Med Chem Res
DOI 10.1007/s00044-015-1489-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

An expeditious one-pot microwave facilitated versus conventional syntheses: in vivo biological screening and molecular docking studies of some 3,5-disubstituted-4,5-dihydro-(1*H*)-pyrazole derivatives

Avinash C. Tripathi[1] · Savita Upadhyay[1] · Sarvesh Paliwal[2] · Shailendra K. Saraf[1]

Received: 15 May 2015 / Accepted: 15 December 2015

Abstract A series of 3,5-disubstituted-2-pyrazoline derivatives (**2a**–**2t**) were synthesized by reacting different aromatic/heteroaromatic aldehydes and ketones, in a two-step reaction through Claisen Schmidt condensation, followed by cyclization of the resulted chalcones with hydrazine hydrate in the presence of a base using conventional and microwave approaches. The synthesized derivatives were characterized by various physicochemical methods, and their chemical structures were established by IR, Mass, ^{1}H-NMR, ^{13}C-NMR spectroscopic data and elemental analysis. The antidepressant with tail suspension test and forced swim test and anti-anxiety with Elevated Plus Maze Test activities were evaluated using suitable animal models. Compounds **2i**, and **2j** showed noticeable antidepressant activity, by reducing the duration of immobility in both the tests, while compounds **2a** and **2b** were found to possess good anxiolytic activity, by increasing the number of arm entries and open arm exploratory time at the tested doses (50 and 100 mg/kg b.w.), when compared to the standard drugs imipramine and diazepam, respectively. In order to ascertain the binding interactions of the synthesized derivatives to the MAO-A target protein, molecular docking was employed which demonstrated the key interactions with the amino acid residues Asn181, Phe208, Tyr69, Tyr197, Tyr444 and Met445 at the binding site. In addition, the most active derivatives **2i** and **2b** showed some imperative conserved interactions of the PDB co-crystal ligand 2Z5X with the amino acid residues at the binding site of MAO-A protein. The results of the study also demonstrated that the Glide gscores of the synthesized derivatives were in close correlation with the in vivo biological activity data, in particular with the forced swim test of the antidepressant activity with a very good correlation coefficient of 0.754103. Furthermore, the ADME properties of the synthesized derivatives were predicted and found to be within the affirmed limits.

Keywords 2-Pyrazolines · Antidepressant · Anti-anxiety · MAO inhibitors · Neurotoxicity · Microwave synthesis · Molecular docking · In silico ADME prediction

✉ Shailendra K. Saraf
dirpharmniec@gmail.com

Avinash C. Tripathi
aviniec31@gmail.com

Savita Upadhyay
savvypharma@gmail.com

Sarvesh Paliwal
paliwalsarvesh@yahoo.com

[1] Division of Pharmaceutical Chemistry, Faculty of Pharmacy, Babu Banarasi Das Northern India Institute of Technology, BBD City, Faizabad Road, Chinhat, Lucknow, UP 226028, India

[2] Department of Pharmacy, Banasthali Vidyapith, Banasthali, Tonk, Rajasthan 304022, India

Introduction

Monoamine oxidase (MAO) [E.C. 1.4.3.4] is a flavin-containing key enzyme located on the outer membrane of the mitochondria, bound via a C-terminal transmembrane polypeptide segment (Mitoma and Ito, 1992) and inserted in the membrane by means of ubiquitin, with energy provided by ATP (Zhuang *et al.*, 1992), in neuronal, glial, and other cells, regulating monoaminergic homeostasis and possibly neurotransmission. Low level of certain

Published online: 04 January 2016

neurotransmitters (NTs) such as serotonin, norepinephrine, dopamine and gamma amino butyric acid (GABA) in the brain is the cause of depression and other mental disorders. NTs are released by the process of neurotransmission and are broken down by the MAOs. Monoamine oxidase inhibitors (MAOIs) block this enzyme, and hence, the concentration of NTs is increased in the brain (Meyer *et al.*, 2006).

Serendipitous finding of antidepressant effects in patients treated with iproniazid, a hydrazine-based antitubercular agent structurally similar to isoniazid, started the journey of development of MAO inhibitors. This discovery, together with the demonstration that iproniazid was a potent MAO inhibitor (Zeller and Barsky, 1952), led to the design and production of other MAO inhibitors such as phenelzine. MAOIs were the first-generation antidepressants used for decades in the treatment for patients with atypical depression (Pletscher, 1991), high level of anxiety, anergic bipolar depression and treatment resistant depression (Thase, 2012), specific phobias, posttraumatic stress disorder and migraine headaches resistant to other therapies (Gareri *et al.*, 2000). Nowadays, the therapeutic interest in MAOIs falls into two major categories. MAO-A inhibitors have been used mostly in the treatment of mental disorders, in particular depression and anxiety (Amrein *et al.*, 1999), while MAO-B inhibitors have shown therapeutic value in a variety of diseases (Youdim *et al.*, 2006), especially neurodegenerative (Foley *et al.*, 2000) such as Parkinson's (Cesura and Pletscher, 1992) and Alzheimer's (Volz and Gleiter, 1998). Hydrazine-based MAO inhibitors were devoid of serious side effects such as liver toxicity and "cheese reaction" (potentially lethal hypertensive crises with cerebral hemorrhages, following the consumption of cheese, wine and other fermented foods, typically rich in tyramine and sympathomimetic amines) (Brown *et al.*, 1989). These side effects were hypothesized to be related to nonselective and irreversible MAO inhibition. The quest for MAO inhibitors devoid of untoward effects prompted research to characterize selective MAO-A and MAO-B inhibitors.

The synthesis of pyrazolines and their derivatives have engrossed substantial attention for many years. Increasing evidence suggests that pyrazoline is an important scaffold since it is known to be associated with multiple biological activities such as tranquilizer, muscle relaxant, anticonvulsant and antidepressant (Bilgin *et al.*, 1993; Kaplancikli *et al.*, 2010; Ozdemir *et al.*, 2008; Palaska *et al.*, 2001; Rajendra Prasad *et al.*, 2005; Ruhoglu *et al.*, 2005), psychoanaleptic and MAO inhibitory (Chimenti *et al.*, 2004, 2010; Gokhan-Kelekci *et al.*, 2009, 2007; Gokhan *et al.*, 2003; Jagrat *et al.*, 2011; Jayaprakash *et al.*, 2008; Karuppasamy *et al.*, 2010; Maccioni *et al.*, 2010; Manna *et al.*, 1998, 2002; Mishra and Sasmal, 2011; Sahoo *et al.*, 2010). Pyrazolines are also well-established pharmacophores for activities other than nervous system such as anti-amebic (Bhat *et al.*, 2009; Budakoti *et al.*, 2009; Husain *et al.*, 2008; Wanare *et al.*, 2010), anti-cancer (Congiu *et al.*, 2010; Havrylyuk *et al.*, 2009; Insuasty *et al.*, 2011; Parekh *et al.*, 2011), anti-viral (Diamond 2009; Ramajayam *et al.*, 2010), anti-malarial (Acharya *et al.*, 2010; Wanare *et al.*, 2010), anti-inflammatory-analgesic (Fioravanti *et al.*, 2010; Girisha *et al.*, 2010; Khode *et al.*, 2009), anti-microbial (Abdel-Wahab *et al.*, 2009; Dawane *et al.*, 2010; Ozdemir *et al.*, 2007; Siddiqui *et al.*, 2011; Sivakumar *et al.*, 2010) etc.

Studies by different workers have demonstrated 1,3,5-trisubstituted-2-pyrazolines to be very promising scaffolds for CNS activities such as antidepressant, anti-anxiety and anticonvulsant. In view of these observations, it was decided to synthesize and characterize a series of 3,5-disubstituted-2-pyrazolines, with novel aromatic and heteroaromatic substitutions at 3rd and 5th positions, respectively, and to evaluate them as antidepressant and anti-anxiety agents using suitable models. In addition to the conventional heating methods, microwave-assisted synthetic approach was employed to prepare the proposed derivatives and the results were compared with those of the former method. It has been observed that most of the conventional heat reactions could be performed more conveniently using this technique, at a faster rate and in good yields (Lidstrom *et al.*, 2001). Finally, the molecular docking studies were performed to predict the binding affinity and ascertain the interactions of the proposed derivatives with the biological target.

Results and discussion

A series of fourteen 2-pyrazoline derivatives (**2a**–**2t**) were synthesized via Claisen-Schmidt condensation and characterized by physicochemical (Table 1), spectral and elemental analysis methods. The IR spectra of the synthesized compounds afforded absorption bands in the regions corresponding to C=N stretching (1509–1612 cm^{-1}), N–H stretching (3456–3105 cm^{-1}) and C–H deformation (1428–1357 cm^{-1}). ^{1}H-NMR spectra of the compounds exhibited the presence of two non-equivalent protons of a methylene group (H_a/H_b) at δ 2.92–3.38 ppm, 3.70–3.93 coupled with each other and in turn with the vicinal methine proton (H_x) at δ 6.68–7.04. All the other aliphatic, aromatic and heteroaromatic protons were also observed at their expected ppm values.

The results of pharmacological studies, presented in Table 2 and Fig. 1, showed that compounds **2i** and **2j** exhibited very good antidepressant activity at the tested doses in both, FST and TST models (Fig. 1a, b).

