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Synthesis, *in silico* analysis and antidepressant activity of pyrazoline analogs

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ABSTRACT

Objective: This study is undertaken to design and synthesize new pyrazoline derivatives and to evaluate their antidepressant efficacy by *in silico* and *in vivo* studies. **Materials and Methods:** The title compounds pyrazoline derivatives (PY1-PY9) were yielded by the cyclization of chalcones (C1-C9) with phenylhydrazine HCl in glacial acetic acid. FT-IR, ^{1}H-NMR, and mass spectral data established the structures of the newly synthesized compounds. *In silico* analysis was carried out using Schrodinger 2018-3 suite device Maestro and docked to the binding site of Human MAO-A enzyme (2Z5X). *In vivo* antidepressant study was performed by tail suspension method and forced swimming test. **Results:** Confirmation of the probable mechanism by which the compounds exhibit neuropharmacological interactions, resulted from the interaction of the compounds to the residues of the binding sites, namely, ASP 46. This shows that the compounds have a profound affinity toward MAO-A target protein. In addition, the non-toxic nature of the compounds was established by LAZAR program. Compounds PY3 and PY2 depicted the best docking scores (–9.894 and –9.766, respectively), and subsequent *in vivo* analysis established that substantial antidepressant efficacy on comparison to the standard was demonstrated by compounds PY2 and PY8 in FST and compounds PY2 and PY3 in the TST. **Conclusion:** The data indicated that the synthesized pyrazoline derivatives, namely, 4-(1-ethyl-5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (PY8), 4-(1-ethyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (PY2) and 4-(1-ethyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (PY3) showed good antidepressive activity with potent inhibitory activity against MAO-A target protein.

Keywords: Antidepressant, chalcones, imipramine, molecular docking, pyrazolines

INTRODUCTION

Pyrazolines are five-membered heterocyclic compounds, which contain two nitrogen atoms adjacent to three atoms of carbon.[1] Substituted pyrazolines and their derivatives are active biological agents, and this class has been aimed at a large amount of research activity. The 2-pyrazoline (4,5-dihydro-1H-pyrazole, A) pharmacophore plays an imperative role in medicinal chemistry.[2] There is increasing evidence that pyrazoline derivatives have a wide range of biological activities, including cardiovascular,[3] antimicrobial,[4] anticonvulsant,[5] antidepressant,[6] anti-cholinesterase,[7] antioxidant, and anti-inflammatory.[8]

Among the various methods available to synthesize pyrazoline derivatives, condensation of chalcones with phenyl hydrazines, which yields 4,5-dihydro pyrazoline derivatives, is most commonly used.[9]

Since pyrazolines are developed as a result of cyclization of the early hydrazine derivatives such as isocarboxazid, they are considered as a scaffold to synthesize MAO-A inhibitors. This nucleus has garnered remarkable recognition due to inhibitory effects on MAO-A, which is an effective target for managing depression-like disorders.[10]

Depression is a disorder that is very familiar and has a severe disability causing mental implications with high prevalence

frequency and recurrence.[11] Various symptoms of clinical nature are employed to characterize depression, namely, loss of pleasure/interest, low self-esteem, and fatigue.[12] Homeostasis, as well as the neurotransmission of various biological amines (such as dopamine [DA], norepinephrine [NE], serotonin [5-HT], and gamma aminobutyric acid), is regulated by the enzyme-monoamine oxidase (MAO). A decrease in the level of these neurotransmitters in the brain, as a result of degradation by the MAOs enzymes, is attributed to cause various depressive mental disorders. Inhibitors of monoamine oxidase (MAOIs) are responsible for the arrest of the MAOs activities, thus leading to elevated NTs concentration in the brain.[13] Numerous antidepressant medications presently in the market, such as phenelzine and celecoxib,[14] alleviate the symptoms of depression, but involve a lengthy recovery period and have various side effects[15,16] that warrant the production of new antidepressant agents with higher efficacy and relatively low adverse effects.

Considering the various biological activities exhibited by the pyrazoline moiety and continuing the research carried out,[17-21] it was proposed to synthesize a series of pyrazoline derivatives (PY1-PY9) and to evaluate their antidepressant efficacy *in vivo*.

MATERIALS AND METHODS

Chemistry

Melting points were determined by Equiptronics digital melting point apparatus (Model EQ-730, India). FT-IR spectra were recorded with the help of KBr discs on Alpha Bruker FT-IR Infrared Spectrophotometer (Germany) (cm^{-1}). Agilent 400MR DD 2 spectrometer (USA) was employed to record ^1H NMR spectra at 400 MHz with d_6-DMSO/$CDCl_3$ as a solvent where TMS served as an internal standard. The recording of the mass spectra was carried out by Waters LC-MS/MS (USA). The progress of the reaction and purity of the synthesized compounds were examined by thin-layer chromatography (TLC), using silica gel G plates as stationary phase and Ethyl acetate:acetone (95:5) in various proportions as mobile phase (CAMAG™). High-performance liquid chromatography (HPLC) chromatograms were recorded on Shimadzu LC system (Japan). All the solvents used were of analytical grade and procured from Sigma-Aldrich and HiMedia (India).

