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Designing and Evaluation of Novel Valproic Acid Derivatives by IvLCB: In-vitro Like Computational Bioassay, Predictive Toxicological Study & Docking Analysis for GABA-A Mediated Inhibition of Neural Firing

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Abstract Gamma-aminobutyric acid (GABA) is the brain's primary inhibitory neurotransmitter, and some anti-epileptic drugs work by enhancing its effects. Medications such as Valproic acid and Benzodiazepines increase GABA activity, which inhibits neural firing and suppresses seizure activity. In the present study twelve Valproic acid derivatives were designed and evaluated for their GABA-A mediated inhibition of neural firing by in-silico methods. All novel designed molecules were evaluated by IvLCB: In-vitro like computational bioassay and SwissDock. These molecules were also evaluated for their in-silico biological activity spectrum & predictive toxicological study by MolpredictX and predictive bioactivity score by Molinspiration. As per the analysis performed it was found that designed molecule VAL1 shows strong binding ability to PDB ID: 4COF with 3 hydrogen bonds and binding affinity of -4.4 kcal/mol.

Keywords: *GABA-A, Valproic acid derivatives, computational study, docking, in-silico analysis, in-vitro like computational bioassay*

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1. Introduction

Anti-epileptic drugs (AEDs), also known as anticonvulsants, are a class of medications used to manage seizures in individuals with epilepsy. Epilepsy is a chronic neurological disorder that leads to recurrent, spontaneous seizures caused by abnormal electrical activity in the brain. AEDs are fundamental in controlling these seizures, reducing their frequency and intensity, and allowing individuals with epilepsy to lead more normal lives.

Seizures, which are the hallmark of epilepsy, can vary in nature. These include generalized tonic-clonic seizures, which affect the entire brain, focal (partial) seizures that begin in one area of the brain, and absence seizures, which are brief episodes of impaired awareness. The purpose of AED therapy is to control or prevent these seizures while minimizing adverse effects and improving the overall quality of life for patients.

1.1. History of Anti-Epileptic Drugs

The history of epilepsy treatments stretches back thousands of years, with early civilizations like the Egyptians and Greeks attributing seizures to divine or supernatural forces. Treatments in ancient times were largely symbolic or based on herbal remedies, with little understanding of the neurological mechanisms behind epilepsy.

The first major breakthrough in modern epilepsy treatment occurred in the 19th century with the use of bromides, a class of compounds that helped control seizures, although their use was limited due to toxicity and side effects like sedation.

The true development of modern AEDs began in the early 20th century. One of the first and most significant advances was the introduction of phenytoin (Dilantin) in the 1930s, which provided more effective seizure control without the extreme side effects seen with earlier treatments. This marked the beginning of a new era in

epilepsy treatment, with subsequent generations of AEDs offering greater effectiveness and fewer side effects.

Since then, numerous AEDs have been developed, including carbamazepine, valproic acid, lamotrigine, and levetiracetam, expanding treatment options and providing personalized approaches to seizure management [1].

1.2. Mechanisms of Action of Anti-Epileptic Drugs

AEDs work primarily by influencing the electrical activity of neurons, preventing or reducing the excessive firing that leads to seizures. Seizures are caused by an imbalance between excitatory and inhibitory signals in the brain, and most AEDs target these mechanisms to restore normal brain function [2].

1.2.1. Ion Channel Modulation

Many AEDs function by altering the flow of ions (sodium, potassium, calcium) across neuronal membranes. For example, phenytoin, carbamazepine, and lamotrigine block sodium channels, which stabilizes the neuronal membranes and reduces the likelihood of seizure activity. This helps prevent neurons from firing too rapidly and uncontrollably [3].

1.2.2. Enhancing GABAergic Activity

Gamma-aminobutyric acid (GABA) is the brain's primary inhibitory neurotransmitter, and some AEDs work by enhancing its effects. Medications such as valproic acid and benzodiazepines (e.g., diazepam) increase GABA activity, which inhibits neural firing and suppresses seizure activity [4].

1.2.3. Inhibition of Glutamate

Glutamate is the main excitatory neurotransmitter in the brain. Some AEDs, such as topiramate and felbamate, reduce the action of glutamate, helping to reduce the overstimulation of neurons that leads to seizures [4].

1.2.4. Calcium Channel Modulation:

Certain AEDs, like ethosuximide, target calcium channels involved in the generation of seizures, especially in conditions like absence seizures. By blocking specific calcium channels, these drugs help prevent the abnormal electrical discharges that characterize these types of seizures [5].

1.3. Treatment of Epilepsy with Anti-Epileptic Drugs

The treatment of epilepsy with AEDs is tailored to the specific type of seizures and the individual patient. The goal is to provide effective seizure control while minimizing side effects. The selection of an AED depends on factors such as the type of epilepsy, the patient's age, medical history, and any other coexisting conditions [7].

1.3.1. First-Line Drugs

The first-choice AEDs vary based on the type of seizures a person experiences. For generalized tonic-clonic seizures, drugs like valproic acid, amotrigine, and vetiracetam are commonly prescribed. For focal seizures, carbamazepine, phenytoin, and oxcarbazepine are often preferred [8].

1.3.2. Second-Line Drugs

If first-line medications do not adequately control seizures, second-line drugs may be considered. These include topiramate, gabapentin, and pregabalin. These drugs can be effective for patients who do not respond to initial therapies or for those who experience intolerable side effects from first-line drugs [9].

1.3.3. Adjunctive Therapy

In cases where seizures are not fully controlled with a single AED, combination therapy may be necessary. This involves the use of two or more AEDs, which requires careful monitoring to manage potential drug interactions and avoid excessive side effects [10].

1.3.4. Special Considerations

The choice of AED must also consider factors like pregnancy, age, and other health conditions. For example, lamotrigine is often chosen as a safer option during pregnancy compared to other drugs, but it still requires careful monitoring [11].

1.4. Side Effects and Complications of Anti-Epileptic Drugs

While AEDs are essential for controlling seizures, they can cause a variety of side effects. These vary depending on the specific drug and the individual’s response. Some side effects can be mild, while others may require adjustments to the treatment plan [12].

1.4.1. Central Nervous System Effects

Many AEDs, particularly older ones, can cause drowsiness, dizziness, and cognitive issues like memory problems or difficulty concentrating. Drugs such as phenytoin and phenobarbital are more likely to induce sedation and impair daily functioning.

