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Research Article | Araştırma Makalesi

INVESTIGATION OF IN SILICO PROPERTIES OF [5-HYRDOXY-7-(PENTYLOXY)-2-PHENYL-4H-CHROMEN-4-ONE] MOLECULE AND ITS EFFECTS ON IN-VITRO EPILEPTIFORM ACTIVATION IN BRAIN SLICES

[5-HİDROKSİ-7-(PENTİLOKSİ)-2-FENİL-4H-KROMEN-4-ONE] MOLEKÜLÜNÜN IN SİLİCO ÖZELLİKLERİNİN VE BEYİN DİLİMLERİNDE IN-VİTRO EPİLEPTİFORM AKTİVASYON ÜZERİNE ETKİLERİNİN İNCELENMESİ

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ABSTRACT

Objective: Epilepsy is an incurable disorder, necessitating research into new drug candidates. The molecule 5-hydroxy-7-(pentyloxy)-2-phenyl-4h-chromen-4-one (5H7P4C4O) is available commercially, but its solubility, spectroscopic properties, and biological activity are unknown. This article focuses on the investigation of a molecule called 5H7P4C4O and its potential biological effects in the central nervous system (CNS) for the treatment of epilepsy.

Methods: Initially, the solubility and spectroscopic properties of 5H7P4C4O were determined. Physicochemical, pharmacokinetic and drug-likeness properties were evaluated in silico with the ADME program. Besides, focusing on the potential of the molecule on the neurological activity, interaction potentials of 5H7P4C4O molecule on NMDA, AMPA and GABA-A receptors was investigated by molecular docking method. Binding energies indicated potential affinity for NMDA and GABA-A receptors. Next, the acute effect of the 5H7P4C4O molecule was electrophysiologically examined in the CA3 region of the hippocampus in brain slices of 30-35 days old C57BL/6 mice. Epileptiform activity was induced in Mg+2-free medium or with 4-aminopyridine (4AP, 100 µM).

Results: When applied alone, 5H7P4C4O exhibited no stimulating effect at doses of 5, 10, and 20 µM. However, it extinguished ictal signals and demonstrated a remarkable modulatory effect on the total power of signals within the 0-47 Hz frequency range in Mg+2-free model.

Conclusion: Based on the results obtained, it was concluded that the 5H7P4C4O indicates modulator effect on neuronal stimulation. Showing this effect only in the Mg+2-free model suggests that it has activity on NMDA receptors. Additionally, its ability to gather power signals within a specific frequency range suggest potential effectiveness in cognitive and/or other brain functions.

Keywords: Epilepsy, Brain slice, Mg-free, 4AP, Electrophysiology

ÖZ

Amaç: Epilepsi tam tedavisi olmayan bir hastalıktır ve yeni ilaç adaylarının araştırılmasını gerektirir. 5-hidroksi-7-(pentiloksi)-2-fenil-4h-kromen-4-one (5H7P4C4O) molekülü ticari olarak mevcuttur, ancak çözünürlüğü, spektroskopik özellikleri ve biyolojik aktivitesi bilinmemektedir. Bu makale, epilepsi tedavisi için 5H7P4C4O adlı bir molekülün ve bunun merkezi sinir sistemindeki (MSS) potansiyel biyolojik etkilerinin araştırılmasına odaklanmaktadır.

Yöntem: İlk olarak 5H7P4C4O'nun çözünürlüğü ve spektroskopik özellikleri belirlendi. Fizikokimyasal, farmakokinetik ve ilaca benzerlik özellikleri ADME programı ile in silico olarak değerlendirildi. Ayrıca molekülün nörolojik aktivite üzerindeki potansiyeline odaklanılarak, 5H7P4C4O molekülünün NMDA, AMPA ve GABA-A reseptörleri üzerindeki etkileşim potansiyelleri moleküler yerleştirme yöntemiyle incelenmiştir. Bağlanma enerjileri, NMDA ve GABA-A reseptörleri için potansiyel afinite gösterdi. Daha sonra 5H7P4C4O molekülünün akut etkisi, 30-35 günlük C57BL/6 farelerinin beyin kesitlerinde hipokampusun CA3 bölgesinde elektrofizyolojik olarak incelendi. Epileptiform aktivite, Mg+2 içermeyen ortamda veya 4-aminopiridin (4AP, 100 uM) ile indüklendi.

Bulgular: 5H7P4C4O, tek başına uygulandığında 5, 10 ve 20 µM dozlarda hiçbir uyarıcı etki göstermedi. Bununla birlikte, Mg+2 içermeyen modelde iktal sinyalleri söndürdü ve 0-47 Hz frekans aralığındaki sinyallerin toplam gücü üzerinde dikkate değer bir modülatör etki gösterdi.

Sonuç: Elde edilen sonuçlara göre 5H7P4C4O'nun nöronal stimülasyon üzerinde modülatör etki göstermektedir. Bu etkiyi sadece Mg+2 içermeyen modelde göstermesi NMDA reseptörleri üzerinden etkinlik gösterdiğini düşündürmektedir. Ayrıca, güç sinyallerini belirli bir frekans aralığında toplama yeteneği, bilişsel ve/veya diğer beyin işlevlerinde potansiyel etkilerinin olduğunu gösterir.

Anahtar Kelimeler: Epilepsi, Beyin Kesitleri, Mg içermeyen, 4AP, Elektrofizyoloji

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Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures caused by abnormal and excessive neuronal activity in the brain.[1,2] Seizures occur when there is an imbalance between neuronal excitation and inhibition in the central nervous system (CNS).[3] Only the basic chemical properties of the 5H7P4C4O molecule are known, and best of our knowledge, there is no study in the literature on its biological effects. Our in-situ investigation revealed that 5H7P4C4O may have affinity for N-methyl-D-aspartate (NMDA) and gamma aminobutyric acid-A (GABA-A) receptors in the CNS.