General Procedure for the Synthesis of Pyrazoline Derivatives (PY1-PY9)

Chalcones (C1-C9) (0.01 mol) were dissolved in 25–30 ml of glacial acetic acid. To this mixture, 0.01 M of phenylhydrazine HCl was added, and the reaction mixture was refluxed for 16–18 h. It was then poured into crushed ice accompanied by vigorous stirring, followed by filtration and recrystallization with ethanol.[8] The physical data of the pyrazolines (PY1-PY9) are reported in Table 1.

In silico Analysis

Schrodinger 2018-3 suite device Maestro 11.7.012, (Ligprep, Glide XP docking, QikProp) was used for in silico analysis (Lipinski's RO5, molecular docking, ADMET properties). The synthesized compounds were docked in the groove of binding site of 2Z5X, which is the crystal structure of human monoamine oxidase A. This enzyme is anchored to the outer membrane of mitochondria and is an essential flavoenzyme that helps catalyze the deamination of xenobiotic and biogenic amines.[22,23]

Antidepressant Activity

Animals

Swiss albino mice (weighing 20–25 g) of either sex were procured from Nitte-Deemed to be university, Karnataka, a week before experimentation and acclimatized to the laboratory conditions (27°C ± 2°C under 12 h dark/light cycles). They were housed in polypropylene cages and fed with standard pellet feed and water. Ethical clearance for experimentation on animals was obtained from the IAEC (Certificate reference no: NGSMIPS/IAEC/121) before the beginning of the work.

Acute Toxicity Studies

Acute toxicity studies of pyrazolines (OECD, 2001) on female albino mice (20–25 g) were performed in standard husbandry conditions. The animals were fasted overnight before the experimentation. A single dose of pyrazoline derivatives was administered to the animals and they were observed for a period of 48 h for mortality (short term toxicity). On the basis

Table 1: Physicochemical properties of compounds (PY 1-9)

Comp	Ar-CHO	Molecular Formula	Molecular Weight	MR [cm^3/mol]	tPSA	Polarizability [Å3]	nrobs	Volume [cm^3]
PY1	3-OCH_3	$C_{22}H_{20}N_2O_2$	344.41	103.22	45.06	40.75±0.5 10^{-24}	4	1127
PY2	4-Cl	$C_{21}H_{17}ClN_2O$	348.83	100.58	35.83	39.98±0.5 10^{-24}	3	307.5
PY3	4-F	$C_{21}H_{17}FN_2O$	332.37	96.38	35.83	38.04±0.5 10^{-24}	3	298.64
PY4	3-NO_2	$C_{21}H_{17}N_3O_3$	359.38	103.55	87.64	40.63±0.5 10^{-24}	4	317.05
PY5	2-Cl	$C_{21}H_{17}ClN_2O$	348.83	100.58	35.83	39.98±0.5 10^{-24}	3	307.25
PY6	2,3-$(OCH_3)_2$	$C_{23}H_{22}N_2O_3$	374.43	110.47	54.29	42.12±0.5 10^{-24}	5	344.80
PY7	3,4-$(OCH_3)_2$	$C_{23}H_{22}N_2O_3$	374.43	110.47	54.29	42.23±0.5 10^{-24}	5	344.80
PY8	H	$C_{21}H_{18}N_2O$	314.38	95.98	35.83	40±0.5 10^{-24}	3	293.71
PY9	4-NO_2	$C_{21}H_{17}N_3O_3$	359.38	103.55	87.64	42.37±0.5 10^{-24}	4	317.05
Std (Imipramine)				90.37	6.48	35.25±0.5 10^{-24}	4	287.31

of short- term toxicity profile, the next doses were determined in accordance to OECD guidelines No.425.[24]

Forced swimming test (FST) and tail suspension test (TST) are the widely employed methods for the assessment of the antidepressant activity.[25] For each of these studies, animals are subject to inescapable, short-term pain. In FST, mice are forced to swim in a restricted space and in the later, they are hung from their tails, respectively. At first, they execute escape behaviors and then undergo an "immobile pose" representing the behavioral phenomenon of depression. The behavior of immobility is associated with depression behavior.[26,27]

FST

Imipramine 10 mg/kg was used as a standard antidepressant drug. Suspension of the synthesized compounds to be tested (100 mg/kg) and standard drug in a 1% aqueous solution of tween 80 was administered (p.o) to mice (0.5 ml/body weight). A control (1% aqueous solution of tween 80) was also maintained. The mice were individually forced to swim in an open cylindrical container (25 cm height), 10 cm diameter containing water to a height of 18 cm, and observed for 6 min. Treatment was given before 1 h of the study. The animal will show initial vigorous struggling in the first 2 min. The immobility time was recorded during the last 4 min. The following formula[16] was employed to calculate the percentage change from control [Table 5 and Figure 3a].

