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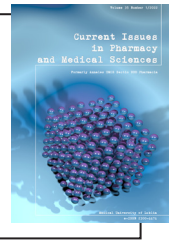

Design, synthesis and biological evaluation of pyrazoline derivatives for antidepressant activity

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ABSTRACT

Pyrazolines constitute a class of privileged scaffolds in modern medicinal chemistry, particularly as central nervous system-active agents, and several pyrazoline-based drugs are used to treat various neurological disorders. In the present study, a series of pyrazoline derivatives bearing different substituents at the 2- and 4-positions were subjected to molecular docking against the human serotonin transporter (PDB ID: 5I6X). Based on the docking results, selected Mannich bases of 3-(heteroaryl)-5-(4-substituted aryl)-1-phenyl-4,5-dihydro-1H-pyrazoles were prioritized for synthesis using substituted anilines and formaldehyde. Reaction progress was monitored by thin-layer chromatography (TLC). Structural characterization of the synthesized compounds was performed using IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Acute toxicity studies were conducted in accordance with OECD guideline No. 425 to determine the LD_{50} values. The antidepressant activity of the synthesized compounds was evaluated using the forced swim test, and statistical analysis was carried out by one-way ANOVA followed by Dunnett's post hoc test.

Compound 2b (percentage change in immobility time: -37.51%), followed by compound 2f (-36.91%) and compound 2e (-36.64%), exhibited significant antidepressant activity at a dose of 10 mg/kg, as evidenced by a marked reduction in immobility time. Structure-activity relationship analysis indicated that the presence of electron-withdrawing groups, such as chloro, bromo, and nitro substituents, at the 2- and/or 4-positions of the phenyl ring at the 5-position of the pyrazoline moiety, as well as on the aniline side chain, enhanced the antidepressant potential of the compounds.

INTRODUCTION

In recent years, there has been a significant increase in interest in nitrogen-containing heterocyclic compounds due to their broad and noteworthy pharmacological activities. Among these, pyrazole and its derivatives have attracted considerable attention, particularly because the pyrazole ring is a key structural component of antipyrine. Pyrazolines are dihydro derivatives of pyrazoles and have long been recognized for their diverse biological and pharmacological properties [1].

Previous studies have demonstrated that the introduction of a phenyl group at the third position of the pyrazoline ring enhances the antidepressant efficacy of certain 1,3,5-triphenyl-2-pyrazoline derivatives [2]. Furthermore, investigations into the antidepressant activity of 3,5-diphenyl-2-pyrazoline derivatives revealed that the presence of methoxy (–OCH_3) and chloro (–Cl) substituents at the para position of the phenyl rings at the third and fourth positions of the pyrazoline core significantly increased their pharmacological efficacy [3]. Similarly, among synthesized 3-(2-furyl)-pyrazoline derivatives, compounds bearing ethyl and allyl thiocarbamoyl substituents at the first position of the pyrazoline ring exhibited substantial antidepressant activity [4].

Several diaryl pyrazoline derivatives substituted with methylsulfonyl groups have also been reported as potent antidepressant agents. The antidepressant potential of newly synthesized dihydropyrazoles was evaluated using activity cage apparatus to assess spontaneous locomotor activity. Some derivatives reduced both vertical and horizontal movements in mice; however, a limited number of compounds significantly decreased immobility time in the tail suspension

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test (TST) and modified forced swim test (MFST) without affecting general locomotor activity. These findings suggest an antidepressant-like effect comparable to that of standard drugs. Notably, similar to the reference antidepressant fluoxetine, these compounds increased swimming time during the MFST without influencing climbing behavior [5].

The therapeutic potential of novel pyrazole derivatives has also been explored in the context of epilepsy and depression. These compounds exhibited comparable antidepressant effects in behavioral tests and demonstrated anticonvulsant activity in pentylenetetrazole (PTZ)-induced seizure models [6]. Additionally, several 2-pyrazoline derivatives have been investigated for antidepressant-like activity, with a subset of evaluated compounds showing significant efficacy in the tail suspension test [7,8]. The observed results suggest that halogen-substituted thiophene moieties play a crucial role in enhancing antidepressant-like effects [9].

