Medicinal Chemistry Research
https://doi.org/10.1007/s00044-018-2167-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Derivatives of 4,5-dihydro (*1H*) pyrazoles as possible MAO-A inhibitors in depression and anxiety disorders: synthesis, biological evaluation and molecular modeling studies

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Received: 20 January 2018 / Accepted: 9 March 2018

Abstract
A series of 1,3,5-trisubstituted-2-pyrazoline derivatives **(3a–3t)** were synthesized in appreciable yields by substituting N1 position of 2-pyrazoline nucleus with 4-nitrobenzenesulfonylchloride using conventional and microwave assisted synthetic approaches. The physicochemical and spectral characterization such as IR, Mass, ^{1}H-NMR and ^{13}C-NMR, and elemental analysis, assured the formation of proposed derivatives. Pharmacological studies revealed that compound **3d** exhibited highest antidepressant activity however, compound **3l** was found to be most effective anxiolytic agent at the tested doses (50 and 100 mg/kg b.w.), when compared to the control group. Molecular docking simulations established the possible mechanism of their neuropharmacological effects, with admirable affinity towards MAO-A protein. This was also evidenced from some of the key interactions of these compounds with the amino acid residues Ala68, Tyr69, Phe208, Tyr407 and Tyr444. Moreover, synthesized derivatives showed encouraging pharmacokinetic (ADME) and toxicological (neurotoxicity, carcinogenicity, mutagenicity, reproductive toxicity, irritancy and acute toxicity) parameters as predicted by computational programs. Some of these toxicity studies were further examined in wet laboratory by accomplishing behavioral neurotoxicity studies and acute toxicity studies as per OECD guidelines.

Key words 2-Pyrazoline · Anxiolytic · Antidepressant · Locomotor and neuromuscular coordination studies · Glide docking · FST and TST

Introduction

In the nervous system, the monoaminergic homeostasis and neurotransmission is regulated by monoamine oxidases (MAOs). Low level of certain neurotransmitters (NTs) in the brain, like-dopamine (DA), norepinephrine, serotonin (5-HT), and gamma amino butyric acid, is the main cause of depressive mental disorders. The concentration of NTs in the brain is increased by blocking the action of MOAs through monoamine oxidase inhibitors (MAOIs) (Meyer et al. 2006; Mitoma and Ito 1992). MAOIs belong to the first generation antidepressants used for decades to treat the patients suffering from high level of anxiety, atypical depression (Pletscher 1991), anergic bipolar depression and treatment resistant depression (Thase 2012), specific phobias, post-traumatic stress disorder and migraine headaches resistant to other therapies (Gareri et al. 2000). The MAO-A inhibitors are employed in the treatment of certain mental disorders such as depression and anxiety (Amrein et al. 1999). However, MAO-B inhibitors have proven their corrective value in neurodegenerative diseases (Foley et al. 2000; Youdim et al. 2006) such as Parkinson's (Cesura and Pletscher 1992) and Alzheimer's (Volz and Gleiter 1998). The initial hydrazine class of MAO inhibitors was associated with some severe adverse effects, such as liver toxicity and cheese reaction (Brown et al. 1989). These side-effects were correlated to nonselective and irreversible MAO inhibition. Pyrazoline derivatives have attracted substantial attention for years, chiefly 1,3,5-trisubstituted-2-pyrazoline pharmacophore has been associated with encouraging neurological activities such as tranquilizer, anticonvulsant, and antidepressant (Kaplancikli et al. 2010;

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Published online: 23 March 2018

Ozdemir et al. 2008; Palaska et al. 2001; Prasad et al. 2005; Ruhoglu et al. 2005), psychoanaleptic, MAO inhibitory and other biological activities (Chimenti et al. 2004, 2010; Gokhan-Kelekci et al. 2009; Gokhan et al. 2003; Jagrat et al. 2011; Jayaprakash et al. 2008; Karuppasamy et al. 2010; Maccioni et al. 2010; Manna et al. 2002; Mishra and Sasmal 2011; Sahoo et al. 2010; Tripathi et al. 2018a). The above findings prompted research to develop selective and reversible type of MAO (A and B) inhibitors. Considering these facts, some 1,3,5-trisubstituted-2-pyrazolines were synthesized by effecting some novel aromatic and hetero-aromatic substitutions at 3rd and 5th positions, respectively. Microwave facilitated synthetic technique was also utilized to prepare the proposed compounds and the results were compared to those of the conventional heating methods (Lidstrom et al. 2001). This work is in continuation to the previous study by our group (Tripathi et al. 2016; Tripathi et al. 2018b; Upadhyay et al. 2017a, b), with some novel derivatives having promising potential in the field.

Results and discussion

Synthesis of a series of fourteen 2-pyrazoline derivatives **(3a–3t)** was undertaken using conventional as well as a green chemistry approach of microwave assisted organic synthesis (MAOS) as given in Fig. 1. The MAOS showed better synthetic efficiency when compared with the conventional procedures. The synthesized derivatives were characterized by various physicochemical (Table 1) and spectral techniques. The spectral analysis data expounded C=N stretching (1509–1612 cm^{-1}), N–H stretching (3456–3105 cm^{-1}) and C–H deformation (1428–1357 cm^{-1}) along with a characteristic peak of sulfonyl group absorption bands (1340–1180 cm^{-1}) in the corresponding regions of IR spectra.

Detailed SAR studies revealed that 4-nitrobenzenesulfonyl substitution at N1 position of 2-pyrazoline nucleus was crucial in exhibiting good anti-depressant and anti-anxiety activities. It was also observed that a polar, 4-hydroxyphenyl, substitution at 3rd position and thiophen-2-yl substitution at 5th position of 2-pyrazoline nucleus was instrumental for the remarkable antidepressant potency of compound **3d**. However, when the polar 4-hydroxyphenyl substituent of the same pharmacophore was replaced by a bulky hydrophobic 1-naphthyl substituent, the spectrum of activity shifted towards anxiolytic, as shown by compound **3l** (Fig. 2). The biological activities of the synthesized compounds were

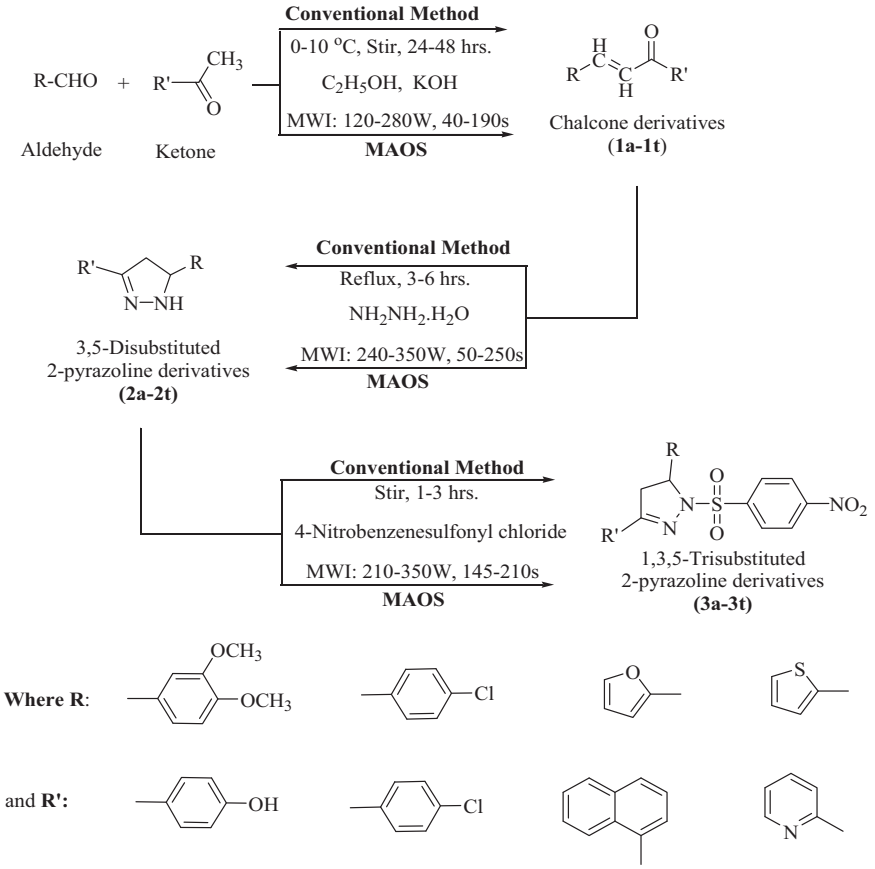


Fig. 1 Synthesis of 1,3,5-trisubstituted-2-pyrazoline derivatives

Table 1 Comparison of physicochemical properties of the synthesized 1,3,5-trisubstituted -2-pyrazoline derivatives **(3a-3t)** using conventional and microwave methods