% Change of immobility = [(Test/control)×100)–100]

TST

In the TST, subjection of mice to an inescapable but moderately stressful situation is carried out. The absence of escape related behavior is regarded as immobility. Imipramine (10 mg/kg) has been used as the standard antidepressant drug. Suspension of the synthesized compounds to be tested (100 mg/kg) and standard drug in 1% aqueous solution of tween 80 was carried out and was then administered (p.o) to mice (0.5 ml/body weight). A control (1% aqueous solution of tween 80) was also maintained. The mice were assigned to different groups on the day of the experiment (n = 6 for each group). Thirty minutes post-administration, individually, the mice were hung using a paper adhesive tape at the height of 65 cm from the benchtop. It was placed 1cm away from the tail tip. Animals were allowed to hang for 6 min, and the immobility duration was observed during the past 5 min. The following formula was employed to calculate the percentage change from control [Table 5 and Figure 3b].[16]

% Change of immobility = [(Test/control)×100)–100]

Statistical Analysis

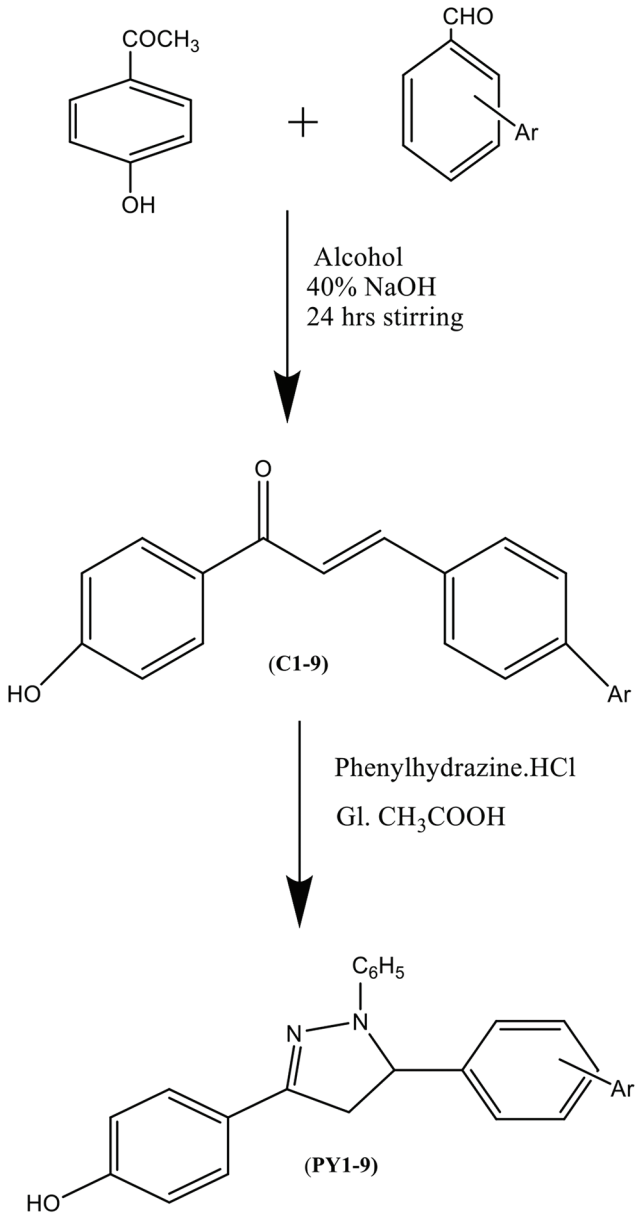
In this study, ANOVA (one-way analysis of variance) and Dunnett test were employed (GraphPad Prism 8 software) to analyze the data and compare the group differences.

RESULTS AND DISCUSSION

The present work demonstrates the synthesis of a new series of pyrazolines (PY1-PY9), where Scheme-01 outlines the synthetic route followed. Claisen Schmidt's condensation reaction was employed to prepare the key intermediate chalcones (C1-C9). The reaction between chalcones and phenylhydrazine HCl in glacial acetic acid medium finally yielded the title compounds in good yield. Table 1 lists the physicochemical features of the final synthesized compounds. The purity of the compound was confirmed through melting points and TLC using silica gel G plates as stationary phase and ethyl acetate:acetone (95:5) in various proportions as mobile phase. They were further purified by recrystallization using appropriate solvents. Based on various spectral data, all the new compounds were established for their assigned structure.

