

Anticonvulsant and Antidepressant Activity Studies of Synthesized Some New 1,3,5-trisubstituted-2-pyrazolines

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Abstract: Thirteen new 1,3,5-trisubstituted-2-pyrazolines were synthesized by reacting 1-(4-aminophenyl)-3-(substituted aryl/heteroaryl)-2-propen-1-one with phenyl hydrazine hydrochloride under basic alcoholic conditions. All structures of the compounds were elucidated by means of their IR, ¹H and ¹³C NMR, Electron Ionization (EI)-mass and microanalyses and screened for their antidepressant and anticonvulsant activities. The antidepressant activity of the compounds was investigated by Porsolt's behavioral despair test (forced swimming) at a dose of 10 mg/kg, on albino mice and compared with tranylcypromine, as reference standard. Among the compounds examined, the compounds **4b** and **4j** showed significant antidepressant activity. Anticonvulsant activity and neuroprotective activity of compounds were determined by maximal electroshock seizures (MES) test, subcutaneous pentylenetetrazole (*scMet.*) induced seizures test and a rotarod test, respectively, in mice. 1-phenyl-3-(4-aminophenyl)-5-(4-dimethylaminophenyl)-2-pyrazoline (**4j**) was found to be protective against MES and *scMet.* induced seizures in between the dose range of 30-300 mg/kg at both half hour and four hour. Compounds **4k**, **4l** and **4m** failed to show neuroprotectivity at a dose of 300 mg/kg, while with the remaining synthesized compounds' exhibited neuroprotectivity at 300 mg/kg dose levels. The results revealed that the compounds possessing electron-releasing groups such as dimethylamino, methoxy, methyl, and chlorine substituents on aromatic ring at position 5 of pyrazolines considerably enhanced the antidepressant and anticonvulsant activities.

Key words: 2-Pyrazolines, Chalcones, Antidepressant activity, Anticonvulsant activity.

Introduction and Experimental

Pyrazoline is five-membered heterocyclic having two adjacent nitrogen atoms within the ring and one endocyclic double bond with basic in nature and are considered as a cyclic hydrazine moiety. It plays a crucial role in the development of theory in heterocyclic chemistry and used extensively as synthons in organic synthesis¹. Literature reveals that pyrazoline derivatives were found to have a broad spectrum of biological activities such as tranquilizing, muscle relaxant, psychoanaleptic, antihypotensive, antipyretic-analgesic, anti-inflammatory, insecticidal, antimicrobial, antidepressant and anticonvulsant activities. Derivatives of 2-pyrazolines also exhibit cytotoxic activity, inhibition of platelet aggregation, herbicidal activity, and cannabinoid CB₁-receptor modulators². Previous reports showed that some 1,3,5-trisubstituted-2-pyrazolines, have monoamine oxidase (MAO) inhibitory properties in rat brain³. Recently an enantioselective MAO-A and MAO-B inhibitory properties of 1-thiocarbamoyl-2-pyrazolines are reported⁴. Isocarboxazid⁵, phenelzine⁶, and

meclobemide⁷ exhibited well known antidepressant activity while inhibiting monoamine oxidase (MAO) enzyme in animals and man because of having hydrazine and amine moiety (Figure 1).

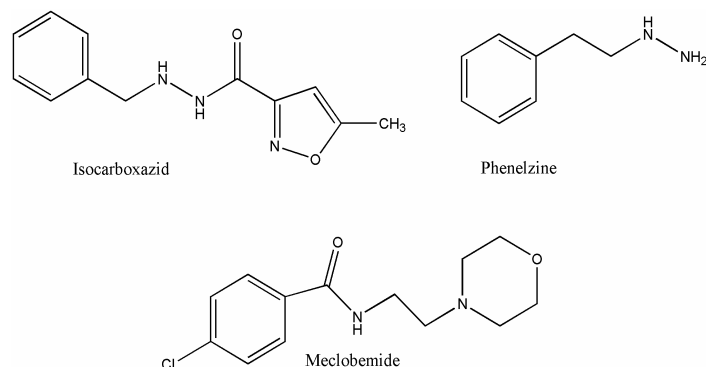


Figure 1. Structures of isocarboxazid, phenelzine and meclobemide.

Additionally, it has been shown that 1,3,5-triphenyl-2-pyrazolines⁸, 3-(2-furyl)pyrazoline derivatives⁹, 3-(2-thienyl)pyrazoline derivatives¹⁰, N,3-(Substituted diphenyl)-5-phenyl-1H-pyrazoline-1-carbothioamide derivatives¹¹, and 1-[(N,N-disubstituted thiocarbamoylthio)- acetyl]-3-(2-thienyl)-5-aryl-2-pyrazolines¹² have antidepressant activity in Porsolt's behavioral despair (forced swimming) test, and 4-(3*H*)-quinazolinone substituted pyrazolines¹³, and 3,5-diarylcarbothioamide pyrazolines¹⁴ have antidepressant activity in MAO inhibitory activity by *in vivo* and *in vitro* tests. Previous studies also reported that the presence of electron-donating substituents on the phenyl ring at position 5 of the pyrazoline ring produced a relatively higher degree of MAO inhibition, whereas electron-withdrawing substituents produced a lesser degree of enzyme inhibition⁸.

