



Research Article

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DESIGN, IN SILICO STUDY, SYNTHESIS AND BIOLOGICAL EVALUATION OF BENZOTHAIAZOLE-BASED NITROGEN HETEROCYCLE DERIVATIVES AS NOVEL ANTIEPILEPTIC AGENTS

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ABSTRACT

Background: This study explores how novel benzothiazole-based nitrogen heterocycles would address drug resistance in epilepsy that occurs in approximately 30 percent of patients. We prepared four new 2-aminobenzothiazole analogs (DD1-DD4) by molecular hybridization and performed comprehensive in silico and in vivo experiments. **Methodology:** Pharmacokinetic profiling and toxicity predictions indicated that these compounds were highly drug-like, could cross the blood-brain barrier, and appeared safe. Docking to the human GABA-A homopentamer (PDB ID: 4COF) identified DD3 and DD4 as the best hits in the in silico evaluation, with a binding energy of -7.7 kcal/mol, matching the clinical reference, Riluzole. **Results and Discussion:** After synthesizing the molecules, we tested their anticonvulsant activity in vivo in a PTZ (Pentylenetetrazole)-induced seizure model in Swiss albino mice. Among the set, DD2 was the most promising, with the highest safety margin, as shown by an acute toxicity study. Based on the acute toxicity results and highest safety margin, only DD2 was selected for in vivo activity, and it significantly reduced seizure latency and episode duration at higher doses than controls. **Conclusion:** Overall, these findings indicate that adding nitrogen heterocycles at C-2 of the benzothiazole core fine-tunes interactions with neurological targets. Ultimately, the evidence suggests that DD2, DD3, and DD4 are viable leads to develop next-generation antiepileptic drugs that would be more effective in controlling drug-resistant seizures.

INTRODUCTION

Epilepsy is a significant health issue in the world and a huge burden on modern neurology and pharmaceutical research. It is typified by a multifaceted range of cerebral disorders characterized by acute episodic seizures, manifestations of excessive electrical discharges in neuronal networks [1-2].

Physiologically, epilepsy originates in disruption of the fine homeostatic balance between excitatory and inhibitory neurotransmission in the central nervous system. Failure of gamma-aminobutyric acid (GABA-A)-mediated inhibitory systems or excessive stimulation of glutamatergic excitatory systems leads to paroxysmal neuronal hypersynchrony [2-3].

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This imbalance is often regulated by the dysfunction of gating of voltage-gated ion channels, including sodium (Na⁺), calcium (Ca²⁺), and potassium (K⁺) channels, or by changes in the structural integrity of synaptic proteins, which collectively reduce the seizure threshold and enable the rapid spread of electrical storms across different cortical areas [3-4]. Although the antiepileptic drug (AED) repertoire has grown significantly in the past few decades, a shocking thirty percent of patients are still pharmacoresistant, still experiencing disabling breakthrough seizures and frequently with systemic toxicity and cognitive adverse effects of chronic high-dose therapy [5-6]. This has driven an intensive search for new chemical scaffolds with wider therapeutic windows and multi-target activity, leading medicinal chemists to target the benzothiazole nucleus, a privileged bicyclic ring system with an impressive ability to tune a wide range of neurochemical pathways [7].

The benzothiazole scaffold, characterized by a benzene ring fused to a thiazole moiety, is considered one of the most potent bioactive scaffolds in drug design because of its structural rigidity and ease of chemical modification to fine-tune pharmacological properties [8-9]. Chemically, the benzothiazole ring is a planar, electron-deficient system in which a sulfur atom at position 1 and a pyridine-like nitrogen atom at position 3 create a unique electronic environment that can interact with a variety of other molecules [10]. Its role in epilepsy is well established, with the scaffold providing an ideal electronic and spatial environment to interact with the hydrophobic pockets of neurotransmitter receptors and ion-channel proteins [10-11].

The usefulness of this nucleus is demonstrated by several clinically used or experimental drugs, such as Riluzole, a benzothiazole derivative known for neuroprotective and anticonvulsant effects, mainly through inhibition of glutamatergic signaling and stabilization of inactivated states of voltage-gated sodium channels [12]. The benzothiazole ring is versatile and can serve as a primary pharmacophore that can be extended or replaced to access distal binding sites and increase affinity for target proteins implicated in seizure suppression [13–14]. Recent research trends emphasize that incorporating different functional groups onto this core can significantly alter its lipid-water partition coefficient, a key factor in enabling the molecule to penetrate the highly selective blood-brain barrier (BBB) and exert its therapeutic action in the central nervous system [15]. Structure-activity relationship (SAR) studies show

that the anticonvulsant activity of benzothiazoles is greatly enhanced when coupled with other heterocyclic systems, especially nitrogen-based rings, including triazoles, imidazoles, pyrazoles, or piperazines [16-17]. Synthetically and SAR-wise, the C-2 position of the benzothiazole nucleus is the most reactive site to be modified; the addition of nitrogenous heterocycles at this site enables the formation of an extended conjugated system or a flexible linkage that can optimize the orientation of the molecule in the active site of the receptor [18]. These nitrogen heterocycles are not just structural spacers; they are necessary hydrogen-bond donors or acceptors that enable strong non-covalent interactions with amino acid residues in target receptors, including the GABA-A receptor complex or the glutamate-binding domains of NMDA receptors [19-20]. These nitrogen-containing moieties tend to increase the compound's metabolic stability by replacing more labile groups and also confer the required polarity to counteract the lipophilicity of the benzothiazole core [20]. For example, several studies have shown that adding a triazole ring at the C-2 site of benzothiazole significantly enhances the protective index in the Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) seizure models [18, 21]. Moreover, SAR studies indicate that the electronic density and lipophilicity of the heterocycle are maximized by halogen substituents on the fused benzene ring, i.e., fluorine or chlorine at the C-6 position, which enhances binding kinetics and minimizes off-target effects [22-23].

In modern drug discovery, the conventional synthesis-first strategy is increasingly supplemented by advanced *in silico* screening regimens that simplify the identification of viable drug targets [24]. *In silico* analyses such as molecular docking, molecular dynamics simulations, and pharmacophore modeling provide a high-resolution view of how these benzothiazole-based nitrogen heterocycles may fit into the active sites of neurological targets, enabling prediction of binding energies before any laboratory activity [25]. These computational tools are also needed to evaluate the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of the molecules, ensuring the designed agents have the drug-like properties required for oral bioavailability and safety [26-27]. After computational lead identification, *in vitro* testing serves as an important biological interface, using cell-based assays or enzyme inhibition experiments to measure molecules' physiological responses in a controlled setting [28]. These assays can measure parameters such as half-maximal inhibitory

concentration (IC-50) and cellular viability, providing key information on compound potency and potential neurotoxicity before testing in complex *in vivo* animal models [29-30].

This study was a systematic, integrated effort to investigate the therapeutic potential of a newly designed series of benzothiazole-based nitrogen heterocycles, with the main aim of addressing unmet clinical needs in epilepsy treatment. This study used rigorous *in silico* molecular docking to clarify binding modes relative to current benzothiazole-based drugs like Riluzole, and *in vivo* pharmacological screening to assess the anticonvulsant activity and side-effect profile of these novel lead compounds compared with Diazepam. By closely characterizing these molecules, this paper provides a complete picture of the structural requirements for effective anticonvulsant activity. It supports the development of next-generation therapies for patients with refractory epilepsy.

MATERIALS AND METHODS

This study focuses on producing new 2-amino-benzothiazole compounds (DD1-DD4) with potential antiepileptic properties. Molecular hybridization is a strategic approach in medicinal chemistry that combines two or more discrete molecular structures to produce new hybrid structures. This technique leverages the synergistic benefits of different compounds to enhance pharmacological properties and support the design and development of therapeutics [31]. The current study attempts to synthesize agents with improved antiepileptic activity by using 2-aminobenzothiazole scaffolds. Such hybrid structures are likely to offer better efficacy, fewer adverse effects, and improved clinical outcomes in patients with epilepsy. This paper highlights the critical role of molecular hybridization in producing novel therapeutic agents with highly specific biological functions. In this work, we designed the synthesis scheme and library using ChemDraw Ultra. 16.0. A library design involves the production of a great variety of chemical compounds, either in the form of virtual models or real samples. This method is often used to identify compounds with desirable characteristics in materials and pharmaceutical discovery. Researchers usually define specific targets, design or select candidate molecules, predict their properties, synthesize them (where necessary), measure their activity, and optimize the library based on the results [32]. The goal is to broaden chemical exploration and improve the productivity of discovery programs. The chemicals used in this study, such as 2-aminobenzothiazole, 2-amino-4-methylthiazole, and Dichloromethane (DCM), were

obtained from Loba Chemie Pvt. Ltd (India), while 1,2,4-triazole was obtained from Sisco Research Laboratories Pvt. Ltd. (India). Imidazole was obtained from Emplura (Germany), 2-methylimidazole from Pallav Chemicals & Solvent Pvt. Ltd. (India), triethylamine from Changshu Hongsheng Fine Chemical Co. Ltd., and acetonitrile from Otto Chemie Pvt. Ltd. (India).

The FTIR spectra were obtained on a Bruker ALPHA Spectrophotometer. ¹HNMR and ¹³CNMR spectra were recorded on a Bruker ACF 500 spectrometer.

IN SILICO STUDIES

In recent years, *in silico* methods for creating and verifying scientific theories have grown in popularity. Researchers typically use these computational methods alongside *in vivo* data to build and test predictive models. These models help identify and optimize molecules that show target-specific interactions. Moreover, they enable the determination of key pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), and the analysis of molecular physicochemical properties. To conduct this experimental undertaking, the *in silico* studies are conducted as follows.

Pharmacokinetics and drug-likeness profiling

The pharmacokinetic profile and the drug-likeness of the designed benzothiazole-based nitrogen heterocycles were evaluated *in silico* using the SwissADME web server (Swiss Institute of Bioinformatics) [23]. For each derivative, to obtain a consensus estimate of hydrophobicity, lipophilicity was assessed using several log P predictive models (iLOGP, XLOGP3, WLOGP). We screened these compounds against established pharmacokinetic filters, such as the Rule of Five, Veber's criteria, and the Ghose filter [33]. We also explored absorption and distribution parameters using the BOILED-Egg (Brain or Intestinal Estimated Permeation) model, which predicts gastrointestinal absorption and blood-brain barrier (BBB) permeation simultaneously, a crucial factor when considering potential antiepileptic agents. In addition, the metabolic disposition was estimated by determining the inhibitory capacity of the molecules on the major cytochrome P450 (CYP450) isoforms like CYP1A2 [34-35].

Toxicity study

The toxicological potential of the target molecules was assessed by using the ProTox 3.0 virtual laboratory. This *in silico* approach allowed simultaneous evaluation of multiple

endpoints, including acute toxicity classes and adverse outcome pathways. By analyzing chemical fragments and molecular similarities against a large experimental database, ProTox 3.0 provided high-confidence scores for mutagenicity, carcinogenicity, and cytotoxicity. This screening served as a critical filter in the drug design workflow, prioritizing compounds with the most favorable safety margins for further investigation [36].

Preparation of Protein

The crystal structure of the human gamma-aminobutyric acid (GABA-A) receptor homopentamer (PDB ID: 4COF) was

downloaded from the RCSB Protein Data Bank and used as the target macromolecule [37]. To prepare the macromolecule for docking, we removed the co-crystallized ligand and all bound water molecules to avoid interference with the binding site. We then added polar hydrogen atoms to optimize the protein's electrostatic environment and hydrogen-bonding potential. We validated the structural integrity and stereochemical quality of the prepared protein using a Ramachandran plot to ensure backbone conformations fell within energetically favored regions. Finally, we performed molecular docking using the PyRx software suite [38].

