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Investigation of structure-activity relationships with molecular docking for some antiepileptic drugs and voltage-gated calcium (CaV) channels

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Abstract: In the study, the active drugs molecules used in the treatment of convulsive seizures occurring in epilepsy disease were used. These molecules; Vigabatrin, Lokosamidin, Zonisamide, Oxcarbazepine, Levetiracetam, Tiagabin, Topiramate, Lamotrigine, Gabapentin, Felbamate, Ethosuximide, Valproic Acid, Mesuximide, Ethotoin, Primidone, Trimethadion, Phenytoin, Remasemide, Mephenytoin. These molecules have been selected considering the physiopathological mechanisms of action of epilepsy. Since the selected molecules are used as a potential antiepileptic agent, they were deemed suitable for molecular insertion studies. In addition, voltage-gated calcium channels, which play an important role in epilepsy, are emphasized. Voltage-gated calcium channels (CaV) act by providing the flow of Ca+ ions during the action potential that triggers seizure formation, and among the ten subtypes of voltage-gated calcium (CaV) channels, CaV3.1- CaV3.3, T-type or abnormal activities are associated with epilepsy, psychiatric form the associated low-voltage-activated subfamily. For this reason, the PDB ID: 6KZP receptor, which acts as an antagonist according to its activity on the channel in the formation of epileptic seizures, was chosen for the molecular insertion study. As a result of molecular placement studies; Oxcarbazepine and Phenytoin gave the best binding affinity for 6KZP with a value of -7.5 kcal/mol. Other results are in descending order (in kcal/mol); Tiagabine (-7.4), Mesuximide (-7.3), Primidone (-7.1), Remacemide (-7.0), Topiramate (-6.9) Mephenytoin (-6.7), Lomotrigine and Ethotoin (-6.4), Locosamide and Zonisamide (-6.1) , Felbamate (-6.0), Levetiracetam and Gabapentin (-5.4), Esuximide (-5.1), Valproic Acid (-4.9), Trimethadione (-4.7), Vigabatrin (-4.4) determined as.

Keywords: Calcium Channels, Epilepsy, Moleculer Docking, Ligand, Receptor, Drug

Öz: Çalışmada epilepsi hastalığında ortaya çıkan konvülsif nöbetlerin tedavisinde kullanılan etken ilaç molekülleri kullanılmıştır. Bu moleküller; Vigabatrin, Lokosamidin, Zonisamid, Okskarbazepin, Levetiracetam, Tiagabin, Topiramat, Lamotrijin, Gabapentin, Felbamat, Ethosuximide, Valproic Asit, Mesuximid, Ethotoin, Primidon, Trimethadion, Phenitoin, Remasemide, Mephenytoin. Bu moleküller epilepsinin fizyopatolojik etki mekanizmaları dikkate alınarak seçilmiştir. Seçilen moleküller potansiyel bir antiepileptik ajan olarak kullanıldığından moleküler yerleştirme çalışmaları için uygun görülmüştür. Ayrıca epilepside önemli rol oynayan voltaj kapılı kalsiyum kanalları üzerinde durulmuştur. Voltaj kapılı kalsiyum kanalları (CaV), nöbet oluşumunu tetikleyen aksiyon potansiyeli sırasında Ca+ iyonlarının akışını sağlayarak hareket eder ve voltaj kapılı kalsiyum (CaV) kanallarının on alt tipi arasından CaV3.1- CaV3.3, T- tipini veya anormal aktivitelerden epilepsi ve psikiyatri ile ilişkili düşük voltajla aktive edilen alt familyayı oluşturmaktadır. Bu nedenle moleküler yerleştirme çalışması için epileptik nöbetlerin oluşumunda kanal üzerindeki aktivitesine göre antagonist görevi gören PDB ID:6KZP reseptörü seçilmiştir. Moleküler yerleştirme çalışmaları sonucunda; Okskarbazepin ve Fenitoin, -7.5 kcal/mol değeri ile 6KZP için en iyi bağlanma afinitesini vermiştir. Diğer sonuçlar azalan sıradadır (kcal/mol olarak); Tiagabin (-7.4), Mesuximide (-7.3), Primidon (-7.1), Remasemide (-7.0), Topiramat (-6.9) Mefenitoin (-6.7), Lomotrigin ve Ethotoin (-6.4), Locosamide ve Zonisamide (-6.1), Felbamat (-6.0), Levetiracetam ve Gabapentin (-5.4), Esuximide (-5.1), Valproik Asit (-4.9), Trimethadion (-4.7), Vigabatrin (-4.4) olarak belirlendi.

Anahtar Kelimeler: Kalsiyum Kanalları, Epilepsi, Moleküler Docking, Ligand, Reseptör, İlaç

1. Introduction

Epilepsy is a neurological disease that affects the majority of the world's population and is characterized by sudden, irregular or excessive neuronal excitation in the gray matter of the brain due to high excitability of the brain and presents symptomatically with seizures [1, 2]. Although there is not much information about the cellular and molecular mechanisms of epileptic seizures, the most well-known mechanism is excessive neuronal firing, which is caused by the disruption of the balance between excitatory and inhibitory voltage-dependent/synaptic transmission. Neurotransmitters are divided into excitatory neurotransmitters and inhibitory neurotransmitters. Excitatory neurotransmitters are acidic amino acids that cause depolarization of the cell by increasing the passage of Na+ and Ca2+ into the cell. Inhibitory neurotransmitters are amino acids that cause hyperpolarization of the cell by increasing the passage of Cl- into the cell

or increasing the outflow of K+ and closing the Na+ and Ca2+ channels in the cell membrane and reducing the entry of Na+ and Ca2+ into the cell. This imbalance in the nervous system causes the development of epilepsy in a certain region of the brain, due to functional disorders in macromolecules involved in excitatory and inhibitory transmission in the epileptic brain [3].

Another mechanism is neuronal membrane and molecular channel changes in ionic conduction [4]. The movement of ions in these synaptic transmissions, which occur in the nervous system through neurotransmitters, creates an action potential in the cell membrane that causes epileptic seizures. The cell membrane has a certain negative voltage (membrane potential) depending on the intracellular and extracellular ion concentration. A nerve cell in the resting phase is polarized and has a membrane potential of about -70 mV to -80 mV. This potential is balanced by ion pumps and ion channels, creating a concentration gradient across the membrane with a greater negative charge inside the cell. With the positive shift of the membrane voltage, the depolarized membrane action potential (AP) is formed, and nerve and muscle cells are stimulated by this potential (Figure 1). AP transmitted along the axon in neurons is transferred to the next neuron via neurotransmitters at the axon tip and neuronal firing is provided. After depolarization, the membrane becomes hyperpolarized by reaching a voltage below the resting potential [5]. This is a response to prevent excessive excitability as a result of successive firings in a healthy nervous tissue, and the membrane quickly returns to the resting phase (polarization). Therefore, the state of extreme excitability; increased excitatory synaptic neurotransmission, decreased inhibitory synaptic neurotransmission, or a change in ion concentration on both sides of the membrane causing depolarization or multiple synchronized sub-threshold excitatory stimuli [6].

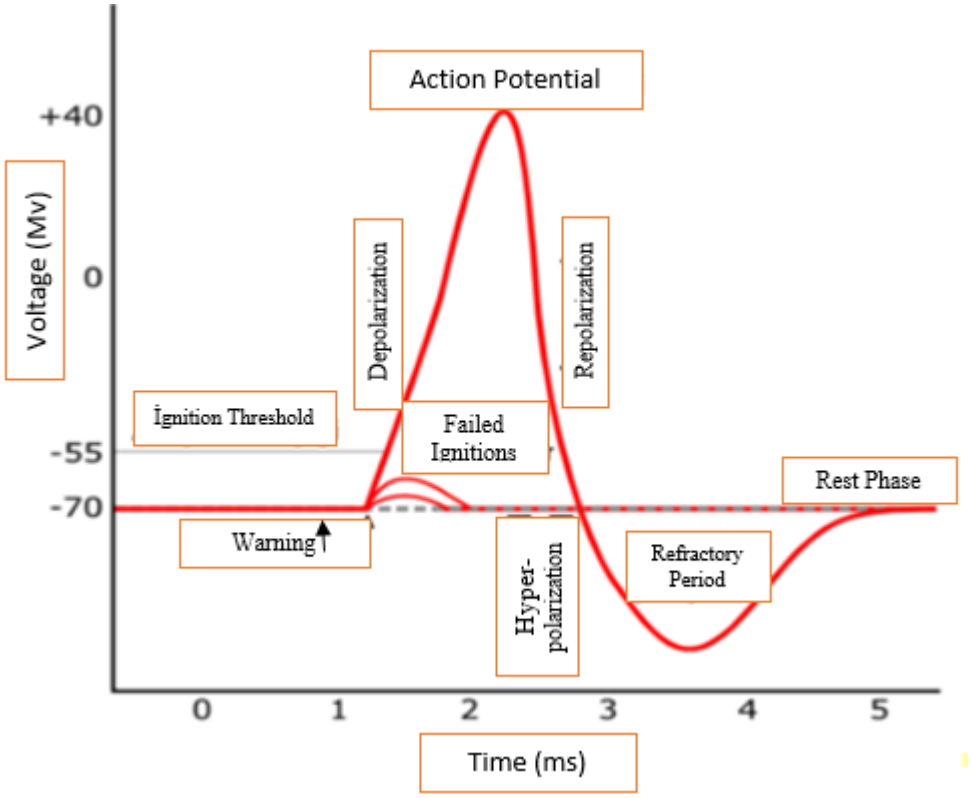


Figure 1. Voltage change during membrane action potential [6]

Treatment methods of epilepsy aim to terminate seizures or reduce the number of seizures with the mentioned mechanisms. Despite the availability of several successful AEDs, some AEDs show toxicity and clinically important drug-drug interactions. Therefore, there is still a need for new drugs with better efficacy and tolerability [7, 8]. Therefore, quantitative structure-activity relationship techniques (QSAR and 3D-QSAR) have been widely used approaches in resolving the action mechanisms of known AEDs or in the design and efficacy of new compounds. These techniques help to predict and improve the activities of different compounds and to identify new compounds with less side-effect profiles [9, 10].

Epilepsy Treatment-Antiepileptic Drugs

The first discovered antiepileptic drugs (bromide, phenobarbital) that suppress the occurrence, spread and severity of seizures have negative properties in terms of keeping seizures under control and side effects [11]. AEDs are classified as first and second generation antiepileptics based on their availability before and after 1990 [12]. Until 1990, only six classical drugs (carbamazepine, ethosuximide, phenobarbital, valproic acid, phenytoin, primidone) could be used for the

treatment of epilepsy, while second generation AEDs (vigabatrin, felbamate, gabapentin, lamotrigine, topiramate, tiagabine, oxcarbazepine, levetire) pregabalin, lacosamide) treatment options have increased [13]. New AEDs have advantages such as higher tolerability, less drug interactions, and no inducing effects on hepatic metabolism enzymes [14]. Sodium channel blockers, calcium channel blockers, glutamate receptor antagonists, GABA receptor agonists and carbonic anhydrase inhibitors are used in the treatment of epilepsy. The part discussed in this study is calcium channels.

Calcium channel blockers; Calcium channels are very important for rhythmic brain activity, especially in the thalamus region. L, N, and T-type calcium channels, especially T-type calcium channels, play a role in absence seizures and their inhibition is important in absence seizures [15]. Voltage-gated calcium channels (CaV) contribute to the general excitability of neurons by transmitting Ca2+ and a small amount of Na+ and play an important role in neuronal firing. It also controls neurotransmitter release from presynaptic nerve endings. In CaV, which has α1, α2, δ1, β subunits, the α1 subunit is functional. The CaV-α1 subunit, four homologous regions (DI-IV), and the selectivity filter with six transmembrane segments (S1-6) forming each region are important components of this subunit. There are several types of the CaV-α1 subunit, and CaV is classified according to the α1 subunit it contains (L-type, P/Q-type, N-type, R-type, T-type). For example, high voltage-dependent channels (type N, P/Q, and R) respond to strong depolarization and control presynaptic neurotransmitter release. Low voltage coupled channels (T-type) respond to normal depolarization and lead to transient currents [16]. Excessive levels of intracellular Ca2+ can lead to neuronal dysfunction (dysfunction) and cell death [17]. Excessive functionality of voltage-gated T-type calcium channels has a negative effect on epilepsy. Calcium ions entering the cell cause excitability and cause seizures. With calcium channel blockade, the formation of epileptic seizures is suppressed by preventing the penetration of Ca2+ ions in high concentrations into the cell.

In the light of the information given in the introduction, the interaction of a total of 19 antiepileptic substances selected according to their active mechanisms with CaV was examined using the computer aided drug design module. These active substances have mechanisms to suppress epileptic seizures. With the molecular docking method, the preferred conformations of one molecule (ligand) to bind to another molecule (receptor) to form a stable complex have been tried to be predicted. Obtained binding energies and resulting bond structures provide important information about the resulting conformations. When we look at the docking studies of known antiepileptics in the literature; Piplani et al. (2016) Homology modelling and molecular docking studies of human placental cadherin protein for its role in teratogenic effects of anti-epileptic drugs article named in the docking study with PDB-1Q55 protein in their anti-epileptic drugs; -5.06 for ethosuximide, -5.27 for felbamate, -6.3 for gabapentin, -5.15 for lacosamide, -6.59 for lamotrigine, -5.00 for leviteracitam, -5.36 for mesuximide, -6.59 for phenytoin, -6.14 for primidone, -9.17 for remacimide, -6.5 for tiagabin, -6.32 for topiramate, -4.63 for valproic acid, -6.13 for vigabatrin and -6.37 Kcal/ for zonisamide , they obtained the mole values [18]. When we compare the results we obtained, it is seen that the values obtained for ethosuximide are almost the same. Felbamate, levetiracetam, mesuximide, phenytoin, primidone, tiagabine, topiramate, valproic acid values gave higher binding affinity in our study; Lower values were obtained for gabapentin, lacosamide, lamotrigine, remacemide, vigabatrin and zonisamide values. In another study, Kundaikar et al. (2015) obtained an affinity value of -3.93 for phenytoin and -3.11 kcal/mol for lamotrigine [19]. Compared to our study, it was seen that our results gave higher binding affinities for both antiepileptics. Results may vary depending on binding sites and target proteins.

