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Synthesis, and anti-depressant evaluation and in silico studies of novel pyrazolines

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Abstract

In the present study, a series of novel pyridine-linked pyrazoline derivatives (5a-e) were synthesized using chalcone intermediates and evaluated for their antidepressant potential. The synthesized compounds were prepared through Claisen-Schmidt condensation followed by cyclization with substituted phenylhydrazines. The synthesized compounds were obtained in satisfactory yields ranging from 70-73%. Acute toxicity studies revealed no signs of toxicity or mortality at an oral dose of 2000 mg/kg, indicating a favorable safety profile. Antidepressant activity was assessed using the Tail Suspension Test (TST) in mice, with fluoxetine serving as the reference standard. Among the synthesized derivatives, compound 5c bearing a para-hydroxyl substituent exhibited the most pronounced antidepressant-like activity, producing the lowest immobility time (15.20 ± 0.663 s) among the test compounds and approaching the activity of fluoxetine. Compounds 5b and 5e demonstrated moderate activity, whereas compound 5d showed minimal antidepressant effect. Structure-activity relationship analysis indicated that para-substituted electron-donating groups enhanced antidepressant activity more effectively than electron-withdrawing substituents. These findings identify compound 5c as a promising lead molecule for further development of novel antidepressant agents.

Keywords: Pyrazoline, anti-depressant, tail suspension test, *in-silico* ADMET, structure activity relationship

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Introduction

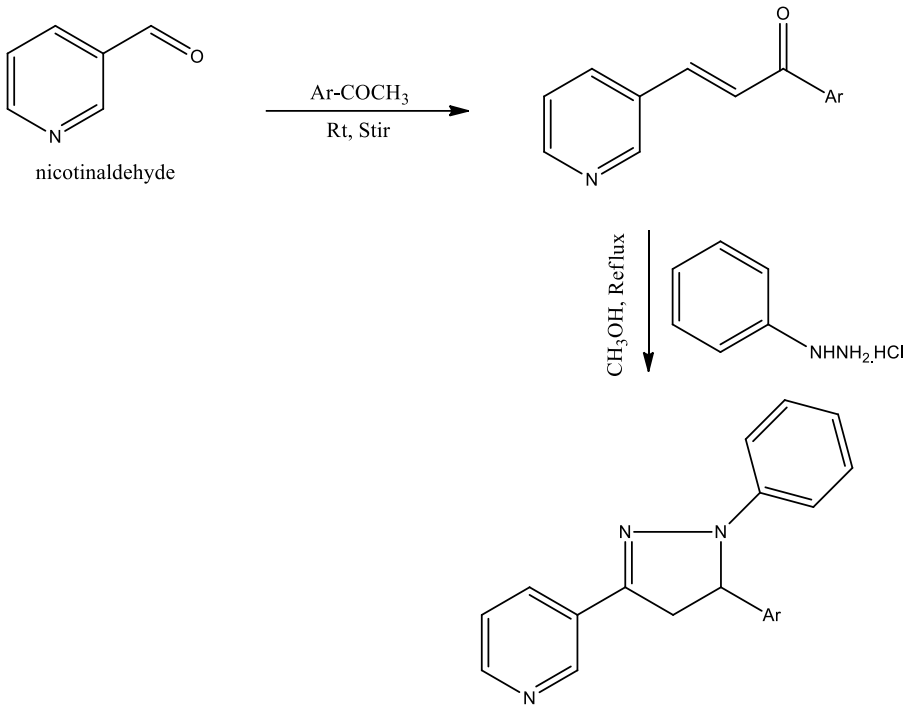
Depression is a common and debilitating psychiatric disorder characterized by persistent feelings of sadness, loss of interest, impaired cognitive function, and reduced quality of life. According to the World Health Organization (WHO), depression affects millions of people worldwide and is a leading cause of disability, contributing significantly to the global burden of disease. Despite the availability of several classes of antidepressant drugs, including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, and monoamine oxidase inhibitors, their clinical utility is often limited by delayed onset of action, incomplete therapeutic response, and numerous adverse effects. Therefore, the search for novel antidepressant agents with improved efficacy and safety profiles remains an important area of pharmaceutical research [1,2].

