}$N$_4$O$_4$S/504 | CHCl$_3$ | 61.68 (61.88) | 7.92 (7.99) | 11.20 (11.10) | 6.40 (6.35) | 174–175/75 |
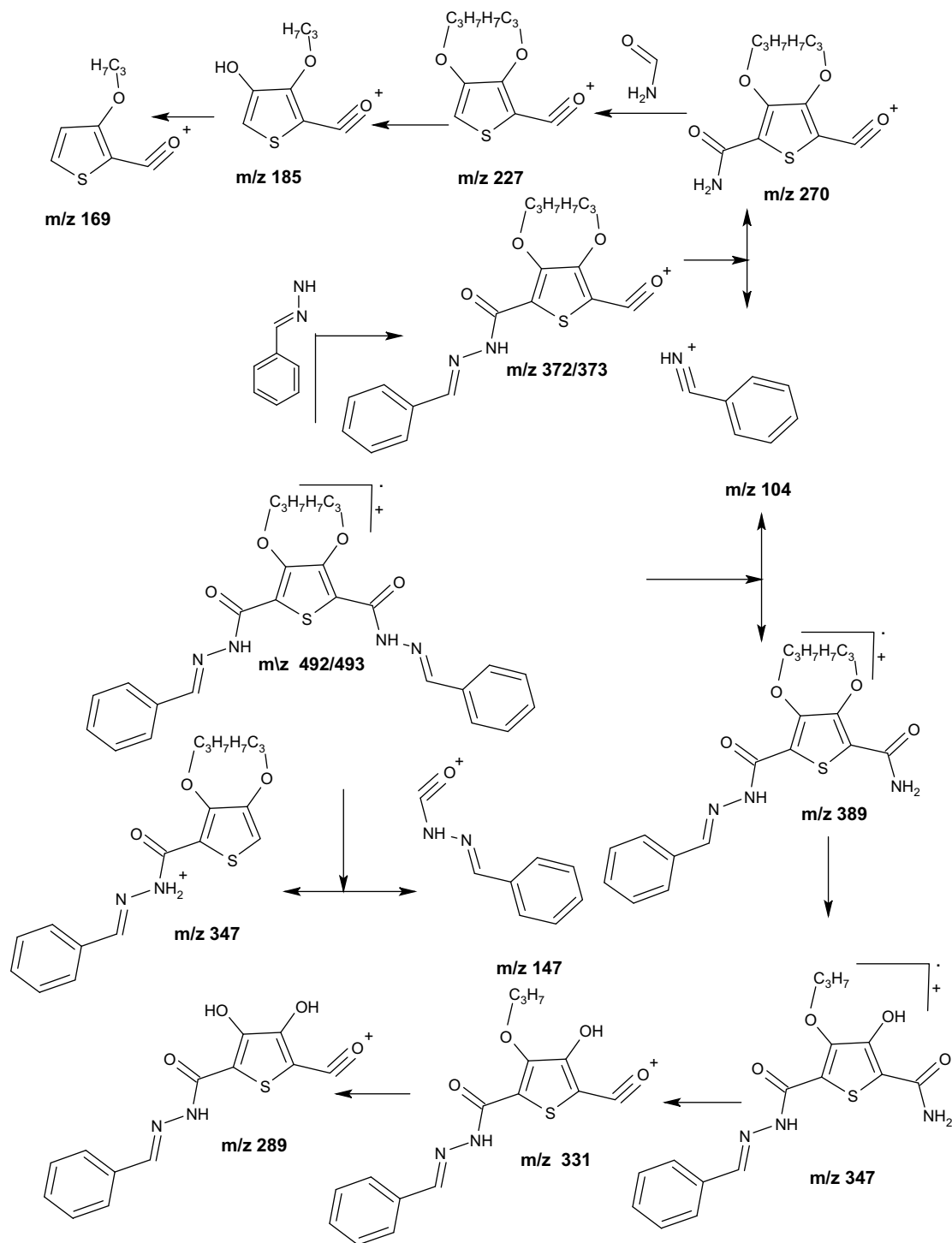
**Scheme 2.** Mass fragmentation pattern of compound **4**.