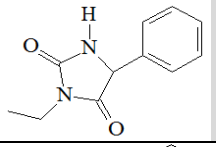
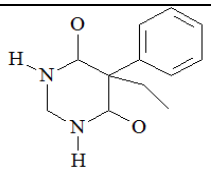
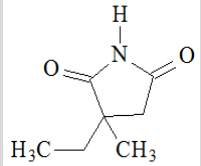
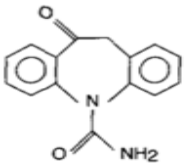
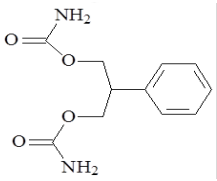
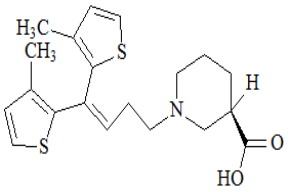
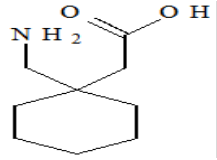
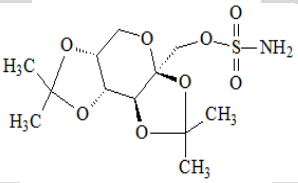
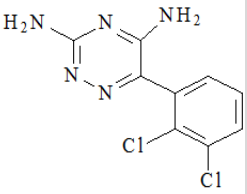
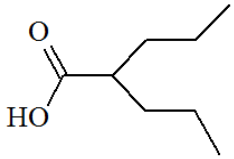
2. Material and Method

Material

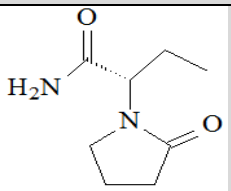
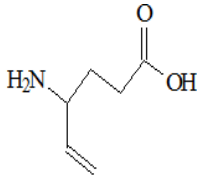
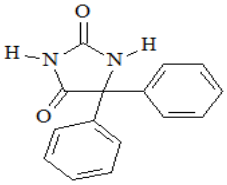
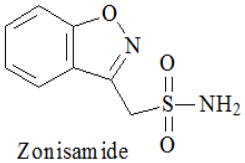
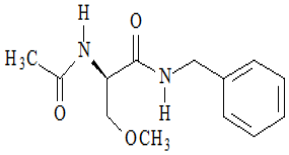
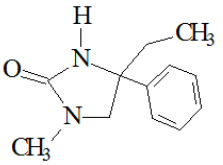
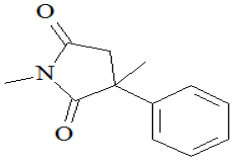
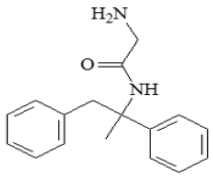
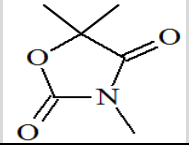
Active Substances Used (Ligand Files)

The 19 active substances selected for the study are listed in Table 1 according to their mechanism of action and their molecular structures are given. The chemical structures of the selected ligand molecules are important. In the results section, it is shown in detail which compound of the ligand molecule and which amino acid structure of the selected macromolecule dock and bond. The molecular structures of 19 active ingredients are listed below. These active ingredients are; Ligands for free download from the database ıt can be listed as [20] It can be listed as; Ethotoin, Primidon, Ethosuximide, Oxcarbazepine, Felbamate, Tiagabine, Gabapentin, Topiramate, Lamotrigine, Valproic acid, Levetiracetam, Vigabatrin, Pheytoin, Zonisamide, Lacosamide, Mephenytoin, Mesuximide, Remadicemide and Trimethadione.

Table 1. Compounds and their action mechanisms of antiepileptic drugs

Compounds	Action Mechanisms	Moleculer Structure
Ethotoin	Na^+ channel inhibition	
Primidone	GABA increase, Glutamate decrease, Na^+, Ca^{2+} channel inhibition, K^+ channel opening	
Ethosuximide	T-type Calcium (Ca^{2+}) channel inhibition	
Oxcarbazepine	Sodium channel inhibition, N, P, R type Ca^{2+} channel inhibition	
Felbamate	Glutamate inhibition, NMDA receptor blockage, increases GABA levels, Na^+ channel blockade, blockade of voltage-gated Ca^{2+} channels	
Tiagabine	Increases GABA concentrations and inhibits GABA-AT	
Gabapentin	Increases GABA levels, Calcium channel modification	
Topiramate	Glutamate reduction, voltage-transition sodium channel inhibition, activation of potassium currents, AMPA and glutamate inhibition, voltage-transition calcium channel inhibition, NMDA inhibition, increase of GABA concentration, inhibition of carbonic anhydrase isoenzyme	
Lamotrigine	Voltage-gated sodium channel inhibition, glutamate reduction, Ca^{2+} channel inhibition	
Valproic acid	Na^+ and T type Ca^{2+} channel inhibition, GABA increase, K^+ channel activity	

continue table….

Compounds	Action Mechanisms	Moleculer Structure
Levetiracetam	Modulation of synaptic vesicle proteins, N, T-type Ca^{2+} Channel inhibition, increases voltage-gated potassium channel conductivity, increases GABA concentration, and inhibits glutamate system by stimulating	
Vigabatrin	GABA increase opens K^+ (potassium) channels, increases Cl^- channel opening, GABA-AT inhibition effect	
Phenytoin	Sodium and calcium channel inhibition activates K^+ channel transmission, increases GABA concentration	
Zonisamide	T-type Calcium channel inhibition, Inhibit Carbonic Anhydrase isoenzyme, voltage-gated Na^+ channel inhibition, inhibit glutamate release	 Zonisamide
Lacosamide	It increases the slow inactivation phase of the Na^+ channel and is responsible for blocking the voltage-gated sodium channel.	
Mephenytoin	Inhibits voltage-gated Na^+ channel conduction	
Mesuximide	T-type Ca^{2+} channel inhibition, partial NMDA inhibition	
Remacemide	Blocks NMDA responses, inhibits voltage-gated Na^+ channel conduction	
Trimethadione	T-type Ca^{2+}, Na^+ channel inhibition, partial NMDA inhibition	

Selected Macromolecule (Receptor Files)

PDB ID: 6KZP was chosen as the receptor in the research conducted with the molecular docking method. It was chosen because it acts as an antagonistic molecule for voltage-gated calcium channels [21]. 6KZP; 3.1 Cryo-EM structures of human CaV bound to Apo and antagonist with its calcium channel ligand structure is a macromolecule studied (Figure 2). Voltage-gated calcium channel subunit 3.1 (CaV3.1) is a macromolecule that will act as a blockade antagonist of epileptic seizures. Among the ten subtypes of voltage-gated calcium (CaV) channels, CaV3.1-CaV3.3 constitute the T-type, or low voltage-activated subfamily, whose abnormal activities are associated with epilepsy, psychiatric.

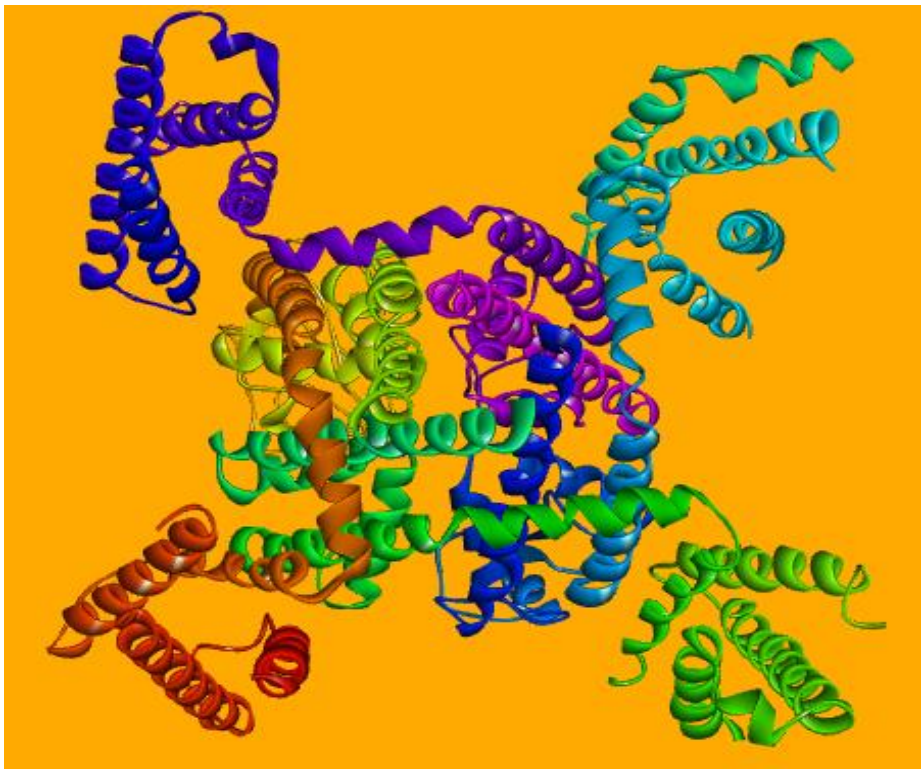


Figure 2. 3D image of 6KZP macromolecule

Method

Working Stages

Firstly, the macromolecule obtained from the databases was saved in the receptor.pdb format by removing the previously studied ligands in the Discovery Studio program, and the ligands were recorded in the same format as ligand.pdb. Afterwards, water is removed from macromolecules in Autodock Tolls program, only polar hydrogens are added and the molecule is saved in receptor.pdbqt format. Then, after the torsion root and the number of rotatable bonds were determined in the ligands, they were recorded in ligand.pdbqt format.

In the next step, the grid was applied to the molecules and the grid dimensions and coordinates were recorded in the configuration file. From the calculated RMSD (root-mean-square deviation) values, the conformation less than 2Å is attributed to the effective binding with the receptor. In the Discovery Studio program, which is a modeling program, 2D and 3D images are obtained, the best value conformation from the out file is placed into the macromolecule. By leaving the conformation with the lowest RMSD value inside the molecule, bond properties and lengths were examined.

Moleculer Docking Analysis

Preparation of Target Protein

As explained in the introduction 6KZP coded molecular structure were selected according to their effect potentials on ligands and active site amino acids were determined by using the DSV (Discovery Studio) [22] program for chelating calculations. After this prepared structure was optimized, it was saved in receptor.pdb format for use in clamping calculations, and the prepared structure was opened with the Autodock Vina Tolls (ADT) program and all crystalline waters in the active region were deleted. Finally, polar hydrogens were added to the structure and saved in .pdbqt format. At the end of the preparation process, active region coordinates were selected to determine the grid structure and conf. saved to file.

The 6KZP macromolecule was selected according to its mechanism of creating an antagonistic effect for neurotransmitters in epilepsy disease, and it shows the potential to interact well on the action mechanisms of certain ligands in the study. In this study, the active binding sites of the 6KZP macromolecule consisting of only the A chain were determined for the docking study and the following amino acid active sites were selected for the binding sites; GLU 354, PHE 917, LEU 920, GLN 922, GLY 951, ASN 952, PHE 956, LYS 1462, ASP 1463, VAL 1505, THR, 1777. The research area was determined as 375Å interval, the grid size was determined as 56Å×54Å×52Å for all ligands. The location of this search area is set for all ligands, the X, Y and Z coordinates of the center are set as

172.839, 171.636 and 186.780 and recorded for each in the conf.txt file. The data obtained for all pdb files are shown later in the results section in the form of tables and figures.

Preparation of Selected Ligands

The 19 ligands in the study were selected according to the features that can convulse seizures in epilepsy disease. First of all, 19 of our substances to be used as ligands were downloaded as .sdf files from the database specified in the material method section. The .sdf file downloaded with the Discovey Studio program has been optimized [20] and the structure saved as ligand.pdb has been prepared for clamping with the help of the ADT user interface. After determining the torsion root of the ligands prepared in DSV in Autodock Vina Tolls program, the number of rotatable ligaments was determined and the file was saved in ligand.pdbqt format.

Modeling Work with Discovery Studio Visualizer After ADT

It has been stated above that ligands are set in ligand.pdbqt format and macromolecules are set in receptor.pdbqt format with the ADT program, which acts as an interface in the Autodock Vina clamping study. In the Discovery studio program, after calling the receptor.pdb file, which was purified from water in the first stage, the out.pdbqt file containing the conformations obtained from the molecular dockingstudy with ADT was opened and the conformation with the lowest affinity value and RMSD value below 2Å was copied from the out.pdbqt file and pasted into the receptor. By performing 3-dimensional and 2-dimensional modeling, bonding properties, bond type and bond lengths were obtained and the data are shown in the results section.

3. Result and Discussion

In the Discovery Studio 2020 Client program, the conformation that gives the best value was placed inside the 6KZP macromolecule to see 2D and 3D structures. The binding affinity values obtained for ligands as a result of this calculation are summarized in Table 2.

Table 2. The binding affinity values of ligands placed in 6KZP at the best conformation

Ligand's	Best Binding affinity (kcal/mol)	Distance from best mode (Å)	
		RMSD l.b	RMSD u.b
Vigabatrin	-4.4	0.000	0.000
Lacosamide	-6.1	0.000	0.000
Zonisamide	-6.1	0.000	0.000
Oxcarbazepine	-7.5	0.000	0.000
Levatiracetam	-5.4	0.000	0.000
Tiagabine	-7.4	0.000	0.000
Topiramate	-6.9	0.000	0.000
Lamotrigine	-6.4	0.000	0.000
Gabapentin	-5.4	0.000	0.000
Felbamate	-6.0	0.000	0.000
Ethosuximide	-5.1	0.000	0.000
Valproic acid	-4.8	0.000	0.000
Mesuximide	-7.3	0.000	0.000
Ethotoin	-6.4	0.000	0.000
Primidone	-7.1	0.000	0.000
Trimethadione	-4.7	0.000	0.000
Phenytoin	-7.5	0.000	0.000
Remacemide	-7.0	0.000	0.000
Mephenytoin	-5.7	0.000	0.000

When the results were examined, Oxcarbazepine and Phenytoin gave the best binding affinity with 6KZP macromolecule -7.5 kcal/mol. Oxcarbazepine and Phenytoin gave the best binding affinity for 6KZP with a value of -7.5 kcal/mol. When the other ligand results are examined, we can rank the results in descending order (as kcal/mol); Tiagabine (-7.4), Mesuximide (-7.3), Primidone (-7.1), Remasemide (-7.0), Topiramate (-6.9) Mephenytoin (-6.7), Lomotrigine and Ethotoin (-6.4), Locosamide and Zonisamide (-6.1), Felbamate (-6.0), Levetiracetam and Gabapentin (-5.4), Esuximide (-5.1), Valproic Acid (-4.9), Trimethadione (-4.7), Vigabatrin (-4.4). The bond types, bond lengths, binding energies and 3D structures of ligand-receptor conformations are presented in detail below.

Vigabatrin

According to the results of the molecular docking analysis, the vigabatrin macromolecule was embedded in 6KZP. The binding affinities and RMSD values for the ligand vigabatrin embedded in the macromolecule 6KZP (Figure 2) are given in Table 2. The binding affinity value at the best binding position of the ligand vigabatrin placed on the macromolecule 6KZP was obtained as -4.4 kcal/mol. The 2D and 3D pictures of the intermolecular interactions between the conformation of vigabatrin in the best binding mode and the macromolecule 6KZP are shown in Figure 3. In this and all other ligands hereafter, the A chain is used for the docking process of the 6KZP macromolecule, and the amino acid active sites for the binding sites are as follows; GLU 354, PHE 917, LEU 920, GLN 922, GLY 951, ASN 952, PHE 956, LYS 1462, ASP 1463, VAL 1505, THR 1777. The ligand-protein interactions obtained were as follows; The LYSA1462 amino acid binding site formed a double bonded oxygen atom with a 3.97 Aº alkyl bond interaction. GLUA354 and GLNA922 amino acid binding sites formed a conventional hydrogen bond interaction with the hydroxyl group of 2.36 Aº and 2.12 Aº lengths, respectively. GLNA922 amino acid active site are 2.55 Å length of with amine they formed a conventional hydrogen bond interaction. And GLNA923 amino acid active site respectively are 2.30 Å and 2.73 Å with amine they formed a conventional hydrogen bond interaction with a length of (Table 3).

