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Original article

Antiarrhythmic, serotonin antagonist and antianxiety activities of novel substituted thiophene derivatives synthesized from 2-amino-4,5,6,7-tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide

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ABSTRACT

A series of novel thiophene derivatives **3**–**17** were synthesized by initial reactions of 2-amino-4,5,6,7-tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide **1** and 2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonitrile **7** with different organic reagents. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, MS spectral data and elemental analysis. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All the compounds were screened for their antiarrhythmic, serotonin antagonist and antianexiety activities and they showed high activity compared with procaine amide, lidocaine, diazepam and buspirone as positive controls. The detailed synthesis, spectroscopic data, LD_{50} and pharmacological activities of the synthesized compounds were reported.

1. Introduction

Aromatic thiophenes play on part in animal metabolism; for examples, in Fig. 1, Biotin®, one of the vitamins (Vitamin H), is a tetrahydrothiophene, however aromatic thiophenes do occur in some plants, in association with polyacetylenes with which they are biogenetically linked and Banminth® (pyrantel), available anthelmintic used in animal husbandry, is one of the thiophene compounds in chemotherapy.

Thiophenes with a wide spectrum of biological activities are known, several of these derivatives possess potent analgesic [1,2], anticonvulsant, anti-inflammatory and antibacterial [3–6], antipyretics [7], antitumor [8,9], antiparasitic [10], antimicrobial [11], antihistaminic (H_1) [12], antianexiety test in mice [13], antiarrhythmic [14] and serotonin antagonist [15]. In previous work we have found that certain substituted pyrimidine and their heterocyclic derivatives show antimicrobial and anti-inflammatory [16–19] and antitumor activities [20–22]. On the other hand, thioxopyrimidine and thiazolopyrimidine derivatives have promising biological and anticancer activities [23–25]. In addition, we have reported that certain of our newly substituted heterocyclic compounds exhibited antiandrogenic [26], anti-inflammatory [27], anticancer [28], anticonvulsant [29] and antiarrhythmic [30] activities. Recently, some new thienopyrimidinone and *p*-methoxyphenyl pyrrolidine derivatives have been synthesized and tested as analgesic, antiparkinsonian, anti-inflammatory, serotonin antagonist and antianexiety activities [31–33].

Arrhythmia is a term for any of a large and heterogeneous group of conditions in which there is abnormal electrical activity in the heart. The heart beat may be too fast or too slow, and may be regular or irregular [34]. Serotonin is a monoamine neurotransmitter, biochemically derived from tryptophan, that is primarily found in the gastrointestinal tract, platelets, and central nervous system of humans and animals. It regulates mood, appetite, sleep, muscle contraction, and some cognitive functions including memory and learning. It is actively taken up by blood platelets, which store it. When the platelets bind to a clot, they disgorge serotonin, where it serves as a vasoconstrictor and helps to regulate hemostasis and blood clotting [35]. Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components. These components combine to create an unpleasant feeling that is typically associated with uneasiness, apprehension, fear, or worry. Anxiety is a generalized mood condition that can often

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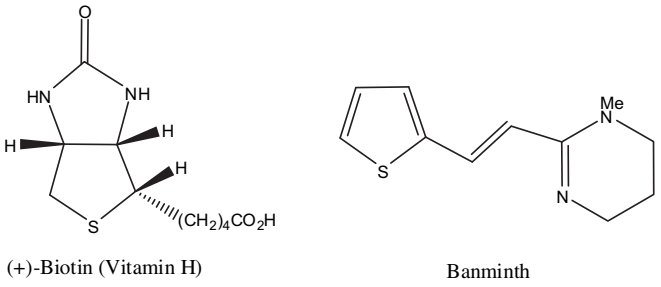


Fig. 1. Chemical structures of (+)-Biotin and Banminth.

occur without an identifiable triggering stimulus [36]. In view of the aforementioned facts, it seemed most interesting to synthesize some condensed tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide derivatives with the aim to evaluate their antiarrhythmic, serotonin antagonist and antianxiety activities.

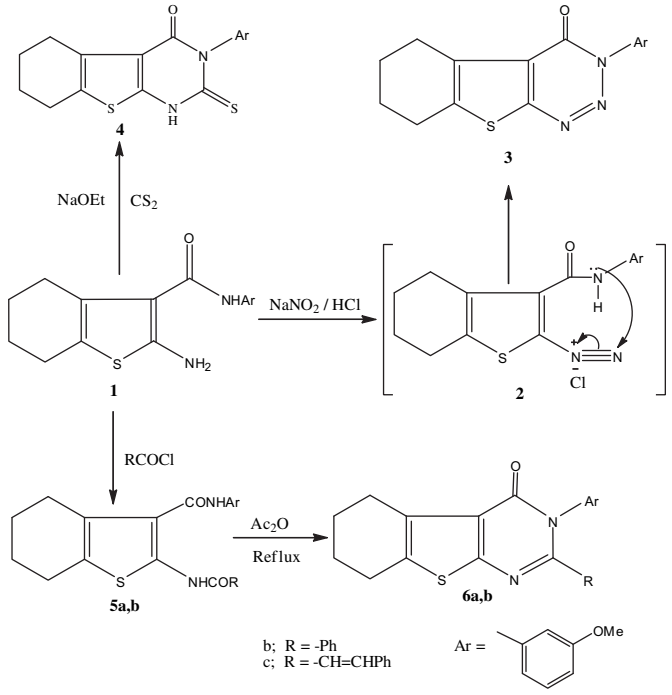
2. Results and discussion

2.1. Chemistry

In the present work, a series of condensed thiophenes via the heterocyclization functionalized thiophene using several reagents. 2-Amino-4,5,6,7-tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide **1** and 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile **7** were synthesized as starting materials according to Gewald procedures [37–39] (Scheme 1).

Nitrosation of thiophene derivative **1** by using $NaNO_2$ in the presence of HCl afforded the corresponding thienotriazine derivative **3**. The formation of **3** from nitrosation of **1** may proceed via the formation of diazonium salt **2** followed by the attack of nucleophilic nitrogen of carboxamide to the electrophilic nitrogen of diazonium salt. Compound **1** undergoes heterocyclization via the addition of amino function of thiophene to carbon disulfide in the presence of sodium ethoxide followed by cycloaddition to produce thienopyrimidine **4**. Also, thiophene derivative **1** was reacted with acid chloride at room temperature to give the acylated derivatives **5a**, **b** which were refluxed with acetic anhydride to afford the cyclized thienopyrimidines **6a**, **b** (Scheme 2). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4).

