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Short communication

Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines

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Abstract – Ten new 3,5-diphenyl-2-pyrazoline derivatives were synthesised by reacting 1,3-diphenyl-2-propen-1-one with hydrazine hydrate. The chemical structures of the compounds were proved by means of their IR, ^{1}H-NMR spectroscopic data and microanalyses. The antidepressant activities of these compounds were evaluated by the 'Porsolt Behavioural Despair Test' on Swiss–Webster mice. 3-(4-Methoxyphenyl)-5-(3,4-dimethoxyphenyl)-2-pyrazoline, 3-(4-methoxyphenyl)-5-(2-chloro-3,4-dimethoxyphenyl)-2-pyrazoline and 3-(4-chlorophenyl)-5-(2-chloro-3,4-dimethoxyphenyl)-2-pyrazoline reduced 41.94–48.62% immobility times at 100 mg kg^{-1} dose level. In addition, it was found that 4-methoxy and 4-chloro substituents on the phenyl ring at position 3 of the pyrazoline ring increased the antidepressant activity; the replacement of these groups by bromo and methyl substituents decreased activity in mice. © 2001 Éditions scientifiques et médicales Elsevier SAS

antidepressant activity / 3,5-diphenyl-2-pyrazolines / Forced-Swimming Test

1. Introduction

Considerable interest has been focused on the pyrazole structure, which has been known to possess a broad spectrum of biological activities such as tranquillising, muscle relaxant, psychoanaleptic, anticonvulsant and antihypotensive activities [1–5]. The discovery of this class of drugs provides an outstanding case history of modern drug development and also points out the unpredictability of biological activity from structural modification of a prototype drug molecule. Prodrug-based monoamine oxidase (MAO) inhibitors have hydrazide, hydrazine and amine moiety such as isocarboxazid [6], phenelzine [7] and moclobemide [8, 9] show prominent antidepressant activity in laboratory animals and man. Additionally, tranylcypromine-like MAO inhibitors are mechanism-based inactivators and they are metabolised by MAO with one electron of the nitrogen pair and to generate an imine, the other residing on a methylene carbon ($R–C=NH_2^+$). The structures of 3-aryl-2-pyrazoline derivatives are very similar to those of isocarboxazid (*figure 1*) and these compounds metabolise easily and show their activity as prodrug. Earlier studies by Parmar et al. [3] and Soni et al. [4] demonstrated monoamine oxidase inhibitory activities of 1,3,5-triphenyl-2-pyrazolines, and we reported some 1,3,5-triphenyl-2-pyrazolines, 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines and bicyclic pyrazolines 8-thiocarbamoyl-7,8-diazabicyclo[4.3.0]non-6-ene derivatives to be active in the behavioural despair test [10–13]. As part of our continuing efforts in this area, a series of some new 3-(4-substituted phenyl)-5-(3,4-dimethoxy- and/or 2-chloro-3,4-dimethoxyphenyl)-2-pyrazolines have been synthesised and evaluated for their antidepressant activities using 'Behavioural Despair Test'.

2. Chemistry

1,3-Diphenyl-2-propen-1-ones (chalcones) (**3a–j**) were synthesised by condensing appropriate acetophenones with benzaldehyde derivatives in dilute ethanolic sodium hydroxide solution at room temperature.

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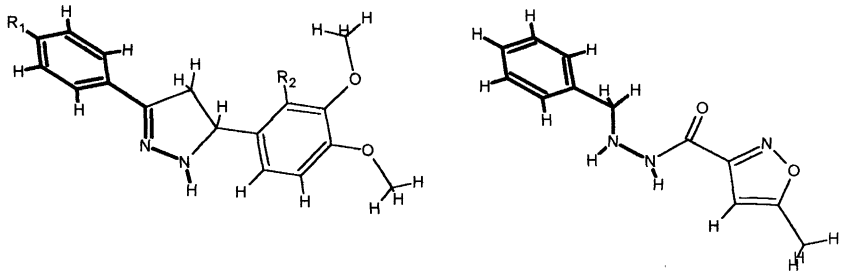


3,5-Diphenylpyrazolines (**4a-j**) Isocarboxazid

Figure 1. Structures of 3,5-diphenyl-2-pyrazolines (**4a**–**j**) and isocarboxazid.

The 3,5-diphenyl-2-pyrazolines (**4a**–**j**) were synthesised by the reaction of appropriate 1,3-diphenyl-2-propen-1-one derivatives (**3a**–**j**) and hydrazine hydrate according to the condensation reaction of α,β-unsaturated ketones with hydrazines in yield varying from 75.3 to 94.3% (*figure 2*).