Table 3. Interactions, types and distances between vigabatrin and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
LYS_A1462	O atom in Vigabatrin	3.97	Alkyl
GLU_A354	Hydroxyl group in Vigabatrin	2.36	Conventional hydrogen bond
GLN_A922	Hydroxyl group in Vigabatrin	2.12	Conventional hydrogen bond
GLN_A922	NH_2 compound in Vigabatrin	2.55	Conventional hydrogen bond
GLN_A923	NH_2 compound in Vigabatrin	2.30	Conventional hydrogen bond
GLN_A923	NH_2 compound in Vigabatrin	2.73	Conventional hydrogen bond

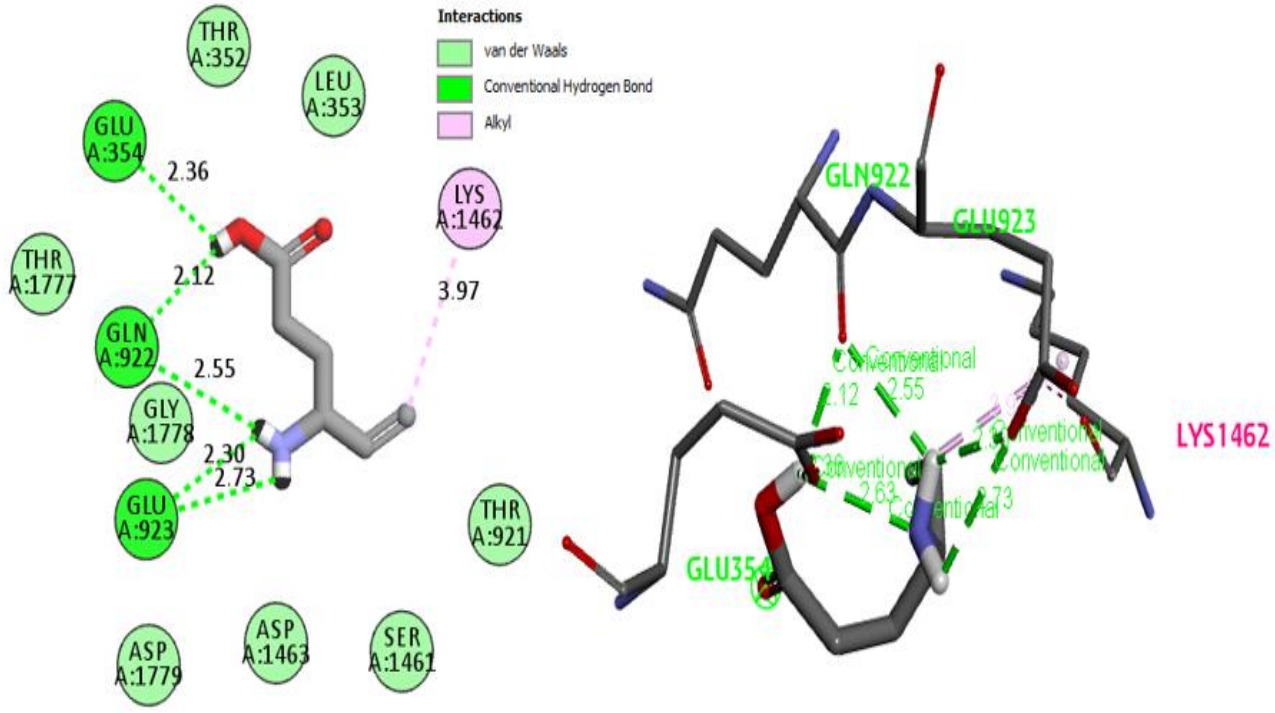


Figure 3. 2D and 3D representation of Vigabatrin and 6KZP macromolecules by molecular docking method

Lacosamide

The binding affinities and RMSD values for the ligand locosamide embedded in the macromolecule 6KZP are given in Table 2. The binding affinity value of the ligand locosamide placed in the macromolecule 6KZP at the best binding position was -6.1 kcal/mol, and the 2D and 3D visualizations of the intermolecular interactions between the locosamide's best binding mode position and the macromolecule 6KZP are shown in in appendix 1. The resulting ligand-protein interactions were as follows; The amino acid active site LEUA1819 formed a 3.79Å long pi-sigma bond interaction with the benzene group (Table 4).

Table 4. Interactions, types and distances between lacosamide and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
LEU_A1819	Benzen group in Lacosamide	3.79	Pi-Sigma

Zonisamide

The binding affinity value at the best binding position of ligand zonosamide placed on the macromolecule 6KZP was -6.1 kcal/mol, and the 2D and 3D visualizations of the intermolecular interactions between the zonosamide best binding mode position and the macromolecule 6KZP are shown in in appendix 2. The resulting ligand-protein interactions were as follows; The THRA921 amino acid binding site formed a 2.37Å-long conventional hydrogen bond interaction with the amine group. The LEUA920 amino acid active site formed a 4.51Aº long pi-alkyl bond interaction with the benzoxoazalin compound. The amino acid active sites of PHEA956 and LEUA872 formed a pi-pi T-shaped and pi-alkyl bond interaction with the benzene group with a length of 5.06Aº and 4.69Aº. Additionally, the types and distances of interactions are summarized in Table 5 in detail.

Table 5. Interactions, types and distances between zonisamide and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
THR_A921	NH_2 compound in Zonisamide	2.37	Conventional hydrogen bond
LEU_A920	Benzaxazoline group in Zonisamide	4.51	Pi-Alkyl
PHE_A956	Benzen group in Zonisamide	5.06	Pi-Pi T- shaped
LEU_A872	Benzen group in Zonisamide	4.69	Pi-Alkyl

Oxcarbazepine

The binding affinity value at the best binding position of the ligand oxcarbazepine placed in 6KZP was obtained as -7.5 kcal/mol. 2D and 3D visualizations of the intermolecular interactions between the oxcarbazepine best binding mode position and the macromolecule 6KZP are shown in appendix 3 and the resulting ligand-protein interactions were as follows; The amino acid active sites ALAA1502 and LEUA872 formed pi-alkyl and pi-sigma bond interactions with the benzene group of 4.52Å and 3.41Å lengths (Table 6).

Table 6. Interactions, types and distances between oxcarbazepine and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ALA_A1502	Benzen group in Oxcarbazepine	4.52	Pi-Alkyl
LEU_A872	Benzen group in Oxcarbazepine	3.41	Pi-Sigma

Levatiracetam

The binding affinity value of levatiracetam at the best binding position of the ligand inserted into 6KZP was -5.4 kcal/mol, and 2D and 3D visualizations of the intermolecular interactions between the levatiracetam best binding mode position and the macromolecule 6KZP are shown in appendix 4. The resulting ligand-protein interactions are as follows; The amino acid binding sites ASNA926, GLUA324 and ASPA924 formed a conventional hydrogen bond interaction with the amine group of 2.67Aº- 2.26Aº and 1.98Å lengths, respectively. ASPA924 amino acid active site formed a carbon-hydrogen bond interaction with the nitrogen atom with a length of 3.73Å, respectively. LYSA927 amino acid active site formed a 3.77Å long carbon hydrogen bond interaction with the O atom (Table 7).

Table 7. Interactions, types and distances between levatiracetam and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ASN_A926	NH_2 compound in levatiracetam	2.67	Conventional hydrogen bond
GLU_A354	NH_2 compound in levatiracetam	2.26	Conventional hydrogen bond
ASP_A924	NH_2 compound in levatiracetam	1.98	Conventional hydrogen bond
ASP_A924	Nitrogen atom in levatiracetam	3.73	Carbon hydrogen bond
LYS_A927	O atom in levatiracetam	3.77	Carbon hydrogen bond

Tiagabine

The binding affinity value at the best binding position of ligand tiagabine placed in 6KZP was -7.4 kcal/mol, and 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of tiagabine and the macromolecule 6KZP are shown in Appendix 5. The resulting ligand-protein interactions were as follows; The PHEA1817 amino acid active site formed a 5.52Å alkyl bond interaction with the S atom. The amino acid binding sites VALA1820 and VALA1505 formed an alkyl bond interaction with the methyl group, with a length of 3.77Aº and 4.92Aº, respectively. The benzene group and VALA1505, LEUA1813 and ALAA1460 amino acid active sites formed 5.49Å-5.25Å and 5.29Å long alkyl bond interactions, respectively. The amino acid binding sites VALA1820, VALA1512 and META1508 formed pi-alkyl, pi-alkyl, pi-sulfur and pi-alkyl bond interactions with the methylthiaphene group in lengths of 4.82Å-5.31Aº- 5.58Å and 5.26Aº, respectively. The amino acid active sites VALA1505 and META1508 formed an alkyl bond interaction of 3.77Å and 4.70Å with the methylene group (Table 8).

Table 8. Interactions, types and distances between tiagabine and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
VAL_A1820	Methyl group in Tiagabine	3.77	Alkyl
VAL_A1820	Methylthiophene group in Tiagabine	4.82	Pi-Alkyl
VAL_A1512	Methylthiophene group in Tiagabine	5.31	Pi-Alkyl
PHE_A1817	S atom in Tiagabine	5.52	Pi-Sulfur
VAL_A1505	Methyl group in Tiagabine	4.92	Alkyl
VAL_A1505	CH_2 group in Tiagabine	3.77	Alkyl
VAL_A1505	Benzen group in Tiagabine	5.49	Alkyl
LEU_A1813	Benzen group in Tiagabine	5.25	Alkyl
ALA_A1460	Benzen group in Tiagabine	5.29	Alkyl
MET_A1508	CH_2 group in Tiagabine	4.70	Alkyl
MET_A1508	Methylthiophene group in Tiagabine	5.58	Pi-Sulfur
MET_A1508	Methylthiophene group in Tiagabine	5.26	Pi-Alkyl

Topiramate

The binding affinity value of the ligand topiramate placed in 6KZP at the best binding position was obtained as -6.9 kcal / mol. The 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of topiramate and the macromolecule 6KZP are shown in appendix 6, and after the molecular docking study, the interactions were as follows; The amino acid active sites PHEA362, PHEA362 and TYRA361 formed an alkyl bond interaction with the methyl group of 4.92Aº- 4.64Aº and 5.33Å lengths. The TYRA361 amino acid binding site formed a 5.05Å long pi-sulfur bond interaction with the S atom (Table 9).

Tablo 9. Interactions, types and distances between topiramate and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
PHE_A362	CH_3 compound in Topiramate	4.92	Alkyl
PHE_A362	CH_3 compound in Topiramate	4.64	Alkyl
TYR_A361	CH_3 compound in Topiramate	5.33	Alkyl
TYR_A361	S atom in Topiramate	5.05	Pi-Sulfur

Lamotrigine

The binding affinity value at the best binding position of the ligand lamotragine placed in 6KZP was obtained as -6.4 kcal/mol. 2D and 3D pictures of the intermolecular interactions between the position in the best binding mode of lamotragine and the macromolecule 6KZP are shown in appendix 7. The resulting ligand-protein interactions are as follows; SERA1461, GLYA1778, GLNA922, THRA352, LEUA353 and GLUA354 amino acid active sites formed a conventional hydrogen bond interaction of 2.45Aº-2.59Aº-2.97Aº-2.27Å-2.88Å and 2.13Å lengths, respectively, with the amine compound. LYSA1482 amino acid active site formed a 3.86Aº alkyl bond interaction with the methyl group (Table 10).

Table 10. Interactions, types and distances between lamotrigine and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
SER_A1461	NH_2 compound in Lamotrigine	2.45	Conventional hydrogen bond
GLY_A1778	NH_2 compound in Lamotrigine	2.59	Conventional hydrogen bond
GLN_A922	NH_2 compound in Lamotrigine	2.97	Conventional hydrogen bond
THR_A352	NH_2 compound in Lamotrigine	2.27	Conventional hydrogen bond
LEU_A353	NH_2 compound in Lamotrigine	2.88	Conventional hydrogen bond
GLU_A354	NH_2 compound in Lamotrigine	2.13	Conventional hydrogen bond
LYS_A1482	CH_3 compound in Lamotrigine	3.86	Alkyl

Gabapentin

The binding affinity value at the best binding position of the ligand gabapentin placed in 6KZP was obtained as -5.4 kcal/mol. 2D and 3D pictures of the intermolecular interactions between the position in the gabapentin best binding mode and the macromolecule 6KZP are presented in appendix 8. The resulting ligand-protein interactions were as follows; The amino acid active sites ASNA952 and LEUA920 formed a conventional hydrogen bond interaction with the amine compound with a length of 2.01Aº and 2.90Aº, respectively. The amino acid active sites LEUA920 and LEUA872 formed an alkyl bond interaction with the benzene group of 5.05Å and 4.88Å lengths, respectively. The GLYA951 amino acid active site formed an unfavorable acceptor-acceptor bond interaction of 2.77Aº with the carbon monoxide group (Table 11).

Table 11. Interactions, types and distances between gabapentin and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ASN_A952	NH_2 compound in Gabapentin	2.01	Conventional hydrogen bond
LEU_A920	NH_2 compound in Gabapentin	2.90	Conventional hydrogen bond
LEU_A920	Benzen group in Gabapentin	5.05	Alkyl
LEU_A872	Benzen group in Gabapentin	4.88	Alkyl
GLY_A951	Carbon monoxide group in Gabapentin	2.77	Unfavorable Acceptor-Acceptor

Felbamate

The binding affinity value at the best binding position of the ligand felbamate placed on the macromolecule 6KZP was obtained as -6.0 kcal/mol. The 2D and 3D visualizations of the intermolecular interactions between the felbamate best binding mode position and the macromolecule 6KZP are shown in appendix 9 and the interactions after the molecular docking study are as follows; The amino acid active sites ILEA387, ASNA388, SERA383, SERA383 and ILEA351 formed a conventional hydrogen bond interaction with the amine group of 2.75Aº-2.01Aº-2.66Aº-2.34Å and 2.74Aº lengths, respectively. PHEA956 amino acid active site formed a 4.53Aº long pi-pi stacked bond interaction with the benzene group. THRA352 amino acid active site formed a 3.57Å long carbon hydrogen bond interaction with the O atom (Table 12).

Table 12. Interactions, types and distances between felbamate and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ILE_A387	NH_2 compound in Felbamate	2.75	Conventional hydrogen bond
ASN_A388	NH_2 compound in Felbamate	2.01	Conventional hydrogen bond
SER_A383	NH_2 compound in Felbamate	2.66	Conventional hydrogen bond
SER_A383	NH_2 compound in Felbamate	2.34	Conventional hydrogen bond
ILE_A351	NH_2 compound in Felbamate	2.74	Conventional hydrogen bond
THR_A352	O atom in Felbamate	3.57	Carbon hydrogen bond
PHE_A956	Benzene group in Felbamate	4.53	Pi-Pi Stacked

Ethosuximide

The binding affinity value at the best binding position for the ligand ethosuximide inserted in 6KZP was -5.1 kcal/mol, and 2D and 3D visualizations of the intermolecular interactions between the best binding mode position and the macromolecule 6KZP in ethosuximide are presented in Appendix 10. After the study, the interactions were as follows; The amino acid active sites ASNB952 and LEUA920 formed a conventional hydrogen bond interaction with the amine group of 2.30Å and 2.28Å lengths, respectively. The amino acid active sites LEUA920 and LEUA872 formed an alkyl

bond interaction with the methylene group of 4.27Å and 4.22Å lengths, respectively. The PHEA956 amino acid active site formed a 5.30Å long pi-alkyl bond interaction with the methyl group (Table 13).

