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DRUG SYNTHESIS

SYNTHESIS AND ANTIDEPRESSANT-LIKE ACTIVITIES OF SOME PIPERIDINE DERIVATIVES: INVOLVEMENTS OF MONOAMINERGIC AND OPIOIDERGIC SYSTEMS

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Abstract: It was aimed to synthesize some novel piperidine derivatives and investigate their antidepressant-like activities, in this study. Targeted compounds were obtained by reacting 1-(4-(substitutedpiperidin-1-yl)phenyl)ethan-1-one and heterocyclic aldehydes in methanol. The structures of the synthesized compounds were determined using data from various spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-NMR, and LCMSMS). The compounds (50 mg/kg) were tested for their antidepressant-like effects by tail suspension and modified forced swimming tests (MFST). In addition, possible alterations in the locomotor activities of mice were evaluated by activity cage measurements. Obtained results showed that compounds **2c**-**2f** reduced the immobility time of mice in both of the antidepressant activity-screening tests indicating that these derivatives have antidepressant-like effects. Moreover, in MFST, compounds **2c** and **2e** significantly increased the swimming times, while compounds **2d** and **2f** significantly prolonged the climbing durations. Antidepressant-like effects of the compounds **2c** and **2e** were reversed with *p*-chlorophenylalanine methyl ester (serotonin depleting agent) pre-treatments while the same effect caused by the compounds **2d** and **2f** was abolished by pretreatment with α-methyl-para-tyrosine methyl ester (catecholamine depleting agent) pre-administrations. Moreover, pre-treatment with naloxone reversed the antidepressant-like effects of these four derivatives. Obtained results indicated that monoaminergic and opioidergic systems mediated the antidepressant-like effects of our novel piperidine derivatives.

Keywords: piperidine, antidepressant, tail suspension test, modified forced swimming test, activity cage test

World Health Organization (WHO) defines depression as a mental disorder characterized by depressive mood, loss of pleasure/interest, low self-esteem, feeling of guilt, loss of energy, poor concentration, unbalanced sleep and appetite (1). It is a common disease worldwide, with an estimated 3.8% of the population affected, including 5.0% among adults and 5.7% among the geriatric population (> 60 age). Approximately 280 million people in the world experience depression, which is a leading cause of disability worldwide and is a major contributor to the overall global burden of disease (2). WHO has predicted that unipolar depressive disorders will be the leading cause of the global burden of disease by 2030 (1). Depression is one of the priority conditions covered by WHO's Mental Health Gap Action Programme (mhGAP) (3).

The causes of depression include complex interactions between social, psychological, and biological factors. Patients with depressive disorders have two treatment options; psychological and pharmacological interventions. Psychological treatments include interpersonal therapy, cognitive behavioral therapy, behavioral activation, and problem-solving counseling. Additionally, pharmacological interventions include antidepressant drug therapy (3). On the other hand, the current antidepressant drugs prescribed for the treatment of depression have some disadvantages, such as the late onset of therapeutic response, resistance to treatment, and various undesirable side effects (4, 5). Therefore, the discovery and development of new antidepressant drugs are of great significance.

Piperidine ring is an important pharmacophore group used in drug development studies. Several

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compounds carrying this ring system in their chemical structure have been reported to possess various *in vitro* and *in vivo* pharmacological activities. Piperidine derivatives have especially been reported for their notable effects on the central nervous system (CNS) such as antidepressant-like (6-9), anxiolytic-like (8), anticonvulsant (10), antinociceptive (11, 12), antipsychotic (13), anti-Alzheimer (14), neuroprotective (15) and hypophagic (16) activities. Nevertheless, the piperidine ring system is also present in the structures of some clinically used psychotropic drugs such as paroxetine, haloperidol, risperidone, methylphenidate, and donepezil (17, 18). In this study, based on CNS-related activity potentials of piperidine derivatives, we aimed to synthesize some novel piperidine derivative compounds and investigate their possible antidepressant-like effects.

EXPERIMENTAL

Chemicals

Fluoxetine hydrochloride, reboxetine mesylate hydrate, α-methyl-para-tyrosine methyl ester (AMPT), *p*-chlorophenylalanine methyl ester (PCPA), naloxone hydrochloride dehydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA); 2-methylpiperidine, 3-methylpiperidine, 4-fluoroacetophenone, pyridine-4-carbaldehyde, dimethylformamide, methanol, potassium hydroxide, ethyl acetate, dimethylsulfoxide (DMSO) were bought from Sigma-Aldrich (Steinheim, Germany); piperidine, 4-methylpiperidine, indole-3-carbaldehyde, potassium carbonate, petroleum ether, silica gel 60 F254 coated aluminum TLC plate were acquired from Merck (Darmstadt, Germany) and ethanol was purchased from Tekkim (Bursa, Türkiye). All reagents were used without further purification.

Chemistry

Melting points (M.p.) were determined on Mettler Toledo-MP90 Melting Point System and were uncorrected. The prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with a Shimadzu DGU-14A degasser, LC-20 A dual piston pump, CTO-10 ASVP column oven, and SPD-MI20A PDA detector Sıl-20ACXR autosampler, and a stainless-steel GL Science Inertsil ODS-3 (4.6 250 mm) column. Solvents (acetonitrile and water, 7 : 3) for the separation of obtained compounds were isocritically mixed at a flow rate of 0.25 mL/min, and injections were performed by volume of 10 µL. IR spectra were recorded on an IR Affinity-*1S* Infrared spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Fourier 300 (Bruker Bioscience, Billerica, MA, USA) respectively, in DMSO-*d6*. (Bruker, USA), respectively. MS studies were performed on an LCMS-8040 tandem mass system (Shimadzu, Tokyo, Japan). The chemical purities of the compounds were checked by classical TLC applications performed on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

Synthesis of 1-(4-(Substitutedpiperidin-1-yl) phenyl)ethan-1-one (1a-1h)

Substituted piperidine (0.017 mol) and 4-fluoroacetophenone (1.82 mL, 0.017 mol) and potassium carbonate (2.35 g, 0.017 mol) were refluxed in DMF (10 mL). When the reaction was complete the reaction content was poured into the iced water, precipitated product was washed with water, filtered, dried, and recrystallized from EtOH (19-21).

Synthesis of target compounds (2a-2h)

A mixture of 1-(4-(Substitutedpiperidin-1-yl) phenyl)ethan-1-one (**1**) (0.002 mol), heterocyclic aldehydes (0.002 mol) and potassium hydroxide (0.112 g, 0.002 mol) was stirred in methanol at 50^{o}C for 36 h. After completion of the reaction, the mixture was cooled, and the precipitated product was filtered, washed with cooled methanol, and dried.

1-(4-(Piperidin-1-yl)phenyl)-3-(pyridin-4-yl) prop-2-en-1-one (2a)

Yield: 75%. M.P.: 123.9-125.6^{o}C. FTIR (ATR, cm^{-1}): 2922 (C-H), 1591 (C=O), 821 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 1.58-1.60 (7H, m, piperidine), 3.43 (3H, br.s., piperidine), 6.99 (2H, d, *J* = 9.12 Hz, Ar-H), 7.60 (1H, d, *J* = 15.63 Hz, Ar-H), 7.82 (2H, d, *J* = 6.24 Hz, Ar-H), 8.06 (2H, d, *J* = 9.03 Hz, Ar-H), 8.14 (1H, d, *J* = 15.63 Hz, Ar-H), 8.65 (2H, d, *J* = 6.06 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 24.44, 25.36, 48.07, 113.35, 122.86, 126.15, 127.13, 131.53, 139.43, 142.69, 150.75, 154.50, 186.27. ESI-MS (*m/z*): [M+H]$^{+}$: 293.10 (22) (100%).

