Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [7] June 2023: 180-185
©2023 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Identification of Novel GABA-A Inhibitor For The Treatment Of Epilepsy: A Computational Approach

Nayana Ravindra Jawale[1], Bandral Sunil Kumar[1], Genne Soujanya[1], Golla Sireesha[1], Agasa Ramu Mahesh[1], Selvaraj Kunjiappan[2], Theivendren Panneerselvam[3], R. Sathish Adithya[4], Parasuraman Pavadai[1]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
M.S. Ramaiah University of Applied Sciences, Bangalore-560054, Karnataka, India
[2]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil-626126, Tamilnadu, India
[3]Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode- 637205, Tamilnadu, India
[4]Department of NanjuMaruthuvam, National Institute of Siddha, Chennai – 600 047, Tamil Nadu, India
E-Mail: pvpram@gmail.com

ABSTRACT

GABA is the most important neurotransmitter in the brain cortex. Seizures may occur whether the equilibrium is upset. The GABA-A receptor is a well-established target for epilepsy therapy. Despite the introduction of multiple new anticonvulsants, certain kinds of seizures are still not carefully regulated with this novel and existing medications, and different adverse effects have been described. To manage the numerous distinct forms of convulsions, newer anticonvulsant medications must be developed.A hypothesis of shared pharmacophore features was formulated to support our study. Structurally different classes of ligands which are active in modulation of Convulsion are used to create pharmacophore model using Pharmagist webserver. The website ZINC Pharmer discovered 1000 top potential ligands with strong pharmacophoric similarity. The molecules have been subsequently screened using the Data warrior software before being utilized towards molecular docking with Autodock vina. The top 5 ligands were submitted to in silico ADMET analyses in PKCSM Webserver based on binding energy and amino acid interactions. ZINC77193731 and ZINC07046228 have good binding affinity and good interactions with amino acids of GABA-A.

Keywords: *Epilepsy, GABA-A, Pharmacophore Modeling, Molecular docking.*

Received 08.05.2023 Revised 15.05.2023 Accepted 27.06.2023

INTRODUCTION

According to statistics, epilepsy is the most common serious neurological disorder. A frequent epileptic symptom is spontaneous seizures caused with transient excess neuron discharge [1]. Despite the development of new anticonvulsants, some types of seizures are still not adequately treated by these new and existing drugs [1-3]. New anticonvulsant drugs are badly required to treat the many types of seizures. Several studies have shown that epilepsy include more than 1 mechanism & may therefore be accountable for the numerous types of seizures recorded.Several novel medications for the treatment of epilepsy have been approved in the recent two decades, namely felbamate, gabapentin, lamotrigine, topiramate, tiagabine, levetiracetam, oxcarbazepine, zonisamide, pregabalin, rufinamide, stiripentol, clobazam, vigabatrin, and lacosamide. In addition, novel formulations of existing medications, such as fosphenytoin, a pro-drug of phenytoin, and a carbamazepine sustained-release preparation, have been released[4-7].Furthermore, vagus nerve stimulation in conjunction with seizure medication has been approved for the treatment of people with partial epilepsy. Currently, research is being conducted not just on chemical substances, but also on implanted antiepileptic devices.

The current antiepileptic medications are ineffective, and the disadvantages restrict its use and complicates patient treatment. Anti - epileptic medications can primarily provide temporary alleviation since drugs reduce convulsions and has little impact upon epileptogenesis. this is the process by which healthy brain circuit is changed into hyperexcitable circuitry, typically following an injury. Long-term use of antiepileptic drugs is limited due to side effects, withdrawal symptoms, bad interactions with other therapies, and economic burden, particularly in developing countries. Ataxia, diplopia, nystagmus,

vertigo, sleepiness, and headache are common side effects. Rashes caused by allergies Hepatoxicity, Weight gain, increased risk of skin rashes Hyponatremia, as well as prenatal abnormalities such as neural tube defects, cognitive impairment, congenital heart problems, orofacial clefts, minor anomalies, growth retardation, developmental delay, and microcephaly [8,9], are associated with hyponatremia.
As a result, the current study attempts to find potent lead compounds for the treatment of epilepsy using computational techniques.

