Molecular Docking an In Vitro Anticonvulsant evaluation of Newly Synthesized Substituted Phthalimide Schiff bases derivatives targeting GABAA receptor

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Received: 23 June 2026; Revised: 28 June 2026; Accepted: 14 July 2026; Available Online: 30 July 2026

ABSTRACT

Epilepsy, a chronic neurological disorder affecting nearly 50 million people worldwide, remains difficult to manage due to drug resistance and adverse effects of existing antiepileptic drugs. This study investigates phthalimide-based Schiff base derivatives as potential anticonvulsant agents targeting the gamma-aminobutyric acid type A ($GABA_A$) receptor. Fifteen derivatives were designed and screened using computer-aided drug design (CADD) and molecular docking simulations against the $GABA_A$ receptor (PDB ID: 6X3X). The top five compounds (A1–A5) were shortlisted based on docking affinity, interaction with key residues, drug-likeness, ADME properties and predicted toxicity. In-silico analyses using Swiss ADME, BOILED-Egg, and Pro Tox-II indicated favorable pharmacokinetic profiles, blood–brain barrier permeability, and low toxicity. Visualization through BIOVIA Discovery Studio revealed stable hydrogen bonding and hydrophobic interactions with residues TYR160, PHE77 and SER205. In-vitro assays using HepG2 cells confirmed dose-dependent activity comparable to phenytoin. The integrated computational and experimental approach demonstrated that these derivatives possess strong binding affinity, good oral bioavailability and minimal toxicity. Overall, the findings highlight phthalimide Schiff base derivatives as promising multifunctional candidates for anticonvulsant therapy, offering improved safety and efficacy profiles for future pharmaceutical development.

KEYWORDS

Phthalimide derivatives; $GABA_A$ receptor; Molecular docking; Anticonvulsant activity; ADME prediction; In-silico toxicity; Blood–brain barrier permeability; Computer-aided drug design

How to cite this article: Kambale AM, Jain BU, Rautmale ST, Kachare PD, Patil AK, Bhandigare SP. Molecular docking and in vitro anticonvulsant evaluation of newly synthesized substituted phthalimide Schiff bases derivatives targeting GABAA receptor. Int J Drug Deliv Technol. 2026;16(72s): 203-215. DOI: 10.25258/ijddt.16.72s.25

INTRODUCTION

Epilepsy is a chronic neurological disorder impacting roughly 50 million individuals globally, defined primarily by the occurrence of recurrent seizures. Although a wide range of antiepileptic drugs (AEDs) is readily available, a significant portion of patients continues to struggle with treatment resistance, debilitating side effects or insufficient clinical outcomes. These challenges have driven an urgent search for innovative compounds that offer improved safety profiles and higher therapeutic indices while effectively targeting the central nervous system. [1]

The core of epilepsy lies in an imbalance between excitatory and inhibitory neurotransmission. A primary target for regulating this activity is the gamma-aminobutyric acid type A ($GABA_A$) receptor, which serves as a critical inhibitory mediator. By modulating these receptors, it is possible to suppress abnormal electrical discharges and reinstate neurological equilibrium.

Within the field of medicinal chemistry, phthalimide-based Schiff bases have emerged as

compelling candidates for anticonvulsant therapy. The phthalimide nucleus is a recognized pharmacophore in various sedative-hypnotic drugs, and its hybridization with a Schiff base (R-1R-2C=NR-3) allows for the precise modulation of lipophilicity. This structural refinement is crucial for improving blood-brain barrier (BBB) permeability and increasing the compound's efficiency in stabilizing hyper-excitable neuronal membranes. [24]

This study employs a computer-aided drug design (CADD) strategy to evaluate the anticonvulsant potential of phthalimide-derived Schiff bases. Using molecular docking simulations, fifteen derivatives were screened for their binding affinity against the $GABA_A$ receptor (PDB ID: 6X3X). Based on superior docking scores and predicted blood-brain barrier (BBB) permeability, five lead compounds were selected for further validation through in vitro neuronal assays and in vivo Maximal Electroshock (MES) seizure models.

These results establish a robust framework for future pharmaceutical development, demonstrating that this integrated computational and experimental approach serves as a resource-efficient predictive model. By streamlining the identification of potent anticonvulsant candidates, this methodology minimizes the time and costs typically associated with traditional drug discovery. [6]

MATERIALS AND METHODS

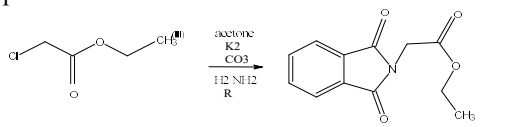
Design strategy

The main scaffold 2-(1H-benzo[d] [1, 2, 3] triazol-1-yl)-N-carbamoyl [R1-Substituted] was altered to produce a special set of fifteen phthalimide based Schiff base derivatives in order to increase potential antidiabetic efficacy. Structure activity connections and pharmacodynamic considerations led to the intro duction of structural variants. Specifically, nitrogen in the ring act s both hydrogen (–H) donor and acceptor, crucial for binding toxicological targets like receptor. Such substitutions are well supported by SAR studies, where electron donating and -withdrawing groups on Schiff bases have been shown to significantly impact enzyme inhibition and pharmacological activity. [14] For com parison, Phenytoin, a clinically approved potentiating $GABA_A$ mediated inhibition widely used for managing the anticonvulsant therapy, was employed as the standard drug (Fig). Chem Draw Professional 8.0 was used to create the SMILES notations for each chemical. For later computer studies, the structures were stored in MDL SDF format. [4]

