Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/d05ey924

EVALUATION OF ANTICONVULSANT PROPERTIES OF NOVEL IMINE DERIVATIVES OF PIPERIDONE OXIMES: *IN-SILICO* AND *IN-VIVO* ACTIVITY IN SEIZURE MODELS

Maryam Inayat[1*], Rehana Perveen[2], Zuneera Akram[2], Ashok Kumar[3], Maryam Akani[4], Asma Elahi[1], Rubina Siddiqui[5], Mahwish Akhtar[6]

[1] *Department of Pharmacology, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
[2] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
[3] Department of Pharmacology, Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan
[4] Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
[5] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan
[6] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Dow college of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan.

***Corresponding Author:** Maryam Inayat
***E-mail:** Maryam_inayat@live.com

ABSTRACT

Epileptic seizures and convulsions are widespread neurological conditions that impact a large number of people worldwide, resulting in a substantial impact on the overall disease and disability burden. Seizures occur worldwide at a rate of approximately 50 million incidents per year, and convulsions make up a significant fraction of these occurrences. These diseases are defined by abrupt and excessive electrical discharges in the brain, frequently leading to uncontrolled muscular spasms and poor consciousness. Although there have been improvements in pharmacotherapy, there is still a significant demand for novel anticonvulsant drugs that can target numerous pathways implicated in the genesis of seizures.

This study assesses the anticonvulsant efficacy of a new chemical, 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime, using a comprehensive methodology that includes in silico docking studies and in vivo efficacy testing with a pentylenetetrazole (PTZ)-induced seizure model. Docking studies evaluated the compound's binding affinities compared to Diazepam, a widely recognized anticonvulsant medication. The results obtained from experiments on living organisms showed that 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime effectively delayed the start of seizures and decreased their intensity. This indicates that the compound has a complex mode of action. The results emphasize the potential of 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime as a viable option for further advancement in treating seizures and convulsions, a pressing requirement in global neurological healthcare.

KEY WORDS: Seizures, Convulsion, Novel Anticonvulsant Agents, Piperidone Oximes, Imine derivatives,

INTRODUCTION:

Seizures and convulsions are prevalent neurological illnesses on a global scale, affecting approximately 50 million individuals annually (Beghi et al., 2023). Convulsions, which involve involuntary muscle spasms and are typically accompanied by changes in awareness, significantly contribute to the worldwide prevalence of neurological illnesses (Riney et al., 2022). The occurrence and frequency of the condition varied significantly between various areas, with low- and middle-income nations experiencing higher rates. This can be attributed to reasons such as insufficient healthcare infrastructure and a more significant occurrence of neurocysticercosis and other infectious causes (Maloney et al., 2020). The ramifications of these disorders go beyond the individuals affected, affecting families, healthcare systems, and society as a whole due to the resulting illness, expenses in healthcare, and decrease in productivity.

The treatment of seizures and convulsions primarily depends on the administration of anticonvulsant drugs, which are designed to prevent or decrease the occurrence and intensity of seizures. Frequently employed anticonvulsants encompass carbamazepine, valproate, phenytoin, and benzodiazepines like diazepam (Sarma et al., 2016). These medications affect different neurotransmitter systems, with several explicitly targeting the GABAergic system to augment inhibitory neurotransmission and stabilize neuronal activity (Perucca et al., 2023).

Although these drugs are commonly used, they do have certain limits. Approximately 20-30% of individuals are unresponsive to current medications, meaning they see minimal to no improvement in controlling seizures (Dang & Silverstein, 2017). Moreover, the adverse effects of numerous medications might be significant, affecting patient adherence and overall well-being. Carbamazepine and phenytoin have been linked to a variety of side effects, including dizziness, ataxia, and gastrointestinal abnormalities. These medications can also cause more serious effects such liver damage, abnormal blood cell counts, and birth defects. Valproate poses hazards of liver damage, inflammation of the pancreas, and considerable harm to fetal development, which restricts its usage, especially in women who are capable of becoming pregnant. Benzodiazepines, although they are useful in the immediate treatment of seizures, are linked to drowsiness, tolerance, dependence, and cognitive decline (Wong & Lhatoo, 2000).