3-(1H-Indol-3-yl)-1-(4-(piperidin-1-yl)phenyl) prop-2-en-1-one (2b)

Yield: 80%. M.P.: 75.8-77.0^{o}C. FTIR (ATR, cm^{-1}): 3361 (N-H), 2926 (C-H), 1651 (C=O), 858 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 1.58 (6H, br.s., piperidine), 3.35-3.37 (4H, m, piperidine), 6.93 (2H, d, *J* = 9.06 Hz, Ar-H), 7.18-7.28 (2H, m, Ar-H), 7.51 (1H, d, *J* = 7.05 Hz, Ar-H), 7.71 (1H, d, *J* = 15.51 Hz, Ar-H), 7.77 (2H, d, *J* = 9.03 Hz, Ar-H), 8.03 (1H, d, *J* = 15.51 Hz, Ar-H), 8.08-8.11 (1H, m, Ar-H), 8.28 (1H, s, Ar-H).

^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 24.44, 25.27, 48.23, 111.07, 113.00, 113.37, 118.59, 121.25, 122.51, 123.84, 124.67, 126.25, 130.67, 137.76, 139.22, 148.87, 154.30, 185.32. ESI-MS (*m/z*): $[M+H]^+$: 331.00 (100%).

1-(4-(2-Methylpiperidin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (2c)

Yield: 79%. M.P.: 178.9-180.1°C. FTIR (ATR, cm^{-1}): 2922 (C-H), 1653 (C=O), 806 (1,4-Disubstitutedbenzene).^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.92 (3H, d, *J* = 6.60 Hz, -CH_3), 1.09-1.20 (2H, m, piperidine), 1.61-1.71 (3H, m, piperidine), 2.82-2.92 (2H, m, piperidine), 3.88-4.00 (2H, m, piperidine), 6.99 (2H, d, *J* = 9.12 Hz, Ar-H), 7.61 (1H, d, *J* =15.63 Hz, Ar-H), 7.84 (2H, d, *J* = 6.03 Hz, Ar-H), 8.07 (2H, d, *J* = 9.00 Hz, Ar-H), 8.15 (1H, d, *J* = 15.66 Hz, Ar-H), 8.67 (2H, d, *J* = 6.00 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 22.15, 30.78, 33.57, 45.14, 47.40, 57.14, 78.73, 113.40, 122.93, 126.17, 127.26, 131.53, 139.36, 142.92, 150.54, 154.37, 186.26. ESI-MS (*m/z*): $[M+H]^+$: 307.10 (98%).

3-(1H-Indol-3-yl)-1-(4-(2-methylpiperidin-1-yl) phenyl)prop-2-en-1-one (3d)

Yield: 81%, M.P.: 122.9-124.7°C, FTIR (ATR, cm^{-1}): 3385 (N-H), 2929 (C-H), 1651 (C=O), 848 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 1.03 (3H, d, *J* = 6.75 Hz, -CH_3), 1.52-1.64 (6H, m, piperidine), 2.85-2.94 (1H, m, piperidine), 3.60-3.64 (1H, m, piperidine), 4.26-4.30 (1H, m, piperidine), 6.89 (2H, d, *J* = 9.15 Hz, Ar-H), 7.19-7.29 (2H, m, Ar-H), 7.50-7.51 (1H, m, Ar-H), 7.69 (1H, d, *J* = 15.51 Hz, Ar-H), 7.77 (2H, d, *J* = 9.06 Hz, Ar-H), 8.02 (1H, d, *J* = 15.51 Hz, Ar-H), 8.08-8.11 (1H, m, Ar-H), 8.29 (1H, s, Ar-H), 12.14 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 13.65, 18.47, 25.67, 26.46, 30.58, 41.22, 48.65, 112.88, 113.13, 118.62, 119.17, 121.28, 122.58, 123.92, 124.57, 125.93, 130.70, 137.51, 138.96, 149.30, 153.93, 185.42. ESI-MS (*m/z*): $[M+H]^+$: 345.30 (100%).

1-(4-(3-Methylpiperidin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (2e)

Yield: 82%. M.P.: >250°C. FTIR (ATR, cm^{-1}): 2924 (C-H), 1651 (C=O), 808 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.92 (3H, d, *J* = 6.60 Hz, -CH_3), 1.09-1.22 (2H, m, piperidine), 1.51-1.75 (4H, m, piperidine), 2.81-2.90 (1H, m, piperidine), 3.83-3.95 (2H, m, piperidine), 6.99 (2H, d, *J* = 9.12 Hz, Ar-H), 7.59 (1H, d, *J* = 15.63 Hz, Ar-H), 7.82 (2H, d, *J* = 6.06 Hz, Ar-H), 8.05 (2H, d, *J* = 9.03 Hz, Ar-H), 8.13 (1H, d, *J* = 15.66 Hz, Ar-H), 8.65 (2H, d, *J* = 6.03 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 19.52, 24.79, 30.62, 33.03, 47.52, 54.67, 78.75, 113.28, 122.91, 126.03, 127.24, 131.56, 139.35, 142.86, 150.61, 154.34, 186.22. ESI-MS (*m/z*): $[M+H]^+$: 307.00 (99%).

3-(1H-Indol-3-yl)-1-(4-(3-methylpiperidin-1-yl) phenyl)prop-2-en-1-one (2f)

Yield: 78%. M.P.: 83.4-85.1°C. FTIR (ATR, cm^{-1}): 3429 (N-H), 2987 (C-H), 1662 (C=O), 848 (1,4-Disubstitutedbenzene).^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.90 (3H, d, *J* = 6.60 Hz, -CH_3), 1.06-1.18 (1H, m, piperidine), 1.50-1.65 (4H, m, piperidine), 2.74-2.83 (1H, m, piperidine), 3.79-3.87 (3H, m, piperidine), 6.93 (2H, d, *J* = 9.09 Hz, Ar-H), 7.18-7.28 (2H, m, Ar-H), 7.50-7.53 (1H, m, Ar-H), 7.69 (1H, d, *J* = 15.48 Hz, Ar-H), 7.77 (2H, d, *J* = 9.06 Hz, Ar-H), 8.01 (1H, d, *J* = 13.98 Hz, Ar-H), 8.07-8.10 (1H, m, Ar-H), 8.28 (1H, s, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 19.54, 24.72, 26.48, 30.48, 33.04, 47.66, 54.91, 111.07, 113.00, 113.28, 118.60, 121.25, 122.51, 123.84, 124.67, 126.13, 130.70, 137.74, 139.19, 147.75, 154.11, 185.32. ESI-MS (*m/z*): $[M+H]^+$: 345.30 (98%).