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

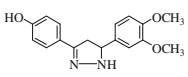
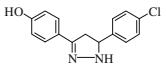
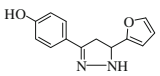
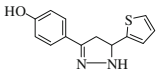
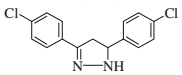
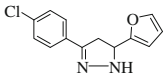
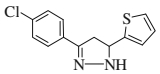
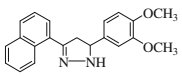
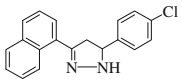
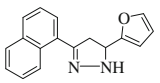
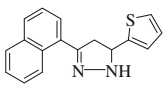
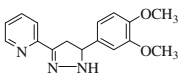
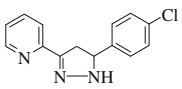
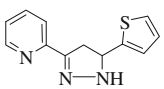
Comps.	Structure	Molecular formula	Color and state	Solubility	R_f value[a]	Melting range (°C)	Conventional synthesis (refluxing)		Microwave assisted synthesis		
							Reaction time (h)	% Yield	Microwave power (W)	Reaction time (s)	% Yield
2a		$C_{17}H_{18}N_2O_3$	Colorless crystalline solid	Hot methanol, chloroform, acetone, DMSO	0.41	178–180	4.5	54.7	240–280	190	86.3
2b		$C_{15}H_{13}ClN_2O$	Colorless crystalline solid	Hot methanol, chloroform, acetone, DMSO	0.38	210–212	4.5	67.2	240	50	90.1
2c		$C_{13}H_{12}N_2O_2$	Cream colored crystalline solid	Methanol, acetone, DMSO	0.48	148–150	5.0	60.5	240–280	110	91.4
2d		$C_{13}H_{12}N_2OS$	Colorless crystalline solid	Methanol, acetone, DMSO	0.31	88–91	6.0	78.8	240–350	230	82.8
2f		$C_{15}H_{12}Cl_2N_2$	Colorless crystalline solid	Hot methanol, Chloroform, acetone, DMSO	0.83	118–120	8.0	68.9	240–280	270	78.6
2g		$C_{13}H_{11}ClN_2O$	Brownish black colored amorphous solid	Hot methanol, Chloroform, acetone, DMSO	0.96	80–82	5.0	57.4	280–350	220	88.2
2h		$C_{13}H_{11}ClN_2S$	Brown colored amorphous solid	Hot methanol, chloroform, acetone, DMSO	0.88	90–92	4.5	59.6	280–350	400	85.0
2i		$C_{21}H_{20}N_2O_2$	Cream yellow colored crystalline solid	Hot methanol, Chloroform, acetone, DMSO	0.91	112–114	6.0	63.09	280–350	200	85.3
2j		$C_{19}H_{15}ClN_2$	Cream White colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.50	118–120	5.0	86.7	280	120	90.8
2k		$C_{17}H_{14}N_2O$	Light brown colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.90	88–90	4.5	71.3	240–350	320	87.4
2l		$C_{17}H_{14}N_2S$	Cream yellow colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.97	98–100	4.5	73.5	280–350	380	94.7
2q		$C_{16}H_{17}N_3O_2$	Brown colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.65	110–113	3.0	93.8	280	160	81.5
2r		$C_{14}H_{12}ClN_3$	Light orange colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.72	170–172	3.0	48.2	280–350	175	68.2
2t		$C_{12}H_{11}N_3S$	Brown colored crystalline solid	Methanol, chloroform, acetone, DMSO	0.54	82–84	4.0	51.6	280–350	300	72.5

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

Comps.	Doses (mg/kg b.w.)	Antidepressant activity[a]		Anti-anxiety activity[b]		Neurotoxicity studies[c]	
		Duration of immobility in FST (s) (mean ± SD)	Duration of immobility in TST (s) (mean ± SD)	Number of entries in closed arm (mean ± SD)	Time spent in closed arms (s) (mean ± SD)	At 1.0 h	At 4.0 h
2a	50	129.17 ± 11.23***	196.17 ± 18.65***	11.17 ± 4.17***	162.00 ± 21.54***	0/6	0/6
	100	97.67 ± 14.19***	196.83 ± 12.56***	15.50 ± 2.35***	126.33 ± 11.50***	0/6	1/6
2b	50	96.67 ± 16.82***	107.17 ± 27.46***	12.33 ± 2.58***	155.33 ± 18.69***	0/6	0/6
	100	85.50 ± 10.50***	100.83 ± 16.19***	**17.67 ± 3.01*** **	**117.33 ± 17.42*** **	0/6	1/6
2c	50	82.67 ± 6.41***	139.33 ± 20.17***	7.00 ± 1.55***	216.50 ± 29.54***	0/6	0/6
	100	74.83 ± 16.82***	125.50 ± 13.75***	6.33 ± 2.16***	242.33 ± 43.58***	0/6	0/6
2d	50	124.33 ± 9.71***	209.17 ± 23.61***	8.83 ± 3.06***	151.17 ± 18.37***	0/6	0/6
	100	97.67 ± 14.19***	160.33 ± 13.44***	10.00 ± 4.77***	143.83 ± 16.04***	1/6	0/6
2f	50	105.17 ± 13.85***	97.50 ± 30.25***	4.50 ± 1.87***	200.67 ± 18.20***	0/6	0/6
	100	87.83 ± 18.79**	65.50 ± 29.61***	6.17 ± 1.83***	228.67 ± 30.90***	0/6	0/6
2g	50	104.17 ± 15.56***	150.50 ± 24.75***	5.83 ± 2.14***	289.00 ± 20.68***	0/6	0/6
	100	99.50 ± 14.64***	155.83 ± 12.80***	9.17 ± 2.64***	283.50 ± 24.81***	0/6	0/6
2h	50	155.00 ± 11.64***	89.83 ± 14.93***	3.17 ± 1.94***	203.00 ± 22.65***	1/6	0/6
	100	101.67 ± 12.26***	149.17 ± 42.10***	4.17 ± 2.14***	260.50 ± 32.12***	0/6	2/6
2i	50	56.33 ± 10.21***	62.33 ± 16.69***	5.50 ± 1.87***	256.17 ± 20.85***	0/6	0/6
	100	**21.17 ± 8.33*** **	**36.83 ± 11.28*** **	7.17 ± 2.93***	211.50 ± 25.10***	0/6	0/6
2j	50	62.17 ± 14.08***	151.33 ± 29.56***	7.67 ± 2.16***	224.17 ± 20.15***	0/6	0/6
	100	43.33 ± 10.98***	**29.67 ± 15.13*** **	8.50 ± 2.43***	193.67 ± 20.22***	1/6	1/6
2k	50	94.33 ± 14.18***	129.50 ± 37.25***	6.50 ± 1.87***	206.33 ± 15.87***	0/6	0/6
	100	73.33 ± 25.40***	132.17 ± 11.32***	6.83 ± 1.72***	174.17 ± 24.24***	0/6	0/6
2l	50	112.50 ± 19.15***	133.33 ± 23.85***	7.67 ± 3.20***	169.17 ± 18.50***	0/6	1/6
	100	84.67 ± 13.34***	107.67 ± 17.96***	9.00 ± 2.37***	134.17 ± 12.73***	0/6	0/6
2q	50	76.50 ± 21.82***	99.17 ± 19.67***	11.17 ± 4.17***	222.50 ± 24.19***	0/6	0/6
	100	68.17 ± 18.88***	76.67 ± 6.59***	15.50 ± 2.35***	162.33 ± 24.08***	0/6	0/6
2r	50	155.00 ± 11.64***	127.67 ± 19.66***	1.00 ± 0.63***	259.50 ± 22.29***	0/6	0/6
	100	101.67 ± 12.26***	98.50 ± 14.27***	2.67 ± 2.80***	233.33 ± 22.65***	0/6	0/6
2t	50	105.50 ± 11.78***	107.83 ± 18.12***	8.17 ± 2.93***	270.17 ± 24.38***	0/6	0/6
	100	78.17 ± 11.39***	76.67 ± 6.59***	5.17 ± 2.40***	244.17 ± 39.50***	0/6	0/6
Control (CMC)	0.5 %	179.67 ± 18.67	219.00 ± 17.56	0.83 ± 0.75	281.67 ± 41.09	0/6	0/6
Standard (Imipramine	10	29.33 ± 8.43***	60.33 ± 9.33***	–9.33 ± 3.08***	–177.33 ± 45.18***	0/6	0/6
Diazepam)	2	–	–			0/6	0/6

Bold data represent 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 (10mg/kg, b.w.) for antidepressant activity and Diazepam (2mg/kg, b.w.) for anti-anxiety activity

[a] The reduction in time of immobility in FST and TST is 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 rotarod test after 1.0 and 4.0 h (number of animals exhibiting toxicity/number of animals tested)

Compounds **2a** and **2b** were found to be the most active compounds for anti-anxiety activity in the elevated plus maze test, with the number of entries in different arms increasing significantly with a lesser closed arm exploration time (Fig. 1c, d). Most of the synthesized derivatives showed biological activities in a dose-dependent manner, with increasing magnitude of the effects at higher tested dose (100 mg/kg b.w.). None of the

