Synthesis And Anticonvulsant Activity Of Some Substituted 3,5-Diphenyl-2-Pyrazoline-1-Carboxamide Derivatives

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Abstract

Some substituted 3,5-diphenyl-2-pyrazoline-1-carboxamide derivatives were synthesized from appropriate substituted 1,3-diphenylprop-2-en-1-one (chalcone) on reaction with semicarbazide hydrochloride. The final compounds were structurally elucidated on the basis of IR, ^{1}H-NMR & mass spectral data and microanalyses. The final compounds were evaluated for anticonvulsant activity by the maximal electroshock seizure (MES) method. The neurotoxicity was determined by rotorod toxicity test on male albino mice. The preliminary results showed that all of the tested compounds were protective against MES at 100-300 mg/kg dose levels. The compounds numbered 4d-4e, 4j-4k, and 4m-4t were most protective against MES even at 30 mg/kg dose levels.

Keywords: 2-Pyrazoline-1-carboxamide; anticonvulsant activity; neurotoxicity test.

1. Introduction

2-Pyrazolines are an important class of five-member heterocyclic compounds and were found to have potential antimicrobial [1], anti-inflammatory [2], antipyretic [3], antidepressant [4], antibacterial [5], tranquillizing [6], anticancer [7], antiviral [8], antihypertensive [9], antiarrhythmic [10], antitubercular [11], psychoanaleptic [12] and antidiabetic [13] activity. However, pyrazolines are still least explored compounds for anticonvulsant profile even, their function is quite stable [14]. In view of this and our continued interest in the synthesis of bioactive heterocyclic compounds with a stable fragment, it was thought of interest to synthesize some new pyrazolines starting from chalcone and semicarbazide [15].

The synthesis of chalcones from substituted benzaldehyde and substituted acetophenone precursors proceeded according to the Claisen-Schmidt condensation. The substituted 3,5-Diphenyl-2-pyrazoline-1-carboxamide derivatives were synthesized from these chalcones using semicarbazide hydrochloride (Scheme 1). The key reactions involved are the intermediate formation of hydrazones and subsequent addition of N-H on the olefinic bond of the propenone moiety that form the ring-closed final products. In this study, 20 new compounds were synthesized (4a-t).

2. Results and Discussion

2.1. Chemistry

The synthesis of chalcones (3a-t) from substituted benzaldehyde 1 and substituted acetophenone 2 was according to the Claisen-Schmidt condensation. The substituted 3,5-Diphenyl-2-pyrazoline-1-carboxamide (4a-t) derivatives were synthesized from these chalcones using semicarbazide hydrochloride (Fig. 1).

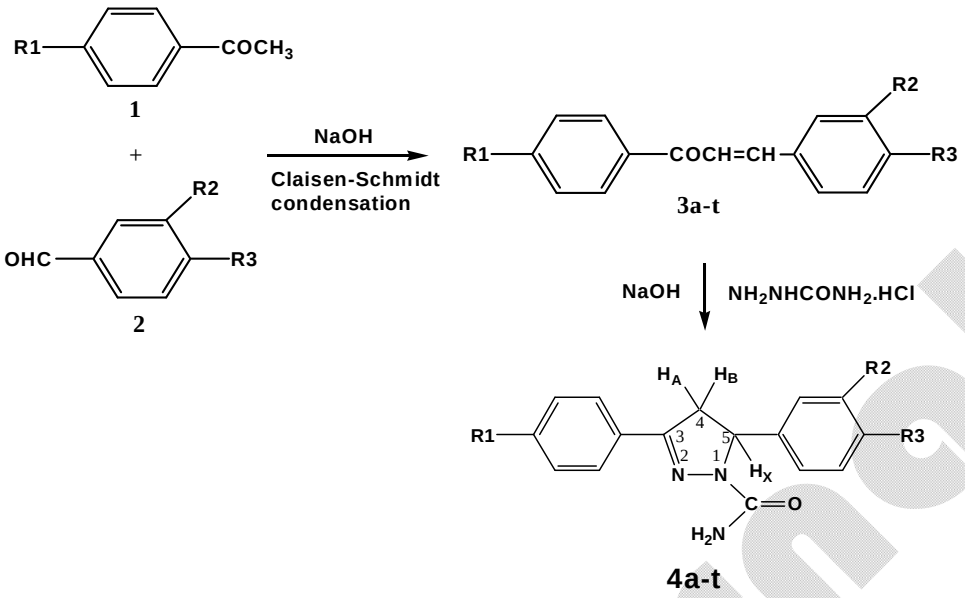


Figure 1. Reaction scheme for synthesis of title compounds.

