Full Paper

Synthesis and Studies on Antidepressant and Anticonvulsant Activities of Some 3-(2-Thienyl)pyrazoline Derivatives

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In this study, the synthesis of twelve 3-(2-thienyl)pyrazoline derivatives are described. The structures of all compounds were confirmed by UV, IR, ^{1}H-NMR, mass spectral data, and microanalyses. In the pharmacological studies, antidepressant and anticonvulsant activities of these compounds have been screened. The antidepressant activities of the compounds were investigated by Porsolt's behavioral despair test (forced swimming) on albino mice and compared with tranylcypromine. Among the compounds examined, the compounds **9** and **12** showed significant antidepressant activity. Anticonvulsant activities of the compounds were determined by maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (metrazol) (scMet.) tests, neurotoxicities were determined by rotarod toxicity test on albino mice. Compound **8** was found to be protective against MES in the range of 30 – 300 mg/kg dose levels at four hours. None of the synthesized compounds showed neurotoxicity at 30 – 300 mg/kg dose levels.

Keywords: Anticonvulsant activity / Antidepressant activity / 3-Pyrazoline derivatives

Received: April 10, 2008; accepted: July 31, 2008

DOI 10.1002/ardp.200800068

Introduction

The chemistry and the synthesis of 2-pyrazoline derivatives have attracted widespread attention in recent years. The present popularity of these derivatives is mainly due to their structural similarity to isocarboxazid (Fig. 1), a monoamine oxidase (MAO) inhibitor, which is well known to show prominent antidepressant activity [1]. In earlier studies, 1,3,5-triphenyl-2-pyrazolines are reported to possess MAO inhibitory activities by Palmar *et al*. [2] and Soni *et al*. [3]. In a recent paper, Chimenti *et al*. [4] reported enantioselective MAO-A and MAO-B inhibiting properties of 1-thiocarbamoyl-2-pyrazolines. MAO inhibitors have been proven to show antidepressant activity both in laboratory animals and man [5, 6].

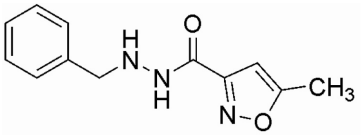

Figure 1. Structure of isocarboxazid(*N*-benzyl-5-methyl-oxazole-3-carbohydrazide).

So far, we synthesized various substituted pyrazolines and their some condensed derivatives and investigated their antidepressant and anticonvulsant activities [7 – 13]. Rajendra Prasad *et al*. [14] also reported the synthesis and antidepressant activity of the compounds with the same ring. Promissing antidepressant or anticonvulsant activities of some derivatives prompted us to investigate them further. In continuation to our earlier studies, we attempted to expand our series of compounds by changing the aryl substituents on the pyrazole ring. In this study, synthesis, structural elucidation, and antidepressant activity as well as anticonvulsant activity of a new series of 3-(2-thienyl)-pyrazoline derivatives are reported.

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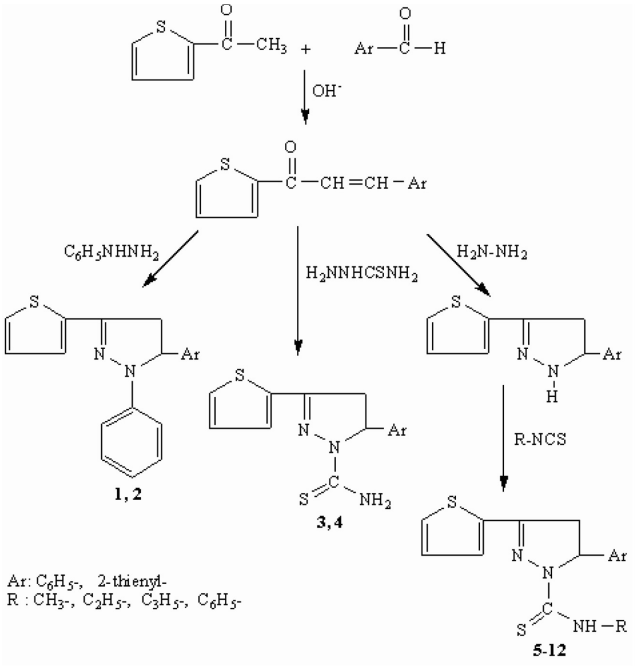
Abbreviations: maximal electroshock seizure (MES); monoamine oxidase (MAO); subcutaneous pentylenetetrazole (metrazol) (scMet.)