Although various substances is used as neurotransmitters in CNS, as an excitatory neurotransmitter glutamate and as an inhibitory neurotransmitter GABA are the major neurotransmitters.[4] Glutamate acts on three types of ionotropic glutamate receptors: NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors. When glutamate binds to these receptors, it triggers the opening of channels that allow the influx of cations resulting in excitatory synaptic transmission. At resting membrane potentials, Mg^{+2} ions strongly block NMDA channels and activation of NMDA channels are dependent on both glutamate release and membrane potentials.[5,6] Removing of Mg^{+2} ions from the extracellular solution resulted in seizure like activity [7], which has been used to generate epileptiform activity in brain slices. Therefore, the discovery of a new NMDA receptor antagonist is valuable for the treatment of epilepsy.

GABA is a major inhibitory neurotransmitter released from GABAergic neurons and binds to both GABA-A and GABA-B receptors. Activation of GABA-A receptors increase inward chloride currents which hyperpolarize the membrane potentials of neurons. On the other hand GABA-B receptor increases potassium permeability, decreases calcium entry, and inhibits the presynaptic release of other transmitters.[8] While drugs that activate the GABA receptor suppress seizures, antagonist drugs cause seizures.

To expedite the drug discovery process, computer models and molecular docking techniques are used to predict the interaction of molecules with target receptors. While the ADME (Absorption, Distribution, Metabolism and Excretion) software used in this study provides important information about the physicochemical and pharmacokinetic properties of a molecule[9], information about the interaction of ligand structures with macromolecular targets is obtained by calculating the ligand-receptor binding free energy by molecular docking method.[10]

Temporal lobe epilepsy (TLE) is a common type of epilepsy, is characterized by recurrent focal seizures originating from mesial temporal lobe, particularly the hippocampus.[11] Investigating the effects of new molecules on GABA-A, NMDA or AMPA channels would be a reasonable approach to explore potential antiepileptic effects. Although 5H7P4C4O (Figure 1) is commercially available, current information is lacking regarding its biological effects, solubility, absorbance wavelength, and fluorescence properties.

In this study, the effects of 5H7P4C4O on GABA-A, NMDA and AMPA channels were investigated for the first time by molecular docking method. The results revealed a stronger allosteric effect of the molecule on the NMDA receptor compared to the other channels, so its effect was electrophysiologically investigated in the both Mg^{2+}-free epilepsy model and 4-aminopyridine (4AP) epilepsy model in the mouse brain slices for the first time. In addition, absorbance and fluorescence measurement results were reported for the first time in this study.

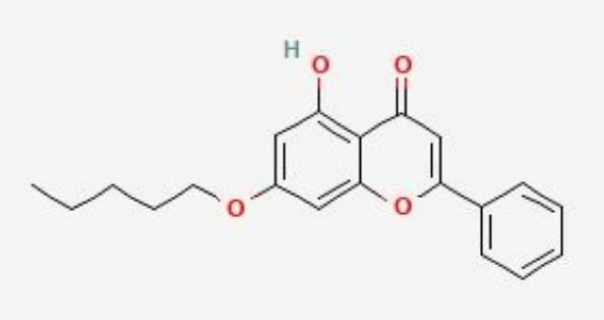


Figure 1. Molecular structure of 5-hydroxy-7-(pentyloxy)-2-phenyl-4h-chromen-4-one (5H7P4C4O) PubChem ID: 5877945

Methods

The animal experiments of this study were approved by Local Animal Care and Ethics Committee (approval number: 2021–108), and certify that the study was performed in accordance with the international ethical standards as declared in the Helsinki Declaration. All the chemicals were purchased from Sigma-Aldrich unless specified.

Absorbance and Fluorescence spectrometric measurement

First 5H7P4C4O cannot be dissolved in water. Therefore 5H7P4C4O was dissolved in pharma grade dimethyl sulfoxide (DMSO (Aromel Kimya)) by sonicating in an ultrasonic bath for 10 min. 100 µl samples were put into 96 well plate to investigate absorbance and fluorescence properties. To determine the absorbance values, the sample was scanned in the wavelength range of 230-1000 nm in the Spectramax Paradigm Spectrometers, Molecular Devices (USA). Then Fluorescence measurements were performed scanning from 400 to 800 nm with 360 nm excitation wavelength which is the minimum wavelength of the device.

Molecular docking and ADME studies

Molecular Docking studies were performed as described elsewhere.[2] Briefly the molecular structure of 5H7P4C4O was obtained from PubChem database (ID: 5877945) and protein structure obtained from protein data bank (PDB), which are NMDA (5B3J), GABA-A (6D6T), AMPA (7LEP) and 5H7P4C4O docked to these receptors by using Autodock 4.2.6 software. For detection of active binding

sites, ifenprodil, bicuculline and perampanel were used as references for NMDA, GABA-A and AMPA receptors, respectively. The protein–ligand complexes were visualized and analyzed using Auto-DockTools and Discovery Studio version 4.0 (Accelrys Software Inc., San Diego, CA, USA). SwissADME web site is used to evaluate physicochemical, water solubility, pharmacokinetic and drug-likeness properties of the molecule.[9]

Slice Preparation

Ex vivo fresh brain slices were prepared from 30 to 35-day-old C57BL/6 female mice as described elsewhere.[12,13] After rapid decapitation mice brain was removed and put into cold (1.5–2 °C) artificial cerebrospinal fluid (ACSF) solution containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM d-glucose, 2 mM $CaCl_2$, and 1.5 mM $MgCl_2$ for 3-4 min. Horizontal hippocampal slices 370-µm-thick were cut using the microtome (Leica VT100S, Germany) and incubated in 30 ± 1°C ACSF solution which was oxygenated with 95% O_2 and 5% CO_2 at pH 7.4, for recovery at least 40 minutes.

Electrophysiological Recordings

First 5, 10 and 20 µM 5H7P4C4O were applied to brain slices to discover whether 5H7P4C4O had a stimulating effects on neurons. The brain slice placed in a submerged type of recording chamber and perfused with oxygen saturated 30 ± 1°C ACSF. Micropipette electrodes were positioned on CA3 region and electrophysiological recording were performed. After being sure there was no abnormal activities or discharges, electrophysiological activities were recorded the slice both in ACSF and in variety concentrations of 5H7P4C4O.

