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Original article

A new class of anticonvulsants possessing 6 Hz activity: 3,4-Dialkyloxy thiophene bishydrazones

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ABSTRACT

Thirty nine new 3,4-di(substituted)oxy-N^2,N^5-bis(substituted)thiophene-2,5-dicarbohydrazides were synthesized starting from ethyl thiodiglycolate through multi-step reactions. In the synthetic sequence, 3,4-dihydroxythiophene-2,5-diester (**1**) was obtained by condensing the ethyl thiodiglycolate with diethyl oxalate. It was derivatized using different alkyl halides to give disubstituted thiophene esters (**2**–**5**), which were then converted to corresponding hydrazides (**6**–**9**) following usual methods. Finally, these hydrazides, on treatment with various substituted carbonyl compounds underwent smooth condensation to yield target hydrazones (**10**–**13**). The new compounds were characterized using FT-IR, ^{1}H NMR and ^{13}C NMR, mass spectral and elemental analyses. The anticonvulsant activity of the title compounds was established after intraperitoneal (ip) administration in three seizure models, which include maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and 6 Hz screens and their neurotoxicity was also evaluated. Compound **11f** has emerged as an active compound with no neurotoxicity in this series. Also, the structure–activity relationship of the tested compounds was discussed.

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1. Introduction

Thiophene derivatives have been reported to possess broad spectrum of biological properties including anti-inflammatory [1], analgesic [2], antidepressant [3], antimicrobial [4] and anticonvulsant activities [5–8]. Presently available active antiepileptic drugs (AEDs) like tiagabine [5], etizolam [7], brotizolam [8] are containing thiophene moiety in their structures as active pharmacophore. Also, it has been established that the higher activity of sodium phethenylate [6] is due to the presence of thiophene ring in its structure. Further, compounds containing hydrazones and thiosemicarbazones with different types of substitution are well documented for their anticonvulsant activity [9–14].

In our earlier work [15], we explored the anticonvulsant property of many hydrazones containing 3,4-dipropyloxythiophene. Amongst them, the compound 3,4-dipropyloxy-N^2,N^5-bis[1-(2-thienyl)ethylidene]thiophene-2,5-dicarbohydrazide (Fig. 1) was found to be lead. In search of potential anticonvulsant agents, it has been thought of designing new hydrazones containing different electron releasing groups at positions 3, 4 of thiophene ring (labeled A in Fig. 1) and various substituted auxiliary thiophenes (labeled B in Fig. 1) attached to azomethine side chains of thiophene, in order to study the effect of molecular dynamics on the activity.

In continuation of our research program on design and synthesis of new anticonvulsant agents, we herein report the multi-step synthesis of hitherto unknown 3,4-di(substitute)oxy-N^2,N^5-bis (substituted)thiophene-2,5-dicarbohydrazides, carrying active groups and evaluation of their anticonvulsant activity by MES, scMET and 6 Hz models. Also, we report their neurotoxicity by rotorod method.

2. Chemistry

The reaction sequence leading to the synthesis of title compounds, viz. 3,4-di(substituted)oxy-N^2,N^5-bis(substituted) thiophene-2,5-dicarbohydrazides (**10**–**13**) is shown in Scheme 1.

The dihydroxythiophene diester (**1**), obtained by condensing diethyl oxalate with ethyl thiodiglycolate in alcoholic sodium ethoxide, was treated with different alkyl halides in the presence of anhydrous potassium carbonate to yield 3,4-disubstituted esters (**2**–**5**). Further, they were condensed with hydrazine hydrate in alcoholic medium to give key intermediates, viz., 3,4-di (substituted)oxythiophene-2,5-dicarbohydrazides (**6**–**9**). These hydrazides were then conveniently converted to hydrazones by refluxing them with appropriate aryl/alkyl aldehydes or ketones in

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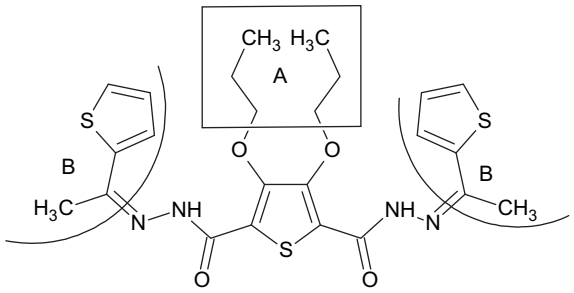


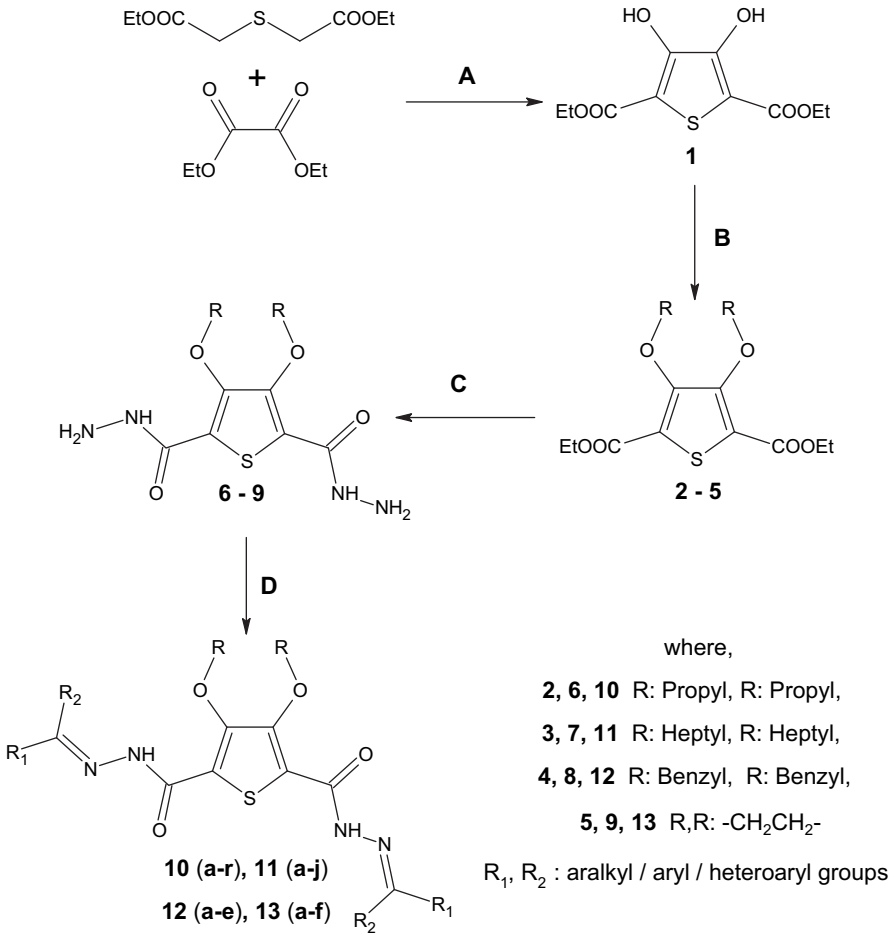
Fig. 1. 3,4-Dipropyloxy-N^2,N^5-bis[1-(2-thienyl)ethylidene]thiophene-2,5-dicarbohydrazide where A and B are the positions of derivatization.

the presence of catalytic amount of conc. HCl. The physiochemical properties and characterization data of the title compounds are summarized in Table 1. 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 3,4-dibenzyloxy-2,5-thiophene diester (**4**) from 3,4-dihydroxy-2,5-thiophene diester (**1**) was confirmed by its IR, ^{1}H NMR and mass spectral studies. Its IR spectrum showed the carbonyl stretching at 1704 cm^{-1} while the stretching of –CH_2– of benzyl group appeared at 2982 cm^{-1}. In its ^{1}H NMR spectrum, –OCH_2– protons resonated at δ 5.1 ppm. This indicated the formation of the compound **4**. Further, its LC–mass spectrum confirmed high purity of **4** and showed its molecular weight that corresponds to $C_{24}H_{24}O_6S$.

