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SYNTHESIS OF SOME NEW 1,3,5-TRISUBSTITUTED PYRAZOLINES WITH ANTIDEPRESSANT AND ANTICONVULSANT ACTIVITIES

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SUMMARY

Twelve 1-phenyl-, 1-thiocarbamoyl-, 1-N-substituted thiocarbamoyl- and 1-(4'-phenylthiazol-2'-yl)-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazoline derivatives were synthesized and tested for their antidepressant and anticonvulsant activities. Their chemical structures were proved by spectral data and microanalysis. The antidepressant activities of the compounds were investigated by the "forced swimming test". Compounds **1, 10, 11** and **12** had equivalent or higher activities than tranylcypromine sulfate that was used as reference antidepressant drug. Anticonvulsant activity and neurotoxicity of the compounds were determined by maximal electroshock seizure (MES), subcutaneous metrazol (ScMet.) and rotarod toxicity tests according to the phase 1 tests of Antiepileptic Drug Development (ADD) programme. Compounds **1, 2, 4, 7, 9, 11** and **12** were active against MES and ScMet. at 30-300 mg/kg dose levels. **7** and **9** were found as the highest protective compounds against ScMet.s at 30 mg/kg dose at four hours.

ÖZET

1-Fenil-, 1-tiyokarbamoil-, 1-N-sübstitüe tiyokarbamoil- ve 1-(4'-feniltiyazol-2'-il)-3-(2-naftil)-5-fenil/(2-furil)-2-pirazolin yapısında oniki bileşiğin sentezi yapılıp, antidepresan ve antikonvülsan aktiviteleri incelenmiştir. Maddelerin yapıları spektral bulgular ve elemantel analizle kanıtlanmıştır. Antidepresan aktiviteler "zorla yüzdürme testi" uygulanarak incelenmiştir. **1, 10, 11** ve **12** numaralı maddeler referans antidepresan ilaç olarak kullanılan tranilsipromine eşit veya daha yüksek aktivite göstermişlerdir. Bileşiklerin antikonvülsan aktiviteleri ve nörotoksisiteleri, antiepileptik ilaç geliştirme (ADD) proğramına uygun olarak maksimal elektroşok kasılma (MES), subkütan metrazol (ScMet.) ve rotarod toksisite testleri ile tayin edilmiştir. **1, 2, 4, 7, 9, 11** ve **12** numaralı bileşikler MES ve ScMet. testlerinde 30-300 mg/kg dozlarda aktif bulunmuştur; **7** ve **9** numaralı bileşikler ScMet.'e karşı 30 mg/kg dozda maksimum koruyucu etki gösteren bileşiklerdir.

Key Words: Pyrazoline, Antidepressant Activity, Anticonvulsant Activity, Synthesis.

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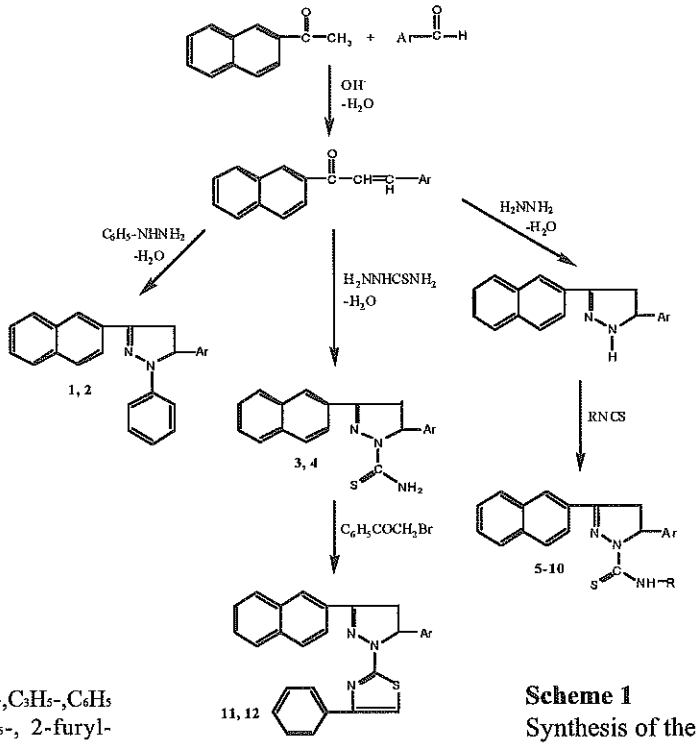
INTRODUCTION