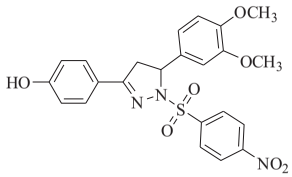
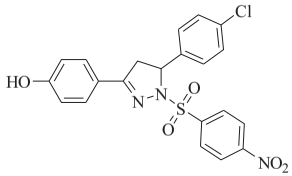
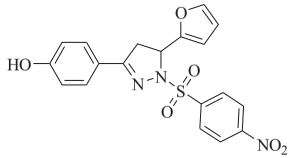
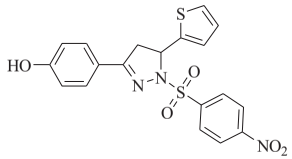
Comps.	Structure	Molecula formula	Color and state	Solubility	*R_f Value	Melting range (^{0}C)	Conventional synthesis refluxing		Microwave assisted synthesis		
							Reaction time (min)	% Yield	Microwave power (W)	Reaction time (min)	% Yield
3a		$C_{23}H_{21}N_3O_7S$	Brown color solid	Methanol, chloroform, acetone, DMSO	0.74	116–118	60	49.2	240–280	3.0	66.8
3b		$C_{21}H_{16}ClN_3O_5S$	Yellow color crystalline solid	Methanol, acetone, DMSO	0.66	168–170	60	43.9	210–280	2.4	73.2
3c		$C_{19}H_{15}N_3O_6S$	Black color solid	Methanol, acetone, DMSO	0.78	182–184	120	50.4	210–280	2.7	70.5
3d		$C_{19}H_{15}N_3O_5S_2$	Cream yellow solid	Methanol, acetone, DMSO	0.70	140–142	120	43.5	280–350	3.3	80.1

Table 1 (continued)

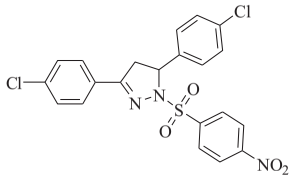
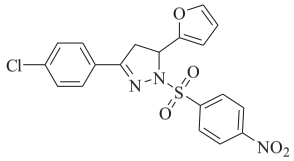
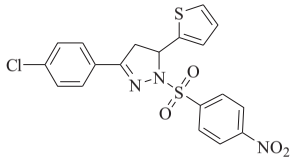
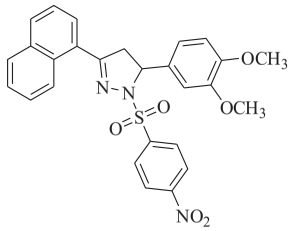
Comps.	Structure	Molecula formula	Color and state	Solubility	$^{*}R_f$ Value	Melting range (^{0}C)	Conventional synthesis refluxing		Microwave assisted synthesis		
							Reaction time (min)	% Yield	Microwave power (W)	Reaction time (min)	% Yield
3f		$C_{21}H_{15}Cl_2N_3O_4S$	Yellowish brown solid	Methanol, acetone, DMSO	0.66	150–152	120	27.88	280–350	2.5	64.6
3g		$C_{19}H_{14}ClN_3O_5S$	Black color solid	DMSO	0.58	196–198	180	61.2	240–350	3.5	81.1
3h		$C_{19}H_{14}ClN_3O_4S_2$	Yellowish grey color solid	Methanol, acetone, DMSO	0.69	152–154	180	38.5	210–350	3.1	77.6
3i		$C_{27}H_{23}N_3O_6S$	Cream yellow color solid	Methanol, acetone, DMSO	0.75	168–170	60	23.8	280	1.8	70.2

Table 1 (continued)

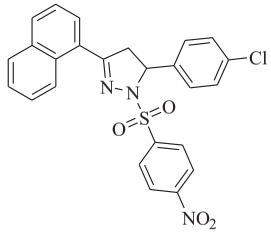
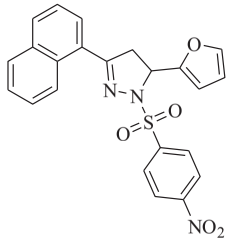
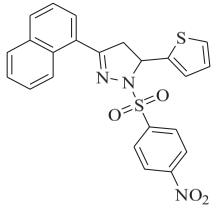
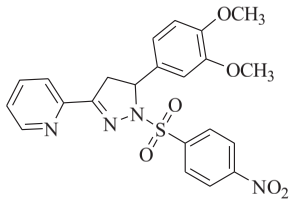
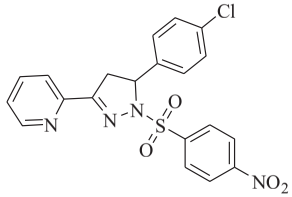
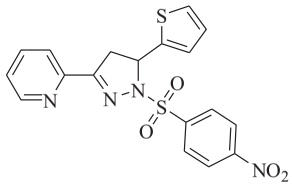
Comps.	Structure	Molecula formula	Color and state	Solubility	$^{*}R_f$ Value	Melting range (^{0}C)	Conventional synthesis refluxing		Microwave assisted synthesis		
							Reaction time (min)	% Yield	Microwave power (W)	Reaction time (min)	% Yield
3j		$C_{25}H_{18}ClN_3O_4S$	Yellowish brown solid	Acetonitrile,DMSO	0.54	140–142	90	78.2	280–350	3.0	92.5
3k		$C_{23}H_{17}N_3O_5S$	Black color solid	Acetonitrile, acetone, DMSO	0.80	132–134	150	73.8	280–350	3.5	95.1
3l		$C_{23}H_{17}N_3O_4S_2$	Brown color solid	Methanol, acetone, DMSO	0.79	158–160	120	68.2	210–280	3.2	83.4

Table 1 (continued)

Comps.	Structure	Molecula formula	Color and state	Solubility	*R_f Value	Melting range (^{0}C)	Conventional synthesis refluxing		Microwave assisted synthesis		
							Reaction time (min)	% Yield	Microwave power (W)	Reaction time (min)	% Yield
3q		$C_{22}H_{20}N_4O_6S$	Yellowish brown solid	Methanol, acetone, DMSO	0.62	132–134	180	55.9	210–280	3.3	63.2
3r		$C_{20}H_{15}ClN_4O_4S$	Brown color solid	Methanol, acetone, DMSO	0.60	180–182	180	48.3	240–350	2.9	80.4
3t		$C_{18}H_{14}N_4O_4S_2$	Brown color sticky solid	Methanol, acetone, DMSO	0.85	Sticky material	150	40.7	280–350	3.2	78.9

*R_f Values were calculated taking solvent system, chloroform (9): methanol (1)

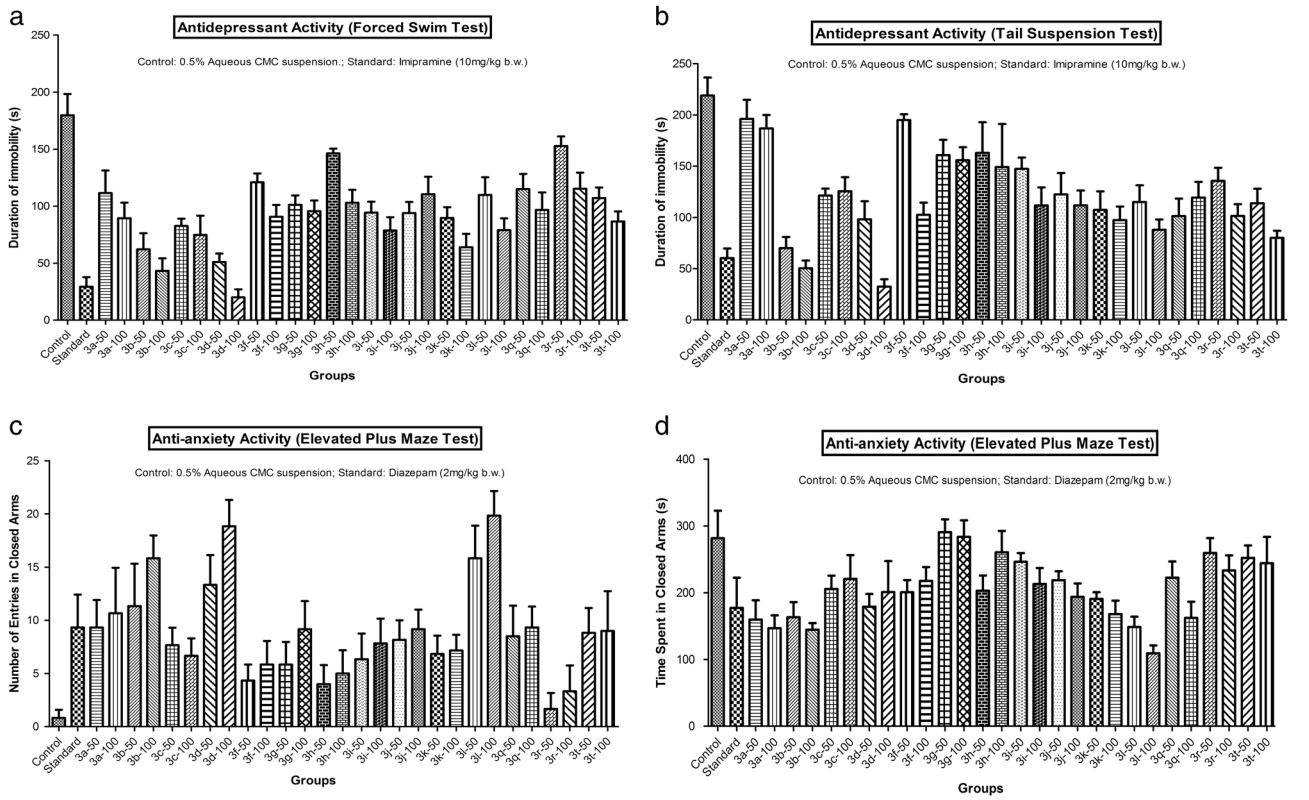


Fig. 2 **a** Graph showing antidepressant activity (Forced Swim Test); **b** Graph showing antidepressant activity (Tail Suspension Test); **c** Graph showing anti-anxiety activity (number of entries in closed arms in Elevated Plus Maze Test); **d** Graph showing anxiety activity (time spent in closed arms in Elevated Plus Maze Test)

dependent upon the doses administered, with increased effects at higher tested dose (100 mg/kg b.w.).