Table 13. Interactions, types and distances between ethosuximide and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ASN_B952	NH_2 compound in Ethosuximide	2.30	Conventional hydrogen bond
LEU_A920	NH_2 compound in Ethosuximide	2.28	Conventional hydrogen bond
LEU_A920	CH_2 compound in Ethosuximide	4.27	Alkyl
LEU_A872	CH_2 compound in Ethosuximide	4.22	Alkyl
PHE_A956	CH_3 compound in Ethosuximide	5.30	Pi-Alkyl

Valproic Acid

The binding affinity value of the ligand valproic acid placed in the 6KZP in the best binding position was obtained as -4.8 kcal/mol, and the 2D and 3D pictures of the intermolecular interactions between the position of the valproic acid in the best binding mode and the macromolecule 6KZP are presented in appendix 11. After molecular chelation, the interactions are as follows; ILEA380 amino acid active sites formed alkyl bond interactions with the methylene group of 4.39Aº and 4.53Aº lengths, respectively. The amino acid active sites PHEA384, PHEA385, PHEA1727 and PHEA1773 formed a pi-alkyl bond interaction with the methylene group of 4.36Å-4.75Å-4.84Aº and 5.12Aº lengths, respectively. The ILEA379 amino acid active site formed a classical hydrogen bond interaction with the hydroxyl group of 2.54Å (Table 14).

Tablo 14. Interactions, types and distances between valproic acid and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ILE_A379	Hydroxyl group in Valproic acid	2.54	Conventional hydrogen bond
ILE_A380	Methylene group in Valproic acid	4.39	Alkyl
ILE_A380	Methylene group in Valproic acid	4.53	Alkyl
PHE_A384	Methylene group in Valproic acid	4.36	Pi-Alkyl
PHE_A1727	Methylene group in Valproic acid	4.84	Pi-Alkyl
PHE_A1773	Methylene group in Valproic acid	5.12	Pi-Alkyl
PHE_A385	Methylene group in Valproic acid	4.75	Pi-Alkyl

Mesuximide

The binding affinity value at the best binding position of the ligand mesuximide placed in 6KZP was obtained as -7.3 kcal/mol. 2D and 3D visualizations of the intermolecular interactions between the mesuximide best binding mode position and the macromolecule 6KZP are presented in appendix 12. The resulting ligand-protein interactions are as follows; Amino acid active sites of ILEA380, LEUA1819 and PHEA1773 formed pi-alkyl, pi-alkyl and pi-pi stacked bond interactions with the benzene group in lengths of 5.01Å-5.08Å and 5.91Å, respectively. PHEA1727, META1728 and GLYA1724 amino acid active sites formed pi-alkyl, alkyl and carbon hydrogen bond interactions with the methyl group of 4.65Å-4.83Å and 3.69Å lengths, respectively. The GLYA1724 amino acid active site formed a 3.29Aº long carbon hydrogen bond interaction with the O atom (Table 15).

Tablo 15. Interactions, types and distances between mesuximide and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ILE_A380	Benzen group in Mesuximide	5.01	Pi-Alkyl
LEU_A1819	Benzen group in Mesuximide	5.08	Pi-Alkyl
PHE_A1773	Benzen group in Mesuximide	5.91	Pi-Pi Stacked
PHE_A1727	CH_3 group in Mesuximide	4.65	Pi-Alkyl
MET_A1728	CH_3 group in Mesuximide	4.83	Alkyl
GLY_A1724	CH_3 group in Mesuximide	3.69	Carbon hydrogen bond
GLY_A1724	O atom in Mesuximide	3.29	Carbon hydrogen bond

Ethotoine

The binding affinity value at the best binding position of the ligand ethotoin placed in 6KZP was obtained as -6.4 kcal/mol. The 2D and 3D pictures of the intermolecular interactions between the position in the best binding mode of ethotoin and the macromolecule 6KZP are shown in appendix 13. After the study, the interactions were as follows; The amino acid active sites of ILEA380, LEUA1819 and PHEA384 formed pi-alkyl, pi-alkyl and pi-pi t-shaped bond interactions with the benzene group of 4.67Aº-5.29Aº and 4.98Å lengths, respectively. GLYA1724 amino acid active site formed a carbon hydrogen bond interaction with the O atom of 3.49Å length (Table 16).

Tablo 16. Interactions, types and distances between ethotoine and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
ILE_A380	Benzen group in Ethotoine	4.67	Pi-Alkyl
LEU_A1819	Benzen group in Ethotoine	5.29	Pi-Alkyl
PHE_A384	Benzen group in Ethotoine	4.98	Pi-Pi T- shaped
GLY_A1724	O atom in Ethotoine	3.49	Carbon hydrogen bond

Primidone

The binding affinity value at the best binding position of the ligand primidone placed in 6KZP was obtained as -7.1 kcal/mol, and the 2D and 3D pictures of the intermolecular interactions between the position in the best binding mode of the primidone and the macromolecule 6KZP are presented in appendix 14. The resulting interactions were as follows; The amino acid active sites PHEA384, PHEA385 and VALA1822 formed pi-alkyl, pi-alkyl and alkyl bond interactions with the methylene group in lengths of 4.33Å-4.93Å and 4.37Å. GLYA1724 amino acid active site formed a carbon hydrogen bond interaction with the O atom of 3.60Å length. The amino acid active sites of ILEA380 and LEUA1819 formed a pi-alkyl bond interaction with the benzene group of 4.66Å and 5.15Å lengths, respectively (Table 17).

Tablo 17. Interactions, types and distances between primidone and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
PHE_A384	CH_2 compound in Primidone	4.33	Pi-Alkyl
PHE_A385	CH_2 compound in Primidone	4.93	Pi-Alkyl
VAL_A1822	CH_2 compound in Primidone	4.37	Alkyl
GLY_A1724	O atom in Primidone	3.60	Carbon hydrogen bond
ILE_A380	Benzen group in Primidone	4.66	Pi-Alkyl
LEU_A1819	Benzen group in Primidone	5.15	Pi-Alkyl

Trimethadione

The binding affinity value at the best binding position of the ligand trimethadione placed on the macromolecule 6KZP was obtained as -4.7 kcal/mol. The 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of trimethadione and the macromolecule 6KZP are shown in appendix 15. After the molecular docking process, the interactions were as follows; VALA1512, VALA1512, ILEA1824, ILEA1389, VALA1820, VALA1820, VALA1505, VALA1508 and PHEA1509 amino acid binding sites with methyl group whit respectivly 3.96Å-4.85Å-5.47 Å-4.84Å-4.22Å-4.51Å-4.53Å- 4.65Å and 5.32Å a length of they have formed alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl and pi-alkyl bond interactions with. The PHEA1817 amino acid active site formed a 3.19Å long carbon hydrogen bond interaction with the O atom (Table 18).

Tablo 18. Interactions, types and distances between trimethadione and 6KZP macromolecule

Resude	**Ligand group**	**Distance (Å)**	**Interaction**
VAL_A1512	CH_3 compound in Trimethadione	3.96	Alkyl
VAL_A1512	CH_3 compound in Trimethadione	4.85	Alkyl
ILE_A1824	CH_3 compound in Trimethadione	5.47	Alkyl
ILE_A1389	CH_3 compound in Trimethadione	4.84	Alkyl
VAL_A1820	CH_3 compound in Trimethadione	4.22	Alkyl
VAL_A1820	CH_3 compound in Trimethadione	4.51	Alkyl
VAL_A1505	CH_3 compound in Trimethadione	4.53	Alkyl
MET_A1508	CH_3 compound in Trimethadione	4.65	Alkyl
PHE_A1509	CH_3 compound in Trimethadione	5.32	Pi-Alkyl
PHE_A1817	O atom in Trimethadione	3.19	Carbon hydrogen bond

Pheytoin

The binding affinity value of the ligand phenytoin placed on the macromolecule 6KZP at the best binding position was obtained as -7.5 kcal/mol. 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of phenytoin and the macromolecule 6KZP are shown in appendix 16 and the interactions after the study were as follows; The amino acid active sites of VALA1822, LEUA1723, PHEA384 and ILEA380 are pi-alkyl, amide pi-stacked, pi-pi t-shaped and pi-alkyl with the benzene group in the lengths of 4.47Å-4.67Å-5.21Å and 4.43Å, respectively they formed a bond interaction (Table 19).

Tablo 19. Interactions, types and distances between phenytoin and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
VAL_A1822	Benzene group in Phenytoin	4.47	Pi-Alkyl
LEU_A1723	Benzene group in Phenytoin	4.62	Amide-Pi Stacked
PHE_A384	Benzene group in Phenytoin	5.21	Pi-Pi T- shaped
ILE_A380	Benzene group in Phenytoin	4.43	Pi-Alkyl

Remacemide

The binding affinity value at the best binding position of the ligand remasemide placed in 6KZP was obtained as -7.0 kcal/mol. 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of remasemide and the macromolecule 6KZP are shown in appendix 17 and the interactions after the study are as follows; The amino acid regions VALA1802, VALA1505 and PHEA1817 formed a pi-sigma, pi-alkyl and pi-pi t-shaped bond interaction with the benzene group, with a length of 3.40Å-4.75Å and 5.15Å, respectively. The META1508 amino acid regions formed an alkyl bond interaction with the methyl group of 4.48Aº and 4.05Aº lengths, respectively. Additionally, the types and distances of interactions are summarized in Table 20 in detail.

Tablo 20. Interactions, types and distances between remacemide and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
VAL_A1820	Benzene group in Remacemide	3.40	Pi-Sigma
VAL_A1505	Benzen group in Remacemide	4.75	Pi-Alkyl
PHE_A1817	Benzen group in Remacemide	5.15	Pi-Pi T- shaped
MET_A1508	CH_3 compound in Remacemide	4.48	Alkyl
MET_A1508	CH_3 compound in Remacemide	4.05	Alkyl

Mephenytoin

The binding affinity value of the ligand mephenytoin placed in the macromolecule 6KZP at the best binding position was obtained as -6,7 kcal/mol. 2D and 3D visualizations of the intermolecular interactions between the position in the best binding mode of mephenytoin and the macromolecule 6KZP are shown in appendix 18, and the interactions after molecular docking are as follows; The PHEA1727 amino acid region formed a 3.18Aº long pi-donor hydrogen bond interaction with the amine group. Amino acid regions ILEA308, PHEA384 and LEUA1819 formed pi-alkyl, pi-pi t-shaped and pi-alkyl bond interactions with the benzene group in length of 4.47Å-5.17Å and 5.45Å, respectively. The amino acid regions PHEA384, LEUA1819 and VALA1822 formed pi-alkyl, alkyl and alkyl bond interactions with the methylene group of 4.93Å-5.33Å and 4.40Aº lengths, respectively (Table 21).

Tablo 21. Interactions, types and distances between mephenytoin and 6KZP macromolecule

Resude	Ligand group	Distance (Å)	Interaction
PHE_A1727	NH_2 compound in Mephenytoin	3.18	Pi-Donor hydrogen bond
ILE_A308	Benzene group in Mephenytoin	4.47	Pi-Alkyl
PHE_A384	Benzen group in Mephenytoin	5.17	Pi-Pi T- shaped
LEU_A1819	Benzene group in Mephenytoin	5.45	Pi-Alkyl
PHE_A384	CH_2 compound in Mephenytoin	4.93	Pi-Alkyl
LEU_A1819	CH_2 compound in Mephenytoin	5.33	Alkyl
VAL_A1822	CH_2 compound in Mephenytoin	4.40	Alkyl

The results of molecular coupling of 19 ligand molecules obtained by their receptor interactions are combined. It was seen that the oxcarbazepine and phenytoin molecule gave the best affinity value, but when we look at the type of bonds it formed, it formed a pi-alkyl, pi-sıgma, pi-pi T shaped and amide-pi T stacked bonds instead of the desired hydrogen bond or van der Waals bond. Hydrogen bond types such as carbon hydrogen bond and classical hydrogen bond are desired bond types. because the stronger the bond between the molecules, the better the interaction. The best interactions in molecular interactions are van der Waals and hydrogen bonding, while the least important interactions are covalent and ionic bonds. One reason is that while cysteine is the only amino acid that will form the covalent disulfide bond, there are many amino acids that will interact with each other through hydrogen bonding and van der Waals interactions. The other reason is that the number of amino acids capable of forming ionic bonds is very few, and the number of units capable of forming hydrogen bonds or van der Waals interactions is innumerable. Although vigabatrin gave the lowest affinity value with the 6KZP macromolecule among 19 molecules, it gave the conventional hydrogen bond with the amino acid GLUA354, GLUA922 and GLUA923. The controversial issue is whether it is the type of bond emerging or the affinity value obtained that matters in docking studies.

When we look at the results, if we compare the bond types formed by the active substances that give the closest or the same affinity values; When the phenytoin (-7.5kcal/mol) and oxcarbazepine (-7.5kcal/mol) ligands were examined, it

was seen that they gave the same affinity values. Although they gave the two highest results among 19 protein-ligand structures, they could not form the desired hydrogen and van der Waals bonds. there are only a few pi bonds, and phenytoin interacted with the four amino acids of the 6KZP protein, while oxcarbazepine interacted with two types of interactions with the two amino acid structures of 6KZP. Then the following ligands with the highest binding affinity are tiagabine (-7.4kcal/mol) and mesuximide (-7.3kcal/mol), respectively. Tiagabin combined the 12 ligand groups in its structure with the 6KZP protein to form various pi and alkyl bonds, while Mesuximide combined the 7 ligand groups and the 6KZP protein in its structure to form the hydrogen bond, which is one of the strong bond structures for various pi bonds, alkyl bonds and molecular interactions.

When we compare primidone (-7.1kcal/mol) and remacamide (-7.0kcal/mol), we see that only a ligand group in the structure of primidone forms hydrogen bonds with the 6KZP macromolecule. Primidone formed hydrogen bonds with 1 of the 6 ligand groups, while remacemide did not form hydrogen bonds with any of the 5 ligand groups. Only various pi and alkyl bonds have occurred. When Topiramate and mephenytoin were compared, it was seen that Topiramate gave -6.9 kcal/mol mephenytoin -6.7kcal/mol. Although ligand-receptor structures gave low results in close binding affinity, only mephenytoin gave the desired hydrogen bond structure with the macromolecule. While mephenytoin interacted with 7 ligand groups, topiramate interacted with 4 ligand groups. And various pi and alkyl bonds are formed. When looking at lamotrigine and ethotoin, it is seen that they give the same binding affinity (-6.4 kcal/mol). Although they showed the same value, lamotrigine formed much more conventional hydrogen bonds with the 6KZP macromolecule than ethotoin. There were 7 types of interactions between the lamotrigine-receptor, and 6 of them were hydrogen bonded. According to this result, we can say that there is a strong interaction. Ethotoin, on the other hand, formed 1 hydrogen bond from 4 types of interactions with the receptor. Among the 19 ligands, we can say that the structure that forms the most hydrogen bonds in ligand-receptor interactions compared so far is the lamotrigine-6KZP structure. The comparison in the total will be made when all results are compared.