It is well known that pyrazoline derivatives have hypoglysemic, antibacterial, antifungal, antihelmintic, local anesthetic, and sedative activities. Parmar et al (1) and Soni et al (2) demonstrated that 1,3,5-triphenyl-2-pyrazolines possess monoamine oxidase (MAO) inhibitory and anticonvulsant activities. In our previous studies, we synthesized several pyrazoline derivatives and tested them for their antidepressant and anticonvulsant activities (3-6). We found that 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines (3) and their condensed analogs 8-thiocarbamoyl-7,8-diazabicyclo [4.3.0]non-6-enes (4) and 1-N-substitued thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazolines (5) have significant antidepressant activity. In our recent study some 1,3-diphenyl-5-(2-furyl)-, 1-thiocarbamoyl-3-phenyl-5-(2-pyrrolyl)- and 1-thiocarbamoyl-3-phenyl-5-(2-thienyl)-2-pyrazolines were found to have significantly high anticonvulsant activity against the MES seizures (6) .

In this study, twelve 1-phenyl-, 1-thiocarbamoyl-, 1-N-substituted thiocarbamoyl and 1-(4[1]-phenylthiazol-2[1]-yl)-3-(2-napthyl)-5-phenyl/furyl-2-pyrazoline derivatives were synthesized according to the scheme 1 and tested for their antidepressant activities by using the forced swimming test (7). Anticonvulsant activities of the synthesized compounds were also determined by maximal electroshock (MES) and subcutaneous metrazol (scMet.) tests. Seizure assays and neurotoxicity determined by rotarod test were performed according to the phase 1 tests of antiepileptic drug development (ADD) programme which were developed by the National Institute of Neurological and Communicative Disorders and Stroke (NINDS)(8, 9).



R :CH_3-,C_3H_5-,C_6H_5
Ar :C_6H_5-, 2-furyl-

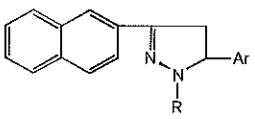
Scheme 1
Synthesis of the compounds

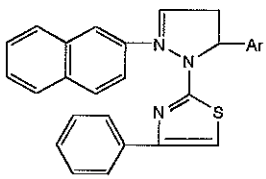
One of the synthesized compounds (1-Phenyl-3-(2-naphtyl)-5-phenyl-2-pyrazoline, Compound 1) has been previously synthesized by Wiley et al (10) and several other research groups but has not been tested for its antidepressant and anticonvulsant activities.

RESULTS AND DISCUSSION

The structures, yields and melting points of synthesized compounds are listed in Table 1.

Table 1. Structures, Yields and Melting Points of Synthesized Compounds



Compounds	R	Ar	Yields (%)	m.p. (°C)
1	C_6H_5	C_6H_5	74.71	214-6[a]
2	C_6H_5	2-furyl	71.00	172-4[a]
3	$CSNH_2$	C_6H_5	55.89	177-9[b]
4	$CSNH_2$	2-furyl	54.52	165-6[b]
5	$CSNHCH_3$	C_6H_5	43.48	236-8[c]
6	$CSNHC_3H_5$	C_6H_5	48.51	86-8[c]
7	$CSNHC_6H_5$	C_6H_5	74.69	126-7[c]
8	$CSNHCH_3$	2-furyl	43.48	236-8[c]
9	$CSNHC_3H_5$	2-furyl	72.00	201-3[c]
10	$CSNHC_6H_5$	2-furyl	71.54	184-5[c]
				