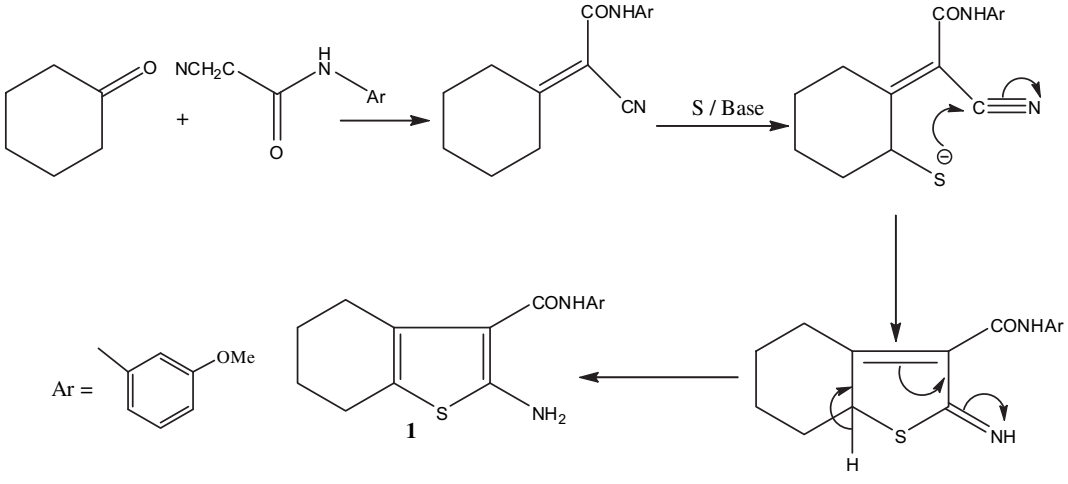
Treatment of aminocyanothiophene derivative **7** with acid chlorides afforded the derivatives **8a**–**c**, which were refluxed sulphuric



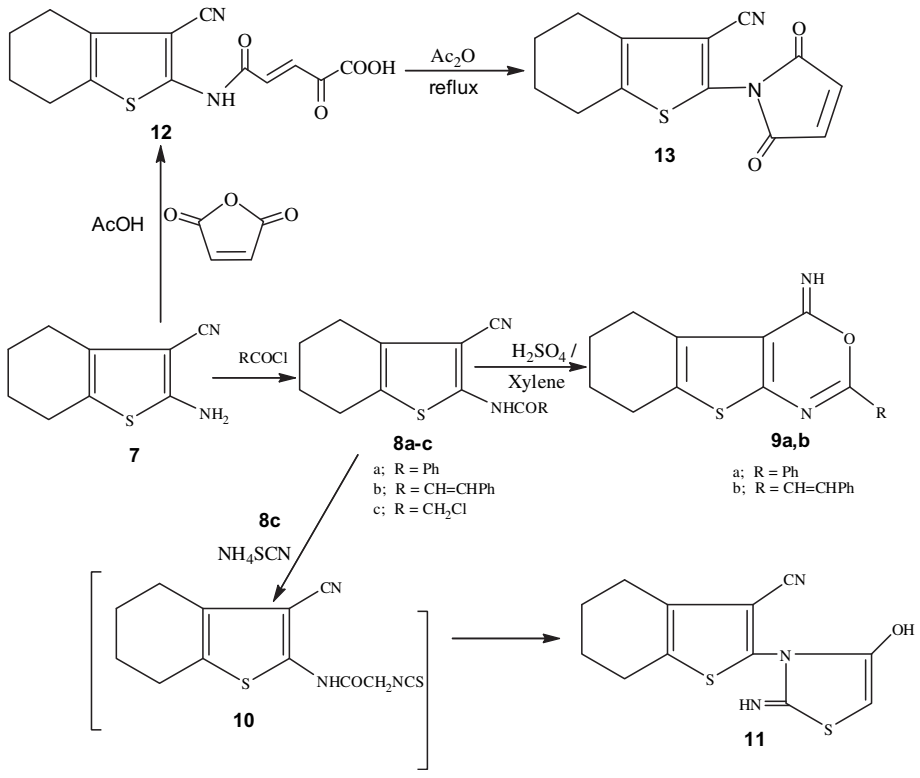
Scheme 2. Synthetic routes of compounds 3–6.

acid in xylene to give cyclized oxazine derivatives **9a**, **b**. Compound **8c** was reacted with ammonium thiocyanate to produce N-thienylthiazole derivative **11** presumably via the non isolable thiocyanate derivative **10**. The reaction of aminocyanothiophene **7** with maleic anhydride afforded the acylated product **12**, which was cyclized with acetic anhydride to give N-thienylmalimide **13** (Scheme 3). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4).

The author investigated the behavior of aminocyanothiophene towards the aroylisothio cyanate. Thus the addition of amino function of thiophene to the electrophilic carbon of isothiocyanate afforded thiourea derivative **14**. The latter compound was cyclized via the addition of nucleophilic nitrogen of thiourea function to the cyano function to afford thienopyrimidine **15**. Condensation of **7** and benzaldehyde afforded Schiff base **16**, which was treated with benzoylisothiocyanate to give the corresponding oxadiazine derivative **17** (Scheme 4). The structures of the synthesized



Scheme 1. Synthetic routes of compound 1.



Scheme 3. Synthetic routes of compounds 8–13.

compounds were assigned on the bases of its spectral data and elemental analysis.

2.2. Pharmacological screening

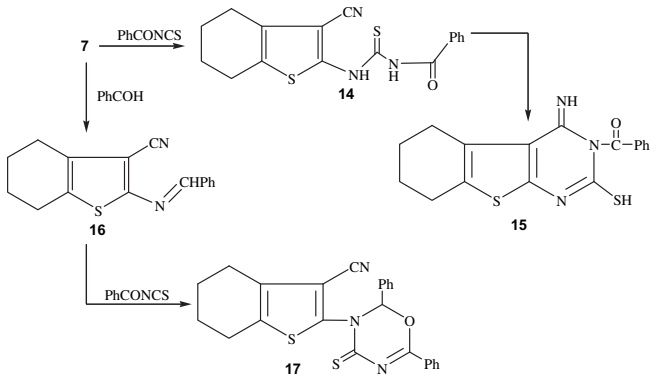
2.2.1. Antiarrhythmic activity

Procaine amide, 5 mg/kg iv and lidocaine 5 mg/kg iv. lead to an increase in LD_{100} by 65%, which corresponds to a LD_{100} of approximately 9 μg/100 mg (Fig. 2).

From Table 1, all the tested compounds showed potent antiarrhythmic activities, where the compounds **6b**, **9b**, **11**, **14**, **15** and **17** are more potent than procaine amide and lidocaine.

2.2.2. Serotonin antagonist activity

Determination of the affinity of tested compounds for the $5HT_{1A}$ receptor in brain may be useful for predicating compounds with novel anxiolytic or atypical antipsychotic profiles. The existence of at least two populations of $5HT_1$ receptors in rat brain was shown by differential sensitivity to spiroperdiol. The spiroperdiol-sensitive receptors were designed as the $5HT_{1A}$ subtype and the insensitive receptors were referred as the $5HT_{1B}$ subtype. *Schlegel* and *Peroutka* identified [^{3}H] *DPAT* as a selective ligand for $5HT_1$ receptors [40].