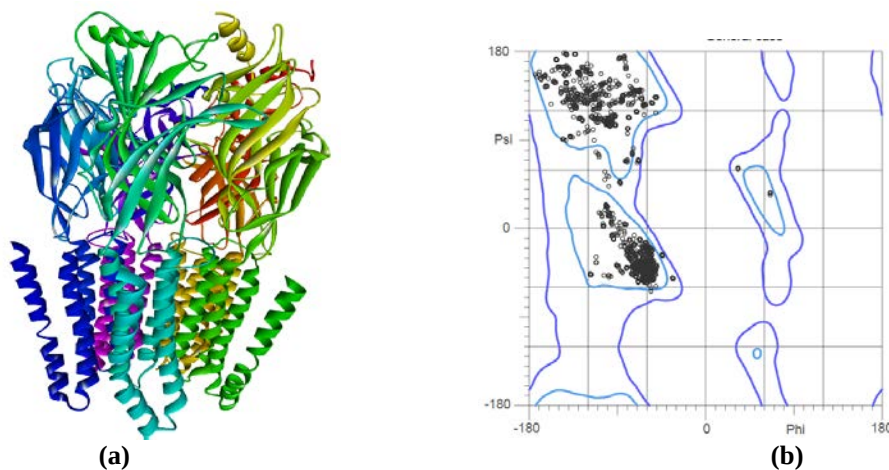


Figure 1: (a) 4COF protein structure with 2.97 Å obtained from the RCSB Protein Data Bank; (b) Ramachandran plot of the protein structure. It shows that most amino acids lie within the most allowed region, indicating structural reliability and good stereochemical quality for molecular docking

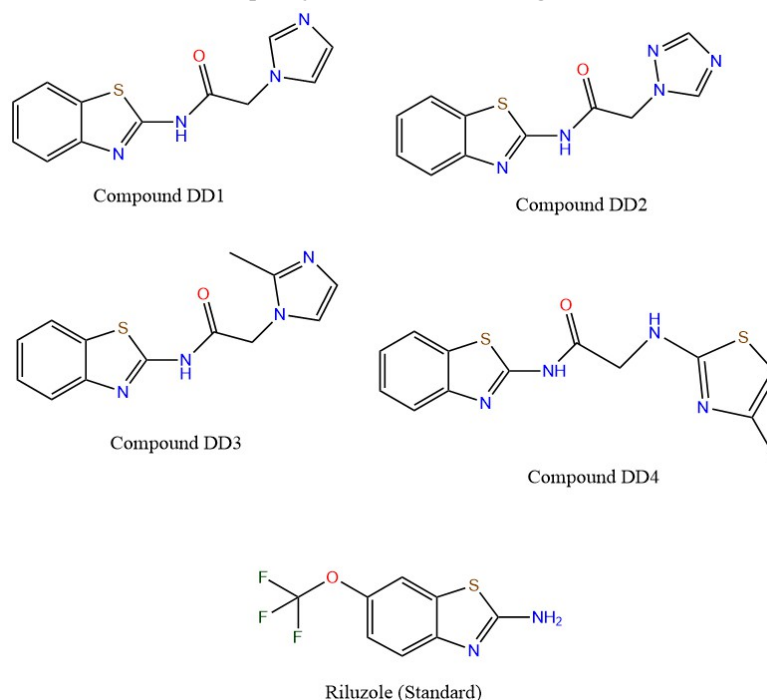
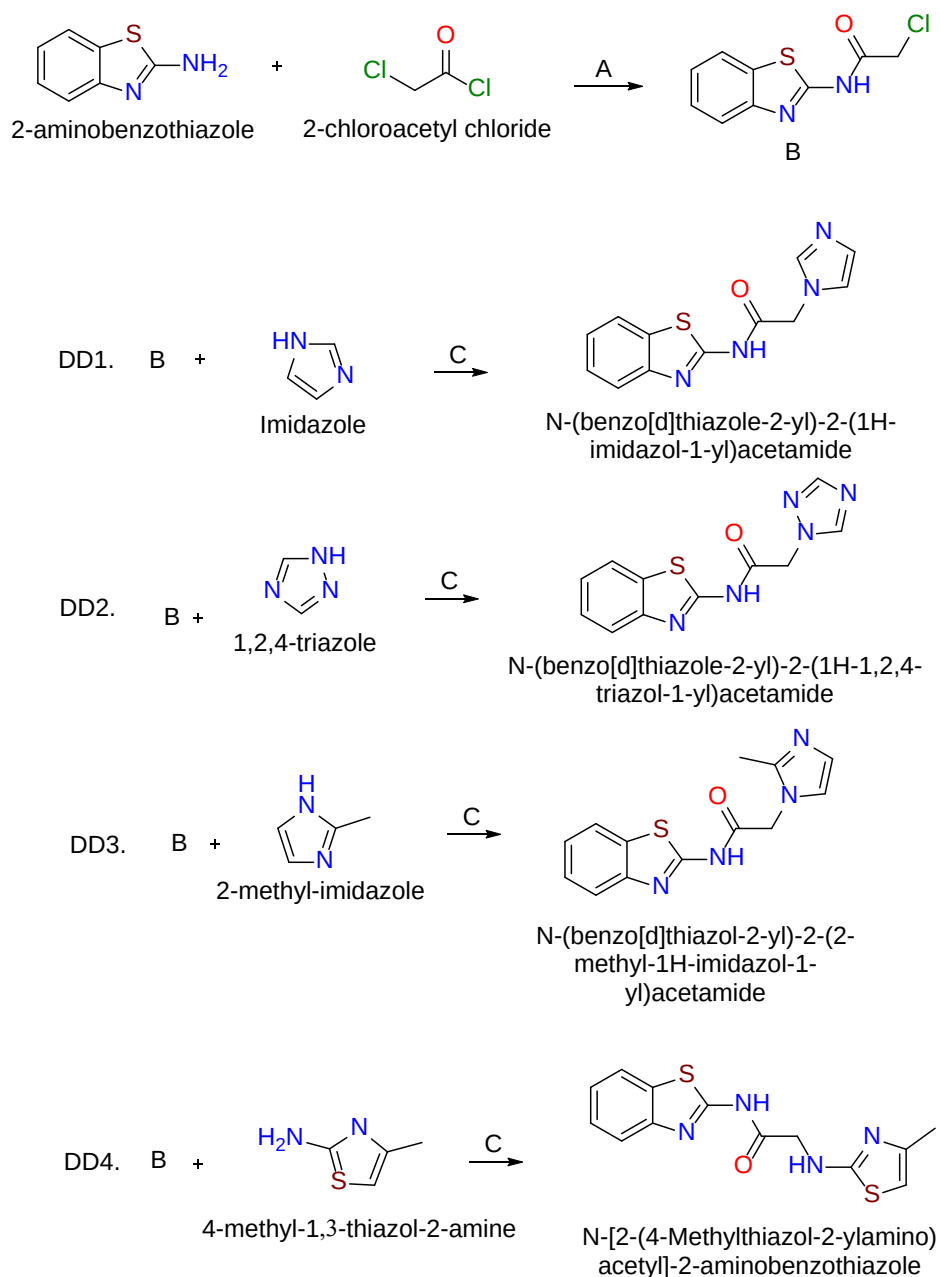


Figure 2: The benzothiazole derivatives (DD1-DD4) along with standard drug Riluzole designed in ChemDraw 16.



A. DCM, trimethyl amine

B. N-(benzo[d]thiazol-2-yl)-2-chloroacetamide

C. Acetonitrile/DMF, Triethyl amine

Figure 3: Synthetic scheme of the compounds DD1-DD4

Preparation of ligands

To select ligand molecules for molecular docking using novel benzothiazole derivatives, we prepared the ligands' two-dimensional (2D) structures in ChemDraw 16.0 and then converted them to three-dimensional (3D) form. A total of four ligands were selected for molecular docking after performing

pharmacokinetic analysis among a library of various designed compounds. The ligands were uploaded to PyRx one by one, and their energy was minimized to ensure a stable 3D conformation before docking. The ligands were converted to 'pdbqt' format using Open Babel to initiate molecular docking [39].

Molecular docking

To elucidate the binding orientation and predict the affinity of the synthesized benzothiazole derivatives, we performed molecular docking. We also docked an FDA-approved anti-epileptic drug, Riluzole, along with the designed compounds to compare results [40]. After preparing the protein and ligands, we subjected them to the docking process. Virtual screening was performed using PyRx 0.8, using the AutoDock Vina wizard as the primary docking engine [41]. After selecting all ligands and proteins, we determined the grid parameters enclosing the binding pocket. A grid box was set up around the active binding site on the GABA-A receptor to allow the benzothiazole scaffold to explore multiple binding orientations. The docking simulations were carried out with an exhaustiveness level of 8, and the binding affinity was measured in terms of the lowest docking score (kcal/mol) [16].

Analysis of Visualization and Interaction

Post-docking analysis was carried out to assess the nature of intermolecular forces that govern the protein-ligand complexes. We analyzed and visualized binding modes such as hydrogen bonding, p-p interactions, and hydrophobic contacts using BIOVIA Discovery Studio Visualizer 2021. This enabled a comparative evaluation of the synthesized compounds with the native ligand and thus prioritized candidates with the most favorable molecular behavior for further evaluation [42].

Molecular Dynamics simulation

The CABS-Flex 3.0 server was used for the molecular dynamics (MD) simulations of four designed compounds and the human gamma-aminobutyric acid receptor (PDB ID: 4COF). These simulations used a coarse-grained modeling approach to observe protein flexibility. Each 10 ns simulation had 50 cycles and 50 trajectory frames. To ensure accuracy, we used distance restraints (global weight of 1.0). The mobility of the resulting complexes was determined using the root-mean-square fluctuation (RMSF) [43].

PROCEDURE FOR SYNTHESIS

Step 1: Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (B):

A solution of 2-aminobenzothiazole (2g) in dichloromethane (10 mL) and triethylamine (1 mL) was stirred in an ice bath (0–5 °C). Chloroacetyl chloride (2 mL) was added dropwise using a dropping funnel while maintaining a constant temperature.

Upon completion, the mixture was poured into ice-cold water; the precipitate formed was filtered, washed with water and dried. The crude product was purified by recrystallization from hot ethanol to give the title compound as purified crystals [44].

Step 2: Preparation of Benzothiazole Derivatives:

Preparation of N-(benzo[d]thiazole-2-yl)-2-(1H-imidazol-1-yl)acetamide (DD1):

The product was dissolved in anhydrous acetonitrile (10mL) and triethylamine (10mL) was added. The mixture was stirred in an ice bath (0–5°C) and imidazole (68mg) was added in portions. At the end of the process, the solvent was evaporated under low pressure in a water bath. The crystalline product obtained was dried and characterized further [45].

Synthesis of N-(benzo[d]thiazole-2-yl)-2-(1H-1,2,4-triazol-1-yl)acetamide (DD2)

In a dry flask, 10 mL of acetonitrile was added to N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (1.2 g). Triethylamine (10 mL) was then put in and the mixture was stirred in an ice bath (0–5°C). Subsequently, 1,2,4-triazole (68 mg) was added to the mixture. Upon completion, the excess solvent was evaporated using a hot water bath. The resulting crystalline products were collected and dried for further analysis [46].

Synthesis of N-(benzo[d]thiazol-2-yl)-2-(2-methyl-1H-imidazol-1-yl)acetamide (DD3)

N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (1.2 g) was dissolved in 10 mL of acetonitrile in a dry flask. Triethylamine (10 mL) was added, and the solution was stirred in an ice bath (0–5°C). Subsequently, 2-methylimidazole (68 mg) was further added to the mixture. After completion, excess solvent was evaporated using a hot water bath. The resulting crystalline product, DD3, was collected and dried for further analysis [47].

Synthesis of N-[2-(4-Methylthiazol-2-ylamino) acetyl]-2-aminobenzothiazole (DD4)

N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (1.2 g) was dissolved in 10 mL of acetonitrile in a dry flask. Triethylamine (10 mL) was added, and the solution was continuously stirred in an ice bath (0–5°C). Subsequently, 2-amino-4-methylthiazole (68 mg) was added to the mixture. Upon completion, the excess solvent was evaporated using a hot water bath. The resulting crystalline product was then collected and dried for further characterization [44, 47].

IN VIVO EVALUATION

Acute Oral Toxicity Study

The acute oral toxicity of the synthetic product will be investigated following the standardized OECD 425 guidelines [44]. Using Swiss Albino mice as the biological model, the study aims to calculate the LD50 value and identify potential clinical signs of toxicity. Following the "Up-and-Down" methodology, the trial will begin with an Initial dose of 55 mg/kg, and the LD50 value was found to be 300 mg/kg. The stepwise dosing protocol enables accurate determination of the compound's toxicological limits and greatly minimizes the number of animals needed to establish scientific rigor and ethical considerations in determining the product's safety margins [45].