Although locasamide and zonisamide (-6.1 kcal/mol) gave the same binding affinity, locasamide formed a single interaction with the 6KZP macromolecule and formed the pi-sigma bond. Zonisamide, on the other hand, formed 4 types of interaction with the receptor and formed 1 hydrogen bond. The conclusion to be drawn from here is that even if they have the same binding energies, the types of bonds, their numbers and strengths can be very different from each other. We can say the following for felbamate (-6.0 kcal/mol) and levetiracetam (-5.4 kcal/mol); Felbamate induced more interactions than levetirecetam through ligand addition to the macromolecule, but appears to form hydrogen bonds to the same extent as levetiracetam. Felbamate formed hydrogen bonds with 6 out of 7 ligand groups, while levetirecetam formed hydrogen bonds with all 6 ligand groups. When gabapentin (-5.4 kcal/mol) and ethosuximide (-5.1 kcal/mol) are examined, it is seen that both ligand structures create 5 types of interaction with the receptor, and both ligand-receptor structures give 2 conventional hydrogen bonds. Considering the bond types made with valproic acid (-4.9 kcal/mol) and trimethadione (-4.7 kcal/mol), which have low binding affinity, it is seen that valproic acid combines 7 ligand groups and trimethadione 10 ligand groups with amino acid sites. The desired hydrogen bond structure was revealed between the 6KZP macromolecule and both ligand structures, and it was observed that various pi and alkyl bonds were also formed.

In addition to the bond structures and interaction types, the IC50 values and protein binding rates of the studied antiepileptic drugs were also examined. The IC50 is the concentration of inhibitor that inhibits 50% of the enzyme, and a low IC50 indicates a high inhibition value. The lower the IC50 concentration values for a drug molecule, the better it inhibits. In the light of this information, when the IC50 and protein binding rates obtained from the Pubchem database for 19 antiepileptic drugs were examined in Table 22, it was seen that the protein binding rate was not suitable for vigabatrin, which gave the lowest affinity value, and the IC50 value on calcium channels could not be determined. For oxcarbazepine, one of the ligands with the highest value, the IC50 value appeared inactive, while the plasma protein binding rate was 40%. For Phenytoin, on the other hand, the IC50 value is 21.9, while the protein binding rate is 90%. Other rates are detailed in the table. As a result of the information given above, since it is known that the high inhibition potential of the drug is proportional to the low IC50 concentration level, the determined IC50 concentration of phenytoin, which gives the best binding affinity and percentage, gave the highest value. Gabapentin, which gave the lowest IC50 concentration, showed lower values for docking affinity and protein binding compared to phenytoin and oxcarbazepine. According to these results, in addition to the inhibitory effect of gabapentin, it know that the bond types and interactions with protein are better than phenytoin and oxcarbazepine.

Table 22. Docking, IC50 and protein binding values of antiepileptics

Ligand's	Best Binding affinity (kcal/mol)	IC50 (μM)	Protein Binding(%)
Vigabatrin	-4.4	-	Protein binding is not amenable
Lacosamide	-6.1	Undefined	<%15
Zonisamide	-6.1	3.3	%40
Oxcarbazepine	-7.5	İnactive	%40
Levatiracetam	-5.4	İnactive	%40
Tiagabine	-7.4	-	%96
Topiramate	-6.9	10	%9-17
Lamotrigine	-6.4	8.1	%55
Gabapentin	-5.4	0.14	<%3
Felbamate	-6.0	İnactive	%20-36
Ethosuximide	-5.1	Undefined	Protein binding is not amenable
Valproic acid	-4.8	Undefined	%10-18.5
Mesuximide	-7.3	-	Protein binding is not amenable
Ethotoin	-6.4	İnactive	Protein binding is not amenable
Primidone	-7.1	Undefined	%10.78-13.70
Trimethadione	-4.7	Undefined	%90
Phenytoin	-7.5	21.9	%90
Remacemide	-7.0	-	Protein binding is not amenable
Mephenytoin	-5.7	-	Protein binding is not amenable

As a result, it was more meaningful to compare the drugs with higher IC50 values -zonisamide, topiramate, lamotrigine, gabapentin, phenytoin-included in Table 22 and whether the ligands showing the desired bond types or the ligands with high chelate affinity had higher IC50 values. Such that, while zonisamide gave an affinity value of -6.1 kcal/mol, it showed inhibition with a value of 3.3 μM. When the binding interaction of zonisamide was examined, it was seen that it made conventional hydrogen bonds, which is one of the desired bond structures. For topitramate, while it showed a binding affinity of -6.9 kcal/mol, it gave an inhibition value with 10 μM, but it did not show a strong interaction. When the lamotrigine ligand was examined, it gave its affinity with a value of -6.4 kcal/mol, and its IC50 value was found to be 8.1 μM. Considering the intermolecular interaction, it was seen that it formed more than one strong interaction. For gabapentin, the binding affinity was -5.4 kcal/mol, while the inhibition value was 0.14 μM, and it is seen that it forms strong bonds in the interaction between the structures. For phenytoin, chelate value was -7.5 kcal/mol, while the IC50 value was found to be 21.9 μM and they did not create a strong and much interaction in the intermolecular interaction. The conclusion to be drawn from this is that having high binding affinity alone is not sufficient. The interpretation we can make according to the data we have is that ligands with an IC50 value of <10 μM can form one or more strong interactions even if they have low affinity, and complex structures with an IC50 value higher than >10 μM show high affinity but do not give the desired bond types.

When looking at the total, it was seen that those with the best affinity values did not make the strongest bonds, while those with the lowest affinity gave the desired hydrogen bond types. And besides, the IC50 values of the structures giving the best affinity were high, while gabapentin, which gave the lowest IC50 concentration value, did not exhibit high binding affinity. The conclusion to be drawn as we discuss it here is that the degree of binding affinity, the number of bonds obtained, and the bond structures are not parallel. And it has been observed that the drug can exhibit low affinity even if the protein binding rates are high or they show the best inhibition concentration..

4. Conclusion

In the study, Discovery Studio 2020 program and AutoDOCK Vina energy scoring were used, and there are various scoring functions. The results show that PDB ID: 6KZP macromolecule, which has an antagonistic effect on voltage-gated calcium channels, also gave good affinity values in studies with 19 active substances. The IC50 values and protein binding rates, as well as the types of bonds that result from the docking study, are important to us. Because even if the affinity value obtained in the ligand-protein docking study is low, considering the low IC50 value and high protein binding rates, we can say that the stronger the bond they establish, the better the interaction.

Competing Interest / Conflict of Interest

The authors declare that they no conflict of interest. The none of the authors have any competing interests in the manuscript.

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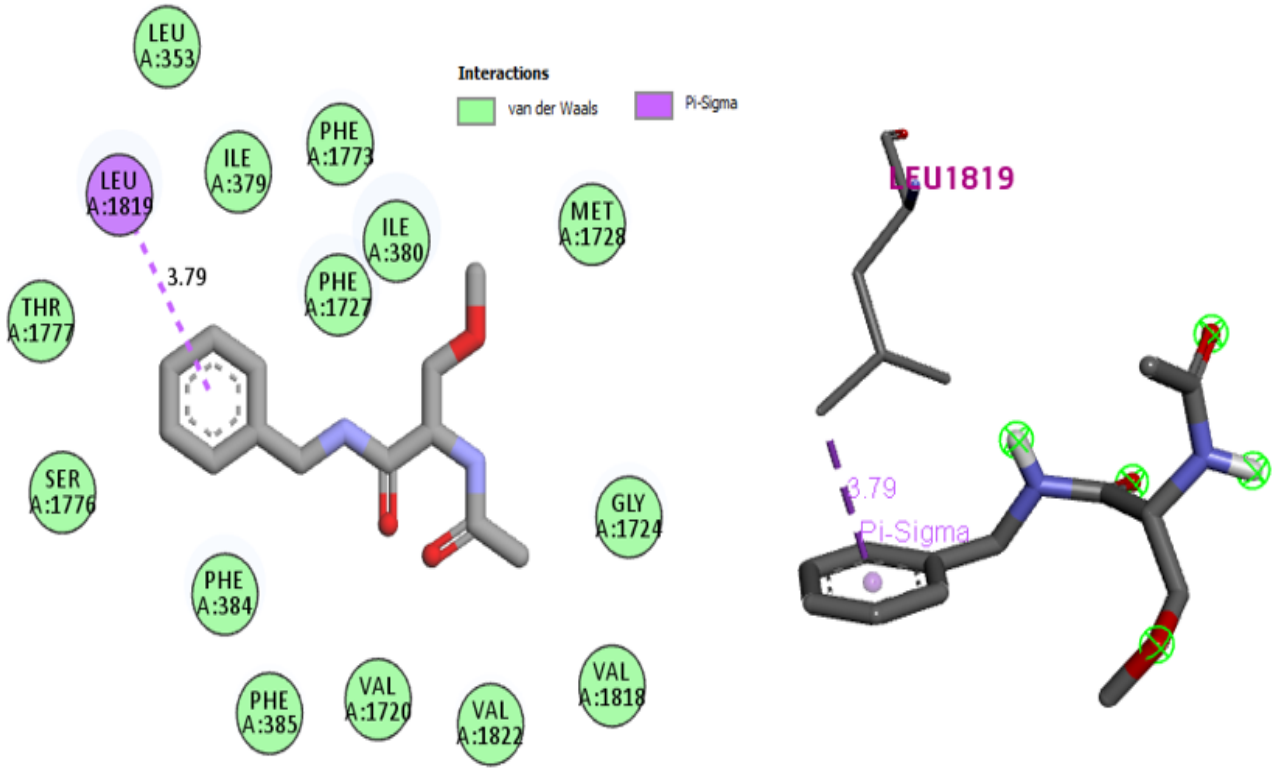
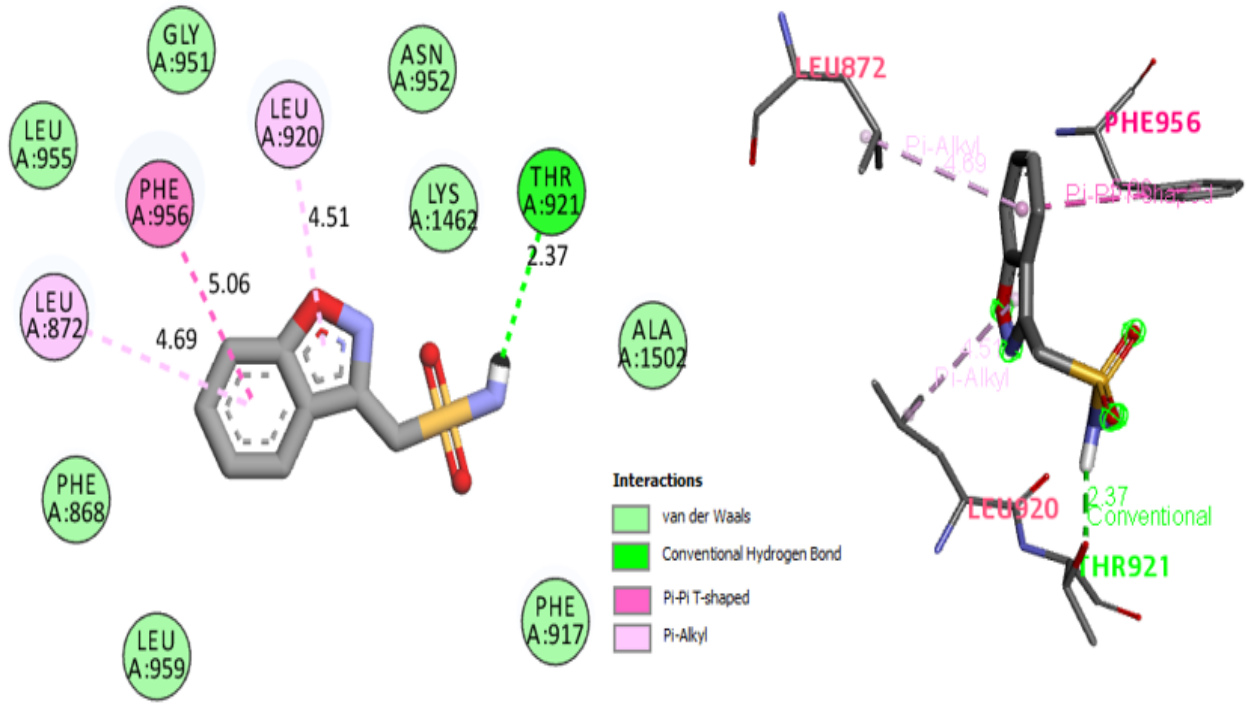
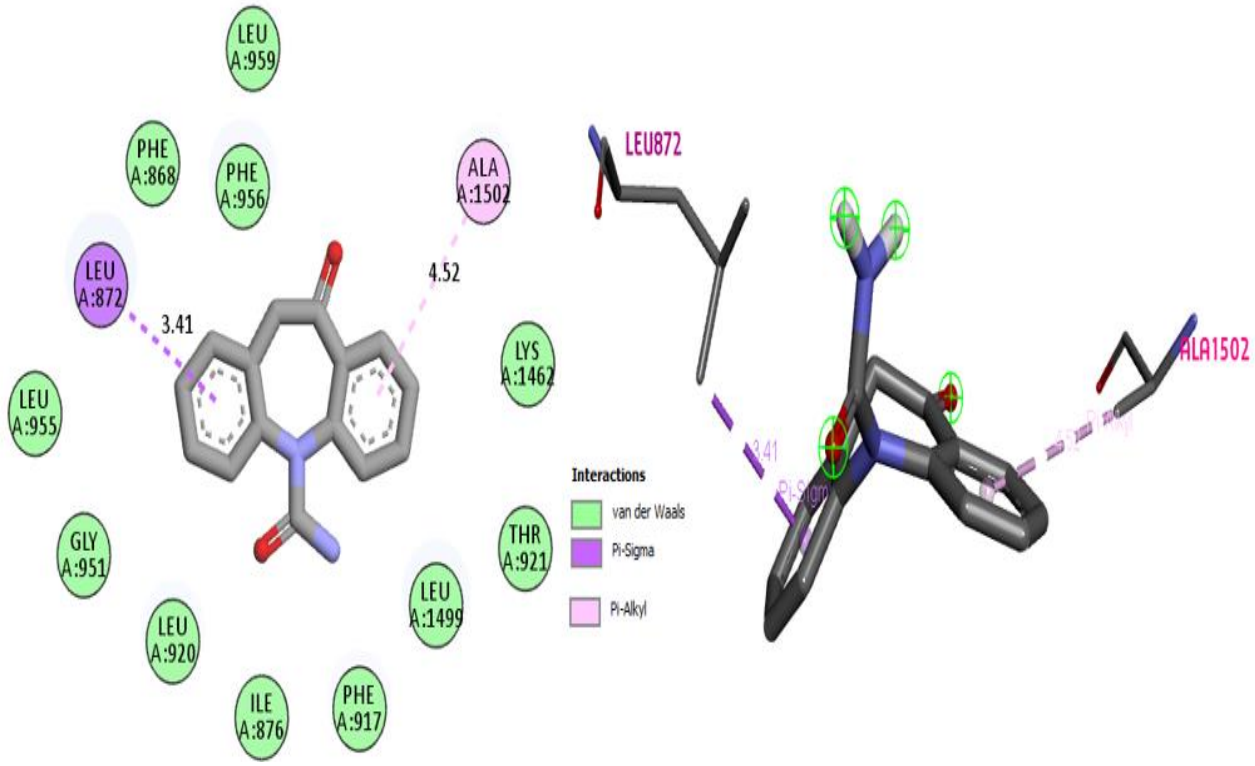
Appendix