Results and Discussion

As shown in Scheme 1, the starting compound, 1-(2-thienyl)-3-phenyl/(2-thienyl)-2-propen-1-one was obtained by the reaction of benzaldehyde and 2-thienylaldehyde with acetylthiophen in a Claisen – Schmidt condensation reaction. The reaction of 1-(2-thienyl)-3-phenyl/(2-thienyl)-2-propen-1-one with phenylhydrazine and thiosemicarbazide in presence of sodium hydroxide in ethanol gave 1-phenyl- (**1** and **2**), and 1-thiocarbamoyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines (**3** and **4**), respectively. Treatment of the starting compound with hydrazine hydrate in ethanol, followed by addition of thiocyanates in the presence of triethylamine in ether provided 1-*N*-substituted thiocarbamoyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines **5** – **12**. Although compounds **1** and **2** have already been reported previously by Ried and Dankert [15], they have been included in our research program to screen their activity. The structures of the isolated compounds were characterized by spectral methods and microanalyses. All spectral data are in accordance with the assumed structures.



Scheme 1. Synthetic route of the 3-(2-thienyl)-pyrazoline derivatives.

In the UV spectra of the compounds, two absorption maxima were observed at 206 – 244 and 327 – 353 nm due to C=N and Ar-N-N=C-Ar groups, respectively. In the IR spectra, all compounds displayed pyrazoline C=N stretching (1501 – 1576 cm^{-1}), C^4-H deformation (1362 – 1464 cm^{-1}), C^5-N^1 streching (1069 – 1189 cm^{-1}), thiocarbamoyl group N-H stretching (3112 – 3481 cm^{-1}), and C=S stretching (1315 – 1357 cm^{-1}) bands.

In the ^{1}H-NMR spectra of the compounds H_A, H_B, and H_X, protons of the pyrazoline ring were observed as doublet of doublet at δ = 2.98 – 3.30 ppm (J_{AB} = 17.44 – 17.69 Hz), 3.45 – 3.75 ppm (J_{Ax} = 3.43 – 3.71 Hz), and 5.98 – 6.97 ppm (J_{Bx} = 11.34 – 11.66 Hz), respectively. N-H protons of the thiocarbamoyl group were generally seen at 7.23 – 9.10 ppm as broad bands. All the other protons belonging methyl, ethyl, allyl groups, benzene and thiophene rings were seen accordingly to the expected chemical shift and integral values.

The mass spectroscopic fragmentation of the compounds was studied under electron ionization. molecular ion peaks [M^+], which were prominent for all the compounds, confirmed the molecular weights of the examined compounds. The fragmentation pattern was essentially identical. Fragments resulting from the loss of the SH ion from the thiocarbamoyl group were observed for all compounds. On the other hand, α-cleavage adjacent to both sides of the C=S group have also been observed causing ejection of NHR or CSNHR type of ions. The fragments resulting by loss of C_5H_4NS and $C_5H_4N_2S$ ions from the molecular ion were observed for almost all compounds. Additionally, the pyrazoline ring has shown a fragmentation pattern giving rise to $C_7H_7N_2S$ and C_5H_4NS type of ions. Microanalyses results were also in accordance with the theoretical amounts.

In-vivo antidepressant activities of the compounds were assessed in mice applying the forced swimming test. The forced swimming test, which is a behavioral test, used to predict the efficacy of antidepressant treatments [16]. It is used effectively in predicting the activity of a wide variety of antidepressants such as MAO inhibitors [17] and typical antidepressants [18]. It also has a good predictive value for the antidepressant potency in humans [19]. The obtained data on the antidepressant activity of the compounds and reference drug were given in Table 1. The compounds **2**, **4**, **9**, **10**, **12** bearing a thienyl group at the 5-position of the pyrazoline ring (except **11**) showed marked antidepressant activity. Among the mentioned derivatives, most promising results were obtained with the compounds carrying 1-*N*-methylthiocarbamoyl- (**9**) and 1-*N*-phenylthiocarbamoyl- (**12**) on the pyrazoline ring. The mentioned derivatives significantly reduced the duration of immobility times at the 10 mg/kg dose level when compared to the control ($p < 0.05$, Table 1).

Anticonvulsant activities of the synthesized compounds were also investigated by maximal electroshock (MES) and subcutaneous pentylenetetrazole (metrazol) (*sc*Met.) tests, and results from these experiments are shown in Table 2. Seizure assays and neurotoxicity were

Table 1. Antidepressant activities of the synthesized compounds.