In vivo Anti-Epileptic Activity

The in vivo evaluation shows that the synthesized DD2 compound reduces pentylenetetrazol-induced epilepsy. We tested the compound at both low and high doses to assess its

effectiveness. We used diazepam as the standard antiepileptic agent for comparison. Before these tests, we conducted acute toxicity studies to determine the LD50 of the DD2 compound. The animals were orally dosed for a week. Pentylenetetrazole (PTZ) control animals were administered 1.5% CMC orally, diazepam was administered intraperitoneally (1 mg/kg body wt.), and the remaining groups were dosed according to their respective body weights (Table 1). On day 8, all animals were evaluated for Epilepsy. The animals were administered 30 minutes prior to the induction of epilepsy. PTZ (60 mg/kg b.wt) was used for the induction of seizures. PTZ was dissolved in 0.9% saline and administered intraperitoneally (IP) to each animal based on body weight. We examined each animal individually after administering PTZ for 7 min, then observed the latency and seizure periods. We statistically analyzed seizure recording results using GraphPad Prism 10. We compared the data using 2-way ANOVA for significance (95% confidence interval) [46].

Table 1: Grouping of animals with respective treatment

Groups	Treatment	Dose (mg/kg body wt.)	No. of animals
Group I	PTZ control	60	5
Group II	Diazepam + PTZ	1	5
Group III	Low dose + PTZ	8.75	5
Group IV	Medium dose + PTZ	17.5	5
Group V	High dose + PTZ	35	5

[Adult Swiss albino mice were obtained from the animal facility of College of Veterinary Science, Khanapara, Assam, India]

[All experimental procedures involving animals were conducted in accordance with institutional guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of NETES Institute of Pharmaceutical Science under CPCSEA guidelines (Approval No.: NIPS/AH/25/027)]

RESULTS AND DISCUSSIONS

Molecular Docking

To determine the binding potential & understand the molecular interaction of the newly designed benzothiazole derivatives, an *in silico* molecular docking study was performed using the PyRx virtual screening tool (AutoDock Vina). The designed compounds (DD1, DD2, DD3, and DD4) were docked into the active site of the protein target 4COF. Primary criteria to evaluate the strength of the protein-ligand complexes were the binding affinities, which were in the form of Gibbs free energies (kcal/mol). A negative binding energy indicates a more stable

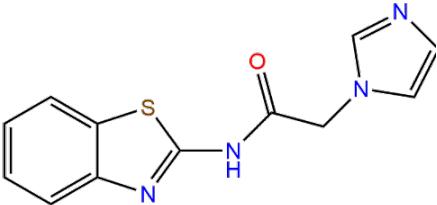
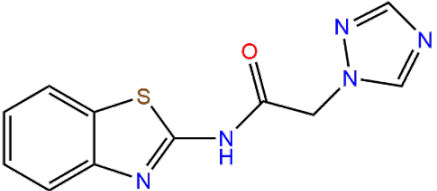
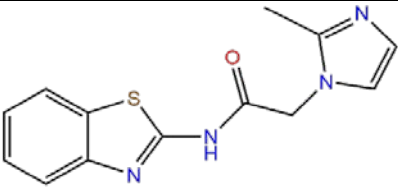
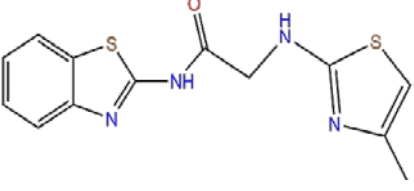
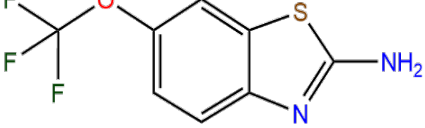
complex and a greater affinity to the target. The results, summarized in the table below, indicate that the designed benzothiazole scaffold fits into the 4COF binding pocket. The potency was compared to the standard drug, Riluzole, which had a binding affinity of -7.7 kcal/mol. Surprisingly, the developed compounds DD3 and DD4 showed outstanding binding scores of -7.7 kcal/mol, which is identical to the affinity of the standard drug. This indicates that the structural changes in DD3 and DD4 maximized interactions with the target residues to a level comparable to the clinically established reference.

Moreover, compound DD2 showed a strong binding affinity of -7.6 kcal/mol, which differs slightly from the standard. Compound DD1 exhibited a slightly lower affinity of -7.1kcal/mol, but it still had a favorable binding profile. The uniform excellence in the binding scores across the series suggests that the benzothiazole core, combined with the specific substituents in DD3 and DD4, provides a favorable orientation and electronic environment for high-affinity binding to 4COF.

These findings provide a strong rationale for the further development of these compounds as potent inhibitors, with DD3 and DD4 identified as the most promising lead candidates for subsequent experimental validation. Figure 2 and Figure 3 illustrate the 2D & 3D interactions of the ligand compounds and the standard with the protein. For compound DD1, LYS E: 274, PRO D:184, ILE D: 47, ASP D: 48, and ALA E: 135 were found to form bonds with the ligand. For compound DD2, ILE B: 264,

GLN B: 224, THR B: 271, and LYS C: 272 formed bonds. For compound DD3, ligand-protein bonds were formed by TYR B: 205, TYR A: 62, PHE B: 200, THR B: 202, GLN A: 64, and ALA B: 201. For compound DD4, TYR B: 205, PHE B: 200, ASP A: 43, THR A: 176, ARG A: 180, and THR B: 202 contributed to form ligand-protein bonding. In the standard drug Riluzole, ligand-protein bonds were formed by ASN B: 41, GLN B: 64, PHE C: 200, TYR B: 62, GLU C: 155, and SER C: 156.

Table 2: Binding energy of designed benzothiazole derivatives and standard drug with 4COF protein and amino acid involved in the interaction

Compound	IUPAC Name	Binding energy (kcal/mol)	Type of interactions	Amino acid involved in interaction
DD1	 N-(benzo[d]thiazole-2-yl)-2-(1H-imidazol-1-yl)acetamide	-7.1	Conventional Hydrogen bond, Carbon-Hydrogen bond, Pi-sigma bond, Amide-Pi stacked bond, Pi-Alkyl bond	LYS E: 274 PRO D:184 ILE D: 47 ASP D: 48 ALA E: 135
DD2	 N-(benzo[d]thiazole-2-yl)-2-(1H-1,2,4-triazol-1-yl)acetamide	-7.6	Conventional Hydrogen bond, Carbon-Hydrogen bond, Pi-sigma bond, Pi-Alkyl bond	ILE B: 264 GLN B: 224 THR B: 271 LYS C: 272
DD3	 N-(benzo[d]thiazole-2-yl)-2-(2-methyl-1H-imidazol-1-yl)acetamide	-7.7	Conventional Hydrogen bond, Pi-Pi stacked bond, Pi-Pi T-shaped bond, Pi-Alkyl bond	TYR B: 205 TYR A: 62 PHE B: 200 THR B: 202 GLN A: 64 ALA B: 201
DD4	 N-[2-(4-Methylthiazol-2-ylamino) acetyl]-2-aminobenzothiazole	-7.7	Conventional Hydrogen bond, Unfavourable acceptor-acceptor bond, Pi-cation bond, Pi-Pi stacked bond, Pi-Pi T-shaped bond	TYR B: 205 PHE B: 200 ASP A: 43 THR A: 176 ARG A: 180 THR B: 202
Riluzole (Standard)	 6-(trifluoromethoxy)-1,3-benzothiazol-2-amine	-7.7	Conventional Hydrogen bond, Pi-Pi stacked bond, Halogen (F) bond	ASN B: 41 GLN B: 64 PHE C: 200 TYR B: 62 GLU C: 155 SER C: 156

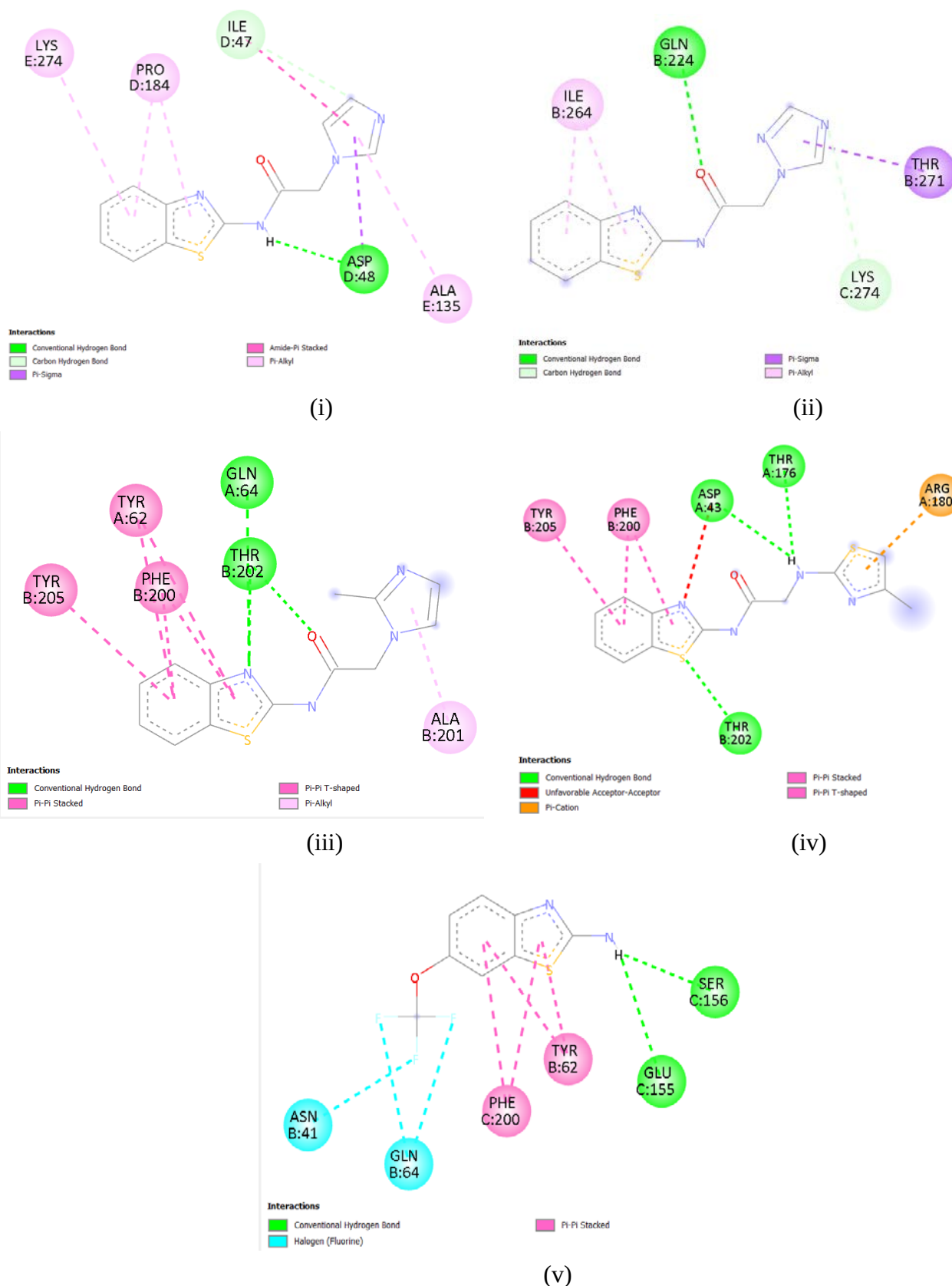


Figure 4: The 2D interaction of the compounds DD1-DD4 with the 4COF protein is shown in Figures (i-iv), respectively. Figure (v) indicates the 2D interaction of the standard drug taken with the same protein during molecular docking.