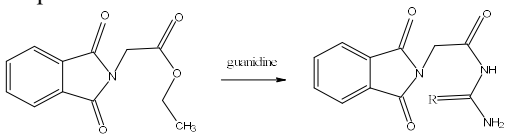
Scheme for phthalimide Schiff based derivative

Step 1: Phthalic anhydride is dissolved in ethanol in a round-bottom flask, and concentrated ammonia solution is added slowly with continuous stirring until a clear solution is obtained, with gentle warming if necessary; the mixture is then heated under reflux for a suitable period, cooled to room temperature, and further chilled in an ice-water bath to induce crystallization, after which the separated white solid is collected by vacuum filtration, washed with cold water, and finally recrystallized from ethanol to yield pure phthalimide.

Step 2: Phthalimide is dissolved in dry acetone containing potassium carbonate and stirred to form a reactive mixture, after which ethyl chloroacetate is added dropwise with continuous stirring; the mixture is then heated under reflux to promote N-alkylation, yielding ethyl (1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl) acetate. Upon completion, the reaction mixture is filtered to remove inorganic salts, the solvent is evaporated under reduced pressure, and the resulting solid is recrystallized from ethanol to obtain the pure product.



Step 3: Ethyl(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acet ate is dissolved in ethanol, and an appropriate guanidine derivative is added with magnetic stirring; a few drops of glacial acetic acid are introduced to catalyze the reaction, and the mixture is heated under reflux to form the final substituted derivatives. After completion, the mixture is cooled, poured onto crushed ice to precipitate the product, which is then collected by vacuum filtration, washed with cold ethanol, and recrystallized to yield the pure final compounds.



Computational docking

a) Ligand Preparation

To prepare the phthalimide Schiff base derivatives for computational analysis, the 2D molecular structures were initially drafted using ChemDraw Pro 8.0 and ChemSketch. These structures were subsequently converted into 3D conformations using PyMOL, followed by energy minimization to ensure the geometries were energetically favorable and stable for docking simulations. The optimized ligands were initially saved in PDB format. To facilitate flexible docking, AutoDock Tools 1.5.7 was used to convert these files into PDBQT format; during this process, Gasteiger charges were assigned, and the specific rotatable bonds were defined to allow for conformational flexibility during the binding simulations. [15]

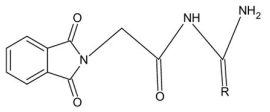


Figure1: General structure of selected series of compounds

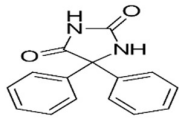


Figure 2: Phenytoin

b) Receptor preparation

The $GABA_A$ receptor (PDB ID: 6X3X) was sourced from the Protein Data Bank and refined for simulation using AutoDock Tools 1.5.7. This preparation included deleting crystallographic water molecules, integrating polar hydrogens, and assigning Gasteiger and Kollman charges to establish the receptor's electrostatic profile. A coordinate grid box was then mapped around the active site to define the search space for ligand interactions. The finalized protein structure was saved in PDBQT format to ensure compatibility with the docking algorithm[16]

c) Docking parameters:

Molecular docking was performed using Auto Dock Vina (integrated with AutoDock Tools). Docking grid boxes were defined around the active site residues, with explicit coordinates and dimensions (X, Y, Z centers and box sizes) optimized for each protein. The exhaustiveness was set to 8, the energy range was set to 4, and the maximum number of binding modes was fixed at 10. The AutoDock Vina scoring function was used to evaluate binding affinity, expressed as binding energy (kcal/ mol). For each receptor, the optimized grid centers were: $GABA_A$ receptor (PDB ID: 6X3X) at (x = 106.8, y = 109.4, z = 114.2 Å) [7]

d) Interaction analysis

The docked complexes were analyzed using BIOVIA Discovery Studio Visualizer and Py MOL. Interactions including hydrogen bonding, hydrophobic contacts, π–π stacking, and electrostatic interactions with catalytically significant residues were examined. The binding modes were visualized through the creation of both 2D and 3D interaction diagrams [17]

e) Selection of top five compounds (A1–A5):

Among the fifteen designed derivatives, a shortlisting strategy was applied to identify the most promising candidates for further validation. The selection criteria were based on multiple computational filters: (i) Docking affinity across all three protein targets, (ii) Interaction with catalytically important residues, (iii) Compliance with drug-likeness and ADME rules, (iv) Predicted toxicity class, and (v) Passes blood brain barrier. Compounds fulfilling these multi-parameter requirements were shortlisted for comparative evaluation. [18]