The key reactions involved are the intermediate formation of hydrazones and subsequent addition of N-H on the olefinic bond of the propenone moiety that forms the ring-closed final products. In this study, 20 new compounds were synthesized. In the nuclear magnetic resonance spectra (^{1}H-NMR) the signals of the respective protons of the final title compounds were verified on the basis of their chemical shifts and multiplicities. Both analytical and spectral data (^{1}H-NMR, IR and Mass) of all the synthesized compounds were in full agreement with the proposed structures.

The IR spectra of the compounds show the disappearance of C=C (olefinic) and N–H stretching bands at 1584–1608 and 3257–3366 cm^{-1} respectively due to the ring closure. The IR spectra of the compounds afforded pyrazoline C=N stretching (1501-1576 cm^{-1}), C-H deformation (1362-1464 cm^{-1}), C_5-N_1 stretching (1069-1189 cm^{-1}), carbamoyl group N-H stretching (3112-3481 cm^{-1}) and C=O stretching (1315-1357 cm^{-1}) bands. In the ^{1}H-NMR spectra, olefinic protons of chalcone appeared as doublets at about 6.75 and 7.18 ppm respectively. After the ring closure, ring protons (H_A and H_B) of the final compounds showed at around 3.07 and 3.8 ppm as a doublet of doublet and also vicinal methine proton H_X showed triplet/multiplet at about 5.4 ppm due to vicinal coupling with the two magnetically nonequivalent protons of the methylene group H_A (upfield H of CH_2) and H_B (downfield H of CH_2). N-H protons of the carbamoyl group were seen at 10.1-10.3 ppm generally as broad bands. The phenyl protons were observed at the expected chemical shifts and integral values. The mass spectrum of the final compounds showed the molecular ion peak in accordance with molecular formula. The compounds were found to be protective against MES induced seizures at 30 mg.kg^{-1} dose levels after half an hour of i.p. administration.

Structure activity relationships based on the observed results indicated that, the type of aryl group substitution attached to the 5-position of pyrazoline nucleus plays a controlling role for anticonvulsant activity. It has been noticed that, attachment of the phenyl group, oriented at the 5-position of pyrazoline heterocycle, with a fluorine atom seems more favorable for an anticonvulsant active agent than the case of using a methoxy residue.

2.2. Anticonvulsant activity

All of the tested compounds were found protective against MES-induced seizures at 100-300 mg/kg dose levels.

Table 1: Data from screen I (anticonvulsant and neurotoxicity identification).

	30 min						4 hr					
Compd.	**30 mg/kg**		**100 mg/kg**		**300 mg/kg**		**30 mg/kg**		**100 mg/kg**		**300 mg/kg**	
	MES	**NT**	**MES**	**NT**	**MES**	**NT**	**MES**	**NT**	**MES**	**NT**	**MES**	**NT**
Phenytoin	4/4	0/4	2/2	1/2	1/1	1/1	2/4	0/4	2/2	0/2	1/1	1/1
4a	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4b	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4c	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4d	3/4	0/4	2/2	1/2	1/1	1/1	1/4	0/4	1/2	0/2	1/1	1/1
4e	4/4	0/4	2/2	1/2	1/1	1/1	2/4	0/4	2/2	0/2	1/1	1/1
4f	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4g	1/4	0/4	1/2	0/2	1/1	1/1	0/4	0/4	0/2	0/2	1/1	1/1
4h	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4i	2/4	0/4	2/2	1/2	1/1	1/1	1/4	0/4	1/2	0/2	1/1	1/1
4j	4/4	0/4	2/2	1/2	1/1	1/1	2/4	0/4	2/2	0/2	1/1	1/1
4k	3/4	0/4	2/2	0/2	1/1	1/1	1/4	0/4	1/2	0/2	1/1	1/1
4l	2/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4m	3/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4n	3/4	0/4	2/2	1/2	1/1	1/1	1/4	0/4	1/2	0/2	1/1	1/1
4o	4/4	0/4	2/2	1/2	1/1	1/1	2/4	0/4	2/2	0/2	1/1	1/1
4p	3/4	0/4	2/2	0/2	1/1	1/1	1/4	0/4	1/2	0/2	1/1	1/1
4q	3/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4r	3/4	0/4	2/2	0/2	1/1	1/1	0/4	0/4	1/2	0/2	1/1	1/1
4s	4/4	0/4	2/2	1/2	1/1	1/1	2/4	0/4	2/2	0/2	1/1	1/1
4t	4/4	0/4	2/2	1/2	1/1	1/1	3/4	0/4	2/2	0/2	1/1	1/1