1.4.2. Gastrointestinal Issues

Nausea, vomiting, and appetite changes are common side effects of AEDs. For example, valproic acid may lead to weight gain, while topiramate can cause weight loss.

1.4.3. Blood Disorders

Certain AEDs, like carbamazepine and phenytoin, can cause blood-related issues, such as a reduction in white blood cell count (leukopenia) or platelets (thrombocytopenia). Patients on these drugs require regular blood tests to monitor for these complications.

1.4.4. Skin Reactions and Allergies

Some AEDs, especially lamotrigine and carbamazepine, can cause rashes, which, in rare cases, may progress to serious allergic reactions such as Stevens-Johnson syndrome. Any unusual skin reactions should be promptly addressed [13].

1.4.5. Teratogenic Effects

Several AEDs, particularly valproic acid, are known to increase the risk of birth defects when taken during pregnancy. Therefore, women of childbearing age must carefully plan their treatment, and alternatives may be considered during pregnancy.

1.4.6. Drug Interactions

AEDs can interact with other medications, altering their effects. For instance, phenytoin and carbamazepine can induce liver enzymes that accelerate the breakdown of other drugs, which might reduce their effectiveness. Monitoring and adjustments are necessary to avoid these interactions [14].

1.5. Common Anti-Epileptic Drug

1.5.1. Phenytoin

One of the oldest and most widely used AEDs, phenytoin is effective for both generalized tonic-clonic and focal seizures. It stabilizes sodium channels, preventing abnormal neural firing. However, it has a narrow therapeutic range and requires careful dosing and regular blood monitoring.

1.5.2. Carbamazepine

This AED is commonly used for focal seizures and generalized tonic-clonic seizures. It also works by stabilizing sodium channels, but like phenytoin, it can interact with other drugs and may require regular blood tests.

1.5.3. Valproic Acid

Valproic acid is a broad-spectrum AED that can treat a wide range of seizure types, including generalized tonic-clonic and absence seizures. While it is highly effective, it has significant side effects, including potential weight gain and teratogenicity.

1.5.4. Lamotrigine

Lamotrigine is effective for both generalized and focal seizures and is associated with a lower risk of side effects compared to older AEDs. It is often used as both a first-line and adjunctive therapy.

1.5.5. Levetiracetam

This AED is favored for its favorable side-effect profile and low likelihood of drug interactions. It can treat a variety of seizure types and is commonly used in combination with other AEDs.

1.5.6. Topiramate

Topiramate is another broad-spectrum AED that is effective for both focal and generalized seizures. It is associated with weight loss as a side effect, which may be beneficial in some patients but cause concerns in others [15,16].

1.6. *In-silico* Study

An *in-silico* study refers to research that utilizes computational models and simulations to investigate scientific phenomena.

These studies typically involve the use of software tools to analyze data, predict biological or chemical interactions, or simulate complex systems, providing valuable insights without the need for physical experimentation [20,21].

1.7. Docking

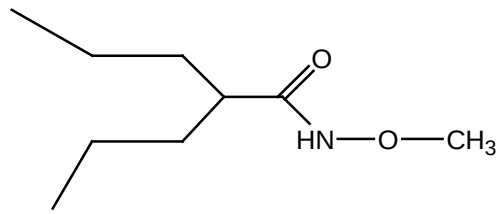
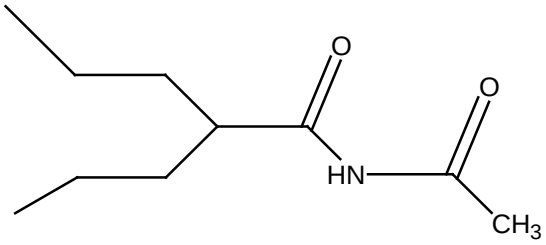
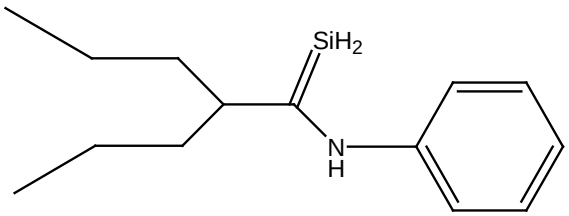
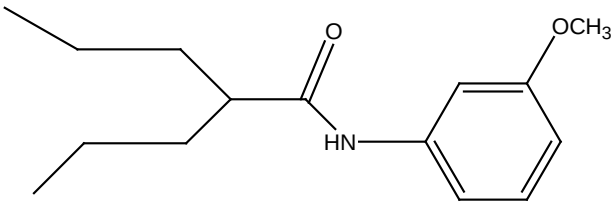
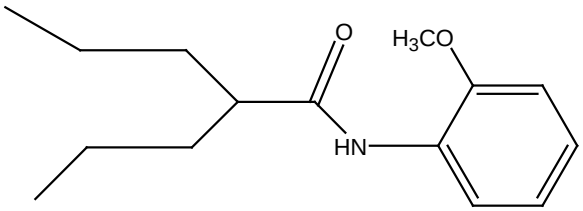
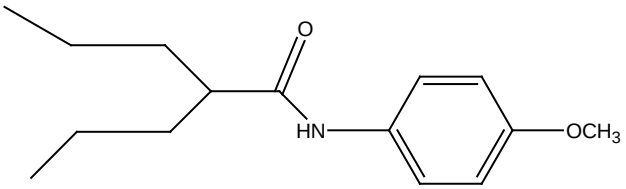
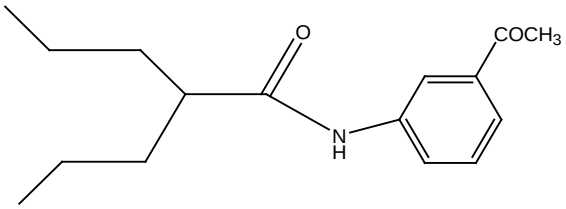
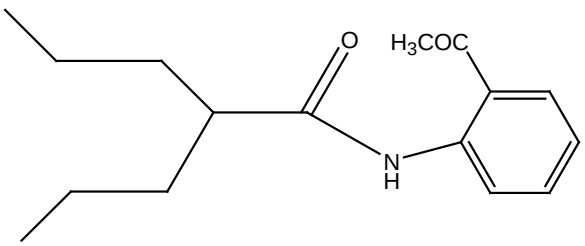
Docking in drug design is a computational approach that helps predict how a small molecule, such as a potential drug, interacts with a target protein or receptor. This method simulates the binding process to determine the most favorable configurations for the molecule's attachment. It takes into account factors like the shape compatibility, electrostatic interactions, and hydrophobic effects to assess how strongly and specifically a molecule may bind to its target [17].

