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Short communication

New pyrazoline derivatives and their antidepressant activity

Zafer Asım Kaplancıklı [a,*], Ahmet Özdemir [a], Gülhan Turan-Zitouni [a], Mehlika Dilek Altıntop [a], Özgür Devrim Can [b]

[a] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Yunus Emre campus Tepebasi, 26470 Eskişehir, Turkey
[b] Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Yunus Emre campus Tepebasi, 26470 Eskişehir, Turkey

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ABSTRACT

Some triazolo-pyrazoline derivatives were synthesized to investigate their potential antidepressant activities. The chemical structures of the compounds were elucidated by IR, NMR and FAB$^+$-MS spectral data and elemental analyses. Antidepressant-like activities of the test compounds (100 mg/kg) were screened using both modified forced swimming and tail suspension 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. The test compounds in the series exhibited different levels of antidepressant activities when compared to reference drug fluoxetine. None of the test compounds changed motor coordination of animals when assessed in the Rota-Rod test. Therefore, experimental results in this study were not interfered with motor abnormalities.

1. Introduction

Depression is a serious disorder with estimates of lifetime prevalence as high as 21% of the general population in some developed countries [1]. Treatment for this disease is possible with antidepressant medications and psychotherapy for some parts of the patients [2]. Tricyclics, monoamine oxidase (MAO) inhibitors, selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors, norepinephrine–dopamine reuptake inhibitors, norepinephrine reuptake inhibitors, serotonin modulators and norepinephrine–serotonin modulators are the major antidepressant drug classes used for the treatment of depressive disorders [3,4]. Although antidepressants have been used in the clinic for several decades, most of them are inadequate in efficiency and have many serious adverse side effects. Therefore, studies for discovering and developing new antidepressant drugs with greater effectiveness and lower adverse effects are still desirable [5,6].

There are some papers reporting the antidepressant-like activities of several 1,2,4-triazole derivative compounds [7–9]. Some triazole derivative drugs are currently in clinical use to treat depression such as nefazodone and etoperidone [10–12]. Both nefazodone and etoperidone have been reported to show their antidepressant action with relation to central serotonergic system [13,14].

Like triazoles, some pyrazolines were also reported as potential antidepressant agents. Several researchers reported MAO inhibitory activity for various pyrazoline derivatives [15–17]. On the other hand, our research group recently suggested the possible involvement of serotonergic system for the antidepressant action of some 2-pyrazoline derivative compounds [18].

In this present study, we aimed to obtain new compounds containing both pyrazoline and triazole rings in the same structure. For this purpose, we synthesized a system combining two biolabile components, 2-pyrazolines and 1,2,4-triazole, and investigated their potential antidepressant effects.

2. Chemistry

In the present work, 4-amino-5-[2-(4-hydroxyphenyl)ethyl]-2,4-dihydro-3H-1,2,4-triazol-3-thione (**1**) has been synthesized by reacting thiocarbohydrazide with 3-(4-hydroxyphenyl)propionic acid. The 1-(2-thienyl)-3-aryl-2-propen-1-ones were prepared by reacting 2-acetylthiophene with aromatic aldehyde [19]. The 1-(2-thienyl)-3-aryl-2-propen-1-ones were treated with hydrazine hydrate to obtain 5-aryl-3-(2-thienyl)-2-pyrazolines [19]. The reaction of 3-(2-thienyl)-5-aryl-2-pyrazolines with chloroacetyl chloride gave 1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**2a**–**f**) [20]. Treatment of equimolar quantities of triazole (**1**) with

* Corresponding author. Tel.: +90 222 335 05 80/37 79; fax: +90 222 335 07 50.
E-mail address: zakaplan@anadolu.edu.tr (Z.A. Kaplancıklı).

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1-(chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**2a–f**) resulted in the formation of the title compounds (**3a–f**) (Scheme 1). Characterization data of compounds **3a–f** are given in Table 1.