The compounds numbered 4d-4e, 4j-4k, and 4m-4t were most protective against MES even at 30 mg/kg dose levels. Neurotoxicity was observed in almost none of the compounds in the dose range of 30-100 mg/kg as shown in Table 1. All of the tested compounds were found protective against MES-induced seizures at 100-300 mg/kg dose levels. The compounds numbered 4d-4e, 4j-4k, and 4m-4t were most protective against MES even at 30 mg/kg dose levels. Neurotoxicity was observed in almost none of the compounds in the dose range of 30-100 mg/kg. The minimal behavioral toxic dose was found to be >30 mg/kg but <100 mg/kg. The test

substances also appear to have a relatively rapid onset and short duration of action because both the anticonvulsant and toxic effects are greater at 30 min than at 4 hours. The compounds with electron withdrawing groups were found to be the most active as an anticonvulsant than those with electron releasing groups.

The compounds with weaker releasing groups were found to be more active as an anticonvulsant than those with strong releasing groups. The anticonvulsant activity is more affected by the electron withdrawing substituent on the benzene 'attached to pyrazoline ring at *5*-position' than those 'attached to pyrazoline ring at *3*-position'. Any substituent on the benzene 'attached to pyrazoline ring at *3*-position' was found to have little effect on the anticonvulsant activity. The 4-fluoro derivatives were found to be most active as an anticonvulsant than the others, because of fluorine (an electron withdrawing group on *p*- position). The 3-chloro derivatives were found to be more active as an anticonvulsant than the 4-chloro derivatives. The compound with both fluorine and bromine substituent at the 'benzenes attached to pyrazoline ring at 5- and 3-positions respectively' was found to be the most active anticonvulsant among all the synthesized compounds.

3. Methods

Chemicals were procured from E. Merck (India) and S. D. Fine Chemicals (India). Melting points were taken in open capillary tubes and are uncorrected. Microanalysis of the compounds was done on a Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA) and the values were found within ± 0.4 % of the theoretical values. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (λ max in cm^{-1}) (Perkin-Elmer, USA) and 1H NMR spectra were recorded on a Varian E-360 MHz (Perkin-Elmer, USA) or Bruker spectrometer DPX-300MHz (Bruker, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument (Jeol, Japan) fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with the assigned structures. The progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent. All solvents were distilled prior to use.

3.1. The title compounds were synthesized in two steps.

3.1.1. Step I. Synthesis of substituted 1,3-Diphenylprop-2-en-1-ones **(3a-t)**

Substituted acetophenone **1** (0.01 mol) was added to equimolar quantity of substituted benzaldehyde **2** (0.01 mol) dissolved in methanol (25 ml). To this solution equimolar NaOH pellets (0.01mol) were added at once and the reaction mixture was stirred for 40 min at room temperature. Excess of methanol was again added and then again stirred for next 40 min at about 40°C temperature. It was cooled and then, diluted with cold water. The product's crystals which separated out were filtered and washed carefully with water untill neutral. The resulting chalcone was purified by recrystallization with methanol.