All the synthesized derivatives were also evaluated for their possible neurotoxicological effects, like- neuromuscular coordination and locomotor activities using rotarod and actophotometer tests, respectively. It was observed that none of the synthesized compounds showed serious neurotoxicity (disturbances in the motor co-ordinations and locomotor activity) threats at the tested doses (Table 2). Therefore, any possibility of CNS stimulating or depressing effects of the tested compounds was ruled out. Additionally, two most potent derivatives (**3d** and **3l**) were screened for their acute toxicities, at the tested doses. During these studies; behavioral pattern, changes in skin and fur, convulsions, tremors and death were not observed in any of the animals, in due course of 14 days of acute toxicity studies as per the OECD guidelines.

The results obtained by animal experimentation were further supported by the outcome of molecular docking experiments. The antidepressant and anxiolytic properties of substituted 2-pyrazoline derivatives may be attributed to their MAO-A inhibiting capabilities. The synthesized compounds were found to possess a strong affinity towards MAO-A enzyme, and may therefore be deemed as imperative targets to develop potential neuropharmacological agents. Docking studies also confirmed that the presence of sulfonyl group at N1 position of pyrazoline nucleus established a hydrogen bonding interaction between the sulfonyl oxygen of the ligands and hydroxyl hydrogen of either Tyr69 or Ala68 side chain residues. Due to these Hydrogen bonding interactions, substituted phenyl ring at C5 and benzenesulfonyl ring at N1 position of pyrazoline nucleus were well placed in the aromatic cage. The most potent compound from N1 benzenesulfonyl substituted series (**3d, 3l**) displayed additional key interactions of this moiety with side chain and backbone residues of the amino acids at the binding pocket. This included- a hydrophobic interaction of thiophene and 4-hydoxyphenyl rings with Tyr 407, Tyr444 residues, and H-bond interactions of 4-nitro and 4-hydroxyphenyl groups with the Gly443, Phe208 and Arg51 binding site residues (Figs. 3 and 4). Also, favorable in silico ADME performances (Table 3) were obtained for all the synthesized compounds. Furthermore, the tested compounds were found to be safe, by not presenting any potential risks of carcinogenicity, mutagenicity, reproductive toxicity, acute toxicity and irritancy as predicted by LAZAR and OSIRIS Property Explorer programs (Table 4). Therefore, the present study clearly demonstrated the

Table 2 Data showing antidepressant, anti-anxiety, and neurotoxicity studies of the synthesized derivatives **(3a–3t)**

Comps.	Doses (mg/kg b.w.)	Antidepressant activity[a]		Anti-anxiety activity[b]		Neurotoxicity studies			
						Rotarod test[c]		Actophotometer test[d] (mean counts)	
		Duration of immobility in FST (s) (Mean ± SD)	Duration of immobility in TST (s) (Mean ± SD)	Number of entries in closed arms (Mean ± SD)	Time spent in closed arms (s) (Mean ± SD)	Before dose	After dose (after 1 h)	Before dose	After dose (after 1 h)
3a	50	111.50 ± 19.81	196.17 ± 1865	9.33 ± 2.58	159.67 ± 29.01	0/6	0/6	292.67	277.67
	100	89.50 ± 13.55	186.83 ± 13.15	10.67 ± 4.27	146.83 ± 19.30	0/6	0/6	289.67	304.67
3b	50	62.17 ± 14.08	70.00 ± 10.86	11.33 ± 3.98	163.17 ± 22.74	0/6	0/6	283.50	282.33
	100	43.33 ± 10.98	50.33 ± 7.63	15.83 ± 2.14	144.67 ± 9.71	1/6	1/6	292.67	294.17
3c	50	82.67 ± 6.41	110.0 ± 16.12	7.67 ± 1.63	205.50 ± 20.00	0/6	0/6	287.17	279.33
	100	74.83 ± 16.82	102.67 ± 17.95	6.67 ± 1.63	220.67 ± 35.68	0/6	0/6	287.67	286.00
3d	50	51.00 ± 7.48	82.50 ± 13.16	13.33 ± 2.80	179.17 ± 19.03	0/6	1/6	285.33	307.67
	100	**20.00 ± 7.07**	**32.50 ± 7.18**	18.83 ± 2.48	200.83 ± 46.50	0/6	0/6	400.67	404.33
3f	50	121.00 ± 7.72	195.00 ± 5.73	4.33 ± 1.51	200.67 ± 18.20	0/6	0/6	390.00	395.33
	100	90.67 ± 10.44	102.50 ± 11.93	5.83 ± 2.23	217.67 ± 20.76	0/6	0/6	223.00	224.67
3g	50	101.17 ± 8.28	147.50 ± 9.18	5.83 ± 2.12	290.50 ± 19.40	0/6	0/6	289.67	294.67
	100	95.67 ± 9.29	144.50 ± 13.40	9.17 ± 2.61	283.50 ± 24.81	0/6	0/6	299.17	301.33
3h	50	146.33 ± 4.13	163.00 ± 29.91	4.00 ± 1.79	203.00 ± 22.65	0/6	1/6	292.67	281.33
	100	103.00 ± 11.40	149.17 ± 42.10	5.00 ± 2.19	260.50 ± 32.12	0/6	0/6	328.00	313.50
3i	50	88.67 ± 12.32	147.50 ± 10.93	6.33 ± 2.42	217.00 ± 30.33	0/6	0/6	390.00	373.33
	100	78.67 ± 11.67	111.67 ± 17.56	7.83 ± 2.32	213.17 ± 23.68	0/6	0/6	289.67	286.33
3j	50	94.00 ± 9.72	122.50 ± 20.91	8.17 ± 1.83	218.67 ± 13.44	1/6	0/6	283.50	249.00
	100	110.50 ± 15.24	111.67 ± 14.65	9.17 ± 1.83	193.67 ± 20.22	0/6	0/6	223.00	234.67
3k	50	89.67 ± 9.44	107.33 ± 18.10	6.83 ± 1.72	190.67 ± 10.11	0/6	0/6	229.17	233.83
	100	64.00 ± 11.75	97.33 ± 13.28	7.17 ± 1.47	168.00 ± 20.09	0/6	0/6	282.50	284.67
3l	50	96.17 ± 15.57	115.00 ± 16.37	15.83 ± 3.06	148.67 ± 15.41	1/6	0/6	289.67	297.33
	100	66.00 ± 11.87	88.00 ± 10.02	**19.83 ± 2.32**	**109.17 ± 11.91**	0/6	0/6	283.50	281.83
3q	50	115.00 ± 13.34	101.33 ± 17.01	8.50 ± 2.88	222.50 ± 24.19	0/6	2/6	292.67	298.33
	100	96.67 ± 15.44	119.33 ± 15.42	9.33 ± 1.97	162.33 ± 24.08	0/6	0/6	285.33	287.17
3r	50	152.83 ± 8.38	135.67 ± 12.75	1.67 ± 1.51	241.00 ± 13.61	0/6	0/6	283.50	279.17
	100	115.33 ± 14.12	101.50 ± 11.47	3.33 ± 2.42	233.33 ± 22.65	0/6	0/6	289.67	286.33
3t	50	107.17 ± 9.24	113.83 ± 14.18	7.67 ± 2.16	252.17 ± 18.69	1/6	1/6	283.50	280.83
	100	86.67 ± 8.78	80.00 ± 6.99	9.00 ± 3.74	244.17 ± 39.49	0/6	0/6	292.67	294.33
Control (CMC)	0.5%	179.67 ± 18.67	219.00 ± 17.56	0.83 ± 0.75	281.67 ± 41.09	0/6	0/6	410.33	394.17

Table 2 (continued)

Comps.	Doses (mg/kg b.w.)	Antidepressant activity[a]		Anti-anxiety activity[b]		Neurotoxicity studies			
						Rotarod test[c]		Actophotometer test[d] (mean counts)	
		Duration of immobility in FST (s) (Mean ± SD)	Duration of immobility in TST (s) (Mean ± SD)	Number of entries in closed arms (Mean ± SD)	Time spent in closed arms (s) (Mean ± SD)	Before dose	After dose (after 1 h)	Before dose	After dose (after 1 h)
Standard (imipramine)	10	29.33 ± 8.43	60.33 ± 9.33	–	–	0/6	1/6	399.50	404.83
Standard (diazepam)	2	–	–	9.33 ± 3.08	177.33 ± 45.18	0/6	0/6	228.00	239.67

Bold data represents the most active compounds in the series; $n = 6$ (Number of animals tested at each dose level); $p < 0.05$.

Control: carboxy methyl cellulose (CMC, 0.5% suspension). Standard: imipramine (10 mg/kg, b.w.) for antidepressant activity and diazepam (2 mg/kg, b.w.) for anti-anxiety activity.

[a]The reduction in time of immobility in FST and TST are established way to evaluate effectiveness of antidepressants.

[b]The number of entries is increased in anxiolytic agents and decrease in anxiogenic agents and the amount of time spent in closed arms is decreased in anxiolytic agents and increase in anxiogenic agents.

[c]Neurotoxicity was evaluated in rota rod test before dosing and after 1 h of dosing (number of animals exhibiting toxicity/number of animals tested).

[d]Neurotoxicity recorded in actophotometer test (counts recorded in 10 min duration).

expediency of synthesized 2-pyrazoline derivatives in neuropharmacological disorders.