11	-	C_6H_5	73.00	221-3[b]
12	-	2-furyl	67.69	219-220[b]

a: ethanol, b: chloroform- ethanol, c: methanol

Elemental analysis and all spectral data are in accordance with assumed structures. In the UV spectra of the compounds two absorption maxima were observed at 235-288 and 326.6-370.6 nm due to C=N and Ar-N-N=C-Ar groups, respectively. The IR spectra of the compounds afforded C=N stretching (1554-1505 cm^{-1}), N-H stretching (3402-3251cm^{-1}) and C=S stretching (1375-1321 cm^{-1}) bands of thiocarbamoyl group. In the ^{1}H-NMR spectra of the compounds H_A, H_B and H_X protons were observed as doublet of doublet at δ 3.27-3.71 (J_{AB}: 16.84-18.07 Hz), 3.63-4.12 (J_{AX}: 3.65-7.18 Hz) and 5.33-6.55 (J_{BX}: 11.45-12.34 Hz) respectively. Aromatic protons appeared at 5.76-

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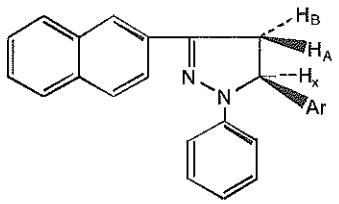
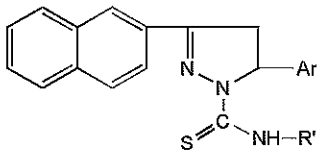
8.25 ppm as expected. The protons of methyl and allyl groups were also observed at expected ppms. N-H proton of the thiocarbamoyl group were seen at 9.15-9.22 ppm in the spectra of compounds **7** and **10**, but could not be observed in the spectra of other thiocarbamoyl compounds, presumably because of exchange processes (Table 2).

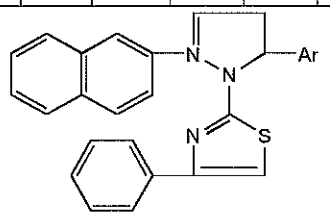
Table 2. Spectral Data



Com.	UV (MeOH,nm)	IR (KBr, cm^{-1})			H^1-NMR ($CDCl_3$, δ)					
		C=N	C=S	N-H	H_A	H_B	H_X	J_{AB}	J_{AX}	J_{BX}
1	236, 370.6	1542	-	-	3.27	3.96	5.33	17.00	7.18	12.34
2	235, 364.6	1558	-	-	3.51	3.82	5.41	16.84	6.74	12.11



3	286,8, 329.6	1569	1373	3402, 3254	3.35	3.95	6.09	17.60	3.65	11.45
4	285.4, 326.6	1597	1375	3385, 3251	3.24-4.00	3.24-4.00	5.95-6.38			
5	287.8, 333.4	1521	1347	3373	3.30	3.90	6.12	17.56	3.78	11.64
6	288, 333.4	1516	1314	3357	3.32	3.92	6.14	17.53	3.73	11.62
7	339	1541	1340	3311	3.25	3.81	6.05	17.23	4.10	11.48
8	330.4	1524	1350	3307	3.31-3.80	3.31-3.80	6.18-6.55			
9	286.8, 331	1505	1321	3295	3.53	3.63	5.76-6.43			
10	335.6	1532	1352	3348	3.38-3.90	3.38-3.90	6.10-6.50			



11	256.6, 364	1554	-	-	3.65	4.12	6.15	18.07	6.25	12.11
12	256, 350.2	1549	-	-	3.71	3.84	5.68-5.98			

PHARMACOLOGY

Antidepressant Activity

The antidepressant activity of the compounds was screened by the "forced swimming test" using tranylcypromine sulfate as reference antidepressant drugs. The forced swimming test is a behavioral test used to predict the efficacy of antidepressant treatments (7). It is used effectively in predicting the activity of a wide variety of antidepressants such as MAO inhibitors and atypical antidepressants (11, 12). As seen in Table 3, compounds **2, 3, 4, 8** significantly decreased the immobility time compared to controls. Compounds **1, 10, 11, 12** showed equivalent or higher antidepressant activity than tranylcypromine sulfate (Table 3).