3.1.2. Step II. Synthesis of substituted 3, 5-phenyl-2-pyrazoline-1-carboxamide derivatives **(4a-t)**

To an aqueous sodium hydroxide (0.017 mol), semicarbazide hydrochloride (0.0085 mol) was dissolved. This solution was added to the solution of substituted 1,3-Diphenylprop-2-en-1-ones (0.005 mol) **(3a-t)** in ethanol (25 ml) and then, refluxed for 2-5 hr. TLC monitoring was extensively done. The product when cool was poured into crushed ice. The solid mass which separated out was filtered, washed carefully with water to neutral reaction, dried and recrystallized from appropriate combination of solvents like ethanol and chloroform (8:2).

3.2.1. 3,5-diphenyl-2-pyrazoline-1-carboxamide **(4a)**

Yield 68%, mp 194°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.50-7.85 (10 H, m, aromatic protons), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3410 (NH_2), 3020 (Ar-CH), 1652 ($C=O_{str}$), 1561 ($C=N_{str}$), 1402 ($C-H_{def}$), 1021 (C_5-N_{1str}), 3216 (carbamoyl group $N-H_{str}$). FABMS m/z 265 (M^+), 266 (M^++1), 267 (M^++2). Anal. Calcd. for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.40; H, 5.69; N, 15.81.

3.2.2. 5-(4'-methoxyphenyl)-3-phenyl-2-pyrazoline-1-carboxamide **(4b)**

Yield 74%, mp 188°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.08 (1H, dd, H_A), 3.72 (1H, dd, H_B), 5.45 (1H, dd, H_X), 6.55-7.9 (9 H, m, aromatic protons), 3.84 (3H, s, $-OCH_3$), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3400(NH_2), 3060 (Ar-CH), 1653 ($C=O_{str}$), 1564 ($C=N_{str}$),

1408 (C-H_{def}), 1016 (C_5-N_{1str}), 3206 (carbamoyl group N-H_{str}). FABMS m/z 295 (M^+), 296 (M^++1), 297 (M^++2). Anal. Calcd. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.10; H, 5.78; N, 14.21.

3.2.3. 5-(4'-chlorophenyl)-3-phenyl-2-pyrazoline-1-carboxamide **(4c)**

Yield 83%, mp 216°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.10 (1H, dd, H_A), 3.84 (3H, s, -OCH_3), 3.73 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.50-7.85 (9H, m, aromatic protons), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3212 (carbamoyl group N-H_{str}), 3070 (Ar-CH), 1688 (C=O_{str}), 1512 (C=N_{str}), 1446 (C-H_{def}), 1176 (C_5-N_{1str}). FABMS m/z 299 (M^+), 300 (M^++1), 301 (M^++2). Anal. Calcd. for $C_{16}H_{14}ClN_3O$: C, 64.10; H, 4.70; N, 14.02. Found: C, 64.08; H, 4.66; N, 14.01.

3.2.4. 5-(3'-chlorophenyl)-3-phenyl-2-pyrazoline-1-carboxamide **(4d)**

Yield 67%, mp 230°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.08 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.56-7.89 (9H, m, aromatic protons), 10.31 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3467(NH_2), 3212 (carbamoyl group N-H_{str}), 3055 (Ar-CH), 1688 (C=O_{str}), 1512 (C=N_{str}), 1446 (C-H_{def}), 1176 (C_5-N_{1str}). FABMS m/z 299 (M^+), 300 (M^++1), 301 (M^++2). Anal. Calcd. for $C_{16}H_{14}ClN_3O$: C, 64.10; H, 4.70; N, 14.02. Found: C, 64.06; H, 4.68; N, 14.04.

3.2.5. 5-(4'-fluorophenyl)-3-phenyl-2-pyrazoline-1-carboxamide **(4e)**

Yield 62%, mp 171°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.56-7.80 (9H, m, aromatic protons), 10.24 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3411(NH_2), 3086 (Ar-CH), 1648 (C=O_{str}), 1559 (C=N_{str}), 1407 (C-H_{def}), 1018 (C_5-N_{1str}), 3208 (carbamoyl group N-H_{str}). FABMS m/z 283 (M^+), 284 (M^++1), 285 (M^++2). Anal. Calcd. for $C_{16}H_{14}FN_3O$: C, 67.83; H, 4.98; N, 14.83. Found: C, 67.80; H, 4.92; N, 14.74.