MATERIAL AND METHODS

Pharmacophore modelling: It is a 3D framework created by mapping the physiologically powerful chemicals necessary for the ligand to attach to the appropriate target protein and interact with it. Molecule are subjected for pharmacophore modelling by uploading to free online tool called pharmacist in Sybyl Mol 2 format [10,11]. The server considers a variety of ways to combine the submitted compounds to produce the pharmacophore in Jmol format [12-14].
Molecular Docking: The chemical structure of each ligand was determined using the ZINC data library. ZINC database is a curated collection of commercially accessible chemical compounds that is developed specifically for virtual screening and is utilised by investigators in pharmaceutical businesses, research universities, and biotech companies. In addition, the structures of FDA-approved medications were obtained from Pub Chem [15].The target protein's typical structure file was retrieved from the RCSB Protein Data Bank, a resource for three-dimensional structural information of major biomolecules like proteins and nucleic acids. The usual structure file from the PDB is not appropriate for immediate use in molecular modelling calculations. PDB structural files typically contain just heavy elements and may also contain water molecules, metal ions, a co-crystallized ligand, and co-factors [16]. As a result, purification was carried out utilising the SPBDV Tool, a web-based platform for interactively building complex systems and preparing their inputs.
Receptor Grid Generation: A predefined structure is required for Receptor Grid Generation, as well as all atom structures with proper bond ordering. AUTODOCK searches for a favourable contact among one or many ligand molecules and a receptor, that's commonly a protein. Considering the form and qualities of the receptors, which are expressed on a grid with multiple distinct sets of fields that give increasingly more precise ligand pose scoring? The possibilities in each tab of the receptor grid generation panel let you customize the framework of the receptor by excluding whatever co-crystallized ligand which might be present, identify the location and size of the active center as it will be displayed by receptor grids, and set up the AUTODOCK constant. Around the binding site of the receptor, a grid was generated [17].
Ligand Docking: AUTODOCK VINA is utilized for docking ligands. AUTODOCK VINA looks for a suitable interaction between one or many ligands and a receptors, which is often a protein. The receptor could contain several molecules, although each ligand operates as a single molecule. A protein as well as a co-factor. AUTODOCK VINA was run in either flexibility but rather rigid docked mode, with the latter generating conformations with every input ligand automatically.Ligand posture in flexible docking relates to the mixture of a ligand's site and alignment related to the receptor, as well as its conformation. The ligand posture produced by AUTODOCK VINA is subjected to several hierarchical filtering that assess the ligand's interactions with the receptor. The first filters assess the spatially fit of the ligands to the designated active site, and the compatibility of ligand receptor interactions is investigated using a structured grid-based technique. Finally, the programme was permitted to operate to begin the docking process, in which each ligand was designed to connect with the active site contained in the binding pocket of the protein of interest under investigation. The final score is then obtained.
Visualization: The pdbqt file which is obtained from the docking is complexed with the protein structure for visualization. In Biovia software, the 2D and 3D structure of the complexes are visualized for studying ligand interactions and enhanced presentation.
ADMET STUDIES: After the molecular docking studies, ADMET STUDIES (physicochemical properties) of the ligand was studied using PKCSM Website after it into smiles by using an online smile translator converting and the obtained data was helpful in determining its efficacy and toxicity[18-20].

RESULT AND DISCUSSION

Ligand-based pharmacophore modelling: Utilizing the PharmaGist website, the pharmacophore hypothesis was built from GABA promoter medications in preclinical trials. The compound synthesized by PharmaGist contains a single aromatic ring and two hydrogen bond acceptors spaced apart. The PharmaGist result is sent to ZINCPharmer, which screens and recovers molecules with similar pharmacophoric characteristics via the ZINC database. The derived pharmacophore modeling aids in determining the conformational need. The screened compounds were submitted to Data Warrior, as well as the compounds have been subsequently sorted depending on the physico - chemical parameters of the

standard GABA boosting medications, including the log p value, molecular weight, hydrogen donor, hydrogen acceptor, plus polar surface area. Each of these compounds have been collected as an SDF format and utilized in molecular docking experiments.

Molecular Docking: After Docking, top 10 candidates that showed certain degree of therapeutic benefits which can be used in the treatment of epilepsy. The ZINC ID of these six were taken from the zinc Database are ZINC07046228, ZINC14129673, ZINC77193731, ZINC90964630, ZINC92106442, ZINC92417055 respectively shown in table 1 and fig 1& 2.