Table 3. Antidepressant Activity Test Results

Compounds	**Antidepressant activities**	
	Duration of immobility (Seconds) Mean ± S.E.M	**Change from control (%)**
1	14±8.20	-92.58
2	150±17.60	-10.71
3	159±29.30	-5.35
4	141±31.50	-16.07
5	213±4.40	26.78
6	200±8.50	19.05
7	168±26.50	0
8	145±30.80	-13.69
9	205±2.50	22.02
10	35±13.70	-79.16
11	31±11.20	-81.55
12	7±3.10	-95.83
Tranylcypromine sulfate (10mg/kg)	24±6.60	-85.71
Control	168±12.10	

Values represents the mean ± S.E.M. (n=6)

*Significantly compared to control (Dunnet's test; $p<0.01$, $p<0.05$)

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Anticonvulsant Activity

The anticonvulsant activities of the compounds were initially evaluated against MES and ScMet. induced seizures using albino male mice (20 ± 2 g). Compound **2** at 300 mg/kg dose level and compound **12** at 100 and 300 mg/kg dose levels were found protective against MES at 0.5 hour and four hours respectively. Compounds **1, 2, 4, 7, 9** and **11** exhibited activity against scMet. induced seizures. Compounds **7** and **9** were found to be protective against ScMet. induced seizures at 30, 100 and 300 mg/kg dose levels at four hours. Neurotoxicity was not observed in the tested compounds at 30-300 mg/kg dose range, except for the compound **10** because of the death of the mice after 3 hours of injection at 100 and 300 mg/kg dose. For this reason, compound **10** could not be evaluated in the rotarod test after 4 hours of injection at these doses (Table 4).

Table 4. Phase 1 Anticonvulsant Screening of the Compounds

Com.	MES						ScMet.						Toxicity					
	1/ 2 hour			4 hours			1/ 2 hour			4 hours			1/ 2 hour			4 hours		
	mg/kg			mg/kg			mg/kg			mg/kg			mg/kg			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	**1/1**	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	**1/1**	**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
3	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
4	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
5	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
6	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
7	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
8	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
9	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2
10	0/1	0/1	0/1	0/1	a)	a)	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	a)	a)
11	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
12	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

MES: maximal electroshock seizure test, **scMet.:** subcutaneous pentylenetetrazole (metrazol) seizure test, **toxicity:** rotorod test.

0/1: no activity at dose level, 1/1: noticeable activity at dose level,

a) Death 3 hrs. after the injection.

EXPERIMENTAL

Chemistry

All chemicals used in this study were supplied by E. Merck (Darmstadt, FRG), Aldrich Chemical Co. (Steinheim, FRG) and Fluka (Buchs, Switzerland). Uncorrected melting points were taken in a Thomas Hooveer Capillary Melting Point Apparatus (Thomas, Philadelphia, PA, USA). UV spectra were obtained on Shimatzu UV 160A (MeOH) Schimatzu, Tokyo, Japan). IR spectra were recorded in a Perkin Elmer FT-IR 1720X Spectrometer (Perkin Elmer, Beaconsfield, UK) using KBr pellets. ^{1}H-NMR spectra were recorded on a Bruker AC 80 (Bruker, Karlsruhe, Germany) 80 MHz Spectrometer in $CDCl_3$ using TMS as internal standart. Microanalyses of compounds were performed at ATAL The Laboratory of Instrumental Analyses-The Scientific and Technical Research Council of Turkey.