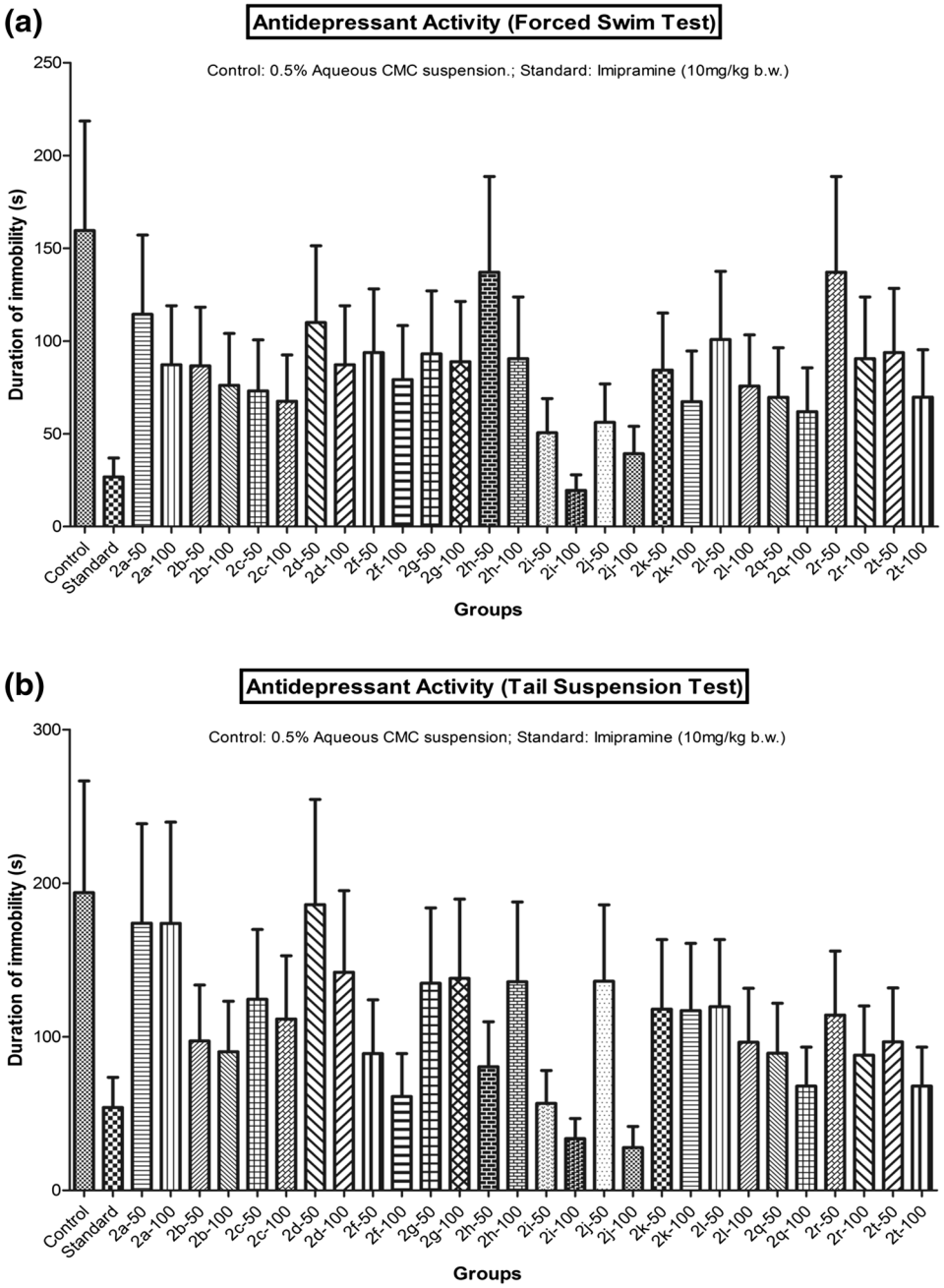
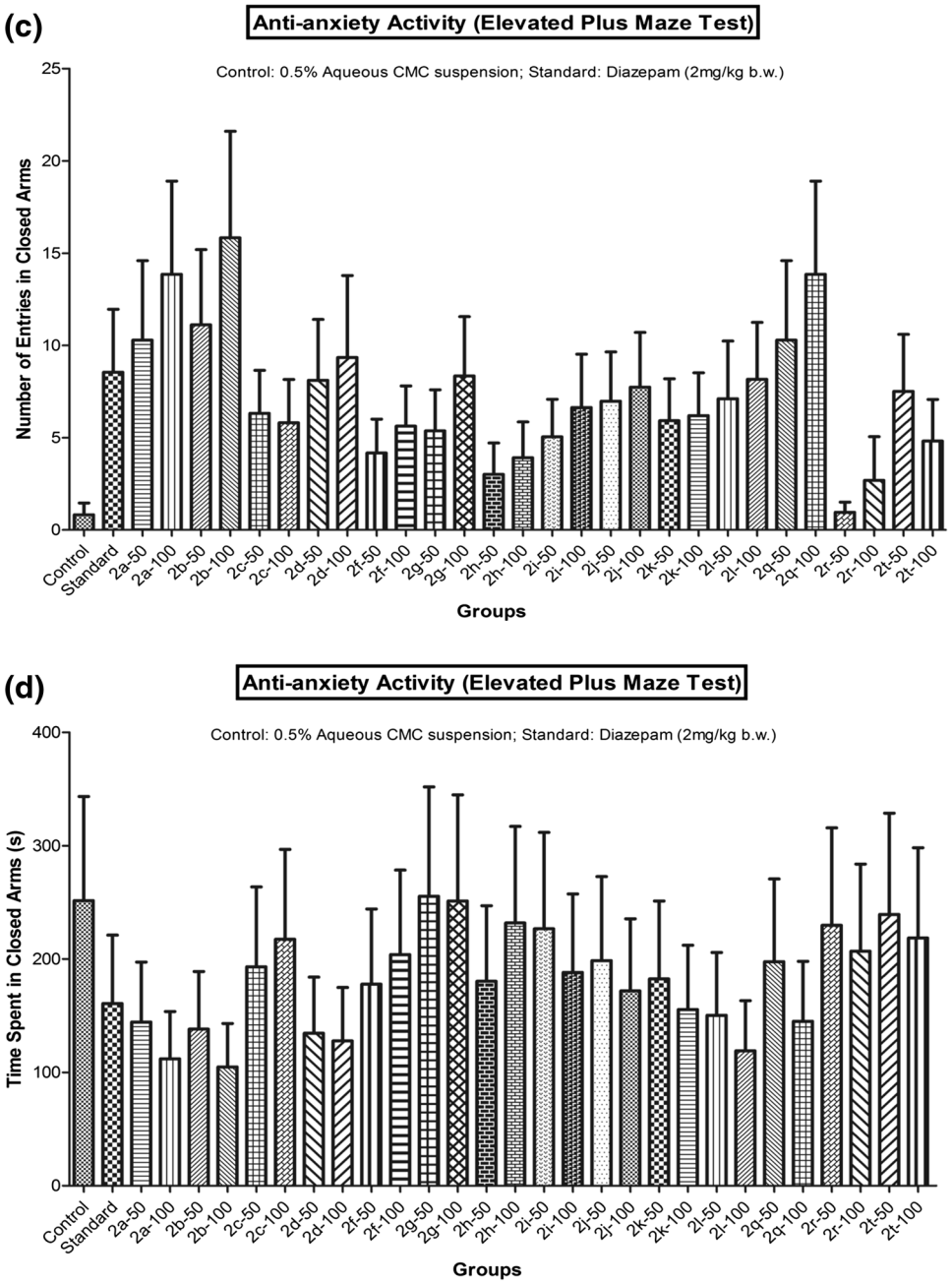


Fig. 1 **a** Graph showing antidepressant activity (Forced Swim Test); **b** graph showing antidepressant activity (Tail Suspension Test); **c** graph showing anti-anxiety activity (number of entries in closed arms in Elevated Plus Maze Test); **d** graph showing anti-anxiety activity (time spent in closed arms in Elevated Plus Maze Test)

synthesized derivatives exhibited neurotoxicity (disturbances in the motor co-ordinations) at the tested doses, as evaluated by the rotarod test (Table 2), since the mean fall off time for all the mice was found to be more than 180 s. All the synthesized derivatives demonstrated excellent in silico ADME properties in predictions using QikProp module (Table 3). The 3,4-dimethoxyphenyl and 4-chlorophenyl substitution at the 5th position (**2i,** and **2j**) and 1-naphthyl substitution at 3rd position of the 2-pyrazoline nucleus was found to be crucial in eliciting antidepressant action, while a polar 4-hydroxyphenyl substitution at 3rd position of the 2-pyrazoline nucleus (**2a** and **2b**) was found to be decisive in exhibiting good anxiolytic properties.

Previous studies have demonstrated that the 2-pyrazoline derivatives possess excellent affinity toward MAO proteins and are therefore considered as important targets for developing antidepressants and anti-anxiety agents (Chimenti *et al.*, 2004; Gokhan-Kelekci *et al.*, 2007; Jayaprakash *et al.*, 2008). In view of these observations, docking studies were performed to gain some insights into the binding mode of these synthesized compounds. The stable ligand–receptor complex conformations and the

Fig. 1 continued



interactions of the most active compounds **2i** and **2b** with amino acid residues have been depicted in Figs. 2, 3 and 4, in the typical binding pocket of MAO-A protein. It may be inferred from the docking studies that Asn181, Phe208, Phe352, Tyr69, Tyr197, Tyr444 and Met445 are the key interacting residues at the binding site of MAO-A protein 2Z5X, making crucial contacts with the 3,5-disubstituted 2-pyrazoline ring. In such an orientation, the bound conformations of compound **2i** engrossed in two hydrogen bond interactions of 2-pyrazoline nitrogen and C5-methoxyphenyl substituent with the side chain residues; Asn181, Tyr197; and two pi–pi stacking interactions of C3 naphthyl ring and 3,4-dimethoxyphenyl ring at C5 position with Phe208 and Tyr444 backbone residues, respectively (Fig. 2a, b). Also, one of the most active compounds **2b** (for anti-anxiety activity) is involved in three pi–pi stacking interactions; first two between 4-chlorophenyl ring at C3 position with Tyr69 and Phe352 residues; and third between 4-hydroxyphenyl ring of 2-pyrazoline nucleus and Tyr444 residue (Fig. 3a, b). In addition, two H-bond interactions are also present between 4-hydroxyphenyl substituent at C3 position Tyr69 and Met445 backbone

Table 3 Glide gscore and in silico ADME prediction data of the synthesized derivatives (**2a–2t**)

Comps.	Glide gscore	MW	donarHB	accptHB	QlogPo/w Predicted	QPlogBB	metab	QPlog Khsa	% Human Oral absorption	Volume	PSA	Violations of Rule of Five
2a	−8.02	298.341	2	4.25	3.079	−0.567	5	0.282	100	978.429	65.316	0
2b	**−8.32**	272.733	2	2.75	3.44	−0.179	3	0.319	100	869.116	50.204	0
2c	−7.15	228.25	2	3.25	2.174	−0.43	4	−0.007	93.135	767.276	61.538	0
2d	−7.15	244.311	2	2.75	2.751	−0.283	4	0.138	100	797.983	51.868	0
2f	−7.40	291.179	1	2	4.587	0.489	2	0.651	100	891.902	28.776	0
2g	−7.60	246.696	1	2.5	3.438	0.335	3	0.268	100	783.703	36.91	0
2h	−7.29	262.756	1	2	4.016	0.382	3	0.471	100	826.674	30.083	0
2i	**−9.70**	332.401	1	3.5	4.852	0.052	4	0.837	100	1096.888	39.532	0
2j	−9.28	306.794	1	2	5.05	0.317	2	0.908	100	984.621	29.11	1
2k	−8.45	262.31	1	2.5	3.871	0.306	3	0.472	100	857.101	36.256	0
2l	−7.80	278.371	1	2	4.352	0.208	3	0.688	100	904.637	28.966	0
2q	−8.07	283.329	1	4.5	3.279	−0.1	5	0.25	100	949	52.796	0
2r	−7.73	257.722	1	3	3.446	0.124	3	0.321	100	837.625	40.379	0
2t	−6.63	229.299	1	3	2.964	0.212	4	0.125	100	762.261	39.19	0
PDB-2Z5X crystal ligand	−7.09	212.251	1	1.75	3.11	0.182	3	0.222	100	733.386	33.499	0
Standard values/ range	–	130–725	0–6	2–20	−2.0 to 6.5	−3.0 to 1.2	1–8	−1.5 to 1.5	>80 % is high; <20 % is poor	500–2000	7–200	Maximum is 4

donarHB: hydrogen bond donar; accptHB: hydrogen bond acceptor; metab: number of likely metabolic reactions; QPlogKhsa: prediction of binding to human serum albumin; QPlogBB: predicted brain/blood partition coefficient; 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; Number of violations of Lipinski's rule of five

residues, respectively. Further, compound **2i** and **2b** were found to possess the conserved interactions (Fig. 4) of the PDB co-crystal ligand 2Z5X with the amino acid residues at the binding site to MAO-A protein. It was also evident from the results that the predicted binding affinity (Glide scores) of the synthesized derivative showed a close correlation with the in vivo biological activity data, in particular with the forced swim test of the antidepressant activity with a very good correlation coefficient of 0.754103 (Table 4; Fig. 5).

Conclusion

In the present study, some new 2-pyrazoline derivatives were synthesized and evaluated in vivo for their antidepressant and anti-anxiety potential. It was observed that the 3,4-dimethoxyphenyl and 4-chlorophenyl substitution at 5th position as well as naphthyl substitution at 3rd position of the 2-pyrazoline nucleus were crucial for the antidepressant activity, as derivatives bearing these substitutions (**2i** and **2j**) were found to be the most active among the series. A polar 4-hydroxyphenyl or pyridin-2-yl substitution at 3rd position (compounds **2a**, **2b** and **2q**) was detrimental in eliciting anxiolytic activity among the synthesized derivatives. Also, favorable in silico ADME performance was observed for all the synthesized derivatives without any significant neurotoxicity. The docking studies established a fair interaction of the synthesized derivatives with the MAO-A protein plays a vital role in neurological disorders such as depression and anxiety. Thus, it may be concluded that the synthesized 2-pyrazoline derivatives could be promising candidates for antidepressant and anxiolytic activities.

