Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 2s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Anticonvulsant Evaluation of 2-Amino Pyrimidine-Based Mannich Base Derivatives Targeting the GABA-A Receptor: In Silico, In Vitro and In Vivo Studies

Sonali Sanjay Nikam[1*], Bhavna Utkarsh Jain[2], Smita Sunil Sawalwade[3], Snehal Prakash Patil[4]

[1*]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-Miraj Road, Sangli (M.S.), India, 416 416.

Email ID: sonalisnikam14@gmail.com

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-Miraj Road, Sangli (M.S.), India, 416 416.

Email ID: drugdesign16@gmail.com

[3]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-Miraj Road, Sangli (M.S.), India, 416 416.

Email ID: smitasawalwade13@gmail.com

[4]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-Miraj Road, Sangli (M.S.), India, 416 416.

Email ID: snehalppatil15@gmail.com

Corresponding Author:

Sonali Sanjay Nikam,

[1*]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-Miraj Road, Sangli (M.S.), India, 416 416.

Email ID: sonalisnikam14@gmail.com

Cite this paper as: Sonali Sanjay Nikam, Bhavna Utkarsh Jain, Smita Sunil Sawalwade, Snehal Prakash Patil, (2025) Anticonvulsant Evaluation of 2-Amino Pyrimidine-Based Mannich Base Derivatives Targeting the GABA-A Receptor: In Silico, In Vitro and In Vivo Studies, *Journal of Neonatal Surgery*, 14 (2s), 158-169

ABSTRACT

Epilepsy affects over 50 million individuals worldwide and is characterized by recurrent seizures that are sometimes unresponsive to current therapies and induce negative effects, underscoring the urgent need for new anticonvulsant medications. This study examines the anticonvulsant effects of 2-Amino Pyrimidine-based Mannich base derivatives that target the GABA-A receptor, a critical inhibitory neurotransmitter site for seizure regulation. Fifteen new derivatives were produced and subjected to molecular docking using Auto Dock Tools 1.5.7 against the GABA-A receptor (PDB ID: 6X3D), indicating strong binding affinities for compounds SSN6, SSN15, SSN10 and SSN12, with docking scores of 14.0, 12.8, 9.2 and 9.0 kcal/mol, respectively. These compounds showed important contacts with the receptor active site residues. In vitro MTT testing on SHSY5Y neuroblastoma cells revealed more than 75% cell viability at 40 µg, suggesting excellent neuroprotection and low cytotoxicity. In vivo confirmation using the maximal electroshock seizure (MES) model in mice revealed that both SSN6 and SSN15 substantially reduced seizure duration ($p < 0.05$), similar to the standard drug Phenobarbitone, with no toxicity or mortality reported. The combined in silico, in vitro and in vivo findings indicate that 2-Amino Pyrimidine Mannich base derivatives, notably SSN6 and SSN15, are promising candidates for developing effective and safer anticonvulsant drugs

Keywords: *2-Amino Pyrimidine, Mannich bases, Anticonvulsant activity, GABA-A receptor, PDB ID: 6X3D, Maximal electroshock seizure (MES) model*

1. INTRODUCTION

Epilepsy is a chronic neurological disease that affects approximately 50 million people worldwide and is characterized by recurrent convulsions. Many people experience treatment resistance, adverse effects, or low effectiveness despite the easy

availability of several antiepileptic drugs (AEDs). This has sparked a quest for novel chemicals with higher therapeutic indices capable of targeting central systems.[1]

Epilepsy is caused by a disruption in the balance between excitatory and inhibitory neurotransmission. One well-known inhibitory target involved in seizure regulation is the gamma-aminobutyric acid type A (GABA-A) receptor. By modifying these receptors, aberrant electrical discharges may be reduced and neurological balance restored.[2]

Since 2-Amino Pyrimidine derivatives are structurally similar to the pharmacophores of well-known antiepileptic medicines (AEDs), they have been demonstrated to have anticonvulsant efficacy among heterocyclic compounds. The Mannich base scaffold enhances the pharmacokinetic and pharmacodynamic properties by adding flexibility.[3]

This work uses a computer-aided medication design strategy to look at the anticonvulsant capabilities of 2-Amino Pyrimidine Mannich base derivatives. Fifteen derivatives were tested for their binding affinity to GABA-A receptor targets using molecular docking techniques (PDB ID: 6X3D). In vitro neuronal experiments and in vivo MES studies were used to validate four chemicals that had high binding interactions and BBB permeability [4]

The findings provide a foundation for future pharmaceutical research and the combination technique provides a predictive model for discovering effective anticonvulsant medications while requiring minimal resources. (See Figure No. 2 for molecular interactions and Table No. 1 for compound structures and docking scores.).