3. Results

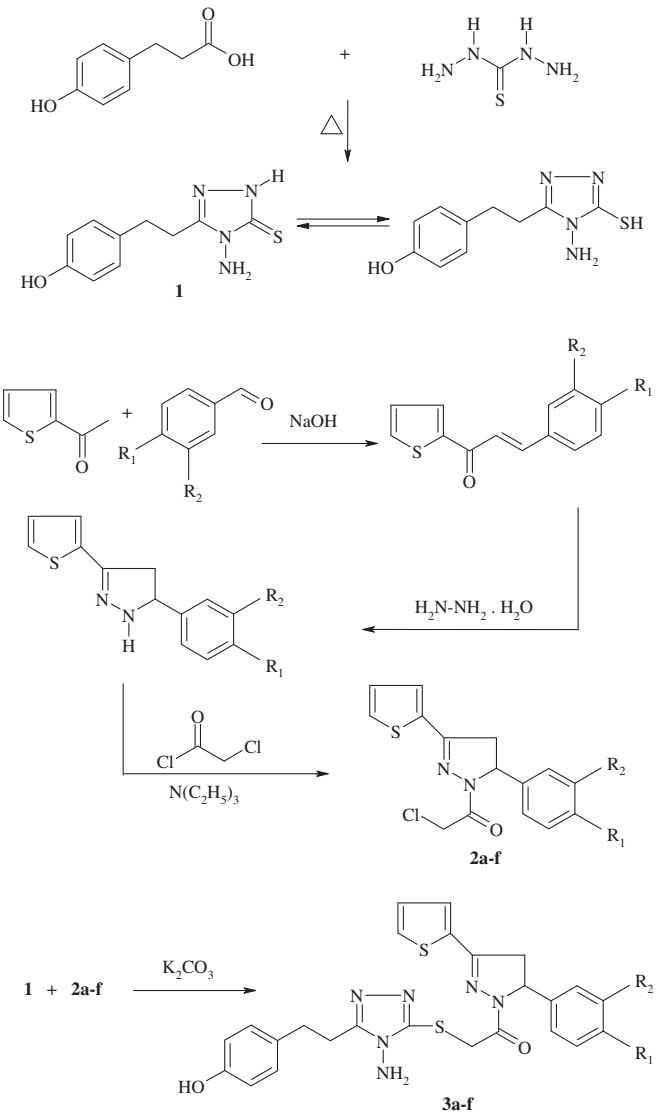
The structures of compounds **1** and **3a–f** were confirmed by elemental analyses, MS-FAB$^+$, IR ^{1}H NMR and ^{13}C NMR spectral data. All compounds gave satisfactory elemental analysis. Mass spectra (MS (FAB)) of compounds showed M + 1 peaks, in agreement with their molecular formula. IR spectra of compounds **3a–f** showed NH, C=O and C=N, C=C bands at 3385–3108 cm^{-1}, 1678–1670 cm^{-1} and 1575–1283 cm^{-1} regions, respectively.

In the 250 MHz ^{1}H NMR spectrum of triazole **1**, NH peak was observed as singlet at 13.45 ppm. Therefore, it was proved that the triazole was found in thionic form.

In the 250 MHz ^{1}H NMR spectrum of compounds **3a–f**, the CH_2–CH_2 protons appeared at 2.70–3.05 ppm. Among the three multiplets of the ABX spin system of the pyrazoline ring, that appearing at 3.10–3.30 ppm with multiplet attributable to the vicinal [$C_4(H_A)$–$C_5(H_x)$] and geminal [$C_4(H_A)$–$C_4(H_B)$] couplings, is assigned to $C_4(H_A)$. The multiplet at 3.80–4.05 ppm is readily assignable to the $C_4(H_B)$. attributable to the geminal coupling with $C_4(H_A)$ and the vicinal coupling with $C_5(H_x)$. The $C_5(H_x)$ proton of pyrazoline appeared as multiplet at 5.45–5.70 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring [22]. The CH_2 protons of acetyl group are observed at 4.35–4.65 ppm as multiplet, it should be noted that this anomaly arises from conformational differences [23]. The all derivatives showed NH_2 and OH protons as singlets at 5.85–6.00 and 9.15–9.25 ppm regions, respectively. But only NH_2 protons of **3a** were observed as multiplet. All the other aromatic and aliphatic protons were observed at expected regions.