Moreover, the antidepressant activity of various pyrrolopyrazoles and their corresponding pyrimidine derivatives has been evaluated and compared with that of fluoxetine. The findings indicated that both pyrazole and pyrazolopyrimidine derivatives possess promising antidepressant potential [10].

In molecular modeling studies, docking techniques are widely employed to predict the preferred orientation of small molecules upon binding to biological macromolecules, thereby forming stable complexes. Interactions with biologically relevant targets are essential for signal transduction, and molecular docking enables the prediction of binding modes, affinity, and potential biological activity of small-molecule therapeutic candidates [11-14].

Based on an extensive literature survey, pyrazoline derivatives have consistently demonstrated antidepressant properties. To enhance antidepressant activity, the pyrazoline scaffold is often combined with heteroaromatic rings such as furan or thiophene. In this context, the present study reports the synthesis and evaluation of selected 4-substituted-N-[5-(4-substituted phenyl)-3-(heteroaryl)-1-phenyl] pyrazoline methyl aniline derivatives for their antidepressant activity.

MATERIALS AND METHODS

General remarks

All chemicals and reagents were of laboratory reagent (LR) grade and were purchased from Loba Chemie and Sigma-Aldrich. Molecular docking studies were performed using Vlife Molecular Design Suite (Vlife MDS, version 4.21). Melting points were determined using an *Elico* melting point apparatus and are reported uncorrected. The progress of reactions and the purity of recrystallized compounds were monitored by thin-layer chromatography (TLC) using a solvent system of petroleum ether and ethyl acetate (4:1).

Infrared (IR) spectra were recorded on a Shimadzu IR Affinity-1 spectrophotometer using KBr pellets, and frequencies are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C) were recorded on a Bruker spectrometer using Bruker Compass Data Analysis software (version 4.2), with DMSO-d_6 as the solvent. All NMR analyses were performed at the Central Instrumentation Facility, Savitribai Phule Pune University, Pune, India.

Molecular docking studies

Protein preparation

The crystal structure of the human serotonin transporter (ts3) complexed with paroxetine (PDB ID: 5I6X) was retrieved from the RCSB Protein Data Bank (www.rcsb.org). The protein structure was preprocessed by removing bound ligands, cofactors, and water molecules. Side chains not involved in salt bridges and not located near the binding cavity were neutralized. Energy minimization of the protein-ligand complex was performed using the AMBER force field, and the minimization process was terminated after 5,000 iterations.

Ligand preparation

The chemical structures of the synthesized 2-pyrazoline derivatives were drawn and saved in .mol2 format. The ligands were converted into three-dimensional structures, followed by geometry optimization using the AMBER force field with default parameters.

Docking protocol

Molecular docking studies were carried out using Vlife MDS version 4.21 on an i3-powered Lenovo computer running Windows XP. The genetic algorithm implemented in the Molecular Design Suite was employed to dock the ligands into the active site of the receptor. Docking scores were used to evaluate the binding affinity of the synthesized compounds and to correlate their predicted interactions with observed biological activity.

Comparative docking studies were conducted between the designed compounds and the reference selective serotonin reuptake inhibitor paroxetine. Batch docking was performed using the X-ray crystal structure of the ts3 human serotonin transporter complexed with paroxetine (PDB ID: 5I6X) at the central binding site.

Synthesis of 3-(heteroaryl)-5-(4-substituted aryl)-N¹-phenyl-2-pyrazolines (1a-1f)

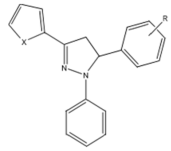
An equimolar mixture of 3-(substituted aryl)-1-(heteroaryl)prop-2-en-1-one (chalcones), synthesized according to a previously reported method [1,15], phenylhydrazine, and anhydrous potassium carbonate was dissolved in an adequate amount of methanol. The reaction mixture was heated under reflux for 8 h. After completion of the reaction, the mixture was cooled and poured into ice-cold water with vigorous stirring to obtain a solid precipitate. The resulting product was filtered, dried, and recrystallized from methanol. The synthesized derivatives are listed in Table 1.