Compounds	Antidepressant activities	
	Duration of immobility (sec) (Mean ± S.E.M.) *	Change from control (%)
1	188 ± 7.7	−6.47
2	96 ± 21.2*	−52.53
3	158 ± 18.3	−21.39
4	84 ± 21.5*	−58.20
5	174 ± 14.2	−13.43
6	190 ± 10	−5.47
7	154 ± 19.9	−23.38
8	148 ± 29.1	−26.36
9	43 ± 15.3*	−78.60
10	88 ± 21.2*	−56.24
11	182 ± 14.9	−9.45
12	48 ± 18.9*	−76.12
Tranylcypromine sulfate (10 mg/kg, ip)	57 ± 11.6	−71.64
Control	201 ± 8.6	

* Values represents the mean ± S.E.M. (n = 6–9).
* Significantly compared to control (Dunnet's test; $p < 0.05$).

determined by rotarod toxicity test according to the phase-I tests of the anti-epileptic drug development (ADD) program which were developed by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) [20, 21]. According to the results of the *in-vivo* experiments, it is difficult to extract a definite structure-anticonvulsant activity relationship between of the tested compounds **1**–**12**. As shown in Table 2, the significant difference in activity was observed depending on both aryl group at 5-position and the substituents on the thiocarbamoyl at the first position of the pyrazoline ring. Compound **4**, 1-thiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline, was found ineffective in the dose range of 30–300 mg/kg, while some remarkable activity were observed with compound **3** having a phenyl at 5-position in the 300 mg/kg-dose level at four hours. Anticonvulsant activity of the compounds bearing a phenyl group at the 5-position are taken into consideration, it could be concluded that the substitution of thiocarbamoyl group always resulted in good activity either at half hour at the 300 mg/kg-dose level (compounds **5** and **7**) or at four hours (compounds **6** and **8**). Among the compounds with phenyl, compound **8** possessed the most prominent and consistent activity against MES in the range of 30–300 mg/kg-dose levels at four hours. It is worth saying that all compounds which exhibited activity were found to be protective against MES-induces seizures at their high dose level (300 mg/kg). However, only two compounds (compounds **6** and **12**) exhibited activity against *sc*Met.–induced seizures at the 300 mg/kg-dose level. Neurotoxicity was observed in none of the synthesized compounds in the dose range of 30–300 mg/kg.

Conclusion

In summary, we have reported the synthesis and biological evaluation of 3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazoline derivatives as novel candidate antidepressant /

Table 2. Phase I anticonvulsant screening of the compounds.

Compounds	MES*						*sc*Met.[a]						Toxicity[b]					
	1/2 h mg/kg			4 h mg/kg			1/2 h mg/kg			4 h mg/kg			1/2 h mg/kg			4 h mg/kg		
	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300
1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
3	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
4	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
5	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
6	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
7	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
8	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
9	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
10	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
11	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
12	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/4	0/4	0/4	0/2	0/2	0/2

* MES: maximal electroshock seizure test.
[a] *sc*Met.: subcutaneous pentylenetetrazole (metrazol) seizure test.
[b] oxicity: rotarod test.
0/1: no activity at dose level, 1/1: noticeable activity at dose level.

anticonvulsant compounds. Generally, the synthesized compounds having a phenyl substituent at the 5^{-} position of the pyrazoline ring (compounds **3**, **5**, **6**, **7**, **8**) possess remarkable anticonvulsant activity. Among the mentioned derivatives, most promising results were obtained with the compound **8** carrying 1-*N*-phenylthiocarbamoyl- against MES in the range of 30 – 300 mg/kg dose levels at four hours. On the contrary, however, the comporids bearing a thienyl at the 5-position of the ring (compounds **2**, **4**, **9**, **10**, **12**, except **11**) attract attention with their antidepressant activity. Two of them (compounds **9** and **12**) showed a larger antidepressant activity than tranylcypromine. It is worth saying that the compounds having a *N*-phenylthiocarbamoyl at 1-position of pyrazoline ring exhibited a remarkable antidepressant and anticonvulsant activity. Therefore, such compounds would represent a fruitful matrix for the development of a new class of antidepressant and anticonvulsant agents and would deserve further investigation and derivatization as a promising scaffold.

This study was supported by Hacettepe University Scientific Research Foundation (Project no: 0302 30100).

The authors have declared no conflict of interest.

Experimental

Chemistry

All chemicals used in this study were supplied by E. Merck (Germany), Aldrich Chemical Co. (Munich, Germany) and Fluka AG (Buchs, Switzerland). Melting points were taken in a Thomas Hoover capillary melting point apparatus (Thomas Hoover, Philadelphia, PA, USA) and are uncorrected. UV spectra were obtained on Agilent 8453 UV-Visible spectrophotometer in methanol. IR spectra were recorded in a Bruker Vector 22 IR Opus Spectroscopic Software Version 2.0 (Bruker Bioscience, Billerica, MA, USA) using KBr pellets. ^{1}H-NMR spectra were recorded on a Bruker Avance 400 MHz FT spectrometer (Bruker) in $CDCl_3$ using TMS as internal standart. Mass spectra were recorded on Scientific Instrument Service HPP7-M (SIS, Ringoes, NJ, USA) direct insertion probe spectrometer using Agilent 5973 network mass selective electron impact detector (Agilent, Palo Alto, CA, USA). Microanalyses of the compounds were performed at The Laboratory of Instrumental Analyses (ATAL). The Scientific and Technical Research Council of Turkey (Instrument: Leco CHNS-932; Leco, St. Joseph, MI, USA).