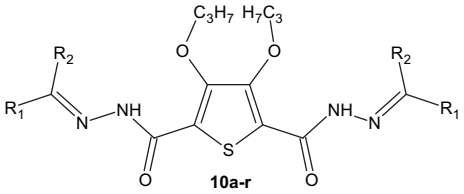
The conversion of the diester (**4**) to the corresponding hydrazide (**8**) was evidenced by its spectral data. IR spectrum of compound **8** showed bands at 3294, 2982 and 1703 cm^{-1} indicating the presence of –NH_2, benzyloxy and >C=O groups respectively. In its ^{1}H NMR spectrum –NH_2 group appeared as broad singlet at δ 4.5 ppm, while –OCH_2– group resonated as a sharp singlet at δ 5.2 ppm. Further, aromatic protons appeared as multiplet at δ 7.3–7.4 ppm and –NH– proton resonated as broad singlet at δ 8.8 ppm. ^{13}C NMR spectrum of the compound **8** showed peak at δ 76 ppm due to –OCH_2– group. The signals appeared at δ 124 and 146 ppm correspond to thiophene carbons. The aromatic carbons showed in the region of δ 128 and 134 ppm and the carbonyl group of the hydrazide resonated at δ 160 ppm. In the mass spectrum, it showed the molecular ion peak at m/z 413 (M + 1, 100%). The peak matches with its molecular formula $C_{20}H_{20}N_4O_4S$. The peaks at m/z 381 and 355 were due to the fragmentation of molecular ion.

The structure of hydrazone **10n** was established by its spectral data. In its IR spectrum, peaks at 1731 and 1679 cm^{-1} clearly showed the presence of ester and amide linkages respectively in the molecule. The structure of hydrazone **10n** was further confirmed by its COSY-^{1}HNMR, which is shown in Fig. 2. Here, the



Scheme 1. Synthesis of 3,4-dialkyloxy bishydrazones. A: NaOEt, ethanol, H^+; B: R–Br, K_2CO_3, DMF; C: $NH_2NH_2 \cdot H_2O$, ethanol; D: aldehyde/ketone, ethanol, H^+.

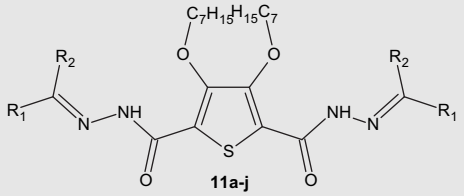
Table 1
Physicochemical and characterization data of compounds **10a–r**.



10a-r

Compound	R_1	R_2	Mp (°C)/Y (%)	Recry. Solven[a]	Elemental analysis (%): Found (cal.)			
					C	H	N	S
10a	CH_3	3-C_4H_3S	256–257/60	MDC	54.02 (54.11)	5.30 (5.19)	10.59 (10.52)	18.15 (18.06)
10b	CH_3	5-Br, 2-C_4H_2S	294–295/72	DMF	41.75 (41.68)	3.85 (3.80)	8.19 (8.11)	13.99 (13.93)
10c	CH_3	5-Cl, 2-C_4H_2S	296–297/70	DMF	47.98 (47.92)	4.31 (4.36)	9.39 (9.31)	16.08 (15.99)
10d	CH_3	2-Br, 3-C_4H_2S	214–215/68	MDC	41.84 (41.75)	3.87 (3.80)	8.18 (8.11)	13.85 (13.93)
10e	CH_3	2,5-Cl, 3-C_4H_2S	241 Dec[b]/75	DMF	42.99 (43.08)	3.69 (3.61)	8.39 (8.36)	14.39 (14.35)
10f	3-isatinyl		>300/65	DMF	58.59 (58.53)	4.59 (4.56)	14.58 (14.63)	5.55 (5.58)
10g	H	3-OCH_3, 4-OH, phenyl	299–300/68	DMF	57.58 (57.52)	5.58 (5.52)	9.51 (9.58)	5.55 (5.48)
10h	H	4-Nitrophenyl	293–294/65	DMF	53.70 (53.60)	4.58 (4.50)	14.45 (14.43)	5.45 (5.50)
10i	H	4-$N(CH_3)_2$ phenyl	259–260/77	MDC	62.35 (62.26)	6.58 (6.62)	14.59 (14.52)	5.51 (5.54)
10j	H	4-COOH phenyl	295 Dec[b]/72	DMF	57.87 (57.92)	4.95 (4.86)	9.58 (9.65)	5.45 (5.52)
10k	H	2-OH phenyl	249–250/67	DMF	59.50 (59.53)	5.48 (5.38)	10.75 (10.68)	6.15 (6.11)
10l	H	2-OCH_3 phenyl	251–252/65	MDC	60.95 (60.85)	5.90 (5.84)	10.20 (10.14)	5.85 (5.80)
10m	H	4-Cl phenyl	261–262/63	MDC	55.69 (55.62)	4.75 (4.67)	9.78 (9.98)	5.64 (5.71)
10n	CH_3	$-CH_2$ $COOC_2H_5$	153–254/68	$CHCl_3$	53.25 (53.32)	6.66 (6.71)	10.45 (10.36)	5.99 (5.93)
10o	H	4-Diacetal phenyl	275–276/68	DMF	62.15 (62.05)	6.99 (6.94)	8.11 (8.04)	4.69 (4.60)
10p	H	2-NH_2, 3-pyridyl	270–271/70	DMF	54.85 (54.95)	5.31 (5.38)	21.42 (21.36)	6.19 (6.11)
10q	H	2-Br, 5-NO_2 phenyl	274–275/63	DMF	42.27 (42.18)	3.35 (3.27)	11.42 (11.35)	4.39 (4.33)
10r	H	N-dodecyl-3-carbazolyl	210–211/60	MDC	73.99 (73.92)	8.11 (8.20)	8.39 (8.34)	3.11 (3.18)

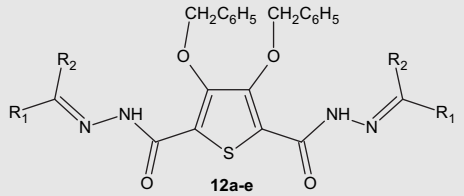
Physicochemical and characterization data of compounds **11a–j.**



