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Original Article

Synthesis, Anticonvulsant, and Molecular Docking Studies of (3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl) (4-chlorophenyl) Methanone Derivatives

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ABSTRACT

Aim/Background: This research work aims to design and synthesize novel compounds containing pyrazoline moiety in their structure with enhanced anticonvulsant activity in comparison with the standard (Phenytoin) drug. In the docking study, the target protein with the active site of human mitochondrial branched-chain aminotransferase (BCATm) (PDB ID: 2A1H) was used against synthesized compounds. Hence the importance of the pyrazoline compounds is thought of as interest for developing a new compound that shows better biological activity with minor side effects. **Materials and Methods:** The new pyrazoline derivatives were synthesized with the help of novel substituted acetophenone react with novel substituted benzaldehyde to form chalcone derivatives. The novel chalcones derivatives react with hydrazine hydrate to form a novel series of (3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl) (4-chlorophenyl) methanone Derivatives and Characterization and identification of each compound were successfully done by IR, ^{1}HNMR, ^{13}CNMR, Mass as well as analytical data. **Conclusion:** In present work has given novel series of (3,5-disubstituted-4,5-dihydro-1H-pyrazol-1-yl) (4-chlorophenyl) methanone derivatives and prepared using a multistep step reaction. Compounds 4a, 4e, and 4f have shown more potent anticonvulsant activity. In the docking study, the target protein against the active site of human mitochondrial branched-chain aminotransferase (BCATm) (PDB ID: 2A1H) was used against synthesized compounds. Among the titled compounds, 4f was found to be most potent and have a high docking score of -6.898 as compared to Gabapentin (-6.013 as Dock Score). **Results:** A series of novel pyrazoline derivatives were synthesized effectively and potential against the anticonvulsant activity.

Keywords: Anticonvulsant, Docking, Pyrazoline, Anticonvulsant, ScPTZ, Phenytoin.

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INTRODUCTION

Pyrazoline is a heterocyclic compound with a wide range of biological applications and the most common fluorescent agent, which absorbs light between 300 and 400 nm and then produces blue fluorescence.[1] It is a five-membered ring with three carbon atoms and two nitrogen atoms within the ring and just one endocyclic bond.[2] Its derivatives possess many activities like antimicrobial,[3] antiviral,[4] anti-tubercular,[5] anti-HIV,[6] molluscicidal,[7] and cerebroprotective.[8] Epilepsy is a type of epilepsy that affects the central nervous system. Which of these is universally recognized by both men and women? Every year, approximately 2,50,000 new cases are recorded worldwide.[9,10] Epilepsy prevalence in India varies between 1710 and 9780 cases per million people, according to two studies.[11] Current antiepileptic medicines (AEDs) have shown that some patients with epilepsy fail to manage their seizures, while others gain seizure control at the risk of severe side effects. The necessity for novel epileptic drugs was underscored by the numerous side effects associated with traditional AEDs.[12] The elderly in poor countries have the highest rate of early-onset seizures of any age group.[13] The elderly is the population segment that is expanding the most steadily as a result of higher life expectancy and the aging of the baby-boomer generation. Both of these factors have contributed to an increase in the prevalence of age-related neurological illnesses like epilepsy, as well as cerebrovascular and neurodegenerative diseases.[14]

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MATERIALS AND METHODS

Instrumentation

All the reagents and solvents were purchased from different companies-CDH (Central Drug House), E. Merck India Ltd, FINAR, RenChem. These reagents and solvents were LR (Laboratory) grade. For thin-layer chromatography (TLC), silica gel G (160-120 lattice) (Merck India Pvt. Ltd.) was used as an adsorbent. The melting points were determined using Lab India Melting Point Apparatus. The proton magnetic resonance spectra (^{1}HNMR) were recorded on a Bruker 300MHz instrument in $CDCl_3$ using tetramethylsilane [$(CH_3)_4Si$] as the internal standard. The ^{13}C NMR spectra were recorded on a Bruker 75 MHz instrument in $CDCl_3$.The infrared spectra of the compound were recorded in KBr on a Perkin-Elmer FTIR spectrometer and the iodine chamber and UV- lamp were used for visualization of TLC spots.

Synthesis of 4-chloro-benzoic acid ethyl ester (ii)

4-chloro benzoic acid (i) (1.56 g, 0.01mol) and with absolute ethanol (35ml) in the presence of H_2SO_4 (0.05ml, 0.01mol) for 2-3hr reflux. TLC was required for cheeked reaction monitoring. After the reaction was completed to neutralize excess organic acid, dropwise additions of concentrated sodium carbonate (Na_2CO_3) were introduced to the reaction mixture. The neutralized reaction takes in the separating funnel and adds 100 ml of distilled water with diethyl ether$(C_2H_5)_2O$ (40ml) was used to extract the final product from the aqueous layer after shaking by using the separating funnel. The two layers had been separated by allowing the solution to stand for some time. To avoid contamination of the higher natural yellow-colored layer containing ethyl 4-chlorobenzoate(ii), it was accumulated from the neck of the separating funnel. Yellow-colored liquid ester was once accumulated through evaporating diethyl ether.[15]

