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Design, In-Silico Studies, Synthesis, Characterization, and Anticonvulsant Activities of Novel Thiazolidin-4-One Substituted Thiazole Derivatives

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Abstract: A series of novel thiazolidine-4-one substituted thiazoles were prepared using multi-step synthesis and screened for their antiepileptic potency. The chemical nature of the prepared derivatives was confirmed using FT-IR, elemental analyses, Mass spectroscopy, and ^{1}H-NMR. Molecular properties and antiepileptic potency of the novel thiazoles were predicted using in-silico models such as molinspiration online tool and molecular docking, respectively. *In-vivo* antiepileptic potency of the entire title compounds was determined using MES and scPTZ method. Additionally, the rotorod test was employed to determine the neurotoxicity of the synthesized derivatives. Entire title analogs displayed -varying degrees of antiepileptic and neurotoxicity potency. The pharmacological potency of tested compounds was compared with their chemical structures. 2-(4-Nitrophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl) thiazolidin-4-one (PTT6) was found to be the very active derivative of this series among thirteen tested derivatives. Thus, this analog may act as a lead molecule to find potent and safer antiepileptic drugs.

Keywords: thiazole; thiazolidin-4-one; epilepsy; seizure; MES method; scPTZ method; neurotoxicity.

1. Introduction

. Epilepsy is a neurological, particularly CNS disorder. The brain activity becomes abnormal in epilepsy, leading to seizures or periods of unusual sensations, behavior, and sometimes loss of awareness. Excessive electrical discharges in a group of brain cells result in seizure episodes. Such electrical discharges may occur in different brain parts [1-3]. Seizures can vary from prolonged and severe convulsions to the briefest lapses of muscle jerks or attention. The frequency of seizures may vary from less than 1/year to several/day. No permanent cure is available for epilepsy to date. The use of AED_S (antiepileptic drugs) and/or direct or indirect electrical stimulation and seizure suppression by surgery (invasive) are the currently available options for treating epilepsy. The major drawbacks of presently existing AEDs are serious side effects and toxicity. Hence, medicinal chemistry has a huge scope for developing novel AED_S with high selectivity and lower toxicity [4].

From the literature review, it was found that the important pharmacophore responsible for producing antiepileptic activity are 1) A or hydrophobic domain (A), 2) HAD or hydrogen

donor or acceptor unit and 3) D or electron donor atom. This pharmacophore pattern was found in many first and second-generation AEDS and preclinical/clinical development stage AEDs [5,6] (Figure 1). Currently, one or more heterocyclic nucleus is present in many available medicinal compounds. Thiazole was found to be one such heterocyclic compound based on its potent biological activities [7-18]. Thiazoles have emerged as anticonvulsant [19,20] agents because of their in-vitro and *In-vivo* broad-spectrum anticonvulsant potency. The anticonvulsant activity of thiazole moiety is ascribed to its ability to act as a constrained pharmacophore at the receptor site. Moreover, thiazolidine-4-one [21-24] is reported to have several biological -properties, specifically anticonvulsant activity. Due to the importance, we aimed to prepare derivatives with thiazolidine-4-one nucleus and thiazole as probable anticonvulsant agents, which might provide improved therapeutic results with less neurotoxicity. In addition, in the organic portal, using the online tool molecular docking, ADME, molecular properties, and toxicity of title compounds were also planned to estimate.

2. Materials and Methods

2.1. Materials.

The reagents and chemicals utilized in this research were procured from several industries like CDH, SD Fine Chem., Qualigens, and E. Merck India Ltd. LR grade solvents were used with purification before their use. From E. Merck India Ltd., silica gel G was obtained for TLC (analytical chromatography). Using an open glass capillary, synthesized compounds' melting points were measured and uncorrected. Jasco FT-IR 410 spectrometer was used to record the IR spectra of synthesized compounds in KBr pellets. ^{1}H-NMR spectra of synthesized derivatives were measured on Bruker FT-NMR spectrometer at 300 MHz. TMS (tetramethylsilane) was used as an internal standard for ^{1}H-NMR. In ppm scale, the chemical shifts are documented. Electron impact ionization was used to measure the mass spectra using the JEOL-SX-102 instrument. 2400CHN analyzer model from Perkin Elmer was utilized for recording microanalysis.

2.2. In-silico studies.

AutoDock 4.2 was used for molecular docking studies. Install Molegro Molecular viewer was used for protein pre-processing, accessing binding pocket interactions, and exploring the docked complex. Additionally, molecular properties and toxicity were evaluated by the online tool in the organic portal web of preADMET and molinspirations. http://www.rcsb.org/pdb (RCSB Protein Data Bank (PDB) database) was used to retrieve the targeted enzyme, i.e., carbonic anhydrase (ID of PDB: 3F8E) and GABAAT (ID of PDB: 1OHW). Biovia Discovery studio visualizer was used to visualize the specific intermolecular interactions with the targets.

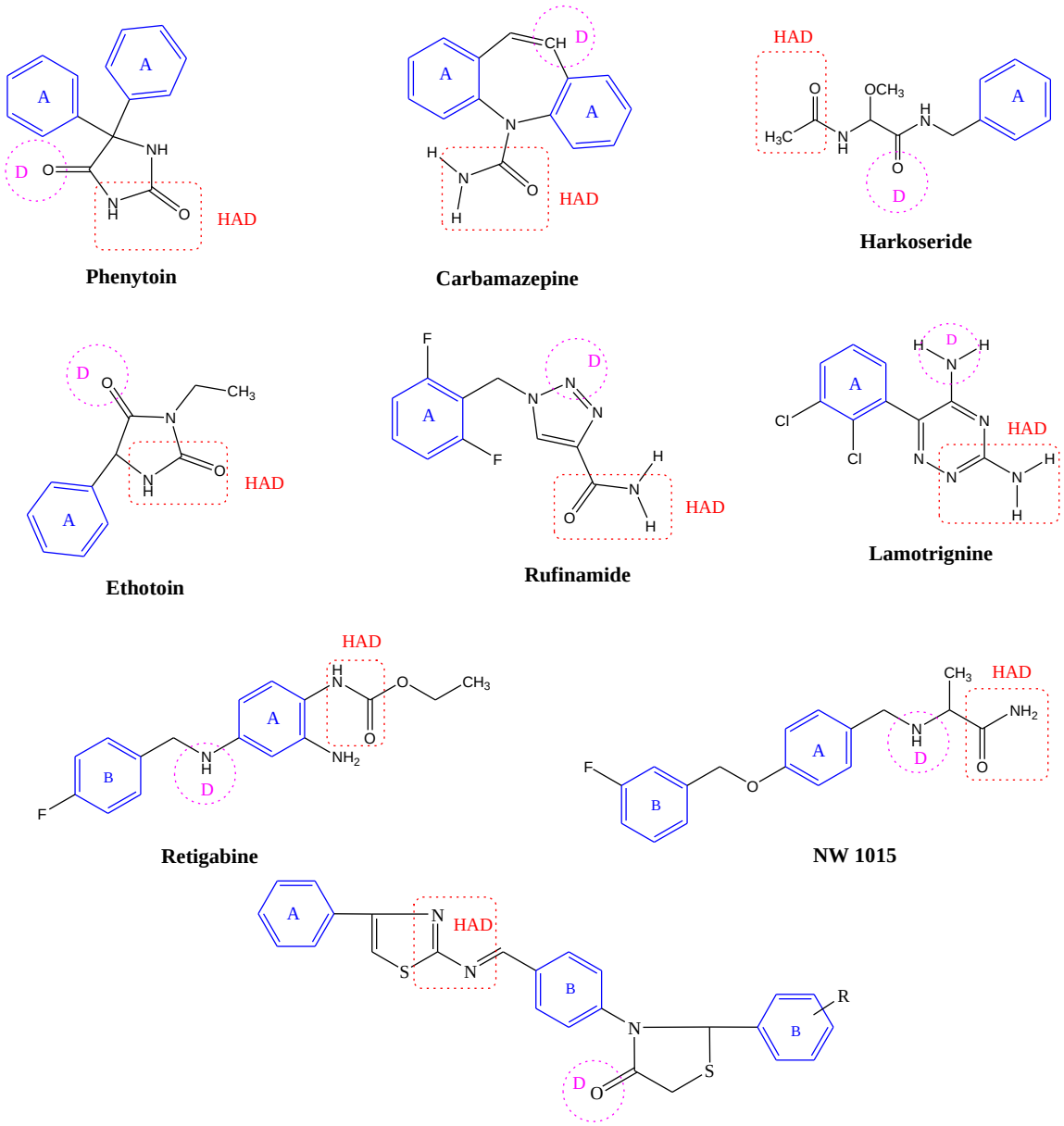


Figure 1. The pharmacophoric pattern of well-known antiepileptics and title compounds with its vital structural features: (**A**) hydrophobic aryl ring system, (HAD) hydrogen bond acceptor/donor domain, (**D**) electron donor moiety and (**B**) distal aryl ring.