Experimental

The chemicals and reagents were procured from Sigma Aldrich and S. D. Fine Chemicals, Mumbai, 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 Systems (Ragatech, Pune, Maharashtra, India).

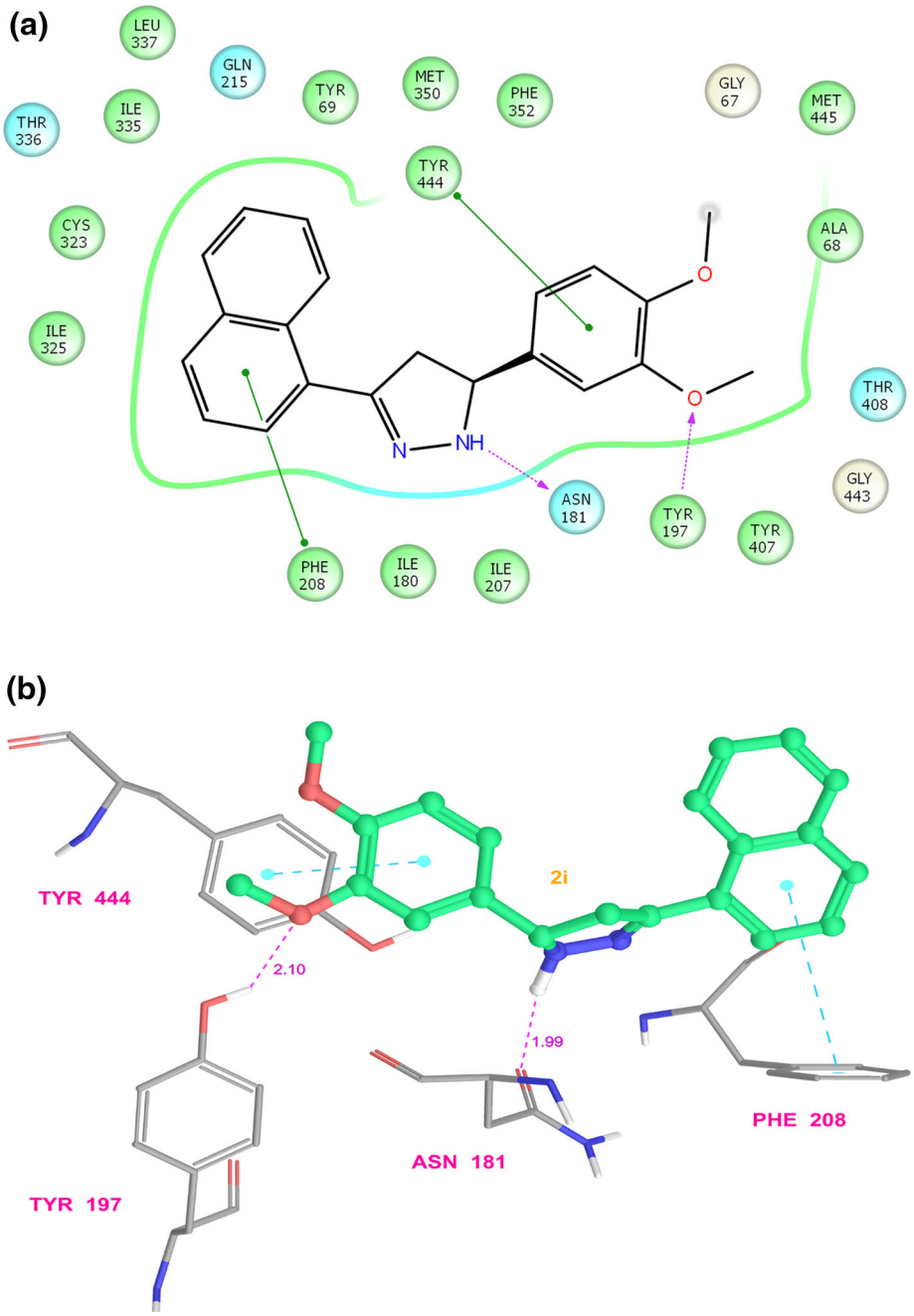


Fig. 2 Ligand–receptor interaction diagram of compound **2i** at the binding site of MAO-A protein (PDB ID: 2Z5X) showing good antidepressant activity. **a** 2D Ligand–receptor interaction diagram. **b** 3D Ligand–receptor interaction diagram

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Shimadzu 8400S and Perkin Elmer RX1 FTIR spectrophotometers (Shimadzu Corporation, Japan) using KBr disks and values are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker DRX-300 spectrometer (Bruker Instruments Inc., USA) at 300 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 Agilent 6520-QTOF LCMS mass spectrometer instrument using ESI technique. Elemental analysis was performed on Vario EL III Elemental analyzer (Elementar, Germany).

Chemistry

In the first step, substituted aldehydes and ketones were reacted in a basic medium to form the substituted chalcones, through Claisen-Schmidt condensation, which in the second step were cyclized into the final derivatives (**2a**–**2t**) using hydrazine hydrate in excess as given in Scheme 1 (Derivatives **2e** and **2s** could not be synthesized by both,

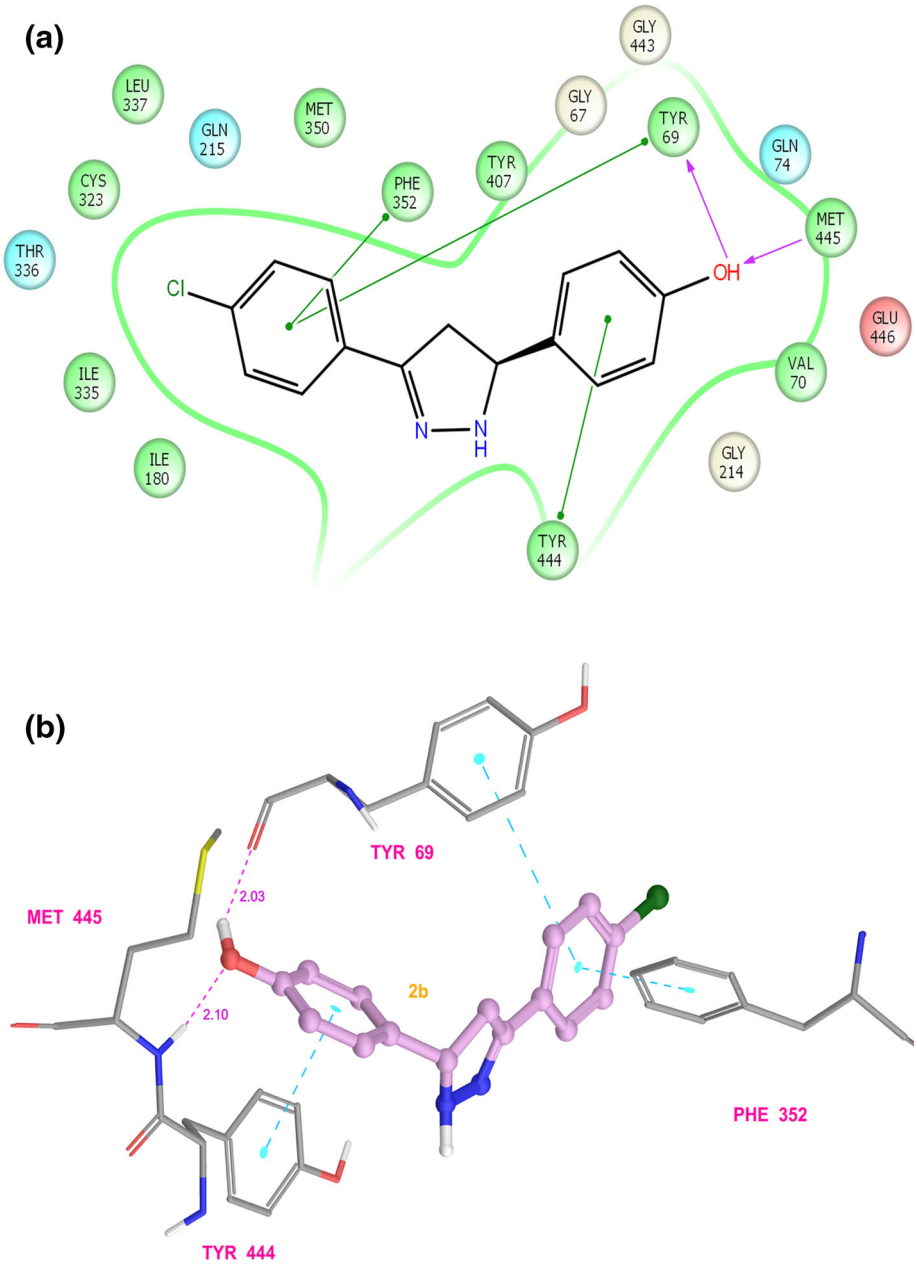


Fig. 3 Ligand–receptor interaction diagram of compound **2b** at the binding site of MAO-A protein (PDB ID: 2Z5X) showing good anti-anxiety activity. **a** 2D Ligand–receptor interaction diagram. **b** 3D Ligand–receptor interaction diagram

conventional and microwave methods). Progress of the reactions was monitored by thin layer chromatography (TLC) on precoated silica gel G plates, using iodine vapors and UV light as the visualizing agents.

General procedure for the synthesis of chalcones

Conventional method 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 hydrochloric acid. The yellow colored intermediates (chalcones) obtained were filtered, washed, dried, and re-crystallized from methanol (Jayaprakash *et al.*, 2008; Karuppasamy *et al.*, 2010; Mishra and Sasmal, 2011; Sahoo *et al.*, 2010; Stirrett *et al.*, 2008).