4-(5-(3-Methoxyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) phenol (PY1)

Yellow Solid. Yield: 82%, MP: 156–158°C. UV (MeOH) λmax=273 nm. HPLC t_R = 3.72 min. FT-IR (KBr, cm^{-1}): 3315 (OH), 3027 (C-H), 1607 (C=N),1508 (C=C), ^{1}H-NMR (DMSO-d_6, 400 MHz) δ:3.33 (s, OCH_3, 3H), 3.67 (dd, H_{AB}, 2H,



Scheme-01: Synthetic scheme of pyrazoline analogs

Table 2: Lipinski's RO5 and Dock scores at the active site of 2Z5X of compounds (PY1-9)

Comp	Molecular weight	Log P	Donor HB	Acceptor HB	Number of violations	Dock score
PY1	344.41	4.28	1	2.5	0	-9.981
PY2	348.83	4.97	1	1.75	0	-9.446
PY3	332.37	4.57	1	1.75	0	-9.894
PY4	359.38	3.54	1	2.75	0	-9.766
PY5	348.83	4.97	1	1.75	0	-8.911
PY6	374.43	4.15	1	3.25	0	-8.305
PY7	374.43	4.15	1	3.25	0	-9.111
PY8	314.38	4.41	1	1.75	0	-9.137
PY9	359.38	4.44	1	2.75	0	-9.475
Std (Imipramine)	280.42	4.32	0	2.5	0	-9.521

Table 3: ADME properties of compounds (PY1-9)

Comp	QlogBB	QlogKp	QPPCaco	QPlogKhsa	Percent human oral absorption
PY1	-0.167	-0.899	2550.9	1.124	100
PY2	0.058	-0.962	2549.2	1.237	100
PY3	0.006	-0.927	2550.1	1.160	100
PY4	-1.159	-2.686	308.91	1.076	100
PY5	0.028	-0.905	2554.2	1.215	100
PY6	-0.241	-0.902	2542.1	1.126	100
PY7	-0.242	-0.992	2566.7	1.137	100
PY8	-0.103	-0.794	2550.2	1.114	100
PY9	-1.169	-2.695	384.96	1.077	100
Standard (Imipramine)	0.658	-2.30	2129.9	0.735	100

Table 4: *In-silico* Toxicity prediction of compounds (PY1-9)

Comp	Toxicity prediction using LAZAR			
	Maximum recommended daily dose(mmol/kg-bw/day)	Mutagenicity	Acute toxicity LC50 (mmol/l) (Fathead Minnow)	Carcinogenic potency
Std (Imipramine)	0.0141	-	0.2	-
PY1	0.00966	+	0.269	-
PY2	0.0079	-	0.0441	-
PY3	0.00452	-	0.141	-
PY4	0.00162	+	0.0956	+
PY5	0.00425	-	0.0554	-
PY6	0.0213	-	0.645	-
PY7	0.0199	-	0.657	+
PY8	0.0232	-	0.0936	-
PY9	0.000798	+	0.119	-

J = 9.2Hz), 3.725 (H_X, dd, 1H, J= 4Hz), 6.72–7.68 (m, Ar-H, 13H), 10.34 (s, OH,1H), MS (m/z): 344.41 (M+).

4-(5-(4-Chloroyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY2)

Brown Solid. Yield: 87%, MP: 165–167°C. UV(MeOH) λmax=232 nm. HPLC t_R = 4.41 min. FT-IR (KBr, cm^{-1}): 3284 (OH), 3090 (C-H), 1592 (C=N), 1496 (C=C), 832 (C-Cl). ^{1}H-NMR (DMSO- d_6, 400 MHz) δ: 3.01 (H_A, dd, 1H, J = 23.6Hz), 3.82 (H_B, dd, 1H, J = 29.6Hz), 5.39 (H_X, dd, 1H, J = 18.4Hz) 6.64–7.92 (m, Ar-H, 13H), 9.76 (s, OH, 1H). MS (m/z): 348.83 (M+).

4-(5-(4-Fluoroyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY3)

White solid. Yield: 81%, MP: 145–147°C. UV(MeOH) λmax=258 nm. HPLC t_R = 3.72 min. FT-IR (KBr, cm^{-1}):

Table 5: Antidepressant activity of Pyrazolines by Forced Swimming Test (FST) and Tail Suspension Test (TST)

Comp[a]	FST		TST	
	Duration of immobility (s)	% change in immobility	Duration of immobility (s)	% change in immobility
PY2	123.6 ± 24.41	-51.30	139±12.19	-55.676
PY3	198.2 ±18.63	-21.91	153±30.99	-51.2117
PY4	199.2±20.89	-21.52	154.6±9.453	-50.7015
PY8	105.4±21.08	-58.47	178±14.63	-43.2398
PY9	140.8±28.22	-44.52	240±25.53***	-23.4694
Control	253.83±26.02	----	313.6±8.4	-
Imipramine	94±9.2	-62.96	120±4.13	-61.7347

[a]Values represent the mean ± S.E.M. (n= 6).

