

# Novel Pyrimidine–Chalcone Hybrids: Design, Synthesis and Evaluation as Potential Anticonvulsant Agents

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## ABSTRACT

Epilepsy is a neurodegenerative disorder distinguish by perennial, unprovoked seizures although antiepileptic remedy aim to prevent seizures, but they often manifest consequential deleterious effects, including cephalalgia, nausea, dizziness, visual disturbances, decompensate Cirrhosis, pancreatitis, and renal disorders. Furthermore, medicament tolerance remains a substantial challenge, highlighting emergent requisite apropos innocuous and efficacious curative conventional, particularly from indigenous medicine, to enhance epilepsy regimen and mitigate its comprehensive impact. This research involves docking, pharmacokinetic and synthesis of 63 pyrimidine-chalcone derivatives, focusing on their biological activity as anticonvulsant agents, molecular docking analysis facilitates the identification of significant binding interactions, contributing to the development of improved medicament; the docking scores of the synthesized compounds range from -9.7 to -7.3 Kcal/mol. In-silico pharmacokinetic studies suggest definitive compounds exhibit promising therapeutic potential, supported by appropriate molecular weight and oral absorption values in humans. The results contribute to the creation of novel pyrimidine-chalcone derivatives with improved anticonvulsant properties, during the initial phase-I the synthesized amalgam were assessed for their anticonvulsant capabilities exploiting the maximal electroshock test (MES) and subcutaneous pentylenetetrazole (scPTZ) in-vivo models most active compounds (5a-5h). Phase II animal screening models, specifically (MES & scPTZ) tests, molecules exhibited significant anticonvulsant effects, with ED<sub>50</sub> values of 17.1 mg/kg and 19.8 mg/kg. These results highlight potential of molecular hybridization regimens in drug discovery, rendering a framework for auxiliary maturation of chalcone–pyrimidine derivatives as promising candidates for anticonvulsant therapy.

**KEYWORDS:** Anticonvulsant, Molecular docking, Pharmacokinetics, Pyrimidine-chalcone, Synthesis.

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## INTRODUCTION:

Epilepsy is a significant neurological disorder that impacts individuals of all ages worldwide, surge risk of mortality up to threefold compared to the general populace, it affects 50 million people globally, with a higher prevalence in low- and middle-income nations<sup>1,2</sup>. The highest incidence rates are observed in adults over 65, attributed to age-related changes in neural networks and cells, characterized by recurrent, unprovoked seizures due to abnormal neuronal activity these condition places a considerable economic, psychosocial, physical, and mental strain on healthcare systems, communities, and those affected<sup>3</sup>. Presently, antiepileptic drugs (AEDs) are the most effective treatment for various seizure types and epileptic syndromes; nonetheless one-third of epilepsy patients is resistant to existing medications and continues to experience seizures<sup>4</sup>. Individuals with drug-resistant epilepsy (DRE) face a heightened risk of sudden death, injury, psychosocial issues, multiple health conditions, diminished quality of life, and economic challenges. Antiepileptic drugs such as phenytoin, carbamazepine, and sulfamate topiramate (TPM) are successful in managing seizures although newer

drugs like pregabalin, gabapentin, lacosamide, vigabatrin, levetiracetam, and lamotrigine have been developed, they come with side effects including liver toxicity, nausea, gastrointestinal problems, excessive hair growth, and weight gain these medications can reduce seizures in 75% of patients or completely control them in 50%<sup>5,6</sup>. The primary challenges with current antiepileptic drugs are resistance and neurotoxicity so there is a pressing need for more effective anticonvulsants with fewer side effects ongoing research aims to develop antiepileptic drugs that offer greater selectivity and lower toxicity.

In recent years, Pyrimidine-chalcone compounds have gained significant attention in medicinal chemistry due to their versatile Pyrimidine-chalcone derivatives, which exhibit antimicrobial, anticancer, anti-inflammatory properties, and more recently, activity in the central nervous system (CNS) through sulfonate inclusion<sup>7</sup>. The incorporation of sulfonate groups in these candidate molecules, along with their varied pharmacokinetic characteristics, membrane permeability, and solubility, facilitates strong interactions with targets. In this context, we

Novel Pyrimidine–Chalcone Hybrids: Design, Synthesis and Evaluation as Potential Anticonvulsant Agents explored the design, synthesis, and comprehensive characterization of a new series of potential antiepileptic Pyrimidine-chalcone agents, utilizing both *in-silico* and *in-vivo* methods.