3.2.6. 3-(4'-methoxyphenyl)-5-phenyl-2-pyrazoline-1-carboxamide **(4f)**

Yield 80%, mp 170°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.828 (3H, s, -OCH_3), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.34-7.84 (9H, m, aromatic protons), 10.15 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3467(NH_2), 3065 (Ar-CH), 1696 (C=O_{str}), 1514 (C=N_{str}), 1370 (C-H_{def}), 1180 (C_5-N_{1str}). FABMS m/z 295 (M^+), 296 (M^++1), 297 (M^++2). Anal. Calcd. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.12; H, 5.68; N, 14.20.

3.2.7. 3,5-bis(4'-methoxyphenyl)-2-pyrazoline-1-carboxamide **(4g)**

Yield 78%, mp 186°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.00 (1H, dd, H_A), 3.70 (3H, s, -OCH_3), 3.75 (3H, s, -OCH_3) 3.80 (1H, dd, H_B), 5.33 (1H, dd, H_X), 6.84-7.77 (8H, m, aromatic protons), 10.05 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3274 (carbamoyl group N-H_{str}), 3071 (Ar-CH), 1689 (C=O_{str}) 1555 (C=N_{str}), 1370 (C-H_{def}), 1101 (C_5-N_{1str}). FABMS m/z 325 (M^+), 326 (M^++1), 327 (M^++2). Anal. Calcd. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.42; H, 5.82; N, 12.88.

3.2.8. 5-(4'-Chlorophenyl)-3-(4'-methoxyphenyl)-2-pyrazoline-1-carboxamide **(4h)**

Yield 78%, mp 210°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.70 (3H, s, -OCH_3), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.50-7.85 (8H, m, aromatic protons), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3400(NH_2), 3039 (Ar-CH), 1585 (C=N_{str}), 1699 (C=O_{str}), 1368 (C-H_{def}), 1031 (C_5-N_{1str}). FABMS m/z 329 (M^+), 330 (M^++1), 331 (M^++2). Anal. Calcd. for $C_{17}H_{16}ClN_3O_2$: C, 61.90; H, 4.89; N, 12.74. Found: C, 61.78; H, 4.85; N, 12.70.

3.2.9. 5-(3'-chlorophenyl)-3-(4'-methoxyphenyl)-2-pyrazoline-1-carboxamide **(4i)**

Yield 67%, mp 220°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.29 (3H, s, -OCH_3), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.55-7.90 (8H, m, aromatic protons), 10.31 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3400(NH_2), 3093 (Ar-CH), 1653 (C=O_{str}), 1564 (C=N_{str}), 1408 (C-H_{def}), 1016 (C_5-N_{1str}), 3206 (carbamoyl group N-H_{str}). FABMS m/z 329 (M^+), 330 (M^++1), 331 (M^++2). Anal. Calcd. for $C_{17}H_{16}ClN_3O_2$: C, 61.90; H, 4.89; N, 12.74. Found: C, 61.88; H, 4.86; N, 12.72.

3.2.10. 5-(4'-fluorophenyl)-3-(4'-methoxyphenyl)-2-pyrazoline-1-carboxamide **(4j)**

Yield 77%, mp 220°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.05 (1H, dd, H_A), 3.29 (3H, s, -OCH_3), 3.80 (1H, dd, H_B), 5.40 (1H, dd, H_X), 6.46-7.81 (8H, m, aromatic protons), 10.21 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3400(NH_2), 3052 (Ar-CH), 1653 (C=O_{str}), 1564 (C=N_{str}), 1408 (C-H_{def}), 1016 (C_5-N_{1str}), 3206 (carbamoyl group N-H_{str}). FABMS m/z 313 (M^+), 314 (M^++1), 315 (M^++2). Anal. Calcd. for $C_{17}H_{16}FN_3O_2$: C, 65.10; H, 5.10; N, 13.41. Found: C, 65.06; H, 4.88; N, 13.32.

3.2.11. 5-phenyl-3-p-tolyl-2-pyrazoline-1-carboxamide **(4k)**

Yield 79%, mp 182°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.70 (3H, s, -CH_3), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.50-7.85 (9H, m, aromatic protons), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3326 (carbamoyl group N-H_{str}), 3066 (Ar-CH), 1646 (C=O_{str}) 1576 (C=N_{str}), 1401 (C-H_{def}), 1071 (C_5-N_{1str}). FABMS m/z 279 (M^+), 280 (M^++1), 281 (M^++2). Anal. Calcd. for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.08; H, 6.08; N, 15.02.