Materials and methods

The chemicals and reagents were procured from Sigma Aldrich and S. D. Fine Chemicals, Mumbai, India and precoated thin layer chromatography (TLC) sheets were purchased from Merck Chemicals, India and were used as such. Solvents were of reagent grade and were purified and dried by standard procedures. Microwave assisted synthesis was performed using Raga's Scientific Microwave System (Ragatech, Pune, Maharashtra, India). Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Bruker FT-IR, ALPHA-T (Eco-ATR) spectrophotometer, (Bruker, Tech. Pvt. Ltd., USA), and the values are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance-400, FTNMR spectrometer (Bruker, Tech. Pvt. Ltd., USA) at 400 MHz and the chemical shifts are reported in parts per million (δ value), taking TMS (δ 0 ppm for ^{1}H NMR) as the internal standard. Mass spectra were recorded on Waters UPLC-TQD Mass Spectrometer instrument (Waters Corporation, USA) using LC-ESI or APCI-MS Technique. Elemental analysis was performed on Perkin Elmer-2400, Series-II Analyzer (Waltham, Massachusetts, USA).

Chemistry

Appropriately substituted aromatic/ heteroaromatic aldehydes and ketones were reacted in an alkaline medium to form the substituted chalcones (**1a**–**1t**), through Claisen-Schmidt condensation, in the first step of the reaction. This was followed by heterocyclization of substituted chalcones to 2-pyrazoline derivatives (**2a**–**2t**) using hydrazine hydrate in excess. Further, the N1 position of the 2-pyrazoline intermediates were substituted by 4-nitrobenzenesulfonylchloride (**3a**–**3t**) in the final step of the reaction, as given in Scheme **1**. Progress of the reactions was monitored by TLC on precoated silica gel G plates, using iodine vapors and UV light as the visualizing agents.

General procedure for the synthesis of chalcones (1a–1t)

Conventional synthesis To a solution of different ketones (0.01 M) and suitably substituted aldehydes (0.01 M) in ethanol (10 mL), aqueous solution of potassium hydroxide (60%) was added drop wise and with continuous stirring at 0 °C over a period of 15 min–2 h. The reaction mixture was stirred at a low temperature (0–10 °C) for about 20–36 h, with occasional shaking. After 36 h, it was poured into ice-cold water and then neutralized to pH 2 using 6 N

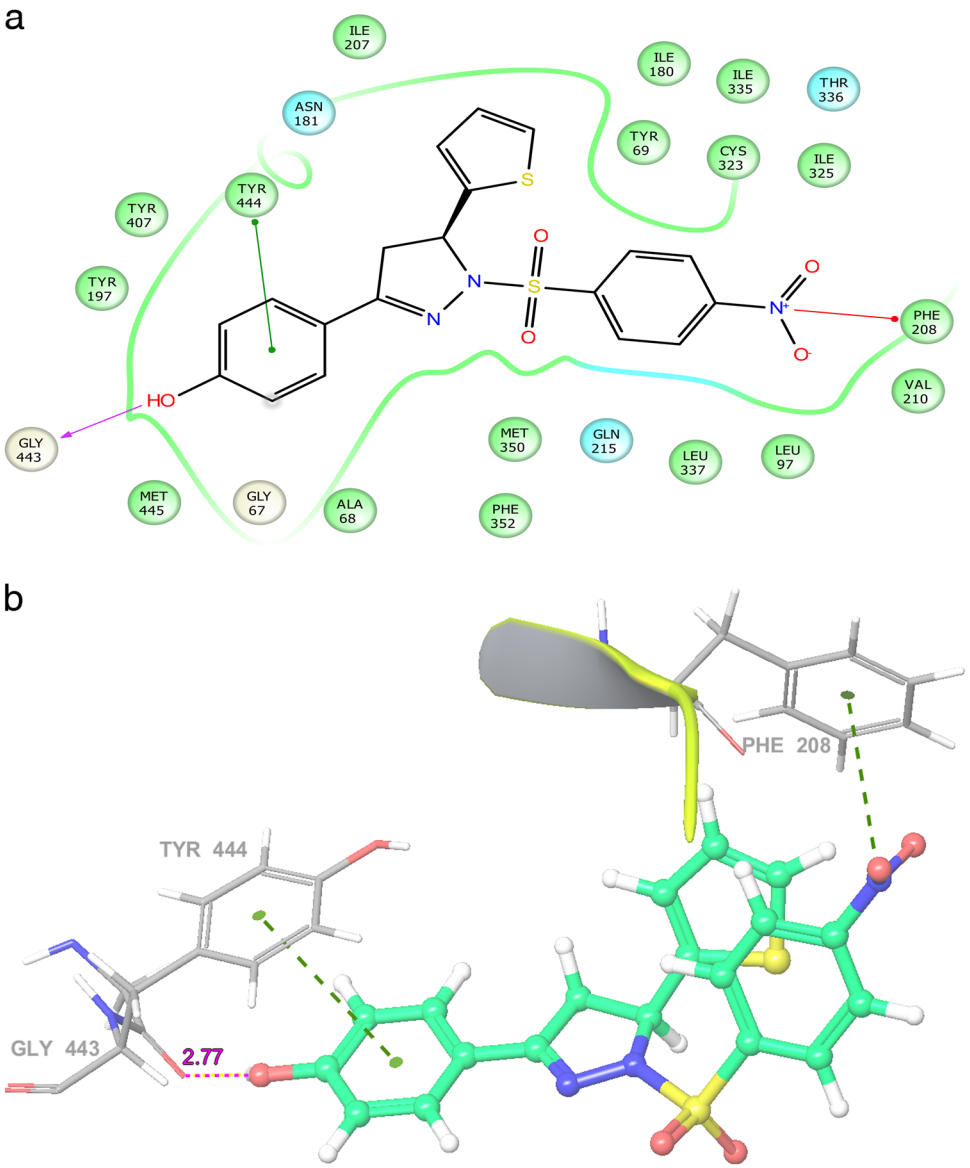


Fig. 3 Ligand receptor interaction diagram of compound **3d** at the binding site of MAO-A protein (PDB ID: 2Z5X) showing best antidepressant activity. **a** 2D Ligand receptor interaction diagram. **b** 3D Ligand receptor interaction diagram

hydrochloric acid. The yellow colored intermediates (chalcones) obtained were filtered, washed, dried, and re-crystallized from methanol (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

Microwave assisted organic synthesis (MAOS) Different aromatic/hetero-aromatic ketones (0.01 M) and suitably substituted aldehydes (0.01 M) were reacted, in the presence of hydro-alcoholic solution of KOH (60%, 10 mL), under microwave irradiation (MWI: 120–280 W, 40–190 s). The reaction mixture was poured into ice-cold water and then neutralized to pH 2 using 6 N hydrochloric acid. The yellow colored intermediates (chalcones) obtained were filtered, washed, dried, and re-crystallized from methanol (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

General procedure for the synthesis of 3,5-disubstituted-2-pyrazoline derivatives (2a–2t)

Conventional synthesis Appropriate chalcone was treated with 10 times excess of hydrazine hydrate (80%) in dry ethanol and refluxed for 3–6 h. The hot reaction mixture was then poured into ice-cold water. The separated out solid was filtered, washed, dried and re-crystallized from ethanol/acetone/ethyl acetate to afford the respective pyrazoline (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

Microwave assisted organic synthesis (MAOS) Appropriate chalcone was treated with 10 times excess of hydrazine hydrate (80%) in dry ethanol, under microwave irradiation (MWI: 240–350 W; 50–250 s). The reaction mixture was