2.3. Synthesis and characterization.

2.3.1. synthesis of 4-phenylthiazol-2-amine (PTA).

In 100 ml RBF, a solution of 0.01 mol thiourea and 0.01 mol acetophenone in 35 ml N-propanol (35 ml) was taken and refluxed for 2 h. Drop by drop with vigorous stirring, 5 ml of pyridine was added to the above solution and refluxed for 5 h further. Evaporated the solution to dryness and mixed it with sodium bicarbonate solution (5%). The obtained product 4-phenylthiazol-2-amine (**PTA**) was filtered, washed with water and dried. The product was recrystallized using methanol. Yield (in %): 83. Melting point (in °C): 216-218. IR (KBr) cm^{-1}: 3347 (N-H stretching), 3031 (Aromatic C-H stretching), 1643 (C=N stretching), 1626 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 6.93-8.06 (m, 5H, Aromatic C-H), 6.59 (s, 1H, =CH of thiazole), 4.71 (s, 2H, NH_2). EI-MS (*m/z*): 176 (M^+). *Anal*. Calcd for $C_9H_8N_2S$: C, 61.34; H, 4.58; N, 15.90. Found: C, 61.52; H, 4.56; N, 15.85.

2.3.2. Synthesis of N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (ABP).

Portion wise, 0.01 mol of 4-phenylthiazol-2-amine (**PTA**) was added with stirring to a finely mixed mixture of 0.01 mol 4-aminobenzaldehyde, 0.5 ml glacial acetic acid, and 30 ml ethanol. In the water bath, refluxed the obtained solution overnight and set it aside for some time. Later the mixture was transferred by stirring into ice-cold water. The separated compound N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (**ABP**) was filtered and dried. Ethanol was employed to recrystallize the products. Yield (in %): 79. Melting point (in °C): 270-272. IR (KBr) cm^{-1}: 3350 (N-H stretching), 3019 (Aromatic C-H stretching), 3012 (N=C-H stretching), 1625 (C=N stretching), 1608 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.79 (s, 1H, N=CH), 7.10-8.34 (m, 9H, Aromatic C-H), 6.92 (s, 1H, =CH of thiazole), 4.15 (s, 2H, NH_2). EI-MS (*m/z*): 279 (M^+). *Anal*. Calcd for $C_{16}H_{13}N_3S$: C, 68.79; H, 4.69; N, 15.04. Found: C, 68.56; H, 4.70; N, 15.09.

2.3.3. Synthesis of 2-(substitutedphenyl-3-(4-((4-phenylthiazol-2-ylimino)methyl) phenyl) thiazolidin-4-one (PTT1-PTT13).

To a mixture of 0.01 mol N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (**ABP**) and 0.01 mol various aromatic aldehydes in ethanol (20 ml), 0.01 mol mecaptoacetic acid were added with stirring slowly. Later, a mixture of zinc chloride (0.5 g) in ethanol (10 ml) was added with vigorous stirring slowly. The contents were refluxed in a water bath at 100°C for 12 h. Finally, the obtained solution was poured into ice-cold water and stirred well. The product-separated **PTT1-PTT13** were filtered, washed with water, and dried. The residue was recrystallized from ethanol.

2-(4-Aminophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT1): Yield (in %): 72. Melting point (in °C): 215-217. IR (KBr) cm^{-1}: 3314 (N-H stretching), 3057 (Aromatic C-H stretching), 3013 (N=C-H stretching), 1719 (C=O stretching), 1656 (C=N stretching), 1602 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.32 (s, 1H, N=CH), 6.85-7.98 (m, 13H, Aromatic C-H), 6.70 (s, 1H, =CH of thiazole), 6.05 (s, 1H, CH of thiazolidinone), 4.59 (s, 2H, NH_2), 3.51 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 456 (M^+). *Anal*. Calcd for $C_{25}H_{20}N_4OS_2$: C, 65.76; H, 4.42; N, 12.27. Found: C, 65.93; H, 4.41; N, 12.30.

2-(4-Hydroxyphenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT2): Yield (in %): 78. Melting point (in °C): 197-199. IR (KBr) cm^{-1}: 3356 (O-H stretching), 3020 (Aromatic C-H stretching), 3015 (N=C-H stretching), 1732 (C=O stretching), 1653 (C=N stretching), 1637 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.57 (s, 1H, N=CH), 7.04-8.11 (m, 13H, Aromatic C-H), 6.50 (s, 1H, =CH of thiazole), 5.86 (s, 1H, CH of thiazolidinone), 5.38 (s, 1H, OH), 3.79 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 457 (M^+). *Anal*. Calcd for $C_{25}H_{19}N_3O_2S_2$: C, 65.62; H, 4.19; N, 9.18. Found: C, 65.79; H, 4.17; N, 9.15.

2-(4-Methoxyphenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT3): Yield (in %): 75. Melting point (in °C): 189-190. IR (KBr) cm^{-1}: 3016 (Aromatic C-H stretching), 3008 (N=C-H stretching), 2892 (Aliphatic C-H stretching), 1735 (C=O stretching), 1620 (C=N stretching), 1614 (C=C stretching), 1039 (C-O-C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.90 (s, 1H, N=CH), 7.12-8.39 (m, 13H, Aromatic C-H), 6.85 (s, 1H, =CH of thiazole), 6.03 (s, 1H, CH of thiazolidinone), 3.97 (s, 3H, OCH_3), 3.46 (s, 2H, CH_2

of thiazolidinone). EI-MS (*m/z*): 471 (M^+). *Anal*. Calcd for $C_{26}H_{21}N_3O_2S_2$: C, 66.22; H, 4.49; N, 8.91. Found: C, 66.41; H, 4.48; N, 8.88.

3-(4-((4-Phenylthiazol-2-ylimino)methyl)phenyl)-2-p-tolylthiazolidin-4-one (PTT4): Yield (in %): 73. Melting point (in °C): 230-231. IR (KBr) cm^{-1}: 3054 (Aromatic C-H stretching), 3001 (N=C-H stretching), 2948 (Aliphatic C-H stretching), 1735 (C=O stretching), 1632 (C=N stretching), 1619 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.22 (s, 1H, N=CH), 6.80-7.96 (m, 13H, Aromatic C-H), 6.54 (s, 1H, =CH of thiazole), 6.02 (s, 1H, CH of thiazolidinone), 3.18 (s, 2H, CH_2 of thiazolidinone), 2.93 (s, 3H, CH_3). EI-MS (*m/z*): 455 (M^+). *Anal*. Calcd for $C_{26}H_{21}N_3OS_2$: C, 68.54; H, 4.65; N, 9.22. Found: C, 68.67; H, 4.66; N, 9.20.

2-(4-Chlorophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT5): Yield (in %): 77. Melting point (in °C): 183-185. IR (KBr) cm^{-1}: 3043 (Aromatic C-H stretching), 3020 (N=C-H stretching), 1707 (C=O stretching), 1634 (C=N stretching), 1629 (C=C stretching), 708 (C-Cl stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.65 (s, 1H, N=CH), 7.07-8.22 (m, 13H, Aromatic C-H), 6.64 (s, 1H, =CH of thiazole), 5.89 (s, 1H, CH of thiazolidinone), 3.73 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 477 (M^{+2}), 475 (M^+). *Anal*. Calcd for $C_{25}H_{18}ClN_3OS_2$: C, 63.08; H, 3.81; N, 8.83. Found: C, 62.89; H, 3.82; N, 8.86.