In the ^{13}C NMR spectra of the compounds, the signal due to the carbonyl carbon appears at 164–165 ppm. ^{13}C NMR chemical shift values of the carbon atoms at 43–44 ppm (C_4), 59–60.31 ppm (C_5) and 151–152 ppm (C_3) corroborate the 2-pyrazoline character deduced from the ^{1}H NMR data. The signals due to the triazole carbons are observed at 150–151 ppm (C_3) and 156–157 ppm (C_5), respectively. The compounds have a signal at 34–35.37 ppm due to S–CH_2 carbon. The other aromatic and aliphatic carbons were observed at expected regions.

As shown in Table 2, reference drug fluoxetine (10 mg/kg) and the test compounds (100 mg/kg) significantly shortened the immobility times of mice in tail suspension test. Similarly, in MFST fluoxetine and all test compounds shortened the immobility and prolonged the swimming times without any change in the climbing times of mice (Table 3). Test compounds **3a** and **3c** were more effective than fluoxetine and some other derivatives as shown in both tables. Neither fluoxetine, nor test compounds significantly altered motor coordination parameters of mice, when compared to the control values (data not shown).



Scheme 1. Synthetic protocol of the title compounds.

Table 1
Some characteristics of the compounds.

Comp.	R_1	R_2	M.p., °C	Yield %	Mol. Formula	M.W.
3a	H	–	244–245	70	$C_{25}H_{24}N_6O_2S_2$	504
3b	F	–	177–179	69	$C_{25}H_{23}FN_6O_2S_2$	522
3c	Cl	–	230–231	75	$C_{25}H_{23}ClN_6O_2S_2$	539
3d	CH_3	–	169–171	69	$C_{26}H_{26}N_6O_2S_2$	518
3e	$N(CH_3)_2$	–	235–237	80	$C_{27}H_{29}N_7O_2S_2$	547
3f	O–CH_2–O		161–162	73	$C_{26}H_{24}N_6O_4S_2$	548

4. Discussion

Experimental data obtained from the tail suspension tests exhibited that all tested triazolo-pyrazoline derivative compounds showed statistically significant antidepressant-like activities as we hypothesized. Besides, MFST results confirmed these findings for all

Table 2
Effects of fluoxetine (10 mg/kg) and test compounds (100 mg/kg) on immobility times of mice in tail suspension test.

Comp.	Dose	Immobility time (s)
Control	–	116.4 ± 4.6
Fluoxetine	10 mg/kg	90.6 ± 5.0^b
3a	100 mg/kg	69.0 ± 2.7$^{c, \#, **, \ddagger}$
3b	100 mg/kg	78.8 ± 3.6^c
3c	100 mg/kg	72.1 ± 3.2$^{c, \#, *, \dagger}$
3d	100 mg/kg	85.9 ± 3.1^c
3e	100 mg/kg	93.5 ± 4.3^b
3f	100 mg/kg	96.1 ± 5.4^a

Significance against control values $^a p < 0.05$, $^b p < 0.01$, $^c p < 0.001$; significance against fluoxetine $^\# p < 0.05$; significance against **3e** $^* p < 0.05$, $^{**} p < 0.01$; significance against **3f** $^\dagger p < 0.01$, $^\ddagger p < 0.001$, $n = 7$.

Table 3
Effects of fluoxetine (10 mg/kg) and test compounds (100 mg/kg) on climbing, swimming and immobility times of mice in MFST.

Comp.	Dose	Climbing time(s)	Swimming time(s)	Immobility time(s)
Control	–	56.8 ± 2.6	95.0 ± 9.7	102.2 ± 7.5
Fluoxetine	10 mg/kg	47.8 ± 3.7	148.5 ± 10.4^{b}	67.6 ± 4.1^{b}
3a	100 mg/kg	43.3 ± 3.5	191.8 ± 9.1$^{c,\ \#,**,\dagger}$	33.3 ± 3.9$^{c,\ \#\#,**,\S}$
3b	100 mg/kg	48.8 ± 5.8	153.3 ± 8.7^{c}	52.5 ± 6.3^{c}
3c	100 mg/kg	45.0 ± 5.9	193.7 ± 8.9$^{c,\ \#,**,\ddagger}$	40.5 ± 5.7$^{c,\ \#,*,\dagger}$
3d	100 mg/kg	54.4 ± 3.5	147.8 ± 7.8^{b}	58.4 ± 5.1^{c}
3e	100 mg/kg	53.5 ± 4.2	140.0 ± 7.7^{a}	66.2 ± 5.5^{b}
3f	100 mg/kg	54.1 ± 3.4	144.7 ± 8.2^{b}	69.9 ± 6.2^{b}