Mg^{2+}-free epilepsy model [14] was modified to mimic epilepsy. Briefly brain slice removed to 30 ± 1 °C oxygenated Mg^{2+}-free ACSF solution containing 125 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM d-glucose, 2 mM $CaCl_2$ and incubated for 80 minutes to repel Mg^{+2} ions from NMDA channels . After that the slice was placed in a recording chamber which was perfused with 30 ± 1 °C, 3 ml/minutes oxygen saturated Mg^{2+}-free ACSF solution. The glass micropipette electrodes were placed on the CA3 region of the hippocampus. Before recording, the slice was allowed to accommodate for 10-15 minutes. Then 30 minutes electrophysiological recording was taken. After that the bath solution was replaced by Mg^{2+}-free ACSF containing 10 µM 5H7P4C4O and 30 minutes recording was obtained.

4-aminopyridine (4AP) induced epilepsy method in brain slices were performed as described elsewhere.[2,12,13,15] Briefly ACSF containing 100 µM 4AP were perfused to the chamber to initiate epileptiform activities. Electrophysiological recordings were taken from the CA3 region for 30 minutes after mature epileptiform activity was obtained. Then, the bath solution was replaced by ACSF containing 100 µM 4AP + 10 µM 5H7P4C4O and the activity was recorded for another 30 minutes.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism software, employing paired t-tests. The data were presented as mean ± standard deviation (SD). A significance level of ($p < 0.05$) was considered statistically significant.

Results

Absorbance and Fluorescence Measurements

Visually 5H7P4C4O is well dissolved in DMSO and absorbance and fluorescence spectra are shown in Figure 2.

ADME Results

Physicochemical properties, lipophilicity, water solubility, pharmacokinetics and drug-likeness properties of 5H7P4C4O are given at the Table 1.

Molecular Docking Results

As a GluN2B selective NMDA antagonist, ifenprodil can change the course of epileptogenesis and ictogenesis in temporal lobe epilepsy.[16] The binding energies of ifenprodil and 5H7P4C4O molecule to the relevant region of the NMDA receptor 5B3J were calculated as -8.20 and -6.97 kcal/mol, respectively, by molecular docking method (Table 2). Bicuculline is a GABA-A receptor antagonist and occupies agonist binding sites.[17] Binding energies of Bicuculline and 5H7P4C4O molecule to 6D6T, the GABA-A receptor, were calculated as -6.76 and -6.34 kcal/mol, respectively (Table 2). The affinity of the

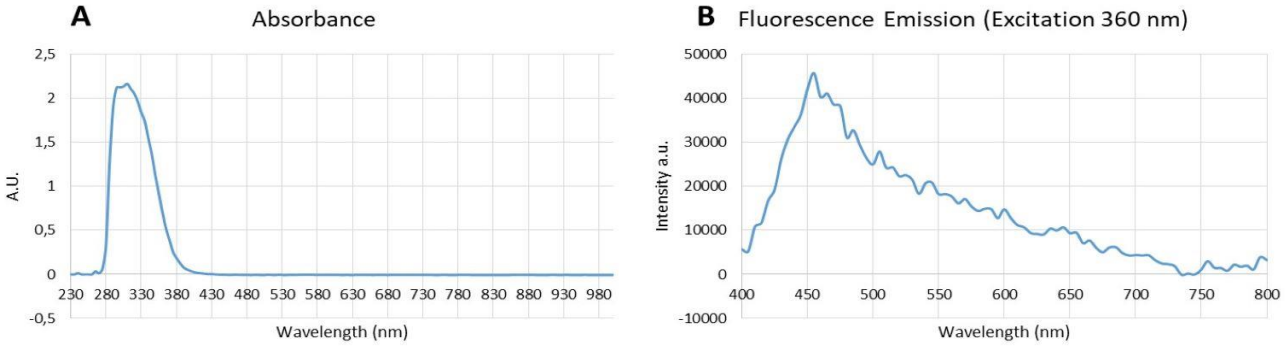


Figure 2. (A) Absorbance and (B) fluorescence spectrum of 5H7P4C4O. Absorbance peak wavelength of 5H7P4C4O is 295-310 nm and fluorescence emission peak value is 455 nm.

Table 1. ADME properties of 5H7P4C4O

Physicochemical Properties		Pharmacokinetics	
Formula	C20H20O4	GI absorption	High
Molecular weight	324.37 g/mol	BBB permeant	Yes
Num. of heavy atoms	24	CYP1A2 inhibitor	Yes
Num. Of rotatable bonds	6	CYP2C19 inhibitor	Yes
Num. of H-bond acceptors	4	CYP2C9 inhibitor	Yes
Num. of H-bond donors	1	CYP2D6 inhibitor	Yes
Molar refractivity	95.66	CYP3A4 inhibitor	Yes
Topolog. Polar Surface Area	59.67 (Å^2)	Log Kp (skin permeation)	-4.27 cm/s
Lipophilicty		**Druglikeness**	
Log $P_{o/w}$ (iLOGP)	3.80	Lipinski	Yes; 0 violation
Log $P_{o/w}$ (WLOGP)	4.73	Ghose	Yes
Log $P_{o/w}$ (XLOGP3)	5.64	Veber	Yes
Log $P_{o/w}$ (MLOGP)	2.27	Egan	Yes
Water solubility		Muegge	No; XLOGP3>5
Log S (ESOL)	-5.50 (Moderately soluble)	Bioavailability Score	0.55
Log S (Ali)	-6.66 (Poorly soluble)		

Table 2. The binding energies, hydrogen bonds, and bond distance of 5H7P4C4O to the relevant receptors (*indicates reference molecules that binds to the proteins)