2-(4-Nitrophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT6): Yield (in %): 74. Melting point (in °C): 237-238. IR (KBr) cm^{-1}: 3057 (Aromatic C-H stretching), 3024 (N=C-H stretching), 1701 (C=O stretching), 1656 (C=N stretching), 1625 (C=C stretching), 1523 and 1307 (NO_2 stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.41 (s, 1H, N=CH), 6.91-8.19 (m, 13H, Aromatic C-H), 6.77 (s, 1H, =CH of thiazole), 5.90 (s, 1H, CH of thiazolidinone), 3.35 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 486 (M^+). *Anal*. Calcd for $C_{25}H_{18}N_4O_3S_2$: C, 61.71; H, 3.73; N, 11.51. Found: C, 61.90; H, 3.74; N, 11.47.

2-Phenyl-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT7): Yield (in %): 70. Melting point (in °C): 193-195. IR (KBr) cm^{-1}: 3049 (Aromatic C-H stretching), 3002 (N=C-H stretching), 1713 (C=O stretching), 1651 (C=N stretching), 1637 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.17 (s, 1H, N=CH), 6.81-7.84 (m, 14H, Aromatic C-H), 6.59 (s, 1H, =CH of thiazole), 5.72 (s, 1H, CH of thiazolidinone), 3.57 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 441 (M^+). *Anal*. Calcd for $C_{25}H_{19}N_3OS_2$: C, 68.00; H, 4.34; N, 9.52. Found: C, 67.77; H, 4.35; N, 9.56.

2-(3-Aminophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT8): Yield (in %): 76. Melting point (in °C): 174-177. IR (KBr) cm^{-1}: 3376 (N-H stretching), 3023 (Aromatic C-H stretching), 3008 (N=C-H stretching), 1726 (C=O stretching), 1647 (C=N stretching), 1614 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.63 (s, 1H, N=CH), 7.07-8.42 (m, 13H, Aromatic C-H), 6.85 (s, 1H, =CH of thiazole), 6.16 (s, 1H, CH of thiazolidinone), 4.48 (s, 2H, NH_2), 3.20 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 456 (M^+). *Anal*. Calcd for $C_{25}H_{20}N_4OS_2$: C, 65.76; H, 4.42; N, 12.27. Found: C, 66.01; H, 4.43; N, 12.22.

2-(3-Hydroxyphenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT9): Yield (in %): 73. Melting point (in °C): 226-228. IR (KBr) cm^{-1}: 3352 (O-H stretching), 3032 (Aromatic C-H stretching), 3016 (N=C-H stretching), 1730 (C=O stretching), 1628 (C=N stretching), 1605 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.36 (s, 1H, N=CH), 7.29-8.14 (m, 13H, Aromatic C-H), 6.86 (s, 1H, =CH of thiazole), 5.81 (s, 1H, CH of thiazolidinone), 5.12 (s, 1H, OH), 3.65 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 457

(M^+). *Anal*. Calcd for $C_{25}H_{19}N_3O_2S_2$: C, 65.62; H, 4.19; N, 9.18. Found: C, 65.45; H, 4.20; N, 9.21.

2-(3-Methoxyphenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT10): Yield (in %): 79. Melting point (in °C): 205-207. IR (KBr) cm^{-1}: 3038 (Aromatic C-H stretching), 3013 (N=C-H stretching), 2960 (Aliphatic C-H stretching), 1734 (C=O stretching), 1639 (C=N stretching), 1602 (C=C stretching), 1028 (C-O-C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.58 (s, 1H, N=CH), 6.73-8.05 (m, 13H, Aromatic C-H), 6.41 (s, 1H, =CH of thiazole), 5.92 (s, 1H, CH of thiazolidinone), 3.84 (s, 3H, OCH_3), 3.49 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 471 (M^+). *Anal*. Calcd for $C_{26}H_{21}N_3O_2S_2$: C, 66.22; H, 4.49; N, 8.91. Found: C, 66.47; H, 4.50; N, 8.89.

3-(4-((4-Phenylthiazol-2-ylimino)methyl)phenyl)-2-m-tolylthiazolidin-4-one (PTT11): Yield (in %): 72. Melting point (in °C): 233-234. IR (KBr) cm^{-1}: 3025 (Aromatic C-H stretching), 3009 (N=C-H stretching), 2954 (Aliphatic C-H stretching), 1708 (C=O stretching), 1647 (C=N stretching), 1621 (C=C stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.20 (s, 1H, N=CH), 6.95-7.92 (m, 13H, Aromatic C-H), 6.68 (s, 1H, =CH of thiazole), 6.14 (s, 1H, CH of thiazolidinone), 3.26 (s, 2H, CH_2 of thiazolidinone), 2.73 (s, 3H, CH_3). EI-MS (*m/z*): 455 (M^+). *Anal*. Calcd for $C_{26}H_{21}N_3OS_2$: C, 68.54; H, 4.65; N, 9.22. Found: C, 68.29; H, 4.67; N, 9.26.

2-(3-Chlorophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT12): Yield (in %): 75. Melting point (in °C): 179-181. IR (KBr) cm^{-1}: 3013 (Aromatic C-H stretching), 3007 (N=C-H stretching), 1715 (C=O stretching), 1641 (C=N stretching), 1620 (C=C stretching), 715 (C-Cl stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.81 (s, 1H, N=CH), 7.20-8.39 (m, 13H, Aromatic C-H), 6.93 (s, 1H, =CH of thiazole), 5.88 (s, 1H, CH of thiazolidinone), 3.64 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 477 (M^{+2}), 475 (M^+). *Anal*. Calcd for $C_{25}H_{18}ClN_3OS_2$: C, 63.08; H, 3.81; N, 8.83. Found: C, 63.27; H, 3.80; N, 8.79.

2-(3-Nitrophenyl)-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl)thiazolidin-4-one (PTT13): Yield (in %): 74. Melting point (in °C): 201-203. IR (KBr) cm^{-1}: 3051 (Aromatic C-H stretching), 3024 (N=C-H stretching), 1726 (C=O stretching), 1639 (C=N stretching), 1603 (C=C stretching), 1525 and 1311 (NO_2 stretching). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 8.44 (s, 1H, N=CH), 7.08-8.16 (m, 13H, Aromatic C-H), 6.71 (s, 1H, =CH of thiazole), 6.07 (s, 1H, CH of thiazolidinone), 3.42 (s, 2H, CH_2 of thiazolidinone). EI-MS (*m/z*): 486 (M^+). *Anal*. Calcd for $C_{25}H_{18}N_4O_3S_2$: C, 61.71; H, 3.73; N, 11.51. Found: C, 61.93; H, 3.72; N, 11.48.

2.4. Biological activities.

2.4.1. Pharmacology.

Using male 18-25 g *Swiss* albino mice and 100-150 g Wistar rats, entire prepared analogs were screened for their antiepileptic potencies. In mice, two epilepsy methods as MES technique and *sc*PTZ technique, are used for two primary qualitative estimations. A standardized rotorod method was employed in mice to examine the acute neurological toxicity induced by the prepared analogs. Initially, 30 mg/kg, 100 mg/kg, and 300 mg/kg, *i.p.* dose was used to assess the antiepileptic potencies of title derivatives using epilepsymodelsl such as MES (produces generalized tonic-clonic seizures) and *sc*PTZ (induces myoclonic seizures) models. The potency was calculated after 0.5 and 4 h of test compounds injection. MES and scPTZ tests are generally used to identify seizure spread prevention and seizure threshold increment, respectively. Standard animal feed was used to feed animals who were grouped as six animals

in all clusters. The animals were preserved at 25 ± 2 °C in colony cages under a 12 h light and dark sequence with 45–55 % relative humidity [25]. Weeks before use, entire animals were acclimatized. The protocol employed for experimentation is properly approved by the Institutional Animal Ethics committee (IAEC).