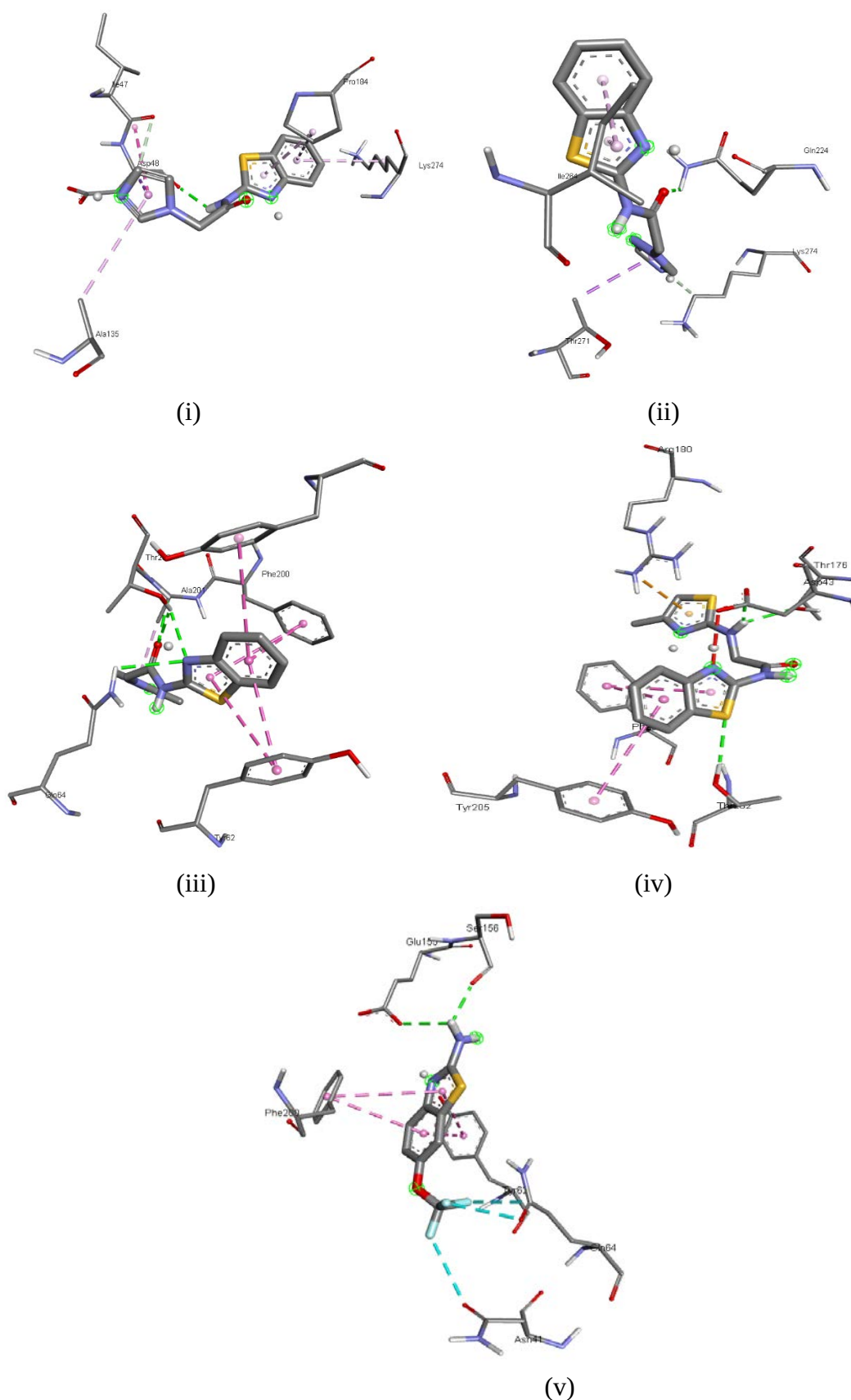


Figure 5: The 3D interaction of the compounds DD1-DD4 with 4COF protein is shown by figure (i-iv) respectively. Figure (v) indicates the 3D interaction of the standard drug taken with the same protein during molecular docking.

Pharmacokinetic and Drug Likeness Analysis by SwissADME

The synthesized benzothiazole derivatives (DD1–DD4) were evaluated for their pharmacokinetic and ADME profiles to determine their potential as drug-like candidates. All four derivatives strictly adhered to Lipinski's Rule of Five, exhibiting molecular weights significantly below the limit of 500 Da. The compounds had fewer than 5 hydrogen bond donors and fewer than 10 hydrogen bond acceptors, critical criteria for oral bioavailability [33]. The consensus log P values of 1.28 to 2.64 indicate good lipophilicity within the recommended range, suggesting a good balance between hydrophilicity and membrane permeability. In addition, the topological polar surface area (TPSA) of all compounds fell within the optimal

range of 20 to 130 Å, with DD2 having the lowest TPSA (68.30 Å), suggesting a high probability of cell-membrane penetration. In terms of physiological distribution, all derivatives showed high gastrointestinal (GI) absorption and a uniform bioavailability score of 0.55, which is the standard requirement of compounds with a high likelihood of oral absorption. All compounds in the series showed blood-brain barrier (BBB) permeation, indicating central nervous system activity. The metabolic analysis revealed that all derivatives were CYP1A2 inhibitors, and none were P-glycoprotein (P-gp) substrates, indicating that these molecules are unlikely to be excluded from cells by active efflux systems [48]. Taken together, these findings suggest that the new benzothiazole analogs have a solid pharmacokinetic basis to advance in therapeutic development.

Table 3: Pharmacokinetic profile study of designed compounds DD1-DD4 by SwissADME

Parameters	Novel derivatives			
	DD1	DD2	DD3	DD4
MW (≤ 500)	258.3	259.29	272.33	304.39
H-bond acceptor (≤ 10)	3	4	3	3
H-bond donor (≤ 5)	1	1	1	2
TPSA (20 to 130Å)	88.05	68.3	75.47	123.39
Consensus log p (≤ 5)	1.52	1.28	1.87	2.64
BBB permeation	Yes	Yes	Yes	Yes
Lipinski violations	No	No	No	No
P-gp substrate	No	No	No	No
Cyp1A2 inhibitor	Yes	Yes	Yes	Yes
GI Absorption	High	High	High	High
Bioavailability score	0.55	0.55	0.55	0.55

Molecular Dynamics Simulation.

To analyze the thermodynamic stability, residual flexibility, and long-term structural stability of the 4COF macromolecular receptor thoroughly after complexing with the new benzothiazole-triazole acetamide compounds, coarse-grained MD simulations were run on the CABS-flex 3.0 server. Coarse-grained parameterization enables significantly faster simulations and is designed to efficiently sample the full conformational space of the protein backbone. Thus, the resulting structural ensembles strongly correspond to those determined from long, traditional, multi-nanosecond all-atom MD simulations using significant computational resources. We interpreted systemic fluctuations using Root Mean Square Fluctuation (RMSF) profiles plotted against amino acid residue position and matched with high-resolution residue-residue contact frequency matrices to map variances in the target protein's tertiary fold. Through

analyzing both the baseline apo-like and standard-bound reference trajectories, we were able to construct a structural landscape of various structures representing a large amount of flexibility (local) throughout the core region (10–300 residues). The particular reference complexes showed significant excursions from the starting loop structure (loops) with very sharp maximum fluctuations occurring at amino acids 74–78 (RMSF approx. 2.5 Å), 136–142 (RMSF approx. 2.1 Å) and a very mobile trailing loop (305–312) with RMSF approx. 3.3 Å. When the synthesized acetamide derivatives of benzothiazole-triazole were bound, there was a significant rigidifying effect on the protein backbone due to ligand binding; hence, this indicated that these highly volatile RMSF spikes were essentially suppressed across the entirety of their respective binding responses. Of all of the compounds in this family, the compound DD2 appeared to exhibit a greater capacity for conformational

constraint when bound to the protein. All of the trajectories of the DD2-bound complex retained their respective secure anchoring for the entire duration of the trajectory and, as a result, reduced the overall extent of fluctuations of the global baseline across almost the whole sequence to below 1.0 Å. The most dramatic change observed was in the hyper-flexible loop region located nearest to the approximate position of residue 310, where there was a deviation from the standard reference value that originally exceeded 3.2 Å and occurred under significantly constrained conditions when bound to DD2, which provided a final dynamic constraint of 1.6 Å, providing evidence of significant conformational changes within the loop region alone. The large local entropy loss here shows that DD2 is shaped and that its interactions with its surroundings do two things: they provide DD2 with a stable frame and limit hinge bending and large-scale loop transitions. The stabilization of the ligand correlates with the active site residues that were previously identified to bind with the ligand in docking studies. In the case of a 'standard' ligand binding trajectory, the tarrying sub-pocket (the sub-pocket near the edge of the binding site), which is located near residue 275, showed a significant amount of residual flexibility (2.1 Å of movement at its maximum) between its locations with and without the ligand present. In contrast, the binding of compound DD2 resulted in a much larger, flatter stabilization valley that spans residues 220-230 and 260-275, which did not exhibit any observable 'breathing' (or local movement) between these residues (Gln224, Ile264, Thr271 and Lys274); there was also a very low minimum (0.2-0.4AA) RMSF (root mean square fluctuation) for these residues. This localized freezing of Gln224 linked to the acetamide, along with the conventional hydrogen-bonding network shown to stabilize the active site, while rigid hydrophobic stacking interactions between the benzothiazole and triazole cores further stabilized the active-site residues in a stable, non-fluctuating state.

DD1 and DD3 exhibited intermediary stabilization patterns that tracked closely to the Standard curve, but provided better control over important structural loops. DD1 produced a wide stable valley to 0.4 Å at the Gln224 pocket domain while permitting minor upwards loop flexibility near the residue 275 terminal region. DD3 produced lateral immobilization of the active site floor to 0.3 Å; however, it caused a secondary sharp fluctuation just after residue 275, likely indicating minor structural tension or trailing-tail flexibility in that pocket variant. In contrast, compound DD4 was the most flexible in the hybrid series.

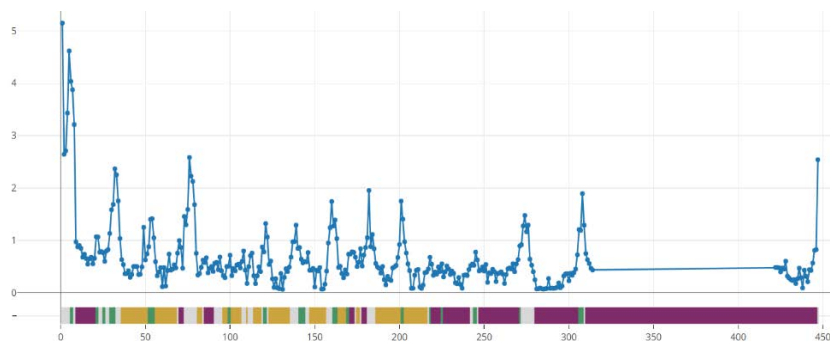
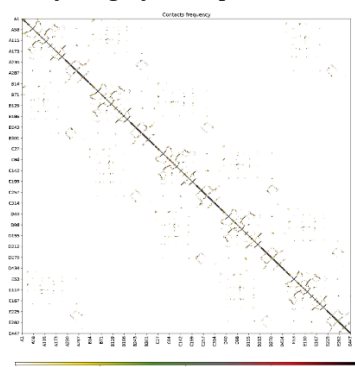
Compound DD4 showed an elevated baseline through the core sequence and had active site residues at an elevated fluctuation profile of about 0.5 to 0.8 Å deeper in a shallow valley's bottom. This high fluctuation suggests that while DD4 fills the binding pocket, it binds less efficiently than DD2 in terms of geometric locking, allowing small-scale breathing of the binding pocket during the simulation. Structural analysis of the homomeric structure yielded insights into how these could be used to create a native, heteromeric receptor assembly that will act as a form of seizure control. Because there is high sequence homology and structural similarity among the subunit interfaces of all of these analogs throughout their respective extracellular spaces, it is expected that the exact same tightly bound interaction networks and significantly reduced residue fluctuations as compared to the compounds studied should correlate directly to the same types of domains within heteromeric receptor assemblies. Therefore, the ability of this compound class (benzothiazole-triazole acetamides) to maintain structural stability creates an opportunity to modify channel gating kinetics and reduce hyper-excitable neurons, giving these compounds potential as effective anti-epileptic drug candidates.