Scheme 4. Synthetic routes of compounds 14–17.

Specific binding is defined as the difference between total binding and binding in the presence of 10 μM 5HT [40,41]. IC_{50} values are calculated from the percent specific binding at each drug concentration (Table 2). Serotonin may play a role in anxiety, since drugs, which reduce serotoninergic function, have anxiolytic effects in animal models. Since buspirone and its analogs have relatively higher affinity for the $5HT_{1A}$ receptor than other receptor and no effect on the benzodiazepine site, their anxiolytic properties are attributed to activity at the $5HT_{1A}$ receptor. So all derivatives were subjected to anxiolytic screening.

From the results in Table 2 all the tested compounds showed potent serotonin antagonist activities, the order of descending activities is **15**, **6b**, (**9b**, **11** & Buspirone), **14**, **17**, **8c**, **9a**, (**8a** & **8b**), **12**, (**4**, **5b** & **13**), **3**, **5a**, **6a**, **16**, **7** and **1**.

2.2.3. Antianxiety test in mice [42–44]

Crawley has described a simple behavior model in mice to detect compounds with anxiolytic effects. Mice tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field. In a two chambered system, where mice can freely move between a brightly-lit open field and a dark corner, animals show more crossing between the two chambers and more locomotors activity after treatment with anxiolytics.

Dose response curves are obtained and the number of crossings through the partition between the light and the dark chambers are compound with total activity compound during 10 min. Dose makes decrease in total activity as that made by diazepam 10 mg/kg s.c. were determined and the relative potencies to diazepam were determined.

From the results in Table 3, all the tested compounds showed potent antianxiety activities, the order of descending activities is **17**, **11**, **14**, **6b**, **9b**, (**5b**, **6a**, **8b** & **15**), (**1**, **3** & **8a**), (**4**, **8c** **9a**, **12**, & **13**), (**5a** & **16**), and **7**.

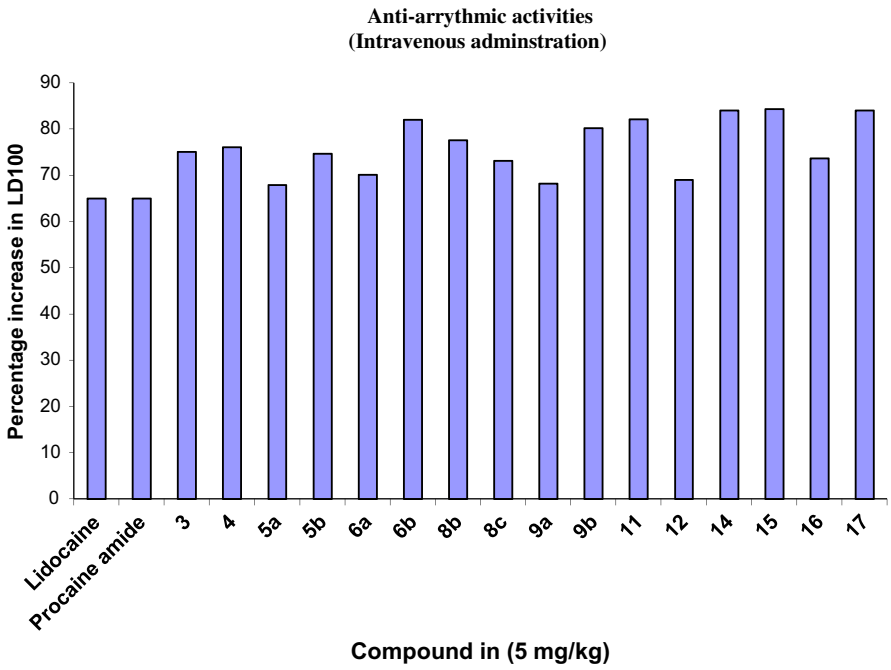


Fig. 2. Antiarrhythmic activities of the newly synthesized compounds.

2.2.4. Structure activity relationship (SAR)

- Thiophene nucleus is essential for antiarrhythmic, serotonin antagonist and antianxiety activities.
- Increasing the number of sulfur and nitrogen atoms sharply increases the activities.
- The side chains containing olefinic bonds are more active than non-olefinic bond substituted (for example compounds **5b**, **6b**, **8b** and **9b** more active than **5a**, **6a**, **8a** and **9a**).

2.2.5. Determination of acute toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with different increasing doses of the synthesized compounds. The dose that killed 50% of the animal was calculated according to *Austen* et al. [45] (Table 4).

Table 1
Antiarrhythmic activities of the newly synthesized compounds.

Compound in (5 mg/kg)	Percentage increase in LD_{100}
Lidocaine	65
Procaine amide	65
3	75.00
4	76.00
5a	67.90
5b	74.60
6a	70.10
6b	82.00
8b	77.50
8c	73.10
9a	68.15
9b	80.10
11	82.10
12	69.00
14	84.00
15	84.30
16	73.60
17	84.00

3. Conclusion

A series of novel substituted thiophene derivatives were synthesized by initial reaction of 2-amino-4,5,6,7-tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide **1** and 2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonitrile **7** with different organic. All the compounds were screened for their antiarrhythmic, serotonin antagonist and antianexiety activities and they showed high activity compared with procaine amide, lidocaine, diazepam and buspirone as positive controls. Thiophene nucleus is essential for activity and increasing the number of sulfur or nitrogen atoms sharply increases the activities. The olefinic bond in the substituted side chain led to increase the activities (for examples, **5b**, **6b**, **8b** and **9b** more active than **5a**, **6a**, **8a** and **9a**).

4. Experimental

4.1. Chemistry

All melting points are uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin–Elmer 1650 spectrophotometer (Perkin–Elmer, Norwalk, CT, USA). ^{1}H NMR was determined on a Jeol-Ex-270 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo electron corporation, USA) Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, and the results were within the accepted range ($\pm$0.2%) of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-coated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany). 2-Amino-4,5,6,7-tetrahydro-N-(3-methoxyphenyl)benzo[b]-thiophene-3-carboxamide (**1**) and 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**7**) were synthesized according to the previous procedures [37–39] as starting materials.

Table 2
Serotonin antagonist activities of known compounds (**1**, **7**) and newly synthesized compounds.