Epilepsy, is a group of disorders of the central nervous system (CNS) characterized by paroxysmal cerebral dysrhythmia, manifesting itself as brief episodes (seizures) of loss or disturbances of consciousness, with or without characteristic body movements, sensory or psychiatric phenomena and inflicts more than 60 million people worldwide¹⁵. About 95% of clinically available drugs for the treatment of epilepsy were approved before 1985 and they could provide seizure control for 60-70% of patient, but their use is often limited by adverse effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anaemia and even life threatening condition. The earlier studies showed that epilepsy is two kinds, one with grandmal and other with petitmal. Anticonvulsant drugs with MES (maximal electroshock) activity are generally useful in grandmal, while ScMET (subcutaneous metrazole) antagonists are effective in petitmal¹⁶. Hence there is need for synthesis of substituted 2-pyrazolines may enhance the chances for producing more number of compounds with specific action and less side effects. And also several substituted derivatives like amide, ameltolide analogues were synthesized and evaluated for their anticonvulsant activity^{17,18,19,20}. Furthermore, N-substituted-N'-(3,5-di/and 1,3,5-trimethylpyrazole-4-yl)thiourea and urea derivatives²¹, Ethyl-1,4,5-trisubstituted-2,5-dihydro-1H-pyrazole-3-carboxylates²², 1-[4,5-dihydro-5-phenyl-3-(phenylamine) pyrazol-1-yl] ethanones²³ and 3,5-dimethylpyrazole derivatives²⁴ have anticonvulsant activity in MES and scMet. test in animal models.

The present study was conducted to synthesize new compounds with specific antidepressant and anticonvulsant activity. The desired target compounds (**4a-m**) were prepared from the 1,3-diarylpropenones (**3a-m**)^{25,26} and phenylhydrazine hydrochloride by refluxing them together in a basic alcoholic media (Scheme 1) and all these synthesized 2-pyrazolines (Table 1) were screened for their antidepressant and anticonvulsant activity in animal models.

Table 1: 1,3,5-Trisubstituted 2-pyrazolines 4a-m.

Compound	Ar	Compound	Ar
4a	2-Chlorophenyl	4h	3,4,5-Trimethoxyphenyl
4b	4-Chlorophenyl	4i	4-Methylphenyl
4c	2,4-Dichlorophenyl	4j	4-Dimethylaminophenyl
4d	4-Bromophenyl	4k	9-Anthracenyl
4e	4-Methoxyphenyl	4l	3-Pyridinyl
4f	3,4-Dimethoxyphenyl	4m	4-Pyridinyl
4g	2,4-Dimethoxyphenyl		

Materials and Methods

Chemistry

Melting points were determined on a standard Boetius apparatus and are uncorrected. The IR spectra were recorded in Perkin-Elmer BXF1 FT-IR spectrophotometer using KBr disc method. ^1H and ^{13}C NMR spectra were recorded in the indicated solvent on a Bruker AMX 400 and 100 MHz respectively with tetramethylsilane (TMS) as internal standard (chemical shifts in δ ppm). The splitting patterns of ^1H -NMR were designed as follows: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. The Mass [EI-MS (70 eV)] spectrometry was performed using Agilent 1100 MSD spectrometer in the electrospray mode. The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within $\pm 0.4\%$ of the theoretical values. TLC was performed on Silica Gel F₂₅₄ plates (Merck) with visualization by UV (254 nm) chamber with protective filters. The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade. All the 2-pyrazolines have been purified by column chromatography performed on silica gel (100-200 mesh, Merck).

Compounds **4a-m** were prepared by reacting the (*E*)-1-(4-aminophenyl)-3-arylsubstituted-2-propen-1-ones (4-aminochalcones) **3a-m** with phenylhydrazine hydrochloride in the presence of pyridine and absolute alcohol. Physicochemical and spectroscopic characterization of the **3a-m** has been described previously^{25,26}.

General procedure for the synthesis of 1,3,5-trisubstituted-2-pyrazolines (**4a-m**)

50 μmole of pyridine was added to a reaction vial containing 50 μmol of corresponding chalcone (**3a-m**) and 400 μl absolute ethanol. The reaction mixture was added with 200 μl of a 0.25 M solution of phenylhydrazine hydrochloride in absolute ethanol. The mixture was capped, shaken to ensure mixing and then allowed to reflux at 70°C for 2 to 6 hours. Completion of the reaction was identified by TLC. Upon completion, the reaction mixture was cooled to room temperature and was shaken to ensure mixing and then concentrated to dryness *in vacuo* to afford the product as a solid. It was purified by column chromatography, using ethyl acetate and hexane mixture as mobile phase obtained pure 2-pyrazoline derivatives (**4a-m**).

1-phenyl-3-(4-aminophenyl)-5-(2-Chlorophenyl)-2-pyrazoline **4a**

Yellow solid, mp: 138 \pm 2°C, Yield = 80.0%. IR (KBr, cm^{-1}): 3432, 3376 (NH_2), 1592 (C=N), 1498 (C=C), 1124 (C-N), 1178 (C-Cl). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.96 (1H, dd, H_A , $J_{AB} = 17.45$ Hz, $J_{AX} = 3.82$ Hz), 3.92 (1H, dd, H_B , $J_{BA} = 17.20$ Hz, $J_{BX} = 11.80$ Hz), 5.54 (1H, dd, H_X , $J_{XA} = 3.74$ Hz, $J_{XB} = 11.82$ Hz), 5.47 (2H, bs, C-4- NH_2), 6.46 (2H, d, $J = 8.44$ Hz, C-3 & 5-H), 6.60 (1H, t, $J = 7.80$ Hz, C-4-H), 6.85 (2H, d, $J = 8.42$ Hz, C-3 & 5-H), 7.12 (3H, m, C-4,5 and 6-H), 7.27 (2H, d, $J = 8.80$ Hz, C-2 & 6-H), 7.42 (2H, d, $J = 8.42$ Hz, C-2 & 6-H), 7.54 (1H, d, $J = 7.66$ Hz, C-3-H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 39.52 (C-4), 44.82 (C-5), 113.5 (C-2 & C-6), 118.05 (C-3 & C-5), 119.2 (C-4), 122.56 (C-1), 126.89 (C-5), 127.2 (C-4), 128.21 (C-6), 128.57 (C-3), 129.8 (C-3 & C-5), 130.6 (C-2 & C-6), 132.53 (C-2), 142.80 (C-1), 144.54 (C-1), 148.64 (C-4), 155.53 (C-3). EI-MS (m/z): 348.3 [(M+H)⁺]. Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_3\text{Cl}$: C, 72.51; H, 5.22; N, 12.08%; Found. C, 72.55; H, 5.22; N, 12.06%.