2.4.2. Antiepileptic activity.

2.4.2.1. The MES (maximal electroshock) test.

In this technique, before inserting the corneal electrodes into the eyes of an animal, a drop of a mixture composed of 0.9 % saline (electrolyte) solution and 0.5 % tetracaine HCl (anesthetic) solution was applied. 50 milli Ampere electrical stimulus was applied for mice at 60 Hz, and 150 milli Ampere electrical stimulus was applied for rats at 60 Hz for a 0.2 sec period using similar earlier documented apparatus. The endpoint of the MES seizure technique was determined from the elimination of the hindleg tonic extensor phase. For the preliminary estimation, mice were used against 30, 100, and 300 mg/kg doses of title analogs by i.p. route of administration. Initially, rats gave a 30 mg/kg dose of the synthesized drug orally. The obtained results were compared with standard phenytoin.

2.4.2.2. The scPTZ (subcutaneous pentylenetetrazole seizure) technique.

In this technique, an 85 mg/kg dose (which produce convulsion in 97 % of animal) of pentylenetetrazole (the chemical which induces convulsion) was injected in the midline of the neck into a loose fold of the skin of mice present to generate convulsion. Test derivatives were injected by *i.p.* injection into the animals before injecting pentylenetetrazole. The stress on animals was minimized by placing the animals in a segregation cage and the presence/absence of a seizure in animals was observed for the next thirty minutes. The endpoint *sc*PTZ technique is a 3-5 sec incident of clonic spasms of the hind and/or forelimbs, jaws, or vibrissae. The animals are considered protected if it does not meet this condition [26-28]. For the estimation, mice were used against 30, 100, and 300 mg/kg dose of title analogs by *i.p.* route of administration. The obtained results were compared with standard ethosuximide.

2.4.3. Acute toxicity-minimal motor impairment.

Obvious signs of damaged muscular or neurological functions of animals are monitored in order to assess the test analogs' toxicity (undesirable side effects). The MNI (minimal neurological impairment) and MMI (minimal muscular impairment) in mice were disclosed using rotorod procedure. The mouse can maintain their equilibrium when the rod rotates at 6 rpm speed for long periods when placed on a rotating rod. During a period of 1 minute, if the mouse falls off three times from this rotating rod, then the corresponding dose of tested analog was considered toxic to animals. Abnormal body posture, a zigzag or circular gait and loss of placing response, the spread of the legs, catalepsy, somnolence, lack of exploratory behavior, changes in muscle tone, stupor, hyperactivity, and tremors also noted in animals in addition to MMI and MNI.code.

3. Results and Discussion

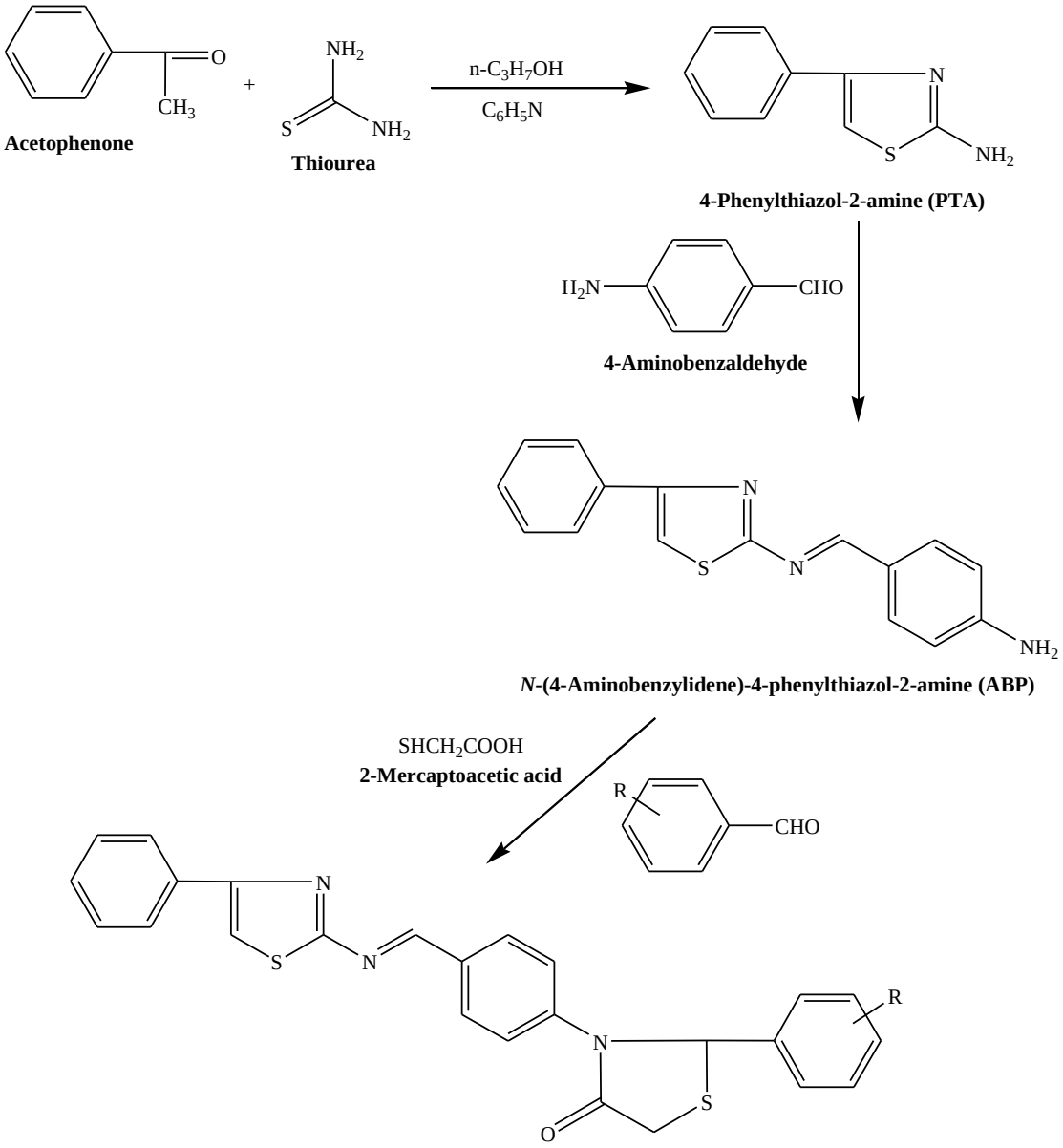
3.1. Chemistry.

A novel thiazolidine-4-one substituted thiazoles (**PTT1-PTT13)** were synthesized according to the protocol specified in the synthetic scheme. In the present research, thirteen novel 2-(substitutedphenyl-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl) thiazolidin-4-one (**PTT1-PTT13**) were prepared from acetophenone and thiourea by multi-step synthesis. Initially, acetophenone and thiourea undergo cyclization in the presence of propanol and pyridine to produce 4-phenylthiazol-2-amine (**PTA**). In the next step, 4-phenylthiazol-2-amine (**PTA**) was reacted with 4-aminobenzaldehyde to produce N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (**ABP**) by a Schiff base reaction with a loss of one water molecule**.** In the final step, various aromatic aldehydes are reacted with 2-mercapto acetic acid and N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (**ABP**) to produce the title compounds, i.e., 2-(Substitutedphenyl-3-(4-((4-phenylthiazol-2-ylimino)methyl)phenyl) thiazolidin-4-one (**PTT1-PTT13**) by ring closure reaction.