Figure 1: (a) 2D Docking interaction of PY3 with 2Z5X, (b) 3D Docking interaction of PY3 with 2Z5X

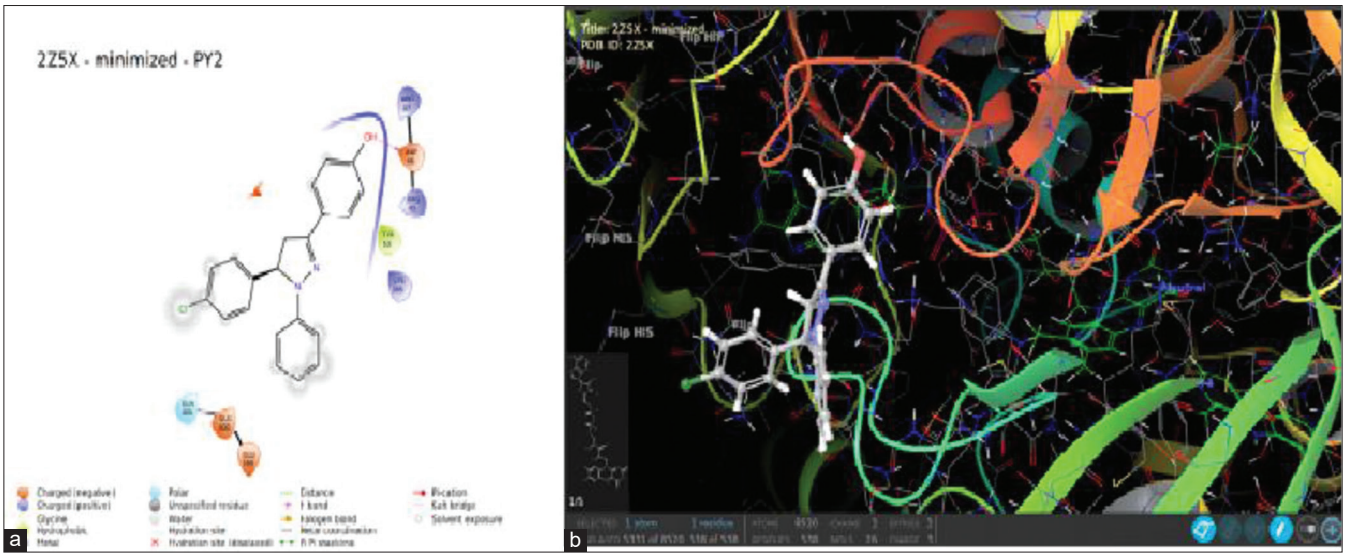


Figure 2: (a) 2D Docking interaction of PY2 with 2Z5X, (b) 3D Docking interaction of PY2 with 2Z5X

3303 (OH), 3050 (C-H), 1595 (C=N), 1494 (C=C), 834 (C-F). ^{1}H-NMR (DMSO- d_6, 400 MHz) δ: 6.75 (H_{AB}, dd, 2H, J = 15.6Hz), 6.81 (H_X, dd, 1H, J = 9.6Hz), 6.80–8.07 (m, Ar-H, 13 H), 10.40 (s, OH, 1H). MS (m/z): 352.37 (M+).

4-(5-(3-Nitroyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY4)

Orange solid. Yield: 80%, MP: 136–138°C. UV(MeOH) λmax=264 nm. HPLC t_R = 3.74 min. FT-IR (KBr, cm^{-1}): 3222

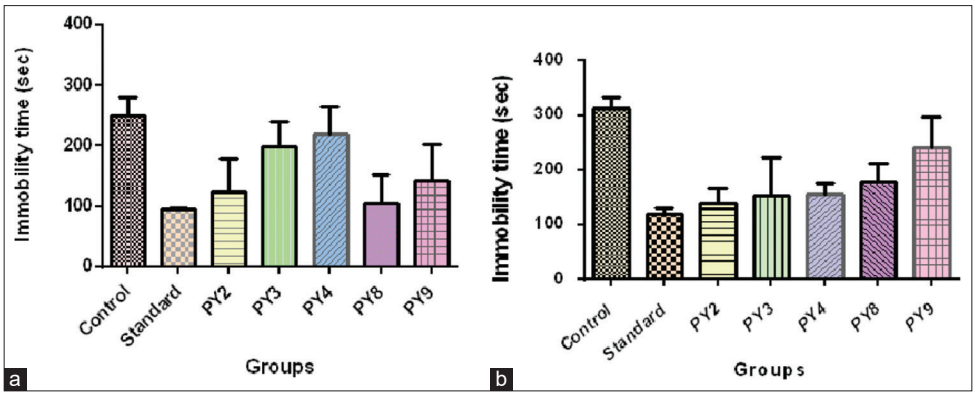


Figure 3: (a) *In vivo* anti depressant activity by forced swimming test, (b) *In vivo* Anti depressant activity by tail suspension test