Synthesis of 4-Choro-benzoic acid hydrazide (iii)

4-chloro-benzoic acid ethyl ester (ii) (6.0ml, 0.01mol) to react with hydrazine hydrate (0.49ml, 0.01mol) in methanol (15ml) along with continuous stirring at room temperature for 1-2hr in a conical flask. After the reaction was completed and the solid product was cooled by the addition of distilled water, filtration, washing, air-dried containing 4-chloro-benzoic acid hydrazide (iii). Finally, the compound was recrystallized from ethanol.[15]

Preparation of disubstituted Chalcones (3a-j)

A mixture of substituted-acetophenone (1a-b) (1.29ml, 0.01mol) and substituted-aromatic benzaldehyde (2a-e) (1.40g, 0.01mol) dissolve in ethanol (15-20ml) and continuously stirred. When 30% Sodium hydroxide (NaOH- 5ml) was added, dropwise and continuously stirring till the precipitation comes about. The precipitate occurred and neutralized with hydrochloric acid (HCL). Then neutralized compound was filtered and air-dried. The dried compound was recrystallized with ethanol to get pure compound substituted Chalcones (3a-j).[16]

Preparation of 3,5-disubstituted (4-chloro-phenyl) -(3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone (4a-j)

The compound substituted chalcones (3a-j) (2.10g, 0.01mol) and 4-chloro-benzoic acid hydrazide (iii) (1.70g, 0.01mol) in ethanol (30-35ml) was refluxed for 30hr. The reaction mixture was cooled and poured onto crushed ice. After filtering the solid, it was recrystallized with ethanol to get pure compound substituted (4-chloro-phenyl) -(3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone (4a-j).

synthesis of (4-chloro-phenyl)-[5-(4-chloro-phenyl)-3-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1yl]-methanone(4a)

Yield (80.5%); m.p. 190-192; R_f = 0.81, IR (KBr), (cm^{-1}): 3462 (NH), 3020 (C-H, Ar), 1750 (C=O), 1610-1426 (C=C, Ar), 1248 (C-N), 1010 (C-F), 750 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.65-7.82 (m, 4H, Ar), 7.27-7.59 (m, 4H, Ar), 7.07-7.26(m, 4H, Ar), 3.29-3.32, 3.61-3.65 (dd, 1H, pyrazoline), 2.22-2.24 (t, 2H, pyrazoline); ^{13}C NMR (75MHz, $CDCl_3$) **δ**(ppm): 134.49(1C, C=O), 130.51(1C, C=N, pyrazoline), 129.27(2C, phenyl"), 128.47 (2C, phenyl"), 128.04 (1C, phenyl"), 117.77 (2C, phenyl'), 117.48 (2C, phenyl'), 115.89 (1C, phenyl'), 115.74 (2C, phenyl), 115.60 (2C, phenyl), 115.45 (1C, phenyl), 78.3 (1C, C-F, phenyl'), 55.9 (1C, phenyl"), 52.3 (1C, C-Cl, phenyl), 44.1 (1C, pyrazoline), 34.1 (1C, C-N, pyrazoline); ESI-MS(*m/z*): 412.05(M^+); Anal. Calcd for $C_{22}H_{15}Cl_2FN_2O$: C, 63.94; H, 3.66; N, 6.78. Found: C, 63.90; H,3.60; N,6.72.

Synthesis of [5-(4-Bromo-phenyl)-3-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl] -(4-chloro-phenyl)-methanone(4b)

Yield 66.5%; m.p.198-200; R_f= 0.85; IR (KBr), (cm^{-1}): 3464 (NH), 3022 (C-H, Ar), 1720 (C=O), 1652 (C=N), 1612-1428 (C=C, Ar), 1250 (C-N), 1012(C-F), 748(C-Cl), 685 (C-Br). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70-7.81 (m, 4H, Ar), 7.05-7.39 (m, 4H, Ar), 7.40 -7.69 (m, 4H, Ar), 3.29-3.36, 3.35-3.36 (dd, 1H, pyrazoline), 2.21-2.23 (t, 2H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 139.96 (1C, C=O), 135.23 (1C, C=N, pyrazoline), 123.20 (2C, phenyl'), 123.15 (2C, phenyl'), 123.02 (1C, phenyl'), 121.88 (2C, phenyl), 120.98 (2C, phenyl), 120.41 (1C, phenyl), 112.16 (2C, phenyl"), 110.12 (2C, phenyl"), 107.42 (1C, phenyl"), 106.53 (1C, C-F, phenyl"), 52.11 (1C, phenyl'), 45.10 (1C, pyrazoline), 34.17 (1C, C-N, pyrazoline), 29.10 (1C, Br, phenyl); ESI-MS(*m/z*): 459.7 (M^+); Anal. Calcd for $C_{22}H_{15}BrClFN_2O$: C, 57.48; H, 3.73; N, 6.09. Found: C, 57.41; H, 3.50; N, 6.70.