1-(4-(4-Methylpiperidin-1-yl)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (2g)

Yield: 81%. M.P.: >250°C. FTIR (ATR, cm^{-1}): 2947 (C-H), 1653 (C=O), 808 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.92 (3H, d, *J* = 6.27 Hz, -CH_3), 1.12-1.23 (3H, m, piperidine), 1.64-1.71 (4H, m, piperidine), 3.88-4.01 (2H, m, piperidine), 6.99 (2H, d, *J* = 9.12 Hz, Ar-H), 7.58 (1H, d, *J* = 15.63 Hz, Ar-H), 7.81 (2H, d, *J* = 6.09 Hz, Ar-H), 8.04 (2H, d, *J* = 9.03 Hz, Ar-H), 8.13 (1H, d, *J* = 15.66 Hz, Ar-H), 8.64 (2H, d, *J* = 6.03 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 22.17, 30.80, 33.58, 47.41, 113.41, 122.86, 126.16, 127.14, 131.53, 139.43, 142.69, 150.74, 154.38, 186.30. ESI-MS (*m/z*): $[M+H]^+$: 307.00 (100%).

3-(1H-Indol-3-yl)-1-(4-(4-methylpiperidin-1-yl) phenyl)prop-2-en-1-one (3h)

Yield: 85%. M.P.: 77.7-79.1°C. FTIR (ATR, cm^{-1}): 3475 (N-H), 2987 (C-H), 1651 (C=O), 848 (1,4-Disubstitutedbenzene). ^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.91 (3H, d, *J* = 6.36 Hz, -CH_3), 1.11-1.20 (2H, m, piperidine), 1.64-1.68 (3H, m, piperidine), 2.77-2.86 (2H, m, piperidine), 3.88-3.93 (2H, m, piperidine), 6.93 (2H, d, *J* = 9.09 Hz, Ar-H), 7.18-7.28 (2H, m, Ar-H), 7.50-7.53 (1H, m, Ar-H), 7.69 (1H, d, *J* = 15.75 Hz, Ar-H), 7.77 (2H,

d, J = 9.06 Hz, Ar-H), 8.02 (1H, d, J = 15.75 Hz, Ar-H), 8.07-8.10 (1H, m, Ar-H), 8.28 (1H, s, Ar-H). ^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 22.19, 30.80, 33.50, 47.58, 112.97, 113.42, 118.60, 121.26, 122.53, 122.98, 123.86, 124.64, 126.26, 130.68, 137.68, 139.14, 148.73, 154.14, 185.35. ESI-MS (*m/z*): $[M+H]^+$: 345.30 (99%).

Prediction of ADME parameters

Physicochemical parameters of compounds (**2a-2h**) were investigated by the online Molinspiration property calculation program (23). The blood-brain barrier (BBB) permeability of the compounds was assessed by using an online BBB Predictor (24).

Pharmacology

Experimental animals

Adult male CD1 mice (30-35 g, 5 months old) obtained from Anadolu University, Center for Animal Experiments and Research, Eskişehir, Turkey were used in the experiments. Animals were housed in a temperature-(25 ± 1°C) and humidity-controlled room on a 12-h light/dark (lights on at 8:00 h AM) schedule. Animals were acclimatized to laboratory conditions at least 48 h prior to experiments. The experimental protocol of this study was approved by the Local Ethical Committee on Animal Experimentation of Anadolu University, Eskişehir, Turkey.

Drug administrations

While all of the test compounds were dissolved in sunflower oil; PCPA and naloxone were dissolved in physiological saline (NaCl, 0.9%). AMPT solution was prepared using saline containing 10% Tween 80.

Test compounds (50 mg/kg) were administered by oral gavage (p.o.) (9), three times at 24, 5, and 1 h before test sessions (25, 26). Reboxetine (20 mg/kg, p.o.) (26) and fluoxetine (20 mg/kg, p.o.) (25), conventional antidepressants, were used as positive controls. All other drugs were administered intraperitoneally (i.p.), at a volume of 10 mL/kg body weight.

Evaluation of spontaneous locomotor activity

The horizontal and vertical locomotor activities of the animals were evaluated with an activity cage device (Ugo Basile, 7420, Varese, Italy), a transparent plexiglass cage measuring 40 x 40 x 31 cm. The device works on the principle that the movements of animals in the horizontal and vertical directions cut off the infrared rays produced by the parts on the two opposite vertical sides. Interruptions in infrared light rays reaching the photocells are recorded by the device. In this study, spontaneous locomotor movements of animals in horizontal and vertical directions were recorded for 4 minutes. The device was cleaned with alcohol after each experiment to avoid the influence of alarm substances (25, 26).

Evaluation of the antidepressant-like effect

Tail suspension test

The tail suspension test was performed to investigate the antidepressant-like effects of the synthesized compounds, as described previously (25, 26). In the test, the mice were suspended 30 cm above the ground with a sticky patch about 1 cm from the tail end. Then, the durations of the immobility behaviors were measured by a blind researcher with a stopwatch. The immobility time measured and recorded in the last 4 of the 6 min test period was considered as a parameter for anhedonic behavior.

Modified forced swimming test

The modified forced swimming test (MFST) was conducted as described previously (25-27). In this test, the mice were forced to swim in a Plexiglas cylinder (12 cm diameter × 30 cm height), which was filled with water to a height of 20 cm. In the "pre-test session", mice were allowed to swim in the cylinder for 15 minutes. 24 h later, in the "test session", each mouse was re-exposed to the water for 5 minutes. The total duration of immobility (only movements necessary to keep the head above the water), swimming (horizontal movement on the surface of the water), and climbing (upward-directed movements with forelegs above the water level) behaviors over 5-s intervals were recorded by a blinded researcher. The water was refilled between the individual mice to avoid the influence of alarm substances and was heated to 25 ± 1ºC. At the end of the training and experimental stages, the animals were placed next to the heat source and dried.

Mechanistic studies

Studies were performed with PCPA to investigate the possible involvement of the serotonergic system in the antidepressant-like effects of test compounds. Mice were injected with PCPA (100 mg/kg, i.p.) for 4 consecutive days and test compounds were administered 24 hours after the last PCPA injection (28). Mechanistic studies were also conducted with AMPT to explore the probable contribution of catecholaminergic systems to the antidepressant-like effects of test compounds. For AMPT studies, mice were injected with 100 mg/kg AMPT intraperitoneally 4 hours prior to the last administration of the test compounds (26, 28). To elucidate the participation of the opioid system in the observed antidepressant-like

activity, naloxone (non-selective opioid receptor antagonist, 1 mg/kg) was used. Mice were injected with naloxone 15 min before administration of the test compounds (29). A tail suspension test was performed 1 hour after the oral administration of test compounds.

Statistical evaluation

Graphpad Prism ver. 8.2.1 software was used for the statistical analysis. The data obtained from the behavioral experiments were evaluated by one-way ANOVA followed by Tukey-HSD multiple comparison tests. The results were given as the mean ± standard error of the mean. A p-value lower than 0.05 was considered significant. Graphs were also prepared by using the same software.