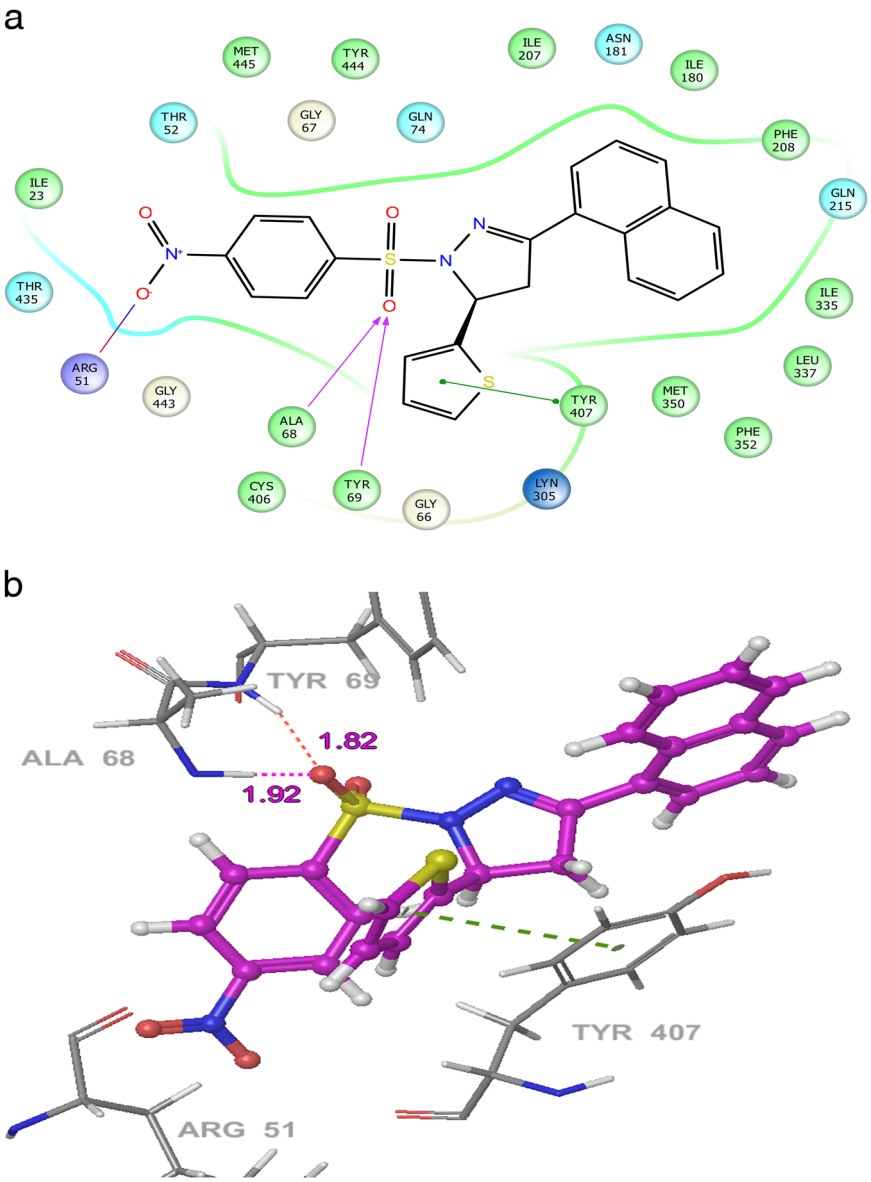


Fig. 4 Ligand receptor interaction diagram of compound **3l** at the binding site of MAO-A protein (PDB ID: 2Z5X) showing best anti-anxiety activity. **a** 2D Ligand receptor interaction diagram. **b** 3D Ligand receptor interaction diagram

then poured into ice-cold water. The separated out solid was filtered, washed, dried and re-crystallized from ethanol/ acetone/ethyl acetate to afford the respective pyrazolines (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

General procedure for the synthesis of 1,3,5-trisubstituted-2-pyrazoline derivatives (3a–3t)

Conventional synthesis Appropriately substituted 3,5-disubstituted-2-pyrazolines (0.001 M) were reacted with 4-chlorobenzenesulfonylchloride (0.002 M) by stirring, taking tetrahydrofuran (THF) (10 mL) as the solvent. Stirring was continued for 1–4 h. After the completion of reaction, the reaction mixture was poured in a petri plate and the solvent was evaporated to dryness. The crude product was re-precipitated using acetonitrile/ methanol and recrystallized from acetonitrile/ methanol to obtain the pure product (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

Microwave assisted organic synthesis (MAOS) Appropriately substituted 3,5-disubstituted-2-pyrazolines (0.001 M) were reacted with 4-chlorobenzenesulfonylchloride (0.002 M) under microwave irradiation (MWI: 210–350 W; 145–210 s), taking THF (10 mL) as the solvent. After the completion of reaction, the reaction mixture was poured in a petri plate and the solvent was evaporated to dryness. The crude product was re-precipitated using acetonitrile/ methanol and recrystallized from acetonitrile/ methanol to obtain the pure product (Tripathi et al. 2016; Upadhyay et al. 2017a, b).

Table 3 Glide gscore and in silico ADME prediction of the synthesized derivatives **(3a–3t)**

Comps.	Glide gscore	#Stars	MW	Volume	PSA	SASA	donorHB	accptHB	CNS	QPlogBB	QlogP o/w	QPlog Khsa	QPP Caco	Site of metab	% Human oral absorption	Violations of rule of five
3a	−7.26	1	483.50	1300.44	129.20	681.17	1	8.75	−2	−1.72	2.7	0.17	107.93	6	79.14	0
3b	−7.86	1	457.89	1208.70	119.70	648.93	1	7.25	−2	−1.56	2.95	0.32	84.59	4	78.69	0
3c	−7.87	1	413.40	1122.82	127.19	616.15	1	7.75	−2	−1.80	1.91	−0.01	65.67	5	70.67	0
3d	**−8.66**	**1**	**429.47**	**1157.76**	**119.53**	**637.02**	**1**	**7.25**	**−2**	**−1.75**	**2.47**	**0.18**	**67.52**	**5**	**74.16**	**0**
3f	−6.39	1	476.33	1230.17	97.16	660.65	0	6.5	−1	−0.84	3.9	0.32	278.62	3	93.54	0
3g	−8.16	1	431.85	1149.78	103.78	628.48	0	7	−2	−1.07	2.85	−0.04	219.93	4	85.55	0
3h	−6.25	1	447.91	1179.21	96.99	648.73	0	6.5	−2	−1.02	3.42	0.18	222.49	4	88.96	0
3i	−6.20	1	517.56	1416.74	106.89	735.05	0	8	−2	−1.15	4.10	0.38	402.27	5	84.62	1
3j	−7.34	1	491.95	1317.87	97.307	699.90	0	6.5	−1	−0.98	4.38	0.58	325.28	3	100	0
3k	−8.07	1	447.46	1232.15	104.81	666.57	0	7	−2	−1.23	3.28	0.20	248.95	4	89.04	0
3l	**−8.23**	**1**	**463.53**	**1300.16**	**97.15**	**720.56**	**0**	**6.5**	**−2**	**−1.42**	**4.03**	**0.53**	**185.96**	**4**	**91.16**	**0**
3q	−7.12	1	468.48	1268.12	119.20	667.26	0	9	−2	−1.28	2.4	−0.32	255.20	6	84.08	0
3r	−6.91	1	398.39	1090.56	117.0	599.20	0	8	−2	−1.33	1.64	−0.48	161.68	5	76.05	0
3t	−6.85	1	414.45	1126.30	109.38	622.82	0	7.5	−2	−1.31	2.24	−0.25	158.17	5	79.41	0
Std. values	–	0−5	130 to 725	500 to 2000	7 to 200	300 to 1000	0 to 6	2 to 20	−2 to +2	−3.0 to 1.2	−2.0 to 6.5	−1.5 to 1.5	< 25poor > 500 great	1 to 8	< 20% low, > 80% high	Maximum is 4

Bold data represents the most active compounds in the series; #stars: Number of property or descriptor values that fall outside the 95% range of similar values for known drugs; Volume: Total solvent accessible volume in cubic angstrom using a probe with 1.4 Å radius; PSA: Van der waals polar surface area of nitrogen and oxygen atoms; SASA: Total solvent accessible surface area in sqare angstrom using a probe with 1.4 Å radius; donorHB: Hydrogen bond donor; accptHB: Hydrogen bond acceptor; CNS: Predicted central nervous system activity at −2 (inactive) to +2 (active) scale; QPlogBB: Predicted brain/blood partition coefficient; QlogPo/w: Predicted octanoo-water partition coefficient; QPlogKhsa: Prediction of binding to human serum albumin; QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec; metab: Number of likely metabolic reactions; Number of violations of Lipinski's rule of five

Table 4 In silico toxicity prediction data of the synthesized derivatives **(3a–3t)**

Compounds	Toxicity prediction							
	Osiris property explorer				LAZAR			
	Mutagenic	Tumorigenic	Irritant	Reproductive effect	Maximum recommended daily dose (mmol)	Mutagenicity	Acute toxicity LC_{50} (mmol)	Carcinogenic potency
3a	No risk	No risk	No risk	No risk	1.82e + 02	Inactive	5.85e + 00	Inactive
3b	No risk	No risk	No risk	No risk	2.53e + 01	Inactive	1.42e + 01	Inactive
3c	No risk	No risk	No risk	No risk	1.19e + 02	Inactive	2.20e + 01	Active
3d	**No risk**	**No risk**	**No risk**	**No risk**	**1.24e + 02**	**Inactive**	**2.10e + 01**	**Active**
3f	No risk	No risk	No risk	No risk	2.81e + 01	Inactive	9.98e + 00	Inactive
3g	No risk	No risk	No risk	No risk	3.64e + 01	Inactive	8.42e + 00	Inactive
3h	No risk	No risk	No risk	No risk	3.78e + 01	Inactive	8.94e + 00	Inactive
3i	Medium risk	No risk	No risk	No risk	4.75e + 01	Inactive	9.88e + 00	Active
3j	Medium risk	No risk	No risk	No risk	3.90e + 01	Inactive	4.13e + 00	Active
3k	Medium risk	No risk	No risk	No risk	5.21e + 01	Inactive	1.34e + 01	Inactive
3l	**No risk**	**No risk**	**No risk**	**No risk**	**6.22e + 01**	**Inactive**	**7.71e + 00**	**Inactive**
3q	No risk	No risk	No risk	No risk	6.92e + 01	Inactive	7.23e + 00	Inactive
3r	No risk	No risk	No risk	No risk	5.19e + 01	Inactive	6.44e + 00	Inactive
3t	No risk	No risk	No risk	No risk	1.38e + 02	Inactive	5.88e + 00	Active

Bold data represents the most active compounds in the series

Characterization of the synthesized derivatives

Intermediates were characterized by TLC, melting point and mass spectra, while the final derivatives were subjected to complete physicochemical (Table 1) and spectral characterization and the values were found to be in accordance with the proposed derivatives.