3.2.12. 5-(4'-methoxyphenyl)-3-p-tolyl-2-pyrazoline-1-carboxamide **(4l)**

Yield 79%, mp 210°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.00 (1H, dd, H_A), 3.70 (3H, s, -OCH_3), 3.15 (3H, s, -CH_3) 3.80 (1H, dd, H_B), 5.33 (1H, dd, H_X), 6.84-7.77 (8H, m, aromatic protons), 10.05 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3241 (carbamoyl group N-H_{str}), 3069 (Ar-CH), 1638 (C=O_{str}), 1551 (C=N_{str}), 1390 (C-H_{def}), 1009 (C_5-N_{1str}). FABMS m/z 309 (M^+), 310 (M^++1), 311 (M^++2). Anal. Calcd. for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.80; H, 6.16; N, 15.52.

3.2.13. 5-(4'-chlorophenyl)-3-p-tolyl-2-pyrazoline-1-carboxamide **(4m)**

Yield 76%, mp 214°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.09 (1H, dd, H_A), 3.13 (3H, s, -CH_3), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.50-7.86 (8H, m, aromatic protons), 10.3 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3209 (carbamoyl group N-H_{str}), 3083 (Ar-CH), 1658 (C=O_{str}), 1587 (C=N_{str}), 1362 (C-H_{def}), 1100 (C_5-N_{1str}). FABMS m/z 313 (M^+), 314 (M^++1), 315 (M^++2). Anal. Calcd. for $C_{17}H_{16}ClN_3O$: C, 65.10; H, 5.14; N, 13.39. Found: C, 65.06; H, 5.12; N, 13.36.

3.2.14. 5-(3'-chlorophenyl)-3-p-tolyl-2-pyrazoline-1-carboxamide **(4n)**

Yield 65%, mp 226°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.08 (1H, dd, H_A), 3.29 (3H, s, -CH_3), 3.80 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.55-7.90 (8H, m, aromatic protons), 10.32 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3465 (carbamoyl group N-H_{str}), 3102 (Ar-CH), 1699 (C=O_{str}), 1586 (C=N_{str}), 1432 (C-H_{def}), 1140 (C_5-N_{1str}). FABMS m/z 313 (M^+), 314 (M^++1), 315 (M^++2). Anal. Calcd. for $C_{17}H_{16}ClN_3O$: C, 65.10; H, 5.14; N, 13.39. Found: C, 65.02; H, 5.10; N, 13.32.

3.2.15. 5-(4'-fluorophenyl)-3-p-tolyl-2-pyrazoline-1-carboxamide **(4o)**

Yield 76%, mp 216°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.01 (1H, dd, H_A), 3.19 (3H, s, -CH_3), 3.82 (1H, dd, H_B), 5.39 (1H, dd, H_X), 6.46-7.81 (8H, m, aromatic protons), 10.22 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3217 (carbamoyl group N-H_{str}), 3040 (Ar-CH), 1719 (C=O_{str}), 1502 (C=N_{str}), 1417 (C-H_{def}), 1082 (C_5-N_{1str}). FABMS m/z 297 (M^+), 298 (M^++1), 299 (M^++2). Anal. Calcd. for $C_{17}H_{16}FN_3O$: C, 68.67; H, 5.42; N, 14.13. Found: C, 68.65; H, 5.38; N, 14.10.

3.2.16. 3-(4'-bromophenyl)-5-phenyl-2-pyrazoline-1-carboxamide **(4p)**

Yield 76%, mp 175°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.08 (1H, dd, H_A), 3.80 (1H, dd, H_B), 5.4 (1H, dd, H_X), 6.49-7.83 (9H, m, aromatic protons), 10.38 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3223 (carbamoyl group N-H_{str}), 3072 (Ar-CH), 1648 (C=O_{str}), 1576 (C=N_{str}), 1464 (C-H_{def}), 1068 (C_5-N_{1str}). FABMS m/z 344 (M^+), 345 (M^++1), 346 (M^++2). Anal. Calcd. for $C_{16}H_{14}BrN_3O$: C, 55.83; H, 4.10; N, 12.21. Found: C, 55.80; H, 4.06; N, 12.18.