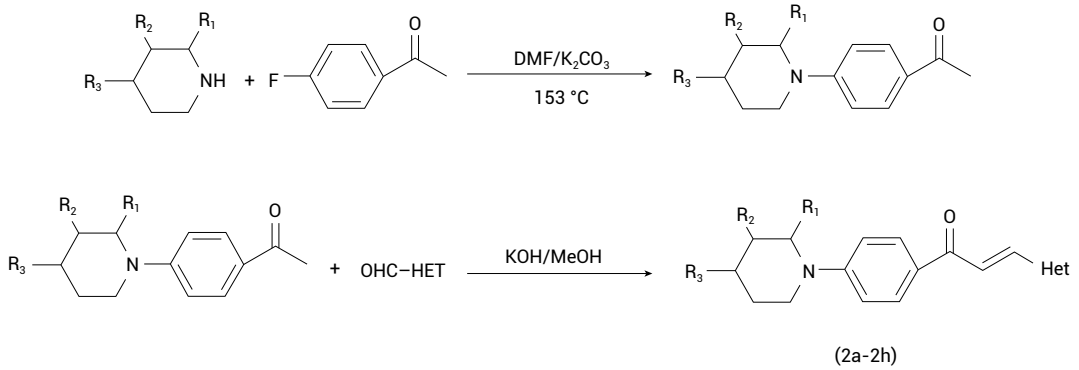
RESULTS

Chemistry

The compounds **2a-2h** were obtained as presented in Figure 1. Initially, ketone derivatives (**1a-1h**) were obtained by means of the reaction between 4-fluoroacetophenone and an appropriate secondary amine. Then, the Claisen–Schmidt reaction was performed between indol-3-carbaldehyde/pyridine-4-carbaldehyde and appropriate ketone derivatives (**1a-1h**) for attaining the final compounds (**2a-2h**). The structures of the obtained compounds were proved by spectroscopic methods like FTIR, ^{1}H-NMR, ^{13}C-NMR, and LCMS-MS.

When the synthesized derivatives are examined, it is seen that the piperidine ring, the 1,4-disubstitutedphenyl ring attached to the piperidine ring, and the chalcone structure are present in all compounds. Apart from these, compounds **2a**, **2c**, **2e,** and **2g** carry a pyridine ring; compounds **2b**, **2d**, **2f,** and **2h** carry an indole ring.

The peaks of methylene (-CH_2-) protons in the piperidine ring were observed in the range of 1.06-4.30 ppm. In derivatives (**2c-2h**) carrying methyl (-CH_3) substituent on the piperidine ring, the peaks belonging to this group were observed as doublets in the range of 0.91-1.03 ppm. Protons of the 1,4-disubstituted phenyl ring in the synthesized compounds were observed as doublets in the range of 6.93-6.99 and 7.77-8.07 ppm, as expected. Protons of the pyridine ring were observed in the range of 7.81-8.65 ppm as two doublets. Protons belonging to the chalcone (prop-2-en-1-one) group in the



(2a-2h)

Compounds	R_1	R_2	R_3	Het
2a	-H	-H	-H	Pyridine-4-yl
2b	-H	-H	-H	1*H*-Indole-3yl
2c	-CH_3	-H	-H	Pyridine-4-yl
2d	-CH_3	-H	-H	1*H*-Indole-3yl
2e	-H	-CH_3	-H	Pyridine-4-yl
2f	-H	-CH_3	-H	1*H*-Indole-3yl
2g	-H	-H	-CH_3	Pyridine-4-yl
2h	-H	-H	-CH_3	1*H*-Indole-3yl

Figure 1. Synthesis pathway of target compounds (2a-2h).

structure were observed in the range of 7.58-7.69 and 8.03-8.13 ppm as two doublets, consistent with the literature data. According to the results of NMR spectroscopy performed with chalcones, the *J* value for the α,β-unsaturated system was 8-9 Hz for the *E* isomer, while this value was determined as *J* = 15-16 Hz for the Z isomer. Hence, our compounds are *Z* isomers (30-32). When the ^{13}C-NMR spectra were examined, the peaks belonging to the carbonyl group of the compounds were observed in the range of 185.32-186.30 ppm. In addition, aliphatic carbons were observed in the range of 13.65-78.73 ppm, while aromatic carbons were observed in the range of 111.07-186.30 ppm.

Prediction of ADME parameters

A drug candidate compound should exhibit ideal pharmacokinetics as well as have high pharmacological activity and low toxicity profile (33). For this reason, we calculated various physicochemical parameters of the compounds in order to have an idea about the absorption, distribution, metabolism, and excretion (ADME) profiles of the derivatives. We used the Molinspiration program for the theoretical calculation of the parameters.

ADME parameters of the synthesized compounds (molecular weight (MW), topological polar surface area (TPSA), predicted octanol/water partition coefficient (log P), the estimated number of hydrogen bonds (HBA), the estimated number of hydrogen donors (HBD), molecular volume (MV)), and the predicted potential to cross the BBB are given in Table 1.

The Molinspiration program is based on the principle of Lipinski's rule of five. These rules evaluate the properties that a candidate drug molecule must have to be active in humans. An orally taken drug should not show more than one parameter violation. The obtained data showed that the compounds *2a-2h* do not violate Lipinski's rule and have a good pharmacokinetic profile that increases the therapeutic potential of the compounds. On the other hand, further studies are needed to obtain definitive results regarding the pharmacokinetic profiles of the compounds.

Pharmacology

Activity cage test results

The effects of the control solution and test compounds (**2a**-**2h**) on the horizontal locomotor activities of the mice in the activity cage tests are shown in Figure 2 [$F(8, 54) = 0.41$, $p > 0.05$]. Tukey HSD multiple comparison tests following one-way ANOVA indicated that test compounds (50 mg/kg) did not cause any significant alteration in the number of horizontal locomotor activities of the animals.

Figure 3 presents the effects of the control solution and test compounds (**2a**-**2h**) on vertical locomotor activities of the mice in the activity cage tests [$F(8, 54) = 1.21$, $p > 0.05$]. Tukey HSD multiple comparison tests showed that test compounds (50 mg/kg) did not cause any significant change in the number of vertical locomotor activities of the animals.

Tail suspension test results

The effects of the control solution, test compounds (**2a**-**2h**), fluoxetine, and reboxetine on the immobility time of mice in the tail suspension test are given in Figure 4 [$F(10, 66) = 7.15$, $p < 0.001$]. Tukey HSD multiple comparison tests following one-way ANOVA indicated that compounds **2c**-**2f** significantly decreased the immobility time of the animals, compared to the control group. As expected, fluoxetine and reboxetine, the reference drugs, significantly reduced the immobility time of the mice. Compounds **2a**, **2b**, **2g,** and **2h** were found to be ineffective on the immobility time of animals.

Table 1. Some physicochemical parameters of the compounds 2a-2h used in the prediction of ADME profiles.

Compounds	MW	TPSA	logP	HBA	HBD	MV	Vio	BBB
2a	292.38	33.20	3.53	3	0	283.65	0	+
2b	330.43	36.10	4.79	3	1	330.43	0	+
2c	306.41	33.20	3.86	3	0	300.23	0	+
2d	344.46	36.10	5.12	3	1	333.37	1	+
2e	306.41	33.20	3.77	3	0	300.23	0	+
2f	344.46	36.10	5.03	3	1	333.37	1	+
2g	306.41	33.20	3.77	3	0	300.23	0	+
2h	344.46	36.10	5.03	3	1	333.37	1	+

