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ORIGINAL PAPER

Design, synthesis, preliminary pharmacological evaluation, and docking studies of pyrazoline derivatives

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Ten derivatives of N1 substituted/unsubstituted 5-(4-chlorophenyl)-3-(2-thienyl) pyrazoline were synthesised from chalcone-like intermediate and substituted phenyl hydrazines, hydrazine hydrate, and semi/thiosemicarbazide. The chemical structure of compounds was confirmed by means of IR, ^{1}H NMR, mass spectroscopy, and elemental analysis. The antidepressant and anticonvulsant activities were investigated by Porsolt's behavioural despair test (forced swimming) and maximum electroshock seizure test, respectively. Rota–Rod test was performed to assess any probable changes in motor coordination induced by the test compounds. Four compounds (*IId*, *IIg*, *IIi*, and *IIj*) exhibited good activity profile against depression and docking studies confirmed their consensual interaction with monoamine oxidase A. In addition, compounds *IIc* and *IIe* showed protection against MES-induced seizures.

Keywords: pyrazoline, antidepressant, anticonvulsant, docking

Introduction

Compounds with pyrazoline ring have received widespread attention in recent years. They have been reported as possessing a wide range of biological activities such as anti-inflammatory, anti-microbial, anti-androgenic, and anti-thrombotic properties (Fioravanti et al., 2010; Rani et al., 2011; El-Wahab et al., 2011; Amr et al., 2006; Casimiro-Garcia et al., 2006). In addition to these effects, in the last decade pyrazolines and substituted pyrazolines have emerged as promising anti-depressant and anti-convulsant agents (Kaplancikli et al., 2010; Gok et al., 2010; Amnerkar & Bhusari, 2010; Siddiqui et al., 2009). Of all the synthesised pyrazoline derivatives, the 1,3,5 tri-substituted derivatives are of particular importance. Prasad et al. (2005) reported that the compounds possessing electron-releasing groups on both aromatic rings in positions 3 and 5 of substituted pyrazolines considerably enhanced the anti-depressant activity. In addition, Ozdemir et al. (2008) adopted the thienyl function (a bio-isostere of phenyl group) in position 3 of the pyrazoline nucleus. The compounds were found to be highly anti-convulsant while di-substitution of 2-thienyl in position 3,5 confirmed them as potent anti-depressants. Chimenti et al. (2004, 2008, 2010) demonstrated that either the N1 acetyl group or N1-propanoyl substitution in position 1 increased the potency and selectivity towards the monoamine oxidase A (MAO-A) inhibition. The authors recently reported on several diverse N1-thiocarbamoyl-3,5-di(hetero)aryl pyrazoline derivatives and observed that the presence of the chlorine atom in position 4 of the 5-phenyl substituent on the pyrazoline ring was significant for the MAO inhibitory activity. Both MAO-A and MAO-B are enzymes which contain flavin adenine dinucleotide (FAD) and catalyse the α-carbon oxidation of a variety of monoamines such as 5-hydroxytryptamine (serotonin, 5-HT), 4-(2-aminoethyl)benzene-1,2-diol (dopamine, DA), and 4-((1*R*)-2-amino-1-hydroxyethyl)benzene-1,2-diol (norepinephrine, NE) localised in human brain neurons (Youdim et al., 2006). Since 5-HT is metabolised by MAO-A in the brain, selective MAO-A inhibitors have

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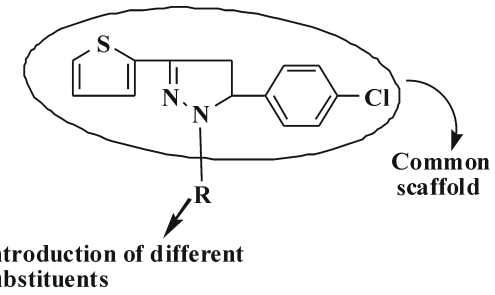

Fig. 1. Scaffold of the designed pyrazoline derivatives.

been used in the treatment of depression. The increase in the levels of the mood-elevating neurotransmitters 5-HT in the brain alleviates the symptoms of mood disorders (Fowler et al., 2010). On the basis of the above findings, we aimed to obtain some N1 substituted/unsubstituted pyrazoline derivatives with 5-(4-chlorophenyl)-3-(2-thienyl) pyrazoline as the common scaffold (Fig. 1), with anticipated anti-depressant and/or anti-convulsant activities.