Significance against control values $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; significance against fluoxetine $^{\#}p < 0.05$, $^{\#\#}p < 0.01$; significance against **3e** $^{*}p < 0.05$, $^{**}p < 0.01$; significance against **3f** $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$, $^{\S}p < 0.001$, $n = 7$.

test compounds and provided additional information related to possible mechanism of this activity. Decrease in immobility and increase in swimming times of mice without any change in the climbing durations indicated that the antidepressant-like effects exhibited in this study may be probably related to serotonergic mechanism rather than noradrenergic mechanism on CNS [24,25]. However, this consideration must be clarified with further detailed studies.

Additionally, none of the test compounds was found to cause significant changes on motor coordination of mice in Rota-Rod tests when applied at 100 mg/kg dose. Therefore, represented experimental results in this study were not affected by probable motor abnormalities.

In the series, unsubstituted derivative **3a** and chlorine substituted derivative **3c** were more effective than the test compounds **3e** and **3f** as well as 10 mg/kg dose of reference drug fluoxetine, with respect to antidepressant action.

5. Conclusion

In conclusion, this study supports the previous findings reporting the antidepressant-like activities of various pyrazoline and/or triazole derivatives [7–9,15–18] and suggests a possible serotonin related mechanism of action for the tested compounds.

6. Experimental

6.1. 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) and ^{13}C NMR, Bruker Avance II 500 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).

6.1.1. General procedure for the synthesis of compounds

*6.1.1.1. 4-amino-5-[2-(4-hydroxyphenyl)ethyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**1**).* Equimolar mixture of thiocarbohydrazide (0.1 mol) and 3-(4-hydroxyphenyl)propionic acid was heated in an oil-bath at 160–170 °C for 2 h. The fused mass thus obtained was dispersed with hot water to obtain the triazole. The product was recrystallized from methanol.

IR: 3319 cm^{-1}, 3269 cm^{-1}, 3207 cm^{-1} NH, 1612–1435 cm^{-1} C=N, C=C, 1220 cm^{-1}, 1178 cm^{-1} C–N, C=S.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.85–2.90 (4H, m, CH_2-triazole, phenol-CH_2), 5.60 (2H, s, NH_2), 6.65–7.00 (4H, m, aromatic protons), 9.20 (1H, s, OH), 13.45 (1H, s, NH).

For $C_{10}H_{12}N_4OS$ calculated: 50.83% C, 5.12% H, 23.71% N; found: 50.87% C, 5.10% H, 23.70% N. MS (FAB) $[M + 1]^{+}$: m/z 237.

6.1.1.2. 1-(2-Thienyl)-3-aryl-2-propen-1-ones (Chalcones). A mixture of 2-acetylthiophene (0.06 mol), aromatic aldehyde (0.06 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (50 mL) was stirred at room temperature for about 3 h. The resulting solid was washed, dried and crystallized from ethanol [20].

6.1.1.3. 3-(2-Thienyl)-5-aryl-2-pyrazolines. A solution of the appropriate chalcone (0.03 mol) and hydrazine hydrate (80%) (0.06 mol) in ethanol (50 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was recrystallized from ethanol [20].

*6.1.1.4. 1-(Chloroacetyl)-3-(2-thienyl)-5-aryl-2-pyrazolines (**2a–f**).* The pyrazoline (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 chloroacetyl chloride (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 filtered; the solvent was evaporated to dryness [21].

*6.1.1.5. 1-[[4-amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-aryl-2-pyrazoline (**3a–f**).* A mixture of the triazole (**1**) (10 mmol), pyrazolines (**2a–f**) and anhydrous potassium carbonate in acetone (20 mL) was mixed at room temperature for 6 h. The mixture was filtered, the filtrate was evaporated until dryness. The residue was washed with water and recrystallized from ethanol.