Synthesis of (4-choro-phenyl)-[5-(3,4-dimethoxy-phenyl)-3-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-methanone (4c)

Yield (57.5%); m.p.182-184; R_f= 0.78; IR (KBr), (cm^{-1}): 3463 (NH), 3026 (C-H, Ar), 1750 (C=O), 1649 (C=N), 1616-1430 (C=C, Ar), 1248 (C-N), 1016 (C-F), 757(C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) **δ** (ppm): 7.68-7.82 (m, 4H, Ar), 7.36 -7.68(m, 4H, Ar), 6.08-7.35(m, 3H, Ar), 3.30-3.33, 3.62-3.66 (dd, 2H, pyrazoline), 3.91 (6H, OCH_3), 2.22-2.23 (t, 1H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ**(ppm): 140.12 (1C, C=O), 135.35 (1C, C=N, pyrazoline), 129.33 (1C, C-F, phenyl), 123.22 (2C, phenyl'), 123.16 (2C, phenyl'), 123.12 (1C, phenyl'), 121.50 (2C, phenyl), 120.90 (2C, phenyl), 120.88 (1C, phenyl), 118.22 (1C, Phenyl"), 116.12 (1C, Phenyl"), 114.55 (1C, phenyl"), 112.25 (1C, phenyl"), 107.15 (1C, phenyl"), 106.50 (1C, phenyl"), 62.24 (2C, -OCH_3), 52.25 (1C, C-Cl, phenyl'), 45.12 (1C, pyrazoline), 34.25 (1C, C-N, pyrazoline). ESI-MS(*m/z*): 438.8(M^+); Anal. Calcd for $C_{24}H_{20}ClFN_2O_3$: C, 65.68; H, 4.59; N, 6.38. Found: C, 64.68; H,3.50; N,5.62.

Synthesis of (4-choro-phenyl)-[5-(4-dimethylamino-phenyl)-3-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-methanone (4d)

Yield (74.5%); m.p. 180-182; R_f = 0.74; IR (KBr), (cm^{-1}): 3463 (NH), 3019 (C-H, Ar), 1752 (C=O),1618-1432 (C=C Ar), 1358 (C-C Ar), 1250 (C-N), 1026 (C-F), 746(C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.09-7.36 (m, 4H, Ar), 7.72-7.86 (m, 4H, Ar), 7.38 -7.69(m, 4H, Ar), 3.80 (6H, N-CH_3), 3.30-3.34, 3.65-3.69 (dd, 2H, pyrazoline), 2.21-2.24 (t, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 141.12 (1C, C=O), 136.35 (1C, C=N, pyrazoline), 122.22 (2C, phenyl'), 122.16 (2C, phenyl'), 122.12 (1C, phenyl'), 121.30 (2C, phenyl), 120.50 (2C, phenyl), 120.10 (1C, phenyl), 113.25 (2C, phenyl"), 109.20 (2C, phenyl"), 108.15 (1C, phenyl"), 107.50 (1C, C-F, phenyl"), 52.60 (1C, C-Cl, phenyl'), 48.10 (1C, C-N, phenyl), 44.12 (1C, pyrazoline), 35.25 (1C, C-N, pyrazoline), 60.78 (2C, N-CH_3). ESI-MS(*m/z*): 421.8(M^+); Anal. Calcd for = $C_{24}H_{21}ClFN_3O$: C, 68.32; H, 5.02; N, 9.96. Found: C, 68.30; H, 5.05; N, 9.92.

Synthesis of (4-chloro-phenyl)-[3-(4-fluoro-phenyl)-5-(2-nitro-phenyl)-4,5-dihydro-pyrazol-1yl]-methanone (4e)

Yield (76.5%); m.p. 212-214; R_f = 0.78; IR (KBr), (cm^{-1}): 3466 (NH), 2925 (C-H, Ar), 1745 (C=O), 1617-1431 (C=C, Ar), 1250 (C-N), 1017 (C-F), 751(C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) **δ** (ppm): 7.69-7.83 (m, 4H, Ar), 7.36 -7.68 (m, 4H, Ar), 6.08-7.35 (m, 4H, Ar), 3.25-3.29, 3.61-3.67 (dd, 2H, pyrazoline), 2.22-2.24 (t, 1H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 139.96(1C, C=O), 135.23 (1C, C=N, pyrazoline), 123.20 (2C, phenyl'), 123.15 (2C, phenyl'), 123.02 (1C, phenyl'), 121.88 (2C, phenyl), 121.20 (1C, phenyl), 120.98 (1C, phenyl), 120.41 (1C, phenyl), 112.16 (2C, phenyl"), 110.12 (2C, phenyl"), 107.42 (1C, phenyl"), 106.53 (1C, C-F, phenyl"), 62.10 (1C, C-NO_2, phenyl), 52.11 (1C, C-Cl, phenyl'), 45.10 (1C, C-2, pyrazoline), 34.17 (1C, C-1, C-N, pyrazoline); ESI-MS(m/z): 423.8(M^+); Anal. Calcd for $C_{22}H_{15}ClFN_3O_3$: C, 62.35; H, 3.57; N, 9.91. Found: C, 63.25; H, 4.57; N, 10.30.