In drug discovery, docking plays an essential role during the initial phases, helping researchers identify promising lead compounds while minimizing the need for costly experimental procedures. It enables the virtual screening of large compound libraries, examining millions of molecules for their potential to interact with the target. This approach not only conserves time and resources but also provides valuable insights into the molecular dynamics between the drug and its target, aiding the further refinement of drugs to improve their effectiveness and safety [18,19].

2. Material and Methods for Valproic Acid derivative

2.1. Designing of Molecules

Identification of pharmacophore group was done from the structure of well-known anti-epileptics and then all the molecules were designed by keeping this pharmacophoric concept in mind. All the novel designed structures are given below in Figure 1.

VAL1 	**VAL2** 
VAL3 	**VAL4M** 
VAL4O 	**VAL4P** 
VAL5M 	**VAL5O** 

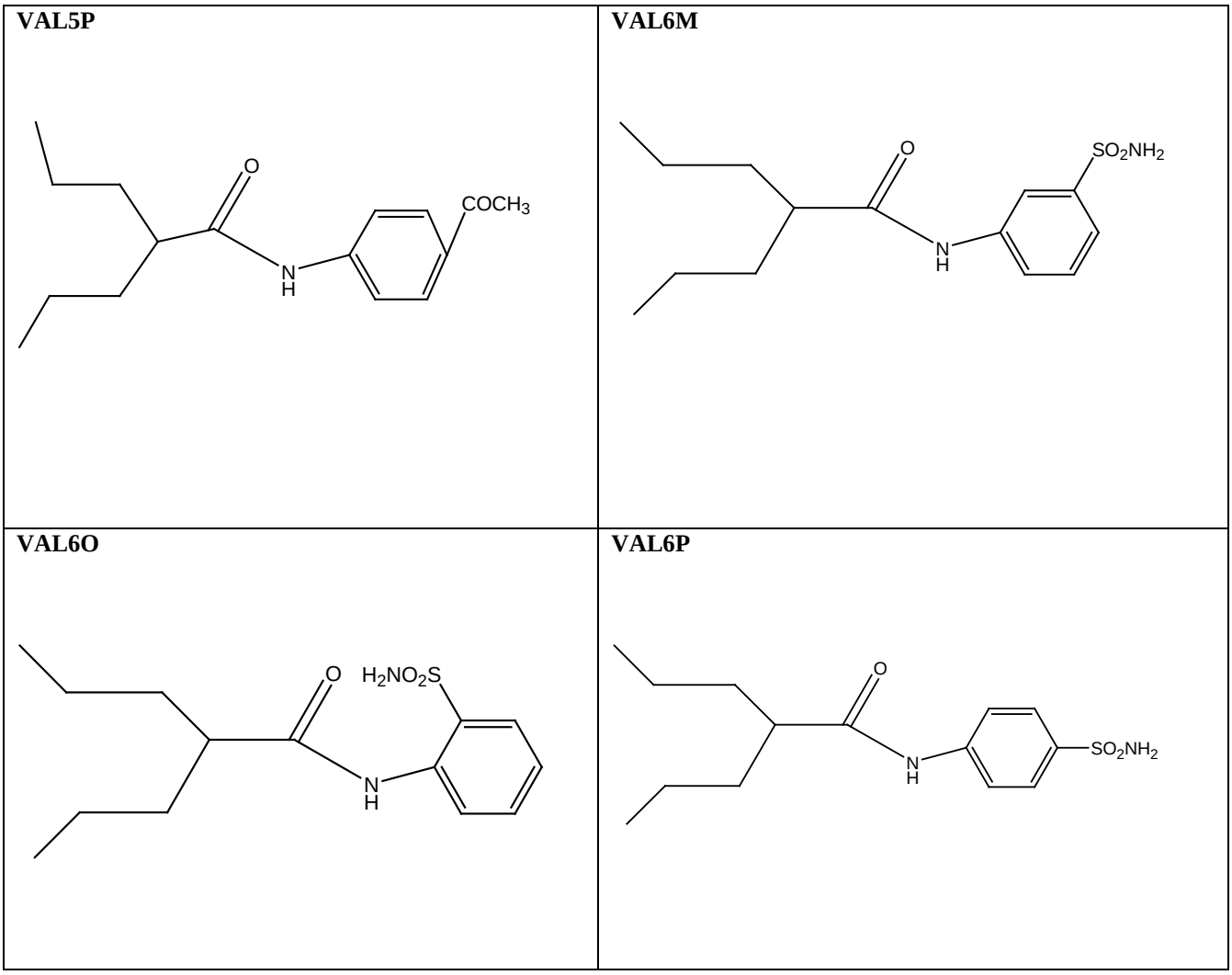


Figure 1. Designed compound of Valproic acid derivatives

2.2. In-Silico Study

In-silico studies are refers to research or experiments conducted by using computer simulations, computational software and models, or data analysis techniques to investigate biological, chemical, or physical phenomenon. The term "*in-Silico*" comes from the Latin word "silicon," referencing the silicon-based computers used to perform these studies. *In-silico* studies often involve the use of software tools, algorithms, and databases to model complex systems, predict outcomes, or analyze large datasets that would be difficult or costly to examine in a laboratory setting [20].

2.2.1. *In-silico* biological activity Spectrum Descriptor of Lipinski rule of 5

A descriptor calculation has been performed in order to observe designed compounds with its drug ability property.

Lipinski rule of five is used to differentiate drug like and non-drug like molecules. It predicts the probability of the two like molecules.

- Molecular mass less than 500Dalton.
- High lipophilicity (AlogP less than 5)
- Less than 5 hydrogen bond donors (nHBD)
- Less than 10 hydrogen bond acceptors (nHBA)
- Molar refractivity (MR) should be between 40-130
- Topological polar surface area (TPSA) should be less than 140

Lipinski rule of five has been performed by the online website named as ADME Calculator which required a smile structure of the compound [20].

2.2.2. *In-silico*/computational Bioassay

Computational Bioassay was performed by the Computational Bioassays for Biochemical Experiments (CBBE) accessed via https:// assay. smallmoles.com/cbbeapps/, I*n-vitro* like computational bioassay (*Iv*LCB); a research product of CBBE (Computational Biology for Biochemical Experiments; www.smallmoles.com) which is a web based software that is designed as a tool for evaluating the newly designed derivatives. [20].