1-phenyl-3-(4-aminophenyl)-5-(4-Chlorophenyl)-2-pyrazoline **4b**

Orange yellow solid, mp: 148 \pm 2°C, Yield = 71.0%. IR (KBr, cm^{-1}): 3445, 3315 (NH_2), 1599 (C=N), 1494 (C=C), 1184 (C-N), 1102 (C-Cl). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.99 (1H, dd, H_A , $J_{AB} = 17.42$ Hz, $J_{AX} = 3.80$ Hz), 3.82 (1H, dd, H_B , $J_{BA} = 17.52$ Hz, $J_{BX} = 11.80$ Hz), 5.36 (1H, dd, H_X , $J_{XA} = 3.86$ Hz, $J_{XB} = 11.64$ Hz), 5.44 (2H, bs, C-4- NH_2), 6.59 (2H, d, $J = 8.28$ Hz, C-3 & 5-H), 6.66 (1H, t, $J = 7.20$ Hz, C-4-H), 6.92 (2H, d, $J = 8.42$ Hz, C-3 & 5-H), 7.22 (2H, d, $J = 7.82$ Hz, C-2 & 6-H), 7.33 (2H, $J = 8.42$ Hz, C-2 & 6-H), 7.41 (4H, m, C-2, 3, 5 & 6-H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 40.4 (C-4), 54.43 (C-5), 112.32 (C-2 & C-6), 116.23 (C-3 & C-5), 119.2 (C-4), 122.56 (C-1), 128.34 (C-2 & C-6), 128.9 (C-3 & C-5), 130.32 (C-3 & C-5), 131.64 (C-2 & C-6), 132.56 (C-4), 141.5 (C-1), 143.64 (C-1), 149.23 (C-4), 155.45 (C-3). EI-MS (m/z): 348.4 [(M+H)⁺]. Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_3\text{Cl}$: C, 72.51; H, 5.22; N, 12.08%; Found. C, 72.56; H, 5.22; N, 12.06%.

1-phenyl-3-(4-aminophenyl)-5-(2,4-dichlorophenyl)-2-pyrazoline **4c**

Orange red solid, mp: 155 \pm 2°C, Yield = 66.0%. IR (KBr, cm^{-1}): 3456, 3332 (NH_2), 1603 (C=N), 1487 (C=C), 1185 (C-N), 1118 (C-Cl). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 2.99 (1H, dd, H_A , $J_{AB} = 17.24$ Hz, $J_{AX} = 4.24$

Hz), 3.92 (1H, dd, H_B, $J_{BA} = 17.60$ Hz, $J_{BX} = 11.86$ Hz), 5.52 (1H, dd, H_X, $J_{XA} = 4.02$ Hz, $J_{XB} = 11.80$ Hz), 5.50 (2H, bs, C-4-NH₂), 6.57 (2H, d, $J = 8.44$ Hz, C-3 & 5-H), 6.70 (1H, t, $J = 8.00$ Hz, C-4-H), 6.84 (2H, d, $J = 8.42$ Hz, C-3 & 5-H), 7.10 (1H, d, $J = 8.02$ Hz, C-6-H), 7.16 (2H, d, $J = 7.64$ Hz, C-2 & 6-H), 7.31 (1H, d, $J = 8.14$ Hz, C-5-H), 7.44 (2H, d, $J = 8.04$ Hz, C-2 & 6-H), 7.72 (1H, s, C-3-H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 41.6 (C-4), 59.78 (C-5), 113.5, (C-2 & C-6), 118.05 (C-3 & C-5), 117.54 (C-4), 122.58 (C-1), 127.2, 128.9, 129.3 (C-5, 3, 6), 130.34 (C-2 & C-6), 130.65 (C-2 & C-6), 132.1, 132.6 (C-2 & C-4), 138.5 (C-1), 144.4 (C-1), 148.64 (C-4), 155.65 (C-3). EI-MS (m/z): 383.3 [(M+H)⁺]. Anal. Calcd for C₂₁H₁₇N₃Cl₂: C, 65.88; H, 4.88; N, 18.85%; Found. C, 65.91; H, 4.87; N, 18.82%.