Synthesis of (4-chloro-phenyl) [5-(4-chloro-phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl] methanone (4f)

Yield (66.2%); m.p. 195-198; R_f= 0.72; IR (KBr), (cm^{-1}): 3460 (NH), 2920 (C-H Ar), 1755 (C=O), 1614-1420 (C=C, Ar), 1250 (C-N), 746 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.72-7.88 (m, 4H, Ar), 7.04-7.33 (m, 4H, Ar), 3.30-3.33, 3.34-3.36 (dd, 1H, Pyrazoline), 2.19-2.24 (t, 2H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 134.55 (1C, C=O), 130.70 (1C, C-3, C=N, pyrazoline), 128.27 (1C, phenyl"), 127.47 (2C, phenyl"), 126.04 (1C, phenyl"), 116.77 (2C, phenyl"), 114.89 (1C, phenyl'), 113.74 (2C, phenyl'), 113.60 (2C, phenyl'), 113.45 (2C, phenyl), 77.3 (2C, phenyl), 55.10 (1C, C-Cl, phenyl'), 54.9 (1C, phenyl), 51.3 (1C, phenyl), 43.10 (1C, pyrazoline), 36.12 (1C, C-N, pyrazoline). ESI-MS(*m/z*): 395.2 (M^+); Anal. Calcd for $C_{22}H_{16}Cl_2N_2O$: C, 66.85; H, 4.08; N, 7.09. Found: C, 65.85; H, 3.57; N, 6.30.

Synthesis of [5-(4-bromo-phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl] (4-chloro-phenyl) methanone (4g)

Yield (66.5%); m.p.194-196; R_f= 0.72; IR (KBr), (cm^{-1}): 3468 (NH), 3115 (C-H, Ar), 1730 (C=O), 1649 (C=N), 1619-1420 (C=C, Ar), 1235 (C-N), 753 (C-Cl), 595 (C-Br); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70-7.81 (m, 4H, Ar), 7.40 -7.70 (m, 5H, Ar), 7.06-7.38 (m, 4H, Ar), 3.28-3.29-,3.34-3.36 (dd, 1H, Pyrazoline), 2.21-2.22 (t, 2H, pyrazoline); ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 140.96 (1C, C=O), 136.23 (1C, C=N), 124.20 (2C, phenyl'), 124.15 (2C, phenyl'), 124.02 (1C, phenyl'), pyrazoline), 122.98 (2C, phenyl), 122.88 (2C, phenyl), 121.31 (1C, phenyl), 113.16 (2C, phenyl"), 111.12 (2C, phenyl"), 108.42 (1C, phenyl"), 107.53 (1C, phenyl"), 53.20 (1C, C-Cl, phenyl'), 45.20 (1C, pyrazoline), 34.15 (1C, C-N, pyrazoline), 30.10 (1C, C-Br, phenyl),; ESI-MS(*m/z*): 439.7(M^+); Anal. Calcd for $C_{22}H_{16}BrClN_2O$: C, 60.09; H, 3.67; N, 6.37. Found: C, 59.80; H, 3.57; N, 6.30.

Synthesis of (4-choro-phenyl)-[5-(3,4-dimethoxy-phenyl)-3-phenyl-4,5-dihydro-pyrazol-1-yl]-methanone (4h)

Yield (80.3%;); m.p.172-174; R_f = 0.79, IR (KBr), (cm^{-1}): 3460 (NH), 3025 (C-H, Ar), 1755 (C=O), 1619-1420 (C=C, Ar), 1250 (C-N), 745 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_{3)}$ δ (ppm): 7.68-7.82 (m, 4H, Ar), 7.36 -7.68 (m, 5H, Ar), 3.91 (6H, OCH_3), 3.29-3.32, 3.62-3.65 (dd, 2H, pyrazoline), 3.07-7.34 (m, 3H, Ar), 2.21-2.22 (t, 1H, pyrazoline. ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 162.04 (C=O), 145.06 (1C, C=N, pyrazoline), 131.70 (2C, phenyl'), 131.54 (2C, phenyl'), 131.30 (1C, phenyl'), 131.08 (2C, phenyl), 130.51 (1C, phenyl), 129.63 (2C, phenyl"), 128.11 (1C, phenyl"), 126.99 (1C, phenyl), 126.75 (1C, phenyl"), 126.50

(2C, phenyl"), 64.12 (2C, OCH_3, phenyl), 61.10 (2C, OCH_3, phenyl), 58.17 (1C, C-Cl, phenyl'), 55.86 (1C, pyrazoline), 41.63 (1C, C-N, pyrazoline). ESI-MS(*m/z*): 420.8(M^+); Anal. Calcd for $C_{24}H_{21}ClN_2O_3$: C, 68.49; H, 5.03; N, 6.66. Found: C, 67.80; H, 4.57; N, 6.30.