1112 series, CHNSO Analyzer (Thermo). Compound **1** and **2** were synthesized from the reported procedures [18–20].

### 6.1.1. Synthesis of 3,4-dipropyloxythiophene-2,5-carbodihydrazide (3)

One gram (0.003 mol) of diethyl 3,4-dipropyloxythiophene 2,5-dicarboxylate was added to a solution of 1.6 ml (0.03 mol) of hydrazine hydrate in 30 ml of ethanol. The reaction mixture was refluxed for 2 h. Upon cooling the reaction mixture in cold water, white needle-like crystals were obtained. The product was filtered, washed with pet. ether (40°–60°) and dried to get 0.7 g of **3** with yield, 78%. Mp 138–139 °C; IR (KBr, cm$^{-1}$) $\upsilon$: 3286 cm$^{-1}$ (–NH$_2$), 3191 cm$^{-1}$ (–NH–), 2964 cm$^{-1}$ (propyl) and 1641 cm$^{-1}$ ($\rangle$C=O); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$), 1.8 (m, 4H, –CH$_2$–), 4.1 (t, 4H, –OCH$_2$–), 5.0 (br, 4H, –NH$_2$) and 8.3 (s, 2H, –NH–).

### 6.1.2. General procedure for synthesis of bishydrazones (4–30)

A clear solution of 1.58 g (0.005 mol) of propyloxy thiophene carbohydrazide in 15 ml of absolute ethanol was mixed with 0.5 ml of conc. Hydrochloric acid. To this 0.010 mol of appropriate

**Table 3**
Anticonvulsant activity and neurotoxicity of compounds **4–30**.

| Cd No | Dose (mg/kg) | MES[a] | | scMET[b] | | Toxicity[c] | |
|---|---|---|---|---|---|---|---|
| | | 05 h | 4 h | 0.5 h | 4 h | 0.5 h | 4 h |
| **4** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 1/5 | 0/4 | 0/2 |
| **5** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 1/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| **6** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 8/8 | 2/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 3/4 | 1/2 |
| **7** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **8** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 1/2 |
| **9** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 3/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 4/4 | 1/2 |
| **10** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 2/8 | 0/4 |
| | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 3/4 | 0/2 |
| **11** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 2/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 2/4 | 0/2 |
| **12** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 1/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| **13** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **14** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **15** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 1/5 | 0/4 | 0/2 |
| **16** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **17** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **18** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **19** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 1/7 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/5 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **20** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 3/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 0/1 | 2/4 | 1/2 |
| **21** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| **22** | 30 | 0/1 | 0/1 | 0/1 | 1/5 | 0/4 | 0/2 |
| | 100 | 1/7 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/5 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **23** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 4/8 | 1/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| **24** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 1/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **25** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| **26** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 1/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 4/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 2/4 | 0/2 |
| **27** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 2/8 | 0/4 |
| | 300 | 0/1 | 0/1 | 0/1 | 0/1 | 2/4 | 1/2 |

*(continued on next page)*

**Table 3** (*continued*)

| Cd No | Dose (mg/kg) | MES[a] | | scMET[b] | | Toxicity[c] | |
|---|---|---|---|---|---|---|---|
| | | 05 h | 4 h | 0.5 h | 4 h | 0.5 h | 4 h |
| **28** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **29** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| **30** | 30 | 0/1 | 0/1 | 0/1 | 0/1 | 0/4 | 0/2 |
| | 100 | 0/3 | 0/3 | 0/1 | 0/1 | 0/8 | 0/4 |
| | 300 | 0/1 | 1/1 | 0/1 | 0/1 | 0/4 | 0/2 |

[a] Maximal electroshock test (number of animals protected/number of animal tested).
[b] Subcutaneous metrazol seizure threshold test (number of animals protected/number of animal tested).
[c] Rotorod toxicity (number of animals exhibiting toxicity/number of animal tested).

carbonyl compound, dissolved in 10 ml of absolute ethanol was added slowly while stirring. The reaction mixture was heated to reflux for 4–5 h and cooled to 10 °C. The precipitated product was separated by filtration and recrystallized from an appropriate solvent. The physical and characterization data of compounds, **4–30** are presented in Tables 1 and 2. Their spectral data are given below.