Synthesis of 4-substituted-N-[5-(4-substituted aryl)-3-(heteroaryl)-N¹-phenyl-2-pyrazolin-4-yl)methyl] anilines (2a-2h) (Figure 1)

A mixture of 3-(heteroaryl)-5-(4-substituted aryl)-N¹-phenyl-2-pyrazolines, formaldehyde, and 4-substituted aniline in a molar ratio of 1:2:1 was dissolved in a sufficient quantity of methanol and heated under reflux for 6 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was poured into ice-cold water and stirred thoroughly. The precipitated product was

collected by filtration, dried, and recrystallized from ethanol. The synthesized derivatives are presented in Table 2.

Table 1. 3-(heteroaryl)-5-(4-substituted aryl)-1-phenyl-4,5-dihydro-1H-pyrazoles derivatives



Code	-X-	R1
1a	-O-	2-Cl
1b	-O-	4-Cl
1c	-S-	4-Cl
1d	-O-	4-N(CH_3)$_2$
1e	-O-	4-OCH_3
1f	-O-	4-OH

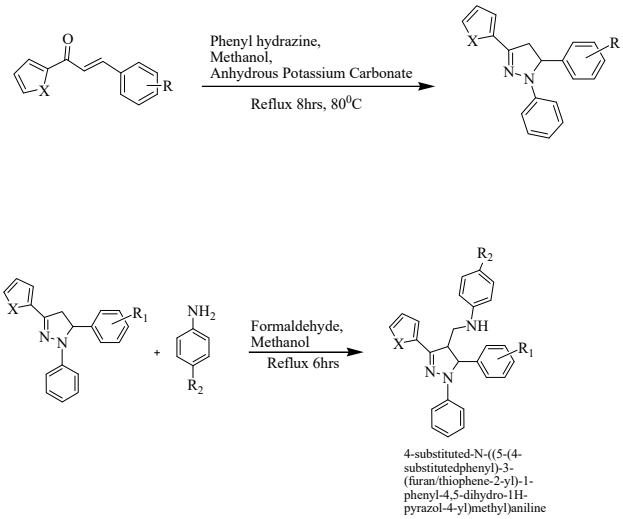
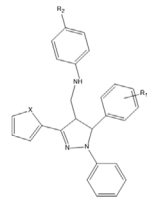


Figure 1. Scheme for synthesis of 4-substituted-N-[5-(4-substituted aryl-3-heteroaryl-N1-phenyl-2-pyrazolines-4-yl) methyl]aniline

Table 2. 4-substituted-N-[5-(4-substituted aryl-3-heteroaryl-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)methyl]aniline derivatives



Compound	-X-	-R1	-R2
2a	-O-	4-OCH_3	4-OCH_3
2b	-O-	4-Cl	4-Br
2c	-S-	4-Cl	4-NO_2
2d	-O-	4-N(CH_3)$_2$	4-NO_2
2e	-O-	2-Cl	4-NO_2
2f	-O-	4-Cl	4-NO_2
2g	-O-	4-OH	4-Cl
2h	-O-	4-OH	4-NO_2

Acute toxicity studies

Acute toxicity studies were conducted in mice in accordance with the Organisation for Economic Cooperation and Development (OECD) guidelines No. 425 to determine the median lethal dose (LD_{50}). Animals were continuously monitored for clinical signs of toxicity and mortality following administration of the test compounds. The number of animals that died within a 48 h observation period was recorded.

Evaluation of antidepressant activity using the forced swim test

Swiss albino mice weighing 22-25 g were used for the study. The animals were randomly divided into groups (n = 6 per group) as described in Table 3. The experimental groups received either imipramine (standard drug) or the test compounds, administered 60 min prior to the forced swim test.

Each mouse was individually placed in a transparent cylindrical container filled with water to a depth of 15 cm, maintained at 25°C. The total duration of the test was 5 min, during which the immobility time was recorded. A mouse was considered immobile when it ceased active movements and remained floating passively in the water without struggling.