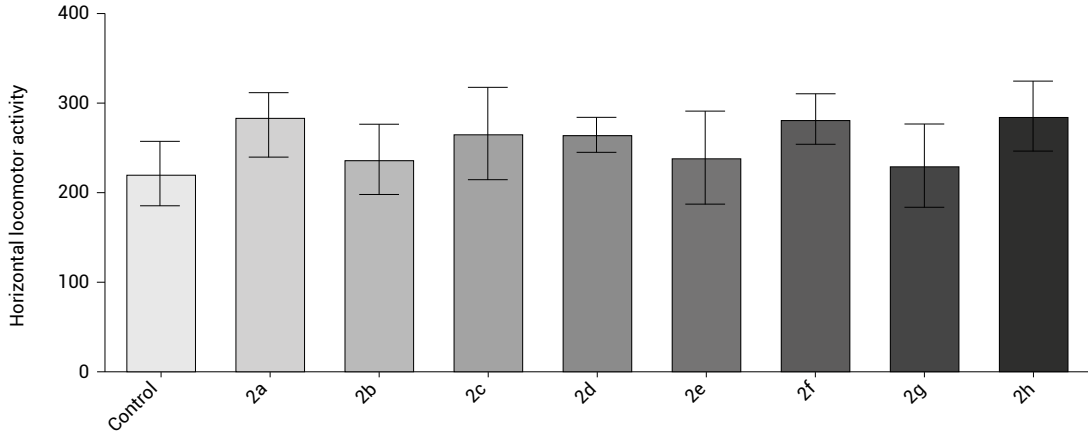


Figure 2. The effect of sunflower oil (control) and 2a-2h (50 mg/kg) administrations on horizontal locomotor activities of animals in the activity cage tests. One-way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

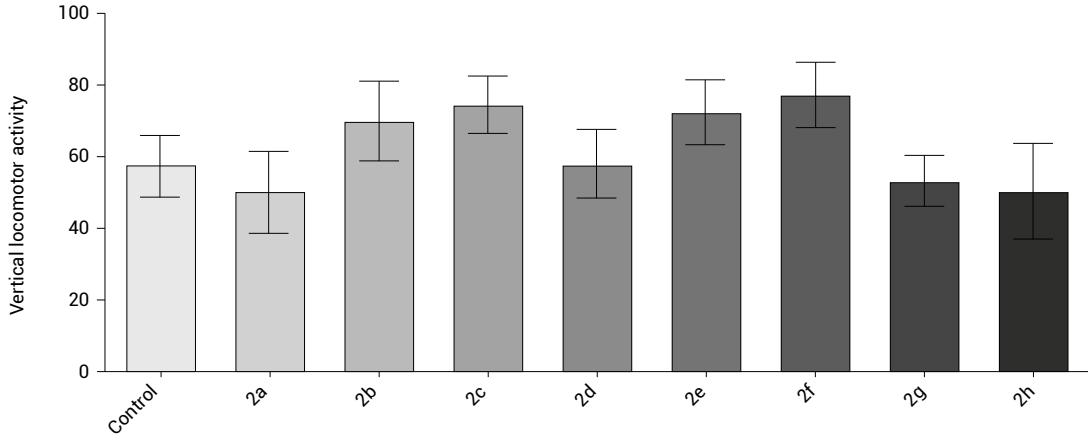


Figure 3. The effect of sunflower oil (control) and 2a-2h (50 mg/kg) administrations on vertical locomotor activities of animals in the activity cage tests. One way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

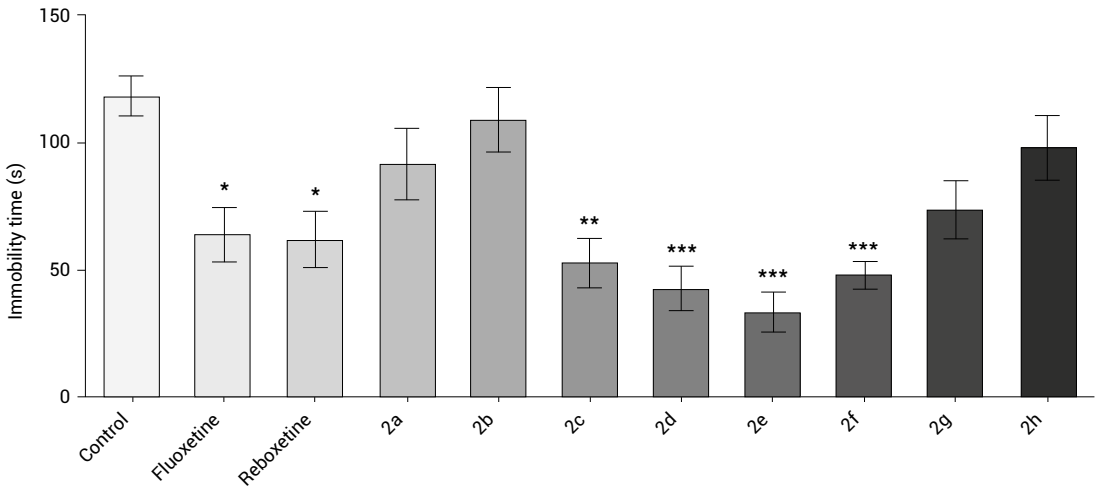


Figure 4. The effect of sunflower oil (control), fluoxetine (20 mg/kg), reboxetine (20 mg/kg) and 2a-2h (50 mg/kg) administrations on immobility time of animals in the tail suspension test. Significant differences compared to the control group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; One way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

Modified forced swimming test results

Figure 5 shows the effects of control solution, test compounds (**2a-2h**), fluoxetine, and reboxetine on the immobility time of mice in the MFST [$F(10, 66) = 8.05$, $p < 0.001$].

Tukey HSD multiple comparison tests showed that compounds **2c-2f** caused a significant reduction in the immobility time of the animals, with respect to the control group. Reference drugs fluoxetine and reboxetine significantly decreased the immobility time of the mice, as expected. On the other hand, compounds **2a**, **2b**, **2g,** and **2h** did not cause any significant change on the same parameter.

The effects of the control solution, test compounds (**2a-2h**), fluoxetine, and reboxetine on the swimming time of mice in the MFST are demonstrated in Figure 6 [$F(10, 66) = 12.91$, $p < 0.001$]. Tukey HSD multiple comparison tests following one-way ANOVA indicated that compounds **2c** and **2e** and reference drug fluoxetine significantly increased the swimming time of the animals, compared to the control group. Compounds **2a**, **2b**, **2d,** and **2f-2h** were ineffective on the swimming time of animals.

Figure 7 displays the effects of control solution, test compounds (**2a-2h**), fluoxetine, and reboxetine on climbing time of mice in the MFST [F(10,

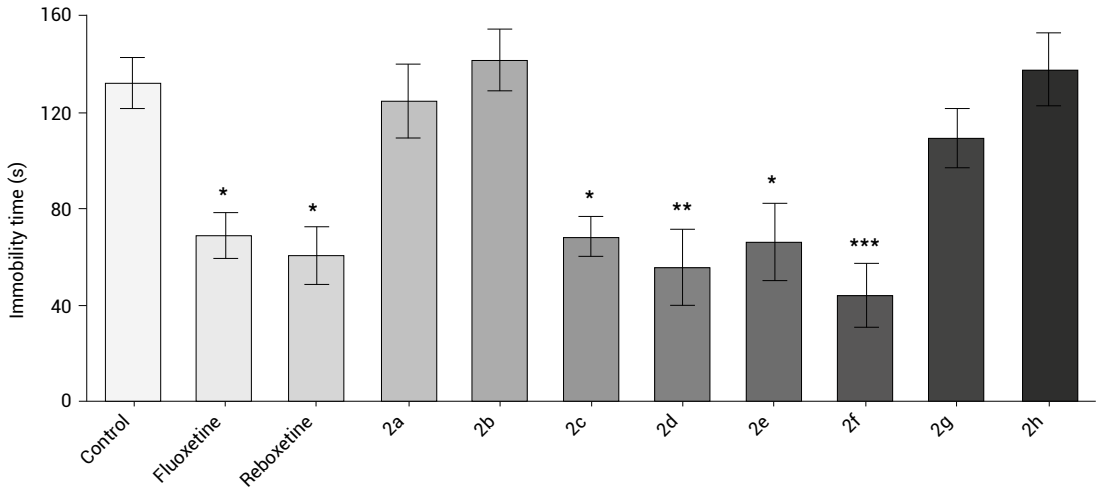


Figure 5. The effect of sunflower oil (control), fluoxetine (20 mg/kg), reboxetine (20 mg/kg) and 2a-2h (50 mg/kg) administrations on immobility time of animals in the MFST. Significant differences compared to the control group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; One way analysis of variance followed by Tukey HSD multiple comparison test, n=7.