## MATERIAL AND METHODS:

### 1. Molecular docking and Designing of amalgams:

Molecular docking simulations were performed using AutoDock4.2 integrated with PyRx software. The enzyme's structure, identified as PDB 1OHW, was sourced from the Protein Data Bank to ensure accurate binding interactions, solvent molecules, ligands, and cofactors were eliminated using Discovery Studio Visualized software. The enzyme structure was then converted into a macromolecule format via PyRx. Ligands, specifically pyrimidine-chalcone, underwent optimization to improve binding efficiency. This optimization was carried out using Spartan 14 v1.14 software, employing density functional theory (DFT) with the B3LYP method and a 6-31G\* basis set. Docking interactions between the enzyme and protein were assessed using PyRx with Auto Dock Vina. Validation involved removing the native ligand, conducting docking, and comparing the predicted binding pose and affinity against experimental data. The success of redocking was evaluated based on Root Mean Square Deviation (RMSD) and docking scores<sup>8</sup>. From the molecular docking studies of Pyrimidine-chalcone derivatives, 63 analogues were designed, with 8 demonstrating favorable scores, as detailed in table 3.

### 2. *In-silico* Pharmacokinetics studies:

The pkCSM web server and SwissADME are network kits available online for studying ADMET these tools are utilized to identify potential unconventional drug candidates, reduce the need for extensive experimental research, and enhance compliance rates in drug discovery<sup>9</sup> as listed in Table 4 among these eight best scores and *in-silico* ADMET derivatives are selected for synthesis and *in-vivo* evaluations.

### 3. Synthesis of selected designed amalgams:

#### Step I- Preparation of Substituted Chalcone:

Substituted acetophenones (0.01 mol) were reacted with benzaldehyde (0.01 mol) in ethanol, the mixture was cooled (0–5 °C), and 10% NaOH was added dropwise with stirring. After stirring for 3–4 hours at room temperature, the reaction completion was checked by TLC. The mixture was then poured into ice water and acidified with dilute HCl to obtain a yellow precipitate, which was filtered, washed, and recrystallized from ethanol to yield substituted chalcone (3a–3h)<sup>10</sup>.

#### Step II- Chalcone–Pyrimidine Derivatives (5a–5h):

Equimolar amounts of chalcone derivatives (3a–3h) and 4, 6-dimethylpyrimidin-2-amine was dissolved in ethanol. A small amount of glacial acetic acid was added, and the mixture was refluxed at 75–80 °C for 6–8 hours. The reaction was monitored by TLC. After completion, it was cooled, poured into ice water, and the solid formed was filtered, washed, and recrystallized to obtain the final chalcone–pyrimidine derivatives (5a–5h)<sup>11</sup>.

**5a.** (Z)-N-((E)-1,3-diphenylallylidene)-4,6-dimethylpyrimidin-2-amine Yield 71%, m.p. 154–156°C,

**IR (KBr cm<sup>-1</sup>):** 3152 (NH), 3054 (Ar–H), 2968, 2930 (aliphatic C–H, CH<sub>3</sub>), 1646 (C=N, azomethine), 1602 (C=C, conjugated), 1512 (Ar C=C), 1460 (C–H bending), 1248 (Ar–O–CH<sub>3</sub>), 1172 (C–O), 1034 (C–N), 832 (p-disubstituted Ar–H), 758 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.69 (6H, s), 6.99–7.18 (2H, 7.06 (d, J = 15.7 Hz), 7.13 (s)), 7.42–7.67 (9H, 7.50 (dddd, J = 7.8, 7.4, 1.5, 0.4 Hz), 7.50 (dddd, J = 7.8, 7.4, 1.2, 0.4 Hz), 7.53 (tt, J = 7.4, 1.6 Hz), 7.55 (tt, J = 7.4, 1.5 Hz), 7.56 (dddd, J = 7.8, 1.6, 1.4, 0.4 Hz), 7.61 (d, J = 15.7 Hz)), 8.31 (2H, dddd, J = 7.8, 1.5, 1.5, 0.4 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 106.5 (1C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.5 (2C, s), 128.7 (2C, s), 129.0 (2C, s), 131.6 (1C, s), 131.9 (1C, s), 134.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 314 (M)<sup>+</sup>, 315 (M+H)<sup>+</sup> **Elemental Analysis:** C<sub>21</sub>H<sub>19</sub>N<sub>3</sub> (314) C, 80.48; H, 6.11; N, 13.