Drug likeness studies and ADME prediction

Calculations of molecular properties and drug-likeness parameters were performed for each of the design compounds (A1 to A5). The drug-likeness study is important because it identifies compounds that fit the criteria as being similar to drugs. It is crucial to investigate ADME qualities because of the pharmacokinetic properties of pharmaceutical compounds, including their oral bioavailability, cell penetration, metabolism, and elimination. Swiss ADME techniques were used to calculate Lipinski's rule of five, and other physicochemical parameters such molecular refractivity, GI absorption, water solubility, and the number of rotatable bonds were predicted. [9] Several software tools were used in this study to ensure accurate projections and in-depth analysis. Using Swiss ADME, which provided crucial details on the pharmacokinetic properties of the medications, predictions for Absorption, Distribution, Metabolism, and Excretion (ADME) were established. Pro Tox 3.0 was used to assess the toxicity profiles of the com pounds and ensure their safety for further studies. Predicted physicochemical and pharmacokinetic parameters of the synthesized derivatives, benchmarked against the reference drug phenytoin, are summarized in table. [8]

Boiled-EGG analysis

The selected drugs gastrointestinal absorption and blood–brain barrier penetration were evaluated using the BOILED-Egg model. The study discovered that chemicals in the white region were linked to greater intestine absorption potential, whereas compounds in the yellow zone were expected to have greater permeability across the blood–brain barrier. The Swiss ADME digital platform was used to conduct the study.[20]

Toxicity studies

To evaluate the safety profile of the synthesized chemicals, a toxicology investigation was conducted. Acute toxicity (LD_{50}), toxicity class, and organ-specific toxicities like hepato toxicity, mutagenicity, and carcinogenicity were estimated using the in-silico prediction platform Pro Tox-II (version 3.0). [3] Using machine learning and molecular similarity techniques, it facilitates effective early-stage screening of possible hazardous effects based on SMILES input. In-silico toxicity profiles of the derivatives in comparison with acarbose are presented in Table. [11]

Selection of the target protein

GABA_A receptor inhibitory modulation:

One important target for controlling neuronal hyperexcitability and seizures in epilepsy is the human gamma-aminobutyric acid type A ($GABA_A$) receptor. [12] The chosen structure (PDB ID: 6X3X) was resolved by cryo-electron microscopy at a resolution of 2.50 Å. [21] Consisting of a heterotetrametric assembly (typically \alpha1\beta2\gamma2 subunits), it offers thorough information on the benzodiazepine and neuro steroid

binding sites. These structural details allow the rational creation of novel phthalimide Schiff base derivatives as potential anticonvulsant agents. Effective GABA receptor modulator development necessitates accurate binding mode and interaction prediction, which is made feasible by docking experiments using 6HUP.

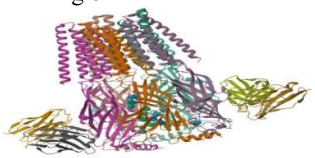


Figure 3: 3D crystal structures of the selected target proteins used in the molecular docking

Interactions between analogs and protein

The complex structures of the $GABA_A$ receptor (gamma-aminobutyric acid type A) were loaded into BIOVIA Discovery Studio Visualizer (v21.1.0.20298) for detailed molecular interaction analysis. In this computational model, the receptor protein served as the biological target, while the synthesized phthalimide-based derivatives were identified as the ligands. To visualize the binding orientation, 2D interaction diagrams were generated to highlight critical molecular forces, including Hydrogen bonds, Hydro phobic contacts (such as pi-pi stacking or alkyl interactions common with the phthalimide ring) Electrostatic interactions. Furthermore, the 3D interaction patterns of the phthalimide ligands were compared against native GABA and clinical standards (e.g., phenytoin) across the selected receptor subunits. The objective of this analysis was to elucidate specific binding motifs and predict the potential anticonvulsant efficacy of the derivatives by identifying ligand-protein interactions essential for modulating the chloride channel. [13] To validate these in silico findings and enhance translational relevance, selected phthalimide derivatives [A1-A5] were further evaluated using in vitro $GABA_A$ binding affinity assays and cellular studies. [1]

In vitro validation of anticonvulsant activity

HepG2 cells. The cell line was maintained in high-glucose DMEM, supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Cells were seeded into 96-well plates at a density of 1 times 10^4 cells/well and incubated at 37°C under a humidified atmosphere of 5% CO2. Test compounds were initially dissolved in DMSO and subsequently diluted in the culture medium to achieve a concentration range of 20, 40, 60, 80, and 100 µM. Following a 24-hour incubation period with the treatments, cell viability was quantified by measuring the absorbance at 550 nm using a microplate reader. [2]

Calculation of % Inhibition.

The percentage inhibition of enzyme activity was calculated using the formula:

$$\%\text{Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

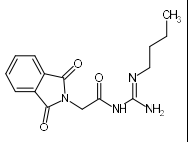
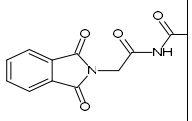
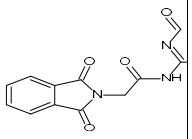
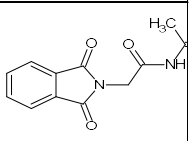
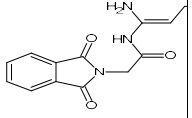
Where: • A control = Absorbance of the control (without test compound).

• A sample = Absorbance in the presence of test compound.

