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Short communication

Synthesis and antinociceptive activities of some pyrazoline derivatives

Zafer Asim Kaplancikli [a,*], Gülhan Turan-Zitouni [a], Ahmet Özdemir [a], Özgür Devrim Can [b], Pierre Chevallet [c]

[a] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470 Eskişehir, Turkey
[b] Anadolu University, Faculty of Pharmacy, Department of Pharmacology, 26470 Eskişehir, Turkey
[c] Institut des Biomolécules Max Mousseron, UMR 5247, CNRS-Université Montpellier 1 et 2, Faculté de Pharmacie, Montpellier Cedex 5, France

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ABSTRACT

In the present study, some pyrazoline derivatives were synthesized to investigate their potential antinociceptive activities. 1-[(Benzoxazole/benzimidazole-2-yl)thioacetyl]pyrazoline derivatives were obtained by reacting 3,5-diaryl-1-(2-chloroacetyl)pyrazolines with 2-marcaptobenzoxazole/benzimidazole. The chemical structures of the compounds were elucidated by IR, ^{1}H NMR and FAB$^+$-MS spectral data and Elemental Analyses.

All of the compounds (100 mg/kg) exhibited significant antinociceptive activities in both hot plate and acetic acid-induced writhing tests. Naloxone (5 mg/kg) pre-treatment reversed the antinociceptive activities suggesting the involvement of opioid system in the analgesic actions. None of the compounds impaired motor coordination of animals when assessed in the Rota-Rod model.

These results support the previous papers reporting the opioid sensitive antinociceptive activities of various benzoxazole/benzimidazole–pyrazoline derivative compounds.

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1. Introduction

Pain is a disagreeable and subjective sensation resulting from a harmful sensorial stimulation that alerts the body about a current or potential damage to its tissues or organs. Despite the painful sensation, which can be efficiently solved by the removal of the main reason, the pain-causing stimulus cannot always be either easily defined or quickly removed. Contemporary analgesics, like opiates and nonsteroidal anti-inflammatory drugs have some limitations in clinical use, especially for opiates, such as addiction, tolerance and side effects [1]. Therefore, experimental researches for the development of safer and more effective analgesic agents are a great deal of interest for many researchers.

In the chemical literature, derivatives of nitrogenated heterocyclic aromatics of five members have been described with the inhibition of prostaglandin biosynthesis. Some of these azole derivatives are pyrrols [2–4], imidazoles [5], pyrazoles [6,7] and pyrazolines, which are pyrazole derivatives [8,9]. It has been suggested that biological evaluation of new bioactive molecules containing pyrazol nucleus is important for the creation of future-promising new analgesic agents [10–12]. Some pyrazoline-derived compounds including dipyrone (metamizol) have been shown to possess analgesic activities mediated by peripheral mechanisms such as inhibitions of cyclooxygenase enzyme activity, arachidonic acid cascade and prostaglandin biosynthesis [2–9]. However, some other pyrazoline-derived compounds have been reported as centrally acting analgesic agents [10–12]. Decrease of on/off cell firing in the periaqueductal gray [11], activation of endogen opioid mechanisms [10] and spinal noradrenergic and serotonergic systems [12] are some of the suggested mechanisms about this centrally mediated analgesia. On the other hand, different pyrazoline derivatives without anti-inflammatory activities or with analgesic actions unrelated to opioids have also been reported [11].

In addition, benzoxazoles/benzimidazoles have been investigated extensively for their analgesic and anti-inflammatory actions and have become a promising group to induce analgesia [13–16]. Some benzoxazole derivatives were reported to inhibit nitric oxide synthase (NOS) activity that contribute to acute and chronic inflammations [17] and they were evaluated as a novel class of non-amino acid based NOS inhibitors [18]. Besides their peripherally mediated antinociceptive actions, some benzimidazole-derived compounds have also been reported to possess centrally mediated analgesic effects like pyrazoline-derived ones [19–21].

In the interest of above, the synthesis of the compact-structured pyrazole system by combining these two bioactive components, benzoxazoles/benzimidazoles and pyrazolines, and investigation of their possible analgesic activities were aimed, in this study.