Synthesis of (4-choro-phenyl)-[5-(4-dimethylamino-phenyl)-3-phenyl-4,5-dihydro-pyrazol-1-yl]-methanone. (4i)

Yield (70.2%); m.p.176-178; R_f = 0.71; IR (KBr), (cm^{-1}): 3467 (NH), 3027 (C-H, Ar), 1755 (C=O), 1617-1430 (C=C, Ar), 1250 (C-N), 756(C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.71-7.84 (m, 4H, Ar), 7.36 -7.70 (m, 5H, Ar), 6.02-7.35 (m, 4H, Ar), 3.89 (6H, N-CH_3), 3.18-3.29, 3.55-3.67 (dd, 2H, pyrazoline), 2.20-2.21 (t, 1H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 142.12 (1C, C=O), 138.35 (1C, C=N, Pyrazoline), 123.12 (1C, phenyl'), 123.16 (2C, phenyl'), 123.22 (2C, phenyl'), 121.50 (2C, phenyl), 121.30 (2C, phenyl), 121.16 (1C, phenyl), 112.25 (2C, phenyl"), 110.20 (2C, phenyl"), 109.15 (1C, phenyl"), 108.50 (1C, phenyl"), 54.60 (1C, C-4, C-Cl, Phenyl'), 49.10, (1C, C-4, C-N, Phenyl), 41.12 (1C, C-2, pyrazoline), 35.27 (1C, C-N, pyrazoline), 27.12 (2C, N-CH_3). ESI-MS(*m/z*): 403.9(M^+); Elem. Calcd for $C_{24}H_{22}ClN_3O$: C, 70.79; H, 5.56; N, 10.40. Found: C, 70.80; H, 4.57; N, 10.39.

Synthesis of (4-chloro-phenyl)-[5-(2-nitro-phenyl)-4,5-dihydro-pyrazol-1yl]-methanone (4j)

Yield (66.6%); m.p.218-220; R_f = 0.62; IR (KBr), (cm^{-1}): 3469 (NH), 3030 (C-H, Ar), 1755 (C=O), 1621-1432 (C=C, Ar), 1260 (C-N), 744 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70-7.83 (m, 4H, Ar), 7.37 -7.69(m, 5H, Ar), 6.09-7.36 (m, 4H, Ar), 3.26-3.31, 3.63-3.68 (dd, 2H, pyrazoline), 2.22-2.24 (t, 1H, pyrazoline). ^{13}C NMR (75 MHz, $CDCl_3$) **δ** (ppm): 137.96 (1C, C=O), 135.50 (1C, C=N, pyrazoline), 125.20 (2C, phenyl'), 123.15 (2C, phenyl'), 122.02 (1C, phenyl'), 121.40 (2C, phenyl), 121.20 (1C, phenyl), 120.48 (1C, phenyl), 120.40 (1C, phenyl), 113.15 (2C, phenyl"), 112.10 (2C, phenyl"), 110.12(1C, Ar-C, phenyl), 107.53 (1C, C-4, phenyl"), 106.40 (1C, phenyl"), 60.10 (1C, Ar-C, phenyl), 53.11 (1C, C-Cl, phenyl'), 44.10 (1C, C-2, pyrazoline), 35.17 (1C, C-1, C-N, pyrazoline). ESI-MS(*m/z*): 405.8(M^+); Elem. Calcd for $C_{22}H_{16}ClN_3O_3$: C, 65.11; H, 3.97; N, 10.35. Found: C, 65.05; H, 3.95; N, 10.30.

Evaluation of Anticonvulsant Activity

Animal selection and maintenance

Adult albino mice (25-30 gm) were used in biological tests for anticonvulsant activity. These animals were taken from the Central Animal House of NIET (Pharmacy institute), Greater Noida. These animals were kept in polypropylene cages at a temperature of 25±5°C under the specified conditions. Animals were given proper light and dark cycles of 12 hr each. Food and water were given to the animals as needed. The CPCSEA registration number 1845/PO/Re/S/16/CPCSEA was used to conduct and execute the animal activity testing data included in protocol number IAEC/NIET/2020/ 01/03.