1-phenyl-3-(4-aminophenyl)-5-(4-bromophenyl)-2-pyrazoline 4d

Orange solid, mp: 120±2⁰C, Yield = 76.0%. IR (KBr, cm⁻¹): 3476, 3324 (NH₂), 1597 (C=N), 1497 (C=C), 1189 (C-N), 1069 (C-Br). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.02 (1H, dd, H_A, $J_{AB} = 17.24$ Hz, $J_{AX} = 4.04$ Hz), 3.82 (1H, dd, H_B, $J_{BA} = 17.02$ Hz, $J_{BX} = 11.84$ Hz), 5.74 (1H, dd, H_X, $J_{XA} = 4.44$ Hz, $J_{XB} = 11.84$ Hz), 5.48 (2H, bs, C-4-NH₂), 6.59 (2H, d, $J = 8.04$ Hz, C-3 & 5-H), 6.67 (1H, t, $J = 7.84$ Hz, C-4-H), 6.95 (2H, d, $J = 8.44$ Hz, C-3 & 5-H), 7.13 (2H, d, $J = 7.60$ Hz, C-3 & 5-H), 7.24 (2H, d, $J = 8.00$ Hz, C-2 & 6-H), 7.43 (2H, d, $J = 8.04$ Hz, C-2 & 6-H), 7.50 (2H, d, $J = 7.86$ Hz, C-2 & 6-H). EI-MS (m/z): 392.3 [(M+H)⁺]. Anal. Calcd for C₂₁H₁₈N₃Br: C, 64.42; H, 4.71; N, 10.76%; Found. C, 64.48; H, 4.71; N, 10.73%.

1-phenyl-3-(4-aminophenyl)-5-(4-methoxyphenyl)-2-pyrazoline 4e

Yellow solid, mp: 138±2⁰C, Yield = 73.0%. IR (KBr, cm⁻¹): 3473, 3366 (NH₂), 1598 (C=N), 1499 (C=C), 1184 (C-N), 1248, 1030 (C-O-C). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.96 (1H, dd, H_A, $J_{AB} = 17.44$ Hz, $J_{AX} = 3.80$ Hz), 3.70 (3H, s, C-4-OCH₃), 3.78 (1H, dd, H_B, $J_{BA} = 17.24$ Hz, $J_{BX} = 12.00$ Hz), 5.26 (1H, dd, H_X, $J_{XA} = 4.08$ Hz, $J_{XB} = 11.88$ Hz), 5.45 (2H, bs, C-4-NH₂), 6.61 (2H, d, $J = 8.46$ Hz, C-3 & C-5-H), 6.65 (1H, t, $J = 7.24$ Hz, C-4-H), 6.88 (4H, d, $J = 8.24$ Hz, C-3 & 5-H), 6.94 (2H, dd, $J = 7.28$ Hz, $J = 7.84$ Hz, C-3 & 5-H), 7.11 (2H, d, $J = 8.62$ Hz, C-2 & 6-H), 7.21 (2H, d, $J = 8.46$ Hz, C-2 & 6-H), 7.43 (2H, d, $J = 8.44$ Hz, C-2 & 6-H). EI-MS (m/z): 344.1 [(M+H)⁺]. Anal. Calcd for C₂₂H₂₁N₃O: C, 76.96; H, 6.14; N, 12.24%; Found. C, 76.90; H, 6.15; N, 12.25%.

1-phenyl-3-(4-aminophenyl)-5-(3,4-dimethoxyphenyl)-2-pyrazoline 4f

Reddish yellow solid, mp:138±2⁰C, Yield = 73.0%. IR (KBr, cm⁻¹): 3453, 3368 (NH₂), 1597 (C=N), 1498 (C=C), 1187 (C-N), 1259, 1024 (C-O-C). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.99 (1H, dd, H_A, $J_{AB} = 17.22$ Hz, $J_{AX} = 3.84$ Hz), 3.70 (6H, s, C-3 & 4-OCH₃), 3.78 (1H, dd, H_B, $J_{BA} = 17.26$ Hz, $J_{BX} = 11.60$ Hz), 5.22 (1H, dd, H_X, $J_{XA} = 4.46$ Hz, $J_{XB} = 11.80$ Hz), 5.45 (2H, bs, C-4-NH₂), 6.59 (2H, d, $J = 8.24$ Hz, C-3 & C-5-H), 6.65 (1H, t, $J = 7.28$ Hz, C-4-H), 6.76 (1H, d, $J = 6.42$ Hz, C-5-H), 6.88 (1H, d, $J = 7.0$ Hz, C-6-H), 6.92 (1H, s, C-2-H), 6.96 (2H, d, $J = 7.60$ Hz, C-3 & 5-H), 7.12 (2H, d, $J = 7.82$ Hz, C-2 & 6-H), 7.43 (2H, d, $J = 8.00$ Hz, C-2 & 6-H). EI-MS (m/z): 374.1 [(M+H)⁺]. Anal. Calcd for C₂₃H₂₃N₃O₂: C, 73.99; H, 6.16; N, 11.26%; Found. C, 73.94; H, 6.16; N, 11.27%.

1-phenyl-3-(4-aminophenyl)-5-(2,4-dimethoxyphenyl)-2-pyrazoline 4g

Reddish yellow solid, mp:132±2⁰C, Yield = 58.0%. IR (KBr, cm⁻¹): 3468, 3365 (NH₂), 1594 (C=N), 1498 (C=C), 1180 (C-N), 1299, 1034 (C-O-C). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.09 (3H, s, C-4-OCH₃), 2.25 (3H, s, C-2-OCH₃), 2.94 (1H, dd, H_A, $J_{AB} = 17.20$ Hz, $J_{AX} = 4.64$ Hz), 3.78 (1H, dd, H_B, $J_{BA} = 17.20$ Hz, $J_{BX} = 11.62$ Hz), 5.27 (1H, dd, H_X, $J_{XA} = 4.26$ Hz, $J_{XB} = 11.60$ Hz), 5.48 (2H, bs, C-4-NH₂), 6.59 (2H, d, $J = 7.84$ Hz, C-3 & 5-H), 6.64 (1H, t, $J = 7.46$ Hz, C-4-H), 6.93 (2H, dd, $J = 6.00$ Hz, $J = 7.02$ Hz, C-5 & 6-H), 7.08 (1H, s, C-3-H), 7.14 (4H, m, C-2, 3, 5 & 6-H), 7.43 (2H, d, $J = 8.24$ Hz, C-2 & 6-H). EI-MS (m/z): 374.2 [(M+H)⁺]. Anal. Calcd for C₂₃H₂₃N₃O₂: C, 73.99; H, 6.16; N, 11.26%; Found. C, 73.98; H, 6.15; N, 11.24%.