* Corresponding author. Tel.: +90 222 335 05 80/3779; fax: +90 222 335 07 50.
E-mail address: zakaplan@anadolu.edu.tr (Z.A. Kaplancikli).

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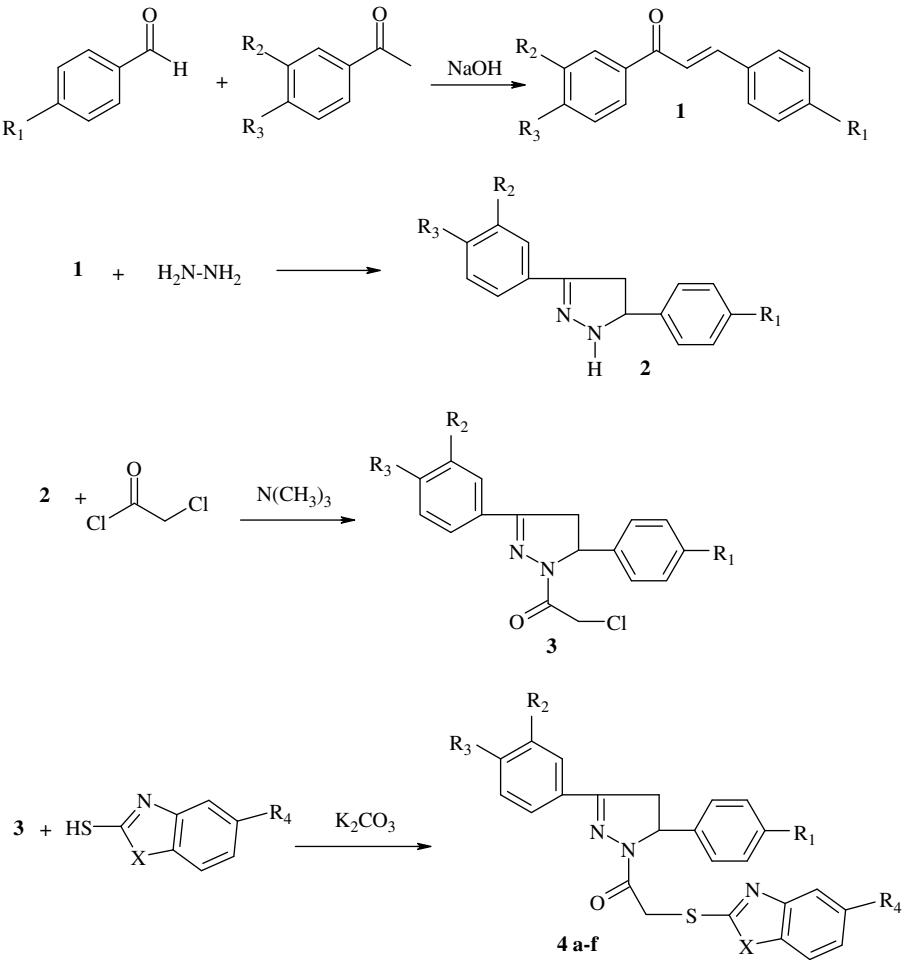

Scheme 1.

2. Chemistry

In the present work, 1,3-diaryl-2-propen-1-ones (**1**) were prepared by reacting acetophenones and benzaldehydes in accordance with the method described in the literature [22]. 3,5-Diaryl-2-pyrazolines (**2**) and 1-(chloroacetyl)-3,5-diaryl-2-pyrazolines (**3**) used in the synthesis were prepared according to the methods reported in the literature [22,23]. The reaction of 1-(chloroacetyl)-3,5-diaryl-2-pyrazoline (**3**), benzazol-2-thiole and anhydrous potassium carbonate in acetone gave the 1-[(benzazole-2-yl)thioacetylamino]-3,5-diaryl-2-pyrazoline derivatives (**4a–f**), as shown in Scheme 1. Some characteristics of the synthesized compounds are shown in Table 1.