RESULTS

Designed phthalimide Schiff base derivatives

ChemDraw Professional 8.0 was employed to design fifteen phthalimide Schiff base derivatives and generate their corresponding SMILES notations. All designed compounds were subjected to molecular docking studies against three selected protein targets. Based on binding affinity scores and the consistency of key molecular interactions, five derivatives exhibiting superior docking performance were selected for detailed comparative analysis. Furthermore, a comprehensive multi-criteria evaluation was conducted by integrating docking scores, interactions with conserved residues, ADME property compliance, toxicity class prediction, and PASS-based bioactivity profiling. Based on this integrated assessment, five derivatives (A1–A5) were ultimately shortlisted as potential lead candidates Table1.

Compound Code	IUPAC Name	Structure
A1	*N*-(*N*'-butylcarbamimidoyl)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) acetamide	
A2	*N*-carbamoyl-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) acetamide	
A3	2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(*N*'-formyl carbamimidoyl) acetamide	
A4	2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-ethanimidoylacetamide	
A5	*N*-[(1*E*)-1-amino-3-oxoprop-1-en-1-yl]-2-(1,3-dioxo-1,3-dihydro-2*H*-	

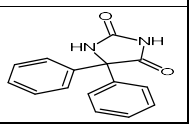
	isoindol-2-yl) acetamide	
STD	5,5-diphenylimidazolidine-2,4-dione	

Table1:2D chemical structures of the top five derivatives with their compound codes3.2 ADME prediction

ADME prediction

The ADME properties of the synthesized phthalimide Schiff base derivatives were evaluated using the Swiss ADME online prediction tool. In silico ADME assessment plays a crucial role in the early stages of drug discovery, as it enables the prediction of pharmacokinetic behaviour prior to in vivo experimentation. Despite demonstrating promising in vitro activity, many drug candidates fail during clinical development due to poor absorption, unfavourable metabolism, or limited bioavailability. Therefore, key parameters including gastrointestinal (GI) absorption, aqueous solubility, Lipinski's Rule of Five, and other relevant physicochemical properties were analyzed to assess the drug-likeness of the synthesized compounds.

Lipinski's Rule of Five provides a widely accepted criterion for predicting oral bioavailability based on molecular weight (MW < 500 g/mol), lipophilicity (Log P ≤ 5), hydrogen bond donors (HBD ≤ 5), and hydrogen bond acceptors (HBA ≤ 10). Evaluation of the synthesized derivatives revealed that the majority of the compounds complied well with these criteria, indicating favourable potential for oral bioavailability. In addition, calculated topological polar surface area (TPSA) values, along with acceptable HBA and HBD counts, further supported good membrane permeability and bioavailability. Collectively, these findings suggest that the selected Schiff base derivatives possess suitable pharmacokinetic profiles for further optimization as drug candidates. The favourable ADME characteristics also justified the selection of derivatives A1–A5 for subsequent analyses. Comparative drug-likeness and ADMET profiles of the synthesized compounds in relation to acarbose are summarized in Table 2 &3

Ligand	**Molecular formula**	**MW (g/mol)**	**LogP**	**HBA**	**HBD**	**Lipinskirule**	**TPSA (Å²)**	**MR (cm³/mol)**	**nRotB**
A1	$C_{15}H_{18}N_4O_3$	308.33	1.23	4	2	Yes	104.86	84.96	7
A2	$C_{11}H_9N_3O_4$	247.21	0.45	4	2	Yes	109.57	62.63	4
A3	$C_{12}H_{10}N_4O_4$	274.23	–0.10	5	2	Yes	121.93	71.13	5
A4	$C_{12}H_{11}N_3O_3$	245.23	1.33	4	2	Yes	90.33	67.73	4
A5	$C_{13}H_{11}N_3O_4$	273.24	0.94	4	2	YES	109.57	71.38	5
Std	$C_{15}H_{12}N_2O_2$	252.27	1.65	2	2	Yes	58.20	77.50	4

Table 2: Drug-likeness of the synthesized schiff base derivatives compared with the standard drug phenytoin

Ligand	**Docking Score (kcal/mol)**	**Water Solubility (logS)**	**Solubility Class**	**BBB Permeability**	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**	**Toxicity (ProTox-II) Class**
A1	-9.6	-3.1	Moderately Soluble	Yes	No	No	No	No	No	IV

A2	-10.8	-2.8	Soluble	Yes	No	No	No	No	No	V
A3	-10.3	-2.5	Soluble	Yes	No	No	No	No	No	V
A4	-9.2	-3.3	Moderately Soluble	Yes	No	No	No	No	No	IV
A5	-9.9	-3.0	Moderately Soluble	Yes	NO	NO	NO	NO	NO	IV
Std	-12.9	-3.3	Soluble	Yes	No	No	No	No	No	V

Table 3: In silico ADMET profiles of the selected schiff base derivatives compared with the standard drug phenytoin

Boiled-EGG analysis/ brain estimated permeation technique

The pharmacokinetic behavior of the five best-performing phthalimide Schiff base derivatives was evaluated using the BOILED-Egg (Brain or Intestinal Esti mated Permeation) model. This graphical method predicts passive gastrointestinal absorption and blood–brain barrier penetration by plotting WLOGP (lipophilicity) on the Y-axis against TPSA (topological polar surface area) on the X-axis. All five molecules were located within the yellow zone, indicating a high likelihood of efficient in crossing of blood brain barrier. Overall, the BOILED Egg results reinforced the favorable ADME findings, further supporting the selection of A1–A5 as promising orally active candidates (Figure 4 &5)

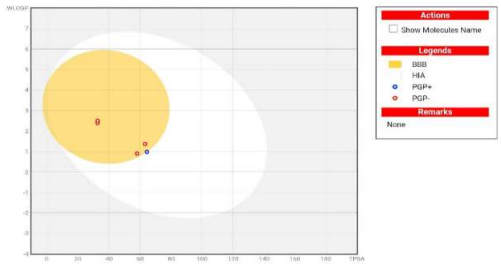


Figure 4: Boiled egg model

In the BOILED-EGG Model, the yellow zone represents the biophysical space of chemicals that are more likely to enter the brain, while the white zone represents the physicochemical region of compounds most likely to be absorbed within the digestive tract.