*6.1.1.5.1. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-phenyl-2-pyrazoline **3a**.* IR: 3385–3110 cm^{-1} NH, 1670 cm^{-1} C=O, 1572–1288 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.75–3.00 (4H, m, CH_2-CH_2), 3.10–3.20 (1H, m, pyrazoline C_4–H_A), 3.90–4.05 (1H, m, pyrazoline C_4–H_B), 4.45–4.65 (2H, m, $COCH_2$), 5.55–5.70 (1H, m, pyrazoline C_5–H_x), 5.90–6.00 (2H, m, NH_2), 6.75–7.70 (12H, m, aromatic protons), 9.15 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 26.39 (CH_2, phenol-CH_2), 31.60 (CH_2, CH_2-triazole), 35.07 (CH_2, S–CH_2), 43.24 (CH_2, pyrazoline C_4), 60.31 (CH, pyrazoline C_5), 115.42 (2CH, aromatic), 125.81 (2CH, aromatic), 127.64 (C, CH aromatic), 128.46 (CH, aromatic), 128.99 (2CH, aromatic), 129.52 (2CH, aromatic), 130.39 (CH, aromatic), 131.30 (CH, aromatic), 134.04 (C, aromatic), 141.88 (C, aromatic), 150.93 (C, triazole C_3), 151.51 (C, pyrazoline C_3), 155.89 (C, aromatic), 156.08 (C, triazole C_5), 164.79 (C, C=O).

For $C_{25}H_{24}N_6O_2S_2$ calculated: 59.50% C, 4.79% H, 16.65% N; found: 59.54% C, 4.82% H, 16.68% N. MS (FAB) $[M + 1]^{+}$: m/z 505.

*6.1.1.5.2. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-(4-florophenyl)-2-pyrazoline **3b**.* IR: 3380–3115 cm^{-1} NH, 1675 cm^{-1} C=O, 1570–1285 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.80–3.00 (4H, m, CH_2-CH_2), 3.15–3.25 (1H, m, pyrazoline C_4–H_A), 3.90–4.00 (1H, m, pyrazoline C_4–H_B), 4.40–4.60 (2H, m, $COCH_2$), 5.60–5.70 (1H, m, pyrazoline C_5–H_x), 5.90 (2H, s, NH_2), 6.70–7.80 (11H, m, aromatic protons), 9.20 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 26.41 (CH_2, phenol-CH_2), 31.56 (CH_2, CH_2-triazole), 35.00 (CH_2, S–CH_2), 43.13 (CH_2, pyrazoline C_4), 59.70 (CH, pyrazoline C_5), 115.42 (2CH, aromatic), 115.63, 115.82 (2CH, aromatic), 128.07 (C, aromatic), 128.48 (3CH, aromatic), 129.49 (2CH, aromatic), 130.42 (CH, aromatic), 131.34 (CH, aromatic), 134.04 (C, aromatic), 138.05 (C, aromatic), 150.89 (C, triazole C_3), 151.50 (C, pyrazoline C_3), 155.91 (C, aromatic), 156.11 (C, triazole C_5), 162.65 (C, aromatic), 164.88 (C, C=O).

For $C_{25}H_{23}FN_6O_2S_2$ calculated: 57.46% C, 4.44% H, 16.08% N; found: 57.45% C, 4.40% H, 16.10% N. MS (FAB) $[M+1]^+$: m/z 523.

6.1.1.5.3. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline ***3c***. IR: 3365–3122 cm^{-1} NH, 1672 cm^{-1} C=O, 1575–1299 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.80–3.05 (4H, m, CH_2–CH_2), 3.20–3.30 (1H, m, pyrazoline C_4–H_A), 3.80–3.95 (1H, m, pyrazoline C_4–H_B), 4.45–4.65 (2H, m, $COCH_2$), 5.65–5.70 (1H, m, pyrazoline C_5–H_x), 5.85 (2H, s, NH_2), 6.75–7.90 (11H, m, aromatic protons), 9.25 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 26.42 (CH_2, phenol-CH_2), 31.56 (CH_2, CH_2-triazole), 34.91 (CH_2, S–CH_2), 43.03 (CH_2, pyrazoline C_4), 59.71 (CH, pyrazoline C_5), 115.40 (2CH, phenol C_3, C_5), 127.91 (C, aromatic), 128.46 (3CH, aromatic), 128.93 (2CH, aromatic), 129.48 (2CH, aromatic), 130.43 (CH, aromatic), 131.34 (CH, aromatic), 132.19 (C, aromatic), 133.95 (C, aromatic), 140.80 (C, aromatic), 150.88 (C, triazole C_3), 151.48 (C, pyrazoline C_3), 155.91 (C, aromatic), 156.10 (C, triazole C_5), 164.90 (C, C=O).