The current anticonvulsant therapies have limitations, and there is a high prevalence of treatment-resistant cases. This highlights the urgent need for new therapeutic agents that are effective and have fewer side effects. Research is now focused on developing compounds that target multiple pathways involved in seizure generation and propagation, aiming for a more comprehensive approach to controlling seizures. Specifically, there is increasing interest in compounds that affect both the GABAergic and cholinergic systems, as these neurotransmitters play crucial roles in maintaining the balance between excitation and inhibition in the central nervous system (Malwaska, 2005. Stefan & Feuerstein, 2007). Recent studies have explored novel compounds that have the potential to be anticonvulsant. Imine derivatives have shown promise because they can modulate neurotransmitter systems and interact with receptor sites that are involved in seizure activity.

Chemistry:

The compound 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime, an imine derivative, has emerged as an exciting candidate due to its unique chemical structure and promising preliminary data in preclinical models.

3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime is characterized by its distinctive chemical framework, which includes a piperidine ring—a six-membered ring with five carbon atoms and one nitrogen atom. This ring is substituted at the 2 and 6 positions with phenyl groups, which enhances the compound's stability and potential interaction with various biological targets (Subbaiah &

Meanwell, 2021). Attached at the 3-position of the piperidine ring is an isopropyl group, which may influence the compound's lipophilicity and ability to cross the blood-brain barrier (Chia *et al*., 2023). Additionally, the oxime group at the 4-position of the piperidine ring is known for its potential to interact with neurotransmitter systems (Banerjee & Sharma, 2012), contributing to the compound's anticonvulsant activity. The unique combination of these structural features positions 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime as a potentially effective anticonvulsant agent, warranting further investigation into its efficacy and safety profile.

MATERIAL AND METHOD:

In-Silico Docking Studies

The docking studies for the novel piperidone derivative (OX) were conducted using AutoDock Vina (version 4.2) to evaluate the compound's binding interactions with specific protein targets associated with antiseizure activity. Diazepam was used as a standard anticonvuslant for comparison (Divya *et al*., 2022).

1. Protein Preparation:

The crystal structure of the target protein associated with antiseizure activity was retrieved from the Protein Data Bank (PDB ID: 6H37) (Kumar *et al*., 2022). The structure had a resolution of 1.90 Å. Using BIOVIA Discovery Studio Visualizer (DSV), excess protein chains, water molecules, and co-crystallized ligands were removed. Polar hydrogen atoms and Kollman charges were added to prepare the protein for docking simulations (Sahoo *et al*., 2022).

2. Ligand Preparation:

The novel piperidone derivative (OX) and the standard compound Diazepam were modeled, and energy was minimized using the MMFF94X force field method with 1000 iterations. The minimized structures were then saved in PDB format and converted to PDBQT files using MGL Tools (Divya *et al*., 2022; Sahoo *et al*., 2022).

3. Docking Procedure:

The docking simulations were performed using AutoDock Vina. A grid box was centered on the protein's active site with dimensions 60 × 76 × 60 points and a spacing of 2.50 Å. The genetic algorithm's default parameters were employed to search for optimal ligand-enzyme binding modes. The binding energies (BE) of the receptor-ligand complexes were calculated to assess the binding affinity ((Divya *et al*., 2022; Sahoo *et al*., 2022).