3. Pharmacology

As stated in Section 1, antinociceptive potential of the some newly synthesized pyrazoline derivatives were investigated, in this study. Centrally and peripherally mediated nociceptive activities were measured by hot plate [24–26] and acetic acid-induced writhing tests [27,28], respectively. Morphine sulphate (Sigma Chemical Company, USA) was used as a reference drug and naloxone used as an opioid antagonist (Sigma Chemical Company, USA) for nociceptive tests. Rota-Rod test was performed for the examination of probable neurological deficits due to the test compounds, which may interfere with the test results to give false positives such as muscle relaxant or impairment of motor coordination [11,29].

4. Result

The structures of compounds **4a–f** were confirmed by elemental analyses, MS-FAB$^+$, IR and ^{1}H NMR spectral data. All compounds gave satisfactory elemental analysis. Mass spectra (MS (FAB)) of compounds showed M + 1 peaks, in agreement with their molecular formula. IR spectra of compounds showed NH, C=O and C=N, C=C bands at 3399–3095 cm^{-1}, 1685–1669 cm^{-1} and 1581–

Table 1
Some characteristics of the compounds

Compound	R_1	R_2	R_3	R_4	X	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
4a	H	H	H	Cl	O	80	155–157	$C_{24}H_{18}ClN_3O_2S$	447
4b	H	CH_3	CH_3	Cl	O	82	143–144	$C_{26}H_{22}ClN_3O_2S$	475
4c	OCH_3	H	H	Cl	O	78	97–99	$C_{25}H_{20}ClN_3O_3S$	477
4d	H	H	H	H	NH	83	127–129	$C_{24}H_{20}N_4OS$	412
4e	H	CH_3	CH_3	H	NH	85	203–205	$C_{26}H_{24}N_4OS$	440
4f	OCH_3	H	H	H	NH	75	152–154	$C_{25}H_{22}N_4O_2S$	442

Table 2
Effects of compounds on hot plate response in mice

Treatment	% Analgesia (mean ± SEM)
Control	−1.74 ± 1.55
4a (10 mg/kg)	59.7 ± 7.4*
4b (10 mg/kg)	72.6 ± 19.9**
4c (10 mg/kg)	47.7 ± 7.9**
4d (10 mg/kg)	61.9 ± 14.1*
4e (10 mg/kg)	82.5 ± 16.3***
4f (10 mg/kg)	60.6 ± 18.3*
Naloxone (5 mg/kg) + **4a** (10 mg/kg)	−1.32 ± 7.94
Naloxone (5 mg/kg) + **4b** (10 mg/kg)	−0.97 ± 6.78
Naloxone (5 mg/kg) + **4c** (10 mg/kg)	−2.95 ± 5.5
Naloxone (5 mg/kg) + **4d** (10 mg/kg)	−1.5 ± 7.7
Naloxone (5 mg/kg) + **4e** (10 mg/kg)	−0.58 ± 9.52
Naloxone (5 mg/kg) + **4f** (10 mg/kg)	−1.34 ± 6.16
Morphine (10 mg/kg)	92.9 ± 9.03***

Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with control. One-way ANOVA, post-hoc Newman-Keul's test, $n = 7$.

1301 cm^{-1} regions, respectively. In the 250 MHz ^1H NMR spectrum of compounds, the C_4 protons of the pyrazoline ring resonated as multiplet at 3.20–3.40 ppm (H_a), 3.80–4.05 ppm (H_b). The CH_2 protons of acetyl which are on 1 position of pyrazolines are observed at 4.55–4.95 ppm as double doublets ($J = 15.54$–16.16 Hz, $J = 15.54$–16.17) this geminal coupling is the result from steric structure of compound. These geminal protons are observed as double doublet because of possible two different conformations since rigid protons occurred. The C_5 (H_x) proton of pyrazoline appeared as multiplet at δ 5.55–5.70 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring. The benzimidazole derivatives showed specific NH proton at 12.55–12.60 ppm as broad or singlet. All the other aromatic and aliphatic protons were observed at expected regions.