(OH), 3091 (C-H), 1594(C=N), 1519 (C=C), 1348 and 1498 (NO_2). ^{1}H -NMR (DMSO- d_6, 400 MHz) δ: 6.80 (H_{ABX}, dd, 3H, J = 20Hz), 6.95–8.13 (m, Ar-H, 13H), 9.78 (s, OH, 1H). MS (m/z): 359.38(M+).

4-(5-(2-Chloroyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY5)

Brown solid. Yield: 81%, MP: 151–153°C. UV(MeOH) λmax=278 nm. HPLC t_R = 3.65 min. FT-IR (KBr, cm^{-1}): 3315 (OH), 3027 (C-H), 1595 (C=N), 1496 (C=C), 744 (C-Cl). ^{1}H-NMR (DMSO- d_6, 400 MHz) δ: 2.98(H_A, dd,1H, J = 17.2 Hz), 3.94(H_B, dd, 1H, J = 29.6Hz), 5.57 (H_X, dd, 1H, J = 18.8 Hz), 6.66–8.08 (m, Ar-H, 13H), 9.80 (s, OH, 1H). MS (m/z): 348.83(M+).

4-(5-(2,3-Dimethoxyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY6)

Orange solid. Yield: 79%, MP: 160–162°C. UV(MeOH) λmax=276 nm. HPLC t_R =3.61 min. FT-IR (KBr, cm^{-1}): 3238 (OH), 3020 (C-H), 1600 (C=N), 1505 (C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ:3.35 (s, 2XOCH_3, 6H), 3.33 (H_{AB}, dd, 2H, J = 28Hz), 3.74 (H_X, dd,1H, J = 20.4 Hz), 3.794–3.832 (m, Ar-H, 12H), 10.39 (s,OH,1H). MS (m/z): 374.43(M+).

4-(5-(3,4-Dimethoxyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY7)

White solid. Yield: 80%, MP: 124–126°C. UV(MeOH) λmax=256 nm. HPLC t_R = 3.58 min. FT-IR (KBr, cm^{-1}): 3458 (OH), 2980 (C-H), 1595 (C=N), 1510 (C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.38 (s, 2XOCH_3, 6H), 2.90 (H_A, dd, 1H, J = 15.2Hz), 6.61 (H_{BX}, dd, 2H, J = 8.4Hz), 6.64–8.48 (m, Ar-H, 12H), 9.64 (s, OH, 1H). MS (m/z): 374.43(M+).

4-(1,5-Phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY8)

Yellow solid. Yield: 85%, MP: 106–108°C. UV(MeOH) λmax=321 nm. HPLC t_R =3.48 min. FT-IR (KBr, $cm^{-1)}$: 3328 (OH), 3027 (C-H), 1595 (C=N), 1498 (C=C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 2.45 (H_{AB}, dd, 1H, J = 12.8Hz), 6.77 (H_X, dd, 2H, J = 13.2Hz), 6.81–8.064 (m, Ar-H, 14H), 10.03 (s, OH, 1H). MS (m/z): 314.38 (M+).

4-(5-(4-Nitroyphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)phenol (PY9)

Brown solid. Yield: 82%, MP: 117–119°C. UV(MeOH) λmax = 278 nm. HPLC t_R =3.11 min. FT-IR (KBr, cm^{-1}): 3315 (OH), 3065 (C-H), 1597 (C=N), 1529 (C=C), 1500 and 1346 (NO2). ^{1}H -NMR (DMSO- d_6, 400 MHz) δ: 2.49 (H_{AB}, dd, 2H, J = 17.6Hz), 6.68 (H_X, dd, 1H, J = 13.2 Hz), 6.70–8.29 (m, Ar-H, 13H), 10.30 (s, OH, 1H).MS (m/z): 359.38 (M+).

The IR spectra of the compound PY2 depicted the absorption bands at 3284 for (OH), 3090 for aromatic (CH), and 1592 (C=N), respectively. In the ^{1}H-NMR spectra of the compound, PY2 reveled the singlet signal for OH at δ 9.76 regions. The aromatic protons were observed as multiplets in the region δ 6.64–7.92. The presence of other protons in the pyrazoline moiety was observed as doublets of doublets showed in the region of δ 2.98–3.04, 3.79–3.86. and 5.37–5.42 corresponding to three protons in HA, HB, and HX pattern, respectively. The mass spectrum of compound PY2 showed molecular ion peak at M/z = 348.8 (M+), which is in agreement with the molecular formula $C_{21}H_{17}ClN_2O$.