In-Vivo Antiseizure Studies

1. Induction Model and Standard Group:

The antiseizure activity of the imine derivative, 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime (OX), was evaluated using the PTZ (pentylenetetrazole)-induced seizure model in male albino mice (Balb/c) (Yuan *et al.*, 2020) which were housed in Baqai Medical University Animal House under standard optimal conditions. The PTZ model is widely recognized for mimicking generalized seizure activity. PTZ was administered at a dose of 60 mg/kg to induce seizures, and the anticonvulsant potential of OX was compared to Diazepam, a standard anticonvulsant, at a dose of 3 mg/kg. Three doses of OX (5 mg/kg, 10 mg/kg, and 20 mg/kg) were tested.

2. Utilization of Modified Racine Scale:

The latency to clonic seizures was recorded, and the severity of seizures was assessed using a modified Racine scale, which categorizes seizures based on their behavioral manifestations. The mice were monitored for the onset of jerks, clonus, and other seizure-related behaviors, and the duration of clonic seizures was documented. Additionally, survival rates were observed to determine the protective effects of OX against PTZ-induced mortality (Van Erum *et al*., 2019).

Results

Docking Analysis:

Docking studies were performed to investigate the binding interactions of the novel piperidone derivative (OX) with antiseizure protein targets using AutoDock Vina software. The compound was docked into the binding sites of the antiseizure protein target (PDB ID: 6H37). The two-dimensional interaction plot, as shown in Figure 1, highlights the interactions between OX and the critical residues within the protein's active site.

The compound 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime displayed a binding energy of -7.8 kcal/mol, forming significant interactions with several key amino acids in the binding pocket. These interactions include two conventional hydrogen bonds with the residues SER-65 and ASN-62, alongside ten hydrophobic interactions with residues such as TRP-5, GLN-67, GLN-92, HIS-96, VAL-143, THR-199, THR-200, PRO-201, PRO-202, and TRP-209.

The native ligand Diazepam also docked into the same protein target, demonstrated a slightly higher binding energy of -7.4 kcal/mol. Diazepam formed hydrogen bonds with the residues ASN-62 and GLN-67 and several hydrophobic interactions involving residues like PHE-131, VAL-143, and THR-199.

The interactions depicted in the two-dimensional plot provided in Figure 1 give a detailed view of how the OX compound aligns within the binding site of the antiseizure protein, engaging in crucial hydrogen bonding and hydrophobic interactions essential for its inhibitory efficacy. These interactions suggest that the novel piperidone derivative could serve as a potent antiseizure agent with the potential to modulate the target protein effectively, similar to or possibly surpassing the efficacy of Diazepam.

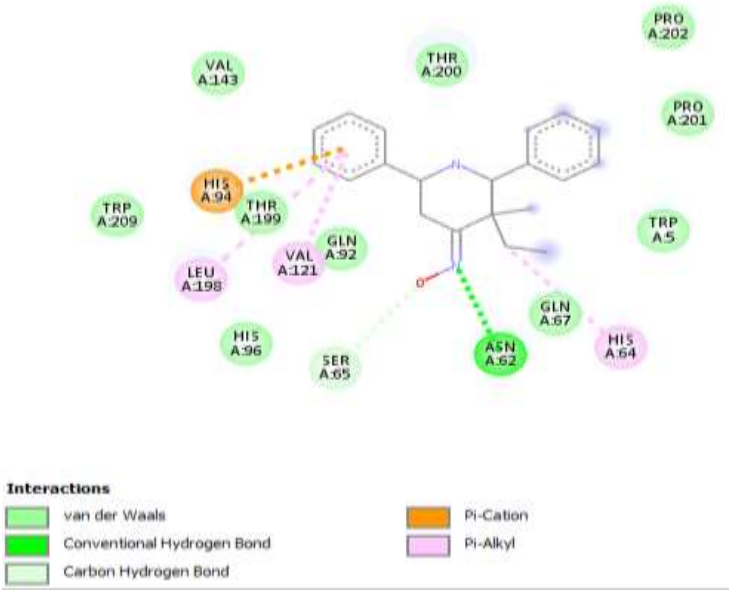


Figure 1: 2D projection of the standard compound OX with an active site for Antiseizure activity using PDB ID 6H37