**5b.** (Z)-N-((E)-1-(4-methoxyphenyl)-3-phenylallylidene)-4,6-dimethylpyrimidin-2-amine

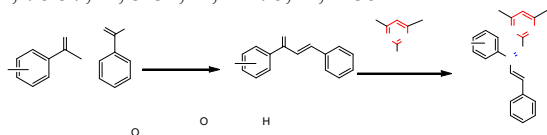
Yield 63%, m.p. 162–164°C, **IR (KBr cm<sup>-1</sup>):** 3152 (NH), 3054 (Ar–H), 2968, 2930 (aliphatic C–H, CH<sub>3</sub>), 1646 (C=N, azomethine), 1602 (C=C, conjugated), 1512 (Ar C=C), 1460 (C–H bending), 1248 (Ar–O–CH<sub>3</sub>), 1172 (C–O), 1034 (C–N), 832 (p-disubstituted Ar–H), 758 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.69 (6H, s), 3.89 (3H, s), 6.34 (1H, s), 6.91 (2H, ddd, J = 8.6, 1.2, 0.5 Hz), 7.04 (1H, d, J = 15.7 Hz), 7.42–7.67 (6H, 7.49 (dddd, J = 7.8, 7.4, 1.5, 0.4 Hz), 7.52 (tt, J = 7.4, 1.6 Hz), 7.54 (dddd, J = 7.8, 1.6, 1.4, 0.4 Hz), 7.60 (d, J = 15.7 Hz)), 7.81 (2H, ddd, J = 8.6, 1.9, 0.5 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 55.3 (1C, s), 106.5 (1C, s), 113.9 (2C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 130.4 (2C, s), 131.6 (1C, s), 134.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 162.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 344 (M)<sup>+</sup>, 345 (M+H)<sup>+</sup> **Elemental Analysis:** C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O (344) C, 76.94; H, 6.16; N, 12.24; O, 4.66.

**5c.** (Z)-N-((E)-1-(4-chlorophenyl)-3-phenylallylidene)-4,6-dimethylpyrimidin-2-amine

Yield 76%, mp 166–168°C, **IR (KBr cm<sup>-1</sup>):** 3150 (NH), 3052 (Ar–H), 2966, 2928 (aliphatic C–H, CH<sub>3</sub>), 1648 (C=N, azomethine), 1604 (C=C, conjugated), 1510 (Ar C=C), 1462 (C–H bending), 1092 (C–N), 1018 (C–Cl), 832 (p-disubstituted Ar–H), 758 (Ar–H, oop), **<sup>1</sup>H NMR (400 MHz):** δ 2.70 (6H, s), 6.98–7.16 (2H, 7.05 (d, J = 15.7 Hz), 7.11 (s)), 7.42–7.68 (8H, 7.49 (dddd, J = 7.8, 7.4, 1.5, 0.4 Hz), 7.52 (tt, J = 7.4, 1.6 Hz), 7.54 (dddd, J = 7.8, 1.6, 1.4, 0.4 Hz), 7.55 (ddd, J = 8.6, 1.5, 0.5 Hz), 7.61 (d, J = 15.7 Hz)), 7.90 (2H, ddd, J = 8.6, 1.6, 0.5 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 106.5 (1C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7–128.9 (4C, 128.7 (s), 128.8 (s)), 130.4 (2C, s), 131.6 (1C, s), 134.7 (1C, s), 138.5 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 164.2 (2C, s) **MS m/z (%):** 347 (M)<sup>+</sup>, 348 (M+H)<sup>+</sup>, 350 (M+H, <sup>37</sup>Cl)<sup>+</sup> **Elemental Analysis:** C<sub>21</sub>H<sub>18</sub>ClN<sub>3</sub> (347) % C, 72.51; H, 5.22; Cl, 10.19; N, 12.08