Microwave-assisted method 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, 60–230 s). The reaction mixture was poured into ice-cold water and then

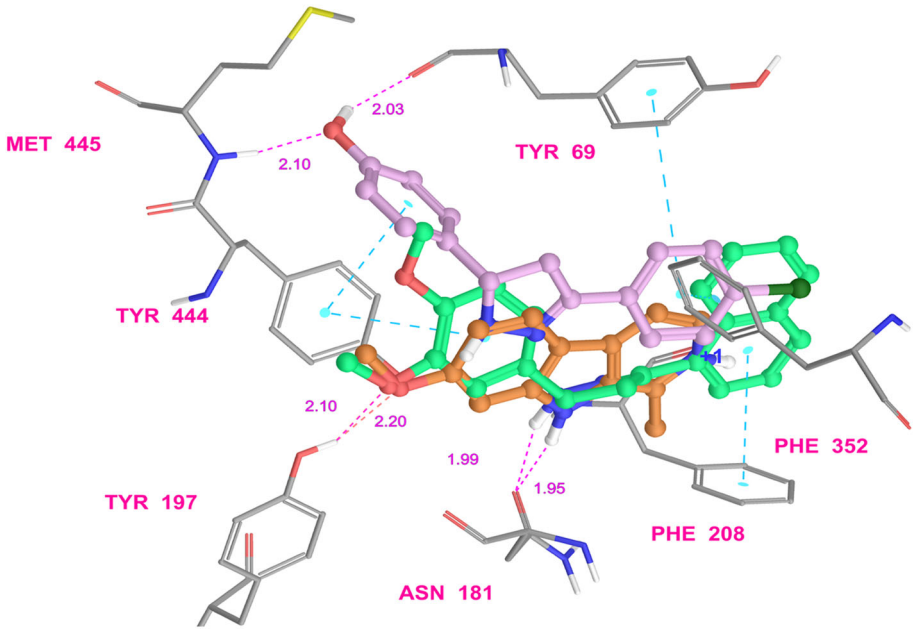


Fig. 4 Compounds **2i** (*spring green color*) and **2b** (*plum color*) showing conserved H-bond interactions of 2Z5X crystal ligand (*orange color*) at the receptor site of MAO-A protein 2Z5X, showing good antidepressant and anti-anxiety activities (Color figure online)

Table 4 Correlation of in vivo biological activity (antidepressant and anti-anxiety) data with Glide scores

Correlation coefficient in relation with	Antidepressant activity		Anti-anxiety activity	
	Duration of Immobility in FST (s)	Duration of immobility in TST (s)	Number of entries in closed	Time spent in closed arm (s)
Glide score	0.754103	0.47171	−0.24201	0.28982

neutralized to pH 2 using 6 N hydrochloric acid. The yellow colored intermediates (chalcones) obtained were filtered, washed, dried, and re-crystallized from methanol (Jayashree *et al.*, 2008).

General procedure for the synthesis of 3,5-disubstituted-2-pyrazoline derivatives

Conventional method 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 (Karuppasamy *et al.*, 2010; Mishra and Sasmal, 2011).

Microwave-assisted method Appropriate chalcone was treated with 10 times excess of hydrazine hydrate (80 %) in dry ethanol, under the exposure of microwave irradiation (MWI: 240–350 W; 50–400 s). The 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 pyrazolines (Chawla *et al.*, 2010; Insuasty *et al.*, 2011).

Characterization of the synthesized 3,5-disubstituted-2-pyrazoline derivatives

Intermediates were characterized by TLC, melting point and mass spectra, while 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)-4,5-dihydro-1H-pyrazol-3-yl]-phenol (**2a**)* IR (KBr, cm^{-1}): N–H str (3278), C–H Ar (3044), C=N str (1597), C–H deform (1429). ^{1}H NMR (DMSO, δ ppm): 2.35–3.00 (dd, J_{ab}: 13.29 Hz, J_{ax}: 4.21 Hz, 1H, H_a), 3.60–3.68 (dd, J_{ab}: 5.60 Hz, J_{bx}: 11.33 Hz, 1H, H_b), 3.72 (m, 6H), 6.59–6.66 (m, 4H, Ar), 7.22 (s, 2H, Ar), 6.88 (s, 1H, NH), 9.27 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, ppm): 41.7 (CH_2 pyrazoline), 48.9 (CH pyrazoline), 57.2 ($2CH_3$ aliphatic), 114.3–116.1 (4CH aromatic), 121.6 (CH aromatic), 125.7 (2C aromatic),

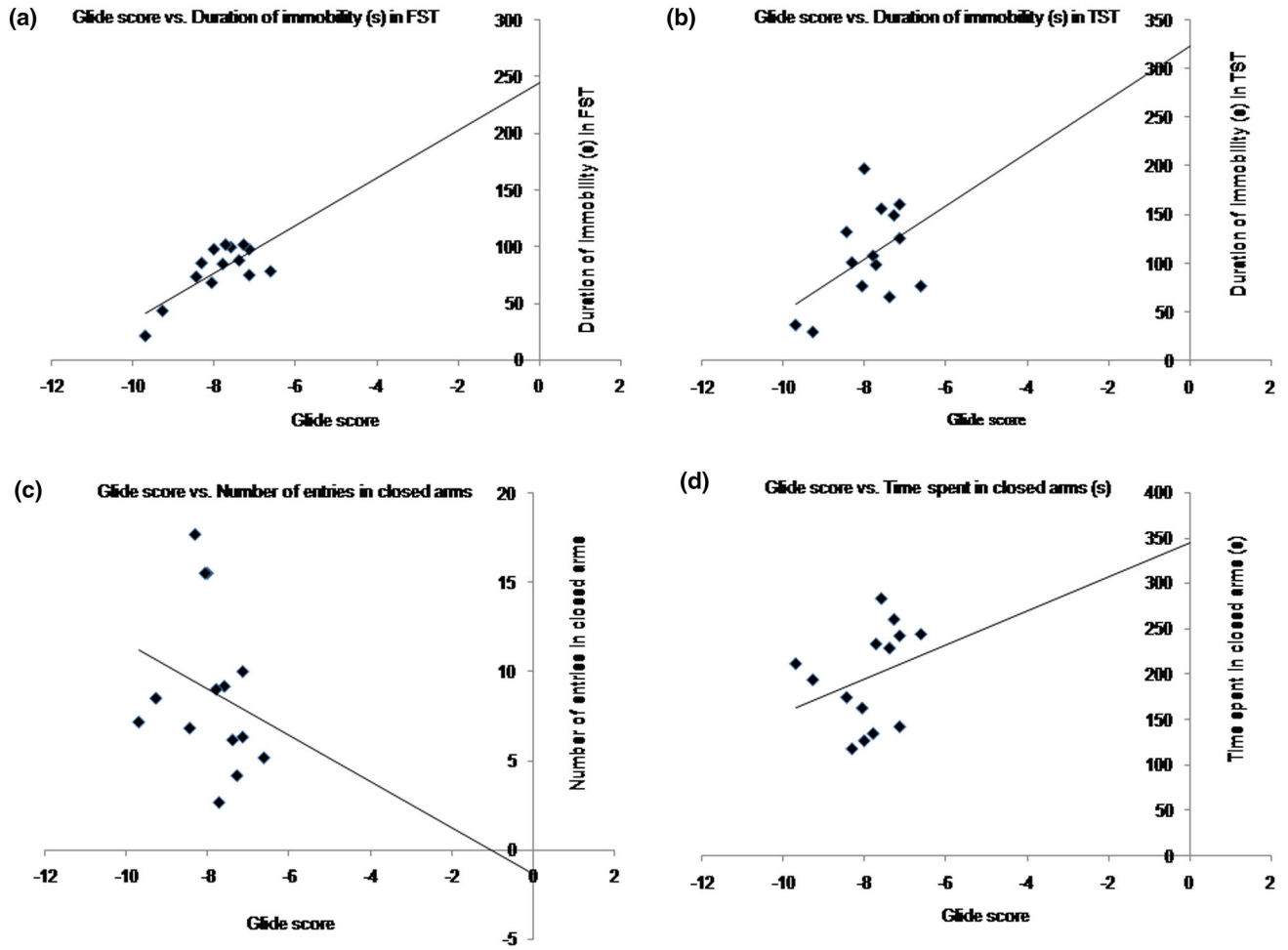
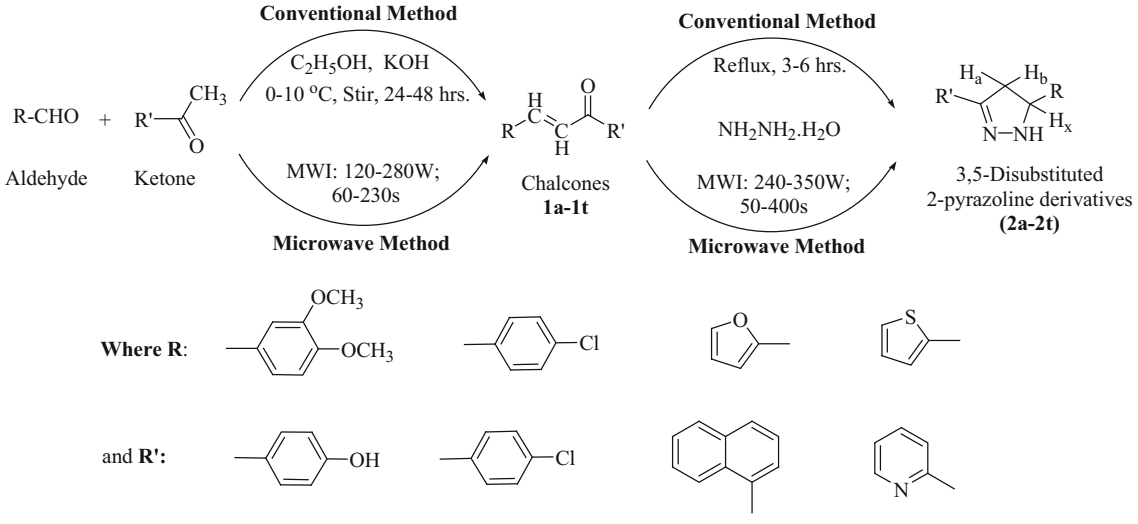


Fig. 5 Graphs correlating in vivo biological activity (antidepressant and anti-anxiety) data with Glide scores. **a** Glide score versus FST. **b** Glide score versus TST. **c** Glide score versus number of entries in closed arms. **d** Glide score versus time spent in closed arms



Scheme 1 Synthesis of 3,5-disubstituted-2-pyrazoline derivatives

128.4–130.1 (3CH aromatic), 151.6 (3C aromatic), 156.8 (C pyrazoline). MS (*m/z*): 299.2 (M + 1). Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.13; H, 6.55; N, 10.30.

4-[5-(4-Chloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-phenol ***(2b)*** IR (KBr, cm^{-1}): N–H str (3359), C–H Ar (3046), C=N str (1572), C–H deform (1461). ^1H NMR (DMSO, δ ppm): 2.76–3.02 (dd, J_{ab}: 17.11 Hz, J_{ax}: 3.62 Hz, 1H, H_a), 3.82–3.89 (dd, J_{ab}: 3.15 Hz, J_{bx}: 12.29 Hz, 1H, H_b), 7.05 (s, 1H, NH), 6.94–7.38 (d, 8H, Ar), 9.22 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, ppm): 42.9 (CH_2 pyrazoline), 49.1 (CH pyrazoline), 113.9–115.1 (2CH aromatic), 120.2 (C aromatic), 141.6 (C aromatic), 129.2–131.1 (6CH aromatic), 133.8 (C aromatic), 154.7 (C pyrazoline), 159.5 (C aromatic). MS (*m/z*): 272.8 (M^+). Anal. Calcd. for $C_{15}H_{13}ClN_2O$: C, 66.06; H, 4.80; N, 10.27. Found: C, 65.97; H, 5.03; N, 10.28.