Anti-seizure Analysis:

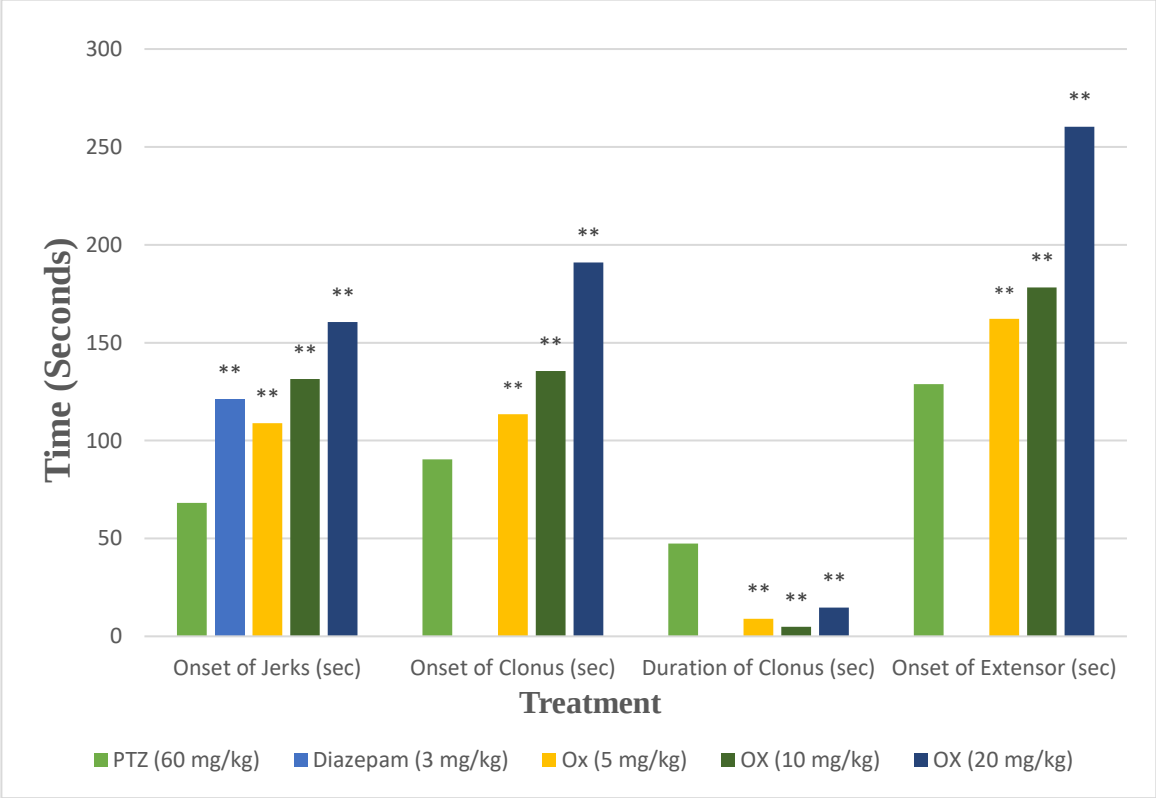
The results demonstrated in Table 2 suggest that all three doses of OX significantly delayed the onset of seizures compared to the PTZ group. The 10 mg/kg dose of OX exhibited the most pronounced anticonvulsant effect, reducing the duration of clonus to 4.83 ± 0.31 seconds, compared to 47.33 ± 0.49 seconds in the PTZ group. The 5 mg/kg and 10 mg/kg doses of OX achieved a 100% survival rate, similar to the Diazepam group. However, the highest dose of 20 mg/kg resulted in a 50% mortality rate, indicating potential dose-dependent toxicity.