2.2.3. Predictive Toxicology

"Predictive Toxicology" was mainly focusing on *in-silico* approaches and applied almost synonymously to computational toxicology it has later been extended to describe models and assays complementary or as replacement to the classical descriptive in vivo toxicology.

Toxicology forms the backbone of the chemical next-generation risk assessment (NGRA), which integrates NAMs to assure human safety without animal testing.

Bioactivity has been performed by the online tool molpredictx (https://www.molpredictx.ufpb.br/home/) which predict the biological activity of the compounds [21].

2.2.4. Prediction of Bioactivity Score

Bioactivity score of the compound has been calculated by the Web Tool of Cheminformatics Community which is https://www.molinspiration.com/cgi/properties [21].

2.2.5. Docking Studies

Docking was performed with the online tool Swissdock. It is virtual online tool for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. It enables medicinal chemists to run virtual screening form any platform. In this tool we directly paste the smile structure of the drug and the PDB Id of the proteins. This tool directly fetches the data from the protein data bank. It helps users in every steps of this process from data preparation to job submission and analysis of the results [18,19].

3. Result and Discussion

All the designed compounds are given in the figure 1.

3.1. Descriptor of Lipinski Rule of Five

A descriptor calculation has been performed in order to observe designed compounds with its drug ability property.

Lipinski rule of five is used to differentiate drug like and non-drug like molecules. It predicts the probability of the two like molecules.

None of the designed molecules are found to be Lipinski failure. (Table 1) Hence, it implies that all the 12 designed compounds have the drug ability properties/drug likeness properties [20].

3.2. Computational Bioassay by *Iv*LCB (*In-vitro* like Computational Bioassay)

A computational evaluation has been performed by licensed subscription of *Iv*LCB (*In-vitro* like computational bioassay) which estimates the comparison of possible **% inhibition** of novel designed molecule at predefined concentration gradient as we do in *in-vitro* experiments (Table 2). It also shows relation between % inhibition variations on the ground of normalized concentration gradient.

It also predicts activity representative of designed molecules in terms of Q-**Score** & C-Score independently for Query and Control compound along with **Activity Pattern.** Among all designed molecules, molecule VAL1 is found with best Q-score of 4.71 which is closest to control drug. As far as its activity pattern is concern *Iv*LCB shows High activity pattern which supports the molecule for its activity.

It gives an **idea about how our current query compound is close to predefined standard drugs** in the form of regression plot (Figure 2). Our designed molecules keep floating in graph very close to Valproic acid which again supports the designed molecules for its better and positive activity as anti-convulsant molecule.

It can **compare our designed molecule's activity among themselves**, i.e. which one is better among all designed molecules. On comparing all the parameters of *Iv*LCB, designed molecule VAL1 is found to be most active molecule among all [20].

3.3. Predictive Toxicology

"Predictive Toxicology" was mainly focusing on *in-silico* approaches and applied almost synonymously to computational toxicology it has later been extended to describe models and assays complementary or as replacement to the classical descriptive *in-vivo* toxicology.

Toxicology forms the backbone of the chemical next-generation risk assessment (NGRA), which integrates NAMs to assure human safety without animal testing [21].

All the designed molecules are checked for their predictive probability against Dengue larvicida, C. albicans, Leishmania amazonensis, Alpjis gossupii, Alzheimer-NADPH, Promastigote Ldonovani etc. The most active designed compound VAL1 is found to be active with all mentioned above. A predictive probability of all designed compounds are given from Table no. 3 to 14.

3.4. Predictive Bioactivity Score

A prediction bioactivity score estimates a compound's potential effectiveness in the human body. It uses computational models to analyze properties like structure, chemical interactions, and bioavailability, predicting how the compound may interact with biological targets. A higher score suggests a greater chance of beneficial effects, helping researchers prioritize promising drug candidates [21]. All the designed molecules were passed from bioactivity score predictor for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors etc. All predicted scores are given in Table 15.

After analysis it was observed that 7 designed molecules (VAL2, VAL4M, VAL4O, VAL4P, VAL5M, VAL5O & VAL5P) were found to be with highest score for Enzyme inhibition. 4 molecules (VAL1, VAL6M, VAL6O & VAL6P) were found to be with higher score as protease inhibitor. 1 molecule (VAL3) was found to be with higher score as ion channel modulator.

3.5. Descriptor Calculation

Some drug design descriptors were also calculated for general idea of designed molecules which are given in Table 16. All the novel designed compounds were found within the prescribed range.

Table 1. Descriptor of Lipinski rule of Five

COMPOUND NAME	MOL. WEIGHT	H. BOND DONOR	H. BOND ACCEPTOR	LOGP	MOL REFRACTIVITY	TSPA
VALPROIC	144.214	1	1	2.287	40.942	37.3
VAL1	173.256	1	2	1.88	48.458	38.33
VAL2	185.267	1	2	1.865	52.13	46.17
VAL3	233.431	1	1	3.078	76.922	12.03
VAL4M	249.354	1	2	3.85	74.93	38.33
VAL4O	249.354	1	2	3.85	74.93	38.33
VAL4P	249.354	1	2	3.85	74.93	38.33
VAL5M	261.365	1	2	4.044	78.382	46.17
VAL5O	261.365	1	2	4.044	78.382	46.17
VAL5P	261.365	1	2	4.044	78.382	46.17
VAL6M	298.408	2	3	2.489	79.751	89.26
VAL6O	298.408	2	3	2.489	79.751	89.26
VAL6P	298.408	2	3	2.489	79.751	89.26

Table 2. Results found by IvLCB (*in-vitro* like computational bioassay)