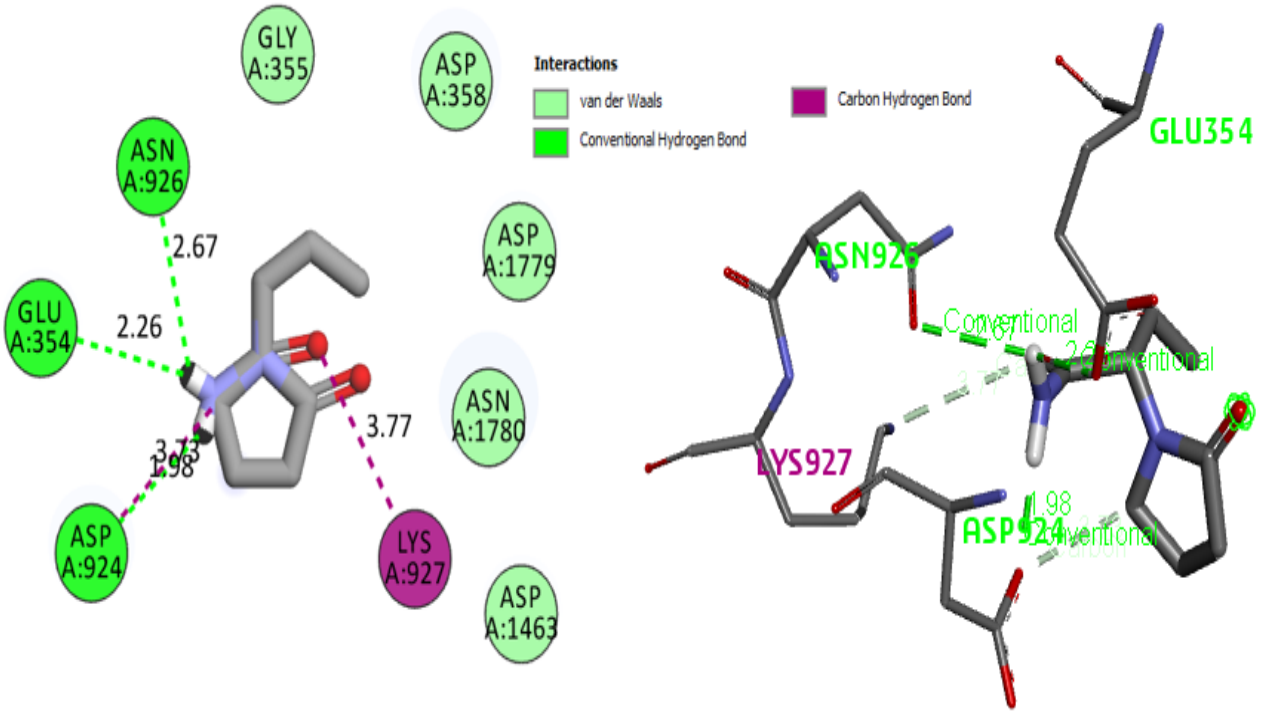
Appendix 1. 2D and 3D display of the interaction of locasamide and macromolecule (6KZP)



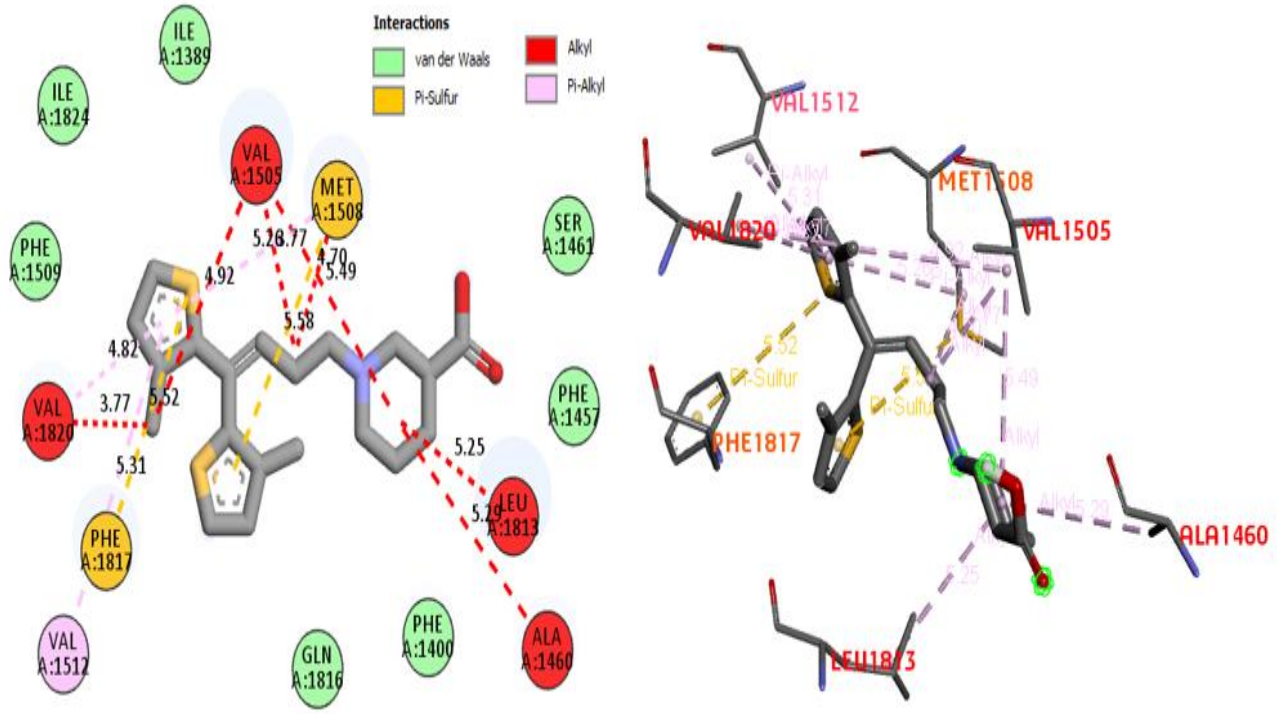
Appendix 2. 2D and 3D display of the interaction of zonisamide and macromolecule (6KZP)



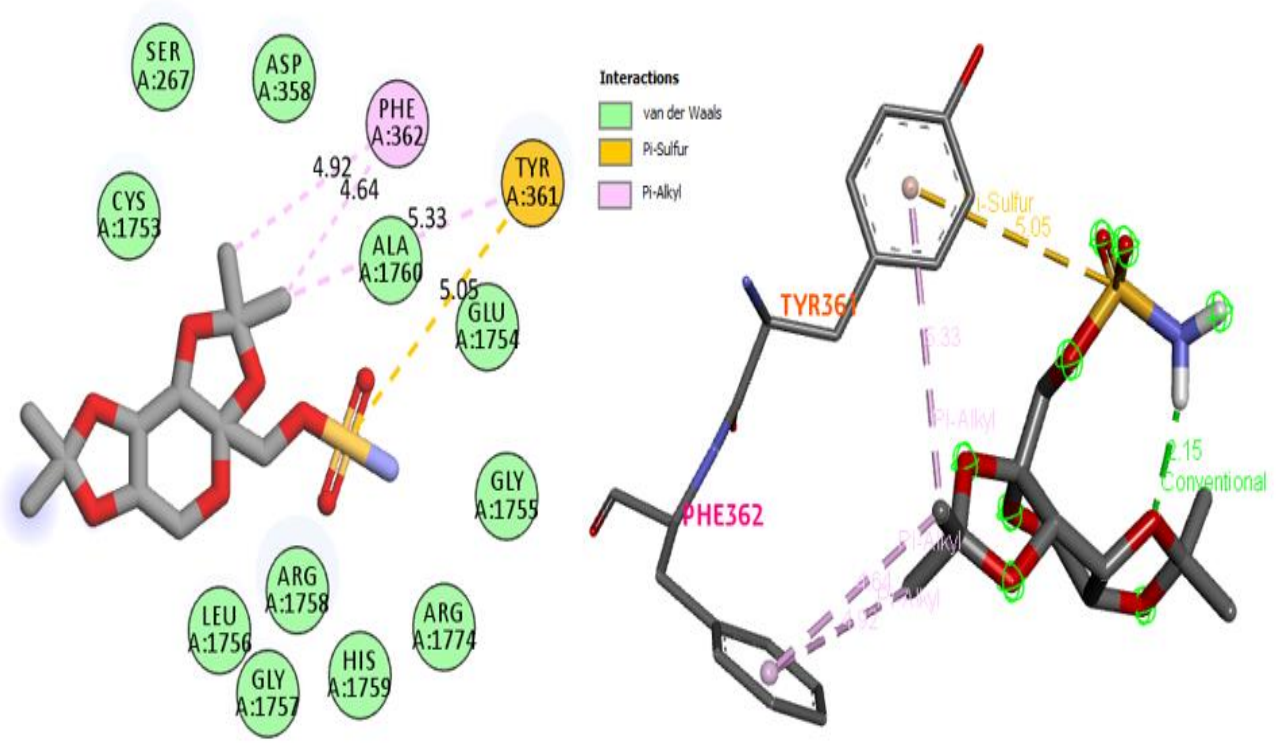
Appendix 3. 2D and 3D display of the interaction of oxcarpazepine and macromolecule (6KZP)



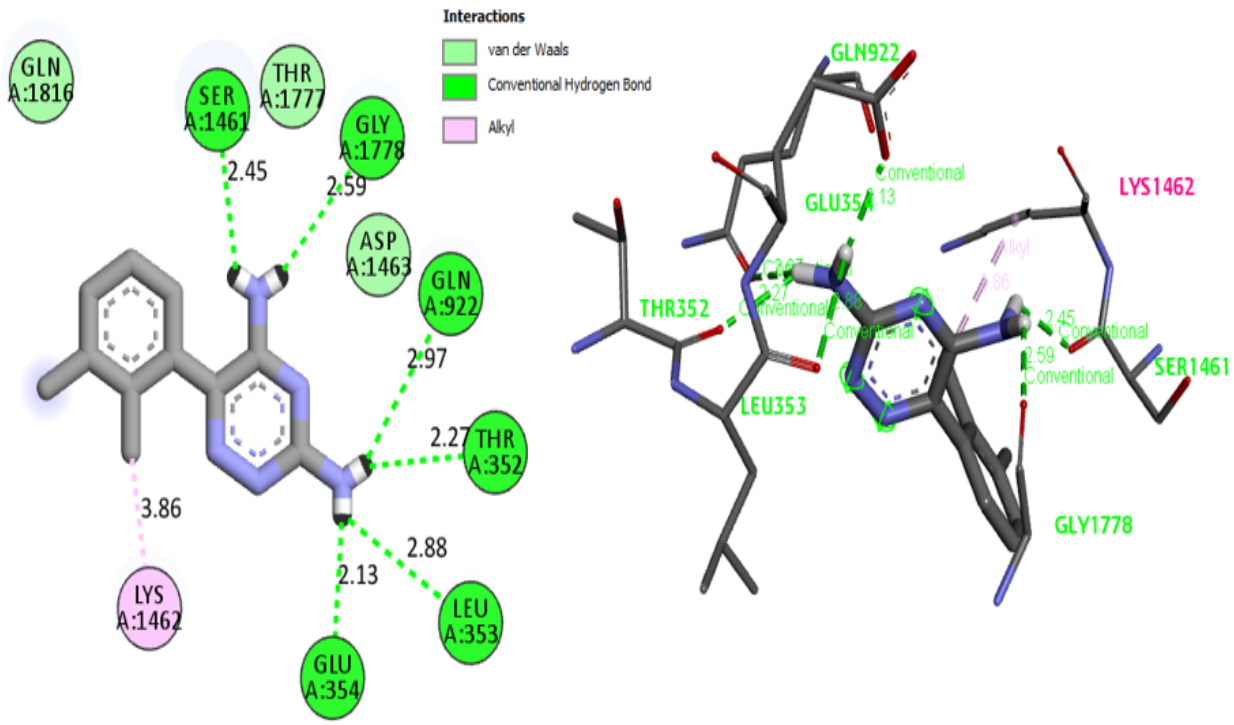
Appendix 4. 2D and 3D display of the interaction of levatiracetam and macromolecule (6KZP)



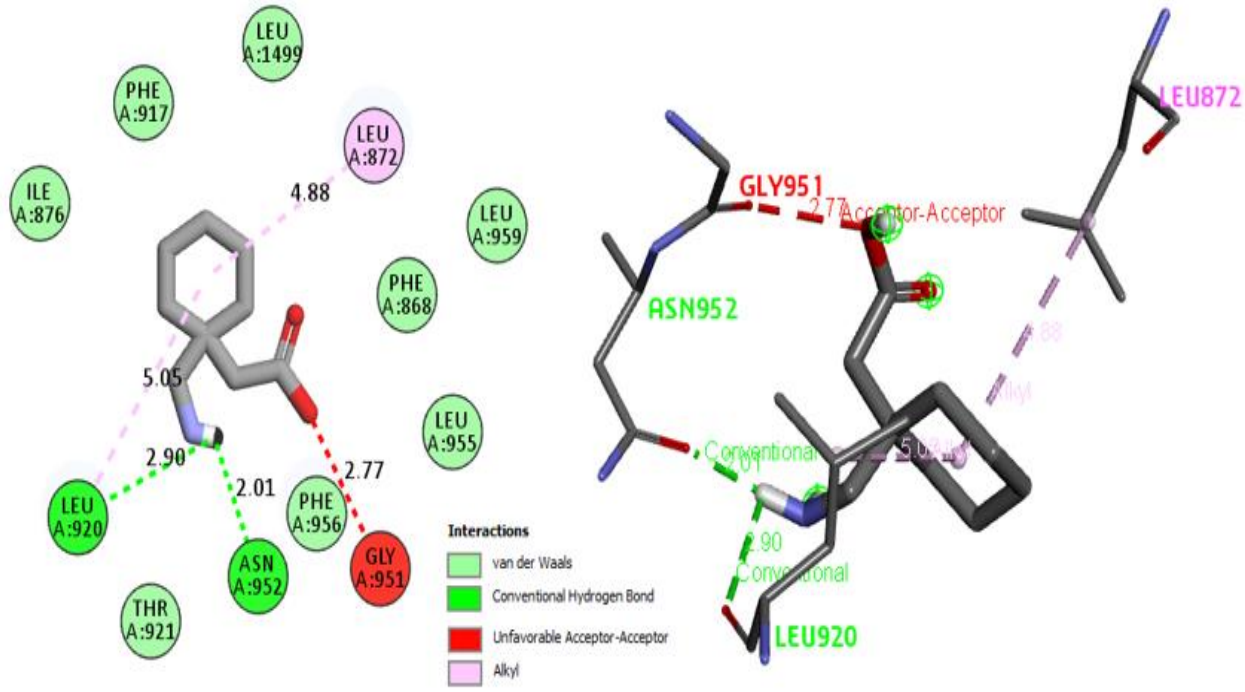
Appendix 5. 2D and 3D display of the interaction of tiagabine and macromolecule (6KZP)