11a-j

Compound	R_1	R_2	Mp (°C)/Y (%)	Recry. Solven[a]	Elemental analysis (%): Found (cal.)			
					C	H	N	S
11a	6-CH_3-5, 6-dihydro-4H-thieno [2,3-b] thiopyran-4-yl		238–239/69	$CHCl_3$	56.74 (56.81)	6.41 (6.36)	7.30 (7.36)	21.12 (21.06)
11b	3-Isatinyl		>300/72	DMF	62.83 (62.95)	6.10 (6.16)	12.30 (12.24)	4.78 (4.67)
11c	H	4-OH, 3-OCH_3 phenyl	194–195/70	MeOH	62.13 (62.05)	6.97 (6.94)	8.09 (8.04)	4.51 (4.60)
11d	H	N-ethyl-3-carbazolyl	202–203/72	$CHCl_3$	71.49 (71.57)	7.07 (6.97)	9.95 (10.02)	3.90 (3.82)
11e	H	Phenyl	159–160/65	MDC	67.59 (67.52)	7.41 (7.33)	9.10 (9.26)	5.21 (5.30)
11f	H	4-NO_2 phenyl	213–214/74	MDC	58.69 (58.77)	6.14 (6.09)	12.05 (12.10)	4.64 (4.62)
11g	H	3,4,5-OCH_3 phenyl	152–153/75	MDC	61.29 (61.20)	7.25 (7.19)	7.19 (7.14)	4.01 (4.08)
11h	H	2-Fluorenyl	223–224/65	DMF	73.89 (73.82)	6.78 (6.71)	7.12 (7.17)	4.15 (4.11)
11i	H	N-dodec-yl-3-carbazolyl	193–194/65	MDC	75.01 (75.09)	8.89 (8.82)	7.59 (7.51)	2.81 (2.86)
11j	CH_3	2-Thienyl	193–194/75	$CHCl_3$	59.53 (59.60)	6.81 (6.88)	8.76 (8.69)	14.91 (14.92)

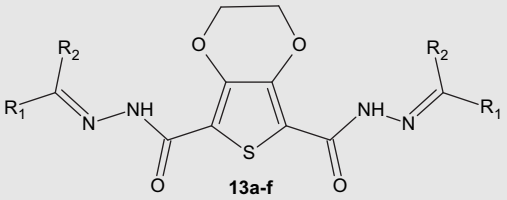
Physicochemical and characterization data of compounds **12a–e.**



12a-e

Compound	R_1	R_2	Mp (°C)/Y (%)	Recry. Solven[a]	Elemental analysis (%): Found (cal.)			
					C	H	N	S
12a	CH_3	2-Thienyl	205–206/68	$CHCl_3$	61.19 (61.12)	4.58 (4.49)	8.96 (8.91)	15.39 (15.30)
12b	H	3,4,5-OCH_3 phenyl	270–271/78	$CHCl_3$	62.52 (62.49)	5.29 (5.24)	7.38 (7.29)	4.20 (4.17)
12c	H	Phenyl	240–241/72	DMF	69.31 (69.37)	4.85 (4.79)	9.59 (9.52)	5.52 (5.45)
12d	H	N-ethyl-3-carbazolyl	272Dec[b]/65	DMF	72.88 (72.97)	5.08 (5.14)	10.17 (10.21)	3.95 (3.90)
12e	H	4-NO_2 phenyl	263 Dec[b]/75	MDC	60.11 (60.17)	3.81 (3.86)	12.32 (12.38)	4.79 (4.72)

Table 1 (*continued*)

Compound	R_1	R_2	Mp (°C)/Y (%)	Recry. Solven[a]	Elemental analysis (%): Found (cal.)			
					C	H	N	S
Physicochemical and characterization data of compounds **13a–f.**								
			 **13a-f**					
Compound	R_1	R_2	Mp (°C)/Y (%)	Recry. Solven[a]	Elemental analysis (%): Found (cal.)			
					C	H	N	S
13a	H	N-ethyl-3-carbazolyl	>300/75	DMF	68.33 (68.25)	4.89 (4.82)	12.65 (12.57)	4.72 (4.79)
13b	CH_3	2-Thienyl	>300/70	DMF	50.51 (50.62)	3.89 (3.82)	11.85 (11.81)	20.21 (20.27)
13c	H	3,4,5-OCH_3 phenyl	>300/68	DMF	54.79 (54.72)	4.99 (4.92)	9.01 (9.12)	5.31 (5.22)
13d	H	Phenyl	>300/68	DMF	60.89 (60.82)	4.25 (4.18)	12.98 (12.90)	7.29 (7.38)
13e	H	2-Fluorenyl	>300/70	DMF	70.72 (70.80)	4.21 (4.29)	9.11 (9.17)	5.29 (5.25)
13f	H	4-NO_2 phenyl	>300/72	DMF	50.21 (50.32)	3.15 (3.07)	16.15 (16.02)	6.01 (6.11)

[a] Recrystallization solvent.
[b] Decomposed.

presence of –OCH_2– group of ester and –OCH_2– group of propyloxy linkage was observed as multiplet at δ 4.2 ppm. Further, the three protons of methyl group of ester and two protons of –CH_2CO– group resonated at δ 1.2 and 3.4 ppm respectively. The appearance of singlet peak at δ 9.9 ppm showed the presence of –NH– group in the molecule. Its structure was further evidenced by its ^{13}C NMR spectrum. The peaks at δ 150 and 156 ppm showed the presence of >C═N– and amide carbonyl groups respectively. In the mass spectrum, it displayed the molecular ion peak at *m*/*z* 540 (M + 1, 100%) that matches with its molecular formula, $C_{24}H_{36}N_4O_8S$. It followed the similar fragmentation pattern as described in our earlier paper [15].

The IR spectrum of compound **11j** showed sharp peaks at 3310, 1679 and 1636 cm^{-1} indicating the presence of –NH–, >C═O and >C═N– groups respectively in its structure. Further, in its ^1H NMR spectrum, the appearance of a triplet at δ 4.3 ppm confirmed the presence of –OCH_2– group of the alkoxy substituent. Also, appearance of a singlet at δ 10.0 ppm showed the presence of –NH– group in the molecule. The formation of **11j** was further confirmed by its ^{13}C NMR spectrum wherein >C═N– appeared at δ 147 ppm while –CONH– resonated at 156 ppm. The mass spectrum of **11j** displayed the molecular ion peak at *m*/*z* 646 (M + 1, 100%), which matches with its molecular formula $C_{32}H_{44}O_4N_4S_3$.

The conversion of the hydrazide **8** to hydrazone **12b** was confirmed by various spectral methods. In its IR spectrum, peaks at 3240 cm^{-1} and 1637 cm^{-1} were due to –NH– and >C═O absorptions respectively. Further, the peaks at 1237 and 1052 cm^{-1} showed the presence of aralkyl ether in the molecule. ^1H NMR spectrum of it displayed two singlets at δ 3.8 and 5.3 ppm

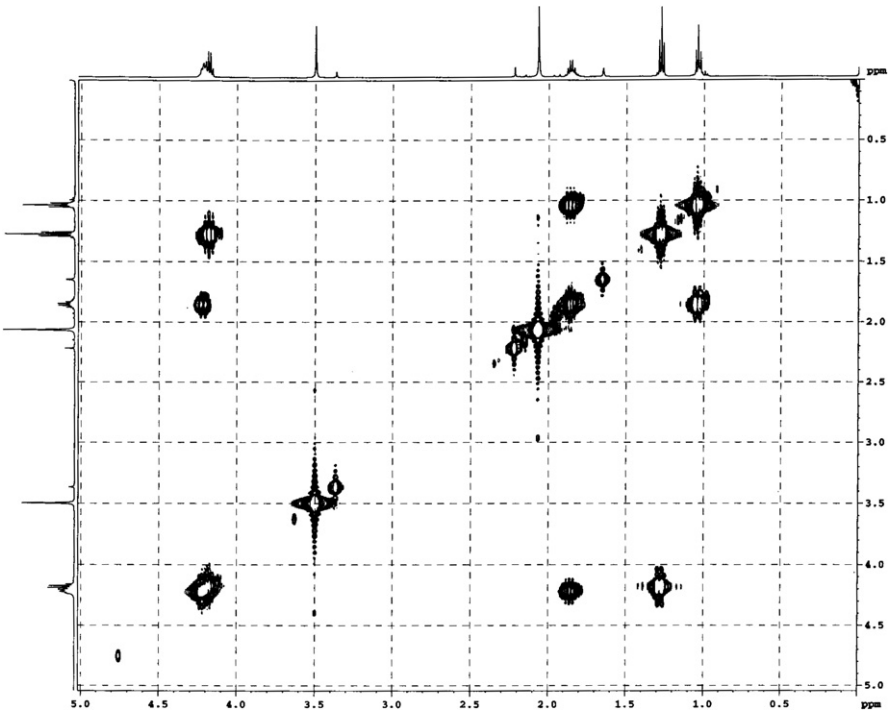


Fig. 2. COSY ^1H NMR spectrum of compound **10n**.