Compound name	Q_Score	C_Score	Assay	Inhibition% (alongwith Normalized Conc. Gradient 0-1)	Q_Activity	C_Activity
VAL1	4.71	2.797	GABAA_HomoSapiens	0,37.20,60.94,77.41,89.49,98.75,106.06,111.98,116.87,12 0.99,124.49	High Activity Pattern Available (0.151)	Low Activity Pattern Available (0.148)
VAL2	29.667	3.149	GABAA_HomoSapiens	0,846.71,891.87,908.01,916.30,921.35,924.74,927.19,92 9.02,930.46,931.61	Low Activity Pattern Available (0.193)	Low Activity Pattern Available (0.149)
VAL3	103.417	2.334	GABAA_HomoSapiens	0,4408.44,4419.64,4423.38,4425.26,4426.38,4427.13,44 27.67,4428.07,4428.38,4428.64	High Activity Pattern Available (0.201)	Low Activity Pattern Available (0.147)
VAL4M	49.215	2.455	GABAA_HomoSapiens	0,1956.01,1980.05,1988.19,1992.29,1994.76,1996.40,19 97.58,1998.47,1999.16,1999.71	High Activity Pattern Available (0.221)	Low Activity Pattern Available (0.148)
VAL4O	67.997	2.61	GABAA_HomoSapiens	0,2567.42,2586.20,2592.52,2595.69,2597.59,2598.87,25 99.78,2600.46,2600.99,2601.42	High Activity Pattern Available (0.232)	Low Activity Pattern Available (0.148)
VAL4P	78.396	2.624	GABAA_HomoSapiens	0,2954.55,2971.01,2976.54,2979.31,2980.97,2982.08,29 82.88,2983.47,2983.94,2984.31	High Activity Pattern Available (0.230)	Low Activity Pattern Available (0.148)
VAL5M	192.44	2.966	GABAA_HomoSapiens	0,6472.82,6480.50,6483.07,6484.35,6485.12,6485.63,64 86.00,6486.27,6486.49,6486.66	Low Activity Pattern Available (0.079)	Low Activity Pattern Available (0.148)
VAL5O	115.965	2.009	GABAA_HomoSapiens	0,5755.00,5763.63,5766.51,5767.95,5768.81,5769.39,57 69.80,5770.11,5770.35,5770.54	Low Activity Pattern Available (0.181)	Low Activity Pattern Available (0.147)
VAL5P	79.351	2.729	GABAA_HomoSapiens	0,2873.71,2890.60,2896.28,2899.12,2900.83,2901.97,29 02.79,2903.40,2903.88,2904.26	High Activity Pattern Available (0.229)	Low Activity Pattern Available (0.148)
VAL6M	197.191	2.155	GABAA_HomoSapiens	0,9139.48,9144.93,9146.75,9147.66,9148.21,9148.57,91 48.83,9149.03,9149.18,9149.30	Low Activity Pattern Available (0.075)	Low Activity Pattern Available (0.147)
VAL6O	133.88	2.687	GABAA_HomoSapiens	0,4962.52,4972.49,4975.83,4977.50,4978.50,4979.17,49 79.64,4980.00,4980.28,4980.50	Low Activity Pattern Available (0.151)	Low Activity Pattern Available (0.148)
VAL6P	85.631	2.927	GABAA_HomoSapiens	0,2891.77,2908.56,2914.21,2917.03,2918.73,2919.87,29 20.68,2921.29,2921.76,2922.14	High Activity Pattern Available (0.224)	Low Activity Pattern Available (0.148)

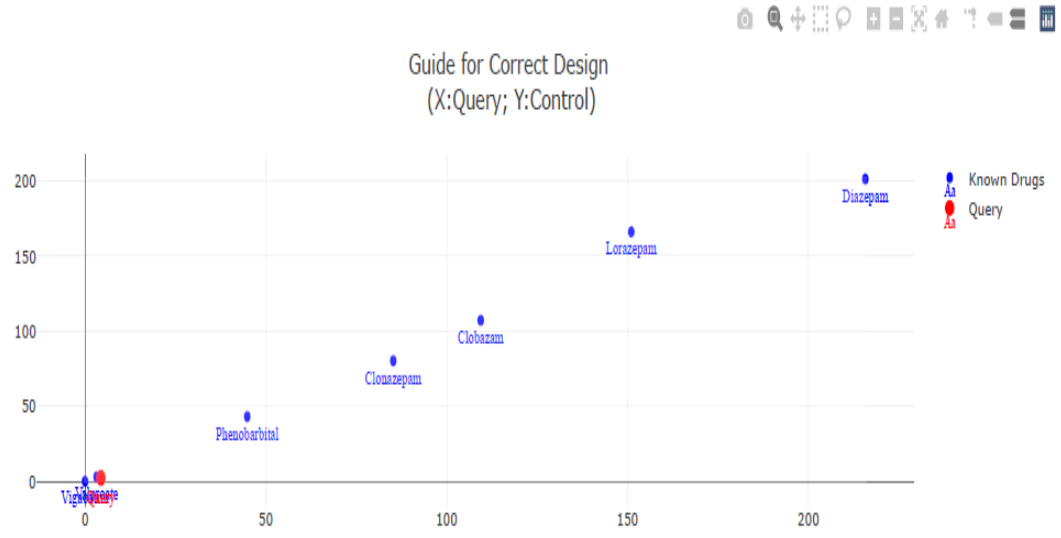


Figure 2. Results found on *Iv*LCB (In-vitro like computational bioassay) web tool for designed molecule VAL1 (Red dot-query) that how close it is to Valproic acid (Blue dot- standard drugs)

Table 3. Predictive Toxicological values for VAL1

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL1	Active	1	1	0	Reliable
2		C_albicans				
	VAL1	Active	1	1	0	Reliable
3		Leishmania amazonensis - Promastigota				
	VAL1	Active	0.8	0.8	0.2	Reliable
4		Alphis gossypii				
	VAL1	Active	0.6	0.6	0.4	Reliable
5		Alzheimer – NADPH				
	VAL1	Active	1	1	0	Reliable
6		Promastigote Ldonovani				
	VAL1	Active	1	1	0	Reliable

Table 4. Predictive Toxicological values for VAL2

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL2	Active	1	1	0	reliable
2		C_albicans				
	VAL2	Active	1	1	0	reliable
3		Alphis gossypii				
	VAL2	Active	1	1	0	reliable
4		Alzheimer - NADPH				
	VAL2	Active	1	1	0	reliable
5		Promastigote Ldonovani				
	VAL2	Active	1	1	0	Reliable
6		Tripomastigote Chagas				
	VAL2	Active	1	1	0	Reliable

Table 5. Predictive Toxicological values for VAL3

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL3	Active	1	1	0	Reliable
2		Leishmania braziliensis				
	VAL3	Active	0.6	0.6	0.4	Reliable
3		Alphis gossypii				
	VAL3	Active	0.8	0.8	0.2	Reliable
4		Promastigote Ldonovani				
	VAL3	Active	0.8	0.8	0.2	Reliable