### 6.1.3. 3,4-Dipropyloxy-$N^2$, $N^5$-bis(phenylmethylene)thiophene-2,5-dicarbohydrazide (**4**)

IR (KBr, cm$^{-1}$) $v$: 3211 cm$^{-1}$ ($>$NH), 2969 cm$^{-1}$ (propyl), 1642 cm$^{-1}$ ($>$C$=$O) and 1601 cm$^{-1}$ ($>$C$=$N–); $^1$H NMR (DMSO-d$^6$, 400 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$, J = 7.2), 1.8 (m, 4H, –CH$_2$–, J = 7.2), 4.2 (t, 4H, –OCH$_2$–, J = 7.2), 7.4 (m, 6H, C$_3$, C$_4$ and C$_5$–phenyl), 7.7 (m, 4H, C$_2$ and C$_6$–phenyl), 8.4 (s, 2H, N$=$CH–), 11.2 (s, 2H, –CONH–); MS (FAB) (m/z, %): 492 (M+, 100), 372 (80), 347 (20), 289 (70), 121 (50).

### 6.1.4. 3,4-Dipropyloxy-$N^2$,$N^5$-bis(1H-indole-2-ylmethylene)thiophene-2,5-dicarbohydrazide (**5**)

IR (KBr, cm$^{-1}$) $v$: 3278 cm$^{-1}$ ($>$NH), 2964 cm$^{-1}$ (propyl), 1662 cm$^{-1}$ ($>$C$=$O) and 1603 cm$^{-1}$ ($>$C$=$N–); $^1$H NMR (DMSO-d$^6$, 400 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$, J = 7.2), 1.8 (m, 4H, –CH$_2$–, J = 6.6), 4.2 (t, 4H, –OCH$_2$–, J = 6.2), 7.2 (m, 4H, C$_5$ and C$_6$–indole), 7.4 (d, 2H, C$_3$–Indole, J = 7.8), 7.8 (d, 2H, C$_7$–indole, J = 2.1), 8.2 (d, 2H, C$_4$–indole, J = 7.5), 8.5 (s, 2H, –N$=$CH–), 10.9 (s, 2H, –CONH–), 11.6 (s, 2H, indole–NH); MS (FAB) (m/z, %): 570 (M+, 40), 492 (50), 459 (10), 372 (10).

### 6.1.5. 3,4-Dipropyloxy-$N^2$,$N^5$-bis[(5-nitro-2-furyl)methylene]thiophene-2,5-dicarbohydrazide (**6**)

IR (KBr, cm$^{-1}$) $v$: 3209 cm$^{-1}$ ($>$NH), 2966 cm$^{-1}$ (propyl), 1646 cm$^{-1}$ ($>$C$=$O) and 1601 cm$^{-1}$ ($>$C$=$N–); $^1$H NMR (DMSO-d$^6$,

**Table 4**
Results of anticonvulsant activity by 6 Hz model.

| Cd No | Dose (mg/kg) | 0.25 h | 0.5 h | 1.0 h | 2.0 h | 4.0 h |
|---|---|---|---|---|---|---|
| **4** | 100 | 0/4 | 2/4 | 2/4 | 0/4 | 0/4 |
| **5** | 100 | 0/4 | 1/4 | 1/4 | 0/4 | 0/4 |
| **7** | 100 | 0/4 | 0/4 | 2/4 | 1/4 | 0/4 |
| **8** | 100 | 0/4 | 1/4 | 1/4 | 1/4 | 0/4 |
| **15** | 100 | 0/4 | 3/4 | 3/4 | 3/4 | 2/4 |
| **23** | 100 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |
| **24** | 100 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |
| **25** | 100 | 0/4 | 0/4 | 1/4 | 0/4 | 0/4 |
| **29** | 100 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |
| **30** | 100 | 0/4 | 1/4 | 0/4 | 0/4 | 0/4 |