indicating the presence of $-OCH_3$ and $-OCH_2-$ groups respectively. The azomethine proton and –NH– proton resonated as singlets at δ 7.5 and 10 ppm respectively, whereas aromatic protons appeared as multiplet in the region of δ 6.8–7.5 ppm. The formation of **12b** was further confirmed by its ^{13}C NMR spectrum. Here the azomethine and amide carbons resonated at 146 and 156 ppm respectively. Finally, its mass spectrum showed the molecular ion peak at m/z 769 (M + 1, 100%). This confirmed its molecular formula $C_{40}H_{40}N_4O_{10}S$.

In the IR spectrum of the hydrazone **13d**, the appearance of peaks at 3306, 1675 and 1577 cm^{-1} clearly indicated the presence of –NH–, >C=O and >C=N- functional groups respectively. Its ^1H NMR spectrum displayed a sharp singlet peak at δ 4.5 ppm due to the presence of ethylenedioxy group. Further, the azomethine proton appeared at δ 8.4 ppm while –NH– proton resonated at δ 10.7 ppm as singlets. The mass spectrum of **13d** showed the molecular ion peak at m/z 435 (M + 1, 100%), which matches with its molecular formula $C_{22}H_{18}N_4O_4S$.

3. Pharmacology

All the new 39 hydrazones were subjected to anticonvulsant screening by maximal electroshock test (MES) and subcutaneous metrazol (scMET) test using doses of 30, 100 and 300 mg/kg and the observation was carried out at two different time intervals (0.5 and 4 h). Only compounds **6**, **7**, **10e**, **10f**, **10h–l**, **10q**, **11e**, **12a**, **12c**, **12e**, **13b**, **13c** and **13e** showed moderate to good activity while the remaining compounds did not show either activity or toxicity at maximum dose and their results are presented in Table 2. Further, some of the selected compounds were tested for their activity at 6 Hz model. The results of screening at five different time points are summarized in Table 3. The detailed experimental procedures are given in the experimental section.

4. Results and discussion

The results of MES study revealed that compounds **12c** and **13b** showed activity at a dose of 300 mg/kg at 0.5 h and compounds **10f** and **12a** displayed resistance at 4 h at the same dose. It has been noticed that the presence of isatin, phenyl and thiophene moieties as a distal ring in their structures is responsible for the observed activity.

In scMET model, compounds **10e**, **10k** and **12c** showed positive response towards induced seizures at a dose of 300 mg/kg and **11e** displayed same level of protection at 0.5 h of time period. Here, the activity is mainly attributed to the presence thiophene and phenyl entities as a distal ring in the designed molecules.

As seen from the results of neurotoxicity screening, compounds **10e**, **10f**, **10q** and **13c** exhibited toxicity at a dose of 300 mg/kg whereas the compounds **10h–j**, **10l** and **12e** showed at a dose of 100 mg/kg. It has been observed that the occurrence of dichlorothiophene, isatin and nitro, trimethoxy substituted phenyl rings in the molecules is responsible for the lower level of toxicity and presence of groups, viz., nitro, N,N-dimethyl, carboxyl and methoxy substituted phenyl rings in the structures caused higher level of neurotoxicity. It is interesting to note that the remaining compounds did not show any neurotoxicity at the maximum administered dose level of 300 mg/kg. These results clearly indicated that toxicity is not due to the presence of the basic moiety, i.e., 3,4-di(substituted)oxy thiophene in the tested compounds.

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 of various tested hydrazones, compounds **10a**, **10f**, **10k**, **10o**, **11e**, **11f**, **11j** and **13b** displayed good activity compared to other compounds. These active compounds contain thiophene, isatin and hydroxy, diacetyl, trimethoxy substituted phenyl rings attached to the 3,4-disubstituted thiophene system. The compound **11f**, carrying 4-nitrophenyl substituted heptyloxythiophene displayed 100% protection against induced convulsions at 0.5 h at the dose of 100 mg/kg, whereas compounds **10b**, **10d**, **11b** and **11g** exhibited moderate activity.

Table 2
Anticonvulsant activity and neurotoxicity of title compounds.

Compound	MES[a]		scMET[b]		Toxicity[c]	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
6	–	–	–	–	100	–
7	–	100	–	–	–	–
10e	–	–	–	300	300	
10f	–	300	–	–	100	300
10h	–	–	–	–	100	–
10i	–	–	–	–	100	300
10j	–	–	–	–	100	–
10k	–	–	–	300	–	–
10l	–	–	–	–	100	–
10q	–	–	–	–	300	–
11e	–	–	300	–	–	–
12a	–	300	–	–	–	–
12c	300	–	–	300	–	–
12e	–	–	–	–	100	–
13b	300	–	–	–	–	–
13c	–	–	–	–	300	–
13e	–	–	300	–	–	–

The figures in the table indicate the minimum dose where bioactivity was observed in at least one or more of the mice. The dash (–) indicates an absence of activity at the maximum dose of 300 mg/kg. *Note*: The remaining compounds in the series did not show either activity or toxicity at a maximum dose of 300 mg/kg.
[a] Maximal electroshock test.
[b] Subcutaneous metrazol seizure threshold test.
[c] Rotorod toxicity.

Table 3
Results of anticonvulsant activity by 6 Hz model.

Compound	Dose (mg/kg)	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h
11e	100	2/4	2/4	1/4	1/4	0/4
10a	100	0/4	3/4	1/4	1/4	0/4
10b	100	1/4	0/4	1/4	1/4	1/4
10d	100	0/4	1/4	1/4	0/4	0/4
10f	100	2/4	1/4	0/4	0/4	0/4
10k	100	0/4	3/4	2/4	0/4	0/4
10o	100	0/4	1/4	2/4	0/4	0/4
10p	100	2/4	0/4	0/4	0/4	0/4
11a	100	0/4	0/4	0/4	1/4	0/4
11b	100	0/4	1/4	0/4	1/4	1/4
11d 11e	50	0/4	0/4	0/4	0/4	0/4
11f	100	0/4	4/4	0/4	2/4	0/4
	60	0/4	0/4	0/4	0/4	0/4
11g	100	0/4	1/4	1/4	1/4	1/4
11j	100	2/4	1/4	0/4	1/4	1/4
	30	0/4	0/4	0/4	0/4	0/4
12c	30	0/4	0/4	0/4	0/4	1/4
13b	100	2/4	2/4	1/4	1/4	0/4
	30	2/4	2/4	1/4	0/4	0/4

5. Structure–activity relationship (SAR)

In our previous article [15], we reported that 3,4-dipropyloxy-N^2,N^5-bis[1-(2-thienyl)ethylidene]thiophene-2,5-dicarbohydrazide emerged as a lead and 3,4-dipropyloxy-N^2,N^5-bis(phenylmethylene) thiophene-2,5-dicarbohydrazide, 3,4-dipropyloxy-N^2,N^5-bis(3,4,5-trimethoxyphenylmethylene)thiophene-2,5-dicarbohydrazide as moderate antiepileptic agents. In the present work, based on the reported results, the active molecules have been further derivatized with different substituents, in order to

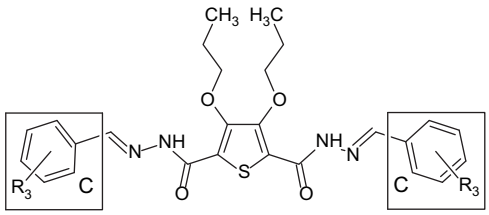


Fig. 3. Positions of derivatization of active molecules.

investigate the pharmacophoric elements, responsible for better activity. In this approach, some of the toxic compounds have been also derivatized.