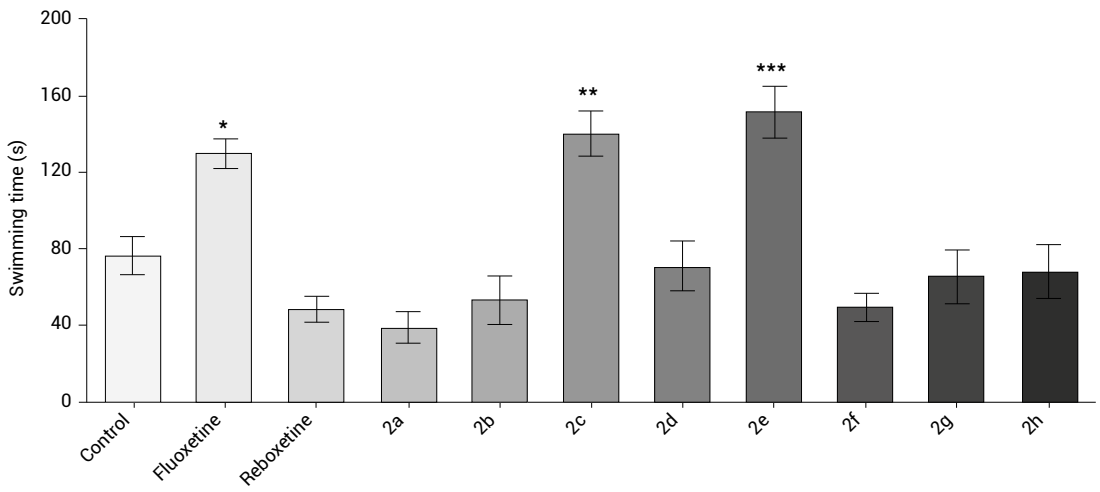


Figure 6. The effect of sunflower oil (control), fluoxetine (20 mg/kg), reboxetine (20 mg/kg) and 2a-2h (50 mg/kg) administrations on swimming time of animals in the MFST. Significant differences compared to the control group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; One way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

66) = 8.88, p < 0.001]. Tukey HSD multiple comparison tests revealed that compounds **2d** and **2f** and reference drug reboxetine caused significant augmentation in the climbing time of the animals with respect to the control group. Compounds **2a-2c, 2e, 2g,** and **2h** did not cause any significant change on the same parameter.

Results of the mechanistic studies

p-Chlorophenylalanine methyl ester studies

The effects of PCPA pre-treatment on anti-immobility effects of compounds **2c** and **2e** in the tail suspension test are demonstrated in Figure 8 [F(5, 36) = 9.31, p < 0.001]. Tukey HSD multiple comparison tests following one-way ANOVA indicated that PCPA pre-treatments significantly reversed the anti-immobility effects induced by compounds **2c** and **2e**.

α-Methyl-para-tyrosine methyl ester studies

Figure 9 shows the effects of AMPT pre-treatment on anti-immobility effects of compounds **2d** and **2f** in the tail suspension test [F(5, 36) = 17.78, p < 0.001]. Tukey HSD multiple comparison tests revealed that the anti-immobility effects of compounds **2d** and **2f** were significantly reversed by AMPT pre-treatments.

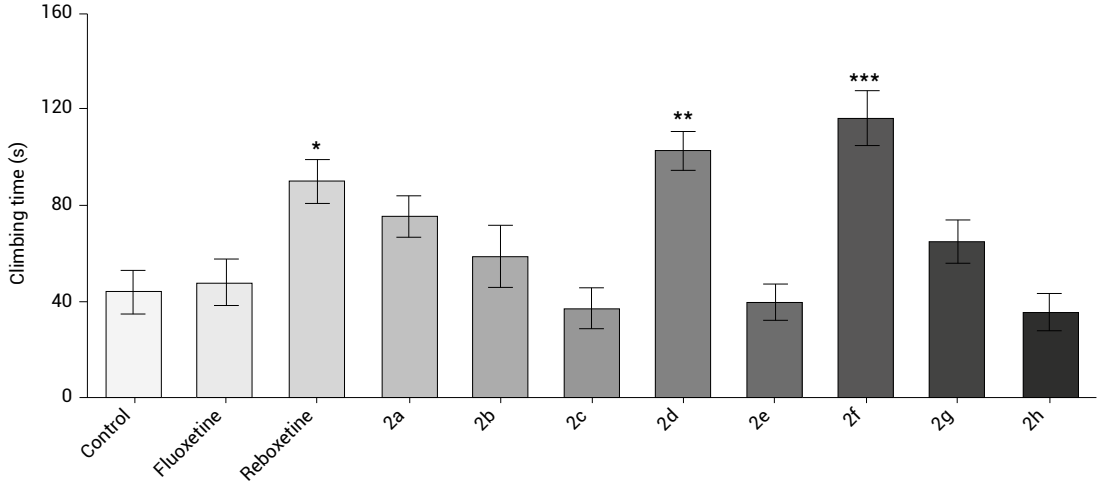


Figure 7. The effect of sunflower oil (control), fluoxetine (20 mg/kg), reboxetine (20 mg/kg) and 2a-2h (50 mg/kg) administrations on climbing time of animals in the MFST. Significant differences compared to the control group $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; One way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

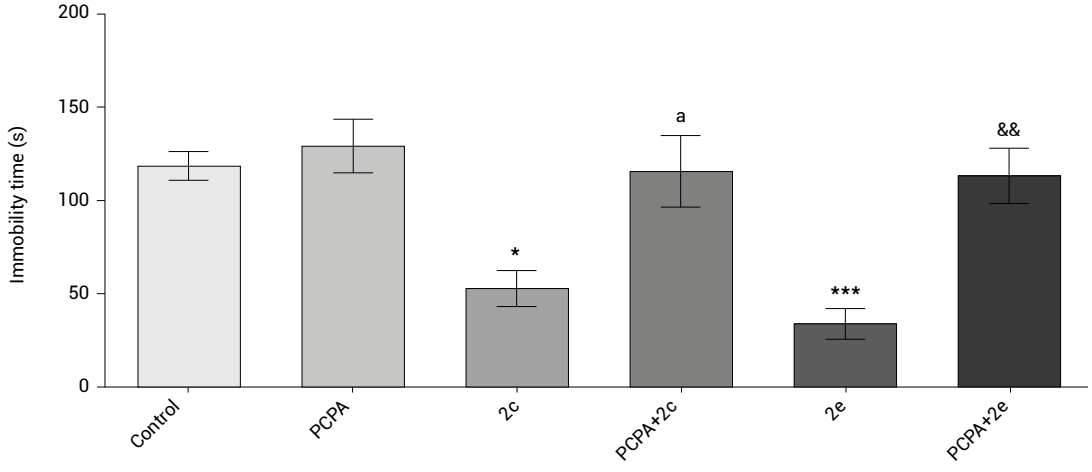


Figure 8. The effect of PCPA pre-treatments on anti-immobility effects of compounds 2c and 2e in the tail suspension test. Significant differences compared to the control group $^{*}p < 0.05$, $^{***}p < 0.001$; Significant differences compared to the compound 2c administrated group $^{a}p < 0.05$; Significant differences compared to the compound 2e administrated group $^{\&\&}p < 0.01$; One way analysis of variance followed by Tukey HSD multiple comparison test, n = 7.