Table 6. Predictive Toxicological values for VAL4M

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL4M	Active	1	1	0	reliable
2		Leishmania amazonensis - Promastigota				
	VAL4M	Active	0.8	0.8	0.2	reliable
3		Alphis gossypii				
	VAL4M	Active	1	1	0	reliable
4		C albicans				
	VAL4M	Active	1	1	0	reliable
5		Alzheimer - NADPH				
	VAL4M	Active	1	1	0	reliable
6		Amastigote Ldonovani				
	VAL4M	Active	0.8	0.8	0.2	reliable
7		Promastigote Ldonovani				
	VAL4M	Active	0.8	0.8	0.2	reliable
8		Tripomastigote Chagas				
	VAL4M	Active	0.6	0.6	0.4	Reliable

Table 7. Predictive Toxicological values for VAL4O

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL4O	Active	1	1	0	reliable
2		Leishmania amazonensis - Promastigota				
	VAL4O	Active	0.8	0.8	0.2	reliable
3		Leishmania braziliensis				
	VAL4O	Active	1	1	0	reliable
4		Alphis gossypii				
	VAL4O	Active	1	1	0	reliable
5		Alzheimer - iNOS				
	VAL4O	Active	0.6	0.6	0.4	reliable
6		Alzheimer - NADPH				
	VAL4O	Active	1	1	0	reliable
7		Amastigote Ldonovani				
	VAL4O	Active	0.8	0.8	0.2	reliable
8		Promastigote Ldonovani				
	VAL4O	Active	1	1	0	reliable
9		Tripomastigote Chagas				
	VAL4O	Active	1	1	0	Reliable

Table 8. Predictive Toxicological values for VAL4P

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL4P	Active	1	1	0	Reliable
2		C_albicans				
	VAL4P	Active	1	1	0	Reliable
3		Alphis gossypii				
	VAL4P	Active	1	1	0	Reliable
4		Amastigote Ldonovani				
	VAL4P	Active	0.8	0.8	0.2	Reliable
5		Promastigote Ldonovani				
	VAL4P	Active	1	1	0	Reliable

Table 9. Predictive Toxicological values for VAL5M

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL5M	Active	1	1	0	Reliable
2		C_albicans				
	VAL5M	Active	0.6	0.6	0.4	Reliable
3		Leishmania amazonensis - Promastigota				
	VAL5M	Active	0.8	0.8	0.2	Reliable
4		Alphis gossypii				
	VAL5M	Active	0.8	0.8	0.2	Reliable
5		Alzheimer – iNOS				
	VAL5M	Active	0.6	0.6	0.4	Reliable
6		Alzheimer – NADPH				
	VAL5M	Active	1	1	0	Reliable
7		Promastigote Ldonovani				
	VAL5M	Active	0.8	0.8	0.2	Reliable
8		Tripomastigote Chagas				
	VAL5M	Active	0.6	0.6	0.4	Reliable
9		Amastigote Ldonovani				
	VAL5M	Active	0.8	0.8	0.2	Reliable

Table 10. Predictive Toxicological values for VAL5O

S. no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL5O	Active	1	1	0	Reliable
2		Alphis gossypii				
	VAL5O	Active	0.8	0.8	0.2	Reliable

3		Alzheimer - iNOS				
	VAL5O	Active	0.6	0.6	0.4	Reliable
4		Alzheimer – NADPH				
	VAL5O	Active	1	1	0	Reliable
5		Amastigote Ldonovani				
	VAL5O	Active	1	1	0	Reliable
6		Promastigote Ldonovani				
	VAL5O	Active	1	1	0	Reliable
7		Tripomastigote Chagas				
	VAL5O	Active	1	1	0	Reliable

Table 11. Predictive Toxicological values for VAL5P

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL5P	Active	1	1	0	Reliable
2		Salmonella				
	VAL5P	Active	0.8	0.8	0.2	Reliable
3		Hepatite C - Type1				
	VAL5P	Active	0.6	0.6	0.4	Reliable
4		C_albicans				
	VAL5P	Active	0.8	0.8	0.2	Reliable
5		Alphis gossypii				
	VAL5P	Active	0.8	0.8	0.2	Reliable
6		Alzheimer - iNOS				
	VAL5P	Active	1	1	0	Reliable
7		Amastigote Ldonovani				
	VAL5P	Active	0.8	0.8	0.2	Reliable
8		Promastigote Ldonovani				
	VAL5P	Active	1	1	0	Reliable
9		Tripomastigote Chagas				
	VAL5P	Active	0.8	0.8	0.2	Reliable

Table 12. Predictive Toxicological values for VAL6M

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL6M	Active	1	1	0	Reliable
2		Hepatite C - Type1				
	VAL6M	Active	0.6	0.6	0.4	Reliable
3		Leishmania amazonensis - Promastigota				
	VAL6M	Active	0.8	0.8	0.2	Reliable
4		Alphis gossypii				
	VAL6M	Active	0.8	0.8	0.2	Reliable
5		Alzheimer - iNOS				
	VAL6M	Active	0.6	0.6	0.4	Reliable
6		Alzheimer - NADPH				
	VAL6M	Active	1	1	0	Reliable
7		Amastigote Ldonovani				
	VAL6M	Active	0.8	0.8	0.2	Reliable
8		Promastigote Ldonovani				
	VAL6M	Active	1	1	0	Reliable
9		Tripomastigote Chagas				
	VAL6M	Active	0.8	0.8	0.2	Reliable

Table 13. Predictive Toxicological values for VAL6O

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL6O	Active	1	1	0	Reliable
2		Leishmania amazonensis - Promastigota				
	VAL6O	Active	0.6	0.6	0.4	Reliable
3		Alzheimer - iNOS				
	VAL6O	Active	0.8	0.8	0.2	Reliable

4		Alzheimer - NADPH				
	VAL6O	Active	1	1	0	Reliable
5		Amastigote Ldonovani				
	VAL6O	Active	0.8	0.8	0.2	Reliable
6		Promastigote Ldonovani				
	VAL6O	Active	1	1	0	Reliable
7		Tripomastigote Chagas				
	VAL6O	Active	1	1	0	Reliable