In our new synthetic design, active groups, viz., heptyl, benzyl and ethylenedioxy side chains have been introduced at positions 3,4 of the thiophene backbone structure and further, the active distal ring has been derivatized with different substituents. It has been observed that the introduction of the heptyl group has not influenced the MES and scMET screening results as seen in **11j**. But, no change in activity was observed in the case of benzyl substituted (**12a**) and ethylenedioxy substituted (**13b**) 2-thienyl hydrazones. When 2-thienyl hydrazone was replaced by benzaldehyde hydrazones (**11e**, **12c**, **13d**), it retained the activity only in the compounds **11e** and **12c**. Further, the introduction of 2-fluorenyl hydrazone groups (**11h**, **13e**) showed resistance at a dose of 300 mg/kg against the induced convulsion, whereas presence of propyloxy substitution in thiophene ring failed to produce any activity. It has been observed that replacement of propyl with heptyl group (**11b**) causes loss of activity in the isatin hydrazones.

In order to study the influence of substituents attached to different positions of secondary aryl group on the activity of various hydrazones, many derivatives as shown in Figs. 3 and 4 have been synthesized. In these figures, labeled parts C and D indicate different secondary aryl groups attached to azomethine functional group of active hydrazones.

From the results, it has been noticed that introduction of 2-hydroxy phenyl group to hydrazone (**10k**) has caused moderate activity while substitution by 4-hydroxy phenyl group (**10g**) has resulted in poor activity. On the other hand, replacement of hydroxyl group (**10k**) by methoxy group (**10l**) in position 2 of phenyl ring has reduced activity (Fig. 3). This may be attributed to the formation of increased extent of hydrogen bonding during its action. Further, incorporation of 4-nitrophenyl (**11f**) ring in the place of 4-hydroxy phenyl (**10g**) has caused the activity to the maximum level (100%) with no neurotoxicity. This clearly indicates the role of electronic factor on the activity. As far as position of linkage is concerned, change in the linkage of azomethine group from second to third position (**10a**) has retained the activity and introduction of halogen groups (**10d** and **10b**) into the thiophene ring, as given in Fig. 4, has reduced the activity when compared to that of unsubstituted 2-thienyl hydrazone.

Keeping in view of the observed fact that N-ethyl carbazole hydrazone of propyloxy substituted thiophene did not show any activity and displayed toxicity as per our previous report [15], in our synthetic design propyloxy group has been replaced by heptyloxy (**11d**) in order to find the structural requirement for better activity. Surprisingly, the activity has enhanced to 75% and toxicity has reduced to the maximum extent. Further, replacement of the heptyloxy group by benzyloxy (**12d**) and ethylenedioxy (**13a**) moieties has showed neither activity nor toxicity.

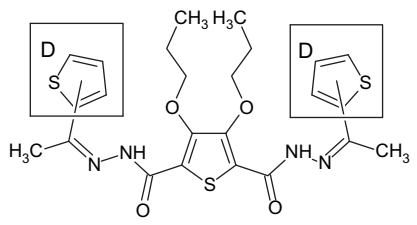


Fig. 4. Positions of derivatization of active molecules.

6. Conclusion

In the present work, thiophene-2,5-dicarbohydrazide containing different types of substituents at its positions 3,4 and various groups attached to the azomethine functional moiety, were synthesized and characterized by spectral and elemental analyses. Based on the findings reported in our earlier work, the position and type of substituents were selected. All the compounds were subjected to antiepileptic screening by standard methods. Amongst the tested models, the 6 Hz screening results were promising. The results revealed that compounds **10a**, **10f**, **10k**, **10o**, **10p**, **11d–f**, **11j** and **13b** exhibited moderate to good activity. The compound **11f** displayed 100% protection and emerged as a lead in this series. Further, compounds **10a** and **10k** came out as potential candidates for further investigations. It can be concluded that increase in hydrophobicity in the molecules brings about some degree of activity in the series. Further, the screening results clearly support that 3,4-dioxy substituted thiophene moiety is a seat of broad spectrum antiepileptic activity and with least neurotoxicity. Finally it can be readily concluded that change in the distal ring influences the activity as well as toxicity of substituted thiophene hydrazones.

7. Experimental

7.1. 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. The FT-IR spectra were recorded on Nicolet Avatar 330 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Varian 300, 400 and 500 MHz NMR spectrophotometers using TMS as an internal standard. Chemical shifts were reported in ppm (δ) 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 1112 series, CHNSO Analyser (Thermo).

Compounds **2–5** were synthesized from diethyl-3,4-diyhdroxythiophene-2,5-dicarboxylate following the reported procedure [16].

7.1.1. Synthesis of 3,4-di(substituted)thiophene-2,5-carbonyldihydrazides (*6–9*)

One gram (0.003 mol) of diethyl-3,4-disubstiutedoxythiophene-2,5-dicarboxylates (**2–5**) 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, white precipitate was obtained. The products (**6–9**) were then filtered, washed with alcohol and dried to get title compounds with good yield. The compounds **6–9** were crystallized using appropriate solvents. The spectral data of compound **8** is given below.

*7.1.2. 3,4-Bis(benzyloxy)thiophene-2,5-dicarbohydrazide (**8**)*

Mp: 159–60 °C; Crystallization Solvent: Ethanol; IR (KBr, cm^{-1}) υ: 3294 cm^{-1} (br, –NH_2), 2982 cm^{-1} (benzyloxy), 1703 cm^{-1} (>C=O); ^1H NMR ($CDCl_3$, 400 MHz) δ in ppm: 4.52 (br, 4H, –NH_2), 5.20 (s, 4H, –OCH_2–), 7.36–7.40 (m, 10H, phenyl), 8.85 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 400 MHz) δ in ppm: 76.4 (–OCH_2–), 124.8 ($C_{3,4}$–thiophene), 128.8–129.4 ($C_{2,3,5,6}$–phenyl), 134.5 (C_1–phenyl), 146.3 ($C_{2,5}$–thiophene), 161.5 (–CONH–). MS (*m*/*z*, %): 413 (M + 1, 100), 381 (20), 355 (20), 279 (10), 265 (10).

The characterization data of compounds **6**, **7** and **9** matched with the reported data [15,17,18].

*7.1.3. General procedure for synthesis of bishydrazones **10a–r**, **11a–j**, **12a–e** and **13a–f***

A clear solution of 0.005 mol of 3,4-disubstituted-thiophenecarbohydrazides (**6–9**) in 15 mL of absolute ethanol was mixed with 0.5 mL of conc. hydrochloric acid. To this 0.01 mol of appropriate carbonyl compound, dissolved in 10 mL of absolute ethanol was added slowly while stirring. The reaction mixture was heated to reflux for 4 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 all the newly synthesized compounds are presented in Table 1. Their spectral data are given below.