4-[5-(3,4-Dimethoxy-phenyl)-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenol (3a) IR (cm^{-1}): 3220 (N–H stretch), 2861 (C–H aromatic), 1637 (C=N stretch), 1516 (C–H deform), 1160, 1350 (sym, asym $S(=O)_2$ stretch). ^1H NMR (DMSO, δ ppm): 2.31–2.62 (d, J_{ab}: 16.88 Hz, J_{ax}: 3.36 Hz, 1H, H_a), 3.63 (m, 6H), 3.71–3.89 (dd, J_{ab}: 3.74 Hz, J_{bx}: 17.12 Hz, 1H, H_b), 5.20 (s, 1H, Ar–OH), 6.29–6.48 (dd, J_{ax}: 3.61 Hz, J_{bx}: 16.23 Hz, 1H, H_x), 6.78–7.22 (m, 3H, Ar), 7.44–7.51 (m, 4H, Ar), 8.65–8.80 (m, 4H, Ar). ^{13}C NMR (ppm, DMSO): 40.12 (CH_2 pyrazoline), 42.76 (CH pyrazoline), 56.80 ($2CH_3$ methyl), 113.7 (4CH benzene), 126.4–130.2 (8CH benzene), 143.5 (5C benzene), 160.3 (C pyrazoline). MS (*m/z*): 483.49 (M^+, 100%). Anal. Calcd. for $C_{23}H_{21}N_3O_7S$: C, 57.14; H, 4.38; N, 8.69. Found: C, 57.13; H, 4.41; N, 8.73.

4-[5-(4-Chloro-phenyl)-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenol (3b) IR (cm^{-1}): 3440 (N-H stretch), 2861 (C-H aromatic), 1516 (C=N stretch), 1437 (C-H deform), 1169, 1351 (sym., asym $S(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 1.99–2.01 (dd, J_{ab}: 17.16 Hz, J_{ax}: 3.43 Hz, 1H, H_a), 3.41–3.46 (dd, J_{ab}: 4.01 Hz, J_{bx}: 16.35 Hz, 1H, H_b), 3.71–3.82 (dd, J_{ax}: 3.26 Hz, J_{bx}: 17.56 Hz, 1H, H_x), 5.39 (s, 1H, Ar-OH), 6.84–7.86 (m, 12H, Ar). ^{13}C NMR (ppm, DMSO): 39.7 (CH_2 pyrazoline), 42.5 (CH pyrazoline), 115.6–128.4 (12CH benzene), 140.3–146.5 (3C benzene), 159.3 (C pyrazoline). MS (*m/z*): 457.89 (M^+, 100%). Anal. Calcd. for $C_{21}H_{16}ClN_3O_5S$: C, 56.38; H, 3.61; N, 6.26. Found: C, 55.56; H, 3.64; N, 6.30.

4-[5-Furan-2-yl-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenol (3c) IR (cm^{-1}): 3486 (N-H stretch), 3185 (C-H aromatic), 1641 (C=N stretch), 1525 (C-H deform), 1165, 1347 (sym., asym $S(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 2.12–2.14 (dd, J_{ab}: 17.08 Hz, J_{ax}: 3.17 Hz, 1H, H_a), 3.33–3.37 (dd, J_{ab}: 3.86 Hz, J_{bx}: 16.65 Hz, 1H, H_b), 3.40–3.82 (dd, J_{ax}: 3.10 Hz, J_{bx}: 17.06 Hz, 1H, H_x), 5.23 (s, 1H, Ar-OH), 6.11–7.45 (m, 11H, Ar). ^{13}C NMR (DMSO, ppm): 37.8 (CH_2 pyrazoline), 42.5 (CH pyrazoline), 106.2 (CH, furan), 114.7 (2CH, benzene), 123.6–130.4 (6CH benzene), 145.3–154.8 (4C benzene), 159.4 (C furan), 160.8 (C pyrazoline). MS (*m/z*): 413.40 (M^{+1}, 80%). Anal. Calcd. for $C_{19}H_{15}N_3O_6S$: C, 55.20; H, 3.66; N, 10.16. Found: C, 54.92; H, 4.17; N, 9.85.

4-[1-(4-Nitro-benzenesulfonyl)-5-thiophen-2-yl-4,5-dihydro-1H-pyrazol-3-yl]-phenol (3d) IR (cm^{-1}): 3268 (N-H stretch), 2876 (C-H aromatic), 1566 (C=N stretch), 1434 (C-H deform), 1212, 1352 (sym., asym $S(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 1.99–2.01 (dd, J_{ab}: 15.74 Hz, J_{ax}:

3.85 Hz, 1H, H_a), 3.40–3.46 (dd, J_{ab}: 3.95 Hz, J_{bx}: 16.69 Hz, 1H, H_b), 3.21–3.84 (dd, J_{ax}: 3.27 Hz, J_{bx}: 17.44 Hz, 1H, H_x), 5.46 (s, 1H, Ar-OH), 6.73–7.81 (m, 11H, Ar). ^{13}C NMR (ppm, DMSO): 39.7 (CH_2 pyrazoline), 43.9 (CH pyrazoline), 113.8–128.6 (9CH benzene), 122.5–126.2 (3CH thiophene), 138.4–151.7 (3C benzene), 161.0 (C thiophene), 162.7 (C pyrazoline). MS (*m/z*): 429.47 (M^{+1}, 80%). Anal. Calcd. for $C_{19}H_{15}N_3O_5S_2$: C, 53.14; H, 3.52; N, 9.78. Found: C, 53.17; H, 3.48; N, 9.75.

3,5-Bis-(4-chloro-phenyl)-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazole (3f) IR (cm^{-1}): 3356 (N-H stretch), 2992 (C-H Aromatic), 1522 (C=N stretch), 1457 (C-H deform), 1275, 1353 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 1.90–1.94 (dd, J_{ab}: 17.49 Hz, J_{ax}: 3.64 Hz, 1H, H_a), 3.27–3.40 (dd, J_{ab}: 4.04 Hz, J_{bx}: 17.24 Hz, 1H, H_b), 3.63–3.80 (dd, J_{ax}: 3.77 Hz, J_{bx}: 16.59 Hz, 1H, H_x), 7.05–7.74 (m, 12H, Ar). ^{13}C NMR (ppm, DMSO,): 39.4 (CH_2 pyrazoline), 42.7 (CH pyrazoline), 124.5–130.2 (12CH benzene), 134.7–141.3 (4C benzene), 160.7 (C pyrazoline). MS (*m/z*): 476.33 (M^+, 95%). Anal. Calcd. for $C_{21}H_{15}Cl_2N_3O_4S$: C, 52.95; H, 3.17; N, 8.82. Found: C, 53.01; H, 3.15; N, 8.83.

3-(4-Chloro-phenyl)-5-furan-2-yl-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazole (3g) IR (cm^{-1}): 3156 (N-H stretch), 3100 (C-H Aromatic), 1588 (C=N stretch), 1399 (C-H deform), 1166, 1345 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 2.08–2.13 (dd, J_{ab}: 15.31 Hz, J_{ax}: 2.98 Hz, 1H, H_a), 3.39–3.44 (dd, J_{ab}: 4.16 Hz, J_{bx}: 15.57 Hz, 1H, H_b), 3.61–3.79 (dd, J_{ax}: 3.58 Hz, J_{bx}: 16.95 Hz, 1H, H_x), 6.53–7.60 (m, 11H, Ar). ^{13}C NMR (DMSO, ppm): 38.9 (CH_2 pyrazoline), 42.5 (CH pyrazoline), 104.5–112.8 (2CH furan), 113.7–129.5 (8CH benzene), 139.2–136.8 (3C benzene), 159.2 (C furan), 161.7 (C pyrazoline). MS (*m/z*): 431.85 (M^{+1}, 70%). Anal. Calcd. for $C_{19}H_{14}ClN_3O_5S$: C, 52.84; H, 3.27; N, 9.73. Found: C, 52.82; H, 3.30; N, 9.75.

3-(4-Chloro-phenyl)-1-(4-nitro-benzenesulfonyl)-5-thiophen-2-yl-4,5-dihydro-1H-pyrazole (3h) IR (cm^{-1}): 3482 (N-H stretch), 3101 (C-H aromatic), 1614 (C=N stretch), 1398 (C-H deform), 1168, 1347 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 2.11–2.18 (dd, J_{ab}: 16.83 Hz, J_{ax}: 3.27 Hz, 1H, H_a), 4.13–4.20 (dd, J_{ab}: 4.11 Hz, J_{bx}: 16.58 Hz, 1H, H_b), 4.20–4.23 (dd, J_{ax}: 3.66 Hz, J_{bx}: 16.85 Hz, 1H, H_x), 6.66–7.79 (m, 11H, Ar). ^{13}C NMR (ppm, DMSO): 39.7 (CH_2 pyrazoline), 41.3 (CH pyrazoline), 121.4–136.5 (8CH benzene), 124.2–127.5 (3CH thiophene), 128.6–143.1 (4C benzene), 139.6 (C thiophene), 156.8 (C pyrazoline). MS (*m/z*): 447.92 (M^{+1}, 100%). Anal. Calcd. for $C_{19}H_{14}ClN_3O_4S_2$: C, 50.95; H, 3.15; N, 9.38. Found: C, 51.01; H, 3.18; N, 9.42.