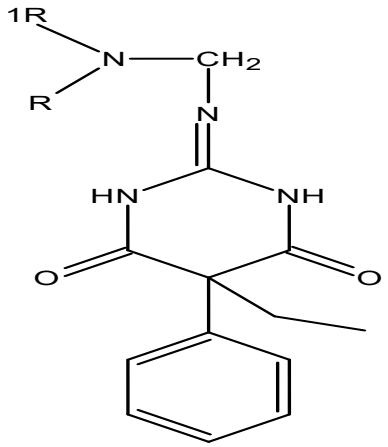


Fig. 1: General structure of selected series of compounds

2. MATERIALS AND METHODS:

1. Computational Docking:

I. **Ligand Preparation:** The 2-Amino Pyrimidine-based Mannich base derivatives were initially sketched using ChemDraw[5] Pro 8.0 and Chem Sketch. These 2D structures were converted to 3D conformations using PyMOL,[6] followed by energy minimization to obtain stable geometries suitable for docking. The ligands were then saved in PDB format and later converted to PDBQT format using AutoDock[7] Tools 1.5.7, with Gasteiger charges assigned and rotatable bonds defined to enable flexible docking.

II. **Receptor Preparation:** The crystal structure of the GABA-A receptor (PDB ID: 6X3D) was retrieved from the Protein Data Bank.[8] Using Auto Dock Tools 1.5.7, water molecules were removed, polar hydrogens were added, and Compute Gasteiger and Kollman charges were applied to the receptor.[9] A grid box was constructed to enclose the active site residues, ensuring adequate space for ligand binding. The receptor was saved in PDBQT format for use in the docking simulation.

III. **Validation of Docking Protocol:** To validate the docking protocol, the co-crystallized ligand from the GABA-A receptor structure was re-docked into its binding site using the same grid and docking parameters. The root-mean-square deviation (RMSD) between the docked pose and the original ligand position was calculated.[10] An RMSD value is 0.821 Å (less than 2.0 Å) confirmed the reliability and accuracy of the docking setup. Additionally, Biovia Discovery Studio 2024 Visualizer was employed to analyse and confirm key molecular interactions, such as hydrogen bonding and hydrophobic contacts, ensuring biological relevance of the docking results.[11].

2. In Vitro Assay:

In Vitro Assay: Neuroblastoma cells of SHSY5Y (CRL-2266)[12] were cultivated in DMEM with high glucose (Cat No-11965-092), supplemented with 1% antibiotic-antimycotic solution (Cat No-15240062) and 10% FBS (Cat No-10270106). After being seeded at a density of 1×10^4 cells/well in 96-well plates, the cells were incubated at 37°C in a humidified environment with 5% CO_2. To reach final concentrations of 20, 40, 60, 80 and 100 µM, a selection of chemicals was diluted in medium after being dissolved in DMSO. Following a 24-hour treatment period,

absorbance was recorded with a microplate reader at 570 nm and cell viability was evaluated using the MTT assay. [13-17]

3. **In Vivo MES Model:** Animal studies were conducted with prior approval of the Institutional Animal Ethics Committee (CPCSEA Reg. Number 843/PO/ReBi/S/04/CCSEA). In addition to having unlimited access to food and drink, healthy adult Swiss albino mice weighing 25–30 g were housed in conditions regulated to include a 12-hour light/dark cycle, with the temperature of 22 ± 2°C and a relative humidity of 50–60%.[18] The two medications with the best in vitro results were selected and administered intraperitoneally. After 30 minutes, maximal electroshock seizures (MES) [19, 20] were induced using an electro-convulsometer with ocular electrodes set to 50 mA current for 0.2 seconds. The degree to which the seizures were severe was determined by the presence or absence of hind limb tonic extension (HLTE).[21] Additional findings included behavioural response, recovery time, delay to HLTE, and mortality monitored for 24 hours following therapy. Phenobarbitone (20 mg/kg, intraperitoneally) was the standard reference drug used. Every procedure closely followed the ethical standards set forth by CPCSEA.[22-24]

3. RESULTS AND DISCUSSION

Table 1: Docking Scores of 2-Amino Pyrimidine Mannich Base Derivatives with GABA-A Receptor.

Compound Name	NRR1	Binding Score (Kcal/Mol)
SSN1	Ethyl amine	-9.4
SSN2	Dimethyl amine	-12.1
SSN3	Diethyl amine	-12.1
SSN4	n-butyl amine	-12.3
SSN5	Cyclohexyl amine	-9.1
SSN6	o-Phenylenediamine	-14
SSN7	2- Aminophenol	-8.3
SSN8	3-Aminophenol	-7.7
SSN9	4- Aminophenol	-8.1
SSN10	p-Amino diphenylamine	-9.2
SSN11	p-Aminopyridine	-8.2
SSN12	2,4-dinitrophenyl hydrazine	-9.0
SSN13	2-Chloro 4-nitro aniline	-8.1
SSN14	Ethyl aniline	-8.0
SSN15	Dibenzyl amine	-12.8

The compounds that can cross the BBB are chosen to synthesise various derivatives

SR. NO.	COMPOUNDS	ANTICONVULSANT DOCKING SCORE
1	SSN6	-14.0
2	SSN15	-12.8
3	SSN10	-9.2
4	SSN12	-9.0

Docking scores ranging from -7.3 to -14.0 kcal/mol were obtained from the molecular docking investigation of fifteen. 2-Amino pyrimidine-based Mannich base derivatives with the GABA-A receptor (PDB ID: 6X3D). The compounds with the highest binding affinities, SSN6, SSN15, SSN10 and SSN12, were –14.0, –12.8, -9.2 and -9.0 kcal/mol, respectively. These substances interacted hydrophobically and established strong hydrogen bonds with important residues in the receptor's active site. Discovery Studio visual analysis verified that the ligand was well accommodated within the binding pocket.