In silico Analysis

The compounds had the desired physicochemical characteristics [Table 1] having no deviations from the standard ranges. The number of rotatable bonds is <10 for all compounds. The tPSA values of all the compounds are within the limit indicating the cell permeability.

The synthesized compounds obey Lipinski's rule of five [Table 2]. The compounds possess one hydrogen bond donor and hydrogen bond acceptors ranging from 1 (PY8) –3(PY6). The affinity of the compounds with receptor –2Z5X is given in Table 2 in terms of dock score. The synthesized compounds have binding free energy in the range of –9.894 to –8.305 kcal/mol. The active residues in 2Z5X are Arg47, Asp 46, Arg 45, Tyr 53, Lys 395, Gln 401, Glu 400, and Glu 399. Highest affinity as well as the binding energy of –9.894 kcal/mol is displayed by Compound PY2 in comparison to the other synthesized compounds. Compounds PY2 and PY3 fit into the binding cleft of the 2Z5X receptor. The hydrogen bond interactions are formed with Asp 46 in the case of PY3 [Figure 1a] and PY2 [Figure 2a]. The hydrophobic interaction between the ligand and the receptor also represents good interaction. The docking conformations of these two compounds are depicted in Figures 1b and 2b.

The ADME studies [Table 3] of the compounds helped in concluding that all the compounds possess good BBB penetration as that of the standard. QPlogKp score indicates that all the test compounds and the standard are having the skin permeability. QPlog Khsa score depicted that all the compounds bound to the human serum albumin.

Toxicity predictions using LAZAR program [Table 4] suggests that while compounds PY1, PY4, PY9, and PY10 were prone to cause mutagenicity and Compounds PY4 and PY7 carcinogenicity, the remaining synthesized compounds were not found to be liable to cause any type of carcinogenicity/mutagenicity. All the synthesized compounds were not prone to cause acute toxicity and hence can be termed as safe.

In vivo Antidepressant Activity

Among the synthesized pyrazoline derivatives, five compounds were selected and screened for *in vivo* antidepressant activity by FST and TST. The results indicate that all the tested compounds showed good activity.

In the FST model of antidepressant activity, however, 4-(1-ethyl-5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol (PY8) displayed the highest antidepressant efficacy, followed by 4-(1-ethyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol(PY2). PY8 reduced the immobility time by 58%, whereas PY2 reduced it by 51%. In the TST model, compounds PY2 and PY3 (4-(1-ethyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol) displayed the highest antidepressant efficacy with a reduction in the immobility by 55.6 and 51.2%, respectively. The data suggest that although both the models show common behavioral measure of despair, the underlying pathophysiology may be different. This can account for the moderate activity depicted by the compounds PY3 and PY8 in FST and TST, respectively.

It can be observed that an electron-withdrawing group at position 3 or 4 of the pyrazoline phenyl ring is responsible for high antidepressant activity. A parachloro/parafluoro substituent on the phenyl ring at position three of the pyrazoline ring highly elevates the antidepressant efficacy. The presence of electron-donating groups at 2, 3, or 4th position of the pyrazoline phenyl ring diminishes antidepressant efficacy.

CONCLUSION

A new series of pyrazoline derivatives were synthesized. Various spectral studies were undertaken to characterize the compounds, and toxicity results depict that nearly all the synthesized compounds were free of carcinogenicity and mutagenicity having a complementary pharmacokinetic efficacy as per the predictions by *in silico* methods. With the aid of molecular docking studies, it was established that the compounds have an imperative interaction with the target protein MAO. The new compounds were evaluated for *in vivo* antidepressant activity. Substantial antidepressant efficacy on comparison to the standard was demonstrated by compounds PY2 and PY8 in FST and compounds PY2 and PY3 by the tail-suspension method.