Experimental

All reagents and solvents used in the study were of analytical grade purity and procured from Sigma–Aldrich (India). The progress of the reaction was monitored by thin layer chromatography with hexane/ethyl acetate (φ_r = 3 : 2) as the mobile phase and performed on Merck silica gel 60 F254 aluminium sheets (Merck, Germany); the products were purified by recrystallisation. Melting points were determined in open capillaries using Stuart SMP10 (Barloworld Scientific Ltd., UK), electrothermal melting point apparatus and were not corrected. IR spectra were recorded on a Shimadzu 8400S FTIR (Shimadzu Corporation, Japan) spectrophotometer using KBr pellets and $\tilde{\nu}$ were recorded in cm^{-1}. ^1H NMR (300 MHz) spectra were acquired on a JEOL AL300 FT-NMR (Jeol Ltd., Japan) in $CDCl_3$ using TMS as the internal standard and the chemical shifts were reported in δ. The mass spectrum was obtained on a Hewlett Packard model GCD-1800A (Hewlett Packard, USA) electron impact mass spectrometer at 70 eV ionising beam and using a direct insertion probe. Elemental analyses for C, H, and N were performed on Exeter CE-440 (Hewlett–Packard, USA) elemental analyser.

Synthesis of (E)-3-(4-chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (I)

Equimolar concentrations (0.04 mol) of 1-thiophen-2-yl-ethanone and 4-chlorobenzaldehyde were added to the 10 % aqueous solution of NaOH and ethanol (30 mL) in order to carry out Claisen–Schmidt condensation. The reaction mixture was then stirred at room temperature for 3 h and the product thus obtained was filtered, dried, and recrystallised from ethanol (Manna et al., 2002).

General procedure for the synthesis of compounds IIa–IIh

Equimolar concentrations (0.1 mol) of *I* and 2-, 4-substituted phenylhydrazine, semi/thiosemicarbazide, and hydrazine hydrate in ethanol were refluxed for 4 h. The reaction mixture was then poured into ice-cold water and the precipitate so obtained was washed with water, dried, and recrystallised from ethanol to afford the target compounds *IIa–IIh*.

General procedure for the Synthesis of compounds IIi–IIj

A mixture of compound *I* (1.0 mol) and semi/thiosemicarbazide (1.0 mol) in ethanolic NaOH (0.02 mol, 50 mL) was refluxed for about 2 h. The reaction mixture was then poured into ice-cold water and the precipitate so obtained was separated by filtration, washed with water, dried, and recrystallised from ethanol to afford the target compounds *IIi* and *IIj*.

Biological assays

The present biological study was approved by the Banaras Hindu University Animal Ethical Committee (No. Dean/10-11/163). All the newly synthesised compounds (*IIa–IIj*) were tested for their anti-depressant and anti-convulsant activities. The Rota–Rod test was performed to assess any probable changes in motor coordination induced by the test compounds. Seizure assay was carried out in accordance with the phase-1 test of the anti-epileptic drug development (ADD) programme which was developed by the National Institute of Neurological and Communicative Disorders and Stroke (Krall et al., 1978).

Anti-depressant activity (Porsolt's behavioural despair test)

The synthesised compounds were screened for their anti-depressant activity using Porsolt's behavioural despair (forced swimming) test (Porsolt et al., 1977). The mice (22–25 g) were housed in groups of six under standard conditions with access to food and water ad libitum. The synthesised compounds (10 mg kg^{-1}), and 3-(10,11-dihydro-5*H*-dibenzo(*b*,*f*)azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine (imipramine) sulphate, as a reference anti-depressant drug (10 mg kg^{-1}), were suspended in a 1 % aqueous solution of polyoxyethylene (20) sorbitan monooleate (Tween 80). On the test-day, the drugs were injected intraperitoneally into mice in a standard volume of 0.5 mL per 20 g body mass, 30 min prior to the test. The reference animals received 1 % aqueous solution of Tween 80. One