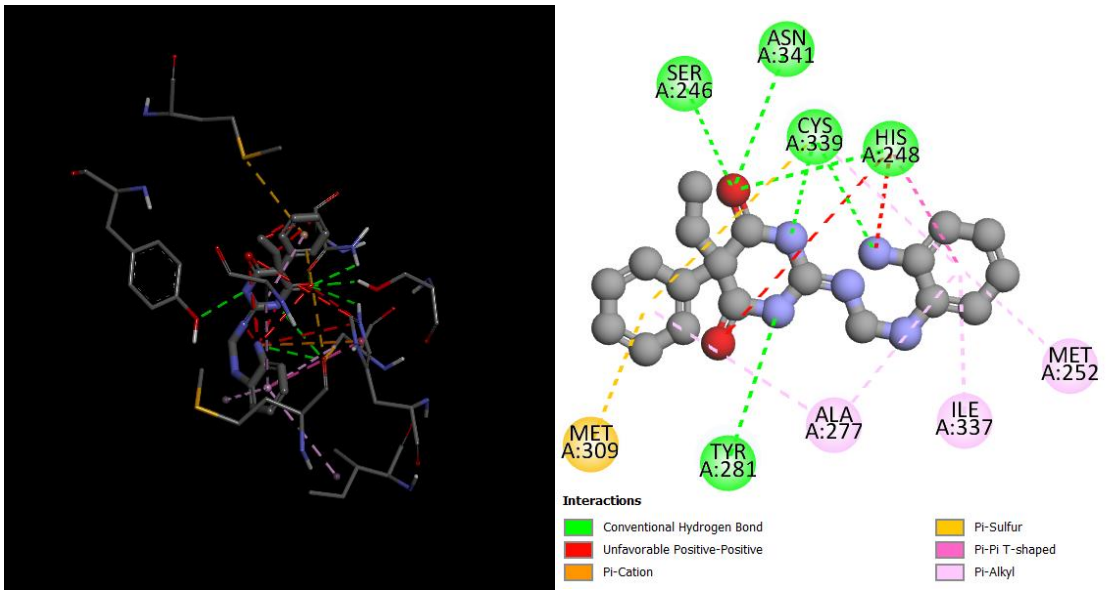


Figure 2: 2D & 3D Representation of compound SSN6 with PDBID: 6X3D

Table 2: Docking interaction of receptor (6X3D) with compound SSN6

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	SER246	2.18256	Hydrogen Bond	Conventional Hydrogen Bond
2	HIS248	2.62	Hydrogen Bond	Conventional Hydrogen Bond
3	ASN341	2.82139	Hydrogen Bond	Conventional Hydrogen Bond
4	CYS339	3.70989	Hydrogen Bond	Conventional Hydrogen Bond
5	CYS339	3.37286	Hydrogen Bond	Conventional Hydrogen Bond
6	ASN341	2.89664	Hydrogen Bond	Conventional Hydrogen Bond
7	TYR281	3.11567	Hydrogen Bond	Conventional Hydrogen Bond
8	HIS248	4.74097	Electrostatic	Pi-Cation
9	MET309	4.24432	Other	Pi-Sulfur
10	CYS339	5.97213	Other	Pi-Sulfur
11	HIS248	4.43629	Hydrophobic	Pi-Pi T-shaped
12	MET252	4.23626	Hydrophobic	Pi-Alkyl
13	ALA277	4.67352	Hydrophobic	Pi-Alkyl
14	ILE337	4.96209	Hydrophobic	Pi-Alkyl
15	CYS339	5.18192	Hydrophobic	Pi-Alkyl