Table 1: Docking score and type of interaction of training set of molecules

Compound	Docking Score	Interacting residues	Type of interaction
ZINC07046228	-8.1	GLY117 HIS438, TRP430, TRP82, TYR440 SER287, LEU286 PHE329 TRP231, GLY116	Van der Waals Conventional Hydrogen Bond Halogen (fluorine) Pi-Sulfur Pi-Pi T-Shaped, Amide-Pi Stacked
ZINC14129673	-7.6	ARG242 LEU286, VAL288	Conventional Hydrogen Bond Alkyl, Pi-Alkyl
ZINC77193731	-8.8	TRP82 TYR332, HIS438 PHE329	Conventional Hydrogen Bond Pi-Pi Stacked, Pi-Pi T-Shaped Pi-Alkyl
ZINC90964630	-7.1	ASP304 TYR396 LEU307, LYS408, PRO401, CYS400, TRP522	Attractive charge Carbon Hydrogen Bond Alkyl, Pi-Alkyl
ZINC92106442	-7.9	GLY116, GLY117, SER198 TRP231 PHE329 LEU286, TRP82	Conventional Hydrogen Bond Pi-Lone Pair Pi-Pi T-Shaped, Amide-Pi Stacked Pi-Alkyl
ZINC92417055	-7.1	TRP82 ALA328	Conventional Hydrogen Bond Pi-Alkyl

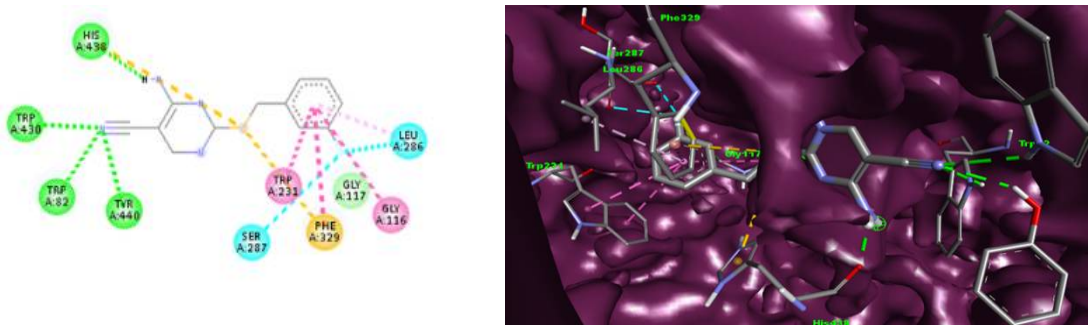

Fig 1: 2D and 3D structures of ZINC07046228

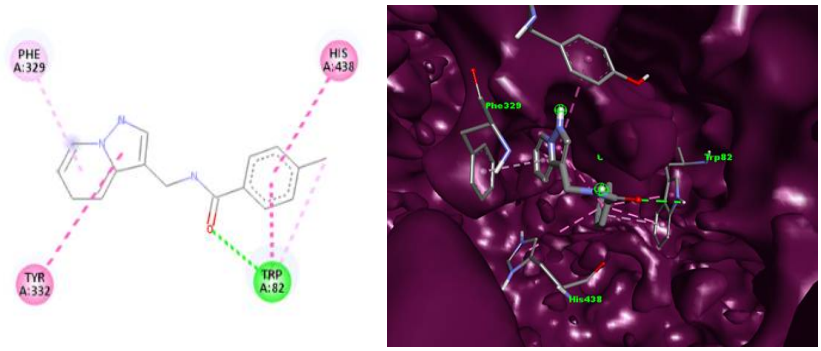

Fig 2: 2D and 3D structure of ZINC77193731

ADMET studies: Drug likeness is predicated based on the Lipinski rule of five, where drug must have molecular weight ≤ 500, A Log P ≤ 5, number of hydrogen donor ≤ 5, and number of hydrogen acceptors ≤ 10, then by these five rules have better activity like permeability, bioavailability, and absorption, shown in table 2-6.

Table 2: Absorption properties of selected test compounds

Compound	Water Solubility	$Caco_2$ Permeability	Intestinal Absorption	Skin Permeability	p-Glycoprotein Substrate	P-Glycoprotein 1st inhibitor	P-Glycoprotein 2nd inhibitor
ZINC07046228	-1.973	1.76	94.966	-2.519	No	No	No
ZINC90964630	-2.986	1.42	96.67	-2.633	No	No	No
ZINC77193731	-3.43	1.268	92.067	-3.467	No	No	No
ZINC92106442	-2.028	1.289	94.218	-2.737	No	No	No
ZINC92417055	-3.551	1.122	92.315	-3.175	No	No	No