Table 1. Characteristics of the prepared compounds

Compound	Formula	M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p.
			C	H	N	%	°C
I	$C_{13}H_9ClOS$	248.81	62.78 63.01	3.65 3.62	–	82	104–106
IIa	$C_{19}H_{15}ClN_2S$	338.12	67.35 67.53	4.46 4.61	8.27 8.25	71	135–137
IIb	$C_{19}H_{14}Cl_2N_2S$	372.67	61.13 60.92	3.78 3.65	7.50 7.48	69	130–132
IIc	$C_{19}H_{14}BrClN_2S$	417.38	54.63 54.81	3.38 3.37	6.71 6.69	70	121–123
IId	$C_{20}H_{17}ClN_2OS$	368.74	65.12 64.89	4.65 4.63	7.59 7.56	67	140–142
IIe	$C_{19}H_{14}ClN_3O_2S$	383.09	59.45 59.53	3.68 3.69	10.95 10.91	63	137–139
IIf	$C_{19}H_{14}Cl_2N_2S$	372.12	61.13 61.24	3.78 3.79	7.50 7.48	66	125–127
IIg	$C_{20}H_{17}ClN_2S$	352.21	68.07 67.42	4.86 4.84	7.94 7.96	68	134–136
IIh	$C_{13}H_{11}ClN_2S$	362.13	59.42 59.27	4.22 4.29	10.66 10.68	70	133–135
IIi	$C_{14}H_{12}ClN_3S_2$	321.04	52.25 52.06	3.76 3.75	13.06 13.01	75	180–182
IIj	$C_{14}H_{12}ClN_3OS$	305.06	54.99 54.85	3.96 3.97	13.74 13.77	87	195–198

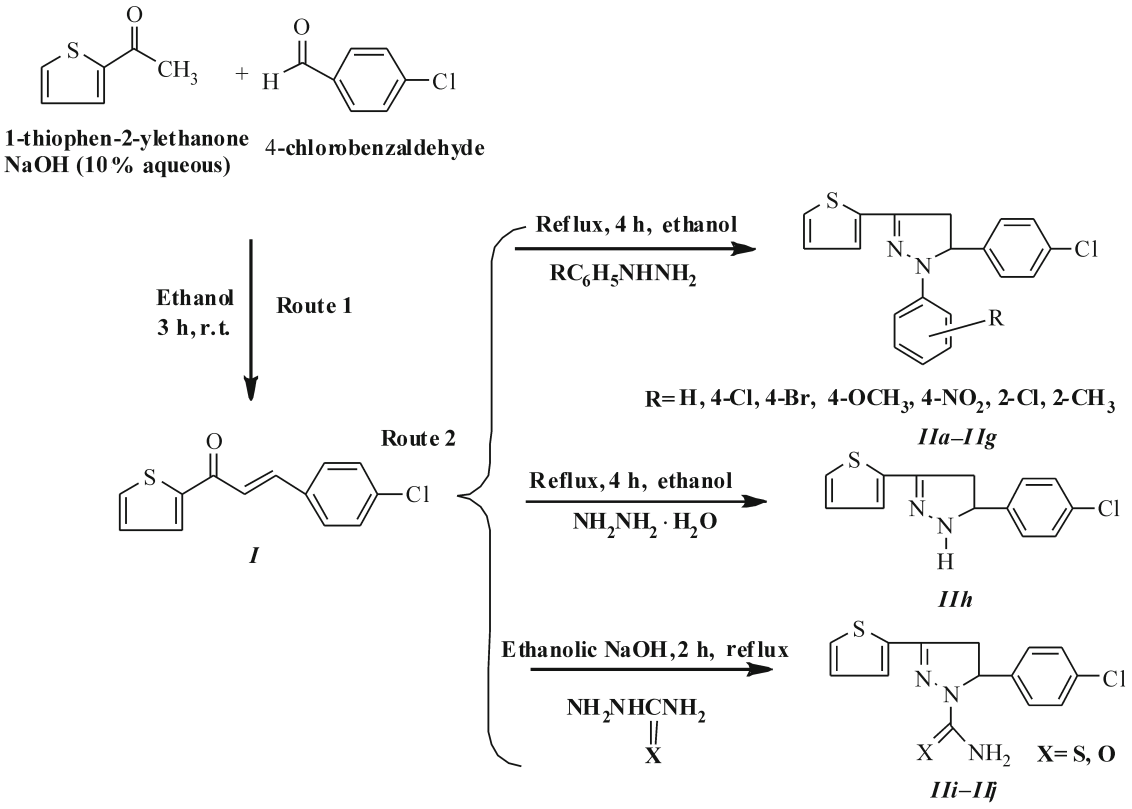

Fig. 2. Synthesis of compounds *IIa–IIj*.