3. Pharmacology

The 3,5-diphenyl-2-pyrazoline derivatives (**4a**–**j**) were screened for their antidepressant activities using a Modified Porsolt Forced Swimming (behavioural despair) test. The synthesised compounds (100 mg kg^{-1}), and the reference antidepressants clomipramine and tranylcypromine (10 and 20 mg kg^{-1}) were suspended in Tween 80 and injected intraperitoneally to mice. One hour later, the mice were dropped one at a time into a Plexiglass cylinder containing water and left for 6 min. At the end of the first 2 min the immobility times of each mouse was measured in the period of 4 min.

4. Results and discussion

The formula, melting points, yields, purification solvents and microanalysis of the compounds are listed in *table I*. IR spectra of the compounds showed C=N stretching band at 1590 cm^{-1}. In the ^{1}H-NMR spectra, H_A, H_B and H_X protons of the pyrazoline ring were seen as doublet of doublets at 3.05–3.10, 3.85–3.95 and 5.40–5.60 ppm ($J_{AB} = 17$, $J_{AX} = 7$, $J_{BX} = 10$ Hz), respectively. The protons belonging to the aromatic ring and methoxy groups were observed with the expected chemical shift and integral values (*table II*).

The Porsolt behavioural despair test is effective in predicting the activity of a wide variety of antidepressants for new molecules [14, 15]. Porsolt forced swimming induced behavioural despair model is capable of predicting a variety of potential antidepressants, yet it is not devoid of biases. However, its validity is unclear, because, it gives false-positive results in cylinders with 10 cm diameter central nervous system (CNS) stimulants, anticholinergics and antihistaminics. Moreover, mice in the 10 cm chambers

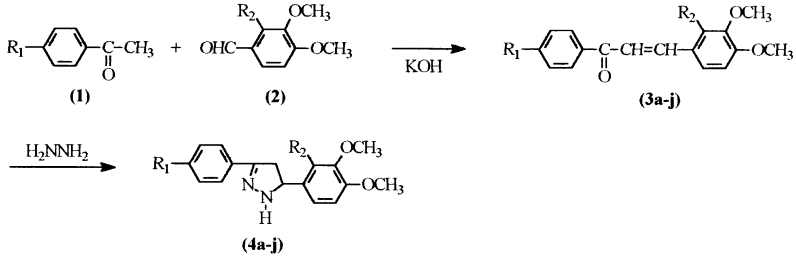
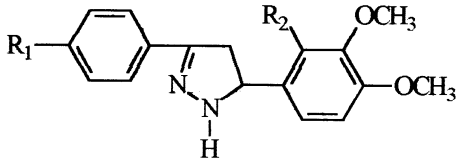


Figure 2. Synthesis of the compounds.

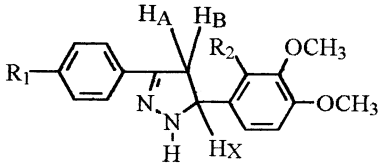
Table I. Structure and chemical data of the compounds **4a–j**.



Compound no.	R_1	R_2	Formula	Melting point (°C)	Yield (%)	Purification
4a	–H	–H	$C_{17}H_{18}N_2O_2$ (C, H, N) [a]	85–88	80.1	ethanol
4b	–Cl	–H	$C_{17}H_{17}ClN_2O_2$ (C, H, N)	79–81	89.5	ethanol–acetone
4c	–Br	–H	$C_{17}H_{17}BrN_2O_2$ (C, H, N)	75–79	90.0	ethanol–acetone
4d	$–CH_3$	–H	$C_{18}H_{20}N_2O_2$ (C, H, N)	66–69	78.3	ethanol
4e	$–OCH_3$	–H	$C_{18}H_{20}N_2O_3$ (C, H, N)	100–105	86.1	ethanol
4f	–H	–Cl	$C_{17}H_{17}ClN_2O_2$ (C, H, N)	95–98	79.8	ethanol–acetone
4g	–Cl	–Cl	$C_{17}H_{16}Cl_2N_2O_2$ (C, H, N)	55–57	94.3	ethanol–acetone
4h	–Br	–Cl	$C_{17}H_{16}BrClN_2O_2$ (C, H, N)	68–69	87.5	ethanol–acetone
4i	$–CH_3$	–Cl	$C_{18}H_{19}ClN_2O_2$ (C, H, N)	78–81	75.3	ethanol–acetone
4j	$–OCH_3$	–Cl	$C_{18}H_{19}ClN_2O_3$ (C, H, N)	71–73	91.8	ethanol–acetone

[a] Elemental analyses for C, H, N are within $\pm 0.4\%$ of the theoretical values.

Table II. Spectral data of the compounds.