*7.1.4. 3,4-Dipropyloxy-N^2,N^5-bis[1-(3-thienyl)ethylidene] thiophene-2,5-dicarbohydrazide (**10a**)*

IR (KBr, cm^{-1}) υ: 3308 cm^{-1} (-NH–), 2969 cm^{-1} (propyl), 1675 cm^{-1} (>C=O), 1535 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.06 (t, 6H, –CH_3– of propyl, $J = 7.2$), 1.88 (m, 4H, –CH_2–), 2.33 (s, 6H, –CH_3), 4.28 (t, 4H, –OCH_2–), 7.31 (d, 2H, C_4– of thiophene, $J = 16.2$), 7.62 (s, 2H, C_2– of thiophene), 7.71 (d, 2H, C_5– of thiophene, $J = 4.5$), 10.13 (s, 2H, –NH–).

*7.1.5. 3,4-Dipropyloxy-N^2,N^5-bis[1-(2-bromo-3-thienyl)ethylidene] thiophene-2,5-dicarbohydrazide (**10d**)*

IR (KBr, cm^{-1}) υ: 3307 cm^{-1} (-NH–), 2967 cm^{-1} (propyl), 1677 cm^{-1} (>C=O), 1548 cm^{-1} (>C=N–); MS (*m*/*z*, %): 691 (M + 1, 100), 473 (30), 387 (20).

*7.1.6. 3,4-Dipropyloxy-N^2,N^5-bis[4-(N,N-dimethyl)phenylmethylene] thiophene-2,5-dicarbohydrazide (**10i**)*

IR (KBr, cm^{-1}) υ: 3293 cm^{-1} (-NH–), 2966 cm^{-1} (propyl), 1666 cm^{-1} (>C=O), 1594 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.11 (t, 6H, –CH_3, $J = 7.5$), 1.91 (m, 4H, –CH_2–), 3.02 [s, 12H, –$N(CH_3)_2$], 4.22 (t, 4H, –OCH_2–, $J = 6.9$), 6.70 (d, 4H, C_3 and C_5– of phenyl), 7.63 (d, 4H, C_2 and C_6– of phenyl), 8.04 (s, 2H, –CH=N–), 10.09 (s, 2H, –NH–).

*7.1.7. 3,4-Dipropyloxy-N^2,N^5-bis(2-hydroxyphenylmethylene) thiophene-2,5-dicarbohydrazide (**10k**)*

IR (KBr, cm^{-1}) υ: 3296 cm^{-1} (-NH–), 3000 cm^{-1} (br –OH), 2973 cm^{-1} (propyl), 1676 cm^{-1} (>C=O), 1616 cm^{-1} (>C=N–); ^1H NMR (DMSO-d_6, 300 MHz) δ in ppm: 0.98 (t, 6H, –CH_3, $J = 6.9$), 1.76 (m, 4H, –CH_2–), 4.19 (t, 4H, –OCH_2–), 6.95 (m, 4H, C_4 and C_3– of phenyl), 7.30 (m, 2H, C_2– of phenyl), 7.67 (d, 2H, C_5– of phenyl), 8.63 (s, 2H, –N=CH–), 11.02 (s, 2H, –OH), 11.44 (s, 2H, –NH–); MS (*m*/*z*, %): 525 (M^+, 100), 389 (70), 363 (20).

*7.1.8. 3,4-Dipropyloxy-N^2,N^5-bis(2-methoxyphenylmethylene) thiophene-2,5-dicarbohydrazide (**10l**)*

IR (KBr, cm^{-1}) υ: 3206 cm^{-1} (-NH–), 2967 cm^{-1} (propyl), 1640 cm^{-1} (>C=O), 1599 cm^{-1} (-N–); ^1H NMR ($CDCl_3$, 400 MHz) δ in ppm: 1.13 (t, 6H, –CH_3– of propyl, $J = 8$), 1.91 (m, 4H, –CH_2– of propyl), 3.89 (s, 6H, –OCH_3, $J = 8$), 4.25 (t, 4H, –OCH_2– of propyl, $J = 8$), 6.90 (d, 2H, C_6– of phenyl, $J = 8$), 7.00 (t, 2H, C_4– of phenyl, $J = 8$), 7.38 (t, 2H, C_5– of phenyl, $J = 8$), 8.13 (d, 2H, C_6– of phenyl, $J = 8$), 8.58 (s, 2H, –CH=N–), 10.28 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 400 MHz) δ in ppm: 10.4 (–CH_3), 23.2 (–CH_2–), 55.5 (–OCH_3), 76.3 (–OCH_2–), 110.8 (C_4–phenyl), 120.9 (C_5–phenyl), 121.8 (C_6–phenyl), 125.4 (C_1–phenyl), 127.1 (C_3–phenyl), 131.9 ($C_{3,4}$–thiophene), 144.1 ($C_{2,5}$–thiophene), 146.8 (C_2–phenyl), 156.9 (–CH=N–), 158.0 (–CONH–).

*7.1.9. 3,4-Dipropyloxy-N^2,N^5-bis(4-chlorophenylmethylene) thiophene-2,5-dicarbohydrazide (**10m**)*

IR (KBr, cm^{-1}) υ: 3217 cm^{-1} (-NH–), 2966 cm^{-1} (propyl), 1643 cm^{-1} (>C=O), 1600 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 1.13 (t, 6H, –CH_3, $J = 7.2$), 1.93 (m, 4H, –CH_2–), 4.26 (t, 4H, –OCH_2–, $J = 6.9$), 7.3 (d, 4H, C_3 and C_5– of phenyl), 7.7 (d, 4H, C_2 and C_6– of phenyl), 8.2 (s, 2H, –CH=N–), 10.2 (s, 2H, –NH–).

*7.1.10. Ethyl 3-{[(3,4-dipropyloxy-5-{[2-(3-ethoxy-1-methyl-3-oxopropylidene)hydrazino] carbonyl}-2-thienyl)carbonyl] hydrazono}butanoate (**10n**)*

IR (KBr, cm^{-1}) υ: 3313 cm^{-1} (-NH–), 2966 cm^{-1} (propyl), 1732 cm^{-1} (ester >C=O), 1660 cm^{-1} (amide >C=O), 1633 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 500 MHz) δ in ppm: 1.0 (t, 6H, –CH_3– of propyl, $J = 4.2$), 1.30 (t, 6H, –CH_3– of ester, $J = 4.2$), 1.85 (m, 4H, –CH_2– of propyl), 2.06 (s, 6H, –CH_3), 3.49 (s, 4H, –$COCH_2$–), 4.20 (m, 8H, –OCH_2– of ester and propyl), 9.97 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 500 MHz) δ in ppm: 10.0 (–CH_3 propyl), 14.1 (–CH_3 ester), 16.1 (–CH_3), 23.4 (–CH_2–propyl), 44.2 (–$COCH_2$–), 61.1 (–OCH_2–), 76.2 (–OCH_2–propyl), 125.5 ($C_{3,4}$–thiophene), 146.7 ($C_{2,5}$–thiophene), 150.5 (–CH=N–), 156.9 (–CONH), 169.6 (–C=O); MS (*m*/*z*, %): 540 (M^+, 100), 510 (5), 396 (100), 227 (10).