Pyrazoline derivatives represent an important class of nitrogen-containing heterocyclic compounds that have attracted considerable interest due to their diverse pharmacological activities. Numerous studies have demonstrated that pyrazoline analogs possess anti-inflammatory, antimicrobial, antioxidant, anticancer, anticonvulsant, analgesic, and central nervous system (CNS) activities. The presence of a pyrazoline nucleus has been associated with favorable interactions with various biological targets, making it a valuable scaffold for the development of novel therapeutic agents. Recent investigations have suggested that structural modification of pyrazoline derivatives can significantly influence their neuropharmacological properties, including antidepressant activity [3-6].

In recent years, computational techniques have become valuable tools in modern drug discovery. *In silico* evaluation of physicochemical and pharmacokinetic properties allows rapid assessment of drug-likeness, oral bioavailability, and absorption characteristics of newly synthesized molecules. These approaches help in predicting the suitability of candidate compounds for further pharmacological development while reducing time and cost associated with experimental screening. Considering the therapeutic significance of pyrazoline derivatives and the continuing need for safer and more effective antidepressant agents, the present study was undertaken to synthesize a series of novel pyridine-linked pyrazoline derivatives via chalcone intermediates.

Material and Methods

The experimental scheme was adapted from the previous reports by modification in conditions and optimized for obtaining highest yield [7,8]. The multistep reaction pathway comprised of one intermediate compound. The intermediate was reacted with various phenylhydrazine leading to the formation of the desired substituted pyrazolines (Scheme 1).



Scheme 1.

Synthesis of chalcone intermediate

A few chalcone compounds were prepared using acetophenone and substituted benzaldehydes in acetic acid and catalytic amount of sulfuric acid using Claisen-Schmidt condensation.

To a solution of nicotinaldehyde (0.0054 mol) and corresponding acetophenone (0.0054 mol) in acetic acid (10 mL) was added conc. H_2SO_4 (0.3 mL) and stirred at 10-20°C until the completion of reaction (≈72 h). The precipitate was filtered off and recrystallized from acetic acid.

Synthesis of pyrazoline derivatives

The pyrazoline compounds were prepared by cyclization reaction of phenylhydrazine with the prepared chalcone compounds. To a solution of 0.001 mol chalcone derivative in 15 mL of acetic acid was added 0.002 mol of the phenylhydrazine and refluxed for 6 hours. The reaction mixture was monitored by thin layer chromatography, then poured onto ice, washed with distilled water, filtered and crystallized from ethanol.

The synthesized compounds were observed for color, practical yield, solubility determination and determination of retention factor (R_f) value by thin layer chromatographic technique (TLC) [9-11].

Anti-depressant activity

Animal used

Mice of any sex weighing between 20-25g were used for the study. The mice were procured from the animal house of the institute and the protocol was approved by the Institutional Animal Ethical Committee. The mice were kept in cages maintained at temperature of 26°C following a 12 h light

and dark cycle. The animals were fed with mice pellet diet (Golden feeds) and had free access to water.

Acute Toxicity Study

A total of three animals were used which received a single oral dose (2000mg/kg) of each compound. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks [12].

Tail Suspension Test

The animal were grouped in seven groups of five animal each. Group 1 was administered normal saline and served as control; group 2 was administered fluoxetine (10 mg/Kg) and served as standard. Groups 3-7 were administered test compounds (25 mg/Kg). The synthesized compounds and fluoxetine were dispersed in DMSO and injected intraperitoneally (0.05 mL per 20 g body weight), 30 minutes prior to the test. To determine the effect of the test compound mice were suspended by tail using clamp (2 cm from the tip of the tail) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Minimal background noise was maintained and the test was done in dark room. The total time of keeping animals suspended by tail was 6 minutes, and the duration of inaction or immobility was noted during the final 4 minutes of the test. Animal was considered immobile when they hung completely motionless [13].