Preparation of stock solution and Dose calculations

Each compound's stock solution was made by dissolving the required amount of standard drugs (phenytoin) and tested compounds were soluble in a vehicle (2% tween 80 + 0.9%NaCl) solution as previously reported.[17,18]

Each volume of the solution was calculated according to the following formula:

$$\text{Volume to be given} = \frac{\text{weight of animal}}{1000} \times \text{Volume concentration}$$

Where dose calculation for each concentration is calculated as:

$$\text{Does to be given} = \frac{\text{Weight of animal}}{1000} \times \text{Dose concentration}$$

Acute Toxicity Study

Acute toxicity research aims to identify the test compound's lethal dose (LD_{50}). Initially, animals were divided into three groups of three according to the dose recommended by the OECD 423 standards. Each compound's dose concentrations were chosen to be 5, 50, 100, 300, and 2000 mg/kg. Doses were given in solutions via the intraperitoneal route. During the first four hours of observation, the mortality mice in each group were recorded, as well as their gross behavioral activity and other changes. After that, they were observed for their mortality.[19]

Anti-convulsant activity

This testing process is used to determine the threshold value that will cause the body to have minimal tonic-clonic seizures. After intraperitoneal administration of control, standard, and test drugs, subcutaneous pentylenetetrazole (PTZ) is administered. The animals will be divided into different groups, each with six animals. The control group administrated (Tween-80 in 0.9%NaCl), the standard group administrated phenytoin 20 mg/kg, and the other groups administrated test compounds solubilized or suspended in the vehicle. Acute toxicity values were used to calculate doses for the chemicals examined, which were 50, 100, and 300 mg/kg. Acute toxicity values were used to calculate doses for the chemicals examined, which were 50, 100, and 300 mg/kg. After 0.5 hr of each administration of drugs, PTZ will be administered 75 mg/Kg via a subcutaneous route. The animals were then monitored for 0.5 hr and then for another 4 hr. The initiation of action (clonus and Tonic) and duration of epilepsy, as well as the signs of Straub tail movement, hind-limb tonic extension, and jerky movement, were all detected in each animal. The absence of a single 5-sec episode of the hind-limb tonic extension will be taken as the end point.[20,21]

Molecular docking

The molecular docking against human mitochondrial branched-chain aminotransferase (hBCATm) complexed with gabapentin was carried out for the ligands 4a-j. The X-ray crystal structure of 2A1H (PDB ID) was downloaded from Protein Data Bank and solved at a resolution of 1.80 Å and R-value 0.208 (obs). The docking protocol was followed as per the reported method.[22]

RESULTS AND DISCUSSION

Synthesis

The reported compound 4-chloro benzoic acid hydrazide (iii) were obtained via two-step reactions.[15] In the (Figure 1) first step of the reaction scheme 4-chloro benzoic acid (i) on reaction with Sulphuric acid in the presence of ethanol gave 4-Chloro-benzoic acid ethyl ester (ii). The 4-Chloro-benzoic acid ethyl ester (ii) reacted with hydrazine hydrate in ethanol to give 4-chloro benzoic acid hydrazide (iii). In the next part scheme, the substituted chalcones (3a-j) was synthesized by the reaction of substituted acetophenone (1a-b) with substituted benzaldehyde (2a-e).[16] The compound (3a-j) on the reaction with 4-chloro benzoic acid hydrazide (iii) in the presence of ethanol gave substituted (4-Chloro-phenyl) -(3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone (4a-j). All the synthesized compounds were characterized by fourier transform infrared (FTIR), proton nuclear magnetic resonance (^{1}HNMR), and elemental analysis.

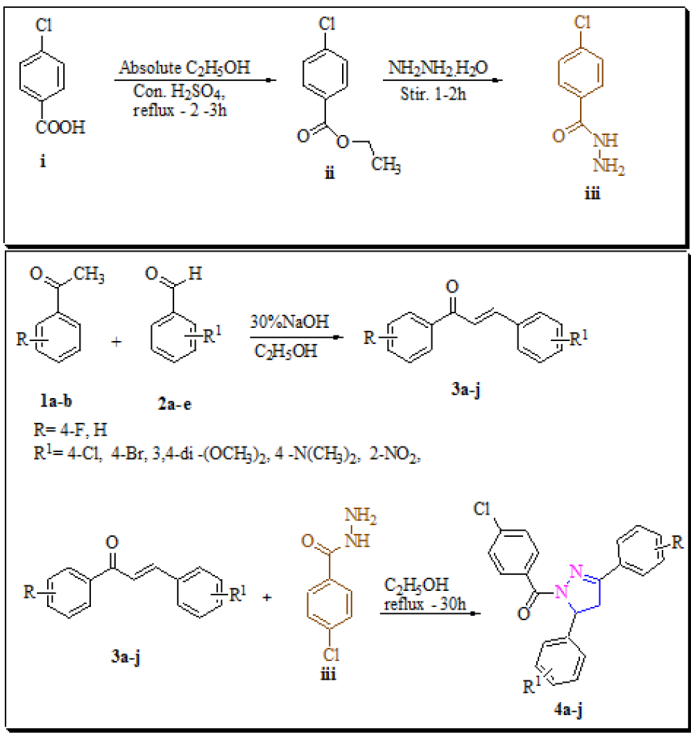


Figure 1: Synthesis of substituted (4-Chloro-phenyl) -(3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone(4a-j).

Table 1: Acute toxicity studies of standard and synthesized compounds.