Assigned structures of the synthesized compounds are confirmed from IR, ^{1}H-NMR, mass spectra, and microanalysis. In IR spectrum of 4-phenylthiazol-2-amine (**PTA**), the appearance of the absorption peak at 3347 cm^{-1} corresponds to N-H stretching and disappearance of the absorption peak around 1700 cm^{-1} corresponds to the carbonyl group (C=O) confirms its formation. It is further supported by the appearance of one proton singlet peak in NMR spectrum at δ 6.59 ppm corresponds to =CH of thiazole. Formation of compound N-(4-aminobenzylidene)-4-phenylthiazol-2-amine (**ABP**) is confirmed by the appearance of an absorption peak at 3012 cm^{-1} corresponding to -N=CH- stretching. It is further supported by the appearance of a singlet for one proton at δ 8.79 ppm due to the -N=CH- group in the ^{1}H-NMR spectrum. The disappearance of the absorption peak around 3300 cm^{-1} corresponds to an amino group in IR spectra, and singlet for two protons around δ 4.50 ppm corresponds to an amino group in the ^{1}H-NMR spectrum, confirming the development of title compounds **PTT1-PTT13.** The IR spectrum of title compounds shows absorption bands between 3009-3057, 3001-3024, 1701-1735, 1620-1656, and 1602-1637 cm^{-1}, which can be assignable to aromatic C-H stretching, N=C-H stretching, C=O stretching, C=N stretching, and C=C stretching, respectively. The appearance of a strong absorption band in the IR spectrum of compounds **PTT1** and **PTT8** at 3314 and 3376 cm^{-1}, respectively, confirm the amino group's presence. The appearance of a strong absorption band in the IR spectrum of compounds **PTT2** and **PTT9** at 3356 and 3352 cm^{-1}, respectively, confirm the hydroxy group's presence. The appearance of a strong absorption band in the IR spectrum of compound **PTT3** and **PTT10** at 1039 and 1028 cm^{-1}, respectively, confirm the presence of C-O-C stretching. A strong absorption band in the IR spectrum of compound PTT3, PTT4, PTT10, and PTT11 at 2892, 2948, 2960, and 2954 cm-1, respectively, can be assigned to aliphatic C-H stretching, confirming its formation. The appearance of the strong absorption band in the IR spectrum of compound **PTT5** and **PTT12** at 708 and 715 cm^{-1}, respectively, which can be assigned to chloro group, confirms its formation. The appearance of a strong absorption band in IR spectrum of compounds **PTT6** and **PTT13** at 1523, 1307, 1525, and 1311 cm^{-1}, respectively, confirm the presence of the nitro group.

The entire title compounds ^{1}H-NMR spectrum comparisons given the following conclusions. a) A singlet for one proton around δ 8.17-8.90 ppm for -N=CH-; b) A multiplet in the range of δ 6.73-8.42 ppm for thirteen/fourteen aromatic proton; c) A singlet for one proton

in the range of δ 6.41-6.93 ppm which can be assignable to C_5-H of thiazole; d) A singlet for one proton in the range of δ 5.72-6.16 ppm for CH of thiazolidinone; e) A singlet for two protons in the range of δ 3.18-3.79 ppm for CH_2 of thiazolidinone. The appearance of an additional singlet peak for 2 protons in NMR spectrum at δ 4.59 and 4.48 ppm corresponds to NH_2 group, which confirms the structure of compounds **PTT1** and **PTT8**, respectively. The appearance of an additional singlet peak for 1 proton in the NMR spectrum at δ 5.38 and 5.12 ppm corresponds to the OH group confirming the structure of compounds **PTT2** and **PTT9**, respectively. The appearance of an additional singlet peak for 3 protons in the NMR spectrum at δ 3.97 and 3.84 ppm corresponds to OCH_3 group and confirms the structure of compounds **PTT3** and **PTT10**, respectively. The appearance of an additional singlet peak for 3 protons in the NMR spectrum at δ 2.93 and 2.73 ppm corresponds to CH_3 group and confirms the structure of compounds **PTT4** and **PTT11**, respectively. Further mass spectrum and elemental analyses confirm the proposed chemical structure of the synthesized compounds.



R = 4-NH_2 (**PTT1**), 4-OH (**PTT2**), 4-OCH_3 (**PTT3**), 4-CH_3 (**PTT4**), 4-Cl (**PTT5**), 4-NO_2 (**PTT6**), H (**PTT7**), 3-NH_2 (**PTT8**), 3-OH (**PTT9**), 3-OCH_3 (**PTT10**), 3-CH_3 (**PTT11**), 3-Cl (**PTT12**), 3-NO_2 (**PTT13**),

Figure 2. Synthetic protocols for the synthesis of the title compound (PTT1-PTT13).

3.2. In-silico studies.

3.2.1. Predictions of molecular properties.

Molecular properties of the test derivatives (**PTT1-PTT13)** were estimated using an online molinspiration tool using the Lipinski rule and were predicted and presented in Table 1. The results depicted that all the synthesized analogs satisfied and obeyed the Lipinski five rule.

Table 1. Molecular properties of synthesized compounds (PTT1-PTT13) by molinspiration.

Compound code	MW (g/mol)	Log P[1]	TPSA[b]	OH-NH interact[c]	O-N interact[d]	nrotb[e]	Volume
PTT1	456.6	4.65	71.59	2	5	5	392.06
PTT2	457.5	5.10	65.79	1	5	5	388.79
PTT3	471.6	5.63	54.8	0	5	6	406.31
PTT4	455.6	6.03	45.5	0	4	5	397.33
PTT5	476.0	6.25	45.56	0	4	5	394.30
PTT6	486.5	5.54	91.39	0	7	6	404.10
PTT7	441.5	5.58	45.56	0	4	5	380.77
PTT8	456.6	4.63	71.59	2	5	5	392.06
PTT9	457.5	5.07	65.79	1	5	5	388.79
PTT10	471.6	5.61	54.80	0	5	6	406.31
PTT11	455.6	6.00	45.56	0	4	5	397.33
PTT12	476.0	6.23	45.56	0	4	5	394.30
PTT13	441.58	5.58	45.56	0	4	5	380.77

[a] Calculated octanol/water partition coefficient; [b] Molecular polar surface area; [c] Number of hydrogen-bond donors; [d] Number of hydrogen-bond acceptors; [e] Number of rotatable bonds.

3.2.2. Predictions of ADME properties and drug-likeness scores.

The selection of prime potential drug molecules was consolidated as a therapeutic agent but may fail in clinical trials and have adverse effects. Synthesized derivatives exhibited significant ADME properties and are presented in Table 2.

Table 2. ADME properties of the synthesized compounds (PTT1-PTT13).

Compound code	BBB[a]	PPB [b]	HIA [c]	CaCo-2 [d]	MDCK [e]
PTT1	0.20	92.84	97.71	48.02	0.11
PTT2	0.07	92.80	97.12	52.23	0.12
PTT3	0.65	94.13	98.00	51.57	0.13
PTT4	0.49	94.84	97.72	19.81	0.27
PTT5	0.41	92.49	97.76	50.59	0.20
PTT6	0.54	93.80	100.00	27.94	0.09
PTT7	0.54	94.03	97.75	51.72	0.47
PTT8	0.22	95.99	97.71	48.06	0.15
PTT9	0.06	98.22	97.12	50.17	0.19
PTT10	0.59	98.00	98.00	51.44	0.18
PTT11	0.48	98.33	97.72	51.88	0.56
PTT12	0.23	92.45	97.76	51.37	0.36
PTT13	0.50	92.55	100.00	30.09	0.07

a Blood-Brain Barrier penetration, b Plasma Protein Binding, c Human Intestinal Absorption, d Caco-2 cell permeability, e MDCK cell permeability

3.2.3. Toxicity prediction.

The toxicity prediction of title compounds was predicted and reported in Table 3, In which the mutagenic property of the test compounds was assessed using the Ames test. From the reports of the Ames test, it was found that the majority of the test compounds were non-mutagenic. In addition, mouse and rat carcinogenicity was also predicted, and it found that most of the test analogs did not exhibit carcinogenicity by nature.

Table 3. Toxicity prediction of synthesized compounds (PTT1-PTT13).

Compound code	Ames test mutagenicity	Mouse carcinogenicity	Rat carcinogenicity
PTT1	Non-Mutagen	Negative	Positive
PTT2	Non-mutagen	Positive	Negative
PTT3	Mutagen	Positive	Negative
PTT4	Non-mutagen	Positive	Positive
PTT5	Non-Mutagen	Positive	Negative
PTT6	Mutagen	Positive	Negative
PTT7	Mutagen	Negative	Positive
PTT8	Non-mutagen	Positive	Positive
PTT9	Non-mutagen	Positive	Positive
PTT10	Non-mutagen	Negative	Positive
PTT11	Non-mutagen	Negative	Positive
PTT12	Non-mutagen	Positive	Negative
PTT13	Non-mutagen	Positive	Negative

[a] Positive = no carcinogenicity in mice; [b] Positive = no carcinogenicity in rats.