400 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$, J = 6.6), 1.7 (m, 4H, –CH$_2$–), 4.2 (t, 4H, –OCH$_2$–), 7.2 (d, 1H, C$_3$–furan), 7.8 (d, 1H, C$_4$–furan, J = 3.4), 8.3 (s, 2H, –N=CH–), 11.7 (s, 2H, –CONH–); MS (FAB) (m/z, %): 562 (M+, 70), 459 (100), 407 (30), 154 (100).

### 6.1.6. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(9H-fluoren-2-ylmethylene)thiophene-2,5-dicarbohydrazide (8)

IR (KBr, cm$^{-1}$) $v$: 3298 cm$^{-1}$ (⟩NH), 2967 cm$^{-1}$ (propyl), 1668 cm$^{-1}$ (⟩C=O) and 1599 cm$^{-1}$ (⟩C=N–); MS (FAB) (m/z, %): 668 (M+, 70), 492 (20), 460 (20), 434 (5), 236 (20), 208 (20), 165 (30), 154 (100).

### 6.1.7. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(2-thienylmethylene)thiophene-2,5-dicarbohydrazide (9)

IR (KBr, cm$^{-1}$) $v$: 3222 cm$^{-1}$ (⟩NH), 2966 cm$^{-1}$ (propyl), 1641 cm$^{-1}$ (⟩C=O) and 1592 cm$^{-1}$ (⟩C=N–); $^1$H NMR (DMSO-d$^6$, 400 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$, J = 6.9), 1.7 (m, 4H, –CH$_2$–, J = 6.7), 4.2 (t, 4H, –OCH$_2$–), 7.1 (t, 2H, C$_4$–thiophene), 7.4 (d, 2H, C$_5$–thiophene) and 7.6 (d, 2H, C$_3$–thiophene, J = 4), 8.6 (s, 2H, –N=CH–), 11.2 (s, 2H, –CONH–); MS (FAB) (m/z, %): 504 (M+, 100), 378 (50), 352 (20), 294 (40), 227 (20), 169 (30), 154 (40).

### 6.1.8. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(butylidene)thiophene-2,5-dicarbohydrazide (10)

IR (KBr, cm$^{-1}$) $v$: 3212 cm$^{-1}$ (⟩NH), 2965 cm$^{-1}$ (propyl), 1645 cm$^{-1}$ (⟩C=O) and 1600 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$ of propyl, J = 7.2), 1.1 (t, 6H, –CH$_3$ of butyl), 1.6 (m, 4H, –CH$_2$–CH$_3$ of butyl, J = 7.5), 1.8 (m, 4H, –CH$_2$– of propyl, J = 7.2), 2.4 (m, 4H, –CH$_2$–CH$_2$– of butyl, J = 6.9), 4.2 (t, 4H, –OCH$_2$–, J = 6.6), 7.6 (t, 2H, –N=CH–CH$_2$, J = 5.4), 9.9 (s, 2H, –CONH–).

### 6.1.9. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(1-phenylethylidene)thiophene-2,5-dicarbohydrazide (14)

IR (KBr, cm$^{-1}$) $v$: 3310 cm$^{-1}$ (⟩NH), 2963 cm$^{-1}$ (propyl), 1674 cm$^{-1}$ (⟩C=O) and 1630 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$ propyl, J = 7.2), 1.9 (m, 4H, –CH$_2$–, J = 7.2), 2.3 (s, 6H, –CH$_3$), 4.3 (t, 4H, –OCH$_2$–, J = 7.2), 7.4 (m, 6H, phenyl), 7.9 (m, 4H, phenyl); MS (FAB) (m/z, %): 523 (M + 2, 100), 388 (40), 304 (20), 161 (30), 135 (20), 118 (60).