mouse at a time was transferred into a plexiglass cylinder (height of 25 cm, diameter of 20 cm), containing water of (25 ± 2)°C up to a height of 15 cm and the mice were observed for 6 min. At the end of the first 2 min, the animals showing initial vigorous struggling became immobile. Then the duration of the

phase of immobility in each mouse was measured in the next 4 min period. The period of immobility was accounted as passive floating without struggling and making only those movements which were necessary to keep its head above the surface of the water. Changes in the duration of immobilisation were evaluated using one-way analysis of variance (ANOVA) Dunnet's post hoc test (GraphPad Instat Version 3.01) expressed as means ± standard error of the mean (SEM) (Motulsky, 1984). A p-value of less than 0.05 was considered statistically significant.

Anti-convulsant activity (maximal electroshock seizure test)

Albino mice of either sex, weighing 20–25 g were used. Food was withdrawn 12–15 h before commencing the experiment while water was withdrawn immediately before the experiment. The synthesised compounds were suspended in 30 % of aqueous solution of poly(ethylene glycol) (PEG 400) and administered to the mice intraperitoneally in a standard volume of 0.5 mL per 20 g body mass at a dose of 30 mg kg^{-1}, 100 mg kg^{-1}, 300 mg kg^{-1}. Reference animals received 30 % aqueous PEG 400 and 5,5-diphenylimidazolidine-2,4-dione (phenytoin) was used as a reference drug (10 mg kg^{-1}). Maximal seizure was induced by application of an electrical stimulus (50 mA at 60 Hz) of 0.2 s in duration transmitted via corneal electrodes across the brain after 30 min and 4 h following the drug administration. After applying the shock, the animals were observed for the type of convulsion produced and the hind limb extensor response was taken as the end point.

Rota–Rod performance test

The Rota–Rod test was carried out in accordance with the method described by Dunham and Miya (Vogel, 2002). The cardinal feature of the test is to ascertain the impairment of motor performance, ataxia, loss of skeletal muscular strength, and acute neurotoxicity produced by drugs in pre-clinical studies. Albino mice weighing between 20–24 g (n = 4–8), where n represents the number of mice in a group, were trained to balance on the knurled wooden rotating rod (3.2 cm diameter) that rotated at 6 min^{-1}. Trained animals were treated with the test compounds at different dose levels (30 mg kg^{-1}, 100 mg kg^{-1}, and 300 mg kg^{-1}) administered intraperitoneally. After 30 min and 4 h, respectively, the mice were placed onto the rotating rod for one minute. Neurological impairment was determined as the inability of the animal to remain on the rod for 1 min.

Molecular docking

In order to gain some structural insights into the binding mode of the ligand with MAO-A, the compounds *IId* and *IIj* were computationally docked into MAO-A (Enzyme Commission number: EC 1.4.3.4) using Glide. The crystal structure of MAO-A (PDB ID: 2BXR) retrieved from the protein data bank was used for molecular docking (De Colibus et al., 2005). 2BXR was subsequently optimised with the "protein preparation wizard" workflow, as implemented in the Schrödinger 2011 package. A cycle of constrained minimisations allowing a maximum root-mean-square deviation (RMSD) of 0.30 Å from the original structure was carried out. The ligands were built using Maestro 9.2 build panel and prepared by LigPrep 2.5 version v25111 (Schrödinger, LLC, USA) application that uses OPLS 2005 force field. OPLS stands for optimised potential liquid simulations and it gave the corresponding energy minima 3D conformers of the ligands. A grid of 17.2612, –25.248, and 13.1624 points in x, y, and z directions was built, centred on the co-factor FAD N5 atom. The default settings were used for all other parameters. The extra precision (GLIDE-XP) protocol implemented in GLIDE was used for docking (Glide 5.7, Schrödinger Inc., USA) (Halgren et al., 2004).