Table 3: Distribution properties of selected test compounds

Compound	Distribution VDss (human)	Distribution fraction unbound (human)	Distribution BBB Permeability	Distribution CNS Permeability
ZINC07046228	0.828	0.542	0.302	-1.833
ZINC90964630	-0.026	0.199	0.371	-1.453
ZINC77193731	-0.191	0.431	0.016	-2.954
ZINC92106442	0.486	0.525	0.411	-2.933
ZINC92417055	-0.027	0.312	-0.432	-2.952

Table 4: Metabolism properties of selected test compounds

Compound	CYP2D6 Substrate	CYP3A4 Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
ZINC07046228	No	Yes	No	No	No	Yes	No
ZINC90964630	No	Yes	Yes	Yes	No	No	No
ZINC77193731	No	No	No	No	No	No	No
ZINC92106442	No	No	Yes	No	No	No	No
ZINC92417055	No	No	Yes	Yes	No	No	No

Table 5: Excretion properties of selected test compounds

Compound	Total clearance	Renal OCT2 Substrate
ZINC07046228	1.041	Yes
ZINC90964630	0.517	No
ZINC77193731	0.403	No
ZINC92106442	0.883	No
ZINC92417055	0.219	No

Compound	AMESToxicity	Max. Tolerated Dose (human)	hERG1 inhibitor	hERG2 inhibitor	Oral rat acute toxicity (LD50)	Oral rat chronic toxicity (LOAEL)	Hepatotoxicity	Skin sensitisation	*T.Pyrifformis*	Minnow toxicity
ZINC07046228	No	0.132	No	No	2.547	1.432	No	No	0.437	0.124
ZINC90964630	No	-0.405	No	Yes	1.938	1.702	Yes	No	0.648	-0.491
ZINC77193731	No	0.413	No	No	2.434	1.107	No	No	0.66	1.57
ZINC92106442	**yes**	0.535	No	No	2.445	1.129	No	No	0.285	2.381
ZINC92417055	No	0.589	No	No	2.274	0.842	Yes	No	0.553	1.018

Table 6: Toxicity properties of selected test compounds

The top 6 compounds were determined and listed based on docking score, and the results fall between -9 and -7 kcal/mol. ZINC77193731 and ZINC07046228 are the two best compounds with the best interactions according to molecular docking for the zinc database. ZINC77193731 has a docking score of -8.8 and the interactions are TRP82 H-bond interaction, HIS438 Π-Π-interaction, and the nest compound ZINC07046228 has a docking score of -8.1 and the interactions are HIS438,TRP430,TRP82 H-bond

interaction, GLY116,TRP231 Π- Π T-shaped interaction, PHE329 Π-sulfur interaction,GLY117 Vander Waals interaction.

CONCLUSION

Drug identification was carried out to identify to discovery of a novel GABA-A inhibitors to treat Epilepsy. Based on the preliminary studies 6 hits was found to be show high binding affinity with GABA-A inhibitor. Diazepam and lorazepam were selected as standard on this study. 6 ligands are subsequently evaluated for ADMET properties using PKCSM webserver**.** ZINC77193731 have high binding affinity with better ADMET properties, which can act as novel leads for GABA inhibitor.

ACKNOWLEGEMENT

The authors are thankful to the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, M S Ramaiah University of Applied Sciences, Bengaluru for providing computational facility for research work.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