1-Phenyl-3-heteroaryl-2-propen-1-ones (Chalcones)

Chalcone derivatives were obtained from 2-acetonaphthone (0.01 mole) and appropriate aldehydes (0.01 mole) by known methods (13-16).

1-Phenyl-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines **(1 and 2)**

The solution of appropriate chalcone (0.01 mole) and phenlyhydrazine (0.02 moles) in ethanolic sodium hydroxide (0.025 moles, 20ml) was refluxed for 4 hrs. The product was poured into ice water and the crude product which was separated out was filtered and crystallized from ethanol.

1-Thiocarbamoyl-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines **(3 and 4)**

1-Thiocarbamoyl-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines were obtained by heating (8 hrs.) thiosemicarbazide (0.012 mole) with appropriate chalcone (0.01 mole) and sodium hydroxide (0.025 mole 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 crytallized from chloroform-ethanol.

1-N-Substituted thiocarbamoyl-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines **(5-10)**

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

1-(4'-Phenylthiazol-2'-yl)-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines **(11 and 12)**

The solution of 1-thiocarbamoyl-3-(2-naphtyl)-5-phenyl/(2-furyl)-2-pyrazolines **(3 and 4)** (0.01 mole) and phenacyl bromide (0.012 mole) in absolute ethanol (50 ml)

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refluxed for 2 hrs. Cooled to room temperature. Precipitated compound was filtered and washed by ether. It was dissolved in water (50 ml) and precipitated in base form by adding sodium bicarbonate portionwise. Filtered and washed with water. Dried in room temperature and purified by crystallization from chloroform-ethanol.

Pharmacology

The ethics committee of Hacettepe University, School of Medicine, Ankara-Turkey (Permission date: 11. 02. 2004, number: 04/ 1-1) approved the animal experimentation.

Antidepressant Activity: Local breed, male albino mice (20±2 g) were used in the forced swimming test with free access to food and water. They were housed in-group of six. On test day mice were dropped one at a time into a plexiglas cylinder (10 cm diameter) containing 15 cm of water at 21-23°C (7). The synthesized compounds were dissolved in a 1% aqueous solution of Tween 80. The drugs were injected intraperitoneally in a standard volume of 0.5 ml/20 g body weight, 1hr before the test. Then, the mice were dropped individually into the plexiglas cylinder and left in water for 6 mins. After the first 2 mins. of the initial vigorous struggling the animals were immobile, the duration of immobility was recorded during the last 4 mins. of the 6 mins. test. For each group of mice, the mean period of immobility and the standard error of the mean (S.E.M.) were calculated. Dunnet's test has been used to evaluate the results, employing Pharmacologic Calculation System Version 4.1. (Microcomputer Specialists, Philadelphia, PA, USA). Tranylcypromine sulfate (10mg/kg) was used as reference drug.

Anticonvulsant Activity: Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Quincy, Mass., USA), and corneal electrode were used for the evaluation of anticonvulsant activity. All synthesized compounds were suspended in 30% aqueous solution of PEG 400 and administered i.p. in a volume of 0.01 mg/kg at body weight to the mice. Twelve male albino mice (20 ± 2 g) were used for each compound according to the NINDS-ADD programme (9). Control animals received 30% aqueous PEG 400. The compounds were tested for their anticonvulsant activity against MES and ScMet. induced seizures and rotarod toxicity test was performed for neurological toxicity according to the phase 1 tests of ADD (Antiepileptic Drug Development) programme (8, 9). Pentylenetetrazole (metrazol) was administered subcutaneously from the back of the neck. Rotarod toxicity test was performed on a 1 inch diameter knurled wooden rod; rotating at 6 rpm (the rotarod used in neurotoxicity test was made by Hacettepe University Technical Department).

Maximal Electroshock Seizure (MES) test: Maximal electroshock seizures are elicitd 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 s.c. in the posterior midline. The animal was observed for 30 mins. 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.

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