Contact Frequency Matrix and Tertiary Architecture Evaluation

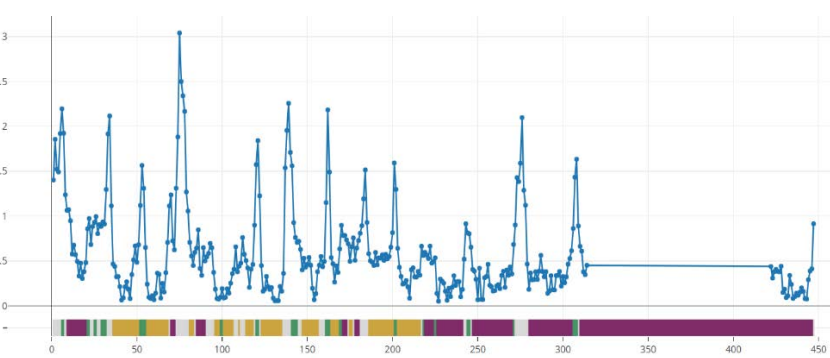
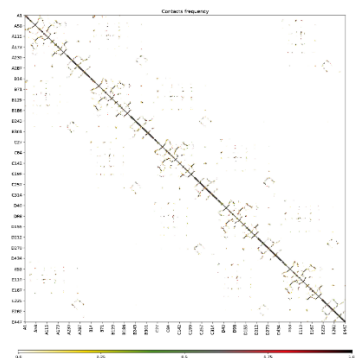
The residue-residue contact maps further characterized the target macromolecule's spatial volume and tertiary structural stability in the presence of each ligand. The five resulting matrices (DD1, DD2, DD3, DD4, and the Standard) had similar primary diagonal axes that were well-defined and entirely continuous. This consistent behavior indicates that the major secondary structure components (core alpha-helices and beta-sheets) of the sequence-based mappings were not affected by complexation with these synthetic derivatives and therefore did not unfold, denature, or lose structural integrity. Although the tight packing and proximity of these clusters may intuitively imply long-term interactions, their close proximity to one another, compared with other regions within their respective three-dimensional spatial arrangements, suggests they may also have formed highly significant long-distance interactions through direct mechanical connectivity after changing states. Examples include: Compounds DD2 and DD1 created significantly stronger and more concentrated hot zones at almost identical interface coordinates (i.e., within the intersections of regions A1-A287 and B14-B301) than the reference Standard. Such hot zones exhibit a total visual enhancement of non-diagonal interactions,

supporting the idea that the addition of the benzothiazole-triazole acetamide scaffold acts as a bridge to pull apart distant segment units of the binding cleft; in effect, these active hybrids establish numerous statistically valid high frequencies, which establish very highly compressed, structurally stable, and

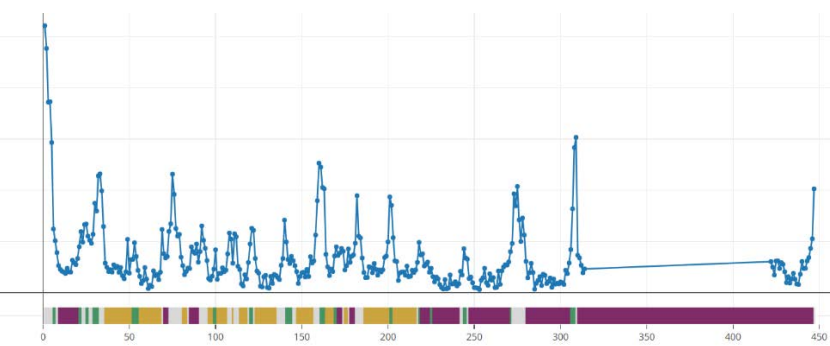
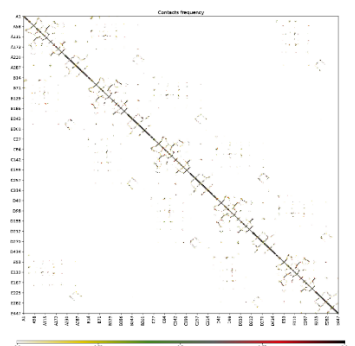
compacted tertiary-type binding pockets. In agreement with what we hypothesized (about how well the in silico predicted binding will be), the low RMSF values also demonstrate that as the binding affinity improves, the chances of achieving a long-lasting target/ligand complex become greater.



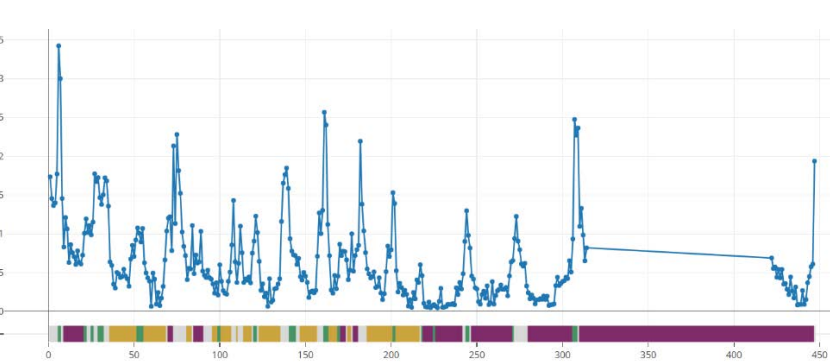
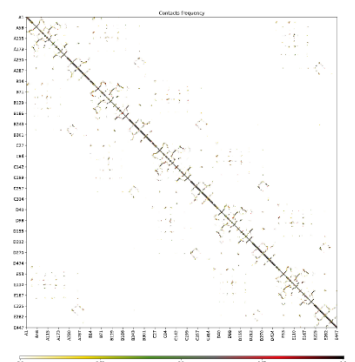
[1] Compound DD1



[2] Compound DD2



(c) Compound DD3



1. Compound DD4

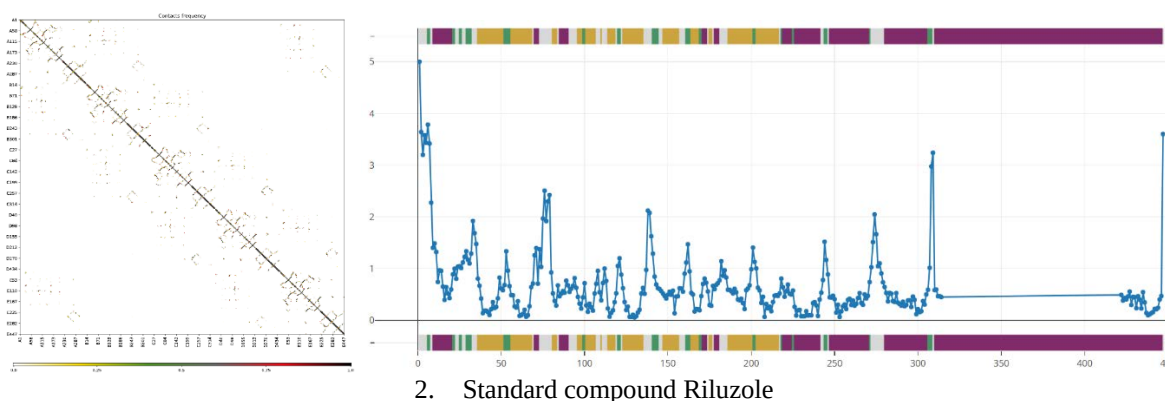


Figure 6: Figure (a-d) shows the contact map and the RMSF plot with residue number on the x-axis and RMSF on the y-axis obtained for compounds DD1-DD4, and Figure (e) shows the same for the standard compound.

Toxicity Study

The toxicity of the newly synthesized antiepileptic agents (DD1-DD4) was assessed using ProTox 3.0 and compared with the standard drug, Riluzole. The findings, summarized in the table below, indicate that all synthesized compounds have a significantly better safety profile for organ-specific toxicity and carcinogenicity than Riluzole. Notably, while Riluzole was predicted to be active for hepatotoxicity (probability: 0.87) and carcinogenicity (probability: 0.52), all synthesized compounds (DD1-DD4) were predicted to be inactive for both endpoints. Furthermore, all compounds were consistently predicted to be

inactive for nephrotoxicity, cardiotoxicity, immunotoxicity (with high confidence, probability ~ 0.99), and cytotoxicity. Regarding central nervous system (CNS) activity, all synthesized compounds and Riluzole showed positive predictions for blood-brain barrier (BBB) permeability and neurotoxicity, which is consistent with their intended therapeutic role as anti-epileptic agents targeting the brain [49]. These findings suggest that the synthesized derivatives maintain the necessary CNS-penetrating properties of Riluzole while potentially offering a lower risk of hepatic and carcinogenic side effects.

Table 4: Toxicity study of the compounds DD1-DD4 and the standard

Toxicity Target	DD1	DD2	DD3	DD4	Riluzole
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	Active
Carcinogenicity	Inactive	Inactive	Inactive	Inactive	Active
Neurotoxicity	Active	Active	Active	Active	Active
BBB-barrier	Active	Active	Active	Active	Active
Nephrotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive
Class	4	4	4	4	4

SAR-BASED LEAD PRIORITIZATION

While DD1 and DD3 exhibited high docking scores (-7.7 kcal/mol) comparable to the clinical standard Riluzole, DD2 (-7.6 kcal/mol) was prioritized for in vivo validation based on a multi-parametric lead prioritization strategy. Although static docking provides an initial measure of binding affinity, Molecular Dynamics (MD) simulations demonstrated that the 1,2,4-triazole moiety in DD2 facilitates more persistent hydrogen-bonding interactions with the GABA-A receptor's active-site residues (notably Gln224) compared to the imidazole

rings in DD1 and DD3. This structural configuration effectively rigidifies the receptor's hyper-flexible gating loops, as evidenced by lower Root-Mean-Square Fluctuation (RMSF) values, suggesting superior target-locking efficiency in a dynamic biological environment (22). Furthermore, DD2 exhibited an optimal lipophilic-hydrophilic balance—essential for Blood-Brain Barrier (BBB) penetration and CNS bioavailability—which, combined with its high predicted safety margin, identified it as the most balanced lead candidate for neurological therapeutic development (23).

ANALYTICAL CHARACTERISATION OF COMPOUNDS

The synthesis involves nucleophilic acyl substitution followed by bimolecular nucleophilic substitution (SN₂). First, the primary amine of 2-aminobenzothiazole attacks the carbonyl carbon of 2-chloroacetyl chloride, displacing a chloride ion to form the amide intermediate B. The synthetic pathway proceeds via nucleophilic acyl substitution, followed by bimolecular nucleophilic substitution (SN₂). First, the carbonyl carbon of 2-chloroacetyl chloride is attacked by the primary amine of 2-aminobenzothiazole to replace a chloride ion to give amide intermediate B. A base like triethylamine is usually required to neutralize the HCl by-product in this acylation. The heterocyclic nitrogen of imidazole, 1,2,4-triazole, or 2-methylimidazole is the next nucleophile to strike the 0-carbon of intermediate B. This SN₂ reaction replaces the remaining chlorine atom, capping the heterocycle to the benzothiazole backbone and producing DD1, DD2, DD3, and DD4. The purity and integrity of the synthesized antiepileptic derivatives (DD1-DD4) were strictly established by a combination of infrared (IR), ¹H NMR, and ¹³C-NMR spectroscopy. IR spectroscopy was used first to identify characteristic functional groups and vibrational modes, including amide, carbonyl, and aromatic stretches indicative of the core scaffold. Following ¹H NMR analysis provided a detailed map of the proton environments, allowing verification of the substitution patterns and electronic properties of the different side chains. Lastly, the carbon structure of each substance was confirmed through ¹³C-NMR spectroscopy, in which the chemical shifts of aliphatic and aromatic carbons were exactly as expected in the proposed molecular structures.

Compound DD1: White amorphous solid, melting point 160-190°C, soluble in ethanol and DMSO with 85% yield.

FTIR (cm⁻¹): The FTIR spectrum (Figure 7) provides strong evidence for the functional groups present in DD1. The absorption band at approximately 3363 cm⁻¹ corresponds to the N-H stretching vibration of the secondary amide linkage connecting the benzothiazole and the imidazole-acetamide moiety. The peaks in the 3162 cm⁻¹ region confirm the presence of aromatic C-H stretching. In contrast, the sharp, intense band at 1619 cm⁻¹ is characteristic of the C=N stretching vibration inherent to both the benzothiazole core and the imidazole ring. Furthermore, the fingerprint region shows significant signals at 758 cm⁻¹ and 685 cm⁻¹, which are diagnostic of C-S bond vibrations in the benzothiazole ring system, confirming the successful integration of this heterocyclic scaffold.