All of the compounds in the serial had statistically significant antinociceptive effects in both hot plate (Table 2) and acetic acid-induced writhing tests (Table 3). Additionally, naloxone pre-treatment reversed the antinociceptive activities of all compounds in both of the tests (Tables 1 and 2). Morphine (10 mg/kg) had both peripheral and central antinociceptive actions supporting its use as a standard agent for both chemical and thermal painful models, causing significant increase in the reaction times in hot plate tests ($P < 0.001$) and decreased the numbers of abdominal contractions in writhing tests ($P < 0.001$).

Among the tested compounds, **4e** ($P < 0.001$), **4b** ($P < 0.001$) and **4f** ($P < 0.001$) inhibited writhing behaviour almost totally. Furthermore, compounds **4e** ($P < 0.001$) and **4b** ($P < 0.01$) also had highest antinociceptive activities in hot plate tests.

In addition, test compounds did not significantly change the fall-latency in Rota-Rod tests (related data not showed here) and none of the animals died when 100 mg/kg doses of the test compounds were applied.

5. Discussion

According to the results of the analgesia experiments, it can be evaluated that 100 mg/kg doses of the all test compounds possess significant antinociceptive activities against chemical and thermal noxious stimuli (Tables 2 and 3). The compounds did not impair the motor performance in Rota-Rod test, indicating that the observed antinociception unlikely occurred due to motor abnormalities.

All of the test compounds caused significant increases in the reaction times of mice against thermal noxious stimulus in hot plate tests (Table 2). Compounds **4b** and **e** showed higher, **4f,d** and a moderate, **4c** relatively lower antinociceptive actions at the applied dose. It is a common knowledge that hot plate test measures centrally mediated transient pain and supraspinally organized responses [30]. Therefore, it can be concluded that the tested compounds in this study may act centrally.

On the other hand, in chemical noxious stimulus-induced writhing test, compounds **4e**, **b**, **f** almost totally and **4d** powerfully protected animals from writhing. **4a** and **c** were the least active compounds in the serial. Compounds **4e**, **b**, **f** and **d** (at 100 mg/kg doses) were as effective as morphine (10 mg/kg) in terms of analgesic action, since no statistically significant differences were seen between morphine and these groups in ANOVA test.

The mouse writhing model involves different nociceptive mechanisms, such as sympathetic system (biogenic amines release), cyclooxygenases (COX) and their metabolites and opioid mechanisms. Acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons sensitive to NSAIDs and/or opioids [31]. When the results of writhing and hot plate tests were considered together, it can be concluded that the antinociceptive activities of the tested compounds may occur by both central and peripheral mechanisms.

Opioids are known to show analgesic activities in both hot plate and writhing tests by acting on central and peripheral nociceptive pathways, respectively [31,32]. So, naloxone was used as an opioid receptor antagonist, for the investigation of the possible involvement of opioid system in the antinociceptive activities of the tested compounds. It was observed that, analgesic activities of all tested compounds were reversed completely by naloxone pre-treatment

Table 3
Effects of compounds on writhing test in mice

Treatment	Number of writhing (10 min) (Mean ± SEM)	% Protection
Control	30.7 ± 3.6	–
4a (10 mg/kg)	13.0 ± 3.57***,[a]	57.72
4b (10 mg/kg)	0.57 ± 0.42***	98.14
4c (10 mg/kg)	17.43 ± 2.32**,[b]	43.32
4d (10 mg/kg)	4.57 ± 1.44***	85.13
4e (10 mg/kg)	0.71 ± 0.56***	97.67
4f (10 mg/kg)	0.85 ± 0.34***	97.21
Naloxone (5 mg/kg) + **4a** (10 mg/kg)	28.43 ± 2.11	7.44
Naloxone (5 mg/kg) + **4b** (10 mg/kg)	24.14 ± 1.91	21.39
Naloxone (5 mg/kg) + **4c** (10 mg/kg)	29.0 ± 1.25	5.58
Naloxone (5 mg/kg) + **4d** (10 mg/kg)	26.86 ± 2.13	12.56
Naloxone (5 mg/kg) + **4e** (10 mg/kg)	24.86 ± 2.45	19.07
Naloxone (5 mg/kg) + **4f** (10 mg/kg)	23.86 ± 1.87	22.32
Morphine (10 mg/kg)	3.85 ± 0.5***	87.45

Values are mean ± SEM. **$P < 0.01$, ***$P < 0.001$ compared with control.
[a] $P < 0.01$ compared with morphine. One-way ANOVA, post-hoc Newman-Keul's test, $n = 7$.
[b] $P < 0.001$ compared with morphine. One-way ANOVA, post-hoc Newman-Keul's test, $n = 7$.