Table 3. Antidepressant effect of N-[5-(4-substituted aryl-3-heteroaryl-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl) methyl] anilines derivatives in mice using forced swim test

Group	Dose mg/kg (i.p)	Immobility time (seconds) (Mean ± SEM)	Percentage change in immobility time
Control (1% Tween 80)	--	149.0 ± 4.19	--
Standard (Imipramine)	10	129.0 ± 3.00	-13.43
2a	100	98.5 ± 3.88	-33.89
2b	100	93.1± 2.10*	-37.51
2c	100	98.0 ± 3.51	-34.22
2d	100	96.0 ± 2.44	-35.57
2e	100	94.4 ±1.90*	-36.64
2f	100	94.0 ± 3.93*	-36.91
2g	100	97.6 ± 4.05	-34.49
2h	100	96.3 ±2.40	-35.36

N=6, in each group; *:P< 0.05 : significant; One Way ANOVA followed Dunnett's test. Values are expressed as Mean ± SEM

The percentage reduction in immobility time for each treated group was calculated relative to the control group. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under Proposal No. MET-IOP-IAEC/2021-22/07.

RESULTS

Docking studies

Docking scores of the 4-substituted N-[(5-substituted phenyl-3-heteroaryl-N¹-phenyl-2-pyrazolin-4-yl)methyl] aniline derivatives are presented in Table 4.

Table 4. Dock scores of 4-substituted-N-(5-substitutedphenyl-3-heteroaryl-1-phenyl-4,5- dihydro-1H-pyrazol-4-yl)methyl)aniline derivatives

Compound _conformer	-R1	-R2	-X-	Dock score
2a_C-10	4-OCH_3	4-OCH_3	-O-	-22.85
2b_C-5	4-Cl	4-Br	-O-	-3.75
2c_C-22	4-Cl	4-NO_2	-S-	-50.21
2d_C-6	4-$N(CH_3)_2$	4-NO_2	-O-	-28.98
2e_C-10	2-Cl	4-NO_2	-O-	-17.46
2f_C-5	4-Cl	4-NO_2	-O-	-11.84
2g_C-5	4-OH	4-Cl	-O-	-25.85
2h_C-5	4-OH	4-NO_2	-O-	-26.02
Paroxetine (Standard)	--	--	--	-65.37

Figure 2 illustrates the binding interactions of paroxetine with cavity 1 of 5I6X. Hydrophobic interactions with residues LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, and LEU565A are shown in blue, while van der Waals interactions with LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, LEU565A, GLU493A, ARG104A, and ARG401A are shown in magenta.

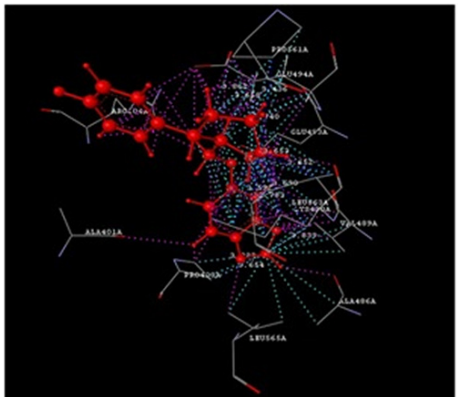


Figure 2. Binding interactions of Paroxetine with cavity # 1 of 5I6X

Figure 3 shows the binding interactions of compound 2b with cavity 1 of 5I6X. Hydrophobic interactions with residues LYS490A and GLU494A are indicated in blue, whereas van der Waals interactions with LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, LEU565A, GLU493A, ARG104A, ARG401A, TYR107A, and ILE179A are shown in magenta.

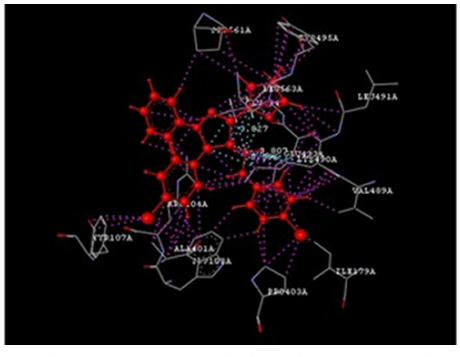


Figure 3. Binding interactions of 2b with cavity # 1 of 5I6X

Figure 4 depicts the binding interactions of compound 2e with cavity 1 of 5I6X. Hydrophobic interactions with LYS490A and GLU494A are shown in blue. Van der Waals interactions with LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, LEU565A, GLU493A, ARG104A, ARG401A, TYR107A, ILE179A, LEU99A, TRP182A, TRP103A, TRP175A, and PHE335A are shown in magenta.