1-phenyl-3-(4-aminophenyl)-5-(3,4,5-trimethoxyphenyl)-2-pyrazoline 4h

Orange yellow solid, mp:172 ±2⁰C, Yield = 51.0%. IR (KBr, cm⁻¹): 3468, 3375 (NH₂), 1594 (C=N), 1497 (C=C), 1181 (C-N), 1228, 1030 (C-O-C). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.04 (1H, dd, H_A, $J_{AB} = 17.60$ Hz, $J_{AX} = 4.64$ Hz), 3.64 (3H, s, C-4-OCH₃), 3.70 (6H, s, C-3 & 5-OCH₃), 3.88 (1H, dd, H_B, $J_{BA} = 17.20$ Hz, $J_{BX} = 12.00$ Hz), 5.18 (1H, dd, H_X, $J_{XA} = 4.26$ Hz, $J_{XB} = 11.68$ Hz), 5.45 (2H, s, C-4-NH₂), 6.59 (2H, d, $J = 8.40$ Hz, C-3 & 5-H), 6.61 (2H, dd, $J = 7.40$ Hz, $J = 7.20$, C-3 & 5-H), 6.69 (1H, t, $J = 7.20$ Hz, C-4-H), 6.98 (2H, d, $J = 7.60$ Hz, C-2 & 6-H), 7.15 (2H, d, $J = 7.86$ Hz, C-2 & 6-H), 7.43 (2H, d, $J = 8.40$ Hz, C-2 & 6-H). EI-MS

(m/z): 404.3 [(M+H)⁺]. Anal. Calcd for C₂₄H₂₄N₃O₃: C, 71.46; H, 6.20; N, 10.42%; Found. C, 76.76; H, 5.83; N, 8.16%.

1-phenyl-3-(4-aminophenyl)-5-(4-methylphenyl)-2-pyrazoline 4i

Yellow solid, mp: 118±2⁰C, Yield = 54.0%. IR (KBr, cm⁻¹): 3461, 3375 (NH₂), 1597 (C=N), 1500 (C=C), 1189 (C-N). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.09 (3H, s, C-4-CH₃), 2.86 (1H, dd, H_A, J_{AB} = 17.20 Hz, J_{AX} = 3.86 Hz), 3.78 (1H, dd, H_B, J_{BA} = 17.48 Hz, J_{BX} = 11.62 Hz), 5.27 (1H, dd, H_X, J_{XA} = 4.04 Hz, J_{XB} = 11.60 Hz), 5.46 (2H, s, C-4-NH₂), 6.61 (2H, d, J = 8.40 Hz, C-3 & 5-H), 6.67 (4H, m, C-2, 3, 5 & 6-H), 6.84 (2H, d, J = 7.60 Hz, C-2 & 6-H), 7.02 (1H, t, J = 7.20 Hz, C-4-H), 7.11 (4H, m, C-2, 3, 5 & 6-H). EI-MS (m/z): 328.3 [(M+H)⁺]. Anal. Calcd for C₂₂H₂₁N₃: C, 80.73; H, 6.42; N, 12.84%; Found. C, 80.69; H, 6.43; N, 12.86%.

1-phenyl-3-(4-aminophenyl)-5-(4-dimethylaminophenyl)-2-pyrazoline 4j

Orange red solid, mp:115±2⁰C, Yield = 50.0%. IR (KBr, cm⁻¹): 3431, 3321 (NH₂), 1601 (C=N), 1497 (C=C), 1185 (C-N). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.79 {6H, s, C-4-N(CH₃)₂}, 2.86 (1H, dd, H_A, J_{AB} = 17.40 Hz, J_{AX} = 3.88 Hz), 3.78 (1H, dd, H_B, J_{BA} = 16.40 Hz, J_{BX} = 11.80 Hz), 5.30 (1H, dd, H_X, J_{XA} = 3.82 Hz, J_{XB} = 11.84 Hz), 5.43 (2H, bs, C-4-NH₂), 6.39 (2H, d, J = 8.60 Hz, C-2 & C-6-H), 6.59 (2H, d, J = 8.40 Hz, C-3 & C-5-H), 6.60 (1H, t, J = 7.4 Hz, C-4-H), 6.64 (2H, d, J = 8.4 Hz, C-3 & C-5-H), 6.98 (2H, d, J = 8.4 Hz, C-2 & C-6-H), 7.24 (2H, d, J = 8.4 Hz, C-3 & 5-H), 7.11 (2H, d, J = 8.2 Hz, C-2 & C-6-H), 7.41 (2H, d, J = 8.4 Hz, C-3 & C-5-H). EI-MS (m/z): 357.0 [(M+H)⁺]. Anal. Calcd for C₂₃H₂₄N₄: C, 77.52; H, 6.74; N, 15.73%; Found. C, 77.02; H, 6.73; N, 15.72%.