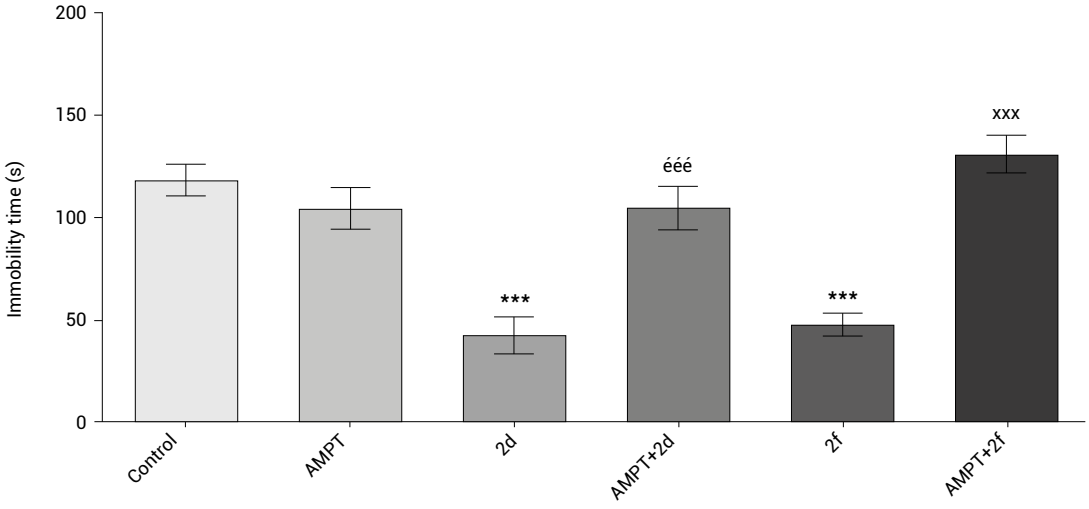


Figure 9. The effect of AMPT pre-treatment on anti-immobility effects of compounds 2d and 2f in the tail suspension test. Significant differences compared to the control group $^{***}p < 0.001$; Significant differences compared to the compound 2d administrated group $^{ééé}p < 0.001$; Significant differences compared to the compound 2f administrated group $^{xxx}p < 0.001$; One-way analysis of variance followed by Tukey HSD multiple comparison test, n=7.

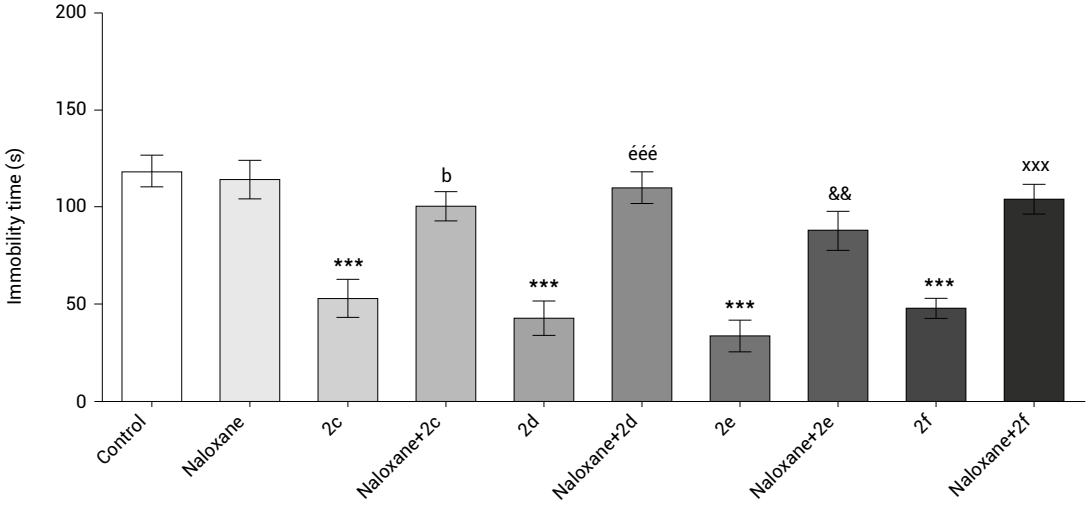


Figure 10. The effect of naloxone pre-treatment on anti-immobility effects of compounds 2c-2f in the tail suspension test. Significant differences compared to the control group $^{***}p < 0.001$; Significant differences compared to the compound 2c administrated group $^{b}p < 0.01$; Significant differences compared to the compound 2d administrated group $^{ééé}p < 0.001$; Significant differences compared to the compound 2e administrated group $^{\&\&}p < 0.01$; Significant differences compared to the compound 2f administrated group $^{xxx}p < 0.001$; One-way analysis of variance followed by Tukey HSD multiple comparison test, n=7.

Naloxone studies

The effects of naloxone pre-treatment on anti-immobility effects of compounds **2c-2f** in the tail suspension test are exhibited in Figure 10 [$F(9, 60) = 15.44$, $p < 0.001$]. Tukey HSD multiple comparison tests following one-way ANOVA indicated that naloxone pre-treatments significantly antagonized the compounds **2c-, 2d-, 2e-** and **2f-**induced decreases in the immobility times of mice.

DISCUSSION

In this study, possible antidepressant-like effects of the test compounds (**2a-2h**) were investigated by tail suspension and MFST.

Before antidepressant-like activity screening tests, we investigated motor performances of animals since agents that affect motor activities may cause false positive or false negative results in behavioral tests (25, 26). In this study, spontaneous locomotor

activities of animals were evaluated by the activity cage tests. Obtained findings showed that test compounds changed neither horizontal nor vertical locomotor activity counts of the animals (Figures 2 and 3). These results eliminated the confounding factors and revealed that the presented antidepressant-like effects are specific.

Tail suspension and MFST are commonly used methods in antidepressant-like activity screening studies (25, 26, 34). In both of these tests, the experimental animals are subjected to short-term and unavoidable stress. Animals, hanged from their tails in the tail suspension test or forced to swim in a restricted area in the MFST, exhibit an immobile posture following some escape-oriented movements. This immobile posture has been associated with an anhedonia-like state of the animals. Therefore, in these tests, agents that reduce the duration or frequency of immobility behavior have been accepted to possess antidepressant-like effects (25, 26, 34, 35).

In this study, compounds **2c**-**2f** significantly reduced the immobility time of mice in both the tail suspension and MFST (Figures 4 and 5), indicating the antidepressant-like effects of these compounds. Moreover, reference drugs fluoxetine and reboxetine also shortened the immobility times of mice and showed their expected antidepressant-like effects.