1-(2-Thienyl)-3-phenyl/(2-thienyl)-2-propen-1-ones chalcones

Chalcone derivatives were obtained from 2-acetylthiophene (0.01 mol) and appropriate aldehydes (0.01 mol) by known methods [22 – 26].

*1-Phenyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines **1, 2***

The solution of appropriate chalcone (0.01 mol) and phenylhydrazine (0.02 mol) in ethanolic sodium hydroxide (0.025 mol, 20 mL) was refluxed for four hours. The product was poured into ice water and the crude product, which was separated out, was filtered and crystallized from a proper solvent.

*1,5-Diphenyl-3-(2-thienyl)-2-pyrazoline **1***

Yield: 65%; m.p.: 128 – 129°C (Crys. solv.: MeOH); UV λ_{Maks}^{MeOH} [nm]: 202 (log ε: 4.31), 253 (log ε: 4.11), 369 (log ε: 4.10); IR ν (KBr) [cm^{-1}]: 1593, 1499 (C=N stretching), 1386 (C^4-H-deformation), 1125 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.15 (dd, J_{AB} = 17.42 Hz, J_{AX} = 7.11 Hz, 1H, H_A), 3.90 (dd, J_{AB} = 17.11 Hz, J_{BX} = 12.41 Hz, 1H, H_B), 5.25 (dd, J_{AX} = 7.13 Hz, J_{BX} = 12.15 Hz, 1H, H_X), 6.80 (m, 1H, thiophene H^4), 7.00 – 7.45 (m, 12H, thiophene H^3, H^5 and benzene); MS m/e: 304 [M$^+$] (100%), 227 [M – C_6H_5] (35%), 91 [C_6H_5N] (50%), 77 [C_6H_5] (35%).

*1-Phenyl-3,5-di-(2-thienyl)-2-pyrazoline **2***

Yield: 88%; m.p.: 107 – 108°C (Crys. solv.: MeOH / H_2O); UV λ_{Maks}^{MeOH} [nm]: 201 (log ε: 4.19), 251 (log ε: 4.13), 365 (log ε: 4.07); IR ν (KBr) [cm^{-1}]: 1591, 1495 (C=N stretching), 1377 (C^4-H-deformation), 1228 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.30 (dd, J_{AB} = 16.83 Hz, J_{AX} = 6.35 Hz, 1H, H_A), 3.85 (dd, J_{AB} = 16.46 Hz, J_{BX} = 12.15 Hz, 1H, H_B), 5.55 (dd, J_{AX} = 6.63 Hz, J_{BX} = 12.21 Hz, 1H, H_X), 6.75 – 7.50 (m, 11H, thiophene and benzene); MS m/e: 310 [M$^+$] (100%), 277 [M – SH] (28%), 227 [M – C_4H_3S] (21%), 218 [M – C_6H_6N] (41%), 200 [M – C_5H_4NS] (38%), 91 [C_6H_5N] (81%).

*1-Thiocarbamoyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines **3, 4***

1-Thiocarbamoyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines were obtained by heating (2 h) thiosemicarbazide (0.012 mol) with the appropriate chalcone (0.01 mol) and sodium hydroxide (0.025 mol in 5 mL water) in ethanol (50 mL). The product was poured into ice water and the crude product, which was separated out, was filtered and crystallized from the proper solvent.

*1-Thiocarbamoyl-3-(2-thienyl)-5-phenyl-2-pyrazoline **3***

Yield: 63%; m.p.: 178 – 179°C (Crys. solv.: MeOH). UV λ_{Maks}^{MeOH} [nm]: 202 (log ε: 4.22), 244 (log ε: 4.20), 338 (log ε: 3.85); IR ν (KBr) [cm^{-1}]: 3356 (N-H stretching), 1571, 1473 (C=N stretching), 1370 (C=S stretching), 1294 (C^4-H-deformation), 1000 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.20 (dd, J_{AB} = 17.65 Hz, J_{AX} = 3.68 Hz, 1H, pyrazoline H_A), 3.90 (dd, J_{AB} = 17.61 Hz, J_{BX} = 11.40 Hz, 1H, pyrazoline H_B), 6.05(dd, J_{AX} = 3.50 Hz, J_{BX} = 11.43 Hz, 1H, pyrazoline H_X), 7.00 – 7.50 (m, 8H, thiophene and benzene); MS m/e: 287 [M$^+$] (96%), 254 [M – SH] (87%), 227 [M – $CSNH_2$] (72%), 177 [M-C_5H_4NS] (100%), 151 [$C_7H_7N_2S$] (65%), 110 [C_5H_4NS] (35%). Anal. Calcd. for $C_{14}H_{13}N_3S_2$: C, 58.51; H, 4.56; N, 14.62; S, 22.31. Found: C, 58.69; H, 5.28; N, 14.75; S, 22.19.