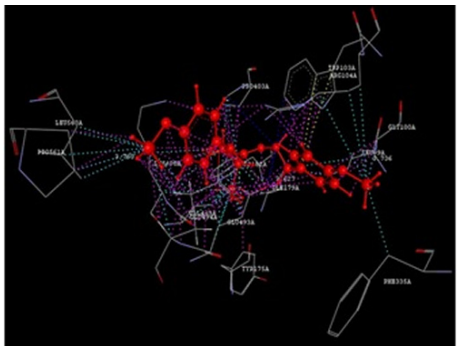


Figure 4. Binding interactions of 2e with cavity # 1 of 5I6X

Figure 5 presents the binding interactions of compound 2f with cavity 1 of 5I6X. Hydrophobic interactions with LYS490A, LEU465A, and PRO403A are shown in blue, while van der Waals interactions with GLY402A, SER404A, VAL489A, TRP182A, PRO403A, ALA486A, ARG104A, LEU565A, ALA401A, TRP103A, ILE108A, LYS490A, ARG564A, TYR107A, GLU494A, ASP400A, and GLU493A are shown in magenta.

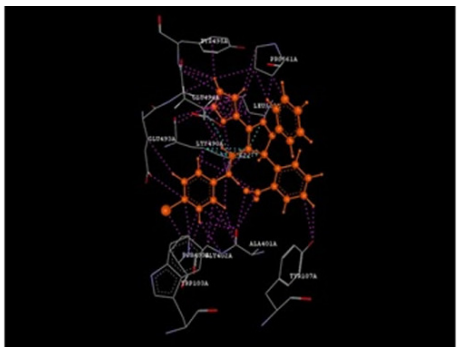


Figure 5. Binding interactions of 2f with cavity # 1 of 5I6X

CHEMISTRY

4-Methoxy-N-[5-(4-methoxyphenyl)-3-(2-furyl)-N^1-phenyl-2-pyrazoline-4-yl)methyl]aniline (2a).

Yellow solid; yield 65.51%; mp 110-112°C; Rf = 0.55. IR (KBr, cm^{-1}): 3367 (N–H str), 1600 (C=N), 1502 (C=C), 1502 (C–N), 1107 (C–O–C), 835 (C–H def).

^{1}H NMR (400 MHz, δ ppm): 3.41-3.52 (2H, d, J = 3.1 Hz), 3.66 (1H, dt, J = 8.1, 3.1 Hz), 5.64 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.78-6.91 (3H, dtd, J = 8.2, 1.2, 0.5 Hz), 6.98-7.41 (6H, tt, J = 7.9, 1.2 Hz), 7.46-7.70 (4H, ddd, J = 8.3, 1.6, 0.5 Hz), 8.07 (2H, ddd, J = 7.9, 1.8, 0.5 Hz).

^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 111.8, 112.0, 116.6, 122.8, 125.0, 127.8, 128.3, 133.0, 136.0, 140.4, 143.6, 147.3, 148.4, 151.7, 152.3.

4-Bromo-N-[5-(p-chlorophenyl)-3-(2-furyl)-N¹-phenyl-2-pyrazoline-4-yl)methyl]aniline (2b).