Compound No. (mg kg^{-1})	Concentration (mg kg^{-1})	IC_{50} (mg kg^{-1})
Buspirone	2×10^{-7}	63
	2×10^{-8}	87
1	2×10^{-7}	47
	2×10^{-8}	56
3	2×10^{-7}	61
	2×10^{-8}	75
4	2×10^{-7}	62
	2×10^{-8}	75
5a	2×10^{-7}	60
	2×10^{-8}	73
5b	2×10^{-7}	62
	2×10^{-8}	75
6a	2×10^{-7}	38
	2×10^{-8}	67
6b	2×10^{-7}	83
	2×10^{-8}	89
7	2×10^{-7}	39
	2×10^{-8}	65
8a	2×10^{-7}	66
	2×10^{-8}	77
8b	2×10^{-7}	66
	2×10^{-8}	78
8c	2×10^{-7}	58
	2×10^{-8}	80
9a	2×10^{-7}	68
	2×10^{-8}	79
9b	2×10^{-7}	63
	2×10^{-8}	87
11	2×10^{-7}	63
	2×10^{-8}	87
12	2×10^{-7}	63
	2×10^{-8}	77
13	2×10^{-7}	62
	2×10^{-8}	75
14	2×10^{-7}	66
	2×10^{-8}	87
15	2×10^{-7}	77
	2×10^{-8}	96
16	2×10^{-7}	39
	2×10^{-8}	67
17	2×10^{-7}	63
	2×10^{-8}	86

Table 3
Antianxiety activities of known compounds (**1**, **7**) and newly synthesized compounds.

Compound No.	Relative potencies to Diazepam
Diazepam	1
1	4
3	4
4	3
5a	2
5b	5
6a	5
6b	7
7	1
8a	4
8b	5
8c	3
9a	3
9b	6
11	12
12	3
13	3
14	10
15	5
16	2
17	16

Table 4
Acute toxicity (LD_{50}) of known compounds (**1**, **7**) and newly synthesized compounds.

Compound No	LD_{50}/mg/kg
Buspirone	113 ± 0.18
Diazepam	102 ± 0.10
1	121 ± 0.10
3	124 ± 0.18
4	210 ± 0.19
5a	236 ± 0.15
5b	276 ± 0.18
6a	136 ± 0.16
6b	243 ± 0.18
7	152 ± 0.12
8a	185 ± 0.17
8b	186 ± 0.18
8c	194 ± 0.19
9a	224 ± 0.18
9b	244 ± 0.11
11	274 ± 0.18
12	224 ± 0.18
13	234 ± 0.17
14	175 ± 0.18
15	221 ± 0.67
16	246 ± 0.16
17	297 ± 0.15

*4.1.1. 3-(3-Methoxyphenyl)-4,5,6,7-tetrahydrobenzo[b]thieno[2,3-d][1,2,3]-triazin-4(3H)-one (**3**)*

To a mixture of compound **1** (0.01 mol) in concentrated HCl (20 ml), sodium nitrite (0.01 mol) was added upon stirring for 2 h at 10 °C. The separated solid was filtrated off, dried and crystallized from acetic acid to give brown crystals of **3**. Yield (95%), m.p. 220 °C, IR (KBr, ν, cm^{-1}): 1654 (C═O), 1602 (C═C). ^1H NMR (DMSO-d_6): δ = 1.20–2.90 (m, 8H, cyclohexane), 3.90 (s, 3H, OCH_3), 6.90–8.40 (m, 4H, Ar_H) ppm. MS: 313 (M^+, 8), 282 (32), 206 (24), 164 (15), 136 (12), 108 (6), 79 (100). Elemental analysis for $C_{16}H_{15}O_2N_3S$ (313.37) Calcd: C, 61.32; H, 4.82; N, 13.41; S, 10.23. Found: C, 61.22; H, 4.76; N, 13.34; S, 10.18

*4.1.2. 3(3-MethoxyPhenyl)-2-thio-5,6,7,8,-tetrahydrobenzo[b] thieno[2,3-d]pyrimidin-4-(3H)-one (**4**)*

A mixture of compound **1** (0.01 mol), carbon disulfide (0.01 mol) and sodium ethoxide (0.01 mol) in ethanol (20 ml) was refluxed for 6 h. The reaction mixture was acidified with HCl (10 ml, 20%) and diluted with water (20 ml). The obtained solid was filtered off, dried and crystallized from acetic acid to give dark brown crystals of **4**. Yield (94%), m.p. 190 °C, IR (KBr, ν, cm^{-1}): 3428 (NH), 1690 (C═O). ^1H NMR (DMSO-d_6): δ = 1.20–2.90 (m, 8H, cyclohexane), 3.90 (s, 3H, OCH_3), 7.00–7.88 (m, 4H, Ar-H), 8.15 (s, 1H, NH) ppm. MS: 344 (M^+, 32), 223 (100). Elemental analysis for $C_{17}H_{16}N_2O_2S_2$ (344.45): C, 59.28; H, 4.68; N, 8.13; S, 18.62. Found: C, 59.22; H, 4.63; N, 8.08; S, 18.56.

*4.1.3. 2-(2-Substituted)-4,5,6,7-tetrahydro-N-(3-methoxyphenyl) benzo[b]thiophene-3-carboxamide (**5a**, **b**)*

A mixture of compound **1** (0.01 mol) and aroyl chloride, namely, benzoyl chloride or cinamoyl chloride (0.0l mol) in acetic acid (20 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and then poured onto water. The formed solid was filtered off, dried and crystallized from acetic acid to give brown crystals of **5a**, **b** respectively.

*4.1.3.1. 2-Benzamido-N-(3-methoxyphenyl)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxamide (**5a**).* Yield (90%), m.p. 190 °C, IR (KBr, ν, cm^{-1}): 3426 (NH), 1666 (C═O). ^1H NMR (DMSO-d_6): δ = 1.70–2.82 (m, 8H, cyclohexane), 3.76 (s, 3H, OCH_3), 7.16–7.86 (m, 9H, Ar-H), 10.65, 12.30 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS: 406

(M^+, 85), 329 (100). Elemental analysis for $C_{23}H_{22}N_2O_3S$ (406.50): Calcd.: C, 67.96; H, 5.46; N, 6.89; S, 7.89. Found: C, 67.90; H, 5.40; N, 6.84; S, 7.85.

*4.1.3.2. 2-Cinnamamido-N-(3-methoxyphenyl)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxamide (**5b**).* Yield (85%), m.p.: 220 °C, IR (KBr, ν, cm^{-1}): 3432 (NH), 1668 (C=O). ^1H NMR (DMSO-d_6): δ = 1.68–2.90 (m, 8H, cyclohexane), 3.70 (s, 3H, OCH_3), 6.65–8.00 (m, 11H, Ar.H + CH=CH), 10.52, 11.85 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS: 432 (M+, 14), 310 (100). Elemental analysis for $C_{25}H_{24}N_2O_3S$ (432.53): Calcd.: C, 69.42; H, 5.59; N, 6.48; S, 7.41. Found: C, 69.34; H, 5.55; N, 6.42; S, 7.35.

*4.1.4. 2-(Supstituted)-3-(3-methoxyphenyl)-4,5,6,7-tetrahydrobenzo[b]thieno[2,3-d]pyrimidin-4(3H)-one (**6a**, **b**)*

A solution of compounds **5a**, **b** (0.01 mol) in acetic anhydride (20 ml) was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, the solid obtained was collected by filtration, dried and crystallized from acetic acid to give brown crystals of **6a**, **b** respectively.