*1-Thiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline **4***

Yield: 66%; m.p.: 157 – 8°C (Crys. solv.: EtOH); UV λ_{Maks}^{MeOH} [nm]: 243 (log ε: 4.23), 337 (log ε: 4.04); IR ν (KBr) [cm^{-1}]: 3438 (N-H stretching), 1571, 1519, 1470 (C=N stretching), 1350 (C=S stretching), 1080 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.35 (dd, J_{AB} = 17.50 Hz, J_{AX} = 3.21 Hz, 1H, H_A), 3.90 (dd, J_{AB} = 17.43 Hz, J_{BX} = 11.40 Hz, 1H, H_B), 6.10 (b, 1H, NH), 6.40 (dd, J_{AX} = 3.45 Hz, J_{BX} =

11.27 Hz, 1H, H_X), 6.90 (b, 1H, NH), 6.90 – 7.50 (m, 6H, thiophene); MS m/e: 293 [M^+] (89%), 260 [M – SH] (70%), 233 [M – $CSNH_2$] (34%), 183 [M – C_5H_4NS] (75%), 169 [M – $C_5H_4N_2S$] (100%), 151 [$C_7H_7N_2S$] (24%), 110 [C_5H_4NS] (57%). Anal. Calcd. for $C_{12}H_{11}N_3S_3$: C, 49.12; H, 3.78; N, 14.32; S, 32.78. Found: C, 49.53; H, 4.20; N, 14.38; S, 32.34.

1-N-substituted-thiocarbamoyl-3-(2-thienyl)-5-phenyl/(2-thienyl)-2-pyrazolines ***5–12***

Hydrazine hydrate (0.02 mol) was added to an ethanolic solution of appropriate chalcone (0.01 mol, 10 mL ethanol) and refluxed for 2 h. The solvent was evaporated at reduced pressure. The residue was dissolved in dry ether. Isothiocyanate (0.01 mol) and four drops of triethylamine were added and stirred at room temperature for 4 h. The mixture was evaporated to dryness and the residue was crystallized from the proper solvent.

1-N-Methylthiocarbamoyl-3-(2-thienyl)-5-phenyl-2-pyrazoline ***5***

Yield: 60%; m.p.: 138 – 139°C (Crys. solv.: EtOH / H_2O); UV λ_{Maks}^{MeOH} [nm]: 203 (log ε: 4.22), 246 (log ε: 4.17), 341 (log ε: 3.89); IR ν (KBr) [cm^{-1}]: 3371 (N-H stretching), 1590, 1435 (C=N stretching), 1388 (C^4-H-deformation), 1343 (C=S stretching), 1110 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.15 (dd, J_{AB} = 17.55 Hz, J_{AX} = 3.70 Hz, 1H, H_A), 3.25 (d, J = 4.91 Hz, 3H, CH_3), 3.82 (dd, J_{AB} = 17.52 Hz, J_{BX} = 11.65 Hz, 1H, H_B), 6.10 (dd, J_{AX} = 3.63 Hz, J_{BX} = 11.68 Hz, 1H, H_X), 7.00 – 7.55 (m, 8H, thiophene and benzene), 7.40 (b, 1H, NH); MS m/e: 301 [M^+] (100%), 268 [M – SH] (75%), 227 [M – $CSNHCH_3$] (96%), 191 [M – C_5H_4NS] (91%), 151 [$C_7H_7N_2S$] (87%), 110 [C_5H_4NS] (37%). Anal. Calcd. for $C_{15}H_{15}N_3S_2$: C, 59.77; H, 5.02; N, 13.94; S, 21.28. Found: C, 60.16; H, 5.07; N, 14.00; S, 20.99.