Macromolecule	Ligand	Binding Energy (kcal/mol)	Inhibition Constant, Ki	Hydrogen Bonds	The distance of hydrogen bonding (Armstrong)
5B3J	5H7P4C4O	-6.97	7.81 µM	H-GLN153:OE1	2.17
	Ifenprodil*	-8.20	979.36 nM	H – SER131:O	2.09
				H – GLU284:OE1	2.07
6D6T	5H7P4C4O	-6.34	22.61 µM	LYS105:NZ - O	2.97
				H – ASP57:O	2.04
	Bicuculline*	-6.76	11.03 µM	-	-
7LEP	5H7P4C4O	-5.09	184.44 µM	H – ALA117:O	1.99
	Perampanel*	-7.22	5.10 µM	VAL514:N - O	2.88

5H7P4C4O molecule for the AMPA (7LEP) receptor was found to be much lower than the reference molecule (Table 2). These results made us think that the 5H7P4C4O molecule may have an effect on NMDA and/or GABA-A receptors. Figure 3 shows the binding sites of both 5H7P4C4O and reference molecules for NMDA and GABA-A receptors. The 2D image of the bindings of 5H7P4C4O and reference molecules with the receptors is shown in the supplementary file (SFigure 1) which indicates possible other bonds with the amino acids.

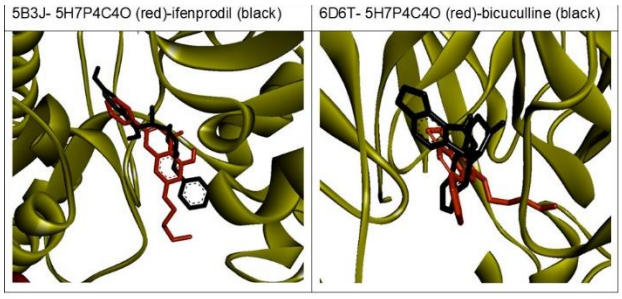


Figure 3. 3D view of the binding sites and molecular position of 5H7P4C4O and reference molecules to the 5B3J (NMDA) and 6D6T (GABA-A) receptors.

Brain Slice Electrophysiological Results

The application of 5H7P4C4O at various concentrations (5, 10, and 20 µM) in brain slices immersed in ACSF did not trigger any spikes or synchronous discharges that could be interpreted as an epileptiform activity. The effects of 5H7P4C4O (10 µM) of epileptiform activities were investigated in two different epilepsy models which are Mg^{2+}- free and 4AP models. Mg^{2+}-free epilepsy model basically depends on the NMDA channels mediated depolarizing currents. In this model, two distinct types of epileptiform activities -both interictal and ictal like activities- were observed in the CA3 region (Figure 4). Signals with amplitude 4 times the initial amplitude, showing sharp rise deviations and lasting less than 4 seconds were called interictal, while activities lasting longer than 4 seconds were called interictal (Figure 4).

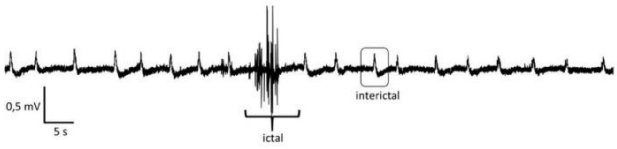


Figure 4. Sample trace of field potential recordings from CA3 region of hippocampus in the Mg^{+2}-free epilepsy model. Interictal sample is shown in rounded rectangle and ictal sample is shown above the bracket.

Interictal frequency, ictal frequency, power of total records were analyzed (Figure 5). Statistical analysis indicated that 5H7P4C4O application did not change the interictal frequency significantly (Figure 5A, D). Although ictal signals were obtained in only 2 samples in Mg^{2+}-free model, they completely disappeared after 5H7P4C4O application. To prevent the inclusion of the 50 Hz frequency, a notch filter was used, and the power of frequencies within the 0-47.6 Hz range was calculated for the total power analysis (Figure 5B), and also average power calculated (Figure 5E). The average total power exhibited a decrease whereas no statistical significant difference was obtained. Upon closer examination of each sample in Figure 5B, it was observed that the total power in the 0-47 Hz range decreased after the application of 5H7P4C4O for samples with initial power higher than 0.011 mV^2/Hz, while lower power signals increased. In other words, the application of 5H7P4C4O in this model exhibited a regulatory effect by reducing high-power signals and increasing low-power signals, resulting in the power of signals gathering between 0.0019 and 0.0090 mV^2/Hz. Although a decrease in the average total power was observed in the 52.4-97.6 Hz frequency range after the application of 5H7P4C4O, the change was not statistically significant (Figure 5C, F).

Application of 5H7P4C4O significantly reduced the interictal frequency of 4AP induced epileptiform activity (Figure 6A). Although there was an increase in ictal events in some brain slices after 5H7P4C4O administration, it was not statistically significant (Figure 6B, F). Moreover it has no significant effect on total power of 0-47.6 Hz and 52.4-97.6 Hz on 4AP model (Figure 6C, D, G, H).