The MFST differs from the tail suspension test in terms of obtaining an idea about the relationship between "analyzed behaviors" and "related neurotransmitter systems". In this test, the durations of the active behaviors such as swimming and climbing together with the immobility times of the animals are also considered. It has been reported that agents that increase serotonergic neurotransmission shorten the immobility times of animals and increase their swimming times, while agents that increase catecholaminergic neurotransmission decrease the immobility and prolong the climbing times (27, 34). In this study, compounds **2c** and **2e** prolonged the swimming times of mice (Figure 6) without any change in the climbing durations (Figure 7) indicating that their antidepressant-like effects might be related to serotonergic rather than catecholaminergic neurotransmission. On the other hand, compounds **2d** and **2f** prolonged the climbing times of the animals (Figure 7) without changing their swimming durations (Figure 6) suggesting that the antidepressant-like effects of these two compounds seem to be associated with the catecholaminergic rather than serotonergic system. Based on these preliminary findings, we further evaluated the involvements of serotonergic and catecholaminergic systems, in the presented antidepressant-like effects by using pharmacologically specific agents.

The possible involvement of the serotonergic system was evaluated with PCPA studies. PCPA, which inhibits the tryptophan hydroxylase enzyme, is a chemical agent that stops the synthesis of serotonin and depletes the endogenous stores of serotonin in the CNS. It has been reported that PCPA administered at a 100 mg/kg dose for 4 consecutive days reduces endogenous serotonin stores by 60-90% in the CNS of mice and does not affect noradrenaline and dopamine levels (36). In this study, compounds **2c** and **2e** induced reductions in the immobility times of mice reversed by PCPA pretreatments (Figure 8). These data suggest that the antidepressant-like effects of compounds **2c** and **2e** are associated with serotonergic neurotransmission and support our prediction obtain from MFST.

The contribution of the catecholaminergic system to antidepressant-like effects of **2d** and **2f** was investigated with AMPT studies. AMPT, another agent used in mechanistic studies, inhibits the tyrosine hydroxylase enzyme, which is a rate-limiting enzyme in the synthesis of noradrenaline and dopamine. It is known that AMPT pre-treatment reduces noradrenaline and dopamine levels in the brains of mice by 53% and 57%, respectively, without affecting the serotonin levels (37). In this study, it was determined that AMPT pre-treatments reversed the compounds **2d-** and **2f**-induced anti-immobility effects (Figure 9) indicating that the antidepressant-like effects of these compounds are related to the catecholaminergic system; once again supports the findings of the MFST.

It has been reported that various ligands/drugs acting on opioid receptors have antidepressant-like effects and that the efficacy of various antidepressant drugs used clinically is mediated by the opioidergic system (38). Therefore, we further investigated the probable involvement of the opioidergic system in the antidepressant-like effect of the active compounds, in the content of this study. Participation of the opioidergic system in the presented antidepressant-like activities was evaluated by naloxone studies. Obtained data indicated that naloxone pretreatments reversed the anti-immobility effects of compounds **2c, 2d, 2e,** and **2f** (Figure 10). These findings revealed that the antidepressant-like effects of these four derivatives are also mediated by the opioidergic system. On the other hand, specific opioid receptor subtypes responsible for the presented antidepressant-like activity should also be revealed by further studies.

Results of this study indicated that, among the tested derivatives, compounds **2c-2f** were active, whereas compounds **2a**, **2b**, **2g,** and **2h** were ineffective. When the structure-activity relationships of the compounds are examined, the differences observed in the pharmacological activities of the derivatives are likely to be related to the steric parameters. Substitution of the piperidine ring with a methyl group from the 2nd or 3rd position eliminates the planarity in the structure. It may be suggested that this structural feature is important for the interaction of the active compounds with the biomolecules in their target regions and is thought to be the reason for the pharmacological activity difference between the compounds in the series.

When the mechanistic studies with PCPA and AMPT are evaluated, it can be suggested that pyridine ring (**2c** and **2e**) may be associated with serotonergic system mediated and indole ring (**2d** and **2f**) may be related with catecholaminergic system mediated antidepressant-like effects of active compounds. In addition, it can also be speculated that both rings may be important for the opioidergic system mediated antidepressant-like activities of the active compounds.

Finally, compounds in the series exhibited negligible toxicity. Any of the test compounds induced death or undesirable side effects such as diarrhea, convulsions, ataxia, or paralysis. These observations pointing to the safety of the tested derivatives increase the potential of these compounds as drug candidate molecules.

In summary, among the newly synthesized original piperidine derivatives, compounds **2c** and **2e** have serotonergic system mediated; compounds **2d** and **2f** have catecholaminergic system mediated antidepressant-like effects. Moreover, the opioidergic system also participates in the presented antidepressant-like activity in this study.

It is well-known that there are physiological interactions between the monoaminergic and opioidergic systems of the CNS. Activation of μ- and δ-opioid receptors has been shown to enhance serotonin (39) and dopamine (40,41) levels in various brain regions. Therefore, based on the data obtained from the mechanistic studies, it can be speculated that our test compounds might have an antidepressant-like effect by increasing the serotonergic or catecholaminergic activity in the limbic system by affecting opioid receptors. However, this idea should be investigated by further studies. Moreover, apart from the monoaminergic and opioidergic systems, there are several endogen mechanisms regulating emotions and mood. Therefore, possible contributions of other endogen mechanisms (such as GABAergic, glutaminergic, and nitrergic) to the presented antidepressant-like effects, should also be investigated in order to clarify the exact pharmacological mechanisms of the active compounds.

CONCLUSION

In conclusion, in this study, antidepressant-like activities of compounds **2c-2f** were demonstrated by tail suspension and MFST. In addition, mechanistic studies with PCPA and AMPT revealed that the antidepressant-like activity of compounds **2c** and **2e** was mediated by the serotonergic system, and the antidepressant-like activity of compounds **2d** and **2f** was related to the catecholaminergic system. Moreover, studies with naloxone indicated that the opioidergic system also participates in the antidepressant-like activities of all effective compounds.

When the structures of piperidine derivatives (**2a**-**2h**) were examined, it was determined that derivatives with antidepressant-like effects carried 2-methyl (**2c** and **2d**) and 3-methyl (**2e** and **2f**) substituents on the piperidine ring. Derivatives containing unsubstituted piperidine (**2a** and **2b**) and 4-methylpiperidine (**2g** and **2h**) were found to be ineffective. In terms of the structure-activity relationship, it can be thought that the antidepressant-like activity of compounds **2c-2f** is related to the methyl substituent brought to the 2nd and 3rd position. Active derivatives, on the other hand, exhibited activity through different mechanisms, depending on whether they contained pyridine and/or indole rings.

This study supports the previous papers reporting the antidepressant activity potentials of piperidine derivatives (6-9). It also provides new insights into the mechanisms that mediate the antidepressant-like effects of piperidine ring-containing compounds. Studies have pointed out that agents affecting various opioid receptor subtypes have been shown to possess some advantages such as the quick onset of the activity or better results in patients with treatment-resistant depression (42, 43). As recent studies have demonstrated the importance of opioidergic ligands in antidepressant drug development studies, it is particularly striking to demonstrate the involvement of the opioidergic system in the antidepressant-like effect presented in this study.

Therefore, the findings of this study indicated that the piperidine ring might be an important pharmacophore used to design novel antidepressant drugs having some clinical advantage potential.

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Conflicts of interest

The authors declare no conflict of interest.

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