4-(5-Furan-2-yl-4,5-dihydro-1H-pyrazol-3-yl)-phenol ***(2c)*** IR (KBr, cm^{-1}): N–H str (3283), C–H Ar (3112), C=N str (1600), C–H deform (1385). ^1H NMR (DMSO, δ ppm): 3.15–3.29 (dd, J_{ab}: 16.74 Hz, J_{ax}: 2.99 Hz, 1H, H_a), 3.67–3.94 (dd, J_{ab}: 3.02 Hz, J_{bx}: 14.23 Hz, 1H, H_b), 6.41–6.75 (dd, J_{ax}: 9.86 Hz, J_{bx}: 15.66 Hz, 1H, H_x), 7.03 (s, 1H, NH), 6.70–7.16 (dd, 4H, aromatic), 6.00–6.26 (s, 2H, Ar), 8.37 (s, 1H, OH). ^{13}C NMR (DMSO, ppm): 41.5 (CH_2 pyrazoline), 49.6 (CH pyrazoline), 79.2 (CH_2, methylene), 102.7–109.8 (4CH aromatic), 118.2 (2CH benzene), 130.7 (2CH aromatic), 136.2 (CH furan), 157.3 (2C pyrazoline, furan), 154.4 (CH pyrazoline). MS (*m/z*): 229.13 (M^+). Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.55; H, 5.33; N, 12.24.

4-(5-Thiophen-2-yl-4,5-dihydro-1H-pyrazol-3-yl)-phenol ***(2d)*** IR (KBr, cm^{-1}): N–H str (3452), C–H Ar (3089), C=N str (1532), C–H deform (1411). ^1H NMR ($CDCl_3$, δ ppm): 3.07–3.24 (dd, J_{ab}: 16.75 Hz, J_{ax}: 4.06 Hz, 1H, H_a), 3.62–3.96 (dd, J_{ab}: 3.11 Hz, J_{bx}: 12.33 Hz, 1H, H_b), 6.12–6.43 (dd, J_{ax}: 11.27 Hz, J_{bx}: 16.93 Hz, 1H, H_x), 6.79–6.85 (s, 5H, Ar), 7.18 (s, 1H, Ar), 8.36 (s, 1H, NH), 9.55 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, ppm): 41.5 (CH_2 pyrazoline), 49.6 (CH pyrazoline), 115.3 (2CH aromatic), 123.2 (CH benzene), 124.1–126.8 (3CH thiophene), 130.7 (2CH aromatic), 138.2 (C thiophene), 159.3 (C aromatic), 154.9 (CH pyrazoline). MS (*m/z*): 243.0 (M − 1). Anal. Calcd. for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47. Found: C, 63.79; H, 5.03; N, 11.52.

3,5-Bis-(4-chloro-phenyl)-4,5-dihydro-1H-pyrazole ***(2f)*** IR (KBr, cm^{-1}): N–H str (32777), C–H Ar (2991), C=N str (1540), C–H deform (1387). ^1H NMR (DMSO, δ ppm): 3.10–3.18 (dd, J_{ab}: 17.55 Hz, J_{ax}: 3.88 Hz, 1H, H_a), 3.76–3.98 (dd, J_{ab}: 4.03 Hz, J_{bx}: 11.64 Hz, 1H, H_b), 6.05–6.21 (dd, J_{ax}: 10.38 Hz, J_{bx}: 17.36 Hz, 1H, H_x), 7.09 (s, 1H, NH), 7.26–7.65 (s, 8H, Ar). ^{13}C NMR (DMSO, ppm): 44.7 (CH_2 pyrazoline), 53.4 (CH pyrazoline), 128.7–129.4 (8CH aromatic), 129.7 (2C aromatic), 135.1 (2C aromatic), 160.1 (C pyrazoline). MS (*m/z*): 288.8 (M + 1). Anal. Calcd. for $C_{15}H_{12}Cl_2N_2$: C, 61.87; H, 4.15; N, 9.62. Found: C, 61.70; H, 4.56; N, 9.41.

3-(4-Chloro-phenyl)-5-furan-2-yl-4,5-dihydro-1H-pyrazole ***(2g)*** IR (KBr, cm^{-1}): N–H str (3315), C–H Ar (3100), C=N str (1586), C–H deform (1397). ^1H NMR ($CDCl_3$, δ ppm): 3.01–3.22 (dd, J_{ab}: 17.04 Hz, J_{ax}: 8.11 Hz, 1H, H_a), 3.73–3.90 (dd, J_{ab}: 6.25 Hz, J_{bx}: 13.38 Hz, 1H, H_b), 5.97–6.04 (dd, J_{ax}: 11.10 Hz, J_{bx}: 15.60 Hz, 1H, H_x), 6.18 (s, 2H, Ar), 7.20 (s, 1H, NH), 7.26–7.42 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$, ppm): 42.7 (CH_2 pyrazoline), 49.5 (CH pyrazoline), 106.3–107.0 (2CH furan), 129.1–130.8 (4CH aromatic), 135.2 (C aromatic), 141.4 (CH furan), 153.6 (C pyrazoline), 158.2 (C furan). MS (*m/z*): 247.0 (M + 1) Anal. Calcd. for $C_{13}H_{11}ClN_2O$: C, 63.29; H, 4.49; N, 11.36. Found: C, 63.13; H, 4.52; N, 11.44.

3-(4-Chloro-phenyl)-5-thiophen-2-yl-4,5-dihydro-1H-pyrazole ***(2h)*** IR (KBr, cm^{-1}): N–H str (3382), C–H Ar (3110), C=N str (1585), C–H deform (1347). ^1H NMR ($CDCl_3$, δ ppm): 3.12–3.28 (dd, J_{ab}: 16.53 Hz, J_{ax}: 6.35 Hz, 1H, H_a), 3.69–3.90 (dd, J_{ab}: 4.16 Hz, J_{bx}: 13.76 Hz, 1H, H_b), 6.22–6.29 (dd, J_{ax}: 10.38 Hz, J_{bx}: 17.36 Hz, 1H, H_x), 6.60–6.73 (m, 3H, Ar), 7.20–7.73 (s, 4H, Ar), 7.05 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): 40.5 (CH_2 pyrazoline), 50.23 (CH pyrazoline), 125.1–125.7 (3CH thiophene), 128.16 (C aromatic), 130.9–131.5 (4CH aromatic), 139.10 (C thiophene), 153.6 (C pyrazoline). MS (*m/z*): 363.0 (M + 1). Anal. Calcd. for $C_{13}H_{11}ClN_2S$: C, 59.42; H, 4.22; N, 10.66. Found: C, 60.02; H, 4.19; N, 10.88.

5-(3,4-Dimethoxy-phenyl)-3-naphthalen-1-yl-4,5-dihydro-1H-pyrazole ***(2i)*** IR (KBr, cm^{-1}): N–H str (3345), C–H Ar (3056), C=N str (1625), C–H deform (1419). ^1H NMR ($CDCl_3$, δ ppm): 3.03–3.10 (dd, J_{ab}: 16.34 Hz, J_{ax}: 5.02 Hz, 1H, H_a), 3.62–3.94 (dd, J_{ab}: 6.76 Hz, J_{bx}: 10.47 Hz, 1H, H_b), 6.55–6.74 (dd, J_{ax}: 11.22 Hz, J_{bx}: 16.43 Hz, 1H, H_x), 3.85 (s, 6H), 6.40–6.55 (s, 3H, Ar), 7.25–7.76 (m, 7H, Ar), 8.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): 40.4 (CH_2 pyrazoline), 51.5 (C pyrazoline), 60.3 ($2CH_3$ aliphatic), 114.6–117.2 (3CH aromatic), 126.4–129.1 (6CH aromatic), 130.6–131.2 (4C aromatic), 157.1 (C pyrazoline). MS (*m/z*): 333.3 (M + 1). Anal. Calcd. for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.06; N, 8.43. Found: C, 75.70; H, 5.91; N, 8.40.

5-(4-Chloro-phenyl)-3-naphthalen-1-yl-4,5-dihydro-1H-pyrazole ***(2j)*** IR (KBr, cm^{-1}): N–H str (3266), C–H Ar (2994), C=N str (1635), C–H deform (1421). ^1H NMR ($CDCl_3$, δ ppm): 2.11 (s, 3H,), 3.00–3.17 (dd, J_{ab}: 15.28 Hz, J_{ax}: 4.77 Hz, 1H, H_a), 3.74–3.89 (dd, J_{ab}:

6.33 Hz, J_{bx}: 11.27 Hz, 1H, H_b), 7.30–8.00 (m, 11H, Ar), 8.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): 40.4 (CH_2 pyrazoline), 51.5 (C pyrazoline), 127.2–128.6 (11CH aromatic), 136.6–138.2 (4C aromatic), 157.1 (C pyrazoline). MS (*m/z*): 307.1 (M + 1). Anal. Calcd. for $C_{19}H_{15}ClN_2$: C, 74.38; H, 4.93; N, 9.13. Found: C, 74.40; H, 4.96; N, 8.99.

5-Furan-2-yl-3-naphthalen-1-yl-4,5-dihydro-1H-pyrazole ***(2k)*** IR (KBr, cm^{-1}): N–H str (3281), C–H Ar (2958), C=N str (1623), C–H deform (1437). ^{1}H NMR ($CDCl_3$, δ ppm): 3.11–3.26 (dd, J_{ab}: 15.09 Hz, J_{ax}: 4.70 Hz, 1H, H_a), 3.77–3.91 (dd, J_{ab}: 6.35 Hz, J_{bx}: 11.54 Hz, 1H, H_b), 6.32–6.44 (dd, J_{ax}: 13.38 Hz, J_{bx}: 18.57 Hz, 1H, H_x), 6.12–6.23 (d, 2H, Ar), 7.25–7.76 (m, 7H, Ar), 8.12 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): 40.4 (CH_2 pyrazoline), 51.5 (C pyrazoline), 108.6 (2CH fruran), 125.8–127.1 (7CH aromatic), 130.6–131.2 (3C aromatic), 138.9 (CH fruran), 157.1 (C pyrazoline), 161.5 (C furan). MS (*m/z*): 263.1 (M + 1). Anal. Calcd. for $C_{17}H_{14}N_2O$: C, 77.84; H, 5.38; N, 10.68. Found: C, 77.72; H, 5.14; N, 10.54.