1-phenyl-3-(4-aminophenyl)-5-(9-Anthracenyl)-2-pyrazoline 4k

Red solid, mp:175±2⁰C, Yield = 35.0%. IR (KBr, cm⁻¹): 3465, 3251 (NH₂), 1597 (C=N), 1495 (C=C), 1197 (C-N). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.91 (1H, dd, H_A, J_{AB} = 16.8 Hz, J_{AX} = 3.86 Hz), 3.92 (1H, dd, H_B, J_{BA} = 17.6 Hz, J_{BX} = 11.60 Hz), 4.15 (1H, dd, H_X, J_{XA} = 4.16 Hz, J_{XB} = 11.58 Hz), 5.52 (2H, bs, C-4-NH₂), 6.65 (2H, d, J = 8.4 Hz, C-3 & 5-H), 6.52 (1H, t, J = 7.0 Hz, C-4-H), 6.91 (2H, d, J = 8.4 Hz, C-2 & 6-H), 7.42 (2H, d, J = 8.0 Hz, C-3 & 5-H), 7.56 (2H, d, J = 8.4 Hz, C-2 & 6-H), 7.66 (4H, m, Anthracenyl C-2,3,6 & 7-H), 8.10 (2H, d, J = 8.0 Hz, Anthracenyl C-4 & 5-H), 8.23 (2H, d, J = 8.0 Hz, Anthracenyl C-1 & 8-H), 8.5 (1H, s, Anthracenyl C-10-H). EI-MS (m/z): 414.2 [(M+H)⁺]. Anal. Calcd for C₂₉H₂₃N₃: C, 84.26; H, 5.56; N, 10.17%; Found. C, 84.23; H, 5.56; N, 10.16%.

1-phenyl-3-(4-aminophenyl)-5-(3-pyridinyl)-2-pyrazoline 4l

Orange yellow solid, mp:170±2⁰C, Yield = 36.0%. IR (KBr, cm⁻¹): 3438, 3298 (NH₂), 1600 (C=N), 1494 (C=C), 1178 (C-N). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.09 (1H, dd, H_A, J_{AB} = 17.20 Hz, J_{AX} = 4.00 Hz), 3.82 (1H, dd, H_B, J_{BA} = 17.20 Hz, J_{BX} = 11.84 Hz), 5.44 (1H, dd, H_X, J_{XA} = 4.28 Hz, J_{XB} = 12.00 Hz), 5.50 (2H, bs, C-4-NH₂), 6.59 (2H, d, J = 8.4 Hz, C-3 & 5-H), 6.67 (1H, t, J = 6.8 Hz, C-4-H), 6.82 (2H, d, J = 8.4 Hz, C-2 & 6-H), 7.13 (2H, d, J = 7.6 Hz, C-3 & 5-H), 7.36 (1H, t, C-5-H), 7.45 (2H, d, J = 8.0 Hz, C-2 & 6-H), 7.61 (1H, d, J = 8.0 Hz, C-6-H), 8.46 (1H, d, J = 6.4 Hz, C-4-H), 8.65 (1H, s, C-2-H). EI-MS (m/z): 315.0 [(M+H)⁺]. Anal. Calcd for C₂₀H₁₈N₄: C, 76.41; H, 5.71; N, 17.82%; Found. C, 76.32; H, 5.70; N, 17.86%.

1-phenyl-3-(4-aminophenyl)-5-(4-pyridinyl)-2-pyrazoline 4m

Orange yellow solid, mp:170±2⁰C, Yield = 36.0%. IR (KBr, cm⁻¹): 3437 (NH₂), 1599 (C=N), 1497 (C=C), 1185 (C-N). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.19 (1H, dd, H_A, J_{AB} = 16.88 Hz, J_{AX} = 4.06 Hz), 3.72 (1H, dd, H_B, J_{BA} = 16.8 Hz, J_{BX} = 12.00 Hz), 5.42 (1H, dd, H_X, J_{XA} = 4.60 Hz, J_{XB} = 11.88 Hz), 5.52 (2H, s, C-4-NH₂), 6.52 (2H, d, J = 8.4 Hz, C-3 & 5-H), 6.64 (1H, t, J = 6.8 Hz, C-4-H), 6.78 (2H, d, J = 8.4 Hz, C-2 & 6-H), 7.08 (2H, d, J = 7.6 Hz, C-3 & 5-H), 7.43 (2H, d, J = 8.0 Hz, C-2 & 6-H), 7.73 (2H, d, J = 8.0 Hz, C-2 & 6-H), 8.56 (2H, d, J = 6.4 Hz, C-3 & 5-H). EI-MS (m/z): 315.2 [(M+H)⁺]. Anal. Calcd for C₂₀H₁₈N₄: C, 76.41; H, 5.71; N, 17.82%; Found. C, 76.34; H, 5.72; N, 17.85%.

Antidepressant activity

The present study was conducted in Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India and the study was approved by Institution Animal Ethical Committee.

The synthesized compounds were screened for their antidepressant activity using Porsolt's behavioral despair (forced swimming test)^{12,27}. Swiss albino mice (20-24 g) of either sex (M/s Ghosh Enterprises Ltd., Calcutta, India) were used in the present study under laboratory conditions with free access to food and water. They were housed in a quiet and temperature- and humidity-controlled room ($22 \pm 3^\circ\text{C}$ and $60 \pm 5\%$, respectively) in which a 12-h light/dark cycle was maintained (08:00-20:00 h light) in groups of six. Mice were handled for 8-10 min for four consecutive days before the experiment. On the test day, mice were dropped one at a time into a plexiglass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at $23 - 25^\circ\text{C}$ ⁸.

On this day, mice were divided into different groups ($n = 6-9$ for each group). Tranylcypromine sulfate was supplied by Sigma Chemical Co. was used as reference standard. The synthesized compounds (10 mg/kg), and tranylcypromine sulfate (10 mg/kg), were suspended in a 1% aqueous solution of Tween 80. The drugs were injected intraperitoneally (i.p.) to mice (22 ± 2 g) in a standard volume of 0.5 ml/20 g body weight, 30 min prior to the test. Controlled animals received 1% aqueous solution of Tween 80. Then, the mice were dropped individually into the plexiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. A mouse was judged immobile if it floated in the 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. The results are summarized in Table 2.