Off-white solid; yield 68.59%; mp 120-124°C; Rf = 0.65.
IR (KBr, cm^{-1}): 3371 (N–H str), 1593 (C=N), 1498 (C=C), 1120 (C–O–C), 812 (C–H def), 742 (C–Cl).
^{1}H NMR (400 MHz, δ ppm): 3.17-3.27 (2H, d, J = 3.1 Hz), 3.61 (1H, dt, J = 8.1, 3.1 Hz), 5.52 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.60 (2H, ddd, J = 8.3, 1.7, 0.5 Hz), 6.78-6.91 (3H, dtd, J = 8.2, 1.2, 0.5 Hz), 7.04 (1H, tt, J = 7.9, 1.2 Hz), 7.21 (2H, ddd, J = 8.3, 1.5, 0.5 Hz), 7.35 (1H, dd, J = 1.8, 0.8 Hz), 7.46-7.69 (6H, ddd, J = 8.4, 1.6, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 111.8, 112.0, 121.9, 122.8, 127.8, 128.7, 131.7, 133.7, 137.2, 140.4, 143.6, 148.4, 151.7, 152.3.

4-Nitro-N-[5-(p-chlorophenyl)-3-(2-thiophenyl)-N¹-phenyl-2-pyrazoline-4-yl)methyl]aniline (2c).

Yellowish-white solid; yield 60.15%; mp 128-130°C; Rf = 0.75.
IR (KBr, cm^{-1}): 3367 (N–H str), 1604 (C=N), 1506 (C–N), 1471 (C=C), 1120 (C–O–C), 856 (C–H def), 752 (C–Cl).
^{1}H NMR (400 MHz, δ ppm): 3.43-3.54 (2H, d, J = 3.1 Hz), 3.68 (1H, dt, J = 8.1, 3.1 Hz), 5.54 (1H, d, J = 8.1 Hz), 6.84 (2H, dtd, J = 8.2, 1.2, 0.5 Hz), 6.91-7.31 (6H, dd, J = 7.3, 1.1 Hz), 7.46-7.69 (6H, ddd, J = 8.4, 1.6, 0.5 Hz), 8.07 (2H, ddd, J = 7.9, 1.8, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 116.6, 122.8, 125.0, 127.5, 128.2, 128.7, 133.7, 137.2, 140.2, 147.3, 148.4, 151.7.

4-(N,N-Dimethylamino)-N-[5-(p-nitrophenyl)-3-(2-furyl)-N¹-phenyl-2-pyrazoline-4-yl)methyl]aniline (2d).

Yellowish solid; yield 62.71%; mp 104-106°C; Rf = 0.58.
IR (KBr, cm^{-1}): 3367 (N–H str), 1602 (C=N), 1500 (C=C), 1188 (C–O–C), 835 (C–H def).
^{1}H NMR (400 MHz, δ ppm): 2.74 (6H, s), 3.36-3.55 (3H, d, J = 3.1 Hz), 5.39 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.59-6.91 (7H, ddd, J = 8.2, 1.2, 0.5 Hz), 6.98-7.20 (3H, tt, J = 7.9, 1.2 Hz), 7.35 (1H, dd, J = 1.8, 0.8 Hz), 7.59 (2H, dddd, J = 8.2, 7.9, 1.5, 0.5 Hz), 8.07 (2H, ddd, J = 7.9, 1.8, 0.5 Hz).
^{13}C NMR (δ ppm): 40.3, 40.7, 49.9, 65.2, 111.8, 112.0, 116.6, 122.8, 125.0, 128.6, 137.2, 140.4, 143.6, 147.3, 148.4, 150.9, 151.7, 152.3.

4-Nitro-N-[5-(o-chlorophenyl)-3-(2-furyl)-N¹-phenyl-2-pyrazoline-4-yl)methyl]aniline (2e).

White solid; yield 61.11%; mp 136-140°C; Rf = 0.68.
IR (KBr, cm^{-1}): 3367 (N–H str), 1654 (C=N), 1598 (C=C), 1114 (C–O–C), 829 (C–H def), 748 (C–Cl), 2908 (C–OCH_3).
^{1}H NMR (400 MHz, δ ppm): 3.07-3.19 (2H), 3.13 (1H, d, J = 5.6 Hz), 3.54 (1H, dt, J = 8.1, 5.6 Hz), 3.68-3.79 (6H, s), 5.39 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.77-7.10 (10H, ddd, J = 8.8, 2.7, 0.4 Hz), 7.21 (2H, ddd, J = 8.8, 1.1, 0.5 Hz), 7.35 (1H, dd, J = 1.8, 0.8 Hz), 7.59 (2H, dddd, J = 8.2, 7.9, 1.5, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 56.0, 56.0, 65.2, 111.8, 112.0, 114.3, 114.5, 120.5, 122.8, 127.8, 128.2, 137.2, 140.4, 143.6, 148.4, 151.7, 152.3, 159.8, 159.8.