3.2.4. Molecular docking study.

Carbonic anhydrase inhibition (CA) has demonstrated interesting pharmacologic applications such as anticonvulsants, anti-glaucoma, and anticancer agents. These enzymes have shown a potential target for designing anticonvulsant drugs with a novel mechanism of action. The enzymes play an important role in the anion exchange processes.

The targeted molecular simulations found that the title analogs produced significant results in all tested targets, and the obtained results are presented in Table 4.

Table 4. Docking score of synthesized compounds (PTT1-PTT13) on *carbonic anhydrase.*

Ligands	Docking score (kcal/mole) COX-1 (3F8E)	Binding site amino acid residues	
		Hydrogen bond Interactions	Hydrophobic bond Interactions
PTT1	-8.6	Trp A: 5; Asn A:11; His A:64; Glu A:236.	His A:4; Lys A:170; Pro A: 201, 202.
PTT2	-7.5	His A:64.	Trp A:5; Gly A:63; Lys A:170; Phe A:131, 231; Leu A:198.
PTT3	-7.7	-	Pro A: 13; His A:15; Phe A: 231; Glu A:239; Asp A: 243.
PTT4	-9.41	Gly A:8.	Trp A:5, 245; Tyr A:7; Gly A:6; Phe A: 231; Glu A: 239; Asp A:243; Pro A: 247.
PTT5	-8.35	Trp A:5; His A:64.	Pro A: 202; Phe A: 231; Glu A:239.
PTT6	-8.5	Arg A:246; Lys A:252.	Glu A: 14; Gln A: 249; Lys A:24, 111, 113; Pro A: 247; Ala A:248.
PTT7	-8.54	Trp A:5; His A:64.	Lys A: 170; Pro A:202; Phe A:231.
PTT8	-7.91	Trp A:5; Thr A:200.	Gly A:63; Leu A:198; Phe A: 131, 231.
PTT9	-7.75	His A:64; Gln A:92	Trp A:5; Phe A: 131, 231; Leu A:198; Pro A:202.
PTT10	-8.1	Trp A:5; Gln A: 92.	Gly A: 63; Phe A: 131, 231; Lys A: 170;Leu A: 198.
PTT11	-8.03	Trp A:5; His A: 64.	Gly A: 63; His A: 94; Val A:121; Phe A: 131, 231; Leu A: 198.
PTT12	-8.53	His A:64	Trp A: 5; His A: 94; Phe A: 131, 231; Leu A: 198; Pro A: 202.
PTT13	-8.49	Trp A:5;	Leu A: 198; Phe A:231.
Phenytoin	-6.65	Trp A:5; Lys A:170; His A:4.	Gly A:6; Trp A:5; Asn A:62.
Ethoximide	-4.78	Asp A:19; His A:15.	Trp A:5; Lys A:18.

The docking score for synthesized derivatives (**PTT1-PTT13)** on *carbonic anhydrase* (PDB ID: 3F8E) produced significant results in the range of **-7.5 to -9.41** Kcal/mole compared with standards ethoximide (-4.78 Kcal/mole) and phenytoin (-6.65 Kcal/mole) were noted in

table 4. Among the thirteen compounds, **PTT4** afforded highly significant results as **-9.41** Kcal/mole. It produced its potency through binding affinity towards the targeted enzyme by hydrophilic and hydrophobic interactions shown in Figures 3A and 3B.

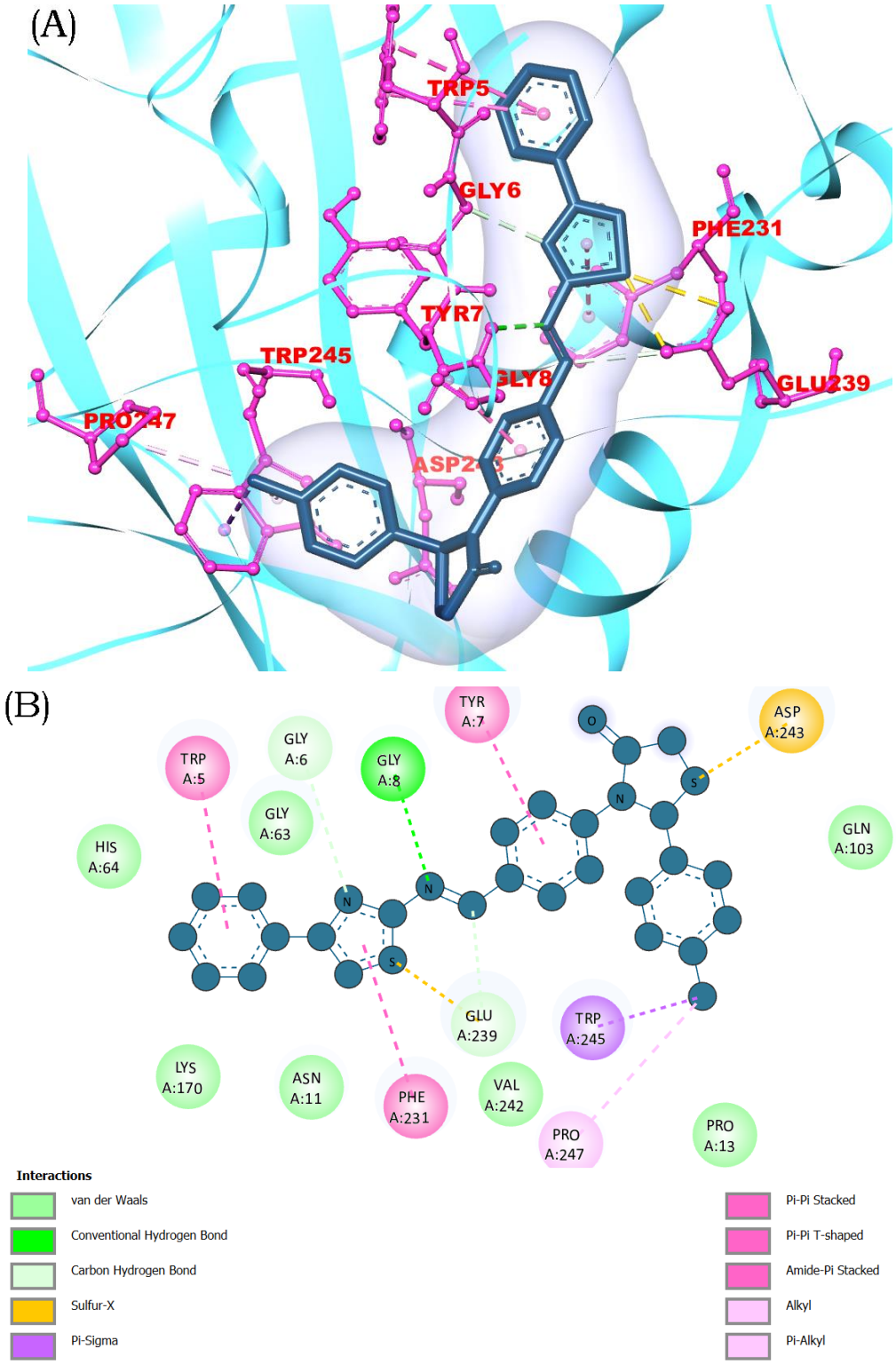
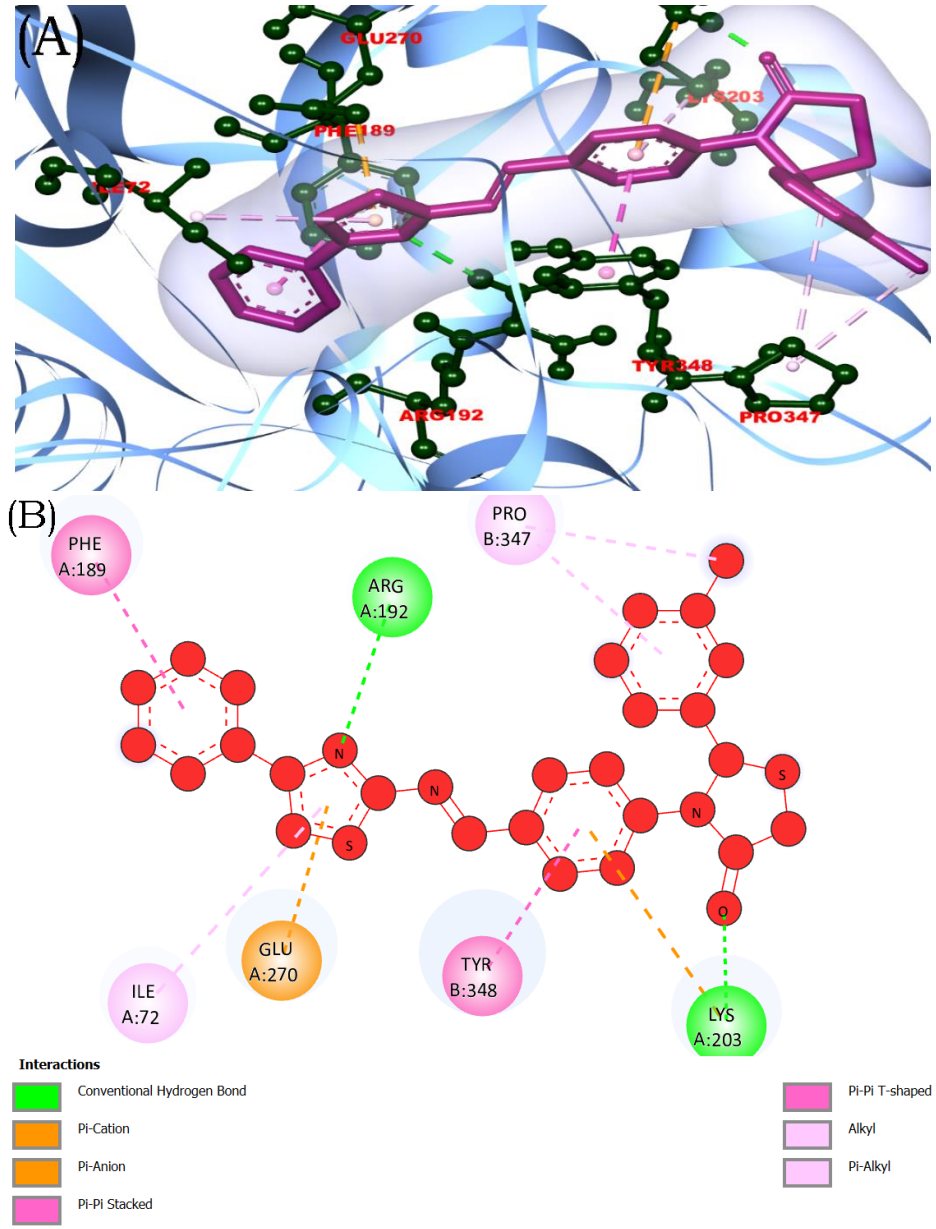