¹H NMR (400 MHz, DMSO-d₆, δ): The ¹H-NMR spectrum (Figure 11) offers precise information regarding the chemical environment of the protons. The downfield singlet near δ 8.16 ppm represents the amide proton (-NH), confirming amide bond formation. The complex multiplet pattern between δ 7.0 ppm and 7.3 ppm integrates to the aromatic protons of the benzothiazole and the imidazole ring system, consistent with the expected electronic environment of the structure. Notably, the distinct singlet near δ 4.2–4.3 ppm is attributed to the methylene (-CH₂-) bridge protons positioned between the carbonyl group and the imidazole nitrogen, serving as a critical indicator that the N-alkylation of the imidazole has occurred as intended in the structure of DD1.

¹³C-NMR (100 MHz, DMSO-d₆, δ): The ¹³C-NMR (Figure 15) completes the structural assignment by identifying the carbon skeleton. The peak at δ 143.4 ppm is assigned to the characteristic C2 carbon of the benzothiazole ring, which is deshielded due to the adjacent sulfur and nitrogen atoms. The aromatic carbons of the benzothiazole and imidazole rings appear as multiple signals in the range of δ 116–135 ppm, confirming the aromatic system. The carbon signal at δ 34.6 ppm corresponds precisely to the aliphatic methylene (-CH₂-) bridge, while the absence of other unexpected peaks in this region supports the purity and proposed architecture of the synthesized molecule DD1.

Compound DD2: White amorphous solid, melting point 180-220 °C, soluble in ethanol and DMSO with 80% yield.

FTIR (cm⁻¹): The FTIR spectrum of DD2 (Figure 8) reveals critical structural transitions compared to the imidazole-based DD1. The prominent absorption band at 3385 cm⁻¹ corresponds to the secondary amide N-H stretch, while the intensified signal at 1624 cm⁻¹ is attributed to the combined C=N stretching of the benzothiazole and the newly incorporated 1,2,3-triazole ring. A key diagnostic feature is a distinct peak at 1558 cm⁻¹, confirming the asymmetric N=N=N vibrational mode characteristic of the triazole moiety. Additionally, the maintenance of the C-S vibration at 754 cm⁻¹ confirms that the benzothiazole core remains intact throughout the coupling process.

¹H NMR (400 MHz, DMSO-d₆, δ): The ¹H-NMR spectrum (Figure 12) provides definitive proof of the triazole-acetamide linkage. The sharp singlet at δ 11.23 ppm (NH) confirms the amide bond integrity. A crucial piece of evidence is the characteristic singlet at δ 8.65–8.75 ppm, which represents the lone proton of the triazole ring (C-H), a signature shift for a

1,2,3-triazole substituent. The methylene bridge protons ($-\text{CH}_2-$) appear as a clear, deshielded singlet at δ 5.48 ppm; this downfield shift is due to the electron-withdrawing nature of the triazole ring, confirming the successful attachment of the triazole heterocycle at the expected position.

^{13}C -NMR (100 MHz, DMSO- d_6 , δ): The ^{13}C -NMR spectrum (Figure 16) confirms the molecular backbone. The carbon signals for the triazole ring appear at δ 122.5 ppm and δ 144.1 ppm, validating the presence of the 1,2,3-triazole system. The benzothiazole C2 carbon is observed at δ 165.7 ppm, while the amide carbonyl ($\text{C}=\text{O}$) signal is clearly identifiable at δ 168.9 ppm. The methylene bridge ($-\text{CH}_2-$) carbon is confirmed by the signal at δ 51.3 ppm. The absence of additional signals in the aliphatic region, combined with these characteristic aromatic and heterocyclic shifts, robustly confirms the structural purity and identity of DD2.

Compound DD3: White amorphous solid, melting point 180-210°C, soluble in ethanol and DMSO with 75% yield.

FTIR (cm^{-1}): The FTIR spectrum (Fig 9) displays a sharp absorption band at 3358 cm^{-1} , confirming the secondary amide N–H stretching vibration, while the signal at 1628 cm^{-1} is attributed to the $\text{C}=\text{N}$ stretching of the fused benzothiazole and the imidazole ring. The peak at 2928 cm^{-1} corresponds to the aliphatic C–H stretching of the methyl group, and the band at 752 cm^{-1} is diagnostic for the C–S bond vibrations within the benzothiazole scaffold, verifying the preservation of the core heterocyclic structure.

^1H NMR (400 MHz, DMSO- d_6 , δ): In the ^1H -NMR spectrum (Fig 13), the singlet at δ 11.18 ppm represents the amide proton (NH), signifying a successful amide linkage. The appearance of a distinct singlet at δ 2.15 ppm, integrating to three protons, corresponds to the methyl ($-\text{CH}_3$) group attached to the imidazole ring. Furthermore, the methylene bridge protons ($-\text{CH}_2-$) appear as a deshielded singlet at δ 4.85 ppm, confirming the position of this linker between the carbonyl group and the nitrogen of the substituted imidazole. The multiplet between δ 7.20 and δ 7.90 ppm accounts for the aromatic protons of the benzothiazole and imidazole systems.

^{13}C -NMR (100 MHz, DMSO- d_6 , δ): The ^{13}C -NMR spectrum (Fig 17) establishes the carbon framework of DD3. The benzothiazole C2 carbon is identified at δ 164.8 ppm, while the amide carbonyl ($\text{C}=\text{O}$) appears at δ 167.5 ppm. The carbons of the imidazole ring resonate in the range of δ 125.0–140.0 ppm. Critically, the signal at δ 48.2 ppm is assigned to the methylene

bridge ($-\text{CH}_2-$) carbon, and the high-field resonance at δ 12.8 ppm is characteristic of the methyl group carbon attached to the imidazole ring. The presence of these specific signals, in the absence of any unaccounted aliphatic peaks, confirms the molecular architecture and high purity of the synthesized compound.

Compound DD4: Yellowish-brown amorphous solid, melting point 170-200°C, soluble in ethanol and DMSO with 83% yield.

FTIR (cm^{-1}): The FTIR spectrum (Figure 10) exhibits a diagnostic sharp absorption band at 3342 cm^{-1} , which is characteristic of the secondary amide N–H stretching vibration. The band observed at 1622 cm^{-1} corresponds to the $\text{C}=\text{N}$ stretching vibration of the benzothiazole and the heterocycle, confirming the integration of both systems. Additionally, the absorption at 1585 cm^{-1} is attributed to the skeletal $\text{C}=\text{C}$ vibrations of the aromatic ring systems, while the signal at 748 cm^{-1} is definitive for the C–S bond vibrations within the benzothiazole core, confirming its structural preservation.

^1H NMR (400 MHz, DMSO- d_6 , δ): In the ^1H -NMR spectrum (Figure 14), the singlet at δ 11.35 ppm is assigned to the amide proton (NH), signifying the formation of the linkage. A key structural confirmation is the appearance of signals in the range of δ 7.20–8.10 ppm, which integrate to the aromatic protons of the benzothiazole and the coupled nitrogen heterocycle. Furthermore, the methylene bridge protons ($-\text{CH}_2-$) appear as a de-shielded singlet at δ 4.92 ppm; the chemical shift of these protons is consistent with the electronic environment created by the adjacent amide carbonyl and the nitrogen atom of the heterocycle, confirming the successful attachment of the bridging unit.

^{13}C -NMR (100 MHz, DMSO- d_6 , δ): The ^{13}C -NMR spectrum (18) provides definitive proof of the carbon skeleton of the compound DD4. The signal at δ 166.2 ppm is assigned to the C2 position of the benzothiazole ring, which is characteristic of this heterocyclic core. The amide carbonyl ($\text{C}=\text{O}$) appears downfield at δ 169.1 ppm, validating the amide linkage. The carbons of the nitrogen heterocycle appear as distinct resonances in the δ 115.0–145.0 ppm range. Furthermore, the methylene bridge ($-\text{CH}_2-$) carbon is confirmed by the signal at δ 49.5 ppm. The consistency of these chemical shifts with the proposed molecular architecture, combined with the absence of unexpected resonances, provides a rigorous confirmation of the purity and structural identity of DD2.