*4.1.4.1. 2-(Phenyl)-3-(3-methoxyphenyl)-4,5,6,7-tetrahydrobenzo[b] thieno[2,3-d]pyrimidin-4(3H)-one (**6a**).* Yield (80%), m.p. >300 °C, IR (KBr, ν, cm^{-1}): 1680 (C=O). ^1H NMR (DMSO-d_6): δ = 1.73–2.92 (m, 8H, cyclohexane), 3.68 (s, 3H, OCH_3), 7.24–8.02 (m, 9H, Ar-H) ppm. MS: 390 (M^++2, 8), 204 (100); Elemental analysis for $C_{23}H_{20}N_2O_2S$ (388.48): Calcd.: C, 71.11; H, 5.19; N, 7.21; S, 8.25. Found: C, 71.05; H, 5.14; N, 7.16; S, 8.20

*4.1.4.2. 2-(Cinnamoyl)-3-(3-methoxyphenyl)-4,5,6,7-tetrahydrobenzo [b]thieno[2,3-d]pyrimidin-4(3H)-one (**6b**).* Yield (78%), m.p. >300 °C, IR (KBr, ν, cm^{-1}): 1676 (C=O). ^1H NMR (DMSO-d_6): δ = 1.70–2.88 (m, 8H, cyclohexane), 3.70 (s, 3H, OCH_3), 7.00–8.18 (m, 11H, Ar-H + CH=CH) ppm. MS: 414 (M^+, 28), 103 (100); Elemental analysis for $C_{25}H_{22}N_2O_2S$ (414.52): Calcd.: C, 72.44; H, 5.35; N, 6.76; S, 7.74. Found: C, 72.36; H, 5.30; N, 6.71; S, 7.68.

*4.1.5. 2-N-Substituted-4,5.6.7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**8a**–**c**)*

A mixture of compound **7** (0.01 mol) and halo compounds, namely, aroyl chloride and/or chloroacetylchloride (0.01 mol) in dry acetone (20 ml) was refluxed for 3 h. The solvent was evaporated under reduced pressure, the obtained residue was solidified with diethyl ether, filtered off, washed with ether, dried and crystallized from acetic acid to give white crystals of **8a**, light green of **8b** and light yellow crystal of **8c** respectively.

*4.1.5.1. N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)benzamide (**8a**).* Yield (95%), m.p. 196 °C, IR (KBr, ν, cm^{-1}): 3464 (NH), 2220 (CN), 1678 (C=O). ^1H NMR (DMSO-d_6): δ = 1.65–2.86 (m, 8H, cyclohexane), 7.23–8.00 (m, 5H, Ar-H), 11.54 (s, 1H, NH exchangeable with D_2O) ppm. MS: 282 (M^+, 32), 205 (100); Elemental analysis for $C_{16}H_{14}N_2OS$ (282.36): Calcd. C, 68.06; H, 5.00; N, 9.92; S, 11.36. Found: C, 68.00; H, 4.96; N, 9.85; S, 11.30.

*4.1.5.2. N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)cinnamamide (**8b**).* Yield (95%), m.p. 196 °C, IR (KBr, ν, cm^{-1}): 3464 (NH), 2220 (CN), 1678 (C=O). ^1H NMR (DMSO-d_6): δ = 1.68–2.90 (m, 8H, cyclohexane), 6.90–7.76 (m, 7H, Ar-H + CH=CH), 11.42 (s, 1H, NH exchangeable with D_2O) ppm. MS: 310 (M^++2, 8), 131 (100); Elemental analysis for $C_{18}H_{16}N_2OS$ (308.40): Calcd.: C, 70.10; H, 5.23; N, 9.08; S, 10.40. Found: C, 70.00; H, 5.18; N, 9.00; S, 10.35.

*4.1.5.3. 2-Chloro-N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)acetamide (**8c**).* Yield (68%); m.p. 287 °C, IR (KBr, ν, cm^{-1}): 3426 (NH), 1680 (C=O). ^1H NMR (DMSO-d_6): δ = 1.72–2.86 (m, 8H, cyclohexane), 4.18 (s, 2H, CH_2), 11.65 (s, 1H, NH exchangeable with D_2O) ppm. MS: 256 (M^++2, 32), 254 (M^+, 100); Elemental analysis for $C_{11}H_{11}ClN_2OS$ (254.74): Calcd.: C, 51.86; H, 4.35; Cl, 13.92; N, 11.00; S, 12.59. Found: C, 51.76; H, 4.30; Cl, 13.84; N, 10.88; S, 12.53.

*4.1.6. 2-Aryl-4,5,6,7-tetrahydrobenzo[b]-4H-thieno[2,3-d][1,3] oxazin-4-imine (**9a**, **b**)*

A mixture of compounds **8a**, **b** (0.01 mol) and conc. H_2SO_4 (0.01 mol) in xylene (20 ml) was refluxed for 8 h. After cooling, the reaction mixture was poured onto ice-water, neutralized with sodium carbonate, the obtained solid was filtered off, washed with water, dried and crystallized from acetic acid to give brown crystals of **9a**, **b** respectively.

*4.1.6.1. 2-Phenyl-4,5,6,7-tetrahydrobenzo[b]-4H-thieno[2,3-d][1,3] oxazin-4-imine (**9a**).* Yield (80%), m.p. >360 °C, IR (KBr, ν, cm^{-1}): 3434 (NH). ^1H NMR (DMSO-d_6): δ = 1.70–2.92 (m, 8H, cyclohexane), 7.05–8.00 (m, 5H, Ar-H), 12.15 (s, 1H, NH exchangeable with D_2O) ppm. MS: 282 (M^+, 18), 179 (100); Elemental analysis for $C_{16}H_{14}N_2OS$ (282.36): Calcd.: C, 68.06; H, 5.00; N, 9.92; S, 11.36. Found: C, 68.00; H, 4.96; N, 9.86; S, 11.30.

*4.1.6.2. 2-Cinnamoyl-4,5,6,7-tetrahydrobenzo[b]-4H-thieno[2,3-d] [1,3]oxazin-4-imine (**9b**).* Yield (78%), m.p. >360 °C, IR (KBr, ν, cm^{-1}): 3438 (NH). ^1H NMR (DMSO-d_6): δ = 1.72–2.90 (m, 8H, cyclohexane), 6.88–7.78 (m, 7H, Ar-H + CH=CH), 12.18 (s, 1H, NH exchangeable with D_2O) ppm. MS: 308 (M^+, 6), 129 (100); Elemental analysis for $C_{18}H_{16}N_2OS$ (308.40): Calcd.: C, 70.10; H, 5.23; N, 9.08; S, 10.40. Found: C, 70.01; H, 5.17; N, 9.00; S, 10.35.