4-Nitro-N-[5-(p-chlorophenyl)-N¹-phenyl-3-(2-furyl)-2-pyrazoline-4-yl)methyl]aniline (2f).

Yellowish-white solid; yield 63.0%; mp 126-128°C; Rf = 0.66.
IR (KBr, cm^{-1}): 3369 (N–H str), 1653 (C=N), 1595 (C–NO_2), 1500 (C=C), 1109 (C–O–C), 837 (C–H def), 742 (C–Cl).
^{1}H NMR (400 MHz, δ ppm): 3.36-3.47 (2H, d, J = 3.1 Hz), 3.62 (1H, dt, J = 8.1, 3.1 Hz), 5.58 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.78-6.91 (3H, dtd, J = 8.2, 1.2, 0.5 Hz), 6.98-7.20 (3H, tt, J = 7.9, 1.2 Hz), 7.35 (1H, dd, J = 1.8, 0.8 Hz), 7.46-7.69 (6H, ddd, J = 8.4, 1.6, 0.5 Hz), 8.07 (2H, ddd, J = 7.9, 1.8, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 111.8, 112.0, 116.6, 122.8, 125.0, 127.8, 128.2, 128.7, 133.7, 137.2, 140.4, 143.6, 147.3, 148.4, 151.7, 152.3.

4-Chloro-N-[5-(p-hydroxyphenyl)-N¹-phenyl-3-(2-furyl)-2-pyrazoline-4-yl)methyl]aniline (2g).

Off-white solid; yield 68.50%; mp 140-142°C; Rf = 0.59.
1H NMR (400 MHz, δ ppm): 3.16-3.27 (2H, d, J = 3.1 Hz), 3.52 (1H, dt, J = 8.1, 3.1 Hz), 5.39 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.61-6.91 (7H, ddd, J = 8.3, 1.6, 0.5 Hz), 7.04 (1H, tt, J = 7.9, 1.2 Hz), 7.15-7.41 (5H, ddd, J = 8.3, 1.0, 0.5 Hz), 7.59 (2H, dddd, J = 8.2, 7.9, 1.5, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 111.8, 112.0, 115.7, 120.5, 122.8, 127.2, 128.9, 133.7, 137.2, 140.4, 143.6, 148.4, 151.7, 152.3, 157.4.

4-Nitro-N-[5-(p-hydroxyphenyl)-N¹-phenyl-3-(2-furyl)-2-pyrazoline-4-yl)methyl]aniline (2h).

Yellowish solid; yield 65.4%; mp 130-134°C; Rf = 0.66.
1H NMR (400 MHz, δ ppm): 3.42-3.59 (3H, d, J = 3.1 Hz), 5.41 (1H, d, J = 8.1 Hz), 6.30 (1H, dd, J = 3.4, 1.8 Hz), 6.67 (2H, ddd, J = 8.3, 1.6, 0.5 Hz), 6.78-6.91 (3H, dtd, J = 8.2, 1.2, 0.5 Hz), 6.98-7.28 (5H, tt, J = 7.9, 1.2 Hz), 7.35 (1H, dd, J = 1.8, 0.8 Hz), 7.59 (2H, dddd, J = 8.2, 7.9, 1.5, 0.5 Hz), 8.07 (2H, ddd, J = 7.9, 1.8, 0.5 Hz).
^{13}C NMR (δ ppm): 40.7, 49.9, 65.2, 112.0, 116.6, 122.8, 125.0, 127.8, 128.2, 137.2, 140.4, 143.6, 147.3, 148.4, 151.7, 152.3, 157.4.