5-(3,4-Dimethoxy-phenyl)-3-naphthalen-1-yl-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazole (3i) IR (cm^{-1}): 3345 (N-H stretch), 2916 (C-H aromatic), 1594 (C=N stretch), 1456 (C-H deform), 1167, 1350 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 1.97–1.98 (dd, J_{ab}: 17.22 Hz, J_{ax}: 3.52 Hz, 1H, H_a), 3.39–3.47 (dd, J_{ab}: 3.87 Hz, J_{bx}: 16.73 Hz, 1H, H_b), 3.54–3.69 (dd, J_{ax}: 3.18 Hz, J_{bx}: 17.26 Hz, 1H, H_x), 3.80–3.94 (m, 6H, methyl), 6.78–7.64 (m, 14H, Ar). ^{13}C NMR (DMSO, ppm): 39.5 (CH_2 pyrazoline), 42.8 (CH pyrazoline), 61.7 ($2CH_3$), 113.7–132.5 (14CH benzene, 131.8–147.2 (8C benzene), 159.3 (C pyrazoline). MS (*m/z*): 517.55 (M^{+1}, 80%). Anal. Calcd. for $C_{27}H_{23}N_3O_6S$: C, 62.66; H, 4.48; N, 8.12. Found: C, 62.70; H, 4.51; N, 8.13.

5-(4-Chloro-phenyl)-3-naphthalen-1-yl-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazole (3j) IR (cm^{-1}): 3260 (N-H stretch), 2992 (C-H aromatic), 1565 (C=N stretch), 1514 (C-H deform), 1169, 1345 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 2.15–2.19 (dd, J_{ab}: 16.68 Hz, J_{ax}: 3.27 Hz, 1H, H_a), 3.83–3.88 (dd, J_{ab}: 3.95 Hz, J_{bx}: 17.10 Hz, 1H, H_b), 3.50–3.67 (dd, J_{ax}: 4.12 Hz, J_{bx}: 16.97 Hz, 1H, H_x), 6.90–7.73 (m, 14H, Ar). ^{13}C NMR (ppm, DMSO): 39.2 (CH_2 pyrazoline), 46.4 (CH pyrazoline), 124.2–129.0 (15CH benzene), 129.5–136.1 (7C benzene), 158.7 (C pyrazoline). MS (*m/z*): 491.95 (M^+, 100%). Anal. Calcd. for $C_{25}H_{18}ClN_3O_4S$: C, 61.04; H, 3.69; N, 8.54. Found: C, 60.98; H, 3.71; N, 8.50.

5-Furan-2-yl-3-naphthalen-1-yl-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazole (3k) IR (cm^{-1}): 3312 (N-H stretch), 3109 (C-H aromatic), 1598 (C=N stretch), 1523 (C-H deform), 1170, 1351 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 2.48–2.51 (dd, J_{ab}: 17.12 Hz, J_{ax}: 3.41 Hz, 1H, H_a), 3.04–3.89 (dd, J_{ab}: 4.01 Hz, J_{bx}: 16.23 Hz, 1H, H_b), 4.88–5.21 (dd, J_{ax}: 3.28 Hz, J_{bx}: 17.46 Hz, 1H, H_x), 6.80–6.93 (d, 2H, Ar), 7.43–7.54 (m, 4H, Ar), 7.66–7.85 (m, 7H, Ar). ^{13}C NMR (DMSO, ppm): 50.7 (CH_2 pyrazoline), 77.2 (CH pyrazoline), 124.4–126.9 (2CH furan), 129.0–138.2 (11CH benzene), 133.7–145.2 (5C benzene), 159.4 (C pyrazoline). MS (*m/z*): 447.46 (M^+, 90%). Anal. Calcd. for $C_{23}H_{17}N_3O_5S$: C, 61.74; H, 3.83; N, 9.39. Found: C, 61.71; H, 3.80; N, 9.33.

3-Naphthalen-1-yl-1-(4-nitro-benzenesulfonyl)-5-thiophen-2-yl-4,5-dihydro-1H-pyrazole (3l) IR (cm^{-1}): 3292 (N-H stretch), 3047 (C-H aromatic), 1580 (C=N stretch), 1513 (C-H deform), 1176, 1352 (sym., asym S(=O)$_2$ stretch). ^{1}H NMR (δ ppm, DMSO): 2.08–2.09 (dd, J_{ab}: 17.48 Hz, J_{ax}: 2.99 Hz, 1H, H_a), 3.10–3.18 (dd, J_{ab}: 3.74 Hz, J_{bx}: 17.04 Hz, 1H, H_b), 3.83–3.86 (dd, J_{ax}: 3.26 Hz, J_{bx}: 16.36 Hz, 1H, H_x), 6.60–7.92 (m, 14H, Ar). ^{13}C NMR (ppm, DMSO): 40.6 (CH_2 pyrazoline), 46.1 (CH pyrazoline), 122.5–129.2

(11CH benzene), 123.4–125.8 (3CH thiophene), 137.8–148.1 (6C benzene), 161.5 (C pyrazoline). MS (*m/z*): 463.53 (M^+, 95%). Anal. Calcd. for $C_{23}H_{17}N_3O_4S_2$: C, 59.60; H, 3.70; N, 9.87. Found: C, 59.57; H, 3.75; N, 9.83.

2-[5-(3,4-Dimethoxy-phenyl)-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-pyridine (3q) IR (cm^{-1}): 3406 (N-H stretch), 3093 (C-H aromatic), 1608 (C=N stretch), 1513 (C-H deform), 1174, 1344 (sym., asym S $(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 1.89–1.91 (dd, J_{ab}: 15.62 Hz, J_{ax}: 3.17 Hz, 1H, H_a), 3.33–3.39 (dd, J_{ab}: 3.74 Hz, J_{bx}: 17.11 Hz, 1H, H_b), 3.65–3.80 (dd, J_{ax}: 3.54 Hz, J_{bx}: 16.91 Hz, 1H, H_x), 3.73–3.94 (m, 6H, methyl), 6.97–8.21 (m, 11H, Ar). ^{13}C NMR (ppm, DMSO): 40.2 (CH_2 pyrazoline), 45.7 (CH pyrazoline), 49.5 (6C, CH_3), 114.9–137.2 (11CH benzene), 143.4–152.8 (6C benzene), 161.9 (C pyrazoline). MS (*m/z*): 468.48 (M^+, 80%). Anal. Calcd. for $C_{22}H_{20}N_4O_6S$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.39; H, 4.32; N, 12.03.

2-[5-(4-Chloro-phenyl)-1-(4-nitro-benzenesulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]-pyridine (3r) IR (cm^{-1}): 3420 (N-H stretch), 3069 (C-H aromatic), 1621 (C=N stretch), 1453 (C-H deform), 1175, 1343 (sym., asym $S(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 1.91–1.94 (dd, J_{ab}: 17.36 Hz, J_{ax}: 3.21 Hz, 1H, H_a), 3.64–3.48 (dd, J_{ab}: 4.12 Hz, J_{bx}: 17.44 Hz, 1H, H_b), 3.69–3.85 (dd, J_{ax}: 3.28 Hz, J_{bx}: 16.55 Hz, 1H, H_x), 7.29–8.69 (m, 12H, Ar). ^{13}C NMR (ppm, DMSO): 40.2 (CH_2 pyrazoline), 44.5 (CH pyrazoline), 124.6–149.2 (12CH benzene), 137.1–146.4 (4C benzene), 154.2 (C pyridine), 160.7 (C pyrazoline). MS (*m/z*): 442.88 (M^+, 100%). Anal. Calcd. for $C_{20}H_{15}ClN_4O_4S$: C, 54.24; H, 3.41; N, 12.65. Found: C, 54.22; H, 3.45; N, 12.67.

2-[1-(4-Nitro-benzenesulfonyl)-5-thiophen-2-yl-4,5-dihydro-1H-pyrazol-3-yl]-pyridine (3t) IR (cm^{-1}): 3493 (N-H stretch), 3116 (C-H aromatic), 1519 (C=N stretch), 1453 (C-H deform), 1164, 1344 (sym., asym $S(=O)_2$ stretch). ^1H NMR (δ ppm, DMSO): 1.90–1.93 (dd, J_{ab}: 17.23 Hz, J_{ax}: 3.32 Hz, 1H, H_a), 3.46–3.50 (dd, J_{ab}: 3.87 Hz, J_{bx}: 16.65 Hz, 1H, H_b), 3.82–3.89 (dd, J_{ax}: 3.14 Hz, J_{bx}: 16.91 Hz, 1H, H_x), 6.53–8.97 (m, 11H, Ar). ^{13}C NMR (ppm, DMSO): 38.6 (CH_2 pyrazoline), 43.2 (C pyrazoline), 115.6–128.3 (4CH benzene), 124.8–127.0 (7CH thiophene, pyridine), 140.5 (C thiophene), 144.9–151.1 (2C benzene), 153.4 (C pyridine), 161.3 (C pyrazoline). MS (*m/z*): 414.46 (M^+, 100%). Anal. Calcd. for $C_{18}H_{14}N_4O_4S_2$: C, 52.16; H, 3.40; N, 13.52. Found: C, 52.18; H, 3.44; N, 13.49.

Biological evaluation

Study animals

Animals were procured from the Animal House, Faculty of Pharmacy, BBDNIIT, Lucknow, U.P., India and housed in polypropylene cages with steel net, in a temperature controlled room under standard living conditions of; 26 ± 2 °C and relative humidity of; 55 ± 5% with regular 12 h light and 12 h dark cycles and allowed free access to standard laboratory food and water. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC). The biological activity studies were approved by the IAEC with the protocol number BBDNIIT/IAEC/009/2014.

Study design

For the study, animals were divided into 16 groups, each group consisting of 6 animals ($n = 6$). All the mice were treated at respective doses according to body weight. Treatments including standard/vehicle/test compounds were given at 1 mL/100 g body weight per oral (p.o.). Imipramine (10 mg/kg body weight) for antidepressant activity, and Diazepam (2 mg/kg body weight) for anti-anxiety activity were respectively taken as the standard drugs. Carboxy methyl cellulose (0.5% CMC solution) was taken as the vehicle (control group). The treatment groups and respective doses are given in Table 2.