Table 2: Antidepressant activity of synthesized compounds 4a-m.

Compounds	Antidepressant activity	
	Duration of immobility (sec) (Mean \pm S.E.M.)*	Change from control (%)
4a	84 \pm 3.24*	-57.92
4b	64 \pm 2.54*	-68.31
4c	143 \pm 5.96	-29.20
4d	127 \pm 9.77	-37.12
4e	89 \pm 3.76*	-21.28
4f	143 \pm 5.87	-29.20
4g	117 \pm 6.81	-42.07
4h	133 \pm 4.86	-34.15
4i	110 \pm 2.31	-45.54
4j	76 \pm 3.78*	-62.37
4k	148 \pm 6.79	-26.73
4l	162 \pm 5.16	-19.80
4m	152 \pm 5.73	-24.75
Tranylcypromine sulfate (10 mg/kg, ip)	56 \pm 1.03*	-72.27
Control	202 \pm 1.64	-----

* Values represents the mean \pm S.E.M. ($n = 6-9$).

* Significantly compared to control (Dunnet's test: $p < 0.05$).

Anticonvulsant activity

The compounds were tested for their anticonvulsant activity against MES and scMet.-induced seizures and rotarod used in the neuroprotective activity test according to the phase-I tests of ADD (Antiepileptic Drug Development) program which were developed by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)^{28,29}. INCO electroconvulsimeter (M/s Instruments & Chemicals (P) Ltd., Ambala City, India) was used for the evaluation of anticonvulsant activity. The rotarod used in the neuroprotective test was made by MKM Enterprises Ltd., Chennai, India. Pentylentetrazole was purchased from Sigma Chemical Corporation. Twelve albino male mice (20-24 g) were used for each compound. The synthesized compounds were suspended in 30% aqueous solution of PEG 400 and injected i.p. in a standard volume of 0.5 ml/20 g body weight to the mice. Control animals received 30% aqueous PEG 400. In Phase-I screening (Table 3), each compound was administered at the dose levels of 30, 100, and 300 mg/kg for evaluating the anticonvulsant activity, and its neurotoxicity (neuroprotectivity) was measured at 30 min and 4 h

intervals after administration. Pentylenetetrazole (metrazol) was administered subcutaneously (sc). Rotarod test was performed on a 1-inch diameter knurled wooden rod; rotating at 6 rpm.

Maximal electroshock seizure (MES) test

Maximal electroshock seizures are elicited with a 60-cycle alternating current of 50 mA intensity (5-7 times that is required to elicit minimal electroshock seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline is instilled in the eye prior to application of the electrode in order to prevent the death of the animal. The MES-convulsions are divided into five phases such as (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor, and (e) recovery or death. The time (sec) spent by the animal in each phase of the convulsions was noted. Abolition of the hind limb tonic extension component of the seizure is defined as protection.

Subcutaneous pentylenetetrazole (metrazol) (scMet) test

85 mg/kg of pentylenetetrazole (produce seizures in greater than 95% of mice) is administered as a 0.5% solution in the posterior midline by sc. The animal was observed for 30 min failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neuroprotectivity test

The rotarod test was used to evaluate neuroprotectivity or neurologic toxicity. The animal was placed on a 1-inch diameter knurled wooden rod rotating at 6 rpm. 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 rotarod for 1 min.

Statistical analysis:

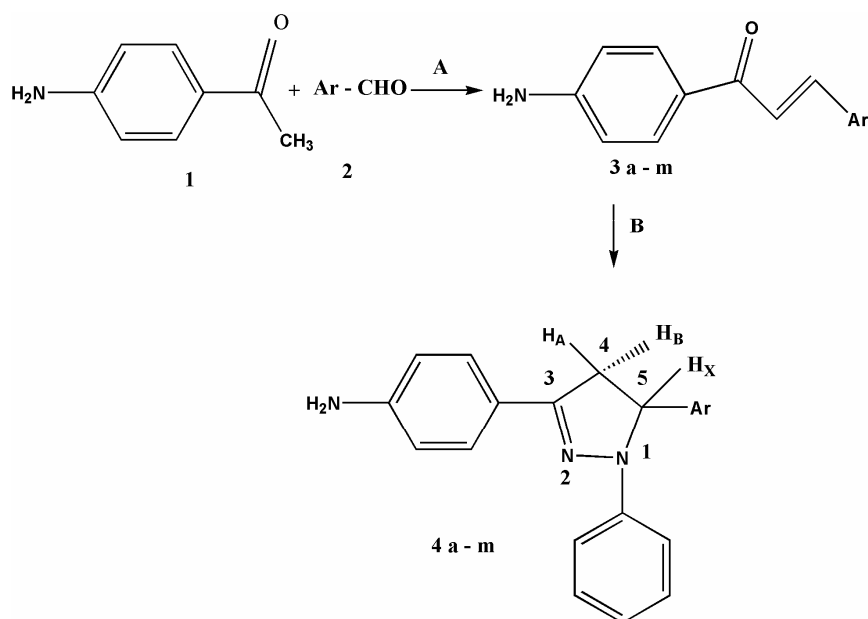
Results are expressed as mean \pm S.E.M.; n represents the number of animals. Data obtained from pharmacological experiments were analyzed with one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test, using Pharmacologic Calculation System Version 4.1. (Microcomputer Specialists). A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Chemistry