***In silico* ADMET study**

The 2D structures of the ligands were sketched on ChemDraw Ultra 12.0 and then transferred on Chem 3D to create the three-dimensional structures. The canonical SMILES (simplified molecular-input line-entry system) were generated using ChemDraw and then submitted to SwissADME online tools for the pharmcokinetic analysis, the prediction of physicochemical parameters and the drug-likeness using the Lipinski rule of five. The so-called Rule-of-five of Lipinski delineated the relationship between pharmacokinetic and physicochemical parameters [14].

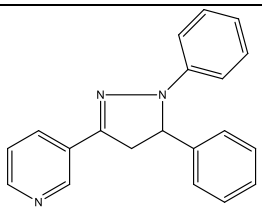
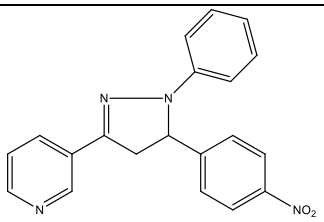
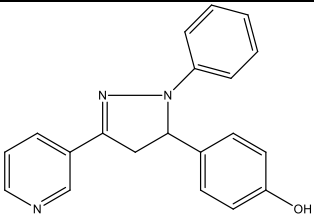
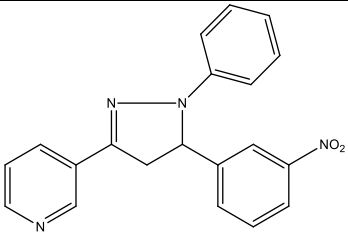
Results and Discussion

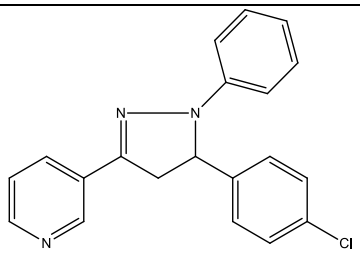
The multistep reaction pathway comprised of synthesizing one intermediate compound (chalcone). The intermediate was reacted with various acetophenones leading to the formation of the desired substituted product.

The first step involved the base catalyzed abstraction of the α-hydrogen from acetophenone, forming an enolate ion. The enolate attacks the carbonyl carbon of nicotinaldehyde, forming a β-hydroxyketone intermediate. This intermediate undergoes dehydration (loss of water) to yield an α,β-unsaturated ketone (chalcone). The chalcone's C=C bond is reduced, and the carbonyl group may undergo nucleophilic attack by the hydrazine leading to the formation of a β-amino ketone. The amine group reacts with the carbonyl carbon to form an imine intermediate. Intramolecular nucleophilic attack and dehydration lead to ring closure, forming the pyrazoline nucleus. Aromatization stabilizes the final product, a substituted pyrazoline attached to the pyridine ring.

The synthesized conjugates were characterized by determining the practical yield, melting point, solubility and spectral studies. The structure of the synthesized compounds and the physicochemical properties are presented in Table 1. The compounds were soluble in chloroform and DMSO.

Table 1. Structure and physical properties of 5a-e

Compound code	**Structure**	**Yield (%)**	**Molecular Weight**	**R_f Value**	**Melting point (°C)**
5a		71	299.37	0.46	174-176
5b		73	344.37	0.63	191-193
5c		72	315.37	0.54	169-171
5d		73	344.47	0.63	193-195

5e		70	333.81	0.61	147-149

Spectral Studies

The structural confirmation of the synthesized compounds was done by interpretation of the IR, and ^{1}H NMR spectra of the compounds. The IR spectrum is an indicator of the relevant functional groups present in the compounds, while the proton NMR spectrum indicates the number and position of the hydrogen atoms present in the molecule.