Compounds	Acute Toxicity Activity Dosage(mg/kg)				
	50mg/kg	100mg/kg	300mg/kg	500mg/kg	2000mg/kg
4a	0/3	0/3	0/3	3/3	3/3
4b	0/3	0/3	1/3	2/3	3/3
4c	0/3	0/3	0/3	2/3	3/3
4d	0/3	0/3	0/3	3/3	3/3
4e	0/3	0/3	1/3	2/3	3/3
4f	0/3	0/3	1/3	1 /3	3/3
4g	0/3	0/3	1/3	2/3	3/3
4h	0/3	0/3	0/3	2/3	3/3
4i	0/3	0/3	0/3	3/3	3/3
4j	0/3	0/3	1/3	1/3	3/3
Phenytoin	0/3	0/3	0/3	2/3	3/3

Acute Toxicity study

Acute toxicity studies were performed to observe the median lethal dose and access the chance of toxic effect of the prepared derivatives. All the prepared compounds were injected intraperitoneally observed median lethal dose of each tested analog was dose concentration given was 50, 100, 300, 500, and 2000 mg/kg, between Swiss albino mice. Compounds were dissolved in (Tween-80 w/v) in 0.9% NaCl solution (vehicle). Each animal was observed for 24hr, after the drug administration. On observation, animals with a dose concentration of 2000mg/kg have shown a full mortality rate. These animals show involuntary movements like unwanted body stiffening and jerky movements. Animals at 500mg/kg dose concentration, show an LD_{50} effect. At 300 mg/kg dose concentration, it shows mortality of a few drugs which do not define LD_{50}. Whereas, at a dose concentration of 50mg/kg, 100 mg/kg animals survived and stay healthy as described in Table 1.

ANTI-CONVULSANT ACTIVITY

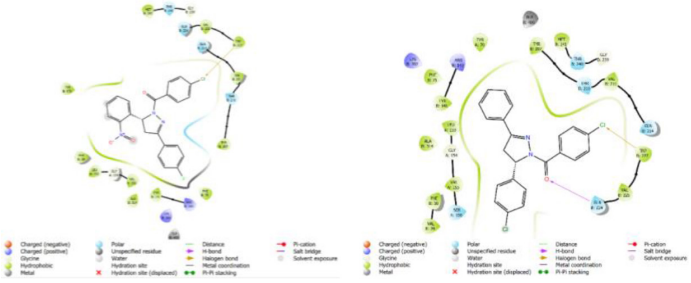
The anticonvulsant activity was screened pentylenetetrazole-induced model using phenytoin as a standard drug. Mice were injected with the control, standard, and tested compounds intraperitoneally, 30 min before the test PTZ injection. On injecting PTZ subcutaneously, animals were observed for anticonvulsant activity. Animals were observed for the onset of tonic and clonic convulsions along with Straub tail and jerky movements. Calculated results from the observed data are shown in the table 2. Most of the synthesized compounds have shown active anticonvulsant activities in mice. Compounds 4a, 4e, and 4f shows the most potent activity against convulsions at the minimum dose of 50 mg/Kg concentration. Compounds 4b, 4c, 4d, 4i, and 4j showed moderate activity within the concentration of 100mg/Kg which was visible as the onset of action within a

Table 2: Anticonvulsant Activity of the tested compounds.

Compounds	Onset of clonus		Onset of tonic	
	0.5	4.0	0.5	4.0
4a	50	100	50	100
4b	100	300	100	300
4c	100	300	100	300
4d	100	300	100	300
4e	50	100	50	100
4f	50	100	50	100
4g	300	-	300	-
4h	300	-	300	-
4i	100	300	100	300
4j	100	300	100	300
Phenytoin	25	-	25	-

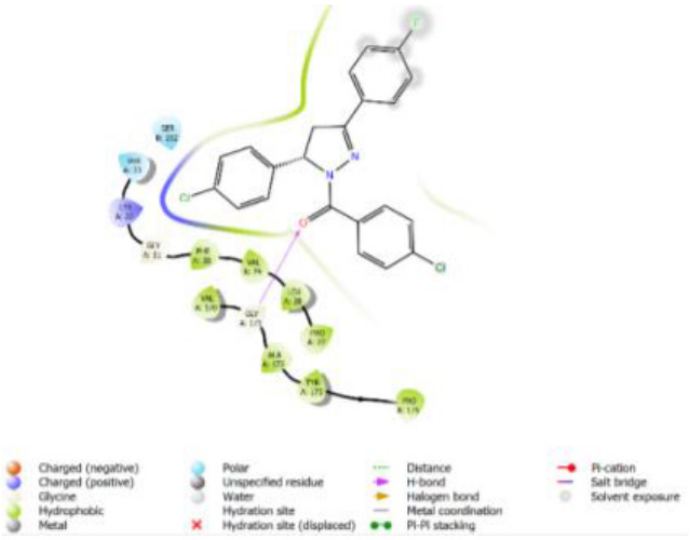
Table 3: The molecular docking scores and emodel score of the compounds 4a-j against human mitochondrial branched-chain aminotransferase (PDB ID: 2A1H).