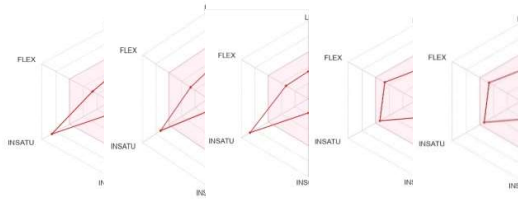


Figure 5: Radar plots showing ADME properties (LIPO, SIZE, POLAR, INSOLU, INSATU, FLEX) of top five 2-mercapto benzimidazole Schiff base derivatives (A1–A5)

Toxicity studies

The toxicity profiles of the designed ligands (A1–A5) were evaluated using the ProTox-II web server (version 3.0). The predicted toxicity classes are presented in Table X. Among the evaluated compounds, A2 and A3 were classified under Class V, indicating relatively low toxicity and a favorable safety profile. In contrast, A1, A4, and A5 were categorized under Class IV, suggesting moderate toxicity with possible harmful effects only at higher exposure levels. Importantly, none of the compounds were predicted to fall under highly toxic categories (Class I–III) indicating an acceptable overall safety margin.

Overall, the toxicity predictions suggest that the designed ligands possess suitable safety profiles and can be considered for further in vivo studies to evaluate their toxicity and safety in biological systems.

Docking study

Docking analysis of fifteen phthalimide Schiff base derivatives with the $GABA_A$ receptor (PDB ID: 6X3X) revealed binding energies ranging from –8.2 to –10.8 kcal/mol. The strongest binding was observed for compounds A11, A7, A3, A6, and A13, which exhibited docking scores of –10.8, –10.3, –9.9, –9.6, and –9.2 kcal/mol, respectively. These derivatives showed favourable interactions within the receptor's active site, mainly through hydrophobic contacts and stable hydrogen bonding with crucial amino acid residues. Further inspection using Discovery Studio confirmed optimal

accommodation of the ligands within the $GABA_A$ receptor binding pocket, supporting their stable and favourable binding conformations Table 4.

Compound Name	-R	Binding Score (Kcal/Mol)
AK1	$–CH_2CH_3$	-9.3
AK2	$–CONH_2$	-8.6
AK3 (A3)	$–OCH_2CH_2–$	-9.9
AK4	–Cl	-8.7
AK5	$–CH_3$	-9.5
AK6 (A4)	$–CH_2CH_2CH_2CH_3$	-9.6
AK7 (A2)	–CHO	-10.3
AK8	–COCl	-8.7
AK9	$–NHCH_3$	-9.8
AK10	$–NHCH_2CH_3$	-8.2
AK11 (A1)	–C(=O)–	-10.8
AK12	–C(=S)–	-9.3
AK13 (A5)	$–CH_3$	-9.2
AK14	$–NHNH_2$	-9.2
AK15	–C=NH	-9.3
Standard	Phenytoin	-12.9

Table 4: Docking Scores of 15 derivatives with GABA-A Receptor.

Statistical validation of docking scores

To strengthen the comparative docking results, statistical analysis was performed between the Schiff base derivatives (A1–A5) and the standard drug acarbose across targets ($GABA_A$). A one-way ANOVA followed by pairwise t-tests was applied to determine the significance of differences in binding affinities.

This statistical validation adds quantitative rigor to the docking outcomes and highlights the relevance of A2 and A3 as promising candidates for crossing BBB and in accordance with toxicity.

Visualization of the selected designed compounds

BIOVIA Discovery Studio Visualizer was used to analyze the 2D and 3D interactions of the top five phthalimide Schiff base derivatives with $GABA_A$ receptor. Strong binding affinities were supported by the identification of important interactions, including hydrophobic contacts and hydrogen bonding. The complexes can be seen in below mentioned Figures & Table.

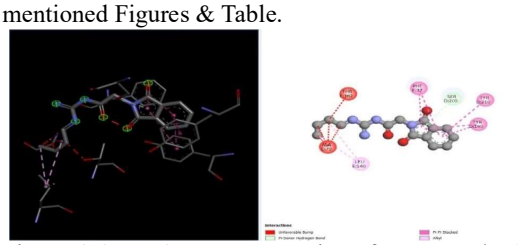


Figure 6: 2D & 3D Representation of compound A1 with PDBID: 6X3X

SN	Residual atom	Distance	Category	Type of Interaction
1	TYR160	5.41415	Hydrophobic	Pi-Pi Stacked
2	LEU140	4.98475	Hydrophobic	Alkyl
3	PHE77	4.24845	Hydrophobic	Pi-Pi Stacked
4	LEU140	4.85726	Hydrophobic	Alkyl
5	SER205	3.67945	Hydrogen Bond	Pi-Donor Hydrogen Bond
6	TYR160	4.48219	Hydrophobic	Pi-Pi Stacked
7	TYR210	4.67628	Hydrophobic	Pi-Pi Stacked
8	PHE77	5.06534	Hydrophobic	Pi-Pi Stacked