3-Naphthalen-1-yl-5-thiophen-2-yl-4,5-dihydro-1H-pyrazole ***(2l)*** IR (KBr, cm^{-1}): N–H str (3292), C–H Ar (3047), C=N str (1560), C–H deform (1353). ^{1}H NMR ($CDCl_3$, δ ppm): 3.09–3.12 (dd, J_{ab}: 17.41 Hz, J_{ax}: 3.65 Hz, 1H, H_a), 3.57–3.84 (dd, J_{ab}: 5.18 Hz, J_{bx}: 16.42 Hz, 1H, H_b), 6.43–6.74 (dd, J_{ax}: 3.38 Hz, J_{bx}: 11.36 Hz, 1H, H_x), 6.50–6.73 (d, 3H, Ar), 7.41–7.88 (m, 7H, Ar), 8.11 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): 40.4 (CH_2 pyrazoline), 51.5 (C pyrazoline), 123.6–124.2 (3CH thiophene), 127.2–129.6 (7CH aromatic), 132.5–134.1 (3C aromatic), 139.2 (CH thiophene), 153.7 (C pyrazoline). MS (*m/z*): 279.1 (M + 1). Anal. Calcd. for $C_{17}H_{14}N_2S$: C, 73.35; H, 5.07; N, 10.06. Found: C, 73.10; H, 5.02; N, 10.37.

2-[5-(3,4-Dimethoxy-phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-pyridine ***(2q)*** IR (KBr, cm^{-1}): N–H str (3321), C–H Ar (2955), C=N str (1647), C–H deform (1473). ^{1}H NMR (DMSO, δ ppm): 2.96–3.10 (dd, J_{ab}: 17.12 Hz, J_{ax}: 3.60 Hz, 1H, H_a), 3.69–3.88 (dd, J_{ab}: 3.14 Hz, J_{bx}: 12.03 Hz, 1H, H_b), 6.05–6.21 (dd, J_{ax}: 11.23 Hz, J_{bx}: 14.36 Hz, 1H, H_x), 3.49 (s, 6H), 6.44–6.62 (m, 3H, Ar), 7.19 (s, 1H, NH), 7.69–8.10 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$, ppm): 42.2 (CH_2 pyrazoline), 50.1 (CH pyrazoline), 62.5 (CH_3 aliphatic), 117.2–120.8 (3CH aromatic), 124.2–126.5 (3CH pyridine), 146.4–149.7 (3C aromatic), 152.6–153.2 (2C pyridine), 159.3 (C pyrazoline). MS (*m/z*): 283.8 (M + 1). Anal. Calcd. for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83. Found: C, 68.05; H, 5.95; N, 14.11.

2-[5-(4-Chloro-phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-pyridine ***(2r)*** IR (KBr, cm^{-1}): N–H str (3400), C–H Ar (3119), C=N str (1635), C–H deform (1374). ^{1}H NMR ($CDCl_3$, δ ppm): 3.02–3.05 (dd, J_{ab}: 17.12 Hz, J_{ax}: 3.41 Hz, 1H, H_a), 3.68–3.90 (dd, J_{ab}: 4.01 Hz, J_{bx}: 16.23 Hz, 1H, H_b), 6.44–6.51 (dd, J_{ax}: 3.28 Hz, J_{bx}: 17.46 Hz, 1H, H_x), 7.0–7.15 (m, 4H, Ar), 7.58 (s, 1H, NH), 7.63–7.80 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$, ppm): 46.3 (CH_2 pyrazoline), 52.5 (C pyrazoline), 126.4–128.7 (5CH aromatic), 131.5–133.2 (4CH pyridine), 153.2 (C pyridine),139.6 (C aromatic), 155.4 (C pyrazoline). MS (*m/z*): 258.0 (M + 1, M − 1). Anal. Calcd. for $C_{14}H_{12}ClN_3$: C, 65.25; H, 4.69; N, 16.30. Found: C, 65.02; H, 4.98; N, 16.42.

2-(5-Thiophen-2-yl-4,5-dihydro-1H-pyrazol-3-yl)-pyridine ***(2t)*** IR (KBr, cm^{-1}): N–H str (3430), C–H Ar (3136), C=N str (1615), C–H deform (1381). ^{1}H NMR ($CDCl_3$, δ ppm): 3.02–3.05 (dd, J_{ab}: 17.12 Hz, J_{ax}: 3.41 Hz, 1H, H_a), 3.68–3.90 (dd, J_{ab}: 4.01 Hz, J_{bx}: 16.23 Hz, 1H, H_b), 6.44–6.51 (dd, J_{ax}: 3.28 Hz, J_{bx}: 17.46 Hz, 1H, H_x), 6.5–6.83 (m, 3H, Ar), 7.58 (s, 1H, NH), 7.79–8.03 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$, ppm): 46.1 (CH_2 pyrazoline), 52.5 (C pyrazoline), 124.4–126.7 (3CH thiophene), 125.2–128.6 (2CH pyridine), 139.6 (C thiophene), 153.2 (C pyridine), 155.4 (C pyrazoline). MS (*m/z*): 229.8 (M^+). Anal. Calcd. for $C_{12}H_{11}N_3S$: C, 62.86; H, 4.84; N, 18.33. Found: C, 62.90; H, 4.98; N, 18.42.

Biological evaluation

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 activities were approved by the IAEC with protocol number BBDNIIT/IAEC/008/2014. The mice were divided into 14 groups, and each group consisted of 6 animals for each dose level in the study.

Antidepressant activity

The antidepressant activity of the synthesized derivatives 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 forced swim test (FST) in mice

The synthesized compounds were screened for their antidepressant activity using Porsolt's behavioral despair test FST (Porsolt *et al.*, 1977; Vogel, 2002). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents, which are therapeutically effective in human depression. Duration of immobility is measured in controls and animals treated with various doses of a test drug or standard.

Tail suspension test (TST) in mice

Another method, TST (Steru *et al.*, 1985; Vogel, 2002) has been described as a facile means of evaluating potential antidepressants. TST, a novel test procedure for antidepressants, was designed in which a mouse is suspended by the tail from a lever and the movements of the animal are recorded. The total duration of the test (6 min) can be divided into periods of agitation and immobility. Antidepressant drugs decrease the duration of immobility and, if coupled with measurement of locomotor activity in different conditions, the test can separate the locomotor stimulant doses from antidepressant doses. The main advantages of this procedure are the use of a simple, objective test situation, the concordance of the results with the validated "behavioral despair" test from Porsolt and the sensitivity to a wide range of drug doses. The immobility displayed by rodents, when subjected to an unavoidable and inescapable stress, has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape, when suspended by the tail. The percentage of animals showing the passive behavior is counted and compared with the vehicle treated controls.

The synthesized compounds (50 and 100 mg/kg b.w.) and imipramine (10 mg/kg b.w.), a reference antidepressant drug, were suspended separately in a 0.5 % aqueous suspension of CMC. The drugs were administered orally in a standard volume of 0.5 ml/20 g body weight, 1 h prior to the test. Control animals received 0.5 % aqueous suspension of CMC. Then, the mice were dropped individually into the Plexiglas cylinder and left in water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. A mice were judged immobile if it floated in water in an upright position and made only slight movements in order to prevent sinking. The duration of immobility was recorded during the last 4 min of the 6-min test.

Anti-anxiety activity

The maze model in mice (Vogel, 2002) 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.

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 × 10 × 40 cm and 2 arms are enclosed 50 × 10 × 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. One hour 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 enclosed arms. During a 6-min test period, the following observations were recorded: time spent in the enclosed arms and total number of the arm entries. The test is rapid and sensitive to the effects of both anxiolytic and anxiogenic agents, anxiolytic agents decreasing, and anxiogenic agents increasing the amount of time spent in closed arms; anxiolytic agents increasing and anxiogenic agents decreasing the number of entries (Lister, 1987; Pellow *et al.*, 1985).

Neurotoxicity study (Rotarod test)

The rotarod 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. One hour 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. The dose which impairs the ability of 50 % of the mice to remain on the revolving rod is considered the endpoint (Vogel, 2002).

Molecular docking studies

In order to gain structural insights into the binding mode of the ligand with biological target, all the synthesized compounds were computationally docked into the MAO-A enzyme crystal structure (PDB ID: 2Z5X) using GLIDE. 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 9.3 build panel and prepared by LigPrep 2.5 version v25111(Schrödinger, LLC, USA) application that uses optimized potential liquid simulations (OPLS) 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 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 ADME (absorption, distribution, metabolism, and excretion) 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 3.5, Schrödinger, LLC, New York, NY, 2012, program was used for in silico prediction of pharmacokinetic properties of the synthesized compounds.

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 significant ($P < 0.05$) identification between various groups. Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA).

Acknowledgments We express our sincere gratitude to Central Drugs Research Institute (CDRI), Lucknow, 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 (RPS), 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 no conflict of interest.

References

Abdel-Wahab BF, Abdel-Aziz HA, Ahmed EM (2009) Synthesis and antimicrobial evaluation of 1-(benzofuran-2-yl)-4-nitro-3-arylbutan-1-ones and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4-(aryl)-1,3-thiazol-2-yl]-1H-pyrazoles. Eur J Med Chem 44:2632–2635

Acharya BN, Saraswat D, Tiwari M, Shrivastava AK, Ghorpade R, Bapna S, Kaushik MP (2010) Synthesis and antimalarial evaluation of 1,3,5-trisubstituted pyrazolines. Eur J Med Chem 45:430–438

Amrein R, Martin JR, Cameron AM (1999) Moclobemide in patients with dementia and depression. Adv Neurol 80:509–519

Bhat AR, Athar F, Azam A (2009) Bis-pyrazolines: synthesis, characterization and antiamoebic activity as inhibitors of growth of Entamoeba histolytica. Eur J Med Chem 44:426–431

Bilgin AA, Palaska E, Sunal R (1993) Studies on the synthesis and antidepressant activity of some 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines. Arzneimittelforschung 43:1041–1044

Brown CS, Kent TA, Bryant SG, Gevedon RM, Campbell JL, Felthous AR, Barratt ES, Rose RM (1989) Blood platelet uptake of serotonin in episodic aggression. Psychiatry Res 27:5–12

Budakoti A, Bhat AR, Azam A (2009) Synthesis of new 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-b]quinoxaline derivatives and evaluation of their antiamoebic activity. Eur J Med Chem 44:1317–1325

Cesura AM, Pletscher A (1992) The new generation of monoamine oxidase inhibitors. Prog Drug Res 38:171–297

Chawla R, Sahoo U, Arora A, Sharma PC, Radhakrishnan V (2010) Microwave assisted synthesis of some novel 2-pyrazoline derivatives as possible antimicrobial agents. Acta Polo Pharm 67:55–61

Chimenti F, Bolasco A, Manna F, Secci D, Chimenti P, Befani O, Turini P, Giovannini V, Mondovi B, Cirilli R, La Torre F (2004) Synthesis and selective inhibitory activity of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives against monoamine oxidase. J Med Chem 47:2071–2074

Chimenti F, Carradori S, Secci D, Bolasco A, Bizzarri B, Chimenti P, Granese A, Yanez M, Orallo F (2010) Synthesis and inhibitory activity against human monoamine oxidase of N1-thiocarbamoyl-