3.2.17. 3-(4'-bromophenyl)-5-(4'-methoxyphenyl)-2-pyrazoline-1-carboxamide **(4q)**

Yield 73%, mp 190°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.29 (3H, s, -OCH_3), 3.79 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.50-7.85 (8H, m, aromatic protons), 10.28 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3301 (carbamoyl group N-H_{str}), 3066 (Ar-CH), 1679 (C=O_{str}), 1572 (C=N_{str}), 1401 (C-H_{def}), 1132 (C_5-N_{1str}). FABMS m/z 374 (M^+), 375 (M^++1), 376 (M^++2). Anal. Calcd. for $C_{17}H_{16}BrN_3O_2$: C, 54.50; H, 4.30; N, 11.23. Found: C, 54.46; H, 4.26; N, 11.18.

3.2.18. 3-(4'-bromophenyl)-5-(4'-chlorophenyl)-2-pyrazoline-1-carboxamide **(4r)**

Yield 79%, mp 190°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.79 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.50-7.85 (8H, m, aromatic protons), 10.28 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3257 (carbamoyl group N-H_{str}), 3061 (Ar-CH), 1632 (C=O_{str}), 1578 (C=N_{str}), 1385 (C-H_{def}), 1169 (C_5-N_{1str}). FABMS m/z 377 (M^+), 378 (M^++1), 381 (M^++4). Anal. Calcd. for $C_{16}H_{13}BrClN_3O$: C, 50.70; H, 3.40; N, 11.00. Found: C, 50.68; H, 3.38; N, 10.96.

3.2.19. 3-(4'-bromophenyl)-5-(3'-chlorophenyl)-2-pyrazoline-1-carboxamide **(4s)**

Yield 63%, mp 222°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.79 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.50-7.85 (8H, m, aromatic protons), 10.28 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3302 (carbamoyl group N-H_{str}), 3078 (Ar-CH), 1694 (C=O_{str}), 1586 (C=N_{str}), 1464 (C-H_{def}), 1140 (C_5-N_{1str}). FABMS m/z 377 (M^+), 378 (M^++1), 381 (M^++4). Anal. Calcd. for $C_{16}H_{13}BrClN_3O$: C, 50.70; H, 3.50; N, 11.10. Found: C, 50.56; H, 3.48; N, 10.98.

3.2.20. 3-(4'-bromophenyl)-5-(4'-fluorophenyl)-2-pyrazoline-1-carboxamide **(4t)**

Yield 74%, mp 190°C; ^{1}H-NMR (300 MHz, DMSO) δ (ppm) 3.07 (1H, dd, H_A), 3.79 (1H, dd, H_B), 5.42 (1H, dd, H_X), 6.50-7.85 (8H, m, aromatic protons), 10.28 (2H, s, $CONH_2$). FTIR (KBr) υ_{max} cm^{-1} 3091 (Ar-CH), 1576 (C=N_{str}), 1412 (C-H_{def}), 1131 (C_5-N_{1str}), 3310 (carbamoyl group N-H_{str}) and 1689 (C=O_{str}). FABMS m/z 362 (M^+), 363 (M^++1), 364 (M^++2). Anal. Calcd. for $C_{16}H_{13}BrFN_3O$: C, 53.10; H, 3.60; N, 11.60. Found: C, 53.08; H, 3.52; N, 11.42.

3.3. Anticonvulsant activity

Animal - Seven albino male mice (25-30 g) in our laboratory were used for each compound. They were kept under standard condition at an ambient temperature of 25 ± 2 ℃. Food and water were withdrawn prior to the experiments [21].

Standard drug (Phenytoin): 5 mg/kg body weight

Test compounds (**4a-t**): equivalent to phenytoin/30 mg/kg body weight

Equipments- Electroconvulsometer [Stimulator (Grass S88, Astro-Med. Inc.), constant current unit (Grass CCU1A, Grass Medical Instruments), and corneal electrode, rotarod used in the neurotoxicity test]. An apparatus with ear electrodes (Woodbury and Davenport 1952) was used to deliver the stimuli [22].