1-N-Ethylthiocarbamoyl-3-(2-thienyl)-5-phenyl-2-pyrazoline ***6***

Yield: 60%; m.p.: 112 – 113°C (Crys. solv.: EtOH / H_2O); UV λ_{Maks}^{MeOH} [nm]: 202 (log ε: 4.24), 247 (log ε: 4.17), 342 (log ε: 3.89); IR ν (KBr) [cm^{-1}]: 3348 (N-H stretching), 1511 (C=N stretching), 1443, 1369 (C^4-H-deformation), 1300 (C=S stretching), 1184 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 1.35 (t, J = 5.40 Hz, 3H, CH_3), 3.15 (dd, J_{AB} = 17.52 Hz, J_{AX} = 3.64 Hz, 1H, H_A), 3.60 – 3.90 (m, 3H, H_B and CH_2), 6.10 (dd, J_{AX} = 3.65 Hz, J_{BX} = 11.68 Hz, 1H, H_X), 7.05 – 7.55 (m, 8H, thiophene and benzene), 7.35 (b, 1H, NH); MS m/e: 315 [M^+] (100%), 282 [M – SH] (61%), 227 [M – $CSNHC_2H_5$] (95%), 205 [M – C_5H_4NS] (47%), 151 [$C_7H_7N_2S$] (73%), 110 [C_5H_4NS] (28%). Anal. Calcd. for $C_{16}H_{17}N_3S_2$: C, 60.92; H, 5.43; N, 13.32; S, 20.33. Found: C, 60.81; H, 6.37; N, 13.23; S, 19.89.

1-N-Allylthiocarbamoyl-3-(2-thienyl)-5-phenyl-2-pyrazoline ***7***

Yield: 38%; m.p.: 101-102°C (Crys. solv.: EtOH / H_2O); UV λ_{Maks}^{MeOH} [nm]: 204 (log ε: 4.78), 248 (log ε: 4.72), 342 (log ε: 4.43); IR ν (KBr) [cm^{-1}]: 3370 (N-H stretching), 1508 (C=N stretching), 1441, 1368 (C^4-H-deformation), 1311 (C=S stretching), 1160 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.20 (dd, J_{AB} = 17.69 Hz, J_{AX} = 3.68 Hz, 1H, H_A), 3.85 (dd, J_{AB} = 17.51 Hz, J_{BX} = 11.67 Hz, 1H, H_B), 4.20 – 4.45 (m, 2H, CH_2), 5.15 – 5.33 (m, 2H, $=CH_2$), 5.90 – 6.00 (m, 1H, =CH), 6.10 (dd, J_{AX} = 3.56 Hz, J_{BX} = 11.60 Hz, 1H, H_X), 7.05 – 7.50 (m, 8H, thiophene and benzene), 7.42 (b, 1H, NH); MS m/e: 327 [M^+] (91%), 294 [M – SH] (18%), 271 [M – NHC_3H_5] (100%), 227 [M – $CSNHC_3H_5$] (77%), 151 [$C_7H_7N_2S$] (66%), 110 [C_5H_4NS] (46%), 104 [C_7H_6N] (86%). Anal. Calcd. for $C_{17}H_{17}N_3OS_2$: C, 62.35; H, 5.23; N, 12.83; S, 19.58. Found: C, 62.72; H, 5.18; N, 12.86; S, 19.91.

1-N-Phenylthiocarbamoyl-3-(2-thienyl)-5-phenyl-2-pyrazoline ***8***

Yield: 67%; m.p.: 122 – 3°C (Crys. solv.: EtOH); UV λ_{Maks}^{MeOH} [nm]: 202 (log ε: 4.47), 251 (log ε: 4.43), 346 (log ε: 4.22); IR ν (KBr) [$cm^{-}1$] 3343 (N-H stretching), 1588, 1513 (C=N stretching), 1451 (C^4-H-deformation), 1343 (C=S stretching), 1169 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.20 (dd, J_{AB} = 17.60 Hz, J_{AX} = 3.40 Hz, 1H, H_A), 3.90 (dd, J_{AB} = 17.62 Hz, J_{BX} = 11.50 Hz, 1H, H_B), 6.20 (dd, J_{AX} = 3.37 Hz, J_{BX} = 11.50 Hz, 1H, H_X), 7.10 – 7.70 (m, 13H, thiophene and benzene), 9.15 (s, 1H, NH); MS m/e: 363 [M^+] (73%), 330 [M – SH] (75%), 271 [M – C_6H_5NH] (77%), 253 [M – C_5H_4NS] (43%), 227 [M – $CSNHC_6H_5$] (79%), 162 [$C_8H_6N_2S$] (100%), 151 [$C_7H_7N_2S$] (64%), 110 [C_5H_4NS] (30%). Anal. Calcd. for $C_{20}H_{17}N_3S_2$: C, 66.08; H, 4.71; N, 11.56; S, 17.64. Found: C, 66.47; H, 5.07; N, 11.57; S, 17.50.