These findings suggest that OX exhibits significant anticonvulsant activity at lower doses, with efficacy comparable to Diazepam, but higher doses may pose safety concerns. Graphs 1 and 2 also depict the interpreted antiseizure potential data of OX.

Groups	**A**	**B**	**C**	**D**	**E**	**F**
Drug Administered	Water	PTZ (60 mg/kg)	Diazepam (3 mg/kg)	OX (5 mg/kg)	OX (10 mg/kg)	OX (20 mg/kg)
Parameters evaluated						
Onset of Jerks (sec)	0	68.16 ± 0.91	121.16 ± 0.79**	108.83 ± 0.60**	131.5 ± 0.72**	160.5 ± 3.32**
Onset of Clonus (sec)	0	90.33 ± 0.84	0**	113.50 ± 0.68**	135.50 ± 1.5**	191.00 ± 8.84**
Duration of Clonus (sec)	0	47.33 ± 0.49	0**	9.00 ± 0.37**	4.83 ± 0.31**	14.67 ± 0.76**
Onset of Extensor (sec)	0	128.83 ± 0.48	0**	162.16 ± 1.22**	178.16 ± 1.47**	260.33 ± 6.06**
Number of Episodes of Jerks	0	11 ± 0.37	1.33 ± 0.22**	7.6 ± 0.22**	4.66 ± 0.21**	15.16 ± 0.48**
Recovery Rate	100%	83%	100%	100%	100%	50%
Death Rate	0%	17%	0%	0%	0%	50%

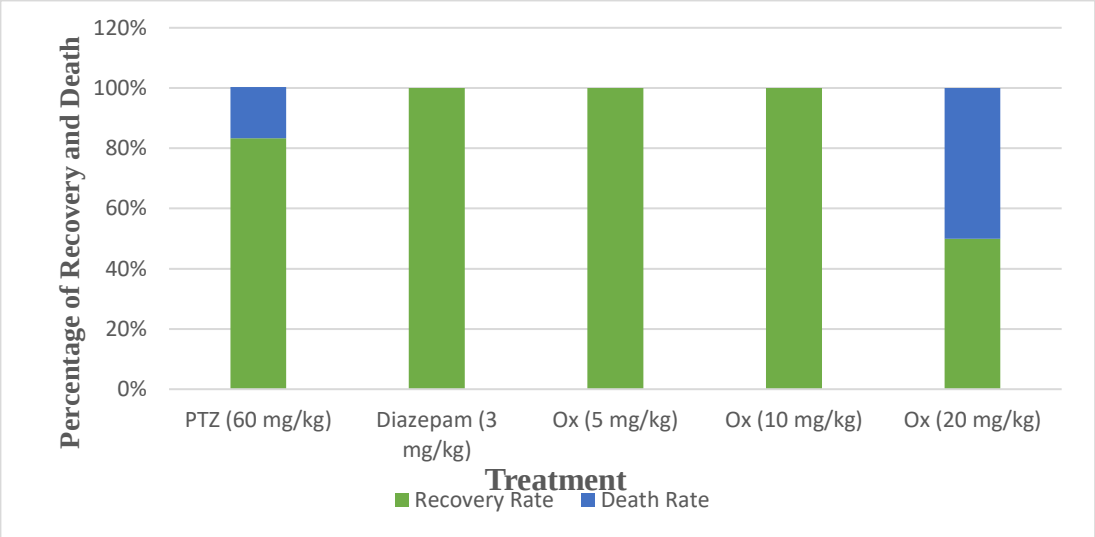
Table 2 The comparison of OX with the positive control group and standard Diazepam

Where: No. of studied animals in each group n=10;
Significant level p<0.01 denoted by **; relative to control.
P-value <0.05 is considered significant.



Graph: 1. The comparison of Anti-seizure activity observed in treatment groups (OX) at doses of 5, 10, and 20 mg/kg with the positive control (PTZ) and Standard Diazepam

Where: No. of studied animals in each group n=10;
Significant level p<0.01 denoted by **; relative to control.
P-value <0.05 is considered significant.