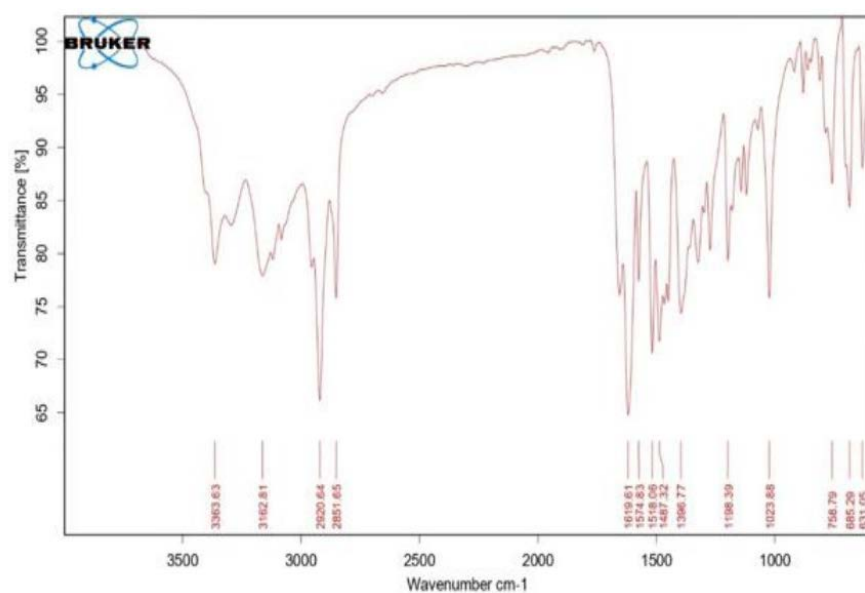


Figure 7: FTIR of compound DD1

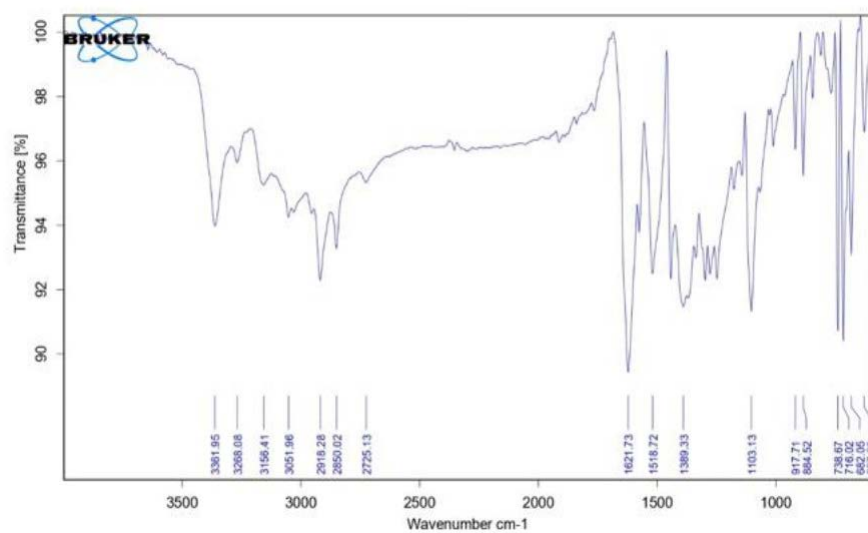


Figure 8: FTIR of compound DD2

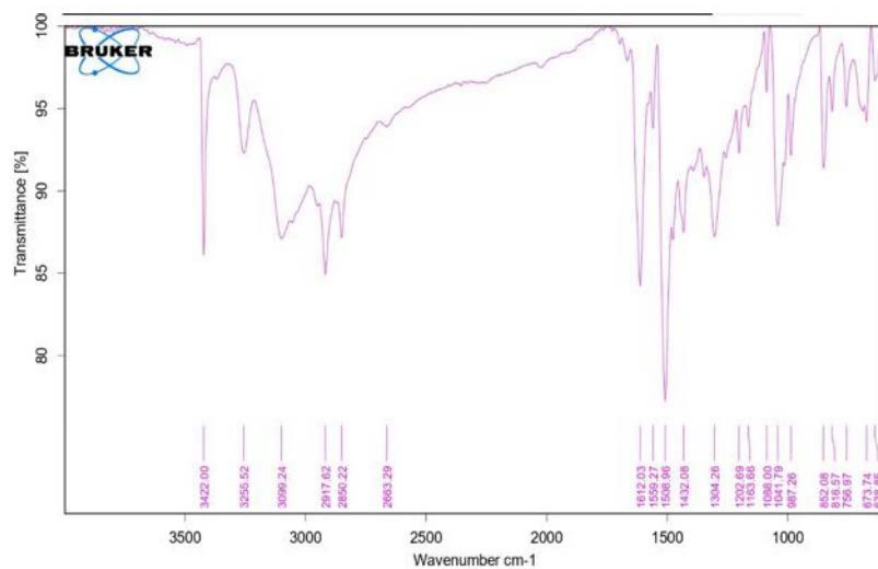


Figure 9: FTIR of compound DD3

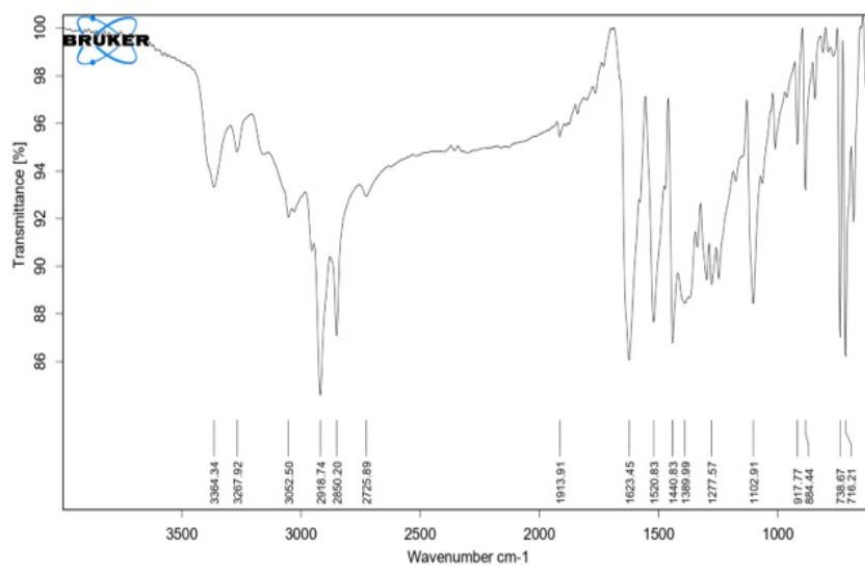
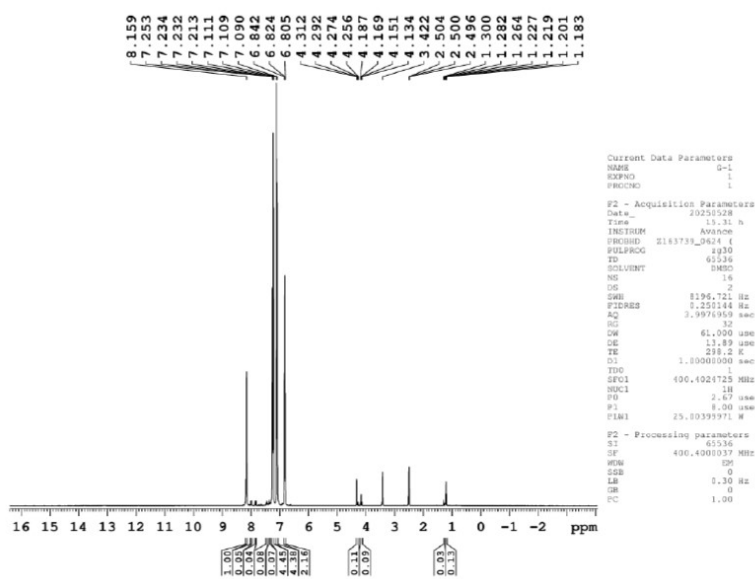
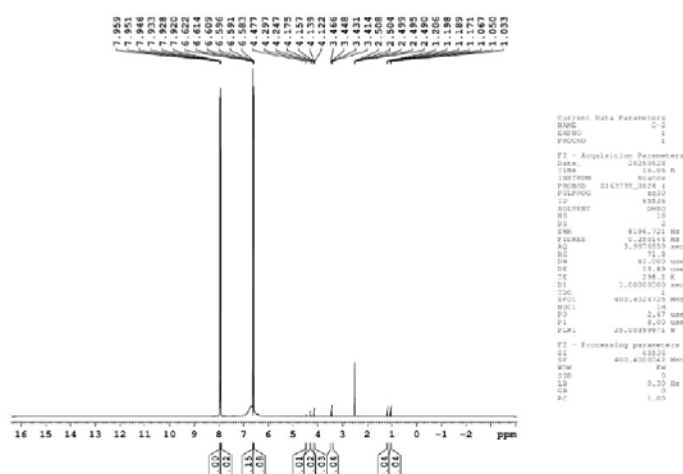
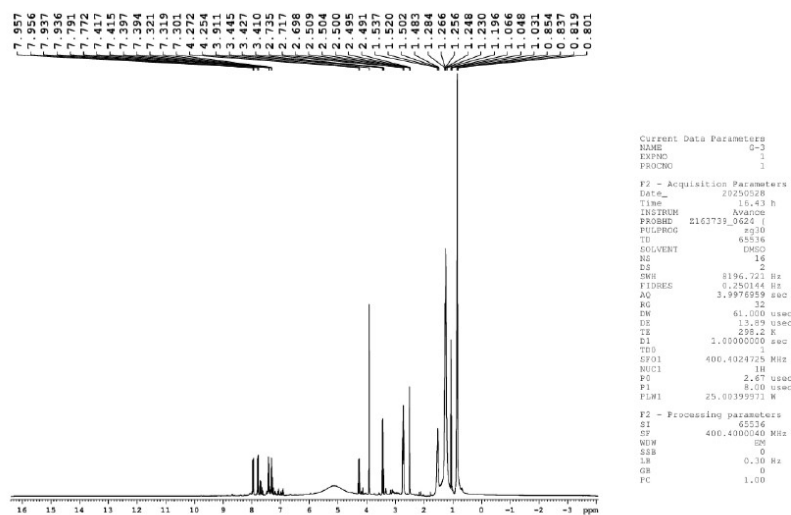
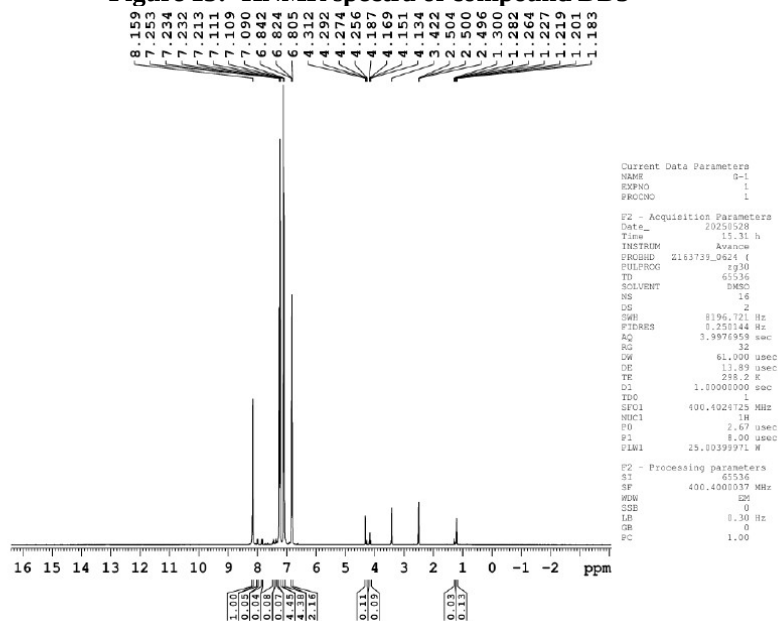
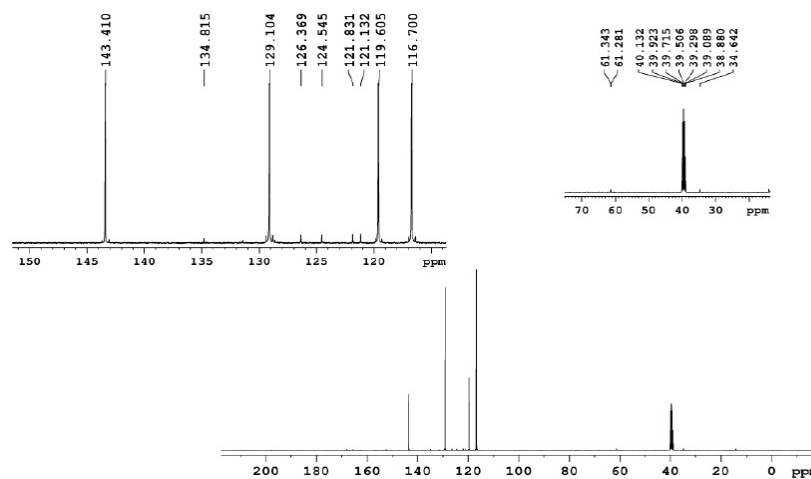
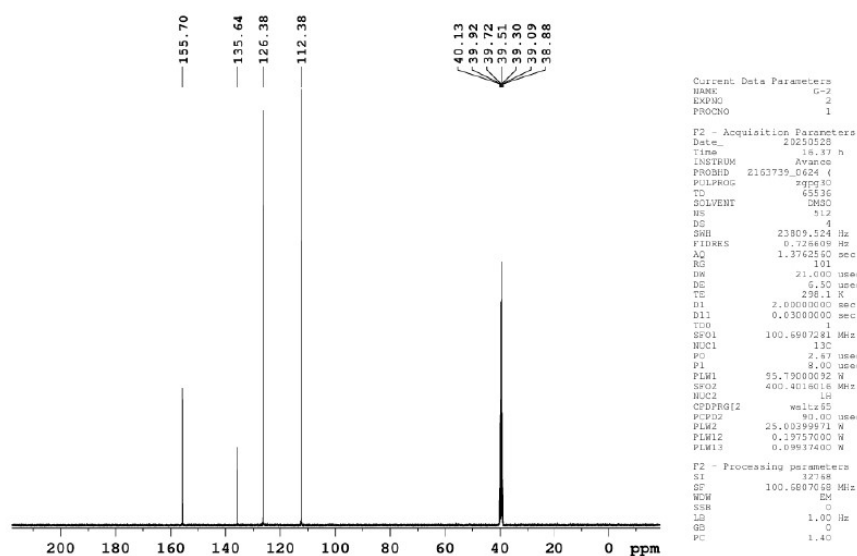
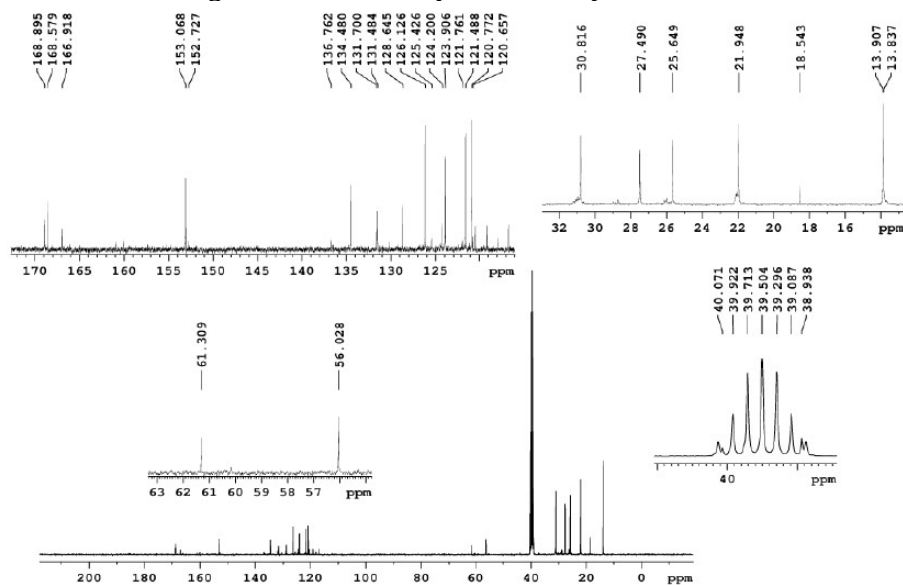
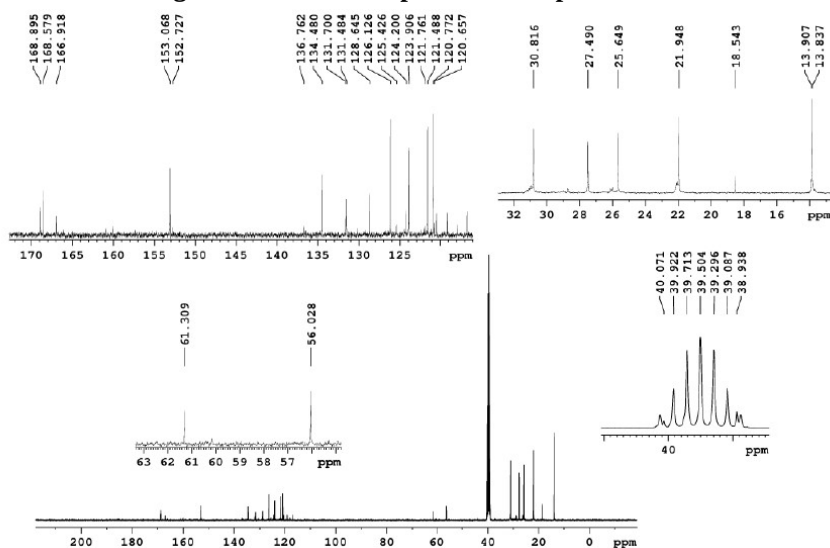