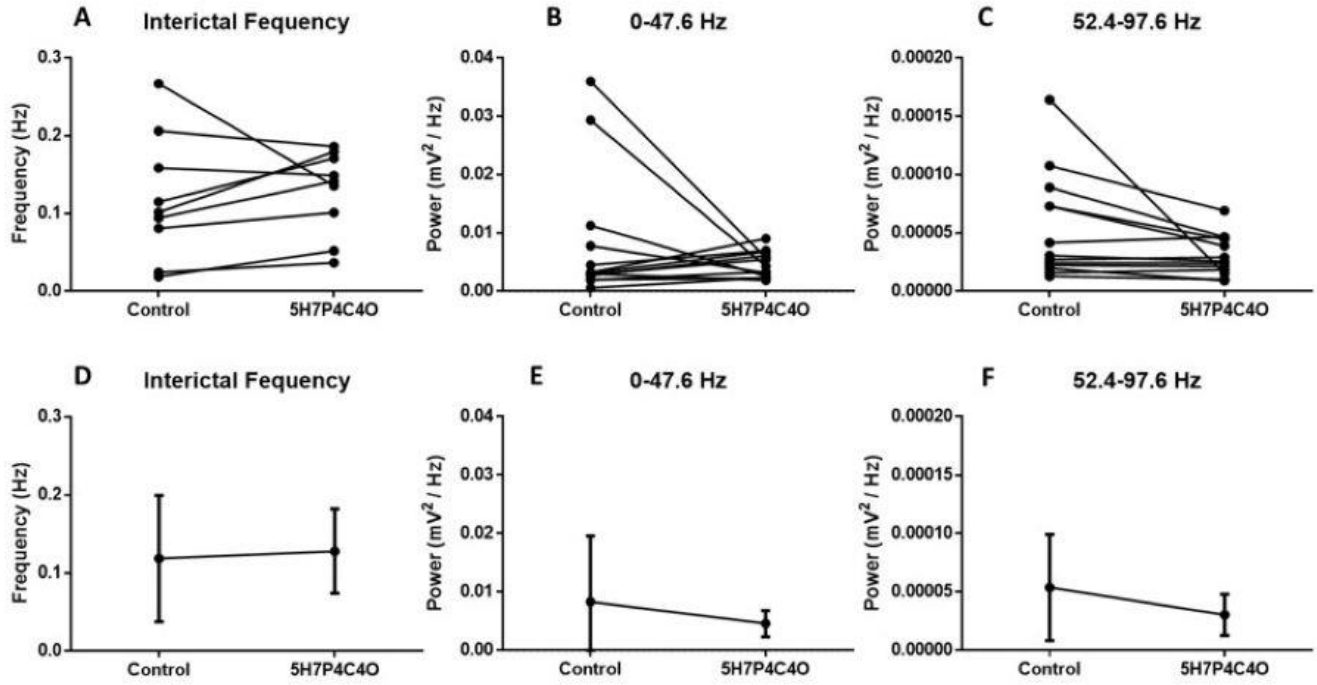


Figure 5. Frequency of interictal activities (A), total power of each sample frequency range 0-47,6 Hz (B) and 52,4-97,6 Hz range (C) in Mg^{+2}- free epilepsy model. Average frequency of interictal activities (D), average power of 0-47,6 Hz frequency range (E) and 52,4-97,6 Hz range (F). (n=9, mean±SD).

Discussion

The investigation of new molecules with potential as drugs becomes increasingly important, considering the lack of effectiveness of commonly used antiepileptic drugs in approximately 40% of patients with temporal lobe epilepsy.[16] Molecular docking analyzes showed that the 5H7P4C4O molecule has a high potential to interact on neuronal targets (NMDA and GABA-A receptors), which are important in excitability in the brain. Furthermore, this study provides valuable insights into the basic physical and chemical properties of the molecule, which have been previously poorly documented. Spectrophotometric methods and ADME analysis were employed to elucidate these properties, offering a comprehensive understanding of the molecule's characteristics. This study provides several important findings regarding the 5H7P4C4O molecule and its effects as a drug for epilepsy treatment.

Firstly, according to the ADME results (Table 1), molecular weight of 5H7P4C4O is smaller than 500, hydrogen bond acceptor is less than 10, hydrogen bone donor is less than 5, molar refractivity is less than 130, number of rotatable bonds are less than 10 and topological polar surface area is less than 140. Lipophilicity values (iLogP, WLogP and MLogP) are less than 5, but XLogP3 is greater than 5. Therefore drug-likeness of 5H7P4C4O is positive based on Lipinski[18], Ghose[19], Veber[20] and Egan[21] filters, whereas it is not good enough for Muegge[22] filter because of the XLogP3 which is greater than 5. Bioavailability (F) score is a percentage number that developed to predict the permeability and

bioavailability properties of compounds which should be greater than 10 % F in rats [23], and since the F value for 5H7P4C4O is 55%, bioavailability can be considered good. Pharmacokinetic properties based on virtual screening indicated that its gastrointestinal (GI) absorption is high and can be able to pass the blood brain barrier (BBB). An increase in the negative Log K_p figure indicates a decrease in the skin permeability of the molecule under investigation.[24] LogS value expresses the estimated solubility of a molecule in water.[25,26] LogS scale; insoluble<-10<Poorly<-6<moderately<-4<soluble<-2 very<0< highly. Two different calculation methods have shown that 5H7P4C4O is moderately or poorly soluble in water. In this study, we also observed that the 5H7P4C4O molecule was insoluble in water, and therefore it was dissolved in DMSO.