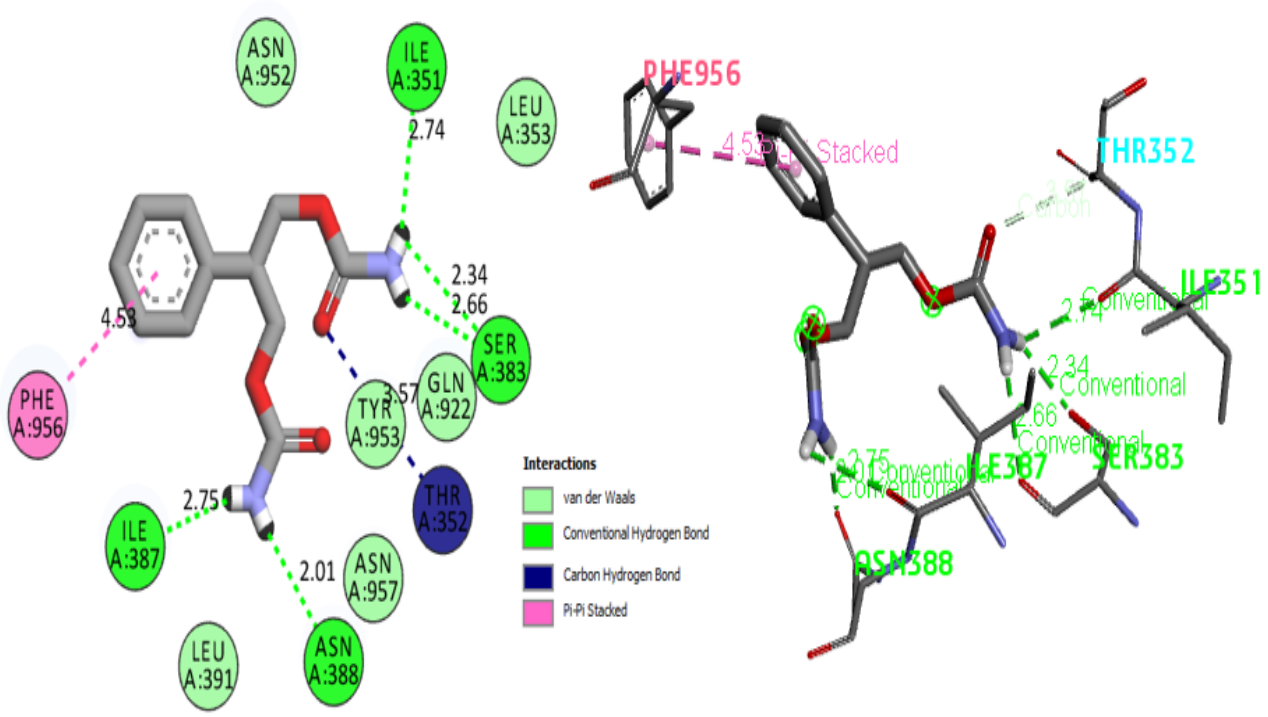
Appendix 6. 2D and 3D display of the interaction of topiramate and macromolecule (6KZP)



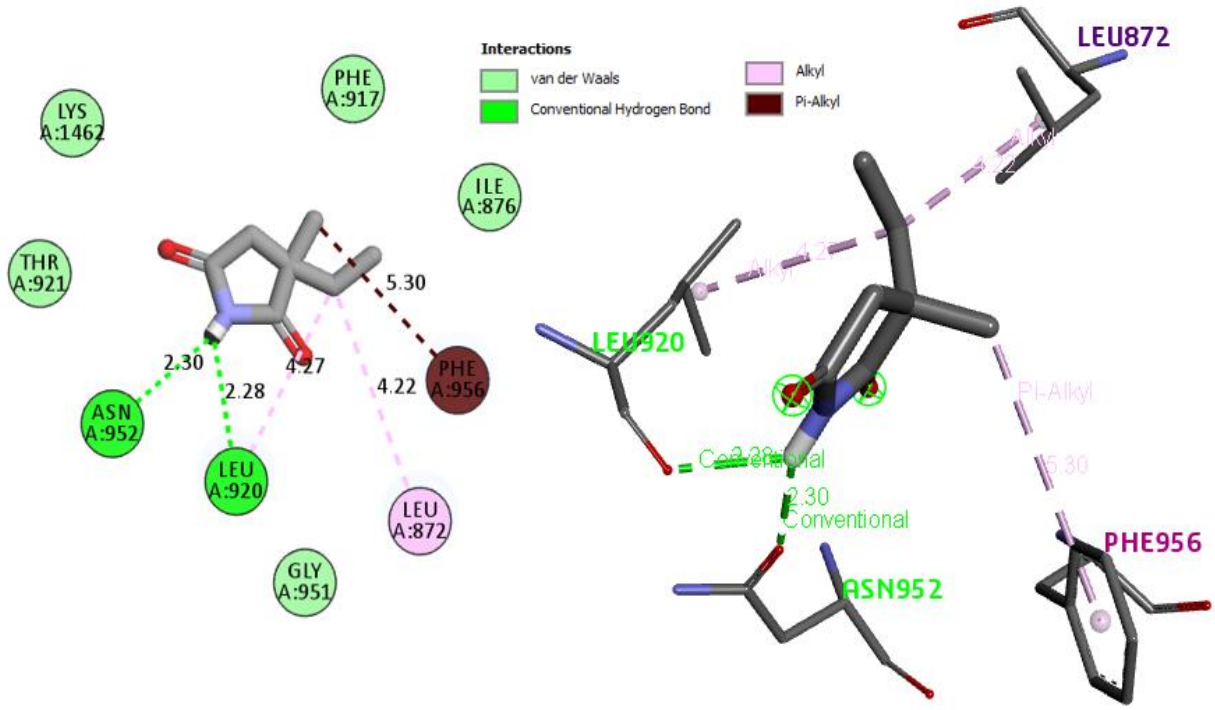
Appendix 7. 2D and 3D display of the Interaction of lamotrigine and macromolecule (6KZP)



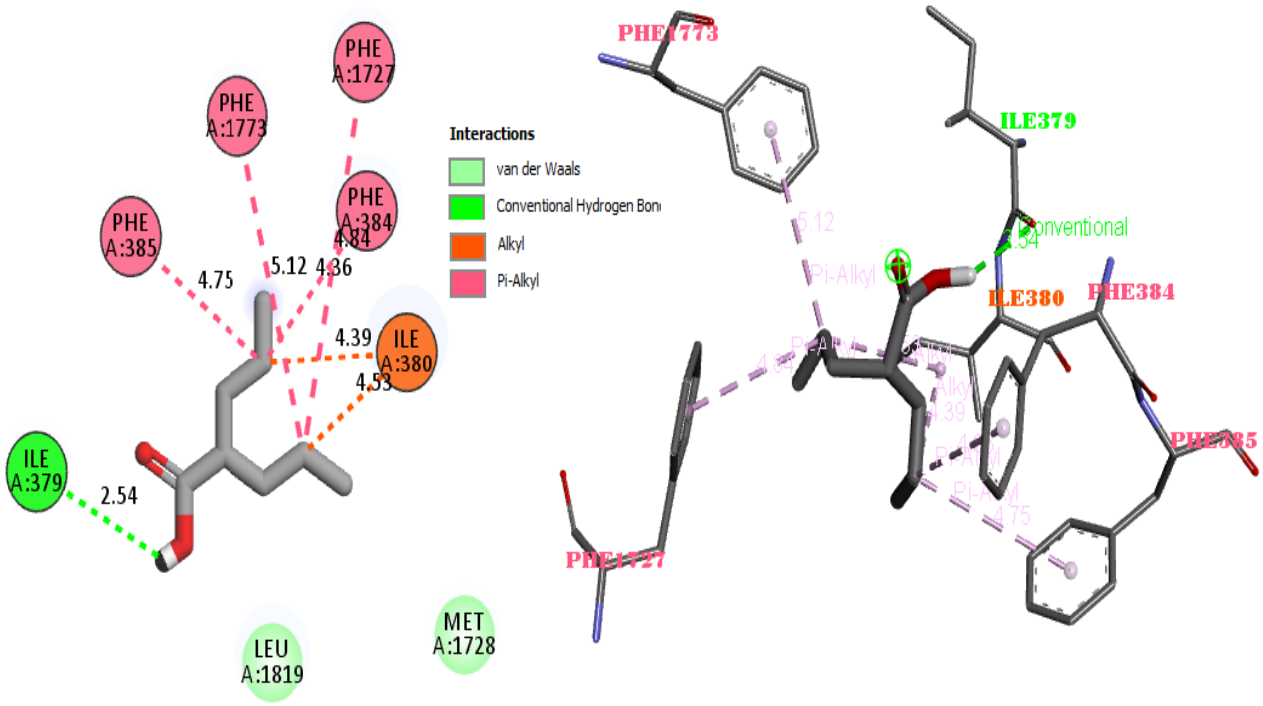
Appendix 8. 2D and 3D display of the interaction of gabapentin and macromolecule (6KZP)



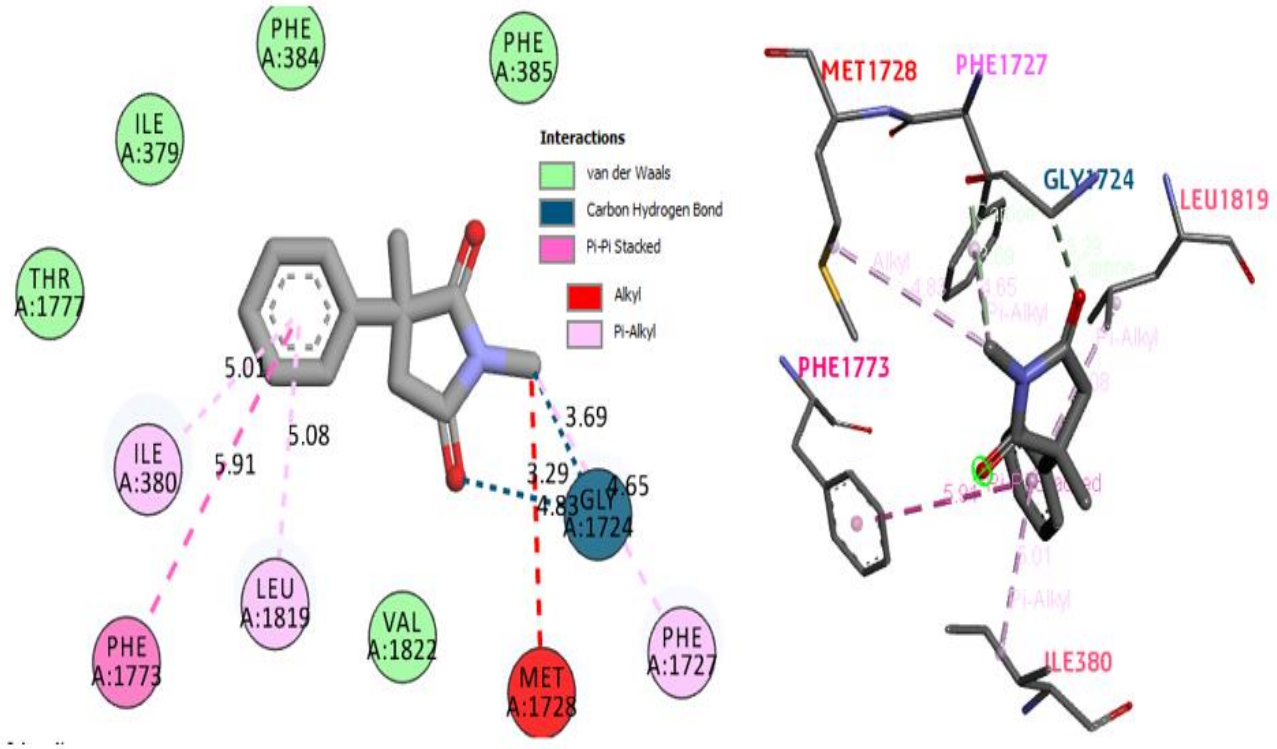
Appendix 9. 2D and 3D display of the interaction of felbamate and macromolecule (6KZP)



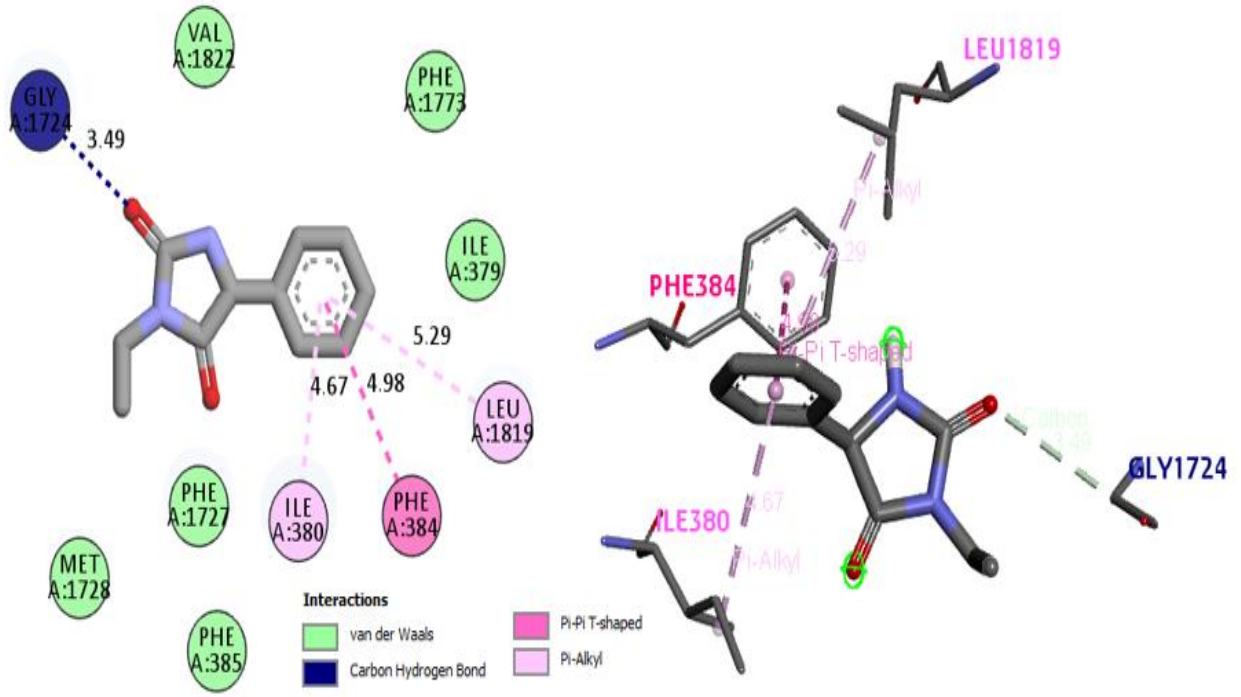
Appendix 10. 2D and 3D display of the interaction of ethosuximide and macromolecule (6KZP)