Results and discussion

Spectral characterisation

The proposed derivatives were synthesised as illustrated in Fig. 2. Physical properties and elemental analysis (Table 1) as well as all the spectral data (Table 2) are in accordance with the structures of the synthesised compounds. The spectra of *I* displayed the characteristic —C═O stretching at 1708 cm^{-1} and a sharp peak at 1658 cm^{-1} due to α,β —CH═CH. The derivatives showed diagnostic infrared absorptions at 1578–1598 cm^{-1} for —C═N stretching of the pyrazoline nucleus. In addition, all the compounds displayed C4—H deformation (1354–1442 cm^{-1}) and C5—N1 stretching (1068–1142 cm^{-1}). Compounds *IIi* and *IIj* showed additional thiocarbamoyl NH stretching vibration (3481 cm^{-1}), carbamoyl NH stretching (3464–3481 cm^{-1}), —C═S stretching at a lower frequency of 1345 cm^{-1}, and —C═O stretching at 1684 cm^{-1}. ^{1}H NMR spectra of *IIa–IIj* exhibited three spectral ranges in which each appears as a double doublet due to the presence of non-magnetically equivalent pyrazoline Ha, Hb, and Hx protons. The geminal pyrazoline proton in position 4 represented by the Ha and Hb methylene protons displayed signals at δ 3.04–3.13 (upfield) and δ 3.13–3.93 (downfield), respectively. The methine proton Hx appeared further downfield at δ 5.16–5.98 and the aromatic and thienyl protons at chemical shift in the range of δ 6.44–7.82. All the other protons belonging to the methyl and methoxy groups were seen according to the expected chemical shift. The mass spec-

Table 2. Spectral characterisation of newly prepared compounds

Compound	Spectral data[a]
I	IR, $\tilde{\nu}$/cm^{-1}: 855 (C—Cl stretching), 1658 (α,β CH═CH), 1708 (C═O) ^{1}H-NMR ($CDCl_3$), δ: 6.65 (d, 1H, —CO—CH═), 7.20–7.68 (m, 7H, Ar—H and thiophene), 7.81 (d, 1H, ═CH—Ar) MS, m/z: 248.81 (M^+)
IIa	IR, $\tilde{\nu}$/cm^{-1}: 1069 (C5—N1 stretching), 1373 (C4—H deformation), 1591 (C═N), 3010 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 3.05 (1H, dd, Ha), 3.89 (1H, dd, Hb), 5.26 (1H, dd, Hx), 6.93–7.71 (12H, m, thiophene and Ar—H) MS, m/z: 338.12 (M^+)
IIb	IR, $\tilde{\nu}$/cm^{-1}: 1076 (C5—N1 stretching), 1089 (C—S—C stretching), 1383 (C4—H deformation), 1597 (C═N), 3012 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 3.07 (1H, dd, Ha), 3.83 (1H, dd, Hb), 5.21 (1H, dd, Hx), 6.94–7.68 (11H, m, thiophene and Ar—H) MS, m/z: 372.67 (M^+)
IIc	IR, $\tilde{\nu}$/cm^{-1}: 1081 (C5—N1 stretching), 1085 (C—S—C stretching), 1367 (C4—H deformation), 1593 (C═N), 3050 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 3.07 (1H, dd, Ha), 3.86 (1H, dd, Hb), 5.24 (1H, dd, Hx), 6.85–7.64 (11H, m, thiophene and Ar—H) MS, m/z: 417.38 (M^+)
IId	IR, $\tilde{\nu}$/cm^{-1}: 1068 (C5—N1 stretching), 1082 (C—S—C stretching), 1132 (C—O stretching), 1358 (C4—H deformation), 1595 (C═N), 3041 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 2.78 (3H, s, OCH_3), 3.04 (1H, dd, Ha), 3.82 (1H, dd, Hb), 5.16 (1H; dd, Hx), 6.73–7.73 (11H, m, thiophene and Ar—H) MS, m/z: 368.74 (M^+)
IIe	IR, $\tilde{\nu}$/cm^{-1}: 1072 (C5—N1 stretching), 1088 (C—S—C stretching), 1350 (N—O stretching), 1359 (C4—H deformation), 1586 (C═N), 3017 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 3.08 (1H, dd, Ha), 3.75 (1H, dd, Hb), 5.53 (1H, dd, Hx), 7.05–7.82 (11H, m, thiophene and Ar—H) MS, m/z: 383.09 (M^+)
IIf	IR, $\tilde{\nu}$/cm^{-1}: 1087 (C—S—C stretching), 1138 (C5—N1 stretching), 1442 (C4—H deformation), 1595 (C═N), 3045 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 3.13 (1H, dd, Ha), 3.71 (1H, dd, Hb), 5.27 (1H, dd, Hx), 6.79–7.60 (11H, m, thiophene and Ar—H) MS, m/z: 372.12 (M^+)
IIg	IR, $\tilde{\nu}$/cm^{-1}: 1079 (C—S—C stretching), 1142 (C5—N1 stretching), 1378 (C4—H deformation), 1578 (C═N), 3032 (CH thienyl) ^{1}H-NMR ($CDCl_3$), δ: 2.12 (1H, s, CH_3), 3.10 (1H, dd, Ha), 3.78 (1H, d, Hb), 5.22 (1H, d, Hx), 6.89–7.65 (11H, m, thiophene and Ar—H) MS, m/z: 352.21 (M^+)
IIh	IR, $\tilde{\nu}$/cm^{-1}: 1075 (C5—N1 stretching), 1091 (C—S—C stretching), 1354 (C4—H deformation), 1597 (C═N), 3012 (CH thienyl), 3290 (NH streching) ^{1}H-NMR ($CDCl_3$), δ: 3.12 (1H, dd, Ha), 3.93 (1H, dd, Hb), 5.29 (1H, dd, Hx), 6.44–7.49 (7H, m, thiophene and Ar—H), 7.51 (1H, s, NH) MS, m/z: 362.13 (M^+)
IIi	IR, $\tilde{\nu}$/cm^{-1}: 1071 (C—S—C stretching), 1085 (C5—N1 stretching), 1345 (C═S stretching), 1425 (C4—H deformation), 1592 (C═N stretching), 3024 (CH thienyl), 3481 (NH stretching) ^{1}H-NMR ($CDCl_3$), δ: 3.12 (1H, dd, Ha), 3.81 (1H, dd, Hb), 5.98 (1H; dd, Hx), 7.07–7.63 (7H, m, thiophene and Ar—H), 7.75 (1H, s, NH_2) MS, m/z: 321.04 (M^+)
IIj	IR, $\tilde{\nu}$/cm^{-1}: 1072 (C—S—C stretching), 1095 (C5—N1 stretching), 1433 (C4—H deformation), 1595 (C═N stretching), 1684 (C═O stretching), 3021 (CH thienyl), 3464 (NH stretching) ^{1}H-NMR ($CDCl_3$), δ: 3.08 (1H, dd, Ha), 3.84 (1H, dd, Hb), 5.29 (1H, dd, Hx), 7.04–7.52 (7H, m, thiophene and Ar—H), 7.56 (2H, s, NH_2) MS, m/z: 305.06 (M^+)