*4.1.7. 4,5,6,7-Tetrahydro-2-(4-hydroxy-2-iminothiazol-3(2H)-yl) benzo[b]thiophene-3-carbonitrile (**11**)*

A mixture of **8c** (0.01 mol) and ammonium thiocynate (0.01 mol) in absolute ethanol (20 ml) was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, then poured onto water, the separated solid was filtered off, dried and crystallized from acetic acid to give brown crystals of **11**. Yield (92%), m.p. >300 °C, IR (KBr, ν, cm^{-1}): 3648 (OH), 3365 (NH), 2208 (CN), 1660 (C=N). ^1H NMR (DMSO-d_6): δ = 1.70–2.87 (m, 8H, cyclohexane), 6.84 (s, 1H, thiazole-H), 10.65 (s, 1H, NH exchangeable with D_2O), 12.05 (s, 1H, OH exchangeable with D_2O) ppm. MS: 277 (M^+, 16), 114 (100); Elemental analysis for $C_{12}H_{11}N_3OS_2$ (277.37): Calcd.: C, 51.96; H, 4.00; N, 15.15; S, 23.12. Found: C, 51.90; H, 3.92; N, 15.08; S, 23.06.

*4.1.8. 4-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylcarbamoyl)-2-oxobut-3-enoic acid (**12**)*

A mixture of **7** (0.01 mol) and malic anhydride (0.01 mol) in glacial acetic acid (50 ml) was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure, after cooling, the obtained solid was filtered off and crystallized from acetic acid to gave yellow crystals of **12**. Yield (85%), m.p. 230 °C, IR (KBr, ν, cm^{-1}): 3464 (OH), 2560 (NH), 2210 (CN), 1692 (C=O). ^1H NMR (DMSO-d_6): δ = 1.68–2.84 (m, 8H, cyclohexane), 6.08, 7.10 (2d, 2H, J = 12.6 Hz, CH=CH), 10.55 (s, 1H, NH exchangeable with D_2O), 12.12 (s, 1H, OH exchangeable with D_2O) ppm. MS: 304 (M^+, 12), 259 (100); Elemental analysis for $C_{14}H_{12}N_2O_4S$ (304.32): Calcd.: C, 55.25; H, 3.97; N, 9.21; S, 10.54. Found: C, 55.19; H, 3.92; N, 9.16; S, 10.50.

*4.1.9. 4,5,6,7-Tetrahydro-2-(2,5-dioxo-2H-pyrrol-1(5H)-yl)benzo[b] thiophene-3-carbonitrile (**13**)*

A solution of compound **12** (0.01 mol) in acetic anhydride (20 ml) was refluxed for 2 h. The solid obtained on concentration

and cooling was collected by filtration and crystallized from acetic acid to give brown crystals of **13**. Yield (80%), m.p. >300 °C, IR (KBr, ν, cm^{-1}): 2208 (CN), 1680 (C=O). ^1H NMR (DMSO-d_6): δ = 1.65–2.90 (m, 8H, cyclohexane), 6.75 (d, 2H, pyrrol-H) ppm. MS: 277 (M^+, 16), 114 (100); Elemental analysis for $C_{13}H_{10}N_2O_2S$ (258.30): Calcd.: C, 60.45; H, 3.90; N, 10.85; S, 12.41. Found: C, 60.40; H, 3.85; N, 10.80; S, 12.35.

4.1.10. N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl) benzoylthiourea (**14**)

A mixture of compound **7** (0.01 mol) and benzoylisothiocyanate (0.01 mol) in dry acetone (20 ml) was refluxed for 1 h. The reaction mixture was poured onto water, the separated solid was filtered off, dried and crystallized from acetic acid to give brown crystals of **14**. Yield (92%), m.p. 180 °C, IR (KBr, ν, cm^{-1}): 3290 (NH), 2210 (CN), 1678 (C=O). MS: 343 (M^++2, 6), 264 (100); Elemental analysis for $C_{17}H_{15}N_3OS_2$ (341.45): Calcd.: C, 59.80; H, 4.43; N, 12.31; S, 18.78. Found: C, 59.74; H, 4.36; N, 12.25; S, 18.70.

4.1.11. (4-Imino-2-mercapto-4,5,6,7-terahydrobenzo[b]thieno[2,3-d]pyrimidin-3(4H)-yl)(phenyl) methanone (**15**)

To a solution of compound **14** (0.01 mol) in dry acetone (20 ml), anhydrous potassium carbonate (0.5 g) was added. The reaction mixture was refluxed for 3 h, after cooling, it was poured onto water. The formed solid was filtered off, dried and crystallized from acetic acid to give yellow crystals of **15**. Yield (78%), m.p. 134 °C, IR (KBr, ν, cm^{-1}): 3344 (NH), 1670 (C=O). ^1H NMR (DMSO-d_6): δ = 1.66–2.92 (m, 8H, cyclohexane), 7.15–7.96 (m, 5H, Ar-H), 9.90 (s, 1H, NH exchangeable with D_2O), 14.10 (s, 1H, SH exchangeable with D_2O), ppm. ^{13}C NMR (DMSO-d_6): δ = 23.10, 23.15, 23.65, 24.55 ($4CH_2$), 127.34, 129.05, 136.85, 143.40 (thiophene ring), 126.12, 128.20, 131.10, 131.86 (Ph-C), 165.75 (C=NH), 164.10 (C-SH), 172.04 (C=O) ppm. MS: 341 (M^+, 6), 164 (100); Elemental analysis for $C_{17}H_{15}N_3OS_2$ (341.45): Calcd.: C, 59.80; H, 4.43; N, 12.31; S, 18.78. Found: C, 59.74; H, 4.36; N, 12.24; S, 18.70.

4.1.12. 2-(Benzylideneamino)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitrile (**16**)

A mixture of compound **7** (0.01 mol) and benzaldehyde (0.01 mol) in absolute ethanol (20 ml) was refluxed for 3 h. The separated solid formed upon dilution with water (20 ml) was filtered off, dried and crystallized from ethanol to give brown crystals of **16**. Yield (95%), m.p. 104 °C, IR (KBr, ν, cm^{-1}): 2204 (CN), 1660 (C=N). ^1H NMR (DMSO-d_6): δ = 1.65–2.88 (m, 8H, cyclohexane), 3.60 (s, 1H, CH=N), 6.98–7.88 (m, 5H, Ar-H) ppm; MS: 268 (M^++2, 16), 104 (100); Elemental analysis for $C_{16}H_{14}N_2S$ (266.36): Calcd.: C, 72.15; H, 5.30; N, 10.52; S, 12.04. Found: C, 72.08; H, 5.23; N, 10.46; S, 11.95.