16	ALA277	4.92372	Hydrophobic	Pi-Alkyl

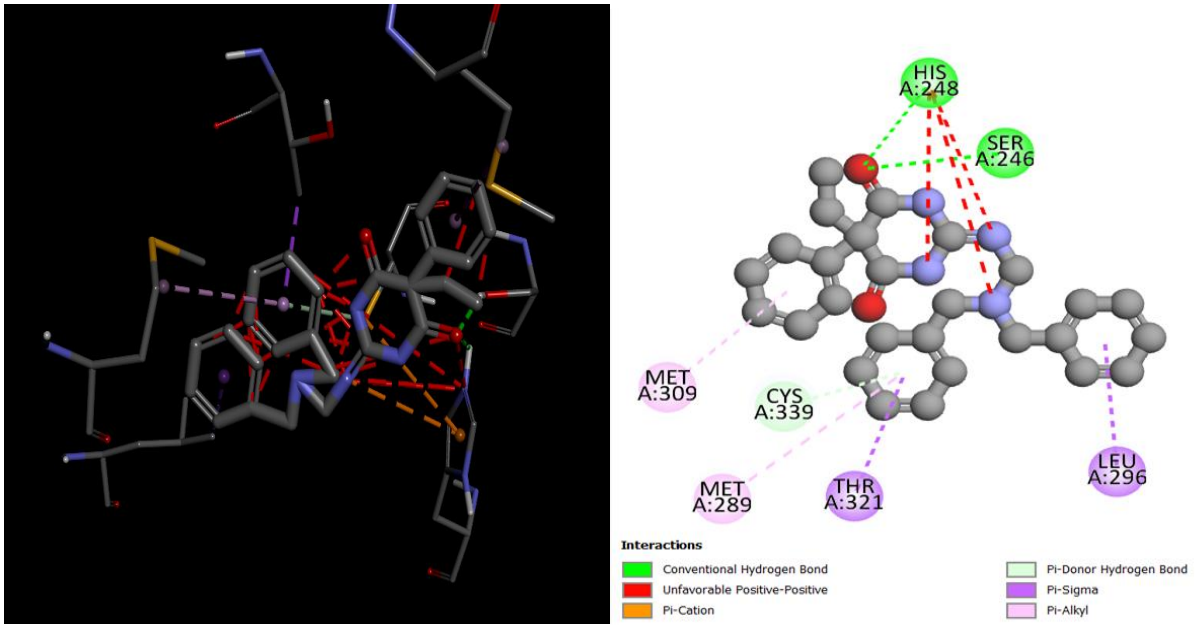


Figure 3: 2D & 3D Representation of compound SSN15 with PDBID: 6X3D

Table 3: Docking interaction of receptor (6X3D) with compound SSN15

Sr. No.	Residue Atom	Category	Distance	Type of Interaction
1	SER246	Hydrogen Bond	2.81808	Conventional Hydrogen Bond
2	HIS248	Hydrogen Bond	2.63903	Conventional Hydrogen Bond
3	HIS248	Electrostatic	4.87301	Pi-Cation
4	HIS248	Hydrogen Bond; Electrostatic	4.07381	Pi-Cation; Pi-Donor Hydrogen Bond
5	CYS339	Hydrogen Bond	4.18297	Pi-Donor Hydrogen Bond
6	LEU296	Hydrophobic	3.83572	Pi-Sigma
7	THR321	Hydrophobic	3.65221	Pi-Sigma
8	MET309	Hydrophobic	4.81271	Pi-Alkyl
9	MET289	Hydrophobic	5.39659	Pi-Alkyl

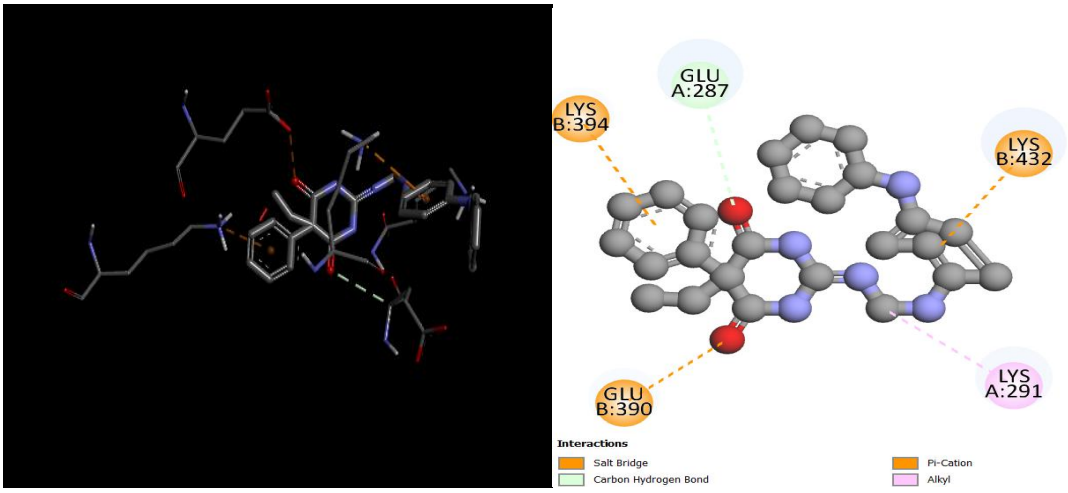


Figure 4: 2D & 3D Representation of compound SSN10 with PDBID: 6X3D

Table 4: Docking interaction of receptor (6X3D) with compound SSN10

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	GLU390	3.08314	Hydrogen Bond; Electrostatic	Salt Bridge
2	GLU287	3.42397	Hydrogen Bond	Carbon-Hydrogen Bond
3	LYS394	3.89121	Electrostatic	Pi-Cation
4	LYS432	4.08583	Electrostatic	Pi-Cation
5	LYS291	4.48321	Hydrophobic	Alkyl