a) Ha and Hb denote the methylene protons and Hx denotes the methine proton of pyrazoline nucleus.

tra of the compounds were studied and the molecular ion peaks ($(M)^+$), which were found consistent for all the compounds. The elemental analysis results were within ± 0.4 % of the theoretical values.

Biological screening

In vivo anti-depressant activity of all the compounds was assessed in mice by applying the forced swimming test. The test is effective in predicting the

activity of a wide variety of anti-depressants including MAO inhibitors (Bourin et al., 2002; Petit-Demouliere et al., 2005). The anti-convulsant activity was evaluated by the MES test and the Rota–Rod test was used to evaluate neurotoxicity. Pharmacological data of the compounds are shown in Table 3 and Table 4.

Anti-depressant activity

A few of the compounds tested (Table 3, Fig. 3) were noted as exhibiting considerable anti-depressant properties, especially *IId*, *IIg*, *IIi*, and *IIj* which exhibited remarkable activities revealing the lowest duration of immobility comparable with 10 mg $kg^{-1}$of imipramine sulphate used as the reference drug. The structure-activity relationship based on the results observed indicated that the type of substituent attached to the N1 of the 5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-(1*H*)-pyrazole scaffold modulated the activity. Attachment of the phenyl group alone does not elicit such favourable activity as compared with the substituted aryl nucleus. Electron-withdrawing substituents decrease the activity in descending order i.e. the higher the electro-negativity the lower the activity,