(Tables 2 and 3), which indicates the involvement of the opioid mechanisms in the analgesic action. This effect could be due to the direct opioid receptor agonistic activities of the constituents in the extract and/or induction of endogenous opioid peptide release. These results support the previous studies suggesting opioid mediated analgesic activities of some benzoxazole/benzimidazole–pyrazoline-derived compounds [10,11,21,33].

6. Conclusions

The synthesis and antinociceptive evaluation of six pyrazoline derivatives were presented in this study. The synthesized compounds exhibited statistically significant antinociceptive activities against thermal and chemical noxious stimuli. As a general consideration, it can be concluded that the compounds **4b** and **e** showed highest antinociceptive activities in both nociception tests. Therefore, it can be suggested that, dimethyl substitution on phenyl at third position of the pyrazole ring increases the antinociceptive activities. Additionally, substitutional changes in benzoxazole/benzimidazole rings on the basic structure did not affect the activity.

The results of this investigation prove the hypothesis that benzoxazoles/benzimidazoles and pyrazoline-combined pyrazole derivatives possess centrally and peripherally mediated antinociceptive activities and supports the previous papers reporting the opioid sensitive antinociceptive activities of various benzoxazole/benzimidazole–pyrazoline derivative compounds.

7. Experimental

7.1. Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK). The compounds were checked for purity by TLC on silica gel 60 F_{254}. Spectroscopic data were recorded on the following instruments: IR, Shimadzu 435 IR spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H NMR, Bruker 250 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6 using TMS as internal standard; MS-FAB, VG Quattro mass spectrometer (Fisons Instruments Vertriebs GmbH, Mainz, Germany), Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin–Elmer, Norwalk, CT, USA).

7.1.1. General procedure for the synthesis of compounds

*7.1.1.1. 1,3-Diaryl-2-propen-1-ones (**1**).* A mixture of acetophenone (0.06 mol), aromatic aldehyde (0.06 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (30 mL) was stirred at room temperature for about 3 h. The resulting solid was washed, dried and crystallized from ethanol.

*7.1.1.2. 3,5-Diaryl-2-pyrazolines (**2**).* A solution of the appropriate chalcone (0.03 mol) (**1**) and hydrazine hydrate (80%) (0.06 mol) in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was recrystallized from ethanol.

*7.1.1.3. 1-(Chloroacetyl)-3,5-diaryl-2-pyrazolines (**3**).* 3,5-Diaryl-2-pyrazoline (**2**) (0.02 mol) and triethylamine (0.02 mol) were dissolved in dry toluene (30 mL) with constant stirring. Later, the mixture was cooled in an ice bath, and chloroacetylchloride (0.02 mol) was added drop wise with stirring. The reaction mixture thus obtained was further agitated for 1 h at room temperature. The precipitate was filtrated, the solvent was evaporated to dryness under reduced pressure and the products were recrystallized from ethanol.

*7.1.1.4. 1-[(Benzazole-2-yl)thioacetylamino]-3,5-diaryl-2-pyrazoline derivatives (**4a–f**).* A mixture of 1-(chloroacetyl)-3,5-diaryl-2-pyrazoline (**3**) (0.01 mol), benzazol-2-thiole (0.01 mol) and K_2CO_3 (0.01 mol) in acetone (50 mL) was refluxed for 8 h. After cooling, the solution was evaporated until dryness. The residue was washed with water and recrystallized from ethanol.

*7.1.1.4.1. Compound **4a**.* IR (KBr, cm^{-1}): 3366 and 3115(NH), 1680 (C=O), 1502–1321 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.20–3.40 (1H, m, pyrazoline C_4-H), 3.90–4.05 (1H, m, pyrazoline C_4-H), 4.70 and 4.90 (2H, 2d [$J = 16.15$ Hz and $J = 16.16$ Hz], $COCH_2$ geminal protons), 5.60–5.70 (1H, m, pyrazoline C_5-H), 7.20–7.90 (13H, m, aromatic protons). MS (FAB) [M + 1]: *m*/*z* 448.