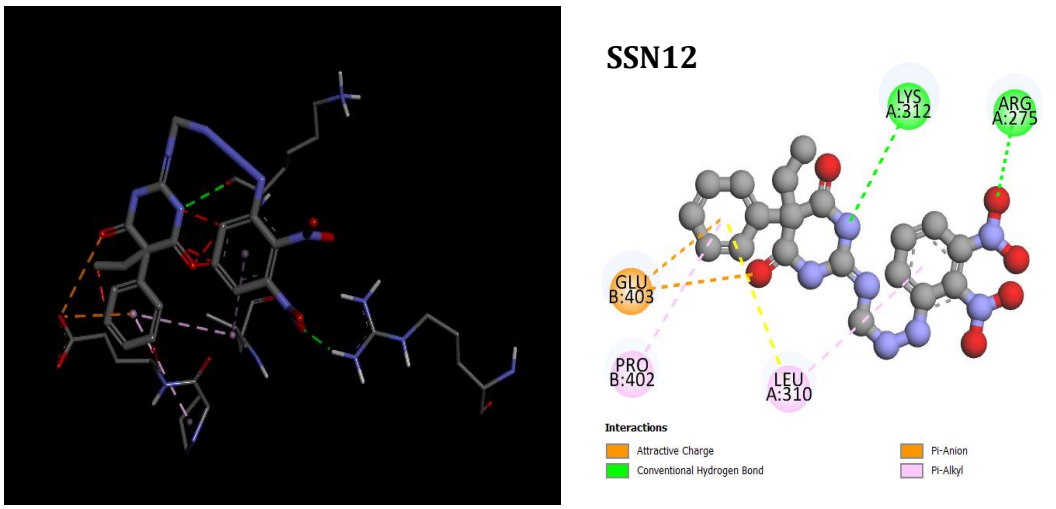


Figure 5: 2D & 3D Representation of compound SSN12 with PDBID: 6X3D

Table 5: Docking interaction of receptor (6X3D) with compound SSN12

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	GLU403	4.731	Electrostatic	Attractive Charge
2	ARG275	2.03334	Hydrogen Bond	Conventional Hydrogen Bond
3	LYS312	3.23192	Hydrogen Bond	Conventional Hydrogen Bond
4	GLU403	4.15885	Electrostatic	Pi-Anion
5	LEU310	5.4426	Hydrophobic	Pi-Alkyl
6	LEU310	5.46867	Hydrophobic	Pi-Alkyl
7	PRO402	5.12288	Hydrophobic	Pi-Alkyl

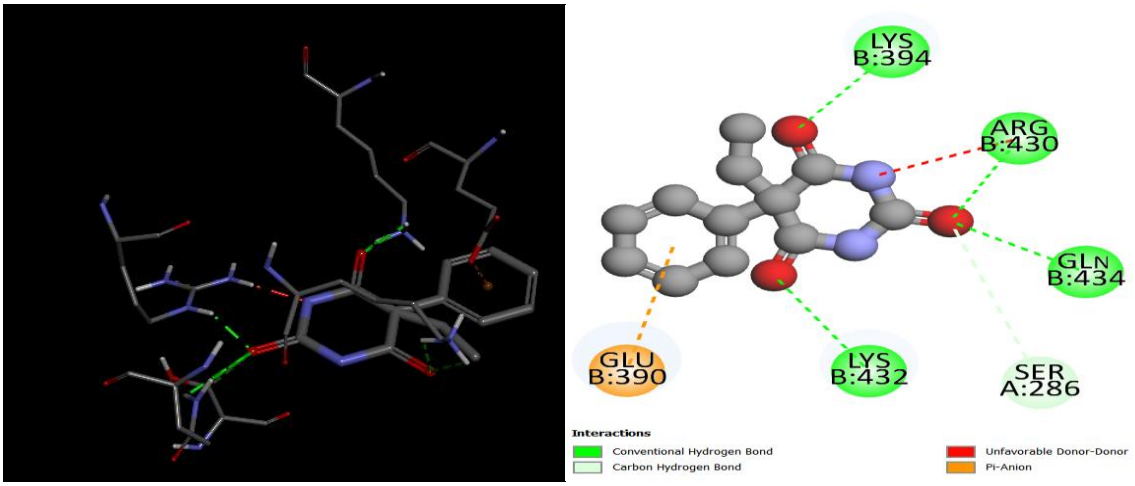


Figure 6: 2D & 3D Representation of standard compound with PDBID: 6X3D

Table 6: Docking interaction of receptor (6X3D) with standard compound

Sr. No.	Residue Atom	Distance	Category	Type of Interaction
1	LYS394	2.2827	Hydrogen Bond	Conventional Hydrogen Bond
2	LYS394	2.34077	Hydrogen Bond	Conventional Hydrogen Bond
3	ARG430	2.0124	Hydrogen Bond	Conventional Hydrogen Bond
4	LYS432	2.95168	Hydrogen Bond	Conventional Hydrogen Bond
5	LYS432	2.65618	Hydrogen Bond	Conventional Hydrogen Bond
6	GLN434	2.79952	Hydrogen Bond	Conventional Hydrogen Bond
7	GLN434	2.88266	Hydrogen Bond	Conventional Hydrogen Bond
8	SER286	3.77754	Hydrogen Bond	Carbon-Hydrogen Bond
9	GLU390	3.65394	Electrostatic	Pi-Anion