3-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine, 5a

IR (cm^{-1}): 3102.68 (C-H), 1713.59 cm^{-1} (C=O), 1453.91 cm^{-1} (C=N), 1158.84 cm^{-1} (C-N) and bending vibrations at around 812.82 cm^{-1} (C-N); ^{1}HNMR (δ ppm): 6.98-8.84 (CH, Aromatic), 5.26 (CH, pyrazoline), 3.44-3.84 (CH, methylene); Mass (m/z): 299.37 (M^+ peak)

3-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine, 5b

IR (cm^{-1}): 3093.00 cm^{-1} (N-H), 1690.78 cm^{-1} (C=O), 1478.91 (C=N), 1157.26 cm^{-1} (C-N) and bending vibrations at around 809.11 cm^{-1} (C-N); ^{1}HNMR (δ ppm): 6.98-8.84 (CH, Aromatic), 8.30 (CH, adjacent to NO_2), 5.29 (CH, pyrazoline), 3.44-3.84 (CH, methylene); Mass (m/z): 344.37 (M^+ peak)

4-(1-phenyl-3-(pyridin-3-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol, 5c

IR (cm^{-1}): 3733.02 cm^{-1} (O-H), 2935.24 cm^{-1} (C-H), 1704.43 cm^{-1} (C=O), 1461.38 (C=N), 1134.02 cm^{-1} (C-N) and bending vibrations at around 820.13 cm^{-1} (C-N); ^{1}HNMR (δ ppm): 6.98-8.84 (CH, Aromatic), 6.73 (CH, adjacent to OH), 5.29 (CH, pyrazoline), 3.45-3.84 (CH, methylene); Mass (m/z): 315.37 (M^+ peak)

3-(5-(3-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine, 5d

IR (cm^{-1}): 3036.76 cm^{-1} (C-H), 1699.89 cm^{-1} (C=O), 1480.66 (C=N), 1157.71 cm^{-1} (C-N) and bending vibrations at around 812.47 cm^{-1} (C-N); ^{1}HNMR (δ ppm): 6.98-8.84 (CH, Aromatic), 8.09-8.18 (CH, adjacent to NO_2), 5.27 (CH, pyrazoline), 3.43-3.87 (CH, methylene); Mass (m/z): 344.37 (M^+ peak)

3-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine, 5e

IR (cm^{-1}): 3103.07 cm^{-1} (C-H), 1693.08 cm^{-1} (C=O), 1141.19 cm^{-1} (C-N) and bending vibrations at around 815.82 cm^{-1} (C-N), and 683.73 (C-Cl); ^{1}HNMR (δ ppm): 6.98-8.84 (CH, Aromatic), 7.23

(CH, adjacent to Cl), 5.29 (CH, pyrazoline), 3.45-3.84 (CH, methylene); Mass (m/z): 525.50 (M^+ peak)

Acute toxicity study

None of the animals presented any symptom of toxicity or mortality and hence the dose of 2000 mg/kg was found to be safe for the animals. A $1/20^{th}$ dose (50 mg/kg) was considered for determination of antidepressant action in mice.

Anti-depressant action

The Tail Suspension Test (TST) is a widely employed behavioral paradigm for assessing antidepressant activity in rodents. The test is based on the principle that when suspended by the tail, mice initially engage in vigorous escape-oriented movements but eventually adopt an immobile posture. The duration of immobility is considered a measure of behavioral despair, and pharmacological agents with antidepressant properties typically reduce immobility time. The immobility time of various synthesized compounds is presented in table 2.

Table 2. Immobility duration by mice in TST

Group	**Immobility time (sec)[#]**
Control	29.60 ± 0.812
Standard	9.00 ± .707
5a	21.20 ± 0.800
5b	18.00 ± 0.548
5c	15.20 ± 0.663
5d	26.00 ± 0.316
5e	20.40 ± 0.400