Table 5: Docking interaction of receptor (6X3X) with compound A1

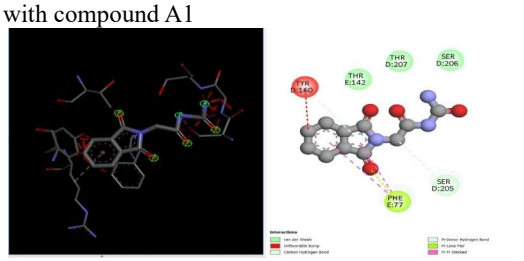


Figure 7: 2D & 3D Representation of compound A2 with PDBID: 6X3X

SN	Residue atom	Distance	Category	Type of Interaction
1	SER205	3.47526	Hydrogen Bond	Carbon Hydrogen Bond
2	TYR160	3.96224	Hydrogen Bond	Pi-Donor Hydrogen Bond
3	PHE77	2.85665	Other	Pi-Lone Pair
4	PHE78	4.85573	Hydrophobic	Pi-Pi Stacked
5	TYR160	4.49353	Hydrophobic	Pi-Pi Stacked
6	PHE77	4.4031	Hydrophobic	Pi-Pi Stacked

Table 6: Docking interaction of receptor (6X3X) with compound A2

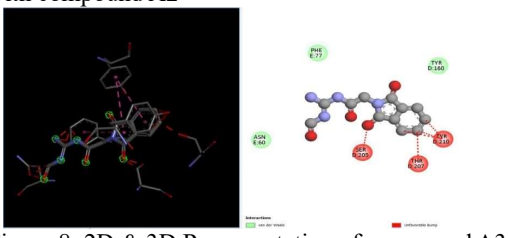


Figure 8: 2D & 3D Representation of compound A3 with PDBID: 6X

SN	Residues atom	Distance	Category	Type of Interaction

1	HIS102	2.87215	Hydrogen Bond	Conventional Hydrogen Bond
2	SER159	2.69566	Other	Pi-Lone Pair
3	TYR210	5.80665	Hydrophobic	Pi-Pi Stacked
4	TYR210	5.16243	Hydrophobic	Pi-Pi Stacked

Table 7: Docking interaction of receptor (6X3X) with compound A3

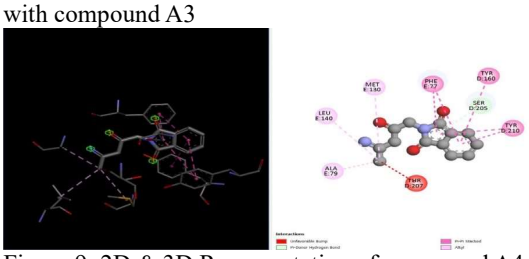


Figure 9: 2D & 3D Representation of compound A4 with PDBID: 6X

SN	Residue atom	Distance	Category	Type of Interaction
1	TYR160	2.22405	Hydrogen Bond	Conventional Hydrogen Bond
2	ARG144	4.47815	Hydrophobic	Pi-Alkyl

Table 8: Docking interaction of receptor (6X3X) with compound A4

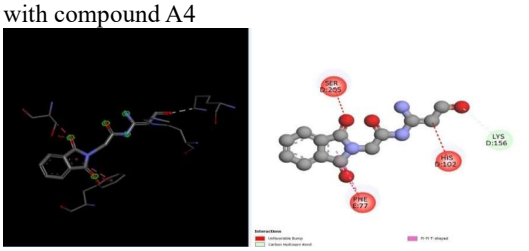


Figure 10: 2D & 3D Representation of compound A5 with PDBID: 6X

SN	Residue atom	Distance	Category	Type of Interaction
1	LYS156	2.47527	Hydrogen Bond	Carbon Hydrogen Bond
2	PHE77	3.99612	Hydrophobic	Pi-Pi T-shaped

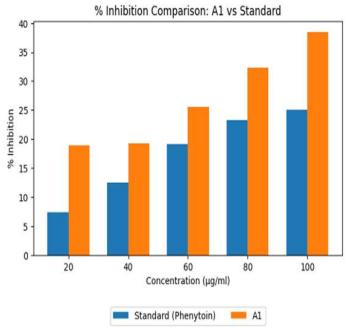
Table 9: Docking interaction of receptor (6X3X) with compound A5

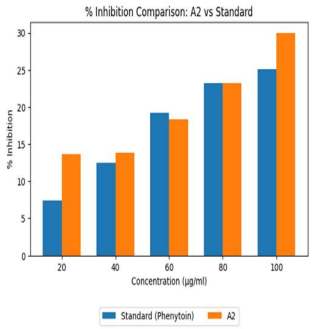
Derivatives	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
A1	1.309	1.303	1.202	1.093	0.994
A2	1.304	1.39	1.318	1.239	1.131
A3	1.387	1.357	1.286	1.177	1.091
A4	1.292	1.261	1.155	1.053	0.971
A5	1.366	1.308	1.195	1.118	1.045
Phenytoin	1.495	1.412	1.305	1.239	1.211