Table 7: Results of the validation process

Parameters	**Ligand (SSN6)**	**Standard**
PDB ID	6X3D	6X3D
Co-crystal ligand	o-Phenylenediamine	Phenobarbitone
Grid box position	X: 19.838110, Y: 10.321411, Z: -14.33406	X:19.838110, Y:10.321411, Z: -14.33406
RMSD (Å)	0.821	0.821
ΔG (kcal/mol)	-14.0	-7.3
Amino Acid Residues 1) Hydrogen Bond	SER246	LYS394
	HIS248	LYS394
	ASN341	ARG430
	CYS339	LYS432
	CYS339	LYS432
	ASN341	GLN434
	TYR281	GLN434
	HIS248	SER286
	HIS248	-
2) Electrostatic	MET252	GLU390
3) Hydrophobic	ALA277	-
	ILE337	-
	CYS339	-
	ALA277	-
	SER246	-

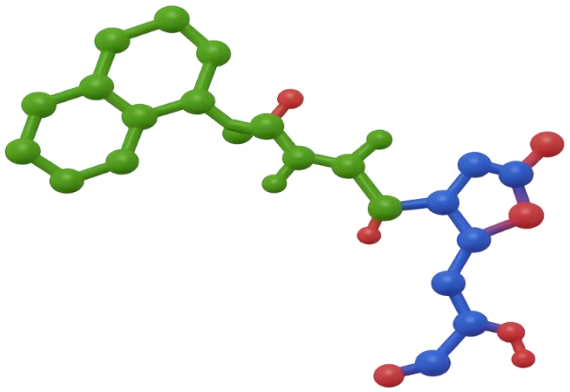
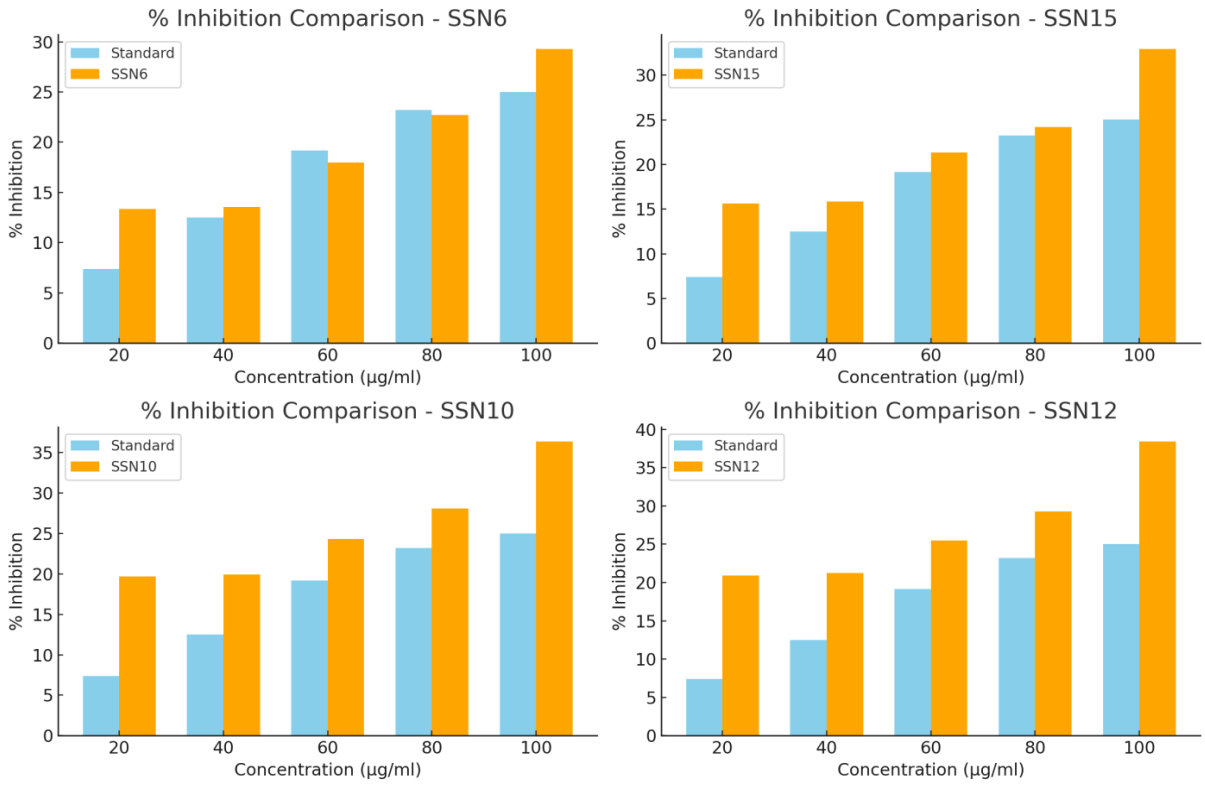