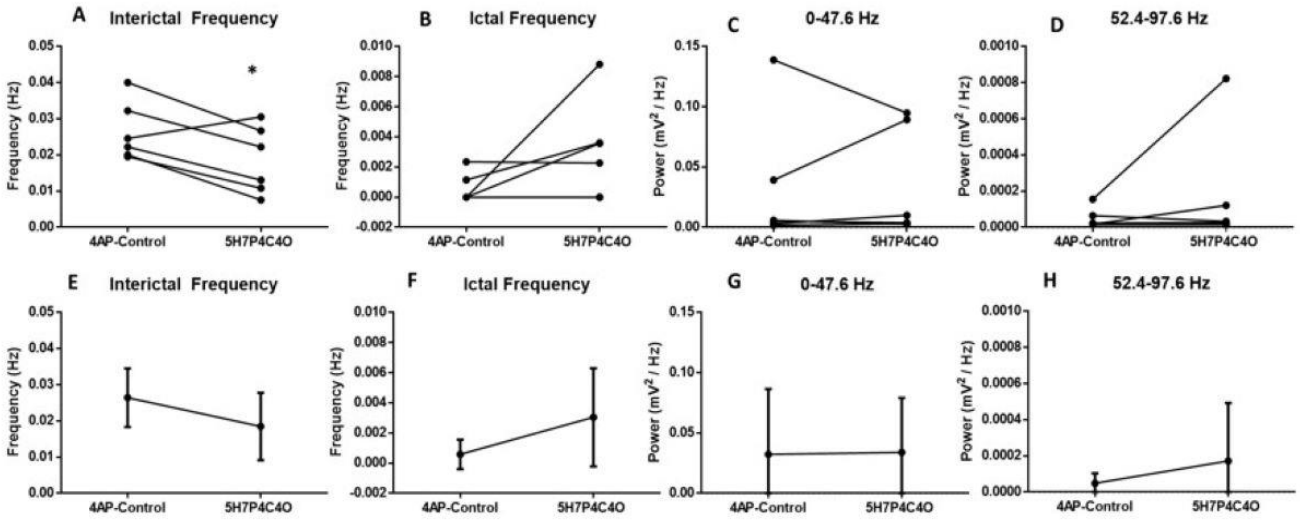


Figure 6. Frequency of interictal (A) and ictal events (B) in 4AP epilepsy model. Interictal frequency significantly decreses after 5H7P4C4O application. Total power of each sample frequency range 0-47,6 Hz (C) and 52,4-97,6 Hz range (D). Average frequency of interictal activities (E) and ictal acitivities (F). Average power of 0-47,6 Hz frequency range (G) and 52,4-97,6 Hz range (H). (n=6, mean±SD).

Second, the study reports the absorbance and fluorescence properties of 5H7P4C4O for the first time. 5H7P4C4O has absorbance peak at ultraviolet wavelength (UV) (315 nm) and also indicates fluorescence properties in visible range with the peak at 455 nm. However, the fluorescence curve is not sharp, indicating limitations in its usefulness for imaging purposes. Moreover, the UV excitation wavelength of the molecule raises concerns about potential harmful effects, such as cancer, in a biological environment.

The molecular docking analyses reveal that 5H7P4C4O has the potential to interact with important neuronal targets involved in excitability, specifically the NMDA and GABA-A receptors. The binding energies suggest that 5H7P4C4O may act as a blocker of the NMDA receptor and potentially have affinity for the GABA-A receptor as a blocker. There are H-bonds between the NMDA receptor 5B3J and the reference molecule ifenprodil at the GLU 284 and SER131 region. On the other hand the 5H7P4C4O molecule, can make H-bond in the GLN153 region. However, 5H7P4C4O has a van der Waals interaction to the GLU 284 and SER131 region (SFigure-1). The binding energies of Ifenprodil and 5H7P4C4O are -8.20 and -6.97, respectively (Table 2). Because of these results, it was thought that 5H7P4C4O might be a blocker of the NMDA receptor. Bicuculline is a well-known GABA-A receptor blocker and has no H-bond with the GABA-A receptor 6D6T, but can Alkyl bond with PRO184, MET58, PRO140 sites and Pi-Anion bond with GLU138 (SFigure-1). The 5H7P4C4O molecule can also Alkyl bond with PRO184 and MET58, and conventional H-bond with GLU138. The binding energies are -6.76 and -6.34 for bicuculline and 5H7P4C4O, respectively (Table 2).

The effect of the 5H7P4C4O molecule in two different epilepsy models (Mg^{+2}- free and 4AP) was investigated for the first time. In the Mg^{2+}-free model, 5H7P4C4O modulates the power of neuronal signals in the frequency range of 0-47 Hz by decreasing high-power signals and amplifying low-power signals. Although ifenprodil is an NMDA antagonist, it is not as potent as Mg or ketamine in reducing the ionic permeability of NMDA channels.

In the studies performed with the patch clamp technique, it was reported that ifenprodil application prolonged the excitatory post-synaptic current decay time in the temporal lobe epilepsy model compared to the control[16] and reduce neuronal excitability on neocortical pyramidal neurons of epilepsy patients in Mg^{+2}-free model.[14] Ifenprodil has been shown to be moderately effective in suppressing seizures in acute seizure models and has an anti–ictogenic effect different from its anti–convulsive effect in acute seizure models.[16,27] In this study 5H7P4C4O has no significant effect on interictal frequency, however, it extinguished the ictal discharges in Mg-free model. The main reason for this result may be that electrophysiological recordings are obtained from numerous cells within the brain slices, rather than focusing on a single cell as in the patch clamp technique. Additionally, in some recordings, interictals were poly-spiked and spike numbers were reduced after 5H7P4C4O

administration. These results suggest that 5H7P4C4O may be moderately effective in epilepsy models, similar to ifenprodil, by reducing interictal activity and potentially acting as a modulator of neuronal signals.

Aminopyridines (4AP) can induce generalized tonic seizures that were associated with neuronal discharges occurring in hippocampus, amygdala and neocortex.[28] 4AP blocks potassium channels and generates both ictal and interictal like events.[29] In this study both ictal and interictal discharges were observed after 4AP bath application to the mice brain slices. However 10µM 5H7P4C4O application significantly reduced the interictal activity, which may be the result of the inability to generate interictal signals due to the predominance of ictal signals after 5H7P4C4O administration. Although the molecular docking results showed that 5H7P4C4O has affinity for the GABA-A receptor, the ex-vivo test results indicated that the molecule had neither antagonist nor agonist effect on the GABA-A receptor.

Overall, the study highlights the unique properties of 5H7P4C4O, including its absorbance and fluorescence characteristics, as well as its effects on epileptiform activity in different models. 5H7P4C4O has no excitatory effect in the brain in ACSF for three concentrations (5, 10 and 20 µM) which indicate that it does not stimulate epileptic activity. However it only indicated modulatory effect on the Mg^{+2}-free epilepsy model. Additionally modulatory effect on the power of neuronal stimulation, specifically gathering the power of the frequency range of 0-47 Hz signals in a certain range suggest that it may be effective through secondary messenger pathways or other pathways that we could not examine in this study such as endocannabinoid pathway or cholecystokinin which modulates intrinsic neuronal excitability and synaptic transmission.[30] The molecule's modulatory effect on neuronal stimulation and its ability to gather power signals within a specific frequency range suggest potential effectiveness in cognitive or other brain functions. Further research is necessary to explore the broader implications and mechanisms of action of 5H7P4C4O in epilepsy and other neurological conditions.