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REFERENCES

1. Tanwar N, Rana D, Kaur R, Singh R, Singh K. Synthesis and characterization of pyrazoline derivatives. J Integrated Sci Tech 2015;3:39-41.
2. Kankanala K, Reddy VR, Devi YP, Mangamoori LN, Rambabu D, Mukkanti K, *et al*. Nonsteroidal anti-inflammatory drug-based N-allylidene benzohydrazides and 1-Acyl-2-pyrazolines: Their synthesis as potential cytotoxic agents *in vitro*. J Heterocyc Chem 2015;52:105-13.
3. Patil PO, Belsare DP, Kosalge SB, Fursule RA. Microwave assisted synthesis and antidepressant activity of some 1, 3, 5-triphenyl-2-pyrazolines. Int J Chem Sci 2008;6:717-25.
4. Azarifar D, Shaebanzadeh M. Synthesis and characterization of new 3, 5-dinaphthyl substituted 2-pyrazolines and study of their antimicrobial activity. Molecules 2002;7:885-95.
5. Parmar SS, Pandey BR, Dwivedi C, Harbison RD. Anticonvulsant activity and monoamine oxidase inhibitory properties of 1, 3, 5-trisubstituted pyrazolines. J Pharm Sci 1974;63:1152-55.
6. Palaska E, Aytemir M, Uzbay IT, Erol D. Synthesis and antidepressant activities of some 3, 5-diphenyl-2-pyrazolines. Eur J Med Chem 2001;36:539-43.
7. Chigurupati S, Selvaraj M, Mani V, Selvarajan KK, Mohammad JI, Kaveti B, *et al*. Identification of novel acetylcholinesterase inhibitors: Indolopyrazoline derivatives and molecular docking studies. Bioorg Chem 2016;67:9-17.
8. Babu VH, Sridevi CH, Joseph A, Srinivasan KK. Synthesis and biological evaluation of some novel pyrazolines. Indian J Pharm Sci 2007;69:470.
9. Lokhande PD, Hasanzadah K. Regioselective formylation and chemoselective oxidation of 1, 3, 5-triaryl pyrazoline: Synthesis of 4-(3, 5-diaryl-1H-pyrazol-1-yl) benzaldehydes. J Chem Pharm Res 2011;3:105-12.
10. Chimenti F, Bolasco A, Manna F, Secci D, Chimenti P, Befani O, *et al*. Synthesis and selective inhibitory activity of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives against monoamine oxidase. J Med Chem 2004;47:2071-4.
11. Amrein R, Martin JR, Cameron AM. Moclobemide in patients with dementia and depression. Adv Neurol 1999;80:509-19.
12. Pletscher A. The discovery of antidepressants: A winding path. Experientia 1991;47:4-8.
13. Tripathi AC, Upadhyay S, Paliwal S, Saraf SK. N1-benzenesulfonyl-2-pyrazoline hybrids in neurological disorders: Syntheses, biological screening and computational studies. Excli J 2018;17:126-48.
14. Siddiqui N, Andalip SB, Ali R, Afzal O, Akhtar MJ, Azad B, *et al*. Antidepressant potential of nitrogen-containing heterocyclic moieties: An updated review. J Pharm Bioallied Sci 2011;3:194-212.
15. Mathew B, Suresh J, Anbazhagan S. Synthesis, preclinical evaluation and antidepressant activity of 5-substituted phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole-1-carbothioamides. Excli J 2014;13:437-45.
16. Kaplancıklı ZA, Özdemir A, Turan-Zitouni G, Altıntop MD, Can ÖD. New pyrazoline derivatives and their antidepressant activity. Eur J Med Chem 2010;45:4383-7.
17. Revanasiddappa BC, Nagendra RR, Subrahmanyam EV, Satyanarayana D. Synthesis and biological evaluation of some novel 1,3, 5-trisusbstituted pyrazolines. E J Chem 2010;7:235-9.

18. Revanasiddappa BC, Subrahmanyam EV, Lakshmi TN. Synthesis and biological evaluation of pyrazolines. Indian J Pharm Educ Res 2011;45:164-67.
19. Satyanarayana D, Revanasiddappa BC, Neema KV. Synthesis and biological evaluation of some novel pyrazolines. Indian J Het Chem 2013;22:353-6.
20. Revanasiddappa BC, Jisha MS, Saira SV, Kalsi J, Jose N. Synthesis and biological evaluation of novel 1,3,5-trisubstituted pyrazolines. Indian J Het Chem 2014;24:51-4.
21. Revanasiddappa BC, Vijay KM, Hemanth K. Synthesis, antibacterial and antifungal evaluation of novel pyrazoline derivatives. Dhaka Univ J Pharm Sci 2018;17:221-6.
22. King AM, Salome C, Salome-Grosjean E, De Ryck M, Kaminski R, Valade A, *et al*. Primary amino acid derivatives: Compounds with anticonvulsant and neuropathic pain protection activities. J Med Chem 2011;54:4815-30.
23. Maas JW. Monoamines in affective disorders. Arch Gen Psychiatry 1975;32:1159-63.
24. OECD Guideline for Testing of Chemicals. The Organization for Economic Co-operation and Development. Paris, France: OECD Guideline for Testing of Chemicals; 2005.
25. Chigurupati S, Shaikh SA, Mohammad JI, Selvarajan KK, Nemala AR, Khaw CH, *et al*. *In vitro* antioxidant and *in vivo* antidepressant activity of green synthesized azomethine derivatives of cinnamaldehyde. Indian J Pharmacol 2017;49:229.
26. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology 1985;85:367-70.
27. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327-36.