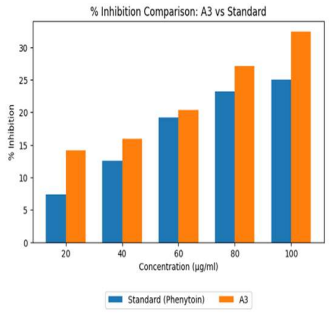
Table 8 In vitro $GABA_A$ inhibitory activity of synthesized phthalimide derivatives at different concentrations

SN	Compounds	Anticonvulsant Docking Score
1	A6	-9.6
2	A11	-10.8
3	A7	-10.3
4	A13	-9.2
5	A3	-9.9

Table 9 The compounds that can cross the BBB are chosen to synthesize various derivatives







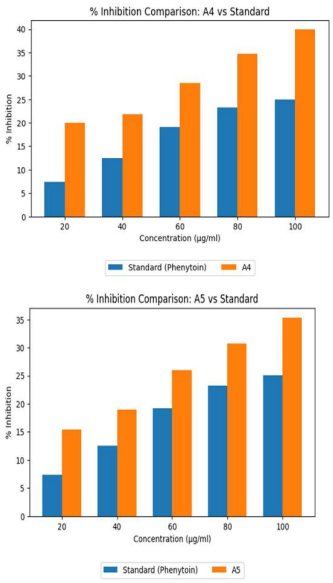
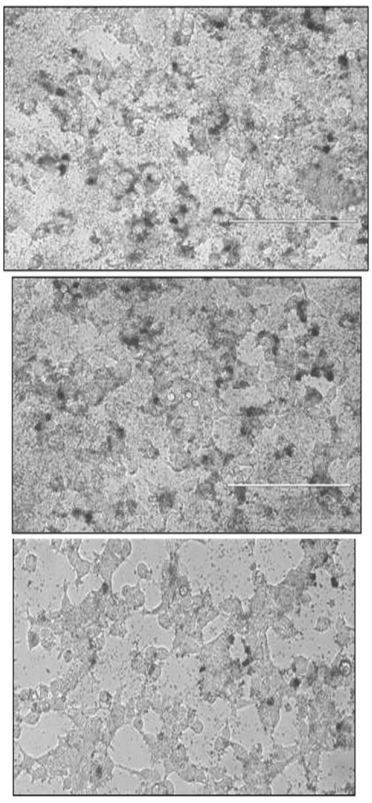


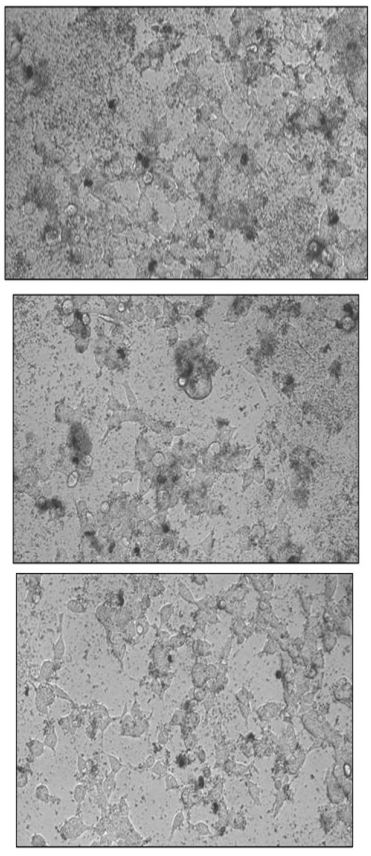
Figure 11: Comparative % inhibition of $GABA_A$ by Schiff base derivatives (A1–A5) and standard drug Acarbose at different concentrations

Sr. No.	Sample Code	Conc. (µg/ml)	OD I	OD II	OD III	OD Mean	% Inhibition	% Viability
1	Control	–	1.614	–	–	1.614	–	–
2	Standard (Phenytoin)	20	1.495	1.497	1.493	1.495	7.38	92.62
		40	1.412	1.414	1.410	1.412	12.51	87.49
		60	1.305	1.307	1.303	1.305	19.16	80.84
		80	1.239	1.241	1.237	1.239	23.22	76.78
		100	1.211	1.213	1.209	1.211	25.02	74.98
3	A1	20	1.309	1.311	1.307	1.309	18.91	81.09
		40	1.303	1.305	1.301	1.303	19.26	80.74
		60	1.202	1.204	1.200	1.202	25.52	74.48
		80	1.093	1.095	1.091	1.093	32.28	67.72
		100	0.994	0.996	0.992	0.994	38.42	61.58
4	A2	20	1.394	1.396	1.392	1.394	13.63	86.37
		40	1.390	1.392	1.388	1.390	13.85	86.15
		60	1.318	1.320	1.316	1.318	18.35	81.65
		80	1.239	1.241	1.237	1.239	23.21	76.79
		100	1.131	1.133	1.129	1.131	29.93	70.07
5	A3	20	1.387	1.389	1.385	1.387	14.09	85.91
		40	1.357	1.359	1.355	1.357	15.95	84.05
		60	1.286	1.288	1.284	1.286	20.32	79.68
		80	1.177	1.179	1.175	1.177	27.10	72.90
		100	1.091	1.093	1.089	1.091	32.38	67.62
6	A4	20	1.2	1.2	1.2	1.292	19.95	80.05