**5d. (Z)-4, 6-Dimethyl-N-((E)-3-phenyl-1-(p-tolyl)allylidene)pyrimidin-2-amine** Yield 79%, mp 160–162°C, **IR (KBr cm<sup>-1</sup>):** 3148 (NH), 3052 (Ar–H), 2968, 2932 (aliphatic C–H, CH<sub>3</sub>), 1646 (C=N, azomethine), 1602 (C=C, conjugated), 1510 (Ar C=C), 1460 (C–H bending), 1376 (CH<sub>3</sub> bending), 1236 (C–N), 820 (p-disubstituted Ar–H), 758 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.23 (3H, s), 2.69 (6H, s), 7.00–7.25 (4H, 7.07 (d, *J* = 15.7 Hz), 7.13 (s), 7.19 (ddd, *J* = 8.0, 1.6, 0.4 Hz)), 7.42–7.67 (6H, 7.50 (dddd, *J* = 7.8, 7.4, 1.5, 0.4 Hz), 7.53 (tt, *J* = 7.4, 1.6 Hz), 7.56 (dddd, *J* = 7.8, 1.6, 1.4, 0.4 Hz), 7.61 (d, *J* = 15.7 Hz)), 7.86 (2H, ddd, *J* = 8.0, 1.6, 0.4 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 21.4 (1C, s), 106.5 (1C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 129.2 (2C, s), 130.4 (2C, s), 131.6 (1C, s), 134.7 (1C, s), 142.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 327 (M)<sup>+</sup>, 328 (M+H)<sup>+</sup>, **Elemental Analysis:** C<sub>22</sub>H<sub>21</sub>N<sub>3</sub> C, 80.70; H, 6.46; N, 12.83

**5e. 4-((1Z, 2E)-1-((4, 6-dimethylpyrimidin-2-yl)imino)-3-phenylallyl) phenol** Yield 77%, m.p. 172–174°C, **IR (KBr cm<sup>-1</sup>):** 3342 (O–H), 3150 (NH), 3054 (Ar–H), 2966, 2930 (aliphatic C–H, CH<sub>3</sub>), 1644 (C=N, azomethine), 1600 (C=C, conjugated), 1512 (Ar C=C), 1460 (C–H bending), 1262 (Ar–O), 1170 (C–O), 1032 (C–N), 832 (p-disubstituted Ar–H), 758 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.72 (6H, s), 6.41 (1H, s), 6.91 (2H, ddd, *J* = 8.4, 1.1, 0.4 Hz), 7.04 (1H, d, *J* = 15.7 Hz), 7.42–7.67 (6H, 7.49 (dddd, *J* = 7.8, 7.4, 1.5, 0.4 Hz), 7.52 (tt, *J* = 7.4, 1.6 Hz), 7.54 (dddd, *J* = 7.8, 1.6, 1.4, 0.4 Hz), 7.60 (d, *J* = 15.7 Hz)), 7.81 (2H, ddd, *J* = 8.4, 1.9, 0.4 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 106.5 (1C, s), 115.3 (2C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 130.4 (2C, s), 131.6 (1C, s), 134.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 160.9 (1C, s), 164.2 (2C, s), **MS m/z (%):** 330 (M+H)<sup>+</sup>, 334 (minor), **Elemental Analysis:** C<sub>21</sub>H<sub>19</sub>N<sub>3</sub>O (30) % C, 76.57; H, 5.81; N, 12.76; O, 4.86.



**Figure 1: Synthesis of Pyrimidine-chalcone derivatives**

**5f. (Z)-4,6-Dimethyl-N-((E)-1-(4-nitrophenyl)-3-phenylallylidene)pyrimidin-2-amine** Yield 75%, m.p. 178–180°C, **IR (KBr cm<sup>-1</sup>):** 3150 (NH), 3052 (Ar–H), 2966, 2928 (aliphatic C–H, CH<sub>3</sub>), 1648 (C=N, azomethine), 1604 (C=C, conjugated), 1522 (NO<sub>2</sub>, asym), 1346 (NO<sub>2</sub>, sym), 1510 (Ar C=C), 1462 (C–H bending), 1238 (C–N), 758 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.69 (6H, s), 3.24 (3H, s), 6.27 (1H, s), 7.05 (1H, d, *J* = 15.7 Hz), 7.21 (2H, ddd, *J* = 8.0, 1.0, 0.5 Hz), 7.41–7.64 (6H, 7.49 (dddd, *J* = 7.8, 7.4, 1.9, 0.4 Hz), 7.52 (tt, *J* = 7.4, 1.6 Hz), 7.53 (dddd, *J* = 7.8, 1.6, 1.4, 0.4 Hz), 7.57 (d, *J* = 15.7 Hz)), 7.81 (2H, ddd, *J* = 8.0, 1.5, 0.5 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 106.5 (1C, s), 119.4 (1C, s), 121.9 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 130.4 (2C, s), 131.6 (1C, s), 133.4 (2C, s), 134.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 358.39 (M)<sup>+</sup>, 359.39 (M+H)<sup>+</sup>

, **Elemental Analysis:** C<sub>21</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub> (358.39) % C, 70.38; H, 5.06; N, 15.63; O, 8.93.