### 6.1.10. 3,4-Dipropyloxy-N$^2$,N$^5$-bis[1-(2-thienyl)ethylidene]thiophene-2,5-dicarbohydrazide (15)

IR (KBr, cm$^{-1}$) $v$: 3304 cm$^{-1}$ (⟩NH), 2962 cm$^{-1}$ (propyl), 1678 cm$^{-1}$ (⟩C=O) and 1626 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$ propyl, J = 7.2), 1.9 (m, 4H, –CH$_2$–, J = 7.2), 2.3 (s, 6H, –CH$_3$), 4.3 (t, 4H, –OCH$_2$–, J = 6.9), 7.0 (t, 2H, C$_4$–thiophene), 7.4 (m, 4H, C$_3$ and C$_5$–thiophene).

### 6.1.11. 3,4-Dipropyloxy-N$^2$,N$^5$-bis[1-(1H-pyrrol-2-yl)ethylidene]thiophene-2,5-dicarbohydrazide (16)

IR (KBr, cm$^{-1}$) $v$: 3311 cm$^{-1}$ (⟩NH), 2971 cm$^{-1}$ (propyl), 1655 cm$^{-1}$ (⟩C=O) and 1592 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$ propyl, J = 7.2), 1.9 (m, 4H, –CH$_2$–, J = 7.2), 2.3 (s, 6H, –CH$_3$), 4.3 (t, 4H, –OCH$_2$–, J = 7.2), 6.9 (d, 2H, pyrrole–C$_5$), 6.5 (d, 2H, pyrrole–C$_3$), 6.2 (d, 2H, pyrrole–C$_4$) 10.1 (s, 2H, –CONH–), 10.7 (s, br, 2H, –NH– pyrrole); $^{13}$C NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 10.00 (–CH$_3$), 12.9 (–CH$_3$), 23.49 (–CH$_2$–), 76.2 (–OCH$_2$–), 109.3 (C$_3$ and C$_4$–pyrrole), 111.8 (C$_5$–pyrrole), 122.4 (C$_2$–pyrrole), 129.9 (C$_2$ and C$_5$–thiophene), 145.4 (C$_3$ and C$_4$–thiophene), 146.6 (–C=N–), 157.2 (⟩C=O); MS (FAB) (m/z, %): 501 (M + 2, 100), 377 (30), 335 (5), 293 (10).

### 6.1.12. 3,4-Dipropyloxy-N$^2$,N$^5$-bis[1-(pyridin-2-yl)ethylidene]thiophene-2,5-dicarbohydrazide (17)

IR (KBr, cm$^{-1}$) $v$: 3314 cm$^{-1}$ (⟩NH), 2969 cm$^{-1}$ (propyl) and 1680 cm$^{-1}$ (⟩C=O); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.1 (t, 6H, –CH$_3$ propyl), 1.9 (m, 4H, –CH$_2$–), 2.5 (s, 6H, –CH$_3$), 4.3 (t, 4H,

–OCH$_2$–), 7.3 (t, 2H, C$_4$–pyridine), 7.7 (t, 2H, C$_5$–pyridine), 8.3 (d, 2H, C$_3$–pyridine), 8.6 (d, 2H, C$_6$–pyridine), 10.4 (s, 2H, –CONH–); MS (FAB) (m/z, %): 525 (M + 2, 100), 389 (40), 136 (20).

### 6.1.13. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(1-methylethylidene)thiophene-2,5-dicarbohydrazide (21)

IR (KBr, cm$^{-1}$) $v$: 3315 cm$^{-1}$ (⟩NH), 2971 cm$^{-1}$ (propyl), 1678 cm$^{-1}$ (⟩C=O) and 1630 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$ propyl, J = 7.2), 1.9 (m, 4H, –CH$_2$–), 2.0 (s, 6H, –CH$_3$), 2.2 (s, 6H, –CH$_3$), 4.2 (t, 4H, –OCH$_2$–, J = 7.2), 9.9 (s, 2H, –CONH–).