The compounds were screened for their anticonvulsant activity by MES method. Supra maximal electroshock of alternating current intensity of 50mA, 60Hz (five to seven times that necessary to elicit minimal seizures) for 0.2 sec. duration was given to mice via ear electrodes. All compounds were solubilized or suspended in 30% aqueous polyethylene glycol 400 (PEG 400), administered with test compounds in a volume of 0.01 ml/g body weight. Control animals received 30% aqueous PEG 400. Mice were tested at 30 minutes and 4 hours following doses of 30, 100 and 300 mg/kg of test compound. The animals were observed closely for 2 min. The abolition of the hind limb tonic extensor spasm indicated the test compound's ability to inhibit MES-induced seizure spread/ discharge through neural tissue and was recorded as an increase of anticonvulsant activity. Percent of inhibition of seizures relative to controls was calculated.

In MES Test: Values represent number of mice protected divided by number of mice tested.

3.4. Neurotoxicity test

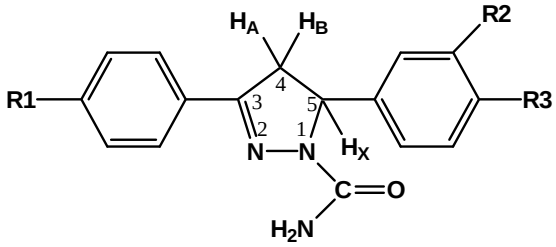
Toxicity induced by a compound was detected in mice using the standardized Rotorod test. All mice were trained to stay on rotating rotorod. Untreated control mice, when placed on a six rpm rotation rod (one inch diameter knurled plastic rod) can maintain their equilibrium for a prolonged period of time. Neurological impairment like ataxia, sedation and hyperexcitability [23] can be demonstrated by the inability of a mouse to maintain equilibrium for one minute in each of three successive trials.

The animal was placed on a one-inch diameter knurled wooden rod rotating at six 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 rod for one min.

In Rotorod Test (NT): Values represent number of mice toxic divided by number of mice tested.

Data from screen I (anticonvulsant & neurotoxicity identification) was described in Table 2.

Table 2: Screening data of synthesized compounds **(4a-t)**.



General structure of synthesized compounds **(4a-t)**.

Compd.	R^1	R^2	R^3	MES (30 min) % Protection (mg/kg) 30	100	300	Compd.	R^1	R^2	R^3	MES (30 min) % Protection (mg/kg) 30	100	300
4a	H	H	H	50*	100	100	**4k**	CH_3	H	H	75**	100	100
4b	H	H	OCH_3	50*	100	100	**4l**	CH_3	H	OCH_3	50*	100	100
4c	H	H	Cl	50*	100	100	**4m**	CH_3	H	Cl	75*	100	100
4d	H	Cl	H	75 **	100	100	**4n**	CH_3	Cl	H	75**	100	100
4e	H	H	F	100***	100	100	**4o**	CH_3	H	F	100***	100	100
4f	OCH_3	H	H	50*	100	100	**4p**	Br	H	H	75**	100	100
4g	OCH_3	H	OCH_3	25	100	100	**4q**	Br	H	OCH_3	75**	100	100
4h	OCH_3	H	Cl	50*	100	100	**4r**	Br	H	Cl	75**	100	100
4i	OCH_3	Cl	H	50*	100	100	**4s**	Br	Cl	H	100***	100	100
4j	OCH_3	H	F	100***	100	100	**4t**	Br	H	F	100***	100	100
Phenytoin	-	-	-	100***	100***	100***							

*p < 0.05, **p < 0.01, ***p < 0.001

3.5. Statistical analysis

Results were expressed as Mean±SEM (Standard Error Mean). Data obtained from pharmacological experiments were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test and used to evaluate the results, employing Pharmacologic Calculation System Version 4.1. (Microcomputer Specialists). A p-value of less than 0.05 was considered statistically significant.

4. Competing Interests

The authors declare that they have no competing interests.

5. Authors' Contributions

AAS and MS developed the project and supervised preparation of the manuscript; MAR carried out the project; RM assisted with preparation of the manuscript; AAS also assisted with spectral analysis and biological activity.

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