1-N-Methylthiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline ***9***

Yield: 53%; m.p.: 149 – 150°C (Crys. solv.: EtOH / H_2O); UV λ_{Maks}^{MeOH} [nm]: 223 (log ε: 4.20), 243 (log ε: 4.03), 340 (log ε: 4.01); IR ν (KBr) [cm^{-1}]: 3321 (N-H stretching), 1531 (C=N stretching), 1431, 1387 (C^4-H-deformation), 1351 (C=S stretching), 1100 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.20 (d, J = 4.65 Hz, 3H, CH_3), 3.40 (dd, J_{AB} = 17.32 Hz, J_{AX} = 3.50 Hz, 1H, H_A), 3.75 (dd, J_{AB} = 17.40 Hz, J_{BX} = 11.48 Hz, 1H, H_B), 6.40 (dd, J_{AX} = 3.41 Hz, J_{BX} = 11.32 Hz, 1H, H_X), 6.80 – 7.50 (m, 6H, thiophene), 7.30 (b, 1H, NH); MS m/e: 307 [M^+] (100%), 274 [M – SH] (80%), 233 [M – $CSNHCH_3$] (70%), 218 [M – $C_2H_5N_2S$] (37%), 197 [M – C_5H_4NS] (74%), 183 [M – $C_5H_4N_2S$] (81%), 110 [C_5H_4NS] (51%). Anal. Calcd. for $C_{13}H_{13}N_3S_3$: C, 50.78; H, 4.26; N, 13.67; S, 31.29. Found: C, 51.04; H, 4.29; N, 13.60; S, 31.67.

1-N-Ethylthiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline ***10***

Yield: 57%; m.p.: 138 – 9°C (Crys. solv.: MeOH); UV λ_{Maks}^{MeOH} [nm]: 200 (log ε: 4.19), 244 (log ε: 4.03), 341 (log ε: 4.01); IR ν (KBr) [cm^{-1}]: 3336 (N-H stretching), 1584, 1511 (C=N stretching), 1440, 1382 (C^4-H-deformation), 1105 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 1.25 (t, J_{AB} = 6.92, 3H, CH_3), 3.30 (dd, J_{AB} = 17.32 Hz, J_{AX} = 3.41 Hz, 1H, H_A), 3.60 – 3.80 (m, 2H, CH_2), 6.40 (dd, J_{AX} = 3.31 Hz, J_{BX} = 11.20 Hz, 1H, H_X), 6.90 – 7.45 (m, 6H, thiophene), 7.30 (b, 1H, NH); MS m/e: 321 [M^+] (100%), 288 [M – SH] (51%), 278 [M – C_2H_5N] (71%), 233 [M – $CSNHC_2H_5$] (79%), 218 [M – $C_3H_7N_2S$] (44%), 211 [M – C_5H_4NS] (39%), 110 [C_5H_4NS] (66%). Anal. Calcd. for $C_{14}H_{15}N_3S_3$: C, 52.30; H, 4.70; N, 13.07; S, 29.92. Found: C, 52.19; H, 4.10; N, 13.17; S, 30.29.

1-N-Allylthiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline ***11***

Yield: 56%; m.p.: 117 – 118°C (Crys. solv.: MeOH); UV λ_{Maks}^{MeOH} [nm]: 224 (log ε: 4.30), 244 (log ε: 4.32), 341 (log ε: 4.02); IR ν (KBr) [cm^{-1}]: 3380 (N-H stretching), 1587, 1506 (C=N stretching), 1441, 1370 (C^4-H-deformation), 1158 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.30 (dd, J_{AB} = 17.31 Hz, J_{AX} = 3.40 Hz, 1H, H_A), 3.75 (dd, J_{AB} = 17.40 Hz, J_{BX} = 11.31 Hz, 1H, H_B), 4.12 – 4.40 (m, 2H, -CH_2), 5.15 – 5.20 (m, 2H, $=CH_2$), 5.90 – 6.10 (m, 1H, -CH=), 6.40 (dd, J_{AX} = 3.40 Hz, J_{BX} = 11.32 Hz, 1H, H_X), 6.90 – 7.50 (m, 6H, thiophene), 7.35 (b, 1H, NH); MS m/e: 333 [M^+] (41%), 300 [M – SH] (18%), 277 [M – NHC_3H_5] (100%), 233 [M – $CSNHC_3H_7$] (50%), 218 [M –

$C_4H_7N_2S$] (32%), 209 [M – $C_5H_4N_2S$] (27%), 168 [$C_6H_4N_2S_2$] (72%), 110 [C_5H_4NS] (57%). Anal. Calcd. for $C_{15}H_{15}N_3S_3$: C, 54.02; H, 4.53; N, 12.60; S, 28.84. Found: C, 54.36; H, 3.93; N, 12.68; S, 29.30.