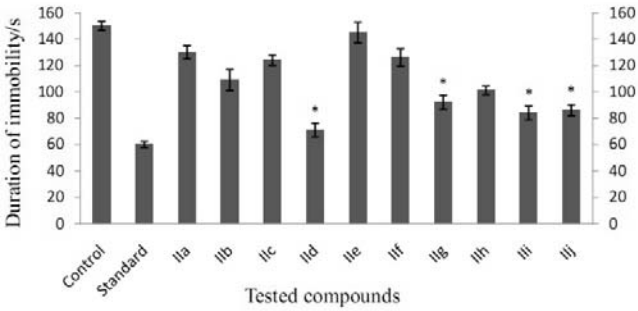

Fig. 3. Duration of immobility by Porsolt's behavioural despair test; ∗ – significant compared with the reference (Dunnet's test; $p < 0.05$) ($n = 6$, dose = 10 mg kg^{-1}).

Table 3. Anti-depressant activities of compounds *IIa–IIj*

Compound	Substituent	Duration of immobility/s	Change from reference/%
		Mean ± SEM	
IIa	H	130 ± 4.9	–13.33
IIb	4-Cl	109 ± 8.1	–27.33
IIc	4-Br	124 ± 3.9	–17.33
IId	4-OCH_3	71 ± 5.3	–52.60[a]
IIe	4-NO_2	145 ± 7.9	–3.33
IIf	2-Cl	126 ± 6.5	–16.00
IIg	2-CH_3	92 ± 5.3	–38.66[a]
IIh	H	101 ± 3.2	–32.66
IIi	S	84 ± 5.2	–44.00[a]
IIj	O	86 ± 4.1	–42.66[a]
Imipramine sulphate[b]		60 ± 2.3	–60.00
Control (vehicle)		150 ± 3.5	–

a) Significant in comparison with the reference (Dunnet's test; $p < 0.05$) ($n = 6$, dose = 10 mg kg^{-1}); *b*) dose = 10 mg kg^{-1}, intraperitoneally.

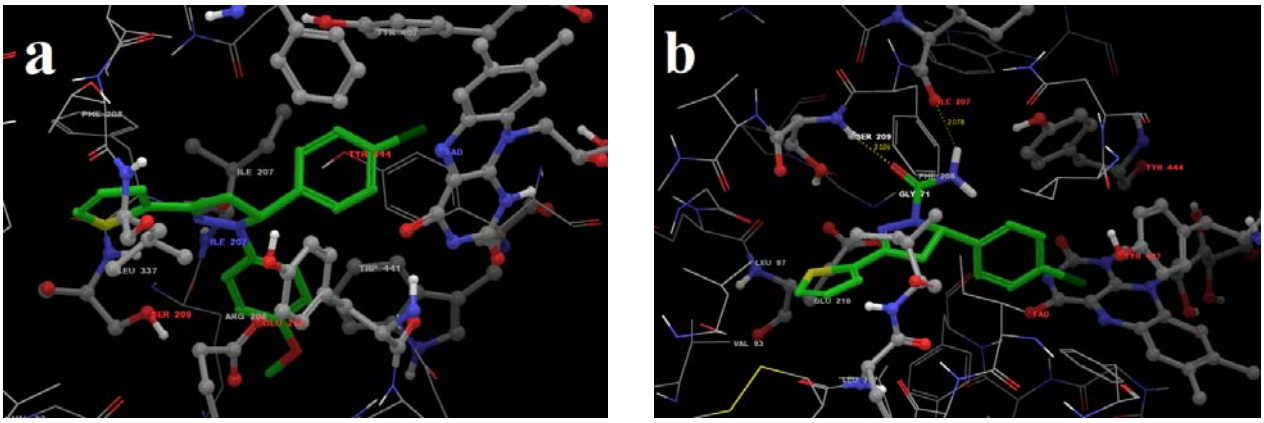

Fig. 4. Interacting mode and orientations of compound (a) *IId* and (b) *IIj* at the active sites of MAO-A. The FAD co-factor and the interacting key amino acid residues are shown in ball and stick models. The hydrogen bonds are displayed as dashed yellow.