Antidepressant activity

The antidepressant activity of the test compounds was evaluated by Porsolt's behavioral despair or forced swim test (FST) and Tail suspension test (TST) in mice. These behavioral tests are used to evaluate the efficacy of antidepressant treatments and in predicting the activity of a wide variety of antidepressants, such as MAO inhibitors and atypical antidepressants (Porsolt 1981). They have good predictive value for the assessment of antidepressant potency in humans (Willner and Mitchell 2002).

Porsolt's behavioral despair or FST in mice

The synthesized compounds were screened for their antidepressant activity using Porsolt's behavioral despair test, i.e, FST. It has been proposed that mice or rats are induced to a characteristic behavior of immobility when they are forced to swim in a restricted space from where they cannot escape. In this test, the mice were plunged individually into a Plexiglas cylinder containing water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. When mice start floating in water in an

upright position with slight movements in order to prevent sinking, they were considered immobile and the duration of immobility was recorded during the last 4 min of the 6 min test. This behavior reflects a state of despair, which can be reduced by several agents, which are therapeutically effective in human depression. Duration of immobility was measured in control and animals treated with various doses of a test drug or standard (Porsolt et al. 1977; Vogel 2002).

TST in mice

Another facile method to evaluate antidepressant potential is TST. It was designed in such a way so that a mouse is suspended by the tail, with the help of a lever, and the movements of the animal are recorded. The complete duration of the test (6 min) can be divided into periods of agitation and immobility. The rodents show the state of immobility, when exposed to an inescapable stress, and this has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. When the mice are suspended by the tail, the state of immobility displayed by the mice after active and unsuccessful attempts to escape is reduced by antidepressants. The percentage of animals showing the passive behavior is counted and compared with the vehicle treated controls. The duration of immobility, in conjunction with measurement of locomotor activity in different conditions, separates the locomotor stimulant doses from antidepressant doses. This procedure is simple, uses objective test situations and the results are in accordance with the validated "behavioral despair" test from Porsolt and the sensitivity to a wide range of drug doses (Steru et al. 1985; Vogel 2002).

Anti-anxiety activity

The maze model in mice is widely used for the evaluation of anti-anxiety activity. Out of the many possibilities to modify maze tests, water maze, the Y-maze, the radial maze, and the elevated plus maze have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time and decrease the time spent in closed arm; anxiogenic compounds have the opposite effect. The values of the treated groups are registered and expressed as percentage of controls. Benzodiazepines and valproates decrease motor activity and increase open arm exploratory time (Vogel 2002).

Elevated plus-maze test

The elevated plus-maze is used to determine the mouse's unconditioned response to a potentially dangerous environment and the anxiety-related behavior is measured by the degree to which the mouse avoids the unenclosed arms of the maze. It is a standard test of fear and anxiety for which the animal was placed in the center of an elevated 4-arm maze, in which 2 arms are open ($50 \times 10 \times 40$ cm) and 2 arms are closed ($50 \times 10 \times 40$ cm), with an open roof. The two open arms were opposite to each other. The maze was elevated to a height of 50 cm. 1 h after oral administration of the standard drug (Diazepam at a dose of 2 mg/kg b.w.), test compound (at doses; 50 and 100 mg/kg b.w.) and control (0.5% aqueous CMC suspension), the mice were placed in the center of the maze, facing one of the closed arms. During a 6 min test period, the following observations were recorded: time spent in the closed arms and total number of the arm entries (Lister 1987; Pellow et al. 1985).

Neurotoxicity study

Neurotoxicity studies are used to evaluate the effect(s) of a test substance on the central nervous system (CNS). Several behavioral studies (such as; rotarod test, open-field/actophotometer test, turning on flat surface, and turning on inclined plane) are employed to identify potential neurotoxicity in mice model. Two most commonly used methods, i.e., rotarod and actophotometer tests, are used to identify the effect of chemical compound (test compound) on neuromuscular coordination and locomotor activities, respectively (Parasuraman 2011).

Neuromuscular coordination study (rotarod test)

The rota rod test is used to evaluate the activity of drugs interfering with motor coordination, i.e. neurotoxicity. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. Male mice, with a weight between 20 and 30 g, undergo a pretest on the apparatus. Only those animals which have demonstrated their ability to remain on the revolving rod for at least 1 min are used for the test. The test compounds are administered intraperitoneally or orally. 1 h after the oral administration of the test compounds/control, the mice are placed for 1 min on the rotating rod. Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min at the tested doses. The number of animals falling from the roller during this time is counted (qualitative test) or one can quantify the values in terms of mean fall off time. The dose which impairs the ability of 50% of the mice to remain on the revolving rod is considered as the endpoint (Bhandari et al. 2013; Vogel 2002).

Locomotor activity (actophotometer/open field test)

Most of the CNS acting drugs influence the locomotor activities in humans and animals; therefore it is used as an index of wakefulness (alertness) of mental activity. The CNS depressant drugs, such as barbiturates and alcohol, reduce the motor activity while the stimulants such as caffeine and amphetamines increase the activity. To rule out these effects of tested compounds on immobility period in FST and TST, the horizontal locomotor activity of control and test animals were recorded for a period of 10 min using Medicraft Actophotometer (Model No. 600-4D, INCO, Ambala, India). The apparatus consists of a square arena ($30 \times 30 \times 25$ cm) with wire mesh bottom, in which an animal moves. The actophotometer operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded (Dhingra and Goyal 2008).

Acute toxicity study

Acute toxicity testing is carried out to determine the effect of a single dose on a particular animal species. In the present study, acute oral toxicity (LD_{50}) of the two most active final derivatives (**3d** and **3l**) was performed as per guidelines laid by Organization for Economic Co-operation and Development (OECD) guideline No 423 "Acute Oral Toxicity-Acute Toxic Class Method". The compounds were administered orally at four fixed dose levels (5, 50, 300, and 2000 mg/kg body weights), after 4 h of fasting. After dose administration, food but not water was withheld for 2 h. The body weight of all the mice were recorded on study days- D_0 (initiation), D_1, D_7 and D_{14}. The animals were observed for 4 h post the treatment and thereafter for 14 days for mortality, including various signs of toxicity such as, changes in skin and fur, behavior patterns, convulsions, tremors and death (OECD Guidelines 2001).

Molecular docking studies

Molecular docking studies were performed on all the synthesized compounds in order to gain structural insights into the binding mode of the ligand with the biological target MAO-A enzyme crystal structure (PDB ID: 2Z5X) using GLIDE (version 6.5, Schrödinger, LLC, New York, NY, 2014). The MAO protein crystal structure used for molecular docking was retrieved from the Protein Data Bank (PDB), and subsequently optimized and minimized with the "protein preparation wizard" workflow. The ligands were built using Maestro 10.0 build panel and prepared by LigPrep 3.2 version v25111(Schrödinger, LLC, New York, NY, 2014) application, that uses optimized potential liquid simulations 2005 force field, and it gave the corresponding energy minima 3D conformers of the ligands. The default settings were used for all the other parameters. All the ligand atoms, but no protein atoms, were allowed to move during the calculations. To validate the docking protocol, re-docking experiment was performed in which the ligand conformation of co-crystal ligand was extracted from the crystal structure of the corresponding MAO-A protein/ligand complex and later docked back into the binding pocket. GLIDE was able to perfectly reproduce the experimental position of the ligand, confirming the ability of the method to accurately predict the binding conformation.

In-silico prediction of pharmacokinetic properties

Nearly 40% of drug candidates fail in clinical trials due to poor absorption, distribution, metabolism, and excretion (ADME) properties. These late-stage failures contribute significantly to the rapidly escalating cost of new drug development. The ability to detect the problematic candidates early can dramatically reduce the amount of wasted time and resources, and streamline the overall development process. QikProp (version 4.2, Schrödinger, LLC, New York, NY, 2014) program was used for in silico prediction of pharmacokinetic properties of the synthesized compounds.

In-silico toxicity prediction

There are a large number of freely available computer programs to envisage the in silico toxicity of the compounds. Two such softwares, used in this study are; LAZAR and OSIRIS Property explorer. LAZAR provides a generic tool for predicting complex toxicological end points, such as- carcinogenicity, long-term toxicity, and reproductive toxicity. Virtual Computational Chemistry Laboratory maintains OSIRIS as a fundamental part of Actelion's in house substance registration system. This calculates various drug-related properties of chemical structures including some toxicity parameters and drug likeness. Predicted outcomes are rated and color coded. Properties such as mutagenicity or a poor intestinal absorption having higher risks of undesired effects are shown in red, whereas a green color indicates drug-conform behavior (Klebe 2000).

Statistical analysis

All the values of the experimental results are expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's test for the possible significance ($P < 0.05$) identification between various groups. Statistical analysis

was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA).

Acknowledgements We express our sincere gratitude to Central Drugs Research Institute (CDRI), Lucknow, India and Department of Chemistry, Banasthali Vidyapith University, Banasthali, Rajasthan, India for providing the library and sophisticated analytical instrument facilities. Authors are thankful to the All India Council for Technical Education (AICTE), New Delhi, India, for providing grant under the Research Promotion Scheme (Grant No.: 8023/RID/RPS/30 (Pvt.) 2011-12), through which the computational software facility has been made available at the host institute. We also acknowledge the technical support team/application scientists of Schrodinger Inc. for their help during computational studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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