Compounds	Docking score	Emodel score
4a	-6.011	-57.309
4b	-5.648	-55.949
4c	-5.154	-49.356
4d	-5.545	-46.766
4e	-6.123	-60.617
4f	-6.898	-63.129
4g	-5.005	-49.619
4h	-5.022	-48.575
4i	-5.445	-53.372
4j	-5.990	-59.994
Gabapentin	-6.013	-50.916



4e **4f**

Figure 2: The 2D interaction of ligands 4a and 4f against the active site of human mitochondrial branched-chain aminotransferase (BCATm) (PDB ID: 2A1H).



4a

Figure 3: The 2D interaction of ligands 4e against the active site of human mitochondrial branched-chain aminotransferase (BCATm) (PDB ID: 2A1H).

half-hour of the PTZ injection. On the alternate side, compound 4g, and 4h showed the least activity within the concentration of 300mg/kg which was visible as the onset of action within a half-hour of the PTZ injection (Table 2).

Molecular docking Analysis

The molecular docking was carried against the active site of human mitochondrial branched-chain aminotransferase (BCATm) to study the binding modes of the analogs 4a-j. The docking scores (ranging between -5.005 and -6.898 kcal/mol) are shown in Table 3. Three types of interactions were observed in the docking studies viz. H-bond, halogen bond, and π-π-stacking. The ligands 4a, 4e, and 4f showed efficient binding among the series. The ligand 4a showed H-bond interaction with the residue Gly171 (with the carbonyl function), while the ligand 4f showed two types of interaction, H-bond interaction with the residue Gln224 (with the carbonyl function) and halogen bond interaction with the residue Trp227 (with chloro function). The ligands 4b and 4e showed a halogen bond with the residue Trp227 (with chloro function), while the ligand 4d showed π-π-stacking interaction with the residue Tyr173 (Figures 2 and 3).

STRUCTURE ACTIVITY RELATIONSHIP

In this study, a new series of substituted (4-Chloro-phenyl) -(3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone (4a-j) were synthesized and evaluated. The Structure-activity and relationship of compound as flows:

The compounds substituted with an electron- withdrawing group (EWG) such as F, Cl, *p*- position and NO_2, *o*-position on phenyl ring showed the most promising of this series.

Replacement of Br with Cl, and F decrease the anticonvulsant activity.

Pyrazoline rings with substituted phenyl ring and acetyl group is essential for anticonvulsant activity. While 4-chlorophenyl ring fused with pyrazoline ring was more stable for clonus tonic seizures.

The phenyl substitution at the p-position of the pyrazoline ring enchance the anticonvulsant activity.

Acetyl linker between pyrazoline and 4-chlorophenhyl ring enchance the anticonvulsant activity.

Substitution with electron donor group on the pyrazoline ring is essential for maintaining anticonvulsant activity.

CONCLUSION

A novel series of Substituted (4-chloro-phenyl)–[3,5-diphenyl-4,5-dihydro-pyrazol-1-yl)-methanone (4a-j) was synthesized. All the synthesized derivatives characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy. Compounds 4a, 4e, and 4f were found to be most potent against convulsions at minimum dose of 50mg/kg. Compounds 4b, 4c, 4d, 4i, and 4j were found to moderately active at dose of 100mg/Kg. Compounds 4g, and 4h were found to be least active against convulsion at dose of 300mg/kg body weight. All the synthesized compounds were also screened by docking using Schrodinger. The docking score of most active compounds 4a, 4e, and 4f found to -6.011, -6.123, and -6.898, respectively, which indicated that these compounds have good anticonvulsant activity. The outcome of this research is that it is very helpful to find out the new lead compound as an antiepileptic drug in drug discovery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AEDs: Antiepileptic Drugs; **GABA:** Gamma aminobutyric acid; **CNS:** Central Nervous System; **N:** Normality; **OECD:** Organisation for Economic Co-operation and Development; **LD_{50}:** Median lethal dose; **i.p.:** Intraperitoneal; **cm:** Centimetre; **T:E:F:** Toluene:Ethyl acetate:Fornic acid; **B:A:** Benzene:Acetone; **IUPAC:** International Union of Pure and Applied Chemistry.

SUMMARY

In the present work synthesized Pyrazoline derivatives were prepared by various types of acetophenone and various type of benzaldehyde via Claisen Schmidt condensation. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral techniques and evaluated for their anticonvulsant activity in swiss albino mice by the ScPTZ model. The synthesized compounds, 4a, 4e, and 4f were found to be most potent and have a high docking score of -6.011, -6.123, and -6.898 as compared to Gabapentin (-6.013 as Dock Score). All the synthesized compounds were found to be active and stable biological activity. We provided a convenient synthetic process for the synthesis of novel pyrazoline derivatives and the results of the anticonvulsant screening are encouraging. Further investigations with suitable structural modifications of novel title compounds may result in therapeutically useful. Among the synthesized derivatives, 4a, 4e, and 4f were found to be more potent against anti-convulsion at a 50 mg/kg dose level.

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