Table 14. Predictive Toxicological values for VAL6P

S no	Molecule	Predicted Outcome	Probability	Probability Active	Probability Inactive	Predicted Reliability
1		Dengue larvicida				
	VAL6P	Active	1	1	0	Reliable
2		Hepatite C - Type1				
	VAL6P	Active	0.6	0.6	0.4	Reliable
3		Leishmania infantum - Promastigota				
	VAL6P	Active	0.6	0.6	0.4	Reliable
4		Alphis gossypii				
	VAL6P	Active	0.8	0.8	0.2	Reliable
5		Alzheimer - iNOS				
	VAL6P	Active	0.8	0.8	0.2	Reliable
6		Amastigote Ldonovani				
	VAL6P	Active	0.8	0.8	0.2	Reliable
7		Tripomastigote Chagas				
	VAL6P	Active	0.6	0.6	0.4	Reliable
8		Promastigote Ldonovani				
	VAL6P	Active	0.8	0.8	0.2	Reliable

Table 15. Predictive bioactivity score for the valproic acid derivatives

Compound	GPCR LIGAND	ION CHANNEL MODULATOR	KINASE INHIBITOR	NUCLEARRECEPTOR LIGAND	PROTEASE INHIBITOR	ENZYME INHIBITOR
VALPROIC	-0.83	-0.3	-1.55	-0.78	-0.74	-0.39
VAL1	-0.53	-0.74	-0.58	-1.21	-0.13	-0.14
VAL2	-0.57	-0.53	-1.01	-0.94	-0.38	-0.32
VAL3	-0.33	-0.1	-0.46	-0.42	-0.41	-0.16
VAL4M	-0.35	-0.36	-0.46	-0.46	-0.36	-0.28
VAL4O	-0.35	-0.4	-0.46	-0.49	-0.42	-0.3
VAL4P	-0.34	-0.34	-0.47	-0.42	-0.34	-0.26
VAL5M	-0.31	-0.34	-0.54	-0.44	-0.31	-0.27
VAL5O	-0.3	-0.3	-0.55	-0.39	-0.33	-0.23
VAL5P	-0.3	-0.32	-0.54	-0.43	-0.3	-0.24
VAL6M	-0.29	-0.37	-0.34	-0.59	0.04	0
VAL6O	-0.27	-0.3	-0.4	-0.57	0.12	0.08
VAL6P	-0.28	-0.35	-0.32	-0.54	0.08	0.02

Table 16. Predictive property for the Valproic acid derivatives

Compound	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume2
VALPROIC	2.8	37.3	10	144.21	2	1	0	5	156.79
VAL1	2.62	38.33	12	173.26	3	1	0	6	186.72
VAL2	2.03	46.17	13	185.27	3	1	0	5	196.72
VAL3	5.94	12.03	16	233.43	1	1	1	7	261.08
VAL4M	4.39	38.33	18	249.35	3	1	0	7	258.13
VAL4O	4.37	38.33	18	249.35	3	1	0	7	258.13
VAL4P	4.42	38.33	18	249.35	3	1	0	7	258.13
VAL5M	4.24	46.17	19	261.37	3	1	0	7	268.13
VAL5O	4.21	46.17	19	261.37	3	1	0	7	268.13
VAL5P	4.26	46.17	19	261.37	3	1	0	7	268.13
VAL6M	3.03	89.26	20	298.41	5	3	0	7	275.31
VAL6O	3.01	89.26	20	298.41	5	3	0	7	275.31
VAL6P	3.06	89.26	20	298.41	5	3	0	7	275.31

3.6. Docking

In drug discovery, docking plays an essential role during the initial phases, helping researchers identify promising lead compounds while minimizing the need for costly experimental procedures. It enables the virtual screening of large compound libraries, examining millions of molecules for their potential to interact with the target. This approach not only conserves time and resources but also provides valuable insights into the molecular dynamics between the drug and its target, aiding the further refinement of drugs to improve their effectiveness and safety [18,19].

For performing docking all the receptor have been downloaded from RCSB website with PDB Id 4COFall the designed ligands have been docked with protein (receptor) with online SwissDock tool. (Table 17 & Figure 3)

On docking analysis, designed compound VAL1 has been found to be strongly docked with GABA (A) with 3 hydrogen bonds and binding affinity of: -4.48Kcal/mol. On residue study Lle47, Glu182 and Glu182 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound VAL2 has been found to be docked with GABA (A) with 1 hydrogen bonds and binding affinity of: -4.976Kcal/mol. On residue study Gln185 was found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound VAL3 has been found to be strongly docked with GABA (A) with 1 hydrogen bonds and binding affinity of: -5.528Kcal/mol. On residue study Val50 was found to be significant. On the account of ligand nitrogen atom is significant in binding with donor bonds, whereas significant element in receptor is oxygen.

On docking analysis, designed compound VAL4M has been found to be docked with GABA (A) with 0 hydrogen bonds and binding affinity of: -6.19Kcal/mol.

On docking analysis, designed compound VAL4O has been found to be docked with GABA (A) with 0 hydrogen bonds and binding affinity of: -5.48Kcal/mol.

On docking analysis, designed compound VAL4P has been found to be strongly docked with GABA (A) with 1hydrogen bonds and binding affinity of: -5.93Kcal/mol. On residue study Lys274 was found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen

On docking analysis, designed compound VAL5M has been found to be docked with GABA (A) with 0 hydrogen bonds and binding affinity of: -5.88Kcal/mol.

On docking analysis, designed compound VAL5O has been found to be docked with GABA (A) with 0 hydrogen bonds and binding affinity of: -5.74Kcal/mol.

On docking analysis, designed compound VAL5P has been found to be docked with GABA (A) with 0 hydrogen bonds and binding affinity of: -5.72Kcal/mol.

On docking analysis, designed compound VAL6M has been found to be strongly docked with GABA (A) with 2 hydrogen bonds and binding affinity of: -6.10Kcal/mol. On residue study Glu52 and Thr271 were found to be significant. On the account of ligand nitrogen atom is significant in binding with acceptor bonds, whereas significant element in receptor is oxygen.

On docking analysis, designed compound VAL6O has been found to be strongly docked with GABA (A) with 2 hydrogen bonds and binding affinity of: -5.91Kcal/mol. On residue study Gln185 and Val50 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound VAL6P has been found to be strongly docked with GABA (A) with 2 hydrogen bonds and binding affinity of: -5.78Kcal/mol. On residue study Thr133 and Thr58 were found to be significant. On the account of ligand nitrogen atom is significant in binding with both type of bonds, whereas significant element in receptor is oxygen [25].