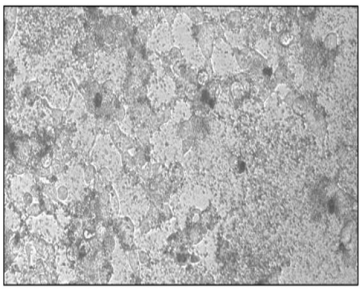
			92	94	90			
		40	1.2611	1.2633	1.2559	1.2611	21.86	78.14
		60	1.1555	1.1557	1.1553	1.1555	28.46	71.54
		80	1.0553	1.0555	1.0551	1.0553	34.75	65.25
		100	0.9771	0.9773	0.9769	0.9771	39.91	60.09
7	A5	20	1.3666	1.3668	1.3664	1.3666	15.34	84.66
		40	1.3088	1.3110	1.3006	1.3088	18.99	81.01
		60	1.1995	1.1997	1.1993	1.1995	25.96	74.04
		80	1.1118	1.1120	1.1116	1.1118	30.71	69.29
		100	1.0445	1.0447	1.0443	1.0445	35.28	64.72



Control
Standard
A1



A2
A3
A4



A5

Figure12: Microscopic images of COS-7 cells showing in vitro GABA A inhibitory activity of Schiff base derivatives (A1–A5) compared with standard and control

DISCUSSION

The present study integrates computational docking, ADME profiling, and in vitro assays to evaluate the therapeutic potential of phthalimide Schiff base derivatives. The docking analysis against the $GABA_A$ receptor (PDB ID: 6X3X) revealed that several derivatives, particularly AK11, AK7, AK3, AK6, and AK13, exhibited strong binding affinities comparable to or approaching the standard drug phenytoin. These compounds demonstrated stable hydrogen bonding and hydrophobic interactions with key residues such as TYR160, PHE77, SER205, and HIS102, which are crucial for receptor modulation. The visualization of ligand–receptor complexes confirmed optimal accommodation within the binding pocket, supporting their role as potential anticonvulsant agents. [19]

The ADME and BOILED-Egg analyses further reinforced the drug-likeness of the selected derivatives. Compliance with Lipinski's Rule of Five, favorable solubility, and predicted blood–brain barrier permeability suggest that these molecules possess suitable pharmacokinetic properties for oral administration. Importantly, the absence of CYP inhibition and toxicity liabilities strengthens their safety profile, making them viable candidates for further development.

In vitro α-amylase inhibitory assays provided an additional dimension to the pharmacological evaluation. The Schiff base derivatives (A1–A5) exhibited significant dose-dependent inhibition, in some cases surpassing the standard drug phenytoin. This dual activity — anticonvulsant potential through $GABA_A$ receptor binding and α-amylase inhibition — highlights the poly pharmacological relevance of these compounds. Such multifunctionality is particularly valuable in drug discovery, as it opens avenues for therapeutic applications in both neurological disorders and metabolic regulation.

Statistical validation using ANOVA and pairwise t-tests added quantitative rigor to the docking outcomes, confirming the significance of observed differences and ensuring reliability of the computational predictions. The integration of computational and experimental approaches thus provides a robust framework for ientifying promising lead candidates. [22][23]

CONCLUSION

The comprehensive in silico and in vitro evaluation of phthalimide Schiff base derivatives highlights their strong potential as multifunctional drug candidates. Docking studies against the $GABA_A$ receptor revealed that several derivatives (notably AK11, AK7, AK3, AK6, and AK13) exhibited excellent binding affinities, supported by stable hydrogen bonding and hydrophobic interactions with key residues such as TYR160, PHE77, and SER205. These findings confirm that the designed ligands are well accommodated within the receptor's binding pocket, suggesting promising anticonvulsant activity.

ADME and BOILED-Egg analyses further validated their drug-likeness, showing compliance with Lipinski's Rule of Five, favourable solubility, and predicted blood–brain barrier permeability. Importantly, none of the shortlisted derivatives showed CYP inhibition or toxicity liabilities, strengthening their pharmacokinetic and safety profiles.

In vitro GABA inhibitory assays demonstrated that compounds A1–A5 exhibited significant dose-dependent inhibition, in some cases surpassing the standard drug phenytoin. This dual activity anticonvulsant potential through $GABA_A$ receptor binding and α-amylase inhibition suggests that these Schiff base derivatives may possess poly pharmacological relevance, opening avenues for therapeutic applications in both neurological disorders and metabolic regulation.

Statistical validation of docking scores through ANOVA and t-tests added quantitative rigor, confirming the significance of observed differences and reinforcing the reliability of the computational predictions. Visualization studies using BIOVIA Discovery Studio provided clear evidence of favourable ligand–receptor interactions, further supporting the docking outcomes.

FUNDING

The authors declare that no external funding was received for this study.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

DECLARATION OF GENERATIVE AI USE

No generative artificial intelligence (AI) tools were used in the preparation of this manuscript. All content, including text, data analysis, and interpretation, was produced entirely by the authors without the assistance of AI technologies.

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