**5g. (Z)-N-((E)-1-(2-methoxyphenyl)-3-phenylallylidene)-4,6-dimethylpyrimidin-2-amine** Yield 80%, m.p. 174–176°C, **IR (KBr cm<sup>-1</sup>):** 3150 (NH), 3054 (Ar–H), 2968, 2930 (aliphatic C–H, CH<sub>3</sub>), 1646 (C=N, azomethine), 1602 (C=C, conjugated), 1512 (Ar C=C), 1460 (C–H bending), 1246 (Ar–O–CH<sub>3</sub>), 1172 (C–O), 1034 (C–N), 750 (Ar–H, oop), **<sup>1</sup>H NMR (400 MHz):** δ 2.69 (6H, s), 3.87 (3H, s), 6.34 (1H, s), 6.92 (1H, ddd, *J* = 8.2, 1.2, 0.5 Hz), 7.07 (1H, d, *J* = 15.7 Hz), 7.29–7.76 (9H, 7.36 (ddd, *J* = 7.9, 7.3, 1.2 Hz), 7.49 (dddd, *J* = 7.8, 7.4, 1.5, 0.4 Hz), 7.52 (tt, *J* = 7.4, 1.6 Hz), 7.54 (dddd, *J* = 7.8, 1.6, 1.4, 0.4 Hz), 7.61 (d, *J* = 15.7 Hz), 7.66 (ddd, *J* = 7.9, 1.5, 0.5 Hz), 7.69 (ddd, *J* = 8.2, 7.3, 1.5 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 56.0 (1C, s), 106.5 (1C, s), 111.4 (1C, s), 121.9 (1C, s), 123.6 (1C, s), 126.7 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 130.3 (1C, s), 132.3 (1C, s), 134.7 (1C, s), 144.3 (1C, s), 157.3–157.5 (2C, 157.3 (s), 157.4 (s)), 160.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 344 (M)<sup>+</sup>, 345 (M+H)<sup>+</sup> (base peak at 345.5), **Elemental Analysis:** C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O (344) % C, 76.94; H, 6.16; N, 12.24; O, 4.66.

**5h. (Z)-N-((E)-1-(2-chlorophenyl)-3-phenylallylidene)-4,6-dimethylpyrimidin-2-amine** Yield 78%, m.p. 182–184°C, **IR (KBr cm<sup>-1</sup>):** 3150 (NH), 3052 (Ar–H), 2966, 2928 (aliphatic C–H, CH<sub>3</sub>), 1648 (C=N, azomethine), 1604 (C=C, conjugated), 1510 (Ar C=C), 1462 (C–H bending), 1094 (C–N), 1016 (C–Cl), 748 (Ar–H), **<sup>1</sup>H NMR (400 MHz):** δ 2.70 (6H, s), 7.00–7.16 (2H, 7.07 (d, *J* = 15.7 Hz), 7.11 (s)), 7.39–7.78 (9H, 7.46 (ddd, *J* = 7.8, 7.5, 1.2 Hz), 7.49 (dddd, *J* = 7.8, 7.4, 1.5, 0.4 Hz), 7.52 (tt, *J* = 7.4, 1.6 Hz), 7.54 (dddd, *J* = 7.8, 1.6, 1.4, 0.4 Hz), 7.56 (ddd, *J* = 8.1, 1.2, 0.5 Hz), 7.57 (d, *J* = 15.7 Hz), 7.71 (ddd, *J* = 8.1, 7.5, 1.6 Hz)), 8.09 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz), **<sup>13</sup>C NMR:** δ 20.9 (2C, s), 106.5 (1C, s), 121.9 (1C, s), 123.6 (1C, s), 126.7 (1C, s), 128.1 (1C, s), 128.4 (2C, s), 128.7 (2C, s), 130.1 (1C, s), 130.3 (1C, s), 132.5–132.7 (2C, 132.6 (s), 132.6 (s)), 134.7 (1C, s), 144.3 (1C, s), 157.3 (1C, s), 160.4 (1C, s), 164.2 (2C, s), **MS m/z (%):** 347 (M)<sup>+</sup>, 348 (M+H)<sup>+</sup> (isotopic peak at 348), **Elemental Analysis:** C<sub>21</sub>H<sub>18</sub>ClN<sub>3</sub> (347.84) % C, 72.51; H, 5.22; Cl, 10.19; N, 12.08.