REFERENCES

1. Kandula, P., & Harden, C. (2009). *Epilepsy. Encyclopedia of Neuroscience, 1147–1149.* doi:10.1016/b978-008045046-9.00586-6
2. Krasowski, M.D., (2010). Therapeutic drug monitoring of the newer anti-epilepsy medications. *Pharmaceuticals*, *3*(6), pp.1909-1935.
3. Treiman, D.M., (2001). GABAergic mechanisms in epilepsy. *Epilepsia*, *42*, pp.8-12.
4. Wahab, A., (2010). Difficulties in treatment and management of epilepsy and challenges in new drug development. *Pharmaceuticals*, *3*(7), pp.2090-2110.
5. Kandratavicius, L., Ruggiero, R.N., Hallak, J.E., Garcia-Cairasco, N. and Leite, J.P., (2012). Pathophysiology of mood disorders in temporal lobe epilepsy. *Brazilian Journal of Psychiatry*, *34*, pp.s233-s245.
6. Wahab, A., (2010). Difficulties in treatment and management of epilepsy and challenges in new drug development. *Pharmaceuticals*, *3*(7), pp.2090-2110.
7. Holmes, G.L., (1995). Role of glutamate and GABA in the pathophysiology of epilepsy. *Mental Retardation and Developmental Disabilities Research Reviews*, *1*(3), pp.208-219.
8. Falco-Walter, J., (2020), December. Epilepsy—definition, classification, pathophysiology, and epidemiology. In *Seminars in Neurology* (Vol. 40, No. 06, pp. 617-623). Thieme Medical Publishers, Inc.
9. Hirose, G., (2013). An overview of epilepsy: its history, classification, pathophysiology and management. *Brain and nerve= Shinkeikenkyu no shinpo*, *65*(5), pp.509-520.
10. Jonathan, H.S. [1992]. Drug Therapy, Vol 8: John A. Oates, MD, Editor, and Alastair J.J. Wood, MD, Associate Editor Boston, MA, New England Journal of Medicine Books, 1991. *Journal of Intensive Care Medicine*, 7[2], pp.109–10doi:https://10.1177/088506669200700207
11. Khedkar, S., Malde, A., Coutinho, E. and Srivastava, S. [2007]. Pharmacophore Modeling in Drug Discovery and Development: An Overview. *Medicinal Chemistry*, 3[2], pp.187–197. doi:10.2174/157340607780059521.
12. Muhammed, M.T. and Esin, A.Y., (2021). Pharmacophore Modeling in Drug Discovery: Methodology and Current Status. *Journal of the Turkish Chemical Society Section A: Chemistry*, *8*(3), pp.749-762.
13. Guner O. (2005). History and Evolution of the Pharmacophore Concept in Computer-Aided Drug Design. Curr Top Med Chem. 2[12]:1321–32. DOI: https://doi.org/10.2174/1568026023392940
14. Sanders MPA, McGuire R, Roumen L, De Esch IJP, De Vlieg J, Klomp JPG, et al.(2012). From the protein's perspective: The benefits and challenges of protein structure-based pharmacophore modeling. Medchemcomm. ;3[1]:28–38. DOI: https://doi.org/10.1039/C1MD00210D
15. Meng, X.-Y., Zhang, H.-X., Mezei, M. and Cui, M. [2011]. Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design, [online] 7[2], pp.146–57. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3151162/.
16. Leelananda SP, Lindert S. (2016). Computational methods in drug discovery. Beilstein J Org Chem. 12:2694–718. DOI: https://doi.org/10.3762/bjoc.12.267.
17. Lin, Shu-Kun Sutter, J.M. (2000). Hoffman R. HypoGen: An automated system for generating predictive 3D pharmacophore models. In: Güner O, editor. Pharmacophore Perception, Development and Use in Drug Design. International University Line; p. 171–89.
18. Noha SM, Schuster D. (2013). Pharmacophore modeling. In: Lill MA, editor. In Silico Drug Discovery and Design. . p. 80–93. ISBN: 9781909453029.
19. Horvath D. (2011). Pharmacophore-Based Virtual Screening. In: Bajorah J, editor. Chemoinformatics and Computational Chemical Biology. Springer; p. 261–97. ISBN: 9781493957934.
20. Gandhi, K. et al. [2022] "Molecular modeling and ADMET predictions of flavonoids as prospective aromatase inhibitors," Indian journal of chemistry, 61[2], pp. 192–200. doi: 10.56042/ijc.v61i2.60719

21. Li, H., Sutter, J. and Hoffmann, R., (2000). HypoGen: An Automated System for Generating 3D Predictive Pharmacophore. *Pharmacophore perception, development, and use in drug design*, *2*, p.171.
22. Sanders, M.P., McGuire, R., Roumen, L., de Esch, I.J., de Vlieg, J., Klomp, J.P. and de Graaf, C., (2012). From the protein's perspective: the benefits and challenges of protein structure-based pharmacophore modeling. *MedChemComm*, *3*(1), pp.28-38.
23. Guner, O.F., (2002). History and evolution of the pharmacophore concept in computer-aided drug design. *Current topics in medicinal chemistry*, *2*(12), pp.1321-1332.
24. Dessai, P. G. *et al.* [2022] "Design, synthesis, graph theoretical analysis and molecular modelling studies of novel substituted quinoline analogues as promising anti-breast cancer agents," *Molecular diversity*. doi: 10.1007/s11030-022-10512-7.

CITATION OF THIS ARTICLE

Nayana R J, Bandral S K, Genne S, Golla S, Agasa R M, Selvaraj K, Theivendren P, R. Sathish Adithya, Parasuraman P. Identification of Novel GABA-A Inhibitor For The Treatment Of Epilepsy: A Computational Approach. Bull. Env. Pharmacol. Life Sci., Vol 12[7] June 2023: 180-185.