Figure 3. Molecular interactions between PTT4 and Carbonic anhydrase (CA) by Biovia discovery studio visualizer: (**A**) 3D-Docked pose of PTT4 (Blue color) and CA binding site (Pink color); (**B**) Hydrogen bond interactions on PTT4 and CA (green dashed lines) and Hydrophobic bonds (Pink dashed lines) and other amino acid residues.

In the CNS the pro-convulsant activity of PTZ is produced by decreasing the intensity of GABA-ergic inhibitory processes and inhibiting the benzodiazepine receptor complex

GABAA-site. In the human brain concentration of GABA is regulated by two pyridoxal-5'phosphate-dependent enzymes, namely *GABA-aminotransferase* (responsible for the inhibitory neurotransmitter degradation) and *glutamate decarboxylase*, (catalyzes glutamate transformation to GABA). Literature review suggests that compounds showing activity on the PTZ-induced seizure model may inhibit *GABA aminotransferase* or act as a GABA agonist. Simultaneously, drugs like gabapentin, benzodiazepine, phenobarbital, felbamate, vigabatrin, etc., displayed potency in the PTZ-induced seizure model because many show a multifactor mechanism of anticonvulsant activity. Hence, the second target of the molecular docking study is GABAAT (*γ-aminobutyrate aminotransferase*), in which all tested derivatives were found to be a noteworthy binding affinity towards this receptor. **PTT11** showed high binding scores such as -7.85 kcal/mole compared with ethoximide and phenytoin -3.08 and -3.78 Kcal/mole, respectively, were mentioned in Table 5, and the binding pose of **PTT11** represented in Figure 4.



4(**A**) 3D-Docked pose of PTT11 (Purple color) and GABAAT binding site (Green color); (**B**) 2D-Docking interaction: Hydrogen bond interactions on PTT11 and GABAAT (green dashed lines) and Hydrophobic bonds (Pink dashed lines) and other amino acid residues.

Table 5. Docking score of synthesized compounds (PTT1-PTT13) on GABAAT.

Ligands	Docking score (kcal/mole) GABAAT (1OHW)	Binding site Amino acid residues	
		Hydrogen bond Interactions	Hydrophobic bond Interactions
PTT1	-6.6	His A:44.	Tyr A:69; Ile A:72; Lys A:203; Val A:300; Lys A:329; Phe B:351; Arg A:445.
PTT2	-6.7	Trp A:215; Arg A:240.	Ile B:171, A: 217; Pro A:219; Arg A:222.
PTT3	-6.3	Ile A:217.	Met A:186; Pro A:219; Arg A:222; Asp A:247; Leu A:248.
PTT4	-6.4	-	Met A: 186; Ile A:217, B:171; Pro A:219.
PTT5	-6.1	-	Met A: 170, 186; Lys A:203; Ala A:204; Pro A:219; Phe A:220.
PTT6	-6.8	Gly A:407, 409.	Tyr A: 225; Pro A:226; His A:315; Lys A:385; Leu A:227, 392; Arg A:406 and 408.
PTT7	-6.3	Ser B:277; Arg B:408.	Leu B:223, 227, 388; Val B:231; Ala B:276; Lys B:385.
PTT8	-6.12	Glu B:41; Asn B: 423.	Tyr A:348; Met B:31; Lys B:32; Ala B:40; His B:44.
PTT9	-5.45	Lys B:86.	Lys A:86; Pro A:91.
PTT10	-6.04	-	Leu A:166; Met A: 170; Ala B:204; Arg B:222; Phe B:220; Asp B:441.
PTT11	-7.85	Arg A:192; Lys A:203.	Ile A:72; Phe A:189; Glu A:270; Pro B:347; Tyr B:348.
PTT12	-6.6	Arg A:240.	Met A: 186; Ile A:217, B: 171; Pro A:219.
PTT13	-7.29	Arg B:408.	Leu B:223, 227; Val B:231; Ala B:381
Phenytoin	-6.78	Val C:60; Arg C:26.	Trp D:5; Arg D:430; Glu D:270; Asn D:62.
Ethoximide	-6.08	Gly A:109; Arg B:26.	Leu B:30.

3.3. Biological activities.

3.3.1. Antiepileptic activity.

The maximal electroshock (MES) technique: MES and *sc*PTZ methods were employed to assess the antiepileptic potency of prepared compounds using mice by *i.p.* route administration. The compounds are considered notably valuable for treating partial, generalized, and even absence seizures if they exhibit good activity in these tests. All initial antiepileptic data of the synthesized compounds are tabulated in Table 6.