Table 4. Phase-1 anti-convulsant screening of compounds *IIa*–*IIj*

Compound	Dose	MES[a]		Toxicity[b]	
	mg kg^{-1}	30 min	4 h	30 min	4 h
IIa	30	0/1	0/1	0/4	0/2
	100	0/3	1/3	0/8	0/4
	300	0/1	1/1	0/4	0/2
IIb	30	0/1	0/1	0/4	0/2
	100	2/3	1/3	0/8	0/4
	300	1/1	0/1	1/4	1/2
IIc	30	1/1	0/1	0/4	0/2
	100	1/3	1/3	2/8	1/4
	300	1/1	1/1	3/4	2/2
IId	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	1/8	1/4
	300	0/1	0/1	2/4	0/2
IIe	30	0/1	1/1	0/4	0/2
	100	3/3	1/3	1/8	0/4
	300	1/1	1/1	1/4	0/2
IIf	30	0/1	0/1	0/4	0/2
	100	0/3	1/3	0/8	0/4
	300	0/1	1/1	1/4	0/2
IIg	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/8	0/4
	300	0/1	0/1	1/4	2/2
IIh	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/8	0/4
	300	0/1	0/1	0/4	0/2
IIi	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/8	2/4
	300	0/1	1/1	1/4	0/2
IIj	30	0/1	0/1	0/4	0/2
	100	0/3	1/3	0/8	0/4
	300	0/1	1/1	1/4	0/2
Phenytoin	30	5/6	5/6	–	–

MES – maximum electroshock seizure. *a*) Number of animals protected/number of animals tested; *b*) Rota–Rod test (number of animals exhibiting toxicity/number of animals tested).

whereas the electron-releasing substituents appear to be more favourable. The bio-isosterically-related compounds *IIi* and *IIj* showed similar and potent anti-depressant activity.

Anti-convulsant activity

Out of all the compounds evaluated, *IIb*, *IIc*, and *IIe* exhibited anti-MES activity at either 100 mg kg^{-1} or 300 mg kg^{-1} in 30 min; in addition, *IIc* was also active at 30 mg kg^{-1}. The compounds *IIb*, *IIc*, and *IIe* were more active within 30 min than in 4 h, indicating that they induced rapid onset of the action while *IIf* and *IIj* elicited late onset of the action. Mostly, the change in motor coordination was observed for *IIb*, *IIc*, *IIg*, and *IIi* at the dose level of 300 mg kg^{-1}. Finally, it may be concluded that *IIc* and *IIe* displayed better activity profiles compared with other derivatives as anti-convulsants with the 4-nitro phenyl derivative (*IIe*) considered as the most potent at a dose level of 100 mg kg^{-1} with a sustained action (Table 4).

Molecular docking

Compounds *IId* and *IIj* representing N1 aryl and carbamoyl substitutions, respectively, were successfully docked at the active site of MAO-A. The binding configuration of *IId* (Fig. 4) shows that the 4-methoxy phenyl is well lodged in the pocket which is formed due to a cavity-shaping loop, characterised by ILE 207, SER 209, ARG 206, GLU 216, and TRP 441, whereas the thienyl ring is accommodated in a pocket surrounded by LEU 337 and PHE 208. The 4-chloro phenyl ring fits into the aromatic cage formed by FAD, TYR 407, and TYR 444. These binding modes were in agreement with most of the suggested ligand–enzyme contact points reported in the literature (Karuppasamy et al., 2010; Jia & Zhu, 2010) and suggested that the compounds might probably act via the MAO-A inhibition. The compound *IIj* (Fig. 4) displayed a similar binding pattern to *IId* except that the N1 carbamoyl group showed hydrogen bonding with SER 209 and ILE 207, the oxygen serving as the hydrogen bond acceptor and the amino hydrogen as the donor. It is also worth noting here that the *S*-configuration of *IIj* binds with both the amino acids while the *R*-configuration binds only with SER 209.

Conclusion

A new series of pyrazoline derivatives with a common skeleton was synthesised and evaluated for their anti-depressant and anti-convulsant properties. A few of them are considered to be promising compounds due to their respective activities. Four compounds (*IId*, *IIg*, *IIi*, and *IIj*) exhibited a good activity profile against depression and docking studies confirmed their consensual interaction with the MAO-A enzyme. Two compounds (*IIc* and *IIe*) showed protection against MES-induced seizures. Therefore, the study deserves further investigation, particularly with respect to that of the in vitro MAO-A inhibitory activity and simultaneous derivatisation of the building block 5-(4-chlorophenyl)-3-(2-thienyl) pyrazoline.

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