In conclusion, Overall, the study highlights the unique properties of 5H7P4C4O, including its absorbance and fluorescence characteristics, as well as its effects on epileptiform activity in different models. 5H7P4C4O has no excitatory effect in the brain in ACSF for three concentrations (5, 10 and 20 µM) which indicate that it does not stimulate epileptic activity. However it only indicated modulatory effect on the Mg+2-free epilepsy model. Additionally modulatory effect on the power of neuronal stimulation, specifically gathering the power of the frequency range of 0-47 Hz signals in a certain range suggest that it may be effective through secondary messenger pathways or other pathways that we could not examine in this study such as endocannabinoid pathway or cholecystokinin which modulates intrinsic neuronal excitability and synaptic transmission.30 The molecule's modulatory effect on neuronal stimulation and its ability to gather power signals within a specific frequency range suggest potential effectiveness in cognitive or other brain functions. Further research is necessary to explore the broader implications and mechanisms of action of 5H7P4C4O in epilepsy and other neurological conditions.

Compliance with Ethical Standards

The study protocol was approved by the Kocaeli University Ethics Committee (Date: 09.05.2023, No: 2023-110).

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

All the authors equally contributed to this work.

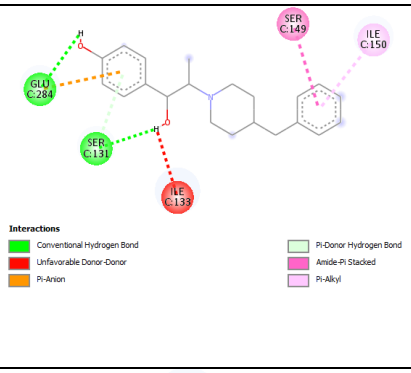
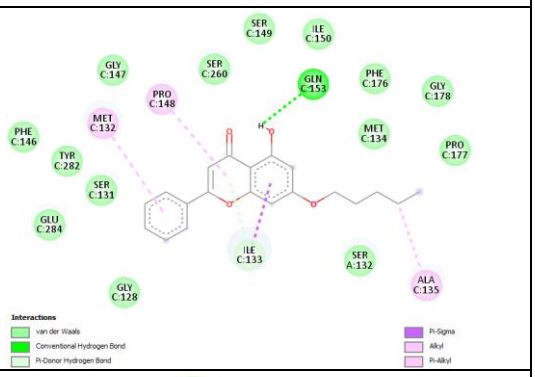
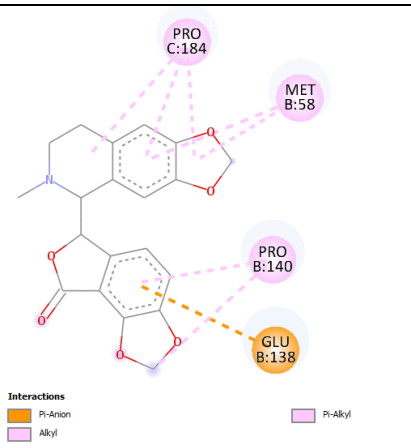
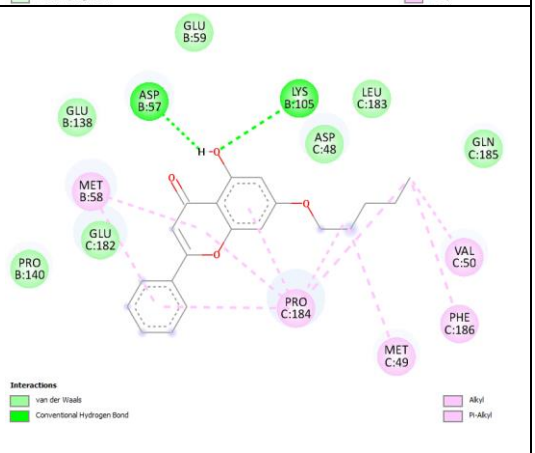
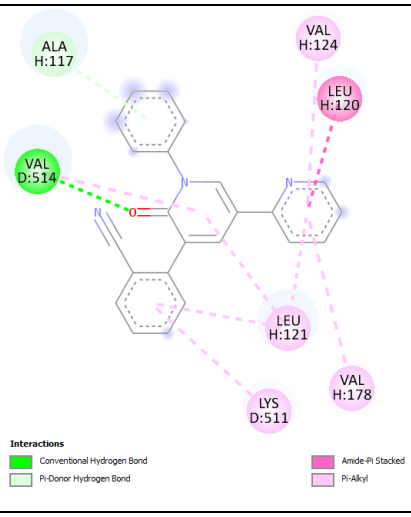
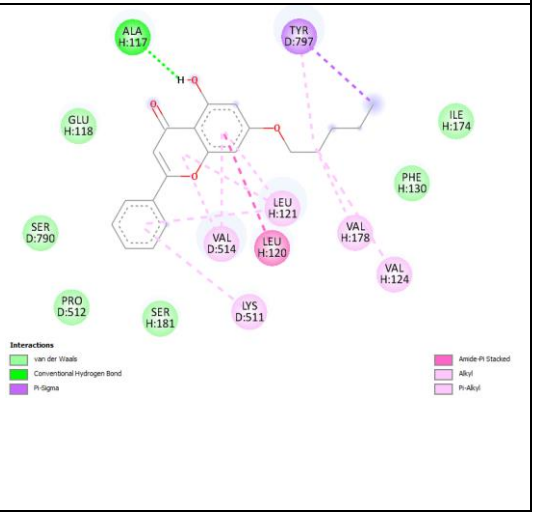
Financial Disclosure