Figure 7. Overlays of redocking ligands (green) with co-crystal ligands from X-crystallography data (blue) at receptor 6X3D with RMSD 0.821

Table 8: In-vitro Anticonvulsant activity study (% Inhibition & % Viability)

Sr. No.	Sample Code	Conc. (µg/ml)	OD			Mean	% Inhibition	% Viability
1	Control	-	1.614			-	-	-
2	Standard (Phenobarbitone)	20	1.495	1.494	1.495	1.495	7.38	92.62
		40	1.412	1.412	1.412	1.412	12.51	87.49
		60	1.305	1.305	1.305	1.305	19.16	80.84
		80	1.239	1.239	1.239	1.239	23.22	76.78
		100	1.211	1.211	1.211	1.211	25.02	74.98
3	SSN6	20	1.399	1.399	1.399	1.399	13.34	86.66
		40	1.395	1.395	1.395	1.395	13.56	86.44
		60	1.324	1.324	1.324	1.324	17.96	82.04
		80	1.247	1.247	1.247	1.247	22.71	77.29
		100	1.141	1.141	1.141	1.141	29.28	70.72
4	SSN15	20	1.362	1.362	1.362	1.362	15.63	84.37
		40	1.358	1.358	1.358	1.358	15.85	84.15
		60	1.269	1.269	1.269	1.269	21.35	78.65
		80	1.223	1.223	1.223	1.223	24.21	75.79
		100	1.083	1.083	1.083	1.083	32.93	67.07
5	SSN10	20	1.297	1.297	1.297	1.297	19.66	80.34
		40	1.292	1.292	1.292	1.292	19.95	80.05
		60	1.221	1.221	1.221	1.221	24.32	75.68
		80	1.16	1.16	1.16	1.16	28.1	71.9

		100	1.027	1.027	1.027	1.027	36.38	63.62
6	SSN12	20	1.277	1.277	1.277	1.277	20.91	79.09
		40	1.271	1.271	1.271	1.271	21.26	78.74
		60	1.202	1.202	1.202	1.202	25.52	74.48
		80	1.141	1.141	1.141	1.141	29.28	70.72
		100	0.994	0.994	0.994	0.994	38.42	61.58



Four compounds were chosen for biological assessment based on projected blood-brain barrier (BBB) permeability and docking scores. Compounds SSN6 and SSN15 showed over 75% survivability at 50 µM concentration in in vitro cytotoxicity tests on SHSY5Y cells, suggesting minimal toxicity and possible neuroprotective qualities. There were no visible morphological alterations or cellular degradation. The compounds SSN6 and SSN15 significantly decreased hind limb tonic extension (HLTE) in comparison to the control in in vivo investigations conducted in Swiss albino mice utilizing the maximal electroshock seizure (MES) model. The outcome was similar to that of the common medication Phenobarbitone. A favourable safety profile is suggested by the absence of behavioural toxicity or death over the trial duration.

These results are consistent with past findings that GABA-A receptor manipulation is a useful tactic for anticonvulsant action. The reported outcomes highlight how predictive computational docking is in directing the selection of lead compounds.

4. CONCLUSION

The current study demonstrates the usefulness of computer-assisted drug design in discovering potential anticonvulsant candidates. According to molecular docking data, the produced 2-Amino Pyrimidine-based Mannich base derivatives had strong binding affinities for the GABA-A receptor, a critical target in seizure modulation. Among the fifteen chemicals tested, SSN6 and SSN15 had the most favourable docking scores and crucial connections with active site residues. These findings were bolstered by in vitro cytotoxicity studies, which showed that both chemicals had neuroprotective potential with more than 75% cell viability at 40 µg in SHSY5Y cells, and in vivo MES model data, which revealed that both compounds considerably reduced seizure duration without causing observable toxicity. These findings, taken together, demonstrate the

promise of SSN6 and SSN15 as lead candidates for the creation of safer and more effective anticonvulsant treatments. Future research will focus on structure-activity relationship (SAR) optimization and thorough mechanistic investigations to confirm and improve their therapeutic potential.

Author's Contribution

Sonali Sanjay Nikam conceptualised the study, designed the research protocol, and performed the molecular docking analysis. She also carried out the in vitro and in vivo experiments. All authors contributed to data analysis, manuscript drafting, and approved the final version, taking full responsibility for its content and integrity. Bhavna U. Jain supervised the research, provided continuous guidance throughout the study, and critically reviewed the manuscript for intellectual and scientific accuracy.