4.1.13. 4,5,6,7-Tetrahydro-2-(2,6-diphenyl-4-thioxo-2H-1,3,5-oxadiazin-3(4H)-yl)benzo[b] thiophene-3-carbonitrile (**17**)

A mixture of compound **16** (0.01 mol) and phenyl isothiocyanate (0.01 mol) in dry acetone (20 ml) was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure, the obtained residue was triturated with n-hexane, the solid obtained was filtered off, washed with n-hexane and diethyl ether, dried and crystallized from acetic acid to give yellow crystals of **17**. Yield (82%), m.p. 156 °C, IR (KBr, ν, cm^{-1}): 2206 (CN), 1670 (C=N). ^1H NMR (DMSO-d_6): δ = 1.62–2.90 (m, 8H, cyclohexane), 5.62 (s, 1H, CH), 7.12–7.86 (m, 10H, Ar-H) ppm. ^{13}C NMR (DMSO-d_6): δ = 22.75, 23.18, 23.65, 24.34 ($4CH_2$), 82.80, 135.50, 136.10, 140.22 (thiophene ring), 116.56 (CN), 125.05, 126.15, 126.45, 128.00, 128.12, 130.60, 134.80, 139.76 (2Ph-C), 86.65 (CH), 179.55 (C=S) ppm. MS: 429 (M^+, 24), 267 (100); Elemental analysis for $C_{24}H_{19}N_3OS_2$ (429.56): Calcd.: C, 67.11; H, 4.46; N, 9.78; S, 14.93. Found: C, 67.01; H, 4.39; N, 9.70; S, 14.86.

4.2. Pharmacological screening

4.2.1. Antiarrhythmic activity [46–50]

4.2.1.1. Purpose and rational. The plant alkaloid aconitine persistently activates sodium channel. Infusion of aconitine in the anesthetized rat causes ventricular arrhythmias. Drugs considered to have antiarrhythmic properties can be tested in aconitine-intoxicated rats.

4.2.1.2. Procedure. Male Ivanovas rats weighing 300–350 g are used. The animals are anesthetized by intra peritoneal injection of 1.25 g/kg urethane: 5 mg/kg aconitine dissolved in 0.1 N HNO_3 is adminstered by continuous infusion into the saphenous vein of 0.1 ml/min and the electrocardiogram (ECG) in lead II is recorded every 30 s. The test compound is injected IV at a screening dose of 5 mg/kg 5 min before the start of the aconitine infusion, 24 animals are used per compound.

4.2.1.3. Evaluation. The antiarrhythmic effect of a test compound is measured by the amount of aconitine/100 g animal. (Duration of infusion) which induces.

- Ventricular extra systoles.
- Ventricular tachycardia.
- Ventricular fibrillation.

Higher doses of aconitine in the treated group as compared to an untreated control group are an indication of antiarrhythmic activity.

Statistical significance between the groups is assessed by the student's *t*-test.

4.2.2. Serotonin antagonist

4.2.2.1. Procedure.

1. Tris buffer pH 7.7
   a. 57.29 Tris HCl, 16.2 g Tris base, q. s to/liter with distilled water (0.5 M Tris buffer, pH 7.7)
   b. Make a 1:10 dilution in deionized H_2O (0.05 M Tris buffer, pH 7.7).
   c. 0.05 M Tris buffer, pH 7.7 containing 10 μM paragyline 4 mM $CaCl_2$ and 0.1% ascorbic acid. 0.49 mg paragyline HCl, 111 mg $CaCl_2$, 20 mg vitamin C, q. s. to 250 ml with 0.05 M Tris buffer, pH 7.7 (reagent 1b).

2. [^{3}H]-*DPAT*(2-N,N-Di[2,3(n)-^{3}propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphth-alene). (160–206 ci/mmol) was obtained from Amersham. For IC_{50} determination = a 10 nM stock solution is made up and 50 mm^3 are added to each tube (final concentration =0.5 nM).

3. Serotonin sulphate. 0.5 nM stock solution is made up in 0.01 N HCl and 20 mm^3 added to 3 tubes for determination of non specific binding (final concentration =10 μM).

4. Test compound 1 mM stock solution is made up in a suitable solvent and serially diluted, such that the final concentrations in the assay range from 2×10^{-5} to 2×10^{-8} M. Seven concentrations are used for each assay. Higher or lower concentrations may be used based on the potency of the drug.

4.2.2.2. Tissue Preparation. Male Wister rats are sacrificed by decapitation. Hippocampus are removed, weighed, and homogenized in 20 volumes of 0.05 M Tris buffer, pH 7.7. The homogenate is

centrifuged at 48000g for 10 min and the supernatant is discarded. The pellet is re-suspended in an equal volume of 0.05 M Tris buffer, incubated at 37 °C for 10 min and re-centrifuged at 48000g for 10 min. The final membrane pellet is re-suspended in 0.05 M Tris buffer containing 4 mM $CaCl_2$, 0.1% vitamin C and 10 μM paragyline.

4.2.2.3. Assay.

- 800 mm^3 Tissue
- 130 mm^3 0.05 M Tris + $CaCl_2$ + paragyline + vitamin C
- 20 mm^3 vehicle/5HT/drug.
- 50 mm^3 [^{3}H] *DPAT.*

Tubes are incubated for 15 min at 25 °C. The assay is stopped by vacuum filtration through whatman GF/B filters, which are then washed 2 times with 5 cm^3 of ice-cold 0.05 M Tris buffer. The filters are then placed into scintillation vials with 10 cm^3 of liquiscint scintillation cocktail and counted.

4.2.3. Antianxiety test in mice

4.2.3.1. Procedure. The testing apparatus consists of a light and a dark chamber that was divided by a photo cell-equipped zone, A polypropylene animal cage 44 × 21 cm, is darkened with black spray over one-third. A partition containing a 13 cm long x5 cm high opening separates the dark on third from the bright two thirds of the cage. The cage rests on an animex activity monitor, which counts total locomotor activity. An electronic system using four sets of photo cells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments. Nanive male albino mice with a body weight of 18–25 g are placed into the cage. The animals are treated (30 min before the experiment) with the tested drugs or the vehicle intraperitoneally and are then observed for 10 min.