3,5-di(hetero)aryl-4,5-dihydro-(1H)-pyrazole derivatives. Eur J Med Chem 45:800–804

Congiu C, Onnis V, Vesci L, Castorina M, Pisano C (2010) Synthesis and in vitro antitumor activity of new 4,5-dihydropyrazole derivatives. Bioorg Med Chem 18:6238–6248

Dawane BS, Konda SG, Mandawad GG, Shaikh BM (2010) Poly(ethylene glycol) (PEG-400) as an alternative reaction solvent for the synthesis of some new 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-imidazol-5yl)-2-pyrazolines and their in vitro antimicrobial evaluation. Eur J Med Chem 45:387–392

Diamond MS (2009) Progress on the development of therapeutics against West Nile virus. Antiviral Res 83:214–227

Fioravanti R, Bolasco A, Manna F, Rossi F, Orallo F, Ortuso F, Alcaro S, Cirilli R (2010) Synthesis and biological evaluation of N-substituted-3,5-diphenyl-2-pyrazoline derivatives as cyclooxygenase (COX-2) inhibitors. Eur J Med Chem 45: 6135–6138

Foley P, Gerlach M, Youdim MB, Riederer P (2000) MAO-B inhibitors: multiple roles in the therapy of neurodegenerative disorders? Parkinsonism Relat Disord 6:25–47

Gareri P, Falconi U, De Fazio P, De Sarro G (2000) Conventional and new antidepressant drugs in the elderly. Prog Neurobiol 61:353–396

Girisha KS, Kalluraya B, Narayana V (2010) Synthesis and pharmacological study of 1-acetyl/propyl-3-aryl-5-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-pyrazoline. Eur J Med Chem 45:4640–4644

Gokhan N, Yesilada A, Ucar G, Erol K, Bilgin AA (2003) 1-N-substituted thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazolines: synthesis and evaluation as MAO inhibitors. Arch Pharm (Weinheim) 336:362–371

Gokhan-Kelekci N, Yabanoglu S, Kupeli E, Salgin U, Ozgen O, Ucar G, Yesilada E, Kendi E, Yesilada A, Bilgin AA (2007) A new therapeutic approach in Alzheimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and antiinflammatory analgesics. Bioorg Med Chem 15:5775–5786

Gokhan-Kelekci N, Koyunoglu S, Yabanoglu S, Yelekci K, Ozgen O, Ucar G, Erol K, Kendi E, Yesilada A (2009) New pyrazoline bearing 4(3H)-quinazolinone inhibitors of monoamine oxidase: synthesis, biological evaluation, and structural determinants of MAO-A and MAO-B selectivity. Bioorg Med Chem 17:675–689

Havrylyuk D, Zimenkovsky B, Vasylenko O, Zaprutko L, Gzella A, Lesyk R (2009) Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. Eur J Med Chem 44:1396–1404

Husain K, Abid M, Azam A (2008) Novel Pd(II) complexes of 1-N-substituted 3-phenyl-2-pyrazoline derivatives and evaluation of antiamoebic activity. Eur J Med Chem 43:393–403

Insuasty B, Garcia A, Quiroga J, Abonia R, Ortiz A, Nogueras M, Cobo J (2011) Efficient microwave-assisted synthesis and antitumor activity of novel 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols]. Eur J Med Chem 46: 2436–2440

Jagrat M, Behera J, Yabanoglu S, Ercan A, Ucar G, Sinha BN, Sankaran V, Basu A, Jayaprakash V (2011) Pyrazoline based MAO inhibitors: synthesis, biological evaluation and SAR studies. Bioorg Med Chem Lett 21:4296–4300

Jayaprakash V, Sinha BN, Ucar G, Ercan A (2008) Pyrazoline-based mycobactin analogues as MAO-inhibitors. Bioorg Med Chem Lett 18:6362–6368

Jayashree BS, Arora S, Venugopala KN (2008) Microwave assisted synthesis of substituted coumarinyl chalcones as reaction intermediates for biologically important coumarinyl heterocycles. Asian J Chem 20:1–7

Kaplancikli ZA, Ozdemir A, Turan-Zitouni G, Altintop MD, Can OD (2010) New pyrazoline derivatives and their antidepressant activity. Eur J Med Chem 45:4383–4387

Karuppasamy M, Mahapatra M, Yabanoglu S, Ucar G, Sinha BN, Basu A, Mishra N, Sharon A, Kulandaivelu U, Jayaprakash V (2010) Development of selective and reversible pyrazoline based MAO-A inhibitors: synthesis, biological evaluation and docking studies. Bioorg Med Chem 18:1875–1881

Khode S, Maddi V, Aragade P, Palkar M, Ronad PK, Mamledesai S, Thippeswamy AH, Satyanarayana D (2009) Synthesis and pharmacological evaluation of a novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents. Eur J Med Chem 44:1682–1688

Lidstrom P, Tierney J, Wathey B, Westman J (2001) Microwave assisted organic synthesis—a review. Tetrahedron 57:9225–9283

Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92:180–185

Maccioni E, Alcaro S, Orallo F, Cardia MC, Distinto S, Costa G, Yanez M, Sanna ML, Vigo S, Meleddu R, Secci D (2010) Synthesis of new 3-aryl-4,5-dihydropyrazole-1-carbothioamide derivatives. An investigation on their ability to inhibit monoamine oxidase. Eur J Med Chem 45:4490–4498

Manna F, Chimenti F, Bolasco A, Bizzarri B, Befani O, Pietrangeli P, Mondovi B, Turini P (1998) Inhibitory effect of 1,3,5-triphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives on activity of amine oxidases. J Enzyme Inhib 13:207–216

Manna F, Chimenti F, Bolasco A, Secci D, Bizzarri B, Befani O, Turini P, Mondovi B, Alcaro S, Tafi A (2002) Inhibition of amine oxidases activity by 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives. Bioorg Med Chem Lett 12:3629–3633

Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson AA, Houle S (2006) Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Arch Gen Psychiatry 63:1209–1216

Mishra N, Sasmal D (2011) Development of selective and reversible pyrazoline based MAO-B inhibitors: virtual screening, synthesis and biological evaluation. Bioorg Med Chem Lett 21:1969–1973

Mitoma J, Ito A (1992) Mitochondrial targeting signal of rat liver monoamine oxidase B is located at its carboxy terminus. J Biochem 111:20–24

Ozdemir A, Turan-Zitouni G, Kaplancikli ZA, Revial G, Guven K (2007) Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. Eur J Med Chem 42:403–409

Ozdemir Z, Kandilci HB, Gumusel B, Calis U, Bilgin AA (2008) Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-thienyl)pyrazoline derivatives. Arch Pharm (Weinheim) 341:701–707

Palaska E, Aytemir M, Uzbay IT, Erol D (2001) Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. Eur J Med Chem 36:539–543

Parekh S, Bhavsar D, Savant M, Thakrar S, Bavishi A, Parmar M, Vala H, Radadiya A, Pandya N, Serly J, Molnar J, Shah A (2011) Synthesis of some novel benzofuran-2-yl(4,5-dihyro-3,5-substituted diphenylpyrazol-1-yl) methanones and studies on the antiproliferative effects and reversal of multidrug resistance of human MDR1-gene transfected mouse lymphoma cells in vitro. Eur J Med Chem 46:1942–1948

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167

Pletscher A (1991) The discovery of antidepressants: a winding path. Experientia 47:4–8

Porsolt RD (1981) Antidepressants. In: Enna SJ, Malick JB, Richelson E (eds) Neurochemical, behavioural and clinical perspectives. Raven Press, New York, pp 129–139

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327–336

Rajendra Prasad Y, Lakshmana Rao A, Prasoona L, Murali K, Ravi Kumar P (2005) Synthesis and antidepressant activity of some 1,3,5-triphenyl-2-pyrazolines and 3-(2″-hydroxy naphthalen-1″-yl)-1,5-diphenyl-2-pyrazolines. Bioorg Med Chem Lett 15:5030–5034

Ramajayam R, Tan KP, Liu HG, Liang PH (2010) Synthesis and evaluation of pyrazolone compounds as SARS-coronavirus 3C-like protease inhibitors. Bioorg Med Chem 18:7849–7854

Ruhoglu O, Ozdemir Z, Calis U, Gumusel B, Bilgin AA (2005) Synthesis of and pharmacological studies on the antidepressant and anticonvulsant activities of some 1,3,5-trisubstituted pyrazolines. Arzneimittelforschung 55:431–436

Sahoo A, Yabanoglu S, Sinha BN, Ucar G, Basu A, Jayaprakash V (2010) Towards development of selective and reversible pyrazoline based MAO-inhibitors: synthesis, biological evaluation and docking studies. Bioorg Med Chem Lett 20:132–136

Siddiqui ZN, Musthafa TN, Ahmad A, Khan AU (2011) Thermal solvent-free synthesis of novel pyrazolyl chalcones and pyrazolines as potential antimicrobial agents. Bioorg Med Chem Lett 21:2860–2865

Sivakumar PM, Prabhu Seenivasan S, Kumar V, Doble M (2010) Novel 1,3,5-triphenyl-2-pyrazolines as anti-infective agents. Bioorg Med Chem Lett 20:3169–3172

Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85:367–370

Stirrett KL, Ferreras JA, Jayaprakash V, Sinha BN, Ren T, Quadri LE (2008) Small molecules with structural similarities to siderophores as novel antimicrobials against *Mycobacterium tuberculosis* and *Yersinia pestis*. Bioorg Med Chem Lett 18:2662–2668

Thase ME (2012) The role of monoamine oxidase inhibitors in depression treatment guidelines. J Clin Psychiatry 73(Suppl 1):10–16

Vogel HG (ed) (2002) Drug discovery and evaluation: pharmacological assays, 2nd edn. Springer, Berlin

Volz HP, Gleiter CH (1998) Monoamine oxidase inhibitors. A perspective on their use in the elderly. Drugs Aging 13:341–355

Wanare G, Aher R, Kawathekar N, Ranjan R, Kaushik NK, Sahal D (2010) Synthesis of novel alpha-pyranochalcones and pyrazoline derivatives as *Plasmodium falciparum* growth inhibitors. Bioorg Med Chem Lett 20:4675–4678

Willner P, Mitchell PJ (2002) The validity of animal models of predisposition to depression. Behav Pharmacol 13:169–188

Youdim MB, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci 7:295–309

Zeller EA, Barsky J (1952) In vivo inhibition of liver and brain monoamine oxidase by 1-isonicotinyl-2-isopropyl hydrazine. Proc Soc Exp Biol Med 81:459–461

Zhuang ZP, Marks B, McCauley RB (1992) The insertion of monoamine oxidase A into the outer membrane of rat liver mitochondria. J Biol Chem 267:591–596