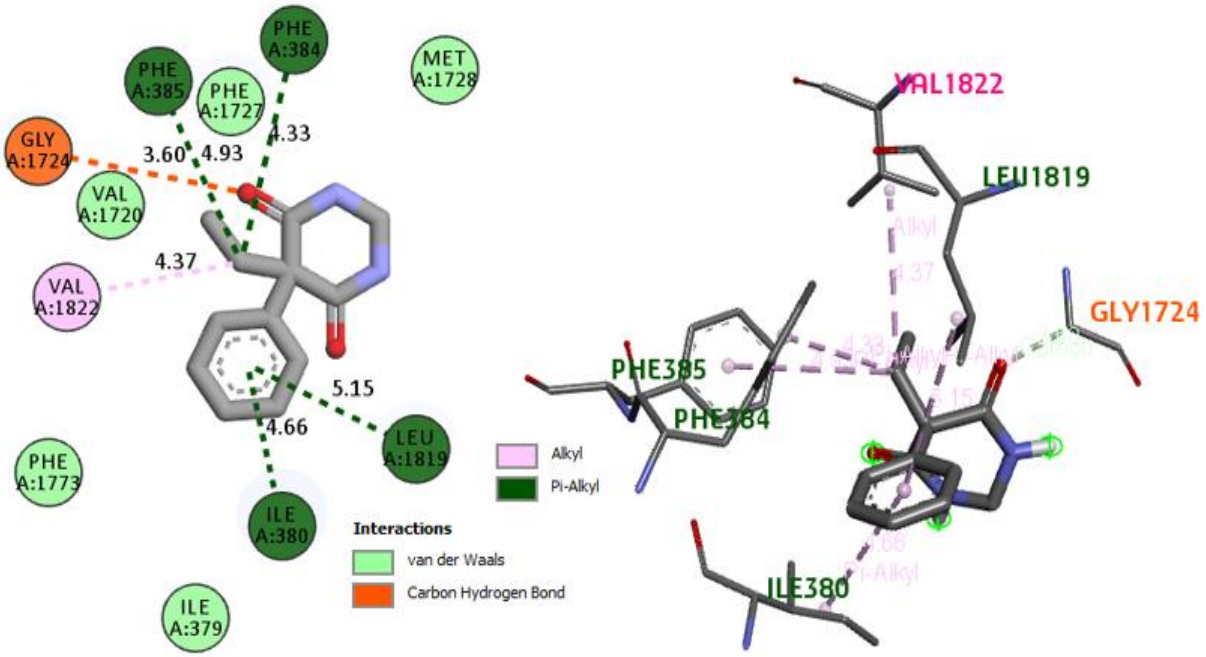
Appendix 11. 2D and 3D display of the interaction of valproic acid and macromolecule (6KZP)



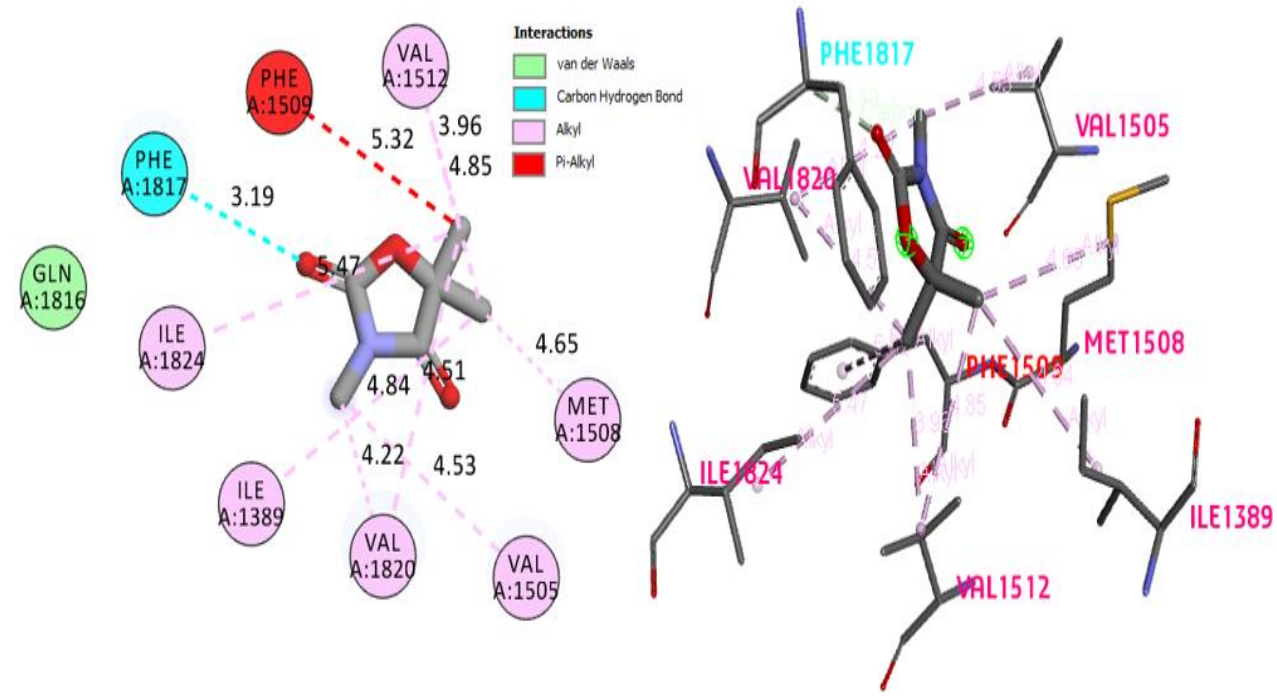
Appendix 12. 2D and 3D display of the interaction of mesuximide and macromolecule (6KZP)



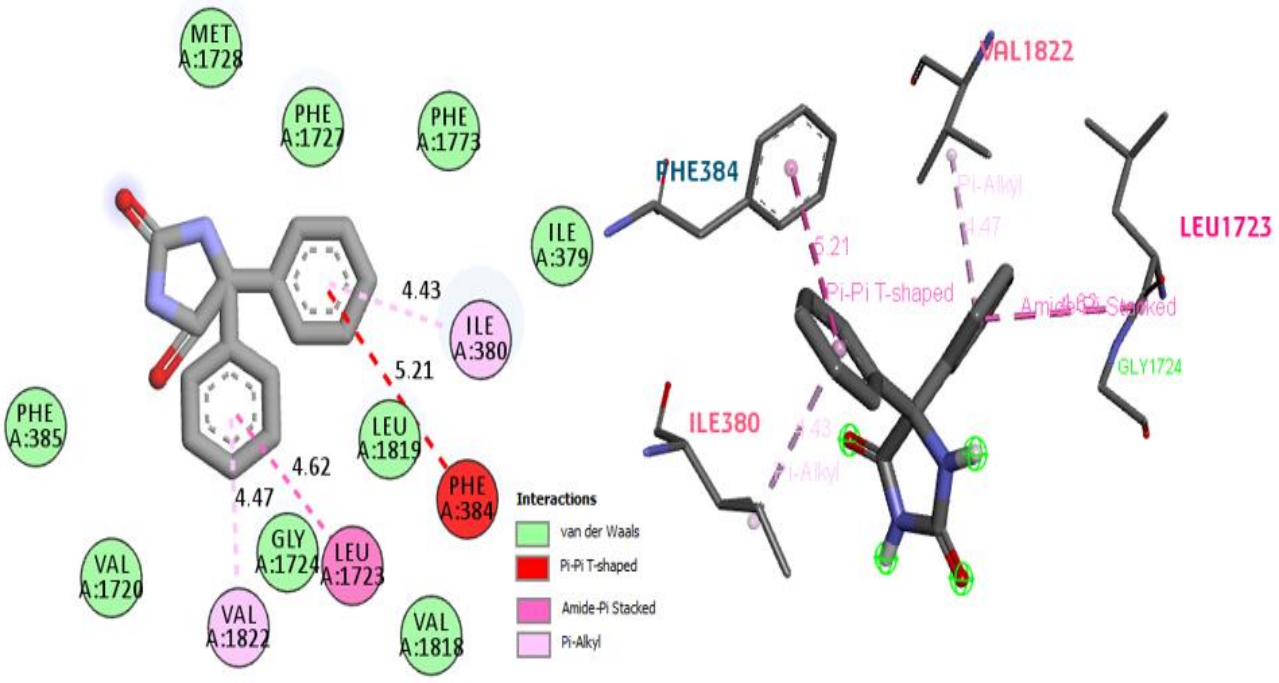
Appendix 13. 2D and 3D display of the interaction of ethotoine and macromolecule (6KZP)



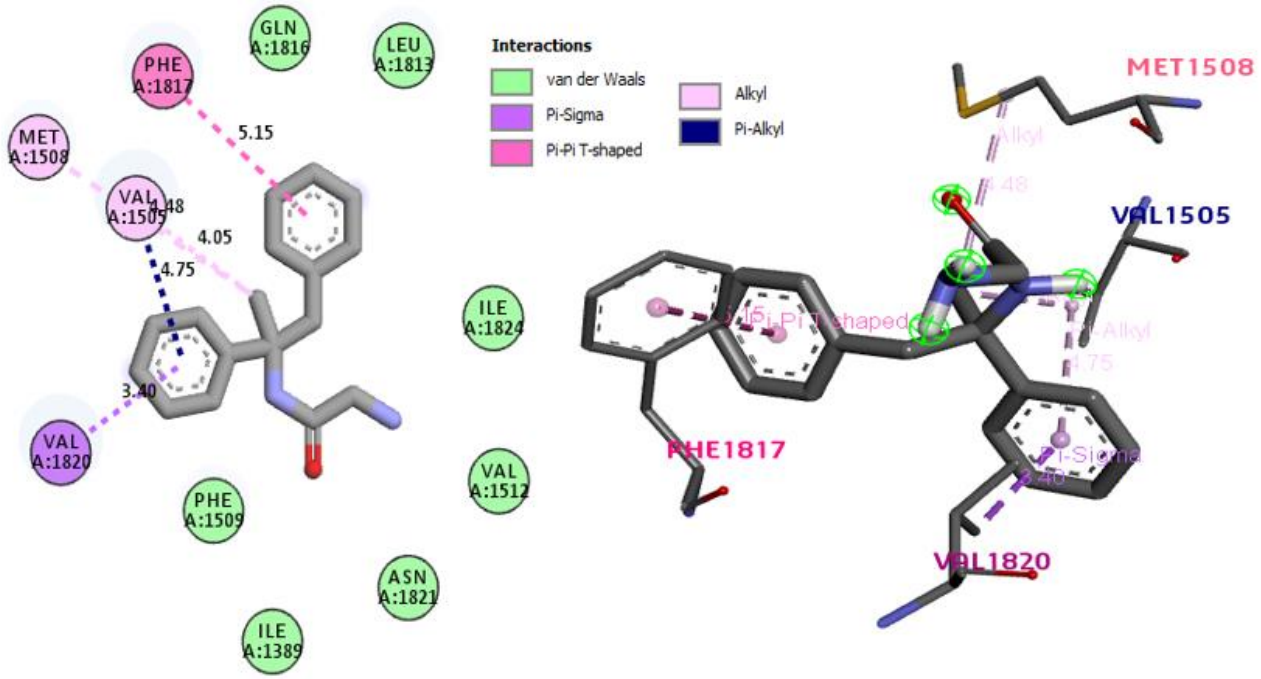
Appendix 14. 2D and 3D display of the interaction of primidone and macromolecule (6KZP)



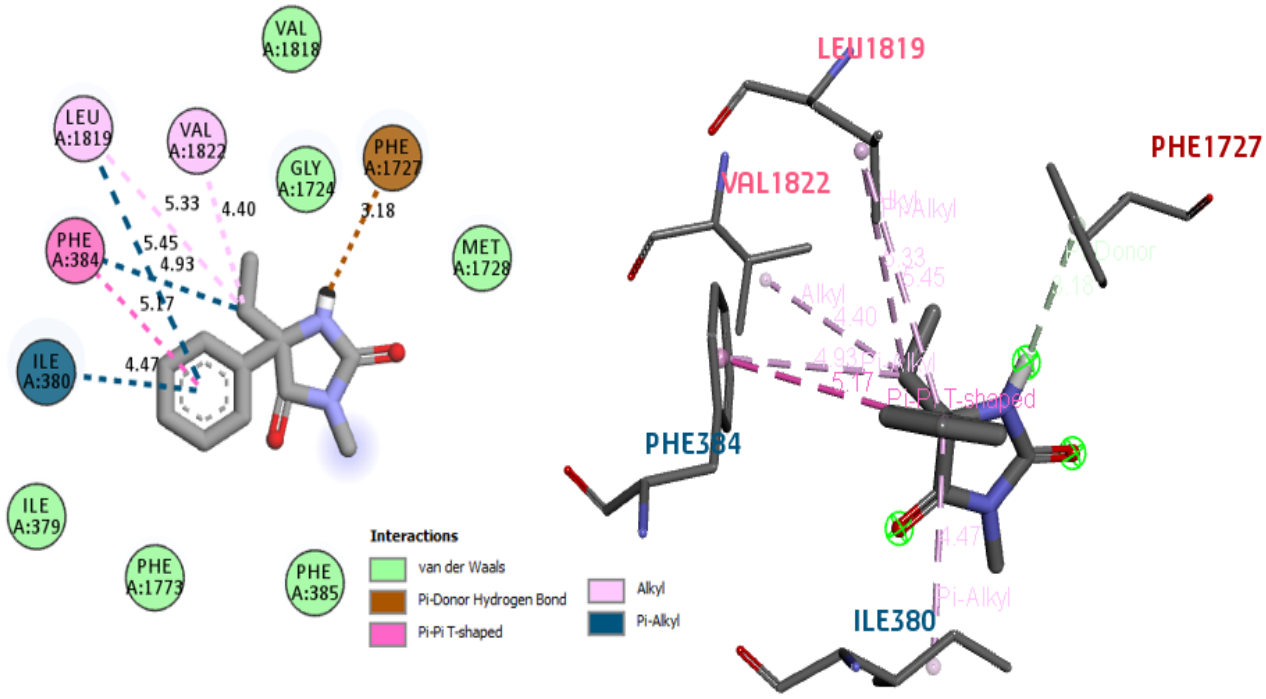
Appendix 15. 2D and 3D display of the interaction of trimethadione and macromolecule (6KZP)



Appendix 16. 2D and 3D display of the interaction of phenytoin and macromolecule (6KZP)



Appendix 17. 2D and 3D display of the interaction of remacemide and macromolecule (6KZP)



Appendix 18. 2D and 3D display of the interaction of mephenytoin and macromolecule (6KZP)