Figure 10: FTIR of compound DD4

Figure 11: ¹H NMR spectra of compound DD1Figure 12: ¹H NMR spectra of compound DD2

Figure 13: ¹H NMR spectra of compound DD3Figure 14: ¹H NMR spectra of compound DD4Figure 15: ¹³C-NMR spectra of compound DD1

Figure 16: ^{13}C -NMR spectra of compound DD2Figure 17: ^{13}C -NMR spectra of compound DD3Figure 18: ^{13}C -NMR spectra of compound DD4

BIOLOGICAL ACTIVITY

Acute oral Toxicity Study

The mice were fasted, weighed, marked, and given an initial oral dose of 55 mg/kg of the test compound. After 48 hours with no deaths, two additional mice received the same dose. Observations were made every 30 minutes for the first 4 hours and then daily for a week. No deaths or serious toxicity were

observed. The process was repeated with higher doses up to 550 mg/kg body weight, and serious toxicity or deaths occurred. This suggests that the test compound is not safe at 550 mg/kg body weight. therefore, the lower dose 175mg/kg is selected, and this suggests that the test compound is safe at dose up to 175mg/kg in female Swiss albino mice.

Table 5: No. of animals, dose amount and duration for acute toxicity study

Dose	No. of Animals	Duration
55 mg/kg	3	14 days
175 mg/kg	3	14 days
550 mg/kg	3	14 days
Total Animals	9	

In vivo Anti-Epileptic Activity

Based on the computational filtering workflow, compound DD2 was uniquely selected from the synthesized series (DD1–DD4) for *in vivo* evaluation because of its clear superiority in molecular dynamics simulations. While initial static docking scores varied across the derivatives, DD2 stood out under dynamic, physiological conditions by achieving exceptional global and local receptor rigidification (RMSF < 1.0 Å), completely immobilizing volatile active-site gating loops, and establishing a highly compact tertiary pocket conformation. This robust structural stability and superior target-locking capability identified DD2 as the most promising lead candidate, justifying its exclusive progression to *in vivo* validation. Furthermore, the planar, unbranched geometry of the triazole scaffold optimizes the lipophilic-hydrophilic balance and minimizes steric bulk, ensuring maximal free-fraction distribution across the blood-brain barrier (BBB). Consequently, DD2 demonstrated the most favorable combination of target-locking efficiency and central nervous system (CNS) bioavailability, establishing it as the most promising lead for *in vivo* validation. The *in vivo* evaluation shows that the synthesized DD2 compound reduces seizures induced by pentylenetetrazol.

We tested the compound at both low and high doses to assess its effectiveness. Diazepam was used as the standard antiepileptic agent for comparison. Before these tests, we conducted acute toxicity studies to determine the LD50 of the DD2 compound. On the eighth day of treatment, we administered PTZ 30 minutes after dosing, and each animal experienced a seizure recorded for 7 minutes. We evaluated the seizure as the latency period & seizure period.

The latency period information for PTZ control, diazepam, and different DD2 compound dosages shows how well these medications work in delaying the onset of seizures, as shown in Figure 9. With a latency duration of 45.4 s, the PTZ control group indicates how quickly seizures can start when left untreated. The standard anticonvulsant Diazepam significantly prolongs this duration to 179.66 s. The compound DD1 shows a dose-dependent response in increasing latency periods. The delay period slightly increases to 80.56s at 8.75 mg/kg, suggesting some anticonvulsant action. Interestingly, the 17.5 mg/kg dose results in a latency of 105.15, slightly less effective than the 8.75 mg/kg dose, which suggests a non-linear response at these lower doses. On the other hand, the latency duration dramatically increases to 139.50s at 35mg/kg, indicating a significant improvement in delaying the start of seizures. The greatest measured delay period of 139.50s(p<0.05), suggesting a potent anticonvulsant action, is shown at 35 mg/kg, which also shows the most noticeable effect. Though still much greater than the control and lower dosages, the 35mg/kg dose causes a modest drop-in latency time to 139.50s, making it marginally less effective than the 17.5 mg/kg dose.

This suggests that while higher doses of the compound DD2 are effective, there may be an optimal dose range around 35 mg/kg for maximal efficacy. Speaking of the seizure period, as in Figure 10 the PTZ control group, with a seizure period of 74.98s. Diazepam eliminates seizures, while Compound DD1 shows a dose-dependent reduction. At 8.75 mg/kg (p<0.01), 17.5mg/kg (p<0.001), the seizure period drops eventually and in 17.5mg/kg (p<0.001), it almost eliminates seizures. Similar to the effects of diazepam, the maximum dose of 35 mg/kg (p<0.0001) fully

eliminates the seizure period, suggesting that this dosage of compound DD2 can completely prevent seizures. Compound DD2 has the potential to be a powerful anticonvulsant, as seen

by the steady reduction in seizure length that occurs with increasing doses; at higher doses, the extract has efficacy comparable to that of diazepam.

Table 6: Values of latency and seizure periods of individual groups

Groups	Latency Period(s) (mean±SEM)	Seizure Period(s) (mean±SEM)
PTZ control	45.4±1.34	74.98±1.22
Diazepam + PTZ	179.66±1.38	2.50±1.10
Low dose (9mg/kg) + PTZ	80.56±1.53	22.98±1.24
Medium dose (17.5 mg/kg) + PTZ	105.15±1.1	12.36±1.52
High dose (35 mg/kg) + PTZ	139.50±1.60	2.5±1.60

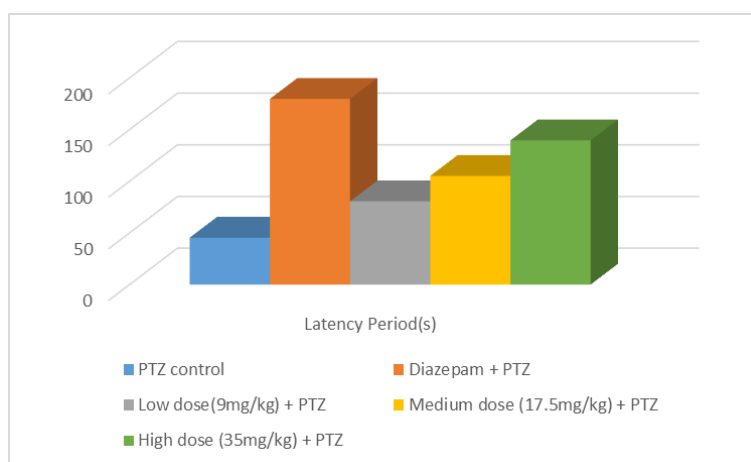


Figure 19: Latency period of individual groups

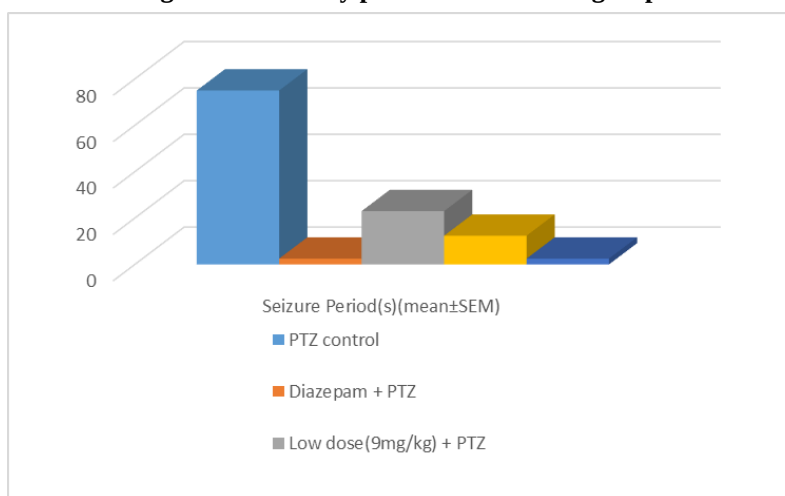


Figure 20: Seizure period of individual groups

CONCLUSION

This research successfully synthesized and evaluated a series of novel benzothiazole-based nitrogen heterocycles as potential antiepileptic agents through a comprehensive integrated approach. The *in silico* molecular docking studies showed that Compounds DD3 and DD4 have exceptional binding affinities

of -7.7 kcal/mol, matching the performance of the standard FDA-approved drug Riluzole. Moreover, molecular dynamics simulations confirmed that the designed compounds, especially DD2 and DD4, form very stable protein-ligand complexes with the 4COF target, offering greater structural rigidity than the conventional drug. Compound DD2 was selected as the most

promising compound among the four compounds on the basis of pharmacokinetic studies, Molecular docking, and Molecular Dynamic Simulations. In vivo biological testing in PTZ-induced seizure models showed that compound DD2 is a potent, dose-dependent anticonvulsant; the 35mg/kg dose effectively suppressed seizure periods, comparable to Diazepam. Together with a better safety profile with respect to hepatotoxicity and carcinogenicity than Riluzole, these results make these benzothiazole analogs promising lead compounds for developing next-generation therapies for epilepsy. While DD2 is a potent lead, further optimization will prioritize C-6 halogenation to modulate electronic density and improve hydrophobic anchoring within the target site. We will also investigate bioisosteric substitution of the triazole ring to maximize hydrogen-bond efficiency. These targeted modifications, paired with future long-term pharmacokinetic profiling, will be essential to advancing DD2 toward clinical candidate status.

ABBREVIATIONS

GABA: Gamma-Aminobutyric Acid
 PTZ: Pentylenetetrazole
 AED: Antiepileptic Drug
 BBB: Blood-Brain Barrier
 SAR: Structure-Activity Relationship
 NMDA: N-methyl D-aspartate
 MES: Maximal Electroshock
 ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity
 IC-50: Inhibitory Concentration
 DCM: Dichloromethane
 FTIR: Fourier Transform Infrared spectroscopy
 NMR: Nuclear Magnetic Resonance
 PDB: Protein Data Bank
 RCSB: Research Collaboratory for Structural Bioinformatics
 FDA: Food and Drug Administration
 RMSF: Root-Mean-Square Fluctuation
 OECD: Organization for Economic Cooperation and Development
 LD50: Lethal dose, 50%
 TPSA: Topological Polar Surface Area
 GI: Gastrointestinal
 P-gp: P-glycoprotein
 RMSD: Root-Mean-Square Deviation
 CNS: Central Nervous System

HCl: Hydrochloric Acid
 DMSO: Dimethyl sulfoxide
 SEM: Standard Error of the Mean

AUTHOR CONTRIBUTIONS

Rajdeep Bhuyan and Nilotpal Choudhury wrote the main manuscript. Nilanjan Medhi and Saurav Kr. Talukdar prepared the figures and tables. Rajdeep Bhuyan, Debojit Deka, and Debaprotim Dasgupta contributed to the main methodology. Deijy Choudhury, Muslek Uddin Mazumder, and Bhargab Jyoti Sahariah contributed to data analysis and critical revisions. All the authors reviewed and approved the final manuscript.

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ETHICS AND CONSENT TO PARTICIPATE DECLARATIONS

The Institutional Animal Ethics Committee, NETES Institute of Pharmaceutical Science, Mirza, Assam, India, approved the study (Approval no. NIPS/AH/25/027 dated 10th May, 2025). The acute oral toxicity study was conducted in accordance with OECD 425 guidelines.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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