*7.1.11. 3,4-Dipropyloxy-N^2,N^5-bis[(N-dodecyl-9H-carbazole-3-yl)-methylene]thiophene-2,5-dicarbohydrazide (**10r**)*

IR (KBr, cm^{-1}) υ: 3263 cm^{-1} (-NH–), 2924 cm^{-1} (propyl and dodecyl), 1661 cm^{-1} (>C=O), 1630 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 400 MHz) δ in ppm: 0.89 (t, 6H, –CH_3– of dodecyl, $J = 8$), 1.23 (t, 6H, –CH_3– of propyl, $J = 8$), 1.31 (m, 36H, –CH_2– of dodecyl), 1.84 (m, 4H, –NCH_2CH_2– of dodecyl), 1.98 (m, 4H, –CH_2– of propyl), 4.30 (m, 8H, –OCH_2– and –NCH_2–), 7.30 (t, 2H, C_4–car), 7.40 (d, 4H, $C_{8,9}$–car, $J = 8$), 7.49 (t, 2H, C_3–car, $J = 8$), 7.90 (d, 2H, C_5–car, $J = 8$), 8.12 (d, 2H, C_2–car, $J = 8$), 8.26 (s, 2H, C_6–car), 8.47 (s, 2H, –CH=N–), 10.19 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 400 MHz) δ in ppm: 10.6 (–CH_3 propyl), 14.0 (–CH_3), 22.6 (–CH_2CH_3), 23.4 (–CH_2–), 27.2–31.8 (–CH_2–dodecyl), 43.1 (–NCH_2–), 76.0 (–OCH_2–), 108.7 (C_1–car), 109.0 (C_8–car), 119.4 (C_4–car), 120.0 ($C_{3,5}$–car), 122.7 ($C_{4a,b}$–car), 124.3 (C_6–car), 125.0 ($C_{3,4}$–thiophene), 126.0 ($C_{2,7}$–car), 140.8 (C_{9a}–car), 141.8 (C_{8a}–car), 146.8 ($C_{2,5}$–thiophene), 148.9 (–CH=N–), 156.7 (–CONH–); MS (*m*/*z*, %): 1008 (M + 1, 100), 631 (30), 605 (15), 588 (15), 546 (10), 361 (80).

*7.1.12. 3,4-Diheptyloxy-N^2,N^5-bis(2-oxo-1,2-dihydro-3H-indol-3-ylidene)thiophene-2,5-dicarbohydrazide (**11b**)*

IR (KBr, cm^{-1}) υ: 3257 cm^{-1} (-NH–), 2925 cm^{-1} (heptyl), 1712 cm^{-1} (>C=O), 1657 cm^{-1} (>C=N–); MS (*m*/*z*, %): 687 (M^+, 100), 656 (20), 604 (60), 527 (50), 383 (10), 355 (10), 339 (20), 312 (20).

*7.1.13. 3,4-Diheptyloxy-N^2,N^5-[bis(4-hydroxy-3-methoxyphenyl)methylene]thiophene-2,5-dicarbohydrazide (**11c**)*

IR (KBr, cm^{-1}) υ: 3290 cm^{-1} (-NH–), 2925 cm^{-1} (heptyl), 1654 cm^{-1} (>C=O), 1591 cm^{-1} (>C=N–); ^1H NMR (MeOD, 300 MHz) δ in ppm: 0.94 (t, 6H, –CH_3), 1.41–1.48 (m, 12H, –$CH_2CH_2CH_2CH_3$), 1.62 (m, 4H, –$OCH_2CH_2CH_2$–), 1.96 (m, 4H, –OCH_2CH_2–), 4.04 (s, 6H, –OCH_3), 4.44 (t, 4H, –OCH_2–), 4.70 (s, 2H, –OH D_2O exchangeable), 6.92 (d, 2H, C_5– of aromatic), 7.20

(d, 2H, C_6– of aromatic), 7.78 (s, 2H, C_2– of aromatic), 8.27 (s, 2H, –N=CH–).

*7.1.14. 3,4-Diheptyloxy-N^2,N^5-bis(phenylmethylene)thiophene-2,5-dicarbohydrazide (**11e**)*

IR (KBr, cm^{-1}) υ: 3263 cm^{-1} (-NH–), 2929 cm^{-1} (heptyl), 1656 cm^{-1} (>C=O), 1603 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 400 MHz) δ in ppm: 0.88 (t, 6H, $-CH_3$– of heptyl, $J = 6$), 1.31 (m, 8H, C_5, C_6– of heptyl), 1.40 (m, 4H, C_4– of heptyl), 1.52 (m, 4H, C_3– of heptyl), 1.88 (m, 4H, C_2– of heptyl), 4.28 (t, 4H, $-OCH_2$–), 7.42 (t, 6H, C_3, C_4, C_5– of phenyl), 7.77 (q, 4H, C_2, C_6– of phenyl), 8.26 (s, 2H, –N=CH–), 10.27 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 400 MHz) δ in ppm:13.9 ($-CH_3$), 22.5 ($-CH_2CH_3$), 26.1–30.0 ($-CH_2$–), 31.78 ($-OCH_2CH_2$–), 74.9 ($-OCH_2$–), 125.0 ($C_{3,4}$–thiophene), 127.7–128.6 ($C_{2,3,5,6}$–phenyl), 130.5 (C_4–phenyl), 133.4 (C_1–phenyl), 147.0 ($C_{2,5}$–thiophene), 148.4 (–CH=N–), 157.0 (–CONH–).

*7.1.15. 3,4-Diheptyloxy-N^2,N^5-bis(4-nitrophenylmethylene) thiophene-2,5-dicarbohydrazide (**11f**)*

IR (KBr, cm^{-1}) υ: 3279 cm^{-1} (-NH–), 2926 cm^{-1} (heptyl), 1665 cm^{-1} (>C=O), 1591 cm^{-1} (>C=N–); ^1H NMR ($CHCl_3$, 300 MHz) δ in ppm: 0.89 (t, 6H, $-CH_3$), 1.32–1.53 (m, 16H, C_3, C_4, C_5 and C_6– of heptyl), 1.90 (m, 4H, $-OCH_2CH_2$–), 4.30 (t, 4H, $-OCH_2$–, $J = 6$), 7.88 (d, 4H, C_2 and C_6– of aromatic), 8.22 (d, 4H, C_3 and C_5– of aromatic), 8.42 (s, 2H, –N=CH–), 10.41 (s, 2H, –NH–).

*7.1.16. 3,4-Diheptyloxy-N^2,N^5-bis[1-(2-thienyl)ethylidene] thiophene-2,5-dicarbohydrazide (**11j**)*

IR (KBr, cm^{-1}) υ: 3310 cm^{-1} (-NH–), 2926 cm^{-1} (heptyl), 1679 cm^{-1} (>C=O), 1636 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 500 MHz) δ in ppm: 0.88 (t, 6H, $-CH_3$– of heptyl, $J = 6.5$), 1.30 (m, 8H, C_5 and C_6– of heptyl), 1.35 (m, 4H, C_4– of heptyl), 1.45 (m, 4H, C_3– of heptyl), 1.85 (m, 4H, C_2– of heptyl), 2.33 (s, 6H, $-CH_3$), 4.30 (t, 4H, $-OCH_2$–), 6.98 (s, 2H, C_5– of thiophene), 7.32 (s, 4H, C_3 and C_4– of thiophene), 10.00 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 500 MHz) δ in ppm: 13.6 ($-CH_3$ hep), 22.0 ($-CH_3$), 25.2 ($-CH_2CH_3$), 28.6–29.8 ($-CH_2CH_2CH_2$–), 31.2 ($-OCH_2CH_2$), 74.4 ($-OCH_2$–), 125.0 ($C_{3,4}$–thiophene), 126.7 ($C'_{3,4}$–thiophene), 128.0 (C'_5–thiophene), 142.4 (C'_2–thiophene), 146.2 ($C_{2,5}$–thiophene), 147.8 (>C=N–), 156.0 (–CONH–); MS (*m*/*z*, %): 646 (M + 1, 100), 521 (5), 505 (50), 477 (5), 309 (50), 167 (40), 124 (50).