1-N-Phenylthiocarbamoyl-3,5-di-(2-thienyl)-2-pyrazoline ***12***

Yield: 72%; m.p.: 147 – 148°C (Crys. solv.: EtOH); UV λ_{Maks}^{MeOH} [nm]: 201 (log ε: 4.25), 247 (log ε: 4.19), 345 (log ε: 4.13); IR ν (KBr) [cm^{-1}]: 3339 (N-H stretching), 1586, 1514 (C=N stretching), 1450, 1344 (C^4-H-deformation), 1240 (C^5-N^1 stretching); ^{1}H-NMR δ ($CDCl_3$) [ppm]: 3.40 (dd, J_{AB} = 17.42 Hz, J_{AX} = 3.30 Hz, 1H, H_A), 3.85 (dd, J_{AB} = 17.45 Hz, J_{BX} = 11.24 Hz, 1H, H_B), 6.55 (dd, J_{AX} = 3.6 Hz, J_{BX} = 12.32 Hz, 1H, H_X), 6.90 – 7.85 (m, 11H, thiophene and benzene), 9.10 (s, 1H, NH); MS m/e: 369 [M^+] (37%), 336 [M – SH] (36%), 277 [M – NHC_6H_5] (38%), 233 [M – $CSNHC_6H_5$] (43%), 218 [M – $C_7H_7N_2S$] (25%), 168 [$C_6H_4N_2S_2$] (100%). Anal. Calcd. for $C_{18}H_{15}N_3S_3$: C, 58.51; H, 4.09; N, 11.37; S, 26.03. Found: C, 58.84; H, 4.51; N, 11.40; S, 25.90.

Pharmacology

The present study was approved by the Hacettepe University Animal Ethics Committee (# 2003/3-3 and 2003/47-1).

Antidepressant activity

The synthesized compounds were screened for their antidepressant activity using Porsolt's behavioral despair (forced swimming) test [16]. Local breed, male albino mice (20 – 24 g) were used in the forced swimming test under standard conditions with free access to food and water. They were housed in groups of six. On the test day, mice were dropped one at a time into a plexiglass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at 23 – 25°C [14]. On this day, mice were assigned into different groups (n = 6 – 9 for each group). Tranylcypromine sulfate was supplied by Sigma Chemical Co. The synthesized compounds (10 mg/kg), and tranylcypromine sulfate, as a reference antidepressant drug (10 mg/kg), were suspended in a 1% aqueous solution of Tween 80. The drugs were injected intraperitoneally (ip) to mice (22 ± 2 g) in a standard volume of 0.5 mL/20 g body weight, 30 min prior to the test. Control animals received 1% aqueous solution of Tween 80. Then, the mice were dropped individually into the plexiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. A mouse was judged immobile if it floated in the water in an upright position and made only slight movements in order to prevent sinking. The duration of immobility was recorded during the last 4 min of the 6-min test.

Anticonvulsant activity

The compounds were tested for their anticonvulsant activity against MES and *sc*Met.-induced seizures and rotarod-toxicity test was performed for neurological toxicity according to the phase-I tests of ADD (Antiepileptic Drug Development) program [20, 21]. Stimulator (Grass S88, Astro-Med. Inc., West Warwick, RI, USA)), constant current unit (Grass CCU1A, Grass Medical Instruments, Quincy, MA, USA)), and corneal electrode were used for the evaluation of anticonvulsant activity. The rotarod used in the neurotoxicity test was made by Hacettepe University Technical Department. Pentylenetetrazole was supplied by Sigma Chemical Co. Twelve albino male mice (20 – 24 g) were used for each compound. The synthesized compounds were suspended in 30% aqueous solution of PEG 400 and administered ip in a volume of 10 mg/kg at body weight to the mice. Control animals received 30% aqueous PEG 400. Pentylenetetrazole (metrazol) was administered subcutaneously (sc) from the back of the neck. Rotarod toxicity test was performed on a 1-inch diameter knurled wooden rod; rotating at 6 rpm.

Maximal electroshock seizure (MES) test

Maximal electroshock seizures are elicited with a 60-cycle alternating current of 50 mA intensity (5 – 7 times that is required to elicit minimal electroshock seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline is instilled in the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extension component of the seizure is defined as protection.

Subcutaneous pentylenetetrazole (metrazol) (scMet) test

85 mg/kg of pentylenetetrazole (produces seizures in greater than 95% of mice) is administered as a 0.5% solution sc in the posterior midline. The animal was observed for 30 min failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neurotoxicity

The rotarod test was used to evaluate neurotoxicity. The animal was placed on a 1-inch diameter knurled wooden rod rotating at 6 rpm. Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min.

Statistical analysis

Results are expressed as mean ± S.E.M.; n represents the number of animals. Data obtained from pharmacological experiments were analyzed with one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test, using Pharmacologic Calculation System Version 4.1. (Microcomputer Specialists). A p-value of less than 0.05 was considered statistically significant.

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