Anal. Calcd for $C_{24}H_{18}ClN_3O_2S$: C, 64.35; H, 4.05; N, 9.38. Found: C, 64.37; H, 4.06; N, 9.35.

*7.1.1.4.2. Compound **4b**.* IR (KBr, cm^{-1}): 3286 and 3095 (NH), 1676 (C=O), 1542–1335 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.30 (6H, s, two CH_3), 3.10–3.25 (1H, m, pyrazoline C_4-H), 3.85–4.00 (1H, m, pyrazoline C_4-H), 4.70 and 4.95 (2H, 2d [$J = 16.16$ Hz and $J = 16.17$ Hz], $COCH_2$ geminal protons), 5.55–5.65 (1H, m, pyrazoline C_5-H), 7.30–7.80 (11H, m, aromatic protons). MS (FAB) [M + 1]: *m*/*z* 476.

Anal. Calcd for $C_{26}H_{22}ClN_3O_2S$: C, 65.61; H, 4.66; N, 8.83. Found: C, 65.63; H, 4.68; N, 8.80.

*7.1.1.4.3. Compound **4c**.* IR (KBr, cm^{-1}): 3391 and 3122 (NH), 1682 (C=O), 1533–1301 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.15–3.30 (1H, m, pyrazoline C_4-H), 3.65 (3H, s, OCH_3), 3.80–4.00 (1H, m, pyrazoline C_4-H), 4.60 and 4.85 (2H, 2d [$J = 16.11$ Hz and $J = 16.12$ Hz], $COCH_2$ geminal protons), 5.50–5.65 (1H, m, pyrazoline C_5-H), 7.10–7.75 (12H, m, aromatic protons). MS (FAB) [M + 1]: *m*/*z* 478.

Anal. Calcd for $C_{25}H_{20}ClN_3O_3S$: C, 62.82; H, 4.22; N, 8.79. Found: C, 62.84; H, 4.25; N, 8.80.

*7.1.1.4.4. Compound **4d**.* IR (KBr, cm^{-1}): 3322 and 3111 (NH), 1669 (C=O), 1566–1385 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.15–3.25 (1H, m, pyrazoline C_4-H), 3.90–4.00 (1H, m, pyrazoline C_4-H), 4.60 and 4.80 (2H, 2d [$J = 15.56$ Hz and $J = 15.57$ Hz], $COCH_2$ geminal protons), 5.60–5.70 (1H, m, pyrazoline C_5-H), 7.10–7.80 (14H, m, aromatic protons), 12.60 (1H, br, benzimidazole N–H). MS (FAB) [M + 1]: *m*/*z* 413.

Anal. Calcd for $C_{24}H_{20}N_4OS$: C, 69.88; H, 4.89; N, 13.58. Found: C, 69.90; H, 4.86; N, 13.59.

*7.1.1.4.5. Compound **4e**.* IR (KBr, cm^{-1}): 3399 and 3215 (NH), 1685 (C=O), 1545–1311 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.35 (6H, s, $2CH_3$), 3.10–3.20 (1H, m, pyrazoline C_4-H), 3.80–3.90 (1H, m, pyrazoline C_4-H), 4.65 and 4.80 (2H, 2d [$J = 15.62$ Hz and $J = 15.62$ Hz], $COCH_2$ geminal protons), 5.60–5.65 (1H, m, pyrazoline C_5-H), 7.10–7.70 (12H, m, aromatic protons), 12.55 (1H, s, benzimidazole N–H). MS (FAB) [M + 1]: *m*/*z* 441.

Anal. Calcd for $C_{26}H_{24}N_4OS$: C, 70.88; H, 5.49; N, 12.72. Found: C, 70.80; H, 5.50; N, 12.72.

*7.1.1.4.6. Compound **4f**.* IR (KBr, cm^{-1}): 3341 and 3130 (NH), 1675 (C=O), 1581–1381 (C=C and C=N).