*7.1.17. 3,4-Dibenzyloxy-N^2,N^5-bis(3,4,5-trimethoxyphenylmethylene) thiophene-2,5-dicarbohydrazide (**12b**)*

IR (KBr, cm^{-1}) υ: 3240 cm^{-1} (-NH–), 2926 cm^{-1} (benzyl), 1637 cm^{-1} (>C=O), 1572 cm^{-1} (-C=N–); ^1H NMR ($CDCl_3$, 400 MHz) δ in ppm: 3.89 (s, 18H, $-OCH_3$), 5.36 (s, 4H, $-OCH_2$–), 6.86 (s, 4H, phenyl), 7.48 (s, 12H, benzyl phenyl and –N=CH–), 10.04 (s, 2H, –NH–); ^{13}C NMR ($CDCl_3$, 400 MHz) δ in ppm: 56.3 ($-OCH_3$), 60.9 ($-OCH_2$–), 104.9 ($C_{2,6}$–trimethoxyphenyl), 126.0 ($C_{3,4}$–thiophene), 128.7 (C_4–benzyl), 129.4 ($C_{2,3,5,6}$–benzyl), 129.4 (C_1–trimethoxyphenyl), 134.8 (C_1–benzyl), 140.4 ($C_{2,5}$–thiophene), 146.7 (–CH=N–), 148.3 (C_4–trimethoxyphenyl), 153.4 ($C_{3,5}$–trimethoxyphenyl), 156.5 (–CONH–).

*7.1.18. 3,4-Dibenzyloxy-N^2,N^5-bis(phenylmethylene)thiophene-2,5-dicarbohydrazide (**12c**)*

IR (KBr, cm^{-1}) υ: 3263 cm^{-1} (-NH–), 2926 cm^{-1} (benzyl), 1673 cm^{-1} (>C=O), 1605 cm^{-1} (>C=N–); ^1H NMR (DMSO-d_6, 300 MHz) δ in ppm: 5.28 (s, 4H, $-OCH_2$–), 7.30–7.61 (m, 20H, aromatic), 7.95 (s, 2H, –CH=N–), 10.86 (s, 2H, –NH–); MS (*m*/*z*, %): 589 (M + 1, 80), 498 (5), 443 (10), 413 (10), 385 (M + Na, 10), 379 (10), 353 (5), 261 (40).

*7.1.19. 3,4-Dibenzyloxy-N^2,N^5-bis[(N-ethyl-9H-carbazole-3-yl)-methylene]thiophene-2,5-dicarbohydrazide (**12d**)*

IR (KBr, cm^{-1}) υ: 3314 cm^{-1} (-NH–), 2980 cm^{-1} (benzyl and ethyl), 1680 cm^{-1} (>C=O), 1596 cm^{-1} (>C=N–); ^1H NMR (DMSO-d_6, 300 MHz) δ in ppm: 1.34 (t, 6H, $-NCH_2CH_3$), 4.4 (d, 4H, $-NCH_2$–, $J = 6$), 5.46 (s, 4H, $-OCH_2$–), 7.2–8.4 (m, 24H, aromatic), 8.51 (s, 2H, –CH=N–), 10.89 (s, 2H, –NH–).

*7.1.20. 3,4-Dibenzyloxy-N^2,N^5-bis(4-nitrophenylmethelene) thiophene-2,5-dicarbohydrazide (**12e**)*

IR (KBr, cm^{-1}) υ: 3266 cm^{-1} (-NH–), 2952 cm^{-1} (benzyl), 1677 cm^{-1} (>C=O), 1632 cm^{-1} (>C=N–); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.51 (s, 4H, $-OCH_2$–), 7.42 (m, 4H, C_2 and C_6– of benzyl), 7.82 (d, 4H, C_2 and C_6– of phenyl), 8.00 (m, 6H, C_3, C_4 and C_6– of benzyl), 8.30 (m, 4H, C_3 and C_4– of phenyl), 8.72 (s, 2H, –N=CH–), 10.55 (s, 2H, –NH–). MS (*m*/*z*, %): 680 (M + 2, 15).

*7.1.21. N^5,N^7-bis(3,4,5-trimethoxyphenylmethylene)-2,3-dihydrothieno[3,4-b][1,4]dioxine-5,7-dicarbohydrazide (**13c**)*

IR (KBr, cm^{-1}) υ: 3303 cm^{-1} (-NH–), 2926 cm^{-1} (EDOT), 1670 cm^{-1} (>C=O), 1577 cm^{-1} (>C=N–); ^1H NMR (DMSO-d_6, 300 MHz) δ in ppm: 3.62 (s, 6H, para-OCH_3), 3.75 (s, 12H, meta-OCH_3), 4.41 (s, 4H, $-OCH_2CH_2O$–), 6.93 (s, 4H, C_2 and C_6– of phenyl), 8.26 (s, 2H, –CH=N–), 10.58 (s, 2H, –NH–).

*7.1.22. N^5,N^7-bis(phenylmethylene)-2,3-dihydrothieno[3,4-b][1,4]dioxine-5,7-dicarbohydrazide (**13d**)*

IR (KBr, cm^{-1}) υ: 3306 cm^{-1} (-NH–), 2926 cm^{-1} (EDOT), 1675 cm^{-1} (>C=O), 1577 cm^{-1} (>C=N–); ^1H NMR (DMSO-d_6, 300 MHz) δ in ppm: 4.50 (s, 4H, $-OCH_2CH_2O$–), 7.43 (s, 6H, C_3, C_4 and C_5– of phenyl), 7.70 (d, 4H, C_2 and C_6– of phenyl), 8.43 (s, 2H, –N=CH–), 10.73 (s, 2H, –NH–); MS (*m*/*z*, %): 435 (M + 1, 100), 391 (20), 332 (10), 315 (10), 289 (10), 232 (20), 107 (5).

*7.1.23. N^2,N^7-bis[(4-nitrophenyl)methylene]-2,3-dihydrothieno[3,4-b][1,4]dioxine-5,7-dicarbohydrazide (**13f**)*

IR (KBr, cm^{-1}) υ: 3290 cm^{-1} (-NH–), 2940 cm^{-1} (EDOT), 1672 cm^{-1} (>C=O), 1580 cm^{-1} (>C=N–); MS (*m*/*z*, %): 525 (M + 1, 20), 482 (10), 378 (20), 361 (40), 232 (20).

7.2. Pharmacology

The evaluation of anticonvulsant activity was 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 by the MES, scMET and neurotoxicity screens. The substances were administered at doses of 30, 100 and 300 mg/kg at two time intervals.

7.2.1. Maximal Electroshock (MES) Test

The MES [19–21] 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.

7.2.2. Subcutaneous Metrazol Seizure Threshold (scMET) Test

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 [22] 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). 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.

7.2.3. Neurotoxicity – minimal motor impairment (MMI)

Rotorod technique [23] is the most widely used method to determine the neurotoxicity of compounds in anticonvulsant studies.

In experimental procedure, the drug treated 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 compound was considered to be toxic, if the treated animal falls off this rotating rod three times during a 1-min period. Similar procedure was followed for all the compounds to evaluate neurotoxicity.

7.2.4. 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 [24,25] 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). The 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 to be protected.

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