Out of thirteen synthesized analogs in the MES method, compounds **PTT5, PTT6** and **PTT13** were found to be considerably potent at the lowest (30 mg/kg) dose itself at a 0.5 h time interval. The activity of derivative **PTT6** and **PTT13** continued at 30 mg/kg dose at the 4.0 h time interval, whereas compounds **PTT5** continued only at 100 mg/kg dose at the 4.0 h time interval. The above statements indicate that these compounds **PTT5, PTT6** and **PTT13** possess long duration and rapid onsetof action. The presence of nitro or chloro group in phenyl ring attached at C-2 of thiazolidinone ring of these derivatives may be responsible for the capable activity. Derivatives **PTT1, PTT4, PTT7, PTT11** and **PTT12** displayed protection after 0.5 h at a dose of 100 mg/kg. This indicates that at a relatively lower dose, these derivatives are capable of guarding against seizures. Compounds such as **PTT7** and **PTT12** were found to exhibit activity at the same dose of 100 mg/kg after a 4.0 h time interval. After 4.0 h, the compounds **PTT1, PTT4** and **PTT11** were found to exhibit activity only at a 300 mg/kg (higher) dose. Either after 0.5 h time interval and/or 4.0 h time interval at a higher tested (300 mg/kg) dose, title compounds **PTT2-PTT3** and **PTT8-PTT10** was found to protect animals from seizure.

Table 6. Antiepileptic and neurotoxicity study of derivatives PTT1-PTT13 administered intraperitoneally to mice.

Derivatives	MES[1] test		*sc*PTZ[2] test		NT[3] test	
	0.5 h[4]	4.0 h[4]	0.5 h[4]	4.0 h[4]	0.5 h[4]	4.0 h[4]
PTT1	100	300	300	300	-	300
PTT2	300	300	300	300	300	-
PTT3	300	-	-	300	300	300
PTT4	100	300	300	-	300	-
PTT5	30	100	300	300	-	-
PTT6	30	30	100	300	-	-
PTT7	100	100	-	300	-	-
PTT8	300	300	300	-	300	300
PTT9	300	300	-	-	300	300
PTT10	-	300	-	-	300	-
PTT11	100	300	-	300	300	-
PTT12	100	100	300	300	-	300
PTT13	30	30	100	300	-	-
Phenytoin[5]	30	30	-	-	100	100
Ethosuximide[6]	-	-	100	300	-	-

[1] 30 or 100 or 300 mg/kg dose through i.p. route administered to mice in maximal electroshock technique. [2] 30 or 100 or 300 mg/kg dose through i.p. route administered to mice in subcutaneous pentylenetetrazole technique. [3] 30 or 100 or 300 mg/kg dose through i.p. route administered to mice in neurotoxicity test. [4] After administration of drug test time. [5] Standard drug for MES technique and neurotoxicity [27]. [6] Standard drugs for scPTZ technique [28]. At maximum dose tested (300 mg/kg) absence of activity was represented by mdash (-) sign.

The subcutaneous pentylenetetrazole (scPTZ) technique: In the *sc*PTZ model, many synthesized derivatives displayed moderate to good antiepileptic activity. Out of 13 prepared compounds, at 100 mg/kg (lowest) dose like standard ethosuximide derivatives, **PTT6** and **PTT13** were found to be considerably potent at 0.5 h time intervals. The activity of the above derivatives continued at a 300 mg/kg dose at the time interval of 4.0 h. Analogs **PTT1, PTT2, PTT5** and **PTT12** showed protection at a dose of 300 mg/kg after 0.5 h and 4.0 h. Either after 0.5 h time interval or 4.0 h time interval rest of the title derivatives **PTT3-PTT4, PTT7-PTT8,** and **PTT11** (except **PTT9** and **PTT10**) were found to be active at a higher tested dose, i.e., 300 mg/kg.

Minimal motor impairment (Acute toxicity): Rotorod method was employed to estimate the neurotoxicity of synthesized derivatives. At higher doses, only most of the title derivatives displayed neurotoxicity when compared to standard ethosuximide or phenytoin. At 300 mg/kg dose, compounds **PTT1-PTT4** and **PTT8-PTT12** were established to be neurotoxic, whereas the remaining title compounds **PTT5-PTT7** and **PTT13** were found to be non-neurotoxic at all doses tested.

The MES (maximal electroshock) test of selected compounds by oral route: The very valuable property of AED is its ability to inhibit epilepsy when administered through the oral route. From the initial screen, four tested derivatives, **PTT5-PTT6** and **PTT12-PTT13,** were identified as potent compounds; hence, these analogs were tested for their oral availability by acute MES seizure test and neurotoxicity test at 30 mg/kg fixed-dose in rats. Table 7 summarizes the obtained data.

Table 7. Antiepileptic and neurotoxicity of selected derivatives (PTT5-PTT6 and PTT12-PTT13) administered orally (30 mg/kg) to rats.

Derivatives	MES[1]					TOX[2]
	0.25 h[3]	0.5 h[3]	1 h[3]	2 h[3]	4 h[3]	
PTT5	One/Four	One/Four	One/Four	One/Four	One/Four	Zero/Four (-)[4]

Derivatives	MES[1]					TOX[2]
	0.25 h[3]	0.5 h[3]	1 h[3]	2 h[3]	4 h[3]	
PTT6	One/Four	Two/Four	Three/Four	Three/Four	Two/Four	Zero/Four (-)[4]
PTT12	One/Four	One/Four	One/Four	One/Four	One/Four	Zero/Four (-)[4]
PTT13	One/Four	Two/Four	Two/Four	Two/Four	Two/Four	Zero/Four (-)[4]
Phenytoin[5]	One/Four	Four/Four	Three/Four	Three/Four	Three/Four	Zero/Four (-)[4]

[1] 30 mg/kg dose administered through oral route to rat in maximal electroshock technique. The data point out: number of protected animals/number of tested animals. [2] 30 mg/kg dose administered through oral route to rat in neurotoxicity test. The data point out: number of protected animals/number of tested animals. [3] After administration of drug test time. [4] (-) at tested dose not neurotoxic. [5] Standard drug [28].

The most active compound of these four derivatives is **PTT6** which protected three rats out of 4 at 1 h and 2 h time points. It protected two rats out of 4 at 0.5 h and 4 h time intervals, and it protected only one rat out of 4 at a time interval of 0.25 h. Similar to standard phenytoin, this derivative exhibited satisfactory activity. Whereas derivatives **PTT13** displayed moderate activity, the rest of the derivatives, **PTT5** and **PTT12,** displayed weaker activity. At 30 mg/kg oral dose, all these five tested compounds were found to be non-toxic.

3.4. Structure-activity relationship.

When comparing the pharmacological potency of prepared derivatives with their chemical structures, it was found that title compounds **PTT5-PTT6** and **PTT12-PTT13** displayed better antiepileptic potency in both MES and *sc*PTZ methods. These compounds have electron-withdrawing moieties like nitro and chloro groups in either para/meta position of the phenyl ring attached at C-2 of thiazolidinon rings. In general, the electron-withdrawing group substituted compounds exhibited better antiepileptic potency than the electron-donating group substituted compounds. But the position of the electron-donating/withdrawing group does not play many roles in antiepileptic potency, as it was evident that both meta / para-substituted compounds exhibited almost equal activity.

4. Conclusions

Multi-step synthesis was used to synthesize thirteen new thiazolidine-4-one substituted thiazoles. All synthesized derivatives were characterized using IR, NMR, mass spectral and elemental analysis data. In silico tools like the molinspiration online tool and molecular docking were utilized to predict molecular properties and the antiepileptic potency of the test analogs. In vivo antiepileptic activity of the entire test, analogs were examined using MES and scPTZ tests. The neurotoxicity of test analogs was estimated using rotorod test. SAR studies reveal that, in general, the electron-withdrawing group substituted compounds exhibited better antiepileptic potency than electron-donating group substituted compounds. But the position of the electron-donating/withdrawing group does not play many roles in antiepileptic potency. Four compounds (PTT5-PTT6 and PTT12-PTT13) were identified as potent compounds from the initial screen, and these compounds were further screened in rats at a 30 mg/kg fixed dose for their oral availability using an acute MES seizure test. Neurotoxicity was also estimated using rotorod test. In the rat MES test, compound PTT6 displayed good antiepileptic activity comparable to reference phenytoin. Hence, this analog PTT6 may emerge as the lead compound without neurotoxicity and a broad spectrum of antiepileptic potency.

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Conflicts of Interest

The authors declare no conflict of interest.

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