For $C_{25}H_{23}ClN_6O_2S_2$ calculated: 55.70% C, 4.30% H, 15.59% N; found: 55.73% C, 4.34% H, 15.62% N. MS (FAB) $[M+1]^+$: m/z 539.

6.1.1.5.4. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-(4-methylphenyl)-2-pyrazoline ***3d***. IR: 3372–3118 cm^{-1} NH, 1678 cm^{-1} C=O, 1562–1283 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.70–3.00 (7H, m, CH_2-CH_2 and CH_3), 3.10–3.25 (1H, m, pyrazoline C_4–H_A), 3.85–4.00 (1H, m, pyrazoline C_4–H_B), 4.45–4.60 (2H, m, $COCH_2$), 5.65–5.70 (1H, m, pyrazoline C_5–H_x), 5.95 (2H, s, NH_2), 6.85–7.80 (11H, m, aromatic protons), 9.15 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 21.02 (CH_3), 26.44 (CH_2, phenol-CH_2), 31.59 (CH_2, CH_2-triazole), 35.14 (CH_2, S–CH_2), 43.27 (CH_2, pyrazoline C_4), 60.10 (CH, pyrazoline C_5), 115.40 (2CH, aromatic), 125.76 (2CH, aromatic), 128.46 (C, aromatic), 129.48 (2CH, aromatic), 129.51 (CH, aromatic), 130.34 (CH, aromatic), 131.23 (2CH, aromatic), 131.33 (CH, aromatic), 134.12 (C, aromatic), 136.84 (C, aromatic), 138.96 (C, aromatic), 150.92 (C, triazole C_3), 151.56 (C, pyrazoline C_3), 155.93 (C, aromatic), 156.09 (C, triazole C_5), 164.73 (C, C=O).

For $C_{26}H_{26}N_6O_2S_2$ calculated: 60.21% C, 5.05% H, 16.20% N; found: 60.24% C, 5.08% H, 16.24% N. MS (FAB) $[M+1]^+$: m/z 519.

6.1.1.5.5. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-(4-dimethylaminophenyl)-2-pyrazoline ***3e***. IR: 3370–3108 cm^{-1} NH, 1674 cm^{-1} C=O, 1571–1306 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.80–3.00 (10H, m, CH_2–CH_2 and $N(CH_3)_2$), 3.10–3.25 (1H, m, pyrazoline C_4–H_A), 3.80–3.95 (1H, m, pyrazoline C_4–H_B), 4.35–4.50 (2H, m, $COCH_2$), 5.45–5.50 (1H, m, pyrazoline C_5–H_x), 5.90 (2H, s, NH_2), 6.65–7.80 (11H, m, aromatic protons), 9.20 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 26.45 (CH_2, phenol-CH_2), 31.66 (CH_2, CH_2-triazole), 35.37 (CH_2, S–CH_2), 41.95 (2CH_3, N$(CH_3)_2$), 43.13 (CH_2, pyrazoline C_4), 60.02 (CH, pyrazoline C_5), 112.79 (2CH, aromatic), 115.41 (2CH, aromatic), 126.69 (C, aromatic), 128.46 (CH, aromatic), 129.40 (2CH, aromatic), 129.47 (2CH, aromatic), 130.24 (CH, aromatic), 131.12 (C, aromatic), 131.33 (CH, aromatic), 134.30 (C, aromatic), 150.16 (C, aromatic), 150.93 (C, triazole C_3), 151.65 (C, pyrazoline C_3), 155.92 (C, aromatic), 156.07 (C, triazole C_5), 164.55 (C, C=O).

For $C_{27}H_{29}N_7O_2S_2$ calculated: 59.21% C, 5.34% H, 17.90% N; found: 59.19% C, 5.34% H, 17.92% N. MS (FAB) $[M+1]^+$: m/z 548.

6.1.1.5.6. 1-[[4-Amino-3-[2-(4-hydroxyphenyl)ethyl]-4H-1,2,4-triazol-5-yl]thioacetyl]-3-(2-thienyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline ***3f***. IR: 3376–3111 cm^{-1} NH, 1677 cm^{-1} C=O, 1560–1289 cm^{-1} C=N, C=C.