### 6.1.14. 3,4-Dipropyloxy-N$^2$,N$^5$-bis[1-phenyl(pyridin-4-yl)methylidene]thiophene-2,5-dicarbohydrazide (22)

IR (KBr, cm$^{-1}$) $v$: 3301 cm$^{-1}$ (⟩NH), 2966 cm$^{-1}$ (propyl), 1670 cm$^{-1}$ (⟩C=O) and 1584 cm$^{-1}$ (⟩C=N–); MS (FAB) (m/z, %): 648 (M + 2, 100), 450 (40), 408 (10), 198 (40).

### 6.1.15. 3,4-Dipropyloxy-N$^2$,N$^5$-bis[(5-bromo-2-thienyl)(1-phenyl)methylidene]thiophene-2,5-dicarbohydrazide (23)

IR (KBr, cm$^{-1}$) $v$: 3290 cm$^{-1}$ (⟩NH), 2968 cm$^{-1}$ (propyl), 1678 cm$^{-1}$ (⟩C=O) and 1636 cm$^{-1}$ (⟩C=N–); MS (FAB) (m/z, %): 814 (M + 2, 100), 535 (50), 449 (20), 266 (60).

### 6.1.16. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(diphenylmethlidene)thiophene-2,5-dicarohydrazide (24)

IR (KBr, cm$^{-1}$) $v$: 3293 cm$^{-1}$ (⟩NH), 2969 cm$^{-1}$ (propyl), 1670 cm$^{-1}$ (⟩C=O) and 1625 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 0.7 (t, 6H, –CH$_3$, J = 7.2), 1.1 (m, 4H, –CH$_2$–, J = 7.5), 3.6 (t, 4H, –OCH2–, J = 7.2), 7.3 (m, 10H, phenyl), 7.6 (m, 10H, phenyl), 10.2 (s, 2H, –CONH–); $^{13}$C NMR (CDCl$_3$, 400 MHz) $\delta$ in ppm: 9.17 (–CH$_3$), 22.0 (–CH$_2$–), 75.1 (–OCH$_2$–), 128–129 (phenyl), 136.9 (C$_2$ and C$_5$–thiophene), 146.1 (C$_3$ and C$_4$–thiophene), 154.5 (⟩C=N), 157.1 (⟩C=O); MS (FAB) (m/z, %): 647 (M + 2, 100), 450 (20), 224 (20).

### 6.1.17. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(6-methyl-5,6-dihydro-4-H-thieno[2,3-b]thiopyran-4-ylidene)thiophene-2,5-dicarbohydrazide (26)

IR (KBr, cm$^{-1}$) $v$: 3322 cm$^{-1}$ (⟩NH), 2965 cm$^{-1}$ (propyl), 1661 cm$^{-1}$ (⟩C=O) and 1592 cm$^{-1}$ (⟩C=N–); $^1$H NMR (DMSO-d$^6$, 400 MHz) $\delta$ in ppm: 0.9 (t, 6H, –CH$_3$ propyl), 1.4 (d, 6H, –CH$_3$), 1.9 (s, 4H, C$_5$–alicyclic), 2.6 (m, 2H, C$_6$–alicyclic), 3.7 (s, 2H, –CH$_2$–), 4.3 (t, 4H, –OCH$_2$–), 7.4 (d, 2H, C$_4$–thiophene, J = 5.24), 7.5 (d, 2H, C$_5$–thiophene, J = 7.5) 10.4 (s, 2H, –CONH–); MS (FAB) (m/z, %): 650 (M + 2, 100), 451 (60), 425 (10), 409 (10), 367 (10), 199 (10), 182 (20).

### 6.1.18. 3,4-Dipropyloxy-N$^2$,N$^5$-bis(dicyclohexylidene)thiophene-2,5-dicarbohydrazide (29)

IR (KBr, cm$^{-1}$) $v$: 3326 cm$^{-1}$ (⟩NH), 2931 cm$^{-1}$ (propyl), 1675 cm$^{-1}$ (⟩C=O) and 1632 cm$^{-1}$ (⟩C=N–); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ in ppm: 1.0 (t, 6H, –CH$_3$), 1.7–1.9 (m, 16H, cyclohexyl), 2.3 (m, 4H, C$_4$–cyclohexyl), 2.5 (m, 4H, –CH$_2$–), 4.2 (t, 4H, –OCH$_2$–), 10.0 (s, 2H, –CONH–); MS (FAB) (m/z, %): 478 (M + 2, 100), 365 (50), 340 (10).