Graph 2. The comparison of the Recovery Rate observed in treatment groups (OX) at doses of 5, 10, and 20 mg/kg with positive control (PTZ) and Standard Diazepam

Where: No. of studied animals in each group n=10.

Discussion

The docking studies revealed that OX exhibited a binding affinity that was slightly better than Diazepam when interacting with the antiseizure target (PDB ID: 6H37). OX formed stable interactions, including hydrophobic interactions and hydrogen bonds with critical residues in the binding pocket. This suggests that OX has a solid potential for binding effectively to the target protein, which could underlie its antiseizure activity. The binding energy of -7.8 kcal/mol for OX, compared to -7.4 kcal/mol for Diazepam, indicates a favorable interaction, suggesting that OX could be a potent inhibitor of the target protein (Nune-Alves *et al*., 2021).

In the PTZ-induced seizure model, OX demonstrated significant antiseizure activity across the tested doses. The compound notably delayed the onset of seizures and reduced the severity and duration of convulsive episodes, particularly at doses of 10 mg/kg. This effect was comparable to that observed with Diazepam, a widely used standard anticonvulsant, indicating that OX has similar, if not superior, efficacy in managing seizure activity.

Interestingly, while lower doses of OX (5 and 10 mg/kg) were associated with 100% survival rates, the highest dose (20 mg/kg) resulted in a 50% mortality rate. This dose-dependent toxicity underscores the importance of careful dosing and further investigation into the safety profile of OX. The reduced duration of clonus and the delayed onset of both jerks and extensor seizures across the lower doses indicate that OX effectively modulates seizure activity, likely through mechanisms that warrant further exploration.

The comparative analysis with Diazepam highlights the potential of OX as a novel antiseizure agent. While Diazepam is well-established for its rapid action in acute seizure management, OX's ability to achieve similar outcomes in the PTZ model with different dosing regimens suggests that it may offer a viable alternative, especially in cases where conventional benzodiazepines are contraindicated or lead to tolerance and dependence issues.

Conclusion

This study provides compelling evidence supporting the antiseizure potential of 3-Isopropyl-2,6-Diphenylpiperidin-4-One Oxime. The compound's efficacy, demonstrated through both *in silico* docking studies and in vivo PTZ-induced seizure models, positions it as a promising candidate for further development. However, the observed dose-dependent toxicity at higher concentrations underscores the need for additional research to optimize dosing strategies and evaluate long-term safety.

Given the high global burden of seizure disorders and the limitations of current pharmacotherapies, the development of new agents like OX could represent a significant advancement in neurology. Future studies should focus on elucidating the precise mechanisms of action, long-term safety, and potential clinical applications of OX, emphasizing its use in treatment-resistant cases.
This research contributes to the growing body of literature on novel antiseizure agents. It highlights the importance of exploring new chemical entities with unique mechanisms of action to address the unmet needs in seizure management.

REFERENCES:

1. Banerjee, P.S. and Sharma, P.K., 2012. New antiepileptic agents: structure-activity relationships. *Medicinal Chemistry Research*, *21*, pp.1491-1508.
2. Beghi, E., Giussani, G., Costa, C., DiFrancesco, J.C., Dhakar, M., Leppik, I., Kwan, P., Akamatsu, N., Cretin, B., O'Dwyer, R. and Kraemer, G., 2023. The epidemiology of epilepsy in older adults: a narrative review by the ILAE task force on epilepsy in the elderly. Epilepsia, 64(3), pp.586-601.
3. Chia, S.Y., Khor, B.K., Tay, Y.J., Liew, K.F. and Lee, C.Y., 2023. Discovery of blood-brain barrier permeant amine-functionalized aurones as inhibitors of activated microglia. *Bioorganic Chemistry*, *135*, p.106509.
4. Dang, L.T. and Silverstein, F.S., 2017. Drug treatment of seizures and epilepsy in newborns and children. Pediatric Clinics, 64(6), pp.1291-1308.
5. Divya, B., Narayanan, J., Chitra, V. and Tamilanban, T., 2022. Evaluation of Anti-Convulsant Activity of Olivetol on Ptz Induced Seizure Model. *NeuroQuantology*, *20*(17), p.372.
6. Kumar, A., Agarwal, P., Rathi, E. and Kini, S.G., 2022. Computer-aided identification of human carbonic anhydrase isoenzyme VII inhibitors as potential antiepileptic agents. *Journal of Biomolecular Structure and Dynamics*, *40*(11), pp.4850-4865.
7. Malawska, B., 2005. New anticonvulsant agents. *Current topics in medicinal chemistry*, *5*(1), pp.69-85.
8. Maloney, E.M., Chaila, E., O'Reilly, É.J. and Costello, D.J., 2020. Incidence of first seizures, epilepsy, and seizure mimics in a geographically defined area. *Neurology*, *95*(5), pp.e576-e590.
9. Nunes-Alves, A., Ormersbach, F. and Wade, R.C., 2021. Prediction of the drug–target binding kinetics for flexible proteins by comparative binding energy analysis. Journal of Chemical Information and Modeling, 61(7), pp.3708-3721.
10. Perucca, E., Bialer, M. and White, H.S., 2023. New GABA-targeting therapies for the treatment of seizures and epilepsy: I. Role of GABA as a modulator of seizure activity and recently approved medications acting on the GABA system. *CNS drugs*, *37*(9), pp.755-779.
11. Riney, K., Bogacz, A., Somerville, E., Hirsch, E., Nabbout, R., Scheffer, I.E., Zuberi, S.M., Alsaadi, T., Jain, S., French, J. and Specchio, N., 2022. International League Against Epilepsy classification and definition of epilepsy syndromes with onset at a variable age: a position statement by the ILAE Task Force on Nosology and Definitions. *Epilepsia*, *63*(6), pp.1443-1474.
12. Sahoo, C.R., Paidesetty, S.K., Sarathbabu, S., Dehury, B., Senthil Kumar, N. and Padhy, R.N., 2022. Molecular dynamics simulation, synthesis and topoisomerase inhibitory actions of vanillin derivatives: a systematic computational structural integument. *Journal of Biomolecular Structure and Dynamics*, *40*(22), pp.11653-11663.
13. Sarma, A.K., Khandker, N., Kurczewski, L. and Brophy, G.M., 2016. Medical management of epileptic seizures: challenges and solutions. *Neuropsychiatric disease and treatment*, pp.467-485.
14. Stefan, H.T.J.F. and Feuerstein, T.J., 2007. Novel anticonvulsant drugs. Pharmacology & therapeutics, 113(1), pp.165-183.
15. Subbaiah, M.A. and Meanwell, N.A., 2021. Bioisosteres of the phenyl ring: recent strategic applications in lead optimization and drug design. Journal of Medicinal Chemistry, 64(19), pp.14046-14128.

16. Van Erum, J., Van Dam, D. and De Deyn, P.P., 2019. PTZ-induced seizures in mice require a revised Racine scale. Epilepsy & Behavior, 95, pp.51-55.
17. Wong, I.C. and Lhatoo, S.D., 2000. Adverse reactions to new anticonvulsant drugs. Drug Safety, 23, pp.35-56.
18. Yuan, X., Fu, Z., Ji, P., Guo, L., Al-Ghamdy, A.O., Alkandiri, A., Habotta, O.A., Abdel Moneim, A.E. and Kassab, R.B., 2020. Selenium nanoparticles pre-treatment reverse behavioral, oxidative damage, neuronal loss, and neurochemical alterations in pentylenetetrazole-induced epileptic seizures in mice. International Journal of Nanomedicine, pp.6339-6353.