Compound no.	IR (KBr, cm^{-1})	^{1}H-NMR ($CDCl_3$, ppm) [a]
4a	1600, 1520 (C=C, C=N); 1238, 1159 (C–O); 1066(C–N)	3.07 (1H, dd, H_A), 3.80 (1H, dd, H_B), 3.85 (3H, s, $–OCH_3$), 3.87 (3H, s, $–OCH_3$), 5.23 (1H, dd, H_X), 6.80–7.90 (8H, m, aromatic prot.), ($J_{AB} = 16.98$, $J_{AX} = 7.50$, $J_{BX} = 9.35$ Hz)
4b	1598, 1520 (C=C, C=N); 1238, 1162 (C–O); 1068(C–N)	3.08 (1H, dd, H_A), 3.90 (1H, dd, H_B), 5.40 (1H, dd, H_X), ($J_{AB} = 17.08$, $J_{AX} = 7.70$, $J_{BX} = 10.45$ Hz)
4c	1599, 1522 (C=C, C=N); 1238, 1162 (C–O); 1066 (C–N)	3.10 (1H, dd, H_A), 3.92 (1H, dd, H_B), 5.42 (1H, dd, H_X), ($J_{AB} = 16.99$, $J_{AX} = 7.40$, $J_{BX} = 9.90$ Hz)
4d	1600, 1500 (C=C, C=N); 1238, 1160 (C–O); 1074(C–N)	2.40 (3H, s, $–CH_3$), 3.06 (1H, dd, H_A), 3.90 (1H, dd, H_B), 5.40 (1H, dd, H_X), ($J_{AB} = 17.00$, $J_{AX} = 7.10$, $J_{BX} = 10.15$ Hz)
4e	1598, 1500 (C=C, C=N); 1265, 1140 (C–O); 1047(C–N)	3.12 (1H, dd, H_A), 3.85 (3H, s, $–OCH_3$), 3.97 (1H, dd, H_B), 5.45 (1H, dd, H_X), ($J_{AB} = 17.01$, $J_{AX} = 6.99$, $J_{BX} = 9.80$ Hz)
4f	1595, 1499 (C=C, C=N); 1231, 1179 (C–O), 1068 (C–N)	3.15 (1H, dd, H_A), 3.95 (1H, dd, H_B), 5.45 (1H, dd, H_X), ($J_{AB} = 16.99$, $J_{AX} = 6.91$, $J_{BX} = 9.99$ Hz)
4g	1597, 1504 (C=C, C=N); 1265, 1143 (C–O); 1047 (C–N)	3.11 (1H, dd, H_A), 3.95 (1H, dd, H_B), 5.45 (1H, dd, H_X), ($J_{AB} = 16.92$, $J_{AX} = 6.98$, $J_{BX} = 9.98$ Hz)
4h	1598, 1504 (C=C, C=N); 1264, 1143 (C–O); 1047 (C–N)	3.08 (1H, dd, H_A), 3.95 (1H, dd, H_B), 5.50 (1H, dd, H_X), ($J_{AB} = 17.01$, $J_{AX} = 7.03$, $J_{BX} = 9.98$ Hz)
4i	1598, 1504 (C=C, C=N); 1259, 1143 (C–O); 1047 (C–N)	2.30 (3H, s, $–CH_3$), 3.07 (1H, dd, H_A), 3.91 (1H, dd, H_B), 5.45 (1H, dd, HX), ($J_{AB} = 17.05$, $J_{AX} = 6.79$, $J_{BX} = 9.91$ Hz)
4j	1595, 1489 (C=C, C=N); 1259, 1131 (C–O); 1044 (C–N)	3.05 (1H, dd, H_A), 3.75 (3H, s, $–OCH_3$), 3.95 (1H, dd, H_B), 5.45 (1H, dd, H_X), ($J_{AB} = 17.35$, $J_{AX} = 6.99$, $J_{BX} = 10.02$ Hz)

[a] For the compounds **4b–j** the protons of the methoxy and phenyl groups are more or less constant. s, singlet; dd, doublet of doublets; m, multiplet.

touch the cylinder wall and bottom with their fore and hind paws. Therefore the data may not reflect the true immobility times. In the modified behavioural despair test method [16], with an increase in the diameter of the cylinder, mice lose their chance to touch the sides and the bottom of the cylinder and, thus, are forced to swim and the duration of immobility in 30 cm diameter cylinders was significantly lower than in 10 cm cylinders. The most striking result obtained by increasing the diameter of the cylinder was that the anticholinergics, antihistaminics and CNS stimulants did not give false-positive results when the duration of immobility was used as the criterion. Parmar et al. [3] investigated the ability of some substituted pyrazolines to inhibit rat brain MAO and indicated that the presence of electron-donating substituent on the phenyl ring present at position 5 of the pyrazoline ring produced a relatively higher degree of MAO inhibition while electron-withdrawing substituents produced a lesser degree of enzyme inhibition. In our study, compounds 3-(4-methoxyphenyl)-5-(3,4-dimethoxyphenyl)-2-pyrazoline (**4e**), 3-(4-methoxyphenyl)-5-(2-chloro-3,4-dimethoxyphenyl)-2-pyrazoline (**4g**) and 3-(4-chlorophenyl)-5-(2-chloro-3,4-dimethoxyphenyl)-2-pyrazoline (**4j**) reduced 41.94–48.62% immobility times at 100 mg kg^{-1} dose level. The 4-methoxy and 4-chloro substituents at phenyl at 3 position of pyrazoline ring enhanced the antidepressant activity and the replacement of these substituents with bromo and methyl groups decreased the activity (*table III*).