## 6.2. Pharmacology

The anticonvulsant evaluations were carried out by the National Institute of Health, National Institute of Neurological Disorders and Strokes (NINDS), USA, following reported procedures.

Male albino mice (CF-1 strain, 18–25 g) were used for experimentation. The animals were housed in metabolic cages and allowed free access to food and water. The synthesized compounds were suspended in 0.5% methyl cellulose/water mixture or in

polyethylene glycol (PEG 200) and injected intraperitoneally into mice and evaluated in the MES, scMET and neurotoxicity screens. The substances were administered at doses of 30, 100 and 300 mg/kg at two time intervals.

### 6.2.1. Maximal electroshock test (MES)

The MES [21–23] is a model for generalized tonic–clonic seizures and it provides an indication of ability of a compound to prevent seizure spread when all neuronal circuits in the brain are maximally active. These seizures are highly reproducible and are electrophysiological consistent with human seizures.

For all tests based on MES convulsions, 60 Hz of alternating current (50 mA) was delivered for 2 s by corneal electrodes, which were primed with an electrolyte solution containing an anesthetic agent (0.5% tetracaine HCl). For Test 1, mice were tested at various intervals following doses of 30, 100 and 300 mg/kg of test compound given by i.p. injection of a volume of 0.01 ml/g. In Test 2, mice were tested after a dose of 30 mg/kg (p.o) in a volume of 0.04 ml/g. Test 3 used varying doses administered via i.p. injection, again in a volume of 0.04 ml/g. An animal was considered "protected" from MES-induced seizures upon abolition of the hind limb tonic extensor component of the seizure.

### 6.2.2. Subcutaneous metrazol seizure threshold test (scMET)

This is one of the commonly used tests to measure the ability of a compound to control seizures produced from subcutaneous injection of the Metrazol [24] in mice.

Animals were pretreated with various doses of the test compound (in a similar manner to the MES test, although a dose of 50 mg/kg (p.o.) was the standard for Test 2 scMET). At the previously determined TPE of the test compound, the dose of Metrazol which would induce convulsions in 97% of animals ($CD_{97}$: 85 mg/kg mice) was injected into a loose fold of skin in the midline of the neck. The animals were placed in isolation cages to minimize stress and observed for the next 30 min to see the absence of a seizure. An episode of clonic spasms, approximately 3–5 s, of the fore and/or hind limbs, jaws or vibrissae was taken as the endpoint. Animals, which do not meet this criterion were considered protected.

### 6.2.3. Acute toxicity-minimal motor impairment (MMI)

Rotorod technique [25] is the most widely used method to determine the acute toxicity of compounds in anticonvulsant studies.

In experimental procedure, the mouse was placed on a rod that rotates at a speed of 6 rpm, where the animal can maintain its equilibrium for long periods of time. The animal was considered toxic if it falls off this rotating rod three times during a 1-min period. Similar procedure was followed for all the compounds to evaluate neurotoxicity.

### 6.2.4. Minimal clonic seizure (6 Hz) test

Some clinically useful AEDs are ineffective in the standard MES and scMET tests but still have anticonvulsant activities in vivo. In order to identify potential AEDs with this profile, some compounds were tested in the minimal clonic seizure (6 Hz or psychomotor) test. Like the maximal electroshock (MES) test, the minimal clonic seizure (6 Hz) test [26,27] was used to assess compound's efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3 s).

Test compounds were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% of animals (32 mA for 3 s). Untreated mice would display seizures characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors described originally as being similar to the aura of human patients with partial seizures. Animals not displaying this behavior were considered protected.

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