The synthesis of 1,3,5-trisubstituted-2-pyrazolines was carried out as presented in Scheme 1. Various substituted benzaldehydes (**2**) were mixed with 4-aminoacetophenone (**1**) in the presence of 10% aqueous NaOH in ethanol to yield chalcones **3a-m**. These derivatives were treated with phenylhydrazine in the presence of ethanol and pyridine, followed by cyclization to give the corresponding 1,3,5-trisubstituted-2-pyrazolines **4a-m**. All the compounds were identified by the spectral methods and microanalyses. All spectral data are in accordance with the assumed structures. In general, IR spectra showed the NH stretching vibrations at 3490-3460 cm^{-1} and 3298-3300 cm^{-1} , the C=N stretching vibrations at 1592-1603 cm^{-1} , and the C-N stretching vibrations at 1124-1185 cm^{-1} which are characteristic of the pyrazoline ring. An absence of absorption band at 930 cm^{-1} indicates the formation of pyrazoline ring.

In the $^1\text{H-NMR}$ spectra of the compounds H_A , H_B , and H_X protons of the pyrazoline ring was observed as doublet of doublet at $\delta = 2.86-3.09$ ppm ($J_{AB} = 17.20-17.45$ Hz), 3.78-3.92 ppm ($J_{AX} = 3.86-4.64$ Hz), and 5.26-5.74 ppm ($J_{BX} = 11.60-12.00$ Hz) respectively. N-H protons of the aminophenyl group was generally seen at 5.43-5.50 ppm as broad band which is disappeared when the deuteriodimethylsulphoxide solution was shaken with deuterium oxide. All the other protons belonging methyl, dimethylamino, methoxyl, benzene and pyridine rings were seen accordingly to the expected chemical shift and integral values. In the $^{13}\text{C-NMR}$ spectrum exhibited characteristic peaks between δ 170-160 ppm for ring carbons adjacent to nitrogen atom in pyrazoline nucleus, and δ 150-120 ppm for other ring carbons confirming the pyrazoline structure. The mass spectra showed the corresponding molecular ion peak $[\text{M}^+]$ or $[\text{M}+\text{H}]^+$ as the base peaks and the fragmentation pattern was characteristic of respective 2-pyrazolines. Microanalyses results were also in accordance with the theoretical amounts.



Scheme 1. Synthetic protocol of the compounds 4a-m: A) ethyl alcohol, aqueous KOH, room temperature, mixing 12-24 hr; B) phenylhydrazine hydrochloride, pyridine, ethyl alcohol, Reflux at 70 – 75 °C, 6-8 hr.

Antidepressant activity

In-vivo antidepressant activity of the compounds was assessed in mice applying the forced swimming test, which is a behavioral test, used to predict the efficacy of antidepressant drugs²⁷. It is used effectively in predicting the activity of a wide variety of antidepressants such as MAO inhibitors³⁰ and typical antidepressants³¹. It also has a good predictive value for the antidepressant potency in humans³². The obtained data on the antidepressant activity of the compounds and reference drug were given in Table 2. The compounds **4a**, **4b** and **4j** showed marked antidepressant activity. Among the mentioned derivatives, most promising results were obtained with the compounds carrying 4-chlorophenyl (**4b**) and 4-dimethyl-aminophenyl (**4j**) group at 5-position on the pyrazoline ring. The mentioned derivatives significantly reduced the duration of immobility times at the 10 mg/kg dose level when compared to the control ($p < 0.05$, Table 2). Compound **4b** was the most promising compound and significantly reduced the duration of immobility by 68.31% at a dose of 10 mg/kg compared with the control; it is having activity as that of tranylcypromine sulfate.

Anticonvulsant activity

The anticonvulsant activities of the synthesized compounds were investigated by maximal electroshock (MES) and subcutaneous pentylenetetrazole (metrazol) (scMet.) tests, and results from these experiments are shown in Table 3. According to the obtained results, the compounds showed less anticonvulsant activity. As shown in Table 3, 1-phenyl-3-(4-aminophenyl)-5-(4-Chlorophenyl)-2-pyrazoline (**4b**), 1-phenyl-3-(4-aminophenyl)-5-(4-methylphenyl)-2-pyrazoline (**4i**), and 1-phenyl-3-(4-aminophenyl)-5-(4-dimethylamino-phenyl)-2-pyrazoline (**4j**) exhibited activity against MES-induced seizures and scMet. induced seizures at 100 mg/kg dose level.

It is difficult to analyze the significant anticonvulsant activity based on their structure. As shown in Table 3, the significant difference in activity was observed depending on the substituent on phenyl ring at 5-position of the 2-pyrazoline ring. Anticonvulsant activity of the compounds bearing a halogen as substituent on phenyl ring at 5-position of pyrazoline are taken into consideration (**4a-d**), it could be concluded that the substitution of chlorine at 4-position of phenyl ring present on 5-position of 2-pyrazoline always resulted in good activity either at half hour or at four hours at the 100 mg/kg and 300 mg/kg-dose level (compound **4b**). Among the compounds with electron releasing groups on phenyl ring present at 5-position of 2-pyrazoline (**4e-j**), compound **4j** possessed the most prominent and consistent activity in the range of 30-300 mg/kg-dose levels at both half hour and four hours. It was observed that all compounds which exhibited activity were found to be protective against MES-induced seizures at their high dose level (300 mg/kg). However, only two compounds (compound **4b** and **4j**) exhibited activity against scMet.-induced seizures at the 100 mg/kg and 300 mg/kg-dose level at both half hour and four hours. And also it was found that compound **4j** was identified as lead

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