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.10–3.20 (1H, m, pyrazoline C_4-H), 3.70 (3H, s, OCH_3), 3.85–4.00 (1H, m, pyrazoline C_4-H), 4.55 and 4.80 (2H, 2d [$J = 15.54$ Hz and $J = 15.54$ Hz], $COCH_2$ geminal protons), 5.55–5.65 (1H, m, pyrazoline C_5-H), 6.80–7.80 (13H, m, aromatic protons), 12.55 (1H, br, benzimidazole N–H). MS (FAB) [M + 1]: *m*/*z* 443.

Anal. Calcd for $C_{25}H_{22}N_4O_2S$: C, 67.85; H, 5.01; N, 12.66. Found: C, 67.84; H, 5.00; N, 12.69.

7.2. Pharmacology

7.2.1. Animals

Swiss albino mice of both sexes, weighing 30–40 g, were housed at room temperature of 24 ± 1 °C with 12/12 h light/dark cycle. Twelve hours before each experiment animals received only water, in order to avoid food interference with substance absorption. The experimental protocols have been approved by the Local Ethical Committee on Animal Experimentation of the Eskişehir Osmangazi University, Turkey.

7.2.2. Assessment of analgesic activity

7.2.2.1. Hot plate test. To evaluate the central component of nociception, hot plate test was applied. Mice ($n = 7$) were placed individually on a hot plate set at 55 ± 1.0 °C and the time of licking the forepaws or eventually jumping out of the glass beaker were recorded as an index of nociception. Maximum cut-off time was chosen as 30 s to avoid tissue damage [24–26]. Response latencies were measured 30 min after the application of sunflower oil as control; morphine sulphate as reference drug and 100 mg/kg doses of the each test compounds.

In six separate groups of animals ($n = 7$ for each), naloxone was administered intraperitoneally (5 mg/kg) 15 min before the injection of the compounds in order to examine possible involvement of opioid system in the analgesic activities. The effects of the compounds on nociception were determined by converting the hot plate latencies to percentage analgesic activity according to the following equation:

$$\%\ \text{Analgesic activity} = [(\text{postdrug latency} - \text{predrug latency}) / \text{predrug latency}] \times 100 \quad [26]$$

7.2.2.2. Writhing test. To investigate the antinociceptive activity against chemical noxious stimulus, acetic acid-induced writhing test was applied. Mice ($n = 7$) were pre-treated with sunflower oil, morphine sulphate or test compounds (100 mg/kg, i.p.) 30 min prior to intraperitoneal injection of 0.6% acetic acid (Merck, Brazil) at a dose of 10 mL/kg. Five minutes after the injection of acetic acid, the number of abdominal contractions and stretches during the following 10 min was recorded [27]. In six separate groups ($n = 7$ for each), animals were pre-treated with naloxone 15 min before administration of test compounds, followed by acetic acid administration after 30 min.

The percentage protection of writhing was calculated according to the following formula:

$$\%\ \text{Protection} = [(\text{control mean} - \text{treated mean}) / \text{control mean}] \times 100 \quad [28]$$

7.2.3. Assessment of motor coordination via Rota-Rod test

For the investigation of possible neurological deficits due to the test compounds, which may interfere with the test results to give false positives such as muscle relaxant or impairment of motor coordination, Rota-Rod test was performed. Before the experimental session, three trials were given for three consecutive days on the Rota-Rod apparatus (Ugo Basile 7560, Milano, Italy) set at a rate of 16 revolutions/min. Mice that were able to remain on the rod longer than 180 s were selected for the test. Five minutes after the hot plate test, each mouse was tested in the Rota-Rod and latency to fall from the rotating mill was recorded [11,29].

7.3. Statistical analysis

Experimental data from hot plate, acetic acid-induced writhing and Rota-Rod tests were analysed by One-way ANOVA followed by Newman-Keul's test. The data used in statistical analysis was obtained from seven animals for each of the groups. Statistical evaluation of the data was performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Experimental results were expressed as mean ± SEM. Differences between given sets of data were considered to be statistically significant when P value was less than 0.05.

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