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References

[1] C.G. Dave, P.R. Shah, K.G. Dave, J. Indian Chem. Soc. 66 (1989) 48–50.
[2] V. Alagarsamy, S. Meena, K.V. Ramseshu, V.R. Solomon, K. Thirumurugan, K. Dhanabal, M. Murugan, Eur. J. Med. Chem. 41 (2006) 1293–1300.
[3] K.M. Dawood, H. Abdel-Gawad, E.A. Rageb, M. Ellithey, H.A. Mohamed, Bioorg. Med. Chem. 14 (2006) 3672–3680.
[4] A.D. Pillai, P.D. Rathod, F.P. Xavier, H. Padh, V.V. Sudarsanam, K.K. Vasu, Bioorg. Med. Chem. 13 (2005) 6685–6692.
[5] J.Y. Gauthier, Y. Leblanc, W.C. Black, C. Chan, W.A. Cromlish, R. Gordon, B.P. Kennedey, C.K. Lau, S. Leger, Z. Wang, D. Ethier, J. Guay, J. Mancini, D. Riendeau, P. Tagan, P. Vickers, E. Wong, L. Xu, P. Prasit, Bioorg. Med. Chem. Lett. 6 (1996) 87–92.
[6] H. Vieweg, S. Leistner, G. Wagner, N. Boehm, U. Krasset, R. Grupe, D. Lohmann, G. Loban, East German Patent DD, 257830; Chem. Abst. 110 (1989) 95262.
[7] E. Bousquet, G. Romero, F. Guerrera, A. Caruso, M.A. Roxas, Farmaco, Ed. Sci. 40 (1985) 869–872.
[8] K. Starcevic, M. Krali, I. Piantanida, L. Suman, K. Pavelic, G. Karminski-Zamola, Eur. J. Med. Chem. 41 (2006) 925–939.
[9] Y.D. Wang, S. Johnson, D. Powell, J.P. McGinnis, M. Miranda, S.K. Rabindran, Bioorg. Med. Chem. 15 (2007) 3763–3766.
[10] J.L. Gonzalez, C.E. Stephens, T. Wenzler, R. Brun, F.A. Janious, W.D. Wilson, T. Barszcz, K.A. Werbovetz, D.W. Boykin, Eur. J. Med. Chem. 14 (2007) 552–557.
[11] A.E. Abdel-Rahman, E.A. Bakhit, E.A. Al-Taifi, J. Chin. Chem. Soc. 49 (2002) 223–227.
[12] C.J. Shishoo, V.S. Shirsath, I.S. Rathod, V.D. Yande, Eur. J. Med. Chem. 35 (2000) 351–358.
[13] T. Kilfoil, A. Michel, D. Montgomry, R.L. Whiting, Neuropharmacology 28 (1989) 901–905.
[14] A. Vaille, A.M. Scotto diTella, J. Maldonado, P. Vanelle, Meth Find Exp. Clin. Pharmacol. 14 (1992) 183–188.
[15] S.J. Peroutka, J. Neurochem. 47 (1986) 925–932.
[16] A.E. Amr, Indian J. Heterocycl. Chem. 10 (2000) 49–58.
[17] A.E. Amr, H.H. Sayed, M.M. Abdalla, Arch. Pharm. Chem. Life Sci. 338 (2005) 433–440.
[18] A.E. Amr, N.M. Sabry, M.M. Abdalla, Monatsh. Chem. 138 (2007) 699–707.
[19] A.E. Amr, Z. Naturforsch. 60 (2005) 990–998.
[20] M.H. Abo-Ghalia, A.E. Amr, Amino Acids 26 (2004) 283–289.
[21] A.E. Amr, O.I. Abd El-Salam, A. Attia, I. Stibor, Collect. Czech. Chem. Commun. 64 (1999) 288–298.
[22] A.G. Hammam, A.F.M. Fahmy, A.E. Amr, A.M. Mohamed, Indian J. Chem. 42B (2003) 1985–1993.
[23] A.E. Amr, M.M. Abdulla, Indian J. Heterocycl. Chem. 12 (2002) 129–134.
[24] A.G. Hammam, A.A. Naglaa, M.H. Wanda, M. Marian, Z. Naturforsch. 55b (2000) 417–424.
[25] M.I. Ali, A.G. Hammam, J. Chem. Eng. Data 26 (1981) 352–355.
[26] A.E. Amr, A.A. Nehad, M.M. Abdalla, Bioorg. Med. Chem. 14 (2006) 373–384.
[27] A.E. Amr, M.M. Abdalla, Bioorg. Med. Chem. 14 (2006) 4341–4352.
[28] A.E. Amr, M.M. Ashraf, F.M. Salwa, A.A. Nagla, A.G. Hammam, Bioorg. Med. Chem. 14 (2006) 5481–5488.
[29] A.A. Nehad, A.E. Amr, A.I. Alhusien, Monatsh. Chem. 138 (2007) 559–567.
[30] F.A.S. Ahmed, M.M. Abdulla, A.E. Amr, A.H. Azza, Monatsh. Chem. 138 (2007) 1019–1027.
[31] N.H. Ouf, A.E. Amr, A.A. Fayed, Monatsh. Chem. 139 (2008) 281–287.
[32] N.H. Ouf, A.E. Amr, Monatsh. Chem. 139 (2008) 579–585.
[33] M.M. Abdalla, B.F. Abdel-Wahab, A.E. Amr, Monatsh Chem. 140 (2009) 129–137.
[34] D. Wyse, A. Waldo, J. DiMarco, M. Domanski, Y. Rosenberg, E. Schron, J. Kellen, H. Greene, M. Mickel, J. Dalquist, S. Corley, N. Engl. J. Med. 347 (2002) 1825–1833.
[35] M. Berger, J.A. Gray, B.L. Roth, Annu. Rev. Med. 60 (2009) 355–366.
[36] D.H. Barlow, Am. Psychol. (2002) 1247–1263.
[37] K. Gewald, E. Schinke, H. Bottcher, Chem. Ber. 99 (1966) 94–100.
[38] K. Gewald, M.J. Hentschel, J. Prakt. Chem. 318 (1976) 343–346.
[39] K. Gewald, Lect. Heterocycl. Chem. 6 (1981) 121–126.
[40] J.R. Schlegel, S.J. Peroutka, Biochem. Pharmacol. 35 (1986) 1943–1949.
[41] N.W. Pedigo, H.I. Yammamura, D.L. Nelson, J. Neurochem. 36 (1981) 220–226.
[42] B. Costall, C.A. Hendrie, M.E. Kelly, R.J. Naylor, Neuropharmacology 26 (1987) 195–200.
[43] J.N. Crawley, K.K. Goodwin, Pharmacol. Biochem. Behav. 13 (1980) 167–170.
[44] J.N. Crawley, Pharmacol. Biochem. Behav. 15 (1981) 695–699.
[45] K.F. Austen, W.E. Brocklehurst, J. Exp. Med. 113 (1961) 521–539.
[46] M.J.A. Walker, M.J. Curtius, D.J. Hearse, R.W.F. Campbell, M.J. Jams, D.M. Yellon, S.M. Coker, J.B. Harness, D.W.G. Harron, A.J. Miggins, D.G. Julian, M.J. Lab, A.S. Manning, B.J. Northover, J.R. Parratt, R.A. Riemrsma, E. Riva, D.C. Russell, D.J. Sheridan, E. Winslow, B. Woodward, Cardiovasc. Res. 22 (1988) 447–455.
[47] C. Bazzani, S. Genedani, S. Tugliavini, A. Bertolini, J. Pharm. Pharmacol. 41 (1989) 651–653.
[48] F. DeClerk, J. Cardiovasc. Pharmacol. 22 (1993) 120–125.
[49] E. Winslow, Br. J. Pharmacol. 71 (1980) 615–622.
[50] E. Winslow, J. Cardiovasc. Pharmacol. 3 (1981) 87–100.