**4. Experimental animals:** Central Animal House Facility, Shobhit University, Saharanpur, India, Swiss albino mice weighing 20–25g with standard housing conditions, which included a controlled temperature (23±1°C), a 12-h light/dark cycle (lights on at 7:00), a relative humidity of 45 %– 55 %, and unrestricted access to food and water". Animals were allowed to acclimate in groups of five to six per cage for at least 1 week. Experiments were conducted between 9:00 AM and 2:00 PM, and the treated groups were compared with their respective control groups recorded on the same day<sup>12,13</sup>. Our work was approved by the Institutional Animal Ethics Committee (1264/CPCSEA). The test compounds were administered intraperitoneally (i.p.) as suspensions in 0.5% polyethylene glycol-200 at doses of 30, 100, and 300 mg/kg.

**4.1 In-vivo anticonvulsant:** Each experimental group consisted of 6 animals; the values reported represent the lowest dose at which bioactivity was observed in at least half of the mice while doses are expressed in mg/kg of body weight. Neurotoxicity was assessed using the rotarod test, conducted 30 minutes after administration of the test compound<sup>14,15</sup>.

**Phase I anticonvulsant screening:** The synthesized compounds 5a–5h were evaluated for their anticonvulsant activity using the MES, scPTZ models, and rotarod neurotoxicity test<sup>16,17,18</sup>, the compounds 5a, 5f, and 5e were found to be the most potent in the MES test at a dose of 30 mg/kg at 0.5 h, indicating their ability to prevent seizure spread at a relatively lower dose while 5g, 5h, and 5b showed protection at 100 mg/kg after 0.5 h, suggesting rapid onset of action at a moderate dose. In scPTZ model, compounds 5a, 5f, and 5e were found active at 100 mg/kg after 0.5 h of drug administration, comparable to carbamazepine, indicating their ability to raise seizure threshold and prevent chemically induced seizures. Compounds 5b and 5c were moderate active at 300 mg/kg at 0.5 h. In rotarod neurotoxicity test, compounds 5b and 5c showed mild neurotoxicity at 300 mg/kg at 0.5 h to 4.0 h respectively, whereas compounds 5a and 5f emerged as the most promising anticonvulsant agents in the series, exhibiting high potency, rapid onset, longer duration of action, and minimal neurotoxicity.

**Table 1. Phase-I Anticonvulsant Screening of Synthesized Compounds Following Intraperitoneal Administration in Mice Using MES, scPTZ and Neurotoxicity Tests**

| Comp. Name          | Intraperitoneal injection in mice <sup>1</sup> |         |              |         |                 |         |
|---------------------|------------------------------------------------|---------|--------------|---------|-----------------|---------|
|                     | MES screen                                     |         | scPTZ screen |         | Toxicity screen |         |
|                     | 0.5 h                                          | 4.0 h   | 0.5 h        | 4.0 h   | 0.5 h           | 4.0 h   |
| 5a                  | 30<br>0                                        | 30<br>0 | —            | —       | —               | —       |
| 5b                  | 10<br>0                                        | 30<br>0 | —            | —       | —               | —       |
| 5c                  | 30                                             | 10<br>0 | 10<br>0      | 30<br>0 | —               | —       |
| 5d                  | 30                                             | 30      | 10<br>0      | 10<br>0 | —               | —       |
| 5e                  | 30                                             | 30      | 10<br>0      | 10<br>0 | —               | —       |
| 5f                  | 30                                             | 10<br>0 | 30<br>0      | 30<br>0 | —               | —       |
| 5g                  | 10<br>0                                        | 30<br>0 | 30<br>0      | 30<br>0 | 30<br>0         | 30<br>0 |
| 5h                  | 10<br>0                                        | 30<br>0 | —            | 30<br>0 | 30<br>0         | 30<br>0 |
| Carbamazepine (CBZ) | 30                                             | 10<br>0 | 10<br>0      | 30<br>0 |                 |         |

|  |  |  |  |  |         |         |
|--|--|--|--|--|---------|---------|
|  |  |  |  |  | 10<br>0 | 30<br>0 |
|--|--|--|--|--|---------|---------|