Table 17. Docking results of all the compounds with receptor 4COF

Ligands	Affinity Kcal/mol	H-Bond	H-Binding Ligands			H-Binding Receptor			
			Elem	At. ID	Type	Residue	Elem	At.ID	Type
VAL1	-4.489	3	N	9	Donor	Glu 182	O	1399	Acceptor
			O	11	Acceptor	lle 47	N	283	Donor
			O	11	Acceptor	Glu 182	N	1396	Donor
VAL2	-4.976	1	O	12	Acceptor	Gln 185	N	1428	Donor
VAL3	-5.528	1	N	9	Donor	Val 50	O	310	Acceptor
VAL4M	-6.192	0	NA	NA	NA	NA	NA	NA	NA
VAL4O	-5.481	0	NA	NA	NA	NA	NA	NA	NA
VAL4P	-5.937	1	O	1	Acceptor	Lys 274	N	2139	Donor
VAL5M	-5.884	0	NA	NA	NA	NA	NA	NA	NA
VAL5O	-5.746	0	NA	NA	NA	NA	NA	NA	NA
VAL5P	-5.728	0	NA	NA	NA	NA	NA	NA	NA
VAL6M	-6.102	2	N	13	Donor	Thr 271	O	2120	Acceptor
			O	12	Acceptor	Glu 52	N	320	Donor
VAL6O	-5.916	2	O	11	Acceptor	Gln 185	N	1428	Donor
			N	13	Donor	Val 50	O	310	Acceptor
VAL6P	-5.780	2	N	13	Donor	Thr 58	O	442	Both
			N	13	Donor	Thr 133	O	1069	Both

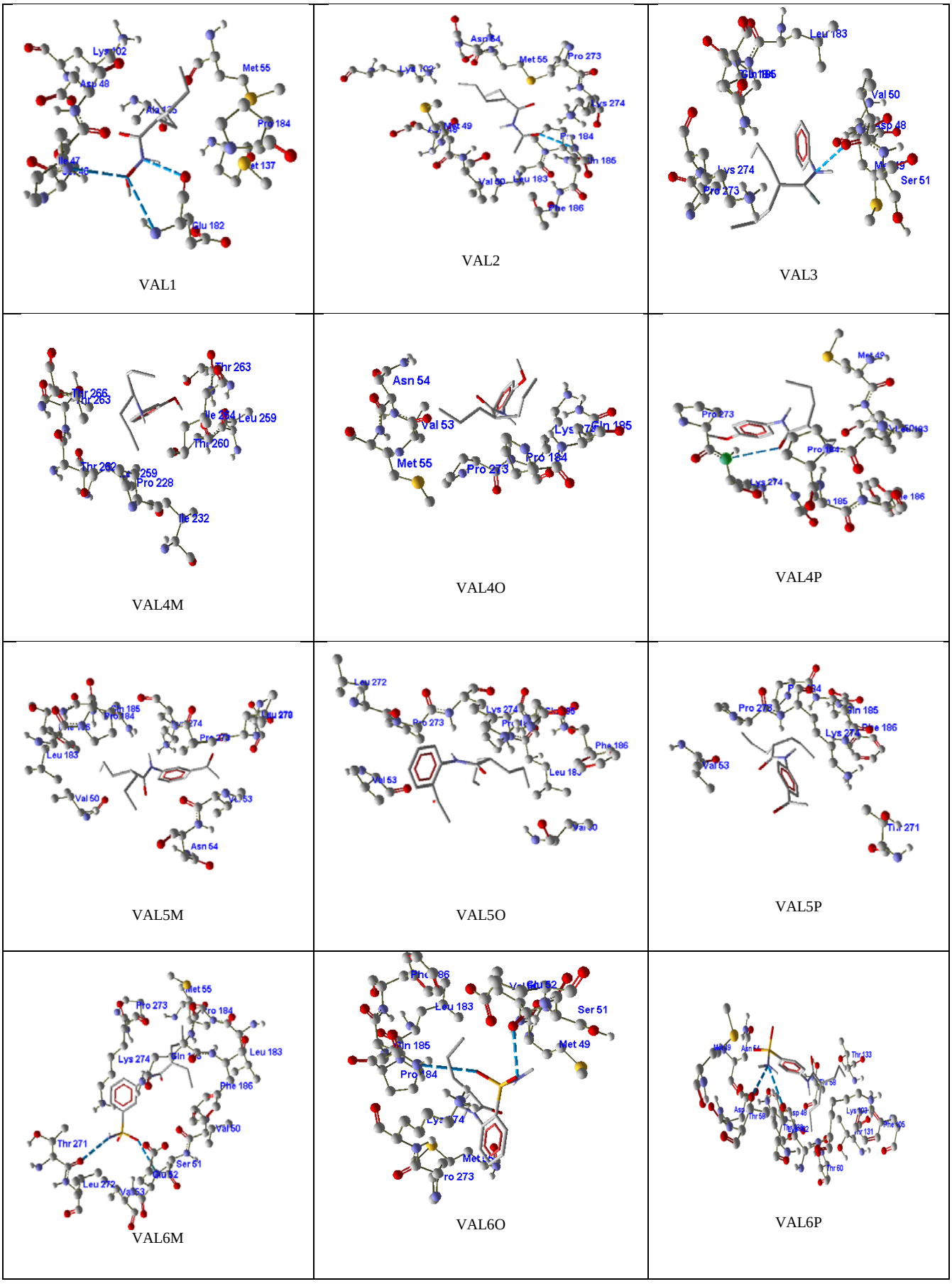


Figure 3. Docking image of the Designed molecules with 4COF

4. Conclusion

Anti-epileptic drugs have dramatically improved the management of epilepsy, allowing many individuals to achieve better control over their seizures. The development of newer AEDs has expanded treatment options and minimized side effects, allowing for more personalized treatment approaches. Gamma-aminobutyric acid (GABA) is the brain's primary inhibitory neurotransmitter, and some anti-epileptic drugs work by enhancing its effects. All novel designed molecules were evaluated by *Iv*LCB: *In-vitro* like computational bioassay and SwissDock. These molecules were also evaluated for their *in-silico* biological activity spectrum & predictive toxicological study by MolpredictX and predictive bioactivity score by Molinspiration. It was found that designed molecule VAL1 shows strong binding ability to PDB ID: 4COF with 3 hydrogen bonds and binding affinity of -4.4 kcal/mol and *Iv*LCB: *In-vitro* like computational bioassay also predicts this compound as active molecule as anti-convulsant molecule. All the above studies supported the molecules for good and potent anti-convulsant activity but then also a biochemical experimental study is required to confirm the findings.

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