None

References

1. Sharifi-Rad J, Quispe C, Herrera-Bravo J, et al. A Pharmacological Perspective on Plant-derived Bioactive Molecules for Epilepsy. *Neurochemical research* 2021;46(9):2205-2225. doi:10.1007/s11064-021-03376-0
2. Ozturk H, Basoglu H, Yorulmaz N, Aydin-Abidin S, Abidin I. Fisetin decreases the duration of ictal-like discharges in mouse hippocampal slices. *Journal of Biological Physics* 2022;48(3):355-368. doi:10.1007/s10867-022-09612-0
3. Ozturk H, Yorulmaz N, Durgun M, Basoglu H. In silicoinvestigation of Alliin as potential activator for AMPA receptor. *Biomedical Physics & Engineering Express.* 2021;8(1). doi:10.1088/2057-1976/ac351c
4. Hanada T. Ionotropic Glutamate Receptors in Epilepsy: A Review Focusing on AMPA and NMDA Receptors. *Biomolecules.* 2020;10(3):464.
5. Hansen KB, Yi F. Structure, function, and allosteric modulation of NMDA receptors. *J Gen Physiol*. 2018;150(8): 1081-1105. doi:10.1085/jgp.201812032
6. Blanke ML, VanDongen AMJ. Frontiers in Neuroscience Activation Mechanisms of the NMDA Receptor. In: Biology of the NMDA Receptor. (Van Dongen AM. ed.) CRC Press/Taylor & Francis Copyright © 2009, Taylor & Francis Group, LLC.: Boca Raton (FL); 2009.
7. Anderson WW, Anderson WW, Lewis DV, et al. Magnesium-free medium activates seizure-like events in the rat hippocampal slice. *Brain research* 1986;398(1):215-219. doi:https://doi.org/10.1016/0006-8993(86)91274-6
8. Treiman DM. GABAergic mechanisms in epilepsy. *Epilepsia* 2001;42 Suppl 3(8-12). doi:10.1046/j.1528-1157.2001.042 suppl.3008.x
9. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 2017;7(1):42717. doi:10.1038/srep42717
10. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. *Int J Mol Sci*. 2019;20(18). doi:10.3390/ ijms20184331
11. Bertram EH. Temporal lobe epilepsy: Where do the seizures really begin? *Epilepsy & Behavior* 2009;14(1, Supplement 1):32-37. doi:https://doi.org/10.1016/j.yebeh.2008.09.017
12. Abidin İ, Aydin-Abidin S, Mittmann T. Neuronal excitability and spontaneous synaptic transmission in the entorhinal

cortex of BDNF heterozygous mice. *Neuroscience letters.* 2019;690(69-75). doi:10.1016/j.neulet.2018.10.019
13. Basoglu H OH, Keser H, Aydin-Abidin S, Abidin I. L-Theanine Reduces Epileptiform Activity In Brain Slices. *Akdeniz Tıp Dergisi*. 2022;8(2):121-127. doi: 10.53394/ akd.1057342
14. Wang X, He X, Li T, et al. Anti-epileptic effect of ifenprodil on neocortical pyramidal neurons in patients with malformations of cortical development. *Experimental and therapeutic medicine.* 2017;14(6):5757-5766. doi:10.3892/ etm.2017.5311
15. Aydin-Abidin S, Abidin İ. 7,8-Dihydroxyflavone potentiates ongoing epileptiform activity in mice brain slices. *Neuroscience letters.* 2019;703(25-31). doi:10.1016/j.neulet. 2019.03.013
16. Gorlewicz A, Pijet B, Orlova K, et al. Epileptiform GluN2B-driven excitation in hippocampus as a therapeutic target against temporal lobe epilepsy. *Experimental neurology*. 2022;354(114087). doi:10.1016/j.expneurol.2022.114087
17. Masiulis S, Desai R, Uchański T, et al. GABA(A) receptor signalling mechanisms revealed by structural pharmacology. Nature. 2019;565(7740):454-459. doi:10.1038/s41586-018-0832-5
18. Lipinski CA, Lombardo F, Dominy BW, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews* 2001;46(1-3):3-26. doi:10.1016/s0169-409x(00)00129-0
19. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *Journal of combinatorial chemistry* 1999;1(1):55-68. doi:10.1021/cc9800071
20. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem*. 2002;45(12):2615-23. doi:10.1021/jm020017n
21. Egan WJ, Merz KM, Jr., Baldwin JJ. Prediction of drug absorption using multivariate statistics. *J Med Chem.* 2000;43(21):3867-3877. doi:10.1021/jm000292e
22. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. *J Med Chem*. 2001;44(12):1841-1846. doi:10.1021/jm015507e
23. Martin YC. A bioavailability score. J Med Chem 2005;48(9):3164-3170. doi:10.1021/jm0492002
24. Potts RO, Guy RH. Predicting skin permeability. *Pharmaceutical research* 1992;9(5):663-669. doi:10.1023/ a:1015810312465
25. Delaney JS. ESOL: estimating aqueous solubility directly from molecular structure. *Journal of chemical information and computer sciences*. 2004;44(3):1000-1005. doi:10.1021/ ci034243x
26. Ali J, Camilleri P, Brown MB, et al. In Silico Prediction of Aqueous Solubility Using Simple QSPR Models: The Importance of Phenol and Phenol-like Moieties. *Journal of Chemical Information and Modeling*. 2012;52(11):2950-2957. doi:10.1021/ci300447c
27. Yen W, Williamson J, Bertram EH, et al. A comparison of three NMDA receptor antagonists in the treatment of prolonged status epilepticus. *Epilepsy Research.* 2004;59(1):43-50, doi:10.1016/j.eplepsyres.2004.03.004
28. Lévesque M, Salami P, Behr C, et al. Temporal lobe epileptiform activity following systemic administration of 4-aminopyridine in rats. *Epilepsia.* 2013;54(4):596-604. doi:10.1111/epi.12041
29. Myers TL, Gonzalez OC, Stein JB, et al. Characterizing Concentration-Dependent Neural Dynamics of 4-Aminopyridine-Induced Epileptiform Activity. *Epilepsy journal*.2018;4(2). doi:10.4172/2472-0895.1000128
30. Lee SY, Soltesz I. Cholecystokinin: a multi-functional molecular switch of neuronal circuits. *Developmental neurobiology.* 2011;71(1):83-91. doi:10.1002/dneu.20815

Reference Molecule-Receptor	2D structure image		2D structure image (5H7P4C4O)
Ifenprodil-5B3J		5B3J	
Bicuculline-6D6T		6D6T	
Perampanel-7LEP		7LEP	

SFigure 1. The 2D image of the bindings of 5H7P4C4O and reference molecules with the receptors