DISCUSSION

The TS3 human serotonin transporter (PDB ID: 5I6X), whose X-ray crystal structure was used as the target, served as the receptor for docking studies of all feasible three-dimensional conformations of 4-substituted-*N*-[5-(4-substituted aryl-3-heteroaryl-*N*¹-phenyl-2-pyrazolin-4-yl) methyl]anilines. Intermolecular interactions between the ligands and the receptor protein were investigated in detail. Table 3 presents the docking scores and binding energies of the conformations of the synthesized compounds.

A subset of compounds exhibiting lower docking scores was selected to analyze their binding modes within cavity 1 of the receptor. The interaction pattern of the standard ligand, paroxetine, was used for comparison and is shown in Figure 2. Hydrophobic and van der Waals interactions were analyzed for compounds with the lowest docking scores, namely compound 2b (Conformer_C5; Figure 3),

compound 2e (Conformer_C10; Figure 4), and compound 2f (Conformer_C5; Figure 5).

The analysis revealed that amino acid residues LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, LEU565A, as well as LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, LEU565A, and GLU493A participated in the interactions with cavity 1 of 5I6X for both the test compounds and the standard ligand, paroxetine. Since the binding patterns of the synthesized compounds were comparable to that of the reference drug, these molecules may serve as potential lead candidates for the development of novel and potent antidepressant agents.

In the ^{1}H NMR spectra, signals observed at δ 5.39-5.64 ppm and 3.36-3.66 ppm corresponded to the two pyrazoline protons at the C2 and C3 positions, respectively. Signals corresponding to aromatic protons of the furyl ring appeared in the range δ 6.29-7.35 ppm. In the ^{13}C NMR spectra, signals at δc 40.7, 65.2, and 141.7 ppm were assigned to the C2, C3, and C4 carbons of the pyrazoline ring, respectively.

The LD_{50} value was calculated to be 981.1 mg/kg. Based on this result, a dose of 100 mg/kg was selected for the evaluation of the antidepressant efficacy of the synthesized compounds.

In the present study, the antidepressant activity of the title compounds was evaluated using the forced swim test model. This method is sensitive and relatively specific for detecting monoamine oxidase inhibitors and commonly used antidepressant agents. Antidepressant activity was assessed by measuring the mean immobility time (in seconds), and the results are summarized in Table 4. Compounds 2b, 2e, and 2f showed the most pronounced reduction in immobility time, reaching values as low as 93.1 seconds. The remaining compounds exhibited moderate antidepressant activity when compared with the control group.

CONCLUSION

A series of 4-substituted-*N*-[5-(4-substituted aryl-3-heteroaryl-*N*^{1}-phenyl-2-pyrazolin-4-yl)methyl]anilines was designed and evaluated for interaction with the serotonin transporter receptor (PDB ID: 5I6X) using VLife MDS version 2.0 software. Docking scores of the synthesized ligands were compared with that of the standard antidepressant, paroxetine. Among all derivatives, compound 2b exhibited the lowest docking score, followed by compounds 2f, 2e, and 2a.

Both the synthesized ligands and the standard compound interacted with the receptor primarily through amino acid residues LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, LEU565A, as well as LYS490A, GLU494A, VAL489A, LEU563A, ALA486A, PRO561A, LEU565A, and GLU493A, mainly via hydrophobic and van der Waals interactions.

Pharmacological evaluation demonstrated that compounds 4-bromo-N-[5-(4-chlorophenyl)-3-(2-furyl)-N^{1}-phenyl-2-pyrazolin-4-yl)methyl]aniline (2b), 2-chloro-N-[5-(4-nitrophenyl)-3-(2-furyl)-N^{1}-phenyl-2-pyrazolin-4-yl) methyl]aniline (2e), and 4-chloro-N-[5-(4-nitrophenyl)-3-(2-furyl)-N^{1}-phenyl-2-pyrazolin-4-yl)methyl]aniline (2f) exhibited significant antidepressant activity at a dose of 10 mg/kg. Compared with the control group, these compounds significantly reduced the immobility time in the forced swim test.

Structure-activity relationship analysis indicated that the presence of chloro, bromo, and nitro substituents at the second and/or fourth positions of the phenyl ring at the fifth position of the pyrazoline moiety and the aniline side chain contributed to enhanced antidepressant activity.

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