Table III. Antidepressant activities of the compounds.

Compound [a]	Duration of immobility (s)	% Change from control [b]
4a	35.8 ± 4.1	−17.51
4b	34.4 ± 5.8	−20.74
4c	36.8 ± 4.1	−15.21
4d	28.3 ± 4.2	−34.79
4e	25.2 ± 5.8	−41.94
4f	37.5 ± 5.2	−13.59
4g	24.5 ± 5.4	−43.55
4h	38.9 ± 5.1	−10.37
4i	28.9 ± 6.3	−33.41
4j	22.3 ± 6.0	−48.62
Clomipramine (10 mg kg^{-1})	26.8 ± 3.6	−38.25
Clomipramine (20 mg kg^{-1})	12.4 ± 2.4	−71.43
Tranylcypromine (10 mg kg^{-1})	23.0 ± 3.1	−47.00
Tranylcypromine (20 mg kg^{-1})	9.6 ± 2.6	−77.80
Control (vehicle)	43.4 ± 8.6	–

[a] Compounds were tested at 100 mg kg^{-1} dose level, i.p.
[b] 95% Confidence limits (Dunnet's test), $n = 6$.

5. Experimental protocols

5.1. Chemistry

All chemicals were supplied by E. Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer 1720X FT-IR spectrometer (KBr pellets). ^{1}H-NMR spectra were recorded on a Bruker AC 200 MHz spectrometer using TMS as internal standard in $CDCl_3$. Microanalyses of the compounds were performed at the Scientific and Technical Research Council of Turkey.

5.1.1. 1,3-Diphenyl-2-propen-1-ones (*3a–j*)

1,3-Diphenyl-2-propen-1-one derivatives were synthesized by condensing appropriate acetophenones (**1**) with 3,4-dimethoxy- and/or 2-chloro-3,4-dimethoxy-benzaldehyde (**2**) according to Claisen–Schmidt condensation [17–19].

5.1.2. 3,5-Diphenyl-2-pyrazolines (*4a–j*)

To the solution of 0.01 mol of the appropriate (**3a–j**) derivative in 15 mL of ethanol, 0.02 mol hydrazine hydrate (80%) was added and the reaction mixture was refluxed for 4 h and left overnight. The reaction mixture was cooled to −18 °C and the solid mass separated out was filtered, washed with cold ethanol and purified from suitable solvents.

5.2. Pharmacology

Adult male albino Swiss–Webster mice (22±2 g) were used as subjects in the study. They were housed in a quiet and temperature and humidity-controlled room (22±3 °C and 60±5%, respectively) in which a 12 h light/dark cycle was maintained (08:00–20:00 h light). Food and water intake of the subjects was not restricted during the study. Clomipramine and tranylcypromine were supplied by Sigma Chemical Co.

5.2.1. Test procedure

The mice were housed in Plexiglass cages with six animals for each cage. 'Porsolt Forced Swimming Test', a behavioural despair test, was used for evaluating if the compounds have antidepressant activity. On the testing day, mice were assigned into different groups ($n = 6$ for each group). The synthesized compounds, clomipramine and tranylcypromine were suspended in aqueous Tween 80 (0.2% w/v, 0.9% NaCl). All the synthesised compounds (100 mg kg^{-1}), clomipramine and tranylcypromine (10 and 20 mg kg^{-1}) were injected intraperitoneally to mice at a volume of 0.5 ml per 100 g body weight. One hour later, the mice were dropped one at a time into a Plexiglass cylinder (25 cm height, 30 cm diameter, containing 20 cm height of water at 21–23 °C) and left for 6 min. At the end of the first 2 min the animals showing initial vigorous struggling were immobile. Then, the immobility times of each mouse was measured in the period of 4 min.

5.2.2. Statistical analysis

Statistical significance was set at $P<0.05$ level. Changes in duration of immobilisations expressed as mean ± SEM were evaluated using by Dunnet's test (Pharmacological Calculation System, Version 4.1).

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