^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.75–3.00 (4H, m, CH_2–CH_2), 3.15–3.30 (1H, m, pyrazoline C_4–H_A), 3.85–3.95 (1H, m, pyrazoline C_4–H_B), 4.35–4.45 (2H, m, $COCH_2$), 5.45–5.55 (1H, m, pyrazoline C_5–H_x), 5.90 (2H, s, NH_2), 6.00–6.15 (2H, m, O–CH_2–O), 6.70–7.85 (10H, m, aromatic protons), 9.25 (1H, s, OH).

^{13}C NMR (125 MHz) (DMSO-d_6) δ (ppm): 26.44 (CH_2, phenol-CH_2), 31.63 (CH_2, CH_2-triazole), 35.19 (CH_2, S–CH_2), 43.18 (CH_2, pyrazoline C_4), 60.09 (CH, pyrazoline C_5), 101.39 (CH_2, O–CH_2–O), 106.41 (CH, aromatic), 108.59 (CH, aromatic), 115.45 (2CH, aromatic), 119.13 (CH, aromatic), 128.50 (C, CH, aromatic), 129.50 (2CH, aromatic), 130.39 (CH, aromatic), 131.23 (CH, aromatic), 134.19 (C, aromatic), 135.86 (C, aromatic), 146.79 (C, aromatic), 147.79 (C, aromatic), 150.92 (C, triazole C_3), 151.59 (2C, pyrazoline C_3, aromatic), 156.10 (C, triazole C_5), 164.81 (C, C=O).

For $C_{26}H_{24}N_6O_4S_2$ calculated: 56.92% C, 4.41% H, 15.32% N; found: 56.95% C, 4.44% H, 15.28% N. MS (FAB) $[M+1]^+$: m/z 549.

6.2. Pharmacology

6.2.1. Animals

Adult male Swiss albino mice (25–30 g) were used for the experiments. The animals were housed in a room with controlled temperature (24 ± 1 °C) for 12 h light/12 h dark cycle. All animals were acclimatized to the laboratory environment for at least 48 h before the experiments. Food and water were allowed *ad libitum*. The experimental protocols have been approved by the Local Ethical Committee on Animal Experimentation of Eskişehir Anadolu University, Turkey.

6.2.2. Chemicals and administration of the compounds

Fluoxetine hydrochloride used in this study was purchased from Sigma–Aldrich Chemical Company, St. Louis, MO.

Fluoxetine (10 mg/kg), test compounds (100 mg/kg) and control solution (sunflower oil) were applied via intraperitoneal (*i.p*) route for three times; 24, 5 and 0.5 h before the applications of the tests [18,26].

6.2.3. Assessment of antidepressant activity

6.2.3.1. Tail suspension test. Antidepressant-like activity of the test compounds was screened using the tail suspension test similar to that described by Steru et al. [27]. Mice were dangled from their tail using adhesive tape placed approximately 1 cm from the tip of the tail attached to a applicator stick and hung approximately 30 cm above a table. Mice were considered immobile only when they fail to make any struggling movements and hung passively. Immobility time for each animal was scored by stopwatch during the last 4 min of a 6 min test [28].

6.2.3.2. Modified forced swimming test (MFST). Antidepressant-like activity of the test compounds was screened using the modified forced swimming test (MFST) as described before [18,24,29,30]. The mice were forced to swim individually in a glass cylinder (diameter, 12 cm; height, 30 cm) containing 20 cm of water at 25 ± 1 °C. A 15-min pretest session was followed 24 h later by a 5-min test session. Swimming, climbing and immobility times of animals over 5-s intervals were recorded by a stopwatch [30].

Following the training and the test sessions, the animals were dried in a heated enclosure. The water was changed and the cylinder rinsed with clean water after each animal to avoid the influence of alarm substances.

6.2.3.3. Assessment of motor coordination in Rota-Rod test. With the aim of investigating test compounds induced any changes in motor coordination of the animals, 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 [31,32].

6.2.4. Statistical analyses

The obtained experimental data were evaluated by the one-way analysis of variance (ANOVA) followed by Tukey's test as post hoc. The results are presented as means ± standard error of means (SEM). Differences between data sets were considered as significant when p value was less than 0.05.

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