**Phase II anticonvulsant screening:** The best compounds 5a and 5f were subjected to Phase II were administered intraperitoneally (i.p.) at different dose levels for the determination of median effective dose (ED<sub>50</sub>), median toxic dose (TD<sub>50</sub>), and protective index (PI) in animals<sup>19,20,21</sup>. 5a and 5f exhibited ED<sub>50</sub> values of 17.1 mg/kg and 19.8 mg/kg respectively, in the MES model while the scPTZ screen, the ED<sub>50</sub> values were observed to be 382.6mg/kg for 5a and 308.9mg/kg for 5f, TD<sub>50</sub> values obtained from the neurotoxicity (rotarod) study were calculated as 528.9mg/kg for 5a and 415.7mg/kg for 5f, which are similar to the standard drugs Phenytoin (PHY) and Carbamazepine (CBZ). The protective index for 5a and 5f was found to be 32.4 and 22.1 in the MES model, and 1.5 and 1.3 in the scPTZ test (Table 2). Thus, it can be concluded that these two compounds are effective and comparatively safer anticonvulsant agents as compared to the standard drugs, particularly in the MES model.

**Table 2. Phase-II Anticonvulsant Evaluation of Selected Compounds in MES and scPTZ Seizure Models Showing ED<sub>50</sub>, TD<sub>50</sub> and Protective Index (PI)**

| Comp d. | <sup>1</sup> ED <sub>50</sub> (mg/kg) |        | <sup>2</sup> TD <sub>50</sub> (mg/kg) | PI   |        |
|---------|---------------------------------------|--------|---------------------------------------|------|--------|
|         | ME S                                  | scPT Z |                                       | ME S | scPT Z |
| 5a      | 17.1                                  | 382.6  | 528.9                                 | 32.4 | 1.5    |
| 5f      | 19.8                                  | 308.9  | 415.7                                 | 22.1 | 1.3    |
| PHY     | 9.7                                   | >300   | 66.2                                  | 7.1  | <0.2   |
| CBZ     | 9.1                                   | >100   | 72.4                                  | 8.4  | <0.2   |

## RESULT AND DISCUSSION:

We utilized pharmacokinetics and molecular docking in this investigation, each of which offered distinct complimentary insights amidst structure-activity connections the best binding score of ligands ranged from –7.3 to –9.7 kcal/mol within the active site of GABA aminotransferase (GABA-AT). Among these compounds two amalgams are demonstrating best score as (5a -9.7) and (5f -8.9) as compared to the standard drug due to the presence of aromatic phenyl ring in compound 5a while 5f demonstrate little less due to presence of NO<sub>2</sub> group which plays an essential role in stabilizing the ligand with in the hydrophobic pocket participated in  $\pi$ - $\pi$  stacking interactions with Phe189A and  $\pi$ -sigma interactions with Val300A. Notably, 5a and 5f respectively formed three hydrogen bonds with Ser137A, Lys329A, and Cys135A, which are key catalytic residues within the active site. In contrast (5e -8.2, 5g -8.1 and 5h -8.1) shows similar docking scores as compared to the standard drug due to the presence of hydroxyl, methoxy and chloride groups, which increases bonding interactions with catalytic residues directly correlate with enhanced binding affinity. Notably compounds (5b -7.4, 5c -7.3, and 5d -7.3) demonstrate similar docking score due to

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the attachment of methoxy, chloride, and methyl group reduces the activity due present at para position and its hydrophobic in nature, although it plays an important role for improving ligand orientation and electrostatic stabilization within the binding cavity.