Future Perspective

The findings support continued optimisation of SSN6 and SSN15 as anticonvulsant candidates. Future studies should explore pharmacokinetics, chronic toxicity, and advanced formulation strategies to facilitate clinical translation.

Acknowledgement

The author gratefully acknowledges the Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, for providing necessary laboratory facilities and technical support throughout the research work. Also, special thanks to Bhavna U. Jain for their valuable guidance and encouragement.

Conflict of Interest

The author declares no conflict of interest regarding the publication of this manuscript

REFERENCES

[1] Thurman DJ, Beghi E, Begley CE, Berg AT, Buchhalter JR, Ding D, et al. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia. 2011;52(Suppl 7):2–26. doi:10.1111/j.1528-1167.2011.03121.x

[2] Olsen RW, Sieghart W. GABAA receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology. 2009;56(1):141–148. doi:10.1016/j.neuropharm.2008.07.045.

[3] Kharb R, Sharma PC, Yar MS. Pharmacological significance of synthetic heterocyclic Mannich bases: a review. Eur J Med Chem. 2011;46(7):2327–2346. doi:10.1016/j.ejmech.2011.03.032.

[4] Rangaswamy CR, Dastagiri Reddy N, Basavanag U, Goudanavar PS, Yaragatti C, Ramesh C. In silico docking and in vivo evaluation of novel 1,2,4-triazole derivatives as potential anticonvulsant agents. Eur J Pharm Sci. 2019;132: 96-106. doi:10.1016/j.ejps.2019.02.021.

[5] Iwata H, Mizutani H, Yamamoto M. Structure drawing programs for chemical structure database: ChemDraw and ChemSketch. J Chem Inf Comput Sci. 2003;43(4):1269–1275.

[6] DeLano WL. The PyMOL Molecular Graphics System, Version 2.5. Schrödinger, LLC; 2020. Available from: https://pymol.org

[7] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30(16): 2785–2791. https://doi.org/10.1002/jcc.21256.

[8] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000; 28(1):235–242. https://doi.org/10.1093/nar/28.1.235

[9] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998; 19(14):1639–1662.

[10] Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: Methods and applications. Nat Rev Drug Discov. 2004;3(11):935–949. https://doi.org/10.1038/nrd1549.

[11] BIOVIA Discovery Studio Visualizer, version 2024. San Diego: Dassault Systèmes; 2024.

[12] Xicoy H, Wieringa B, Martens GJM. The SH-SY5Y cell line in Parkinson's disease research: A systematic review. Mol Neurodegener. 2017;12(1):10. https://doi.org/10.1186/s13024-017-0149-0

[13] Riss TL, Moravec RA, Niles AL, et al. Cell Viability Assays. In: Markossian S, Grossman A, Brimacombe K, et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004.

[14] Kandasamy M, Lehner B, Kraus S, et al. Neuroprotective effect of resveratrol against Aβ-induced oxidative

stress and apoptosis in SH-SY5Y cells. Neurochem Int. 2010;57(6):651–658. https://doi.org/10.1016/j.neuint.2010.07.003

[15] Freshney RI. Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications. 7th ed. Hoboken, NJ: Wiley-Blackwell; 2016.

[16] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods.1983;65(1-2):55–63. https://doi.org/10.1016/0022-1759(83)90303-4

[17] Stepanenko AA, Dmitrenko VV. HEK293 in cell biology and cancer research: Phenotype, karyotype, tumorigenicity, and stress-induced genome-phenotype evolution. Gene. 2015;569(2):182–190. https://doi.org/10.1016/j.gene.2015.05.065

[18] Vohora D, Pillai KK, Khanam R. Evaluation of CNS activity of newer antiepileptic drugs using different animal models. Indian J Pharmacol. 2001;33(4):268–276.

[19] Löscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 1988;2(3):145–181. https://doi.org/10.1016/0920-1211(88)90054-X

[20] Löscher W. Animal models of seizures and epilepsy: Past, present, and future role for the discovery of antiseizure drugs. Neurochem Res. 2020;45(1):187–203. https://doi.org/10.1007/s11064-019-02820-2

[21] Dixit S, Goyal A, Jadhav HR. Evaluation of anticonvulsant activity of novel 1,3,4-oxadiazole derivatives using MES and PTZ models in mice. Eur J Pharm Sci. 2016;89:108–116. https://doi.org/10.1016/j.ejps.2016.03.004

[22] CPCSEA Guidelines for Laboratory Animal Facility. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests, Government of India. 2003.

[23] Bhutada P, Mundhada Y, Bansod K, Ubgade A, Patil S, Mundhada D. Anticonvulsant and anxiolytic activity of hydroalcoholic extract of Delphinium denudatum root. Indian J Pharmacol. 2010;42(3):171–175. https://doi.org/10.4103/0253-7613.68424

[24] LoPachin RM, Lehning EJ. Mechanism of action of the anticonvulsant valproic acid. DrugMetab Rev. 1997;29(1-2):243–267. https://doi.org/10.3109/03602539709037578