[#]mean±SEM

The control group exhibited the highest immobility time, consistent with depressive like behavior. The standard antidepressant significantly reduced immobility, validating the sensitivity of the assay. Compound 5a showed moderate reduction compared to control, indicating baseline activity without substituent influence. Compound 5b displayed stronger activity than 5a, suggesting that the electron-withdrawing nitro group at the para position enhances antidepressant-like effect. Compound 5c produced the lowest immobility time among test compounds (15.20 sec), approaching the standard drug. The electron-donating hydroxyl group at para position likely facilitates hydrogen bonding and improves interaction with the biological target, explaining its superior activity. Compound 5d was largely inactive, with immobility close to control. This highlights that meta substitution of nitro group does not favor activity, possibly due to poor electronic resonance contribution compared to para substitution. Compound 5e showed moderate

activity, better than 5a but weaker than 5b and 5c. The chloro substituent (weakly electron-withdrawing) may contribute partially to activity but not as effectively as nitro or hydroxyl groups (Figure 1).

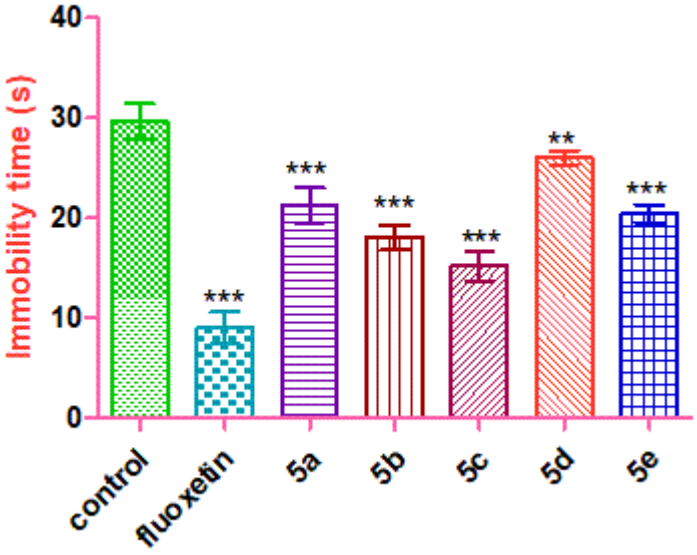


Figure 1. Anti-anxiety action of 5a-e in TST

***In silico* physicochemical properties**

The SMILES of the synthesized derivatives 5a-e was generated using chemdraw ultra 12.0 software. The physicochemical properties of the compounds predicted *in silico* by SwissADME are presented in table 3.

Table 3. Physicochemical properties of 5a-e

Compound Code	HBD	HBA	Log P	NRB	PSA (A)	MR	Log S
5a	0	2	3.70	3	28.49	100.06	-4.57
5b	0	4	3.03	4	74.31	108.8	-4.60
5c	1	3	3.27	3	48.72	102.08	-4.42
5d	0	4	3.03	4	74.31	108.88	-4.60
5e	0	2	4.30	3	28.49	105.07	-5.15

All the compounds exhibited drug likeliness and obeyed the Lipinski's rule of five. The compounds had bioavailability score of 0.55 and possessed high gastric absorption and ability to permeate the blood brain barrier

Conclusion

The present investigation successfully achieved the synthesis and characterization of a series of novel pyrazoline derivatives using chalcone intermediates. Physicochemical evaluation (melting point, solubility, TLC, IR, and NMR) confirmed the structural integrity of the synthesized compounds. In silico studies further supported their drug-likeness and favorable pharmacokinetic properties. Pharmacological evaluation through the Tail Suspension Test (TST) demonstrated that the compounds exhibited varying degrees of antidepressant-like activity, strongly influenced by the nature and position of substituents on the aromatic ring. Among the series, compound 5c (p-OH) showed the most significant reduction in immobility time, approaching the efficacy of the standard drug fluoxetine. Compounds 5b (p-NO_2) and 5e (p-Cl) displayed moderate activity, while 5a (H) showed baseline effect and 5d (m-NO_2) was largely inactive. These findings establish a clear structure–activity relationship (SAR), highlighting that para substitution enhances activity, with electron-donating hydroxyl groups being most favorable. The study thus identifies compound 5c as a promising lead for further development and mechanistic exploration in the search for safer and more effective antidepressant agents.

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