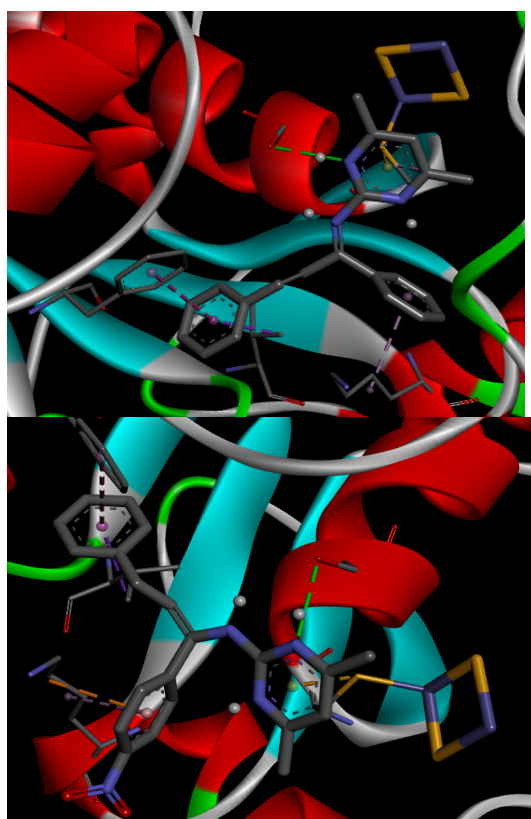
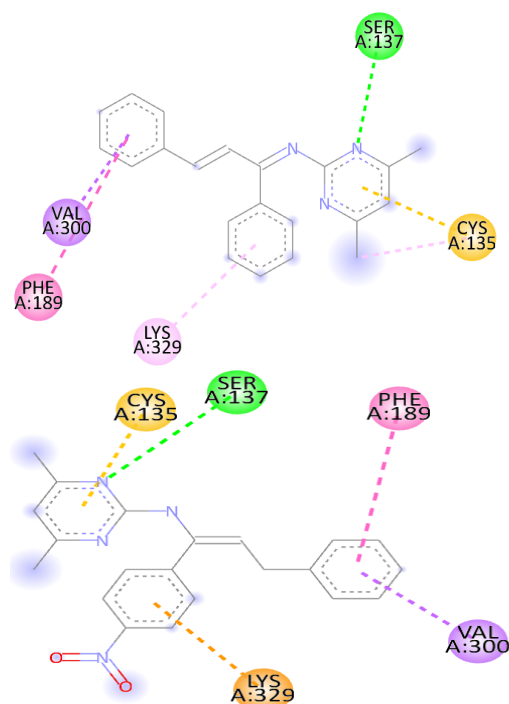


Figure 2: 2D & 3D Molecular docking poses of 5a (Left) and 5f (Right) on active site of target protein

Table 3. Molecular Docking Interaction Summary of Synthesized Compounds with GABA-AT (PDB ID: 1OHV)

| S . N . | Co mp oun d | Sub stit uen t | Bin din g En erg y (kc al/ mol ) | S . N . | Co mp oun d              | Sub stit uen t | Bin din g En erg y (kc al/ mol ) |
|---------|-------------|----------------|----------------------------------|---------|--------------------------|----------------|----------------------------------|
| 1       | 5a          | H              | -9.7                             | 6       | 5f                       | NO 2           | -8.9                             |
| 2       | 5b          | Para - OC H3   | -7.4                             | 7       | 5g                       | Orth o- OC H3  | -8.1                             |
| 3       | 5c          | Para -Cl       | -7.3                             | 8       | 5h                       | Orth o-Cl      | -8.1                             |
| 4       | 5d          | - CH 3         | -7.3                             | 9       | Phenytoin (Standard)     |                | -8.1                             |
| 5       | 5e          | -OH            | -8.2                             | 10      | Carbamazepine (Standard) |                | -8.5                             |

Table 4. *In-silico* ADMET screening of Synthesized compounds

| Co mp Na me | Mol. Wt. | H- Bon d Don or | H- Bon d Accep tors | Topolog ical Polar Surface Area | Rule of 5 |
|-------------|----------|-----------------|---------------------|---------------------------------|-----------|
| 5a          | 313.404  | 0               | 3                   | 142.015                         | 0         |
| 5b          | 343.43   | 0               | 4                   | 153.494                         | 0         |
| 5c          | 347.849  | 1               | 3                   | 142.319                         | 0         |
| 5d          | 327.431  | 2               | 3                   | 138.380                         | 1         |
| 5e          | 329.403  | 3               | 4                   | 126.810                         | 1         |
| 5f          | 358.401  | 0               | 5                   | 136.668                         | 0         |
| 5g          | 343.43   | 1               | 3                   | 123.494                         | 1         |
| 5h          | 347.849  | 0               | 3                   | 132.319                         | 1         |
| Std         | 130-725  | 0-6             | 2-20                | 140                             | 4         |

Through the utilization of molecular docking, pharmacokinetics and *in-vivo* studies, the current study provides evidence that Pyrimidine-chalcone derivatives have the potential to be effective anticonvulsant agents. Docking experiments were extremely helpful in determining the major binding interactions that occurred between the ligands and GABA-AT; this finding is corroborated by the pharmacokinetic studies that were conducted later on, the combination of studies allows for a more comprehensive understanding of the elements that influence anticonvulsant activity. To summarize, the application of molecular modelling approaches has made it possible to rationally design innovative agents that have moderate potential to treat anticonvulsant in a more effective manner.

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