RESEARCH PAPER



Investigation of anti-inflammatory drug usage in the treatment of epilepsy through analyzing the epilepsy-inflammation relation at the molecular level

Buse Nur Maden ¹, Saliha Ece Acuner ^{1,2*}

¹Department of Bioengineering, Istanbul Medeniyet University, 34700, Istanbul, Türkiye ²Science and Advanced Technology Research Center (BİLTAM), Istanbul Medeniyet University, 34700, Istanbul, Türkiye

How to cite:

Maden, B. N., & Acuner, S. E. (2025). Investigation of anti-inflammatory drug usage in the treatment of epilepsy through analyzing the epilepsy-inflammation relation at the molecular level. *Biotech Studies*, *34*(SI), 18-28. <u>http://doi.org/10.38042/biotechstudies.1662530</u>

Article History

Received 22 August 2024 Accepted 11 March 2025 First Online 17 March 2025

Corresponding Author

Tel.: +90 216 280 3333-4035 E-mail: ece.ozbabacan@medeniyet.edu.tr

Keywords

Proteome Transcriptome Protein-protein interaction Subnetwork analysis Molecular docking

Copyright

This is an open-access article distributed under the terms of the <u>Creative Commons Attribution 4.0</u> <u>International License (CC BY).</u>

Introduction

Abstract

Epilepsy is a neurological brain disorder that affects social life by causing seizures, loss of consciousness, and muscle contractions. The causes of epilepsy include tumors, genetic diseases, brain injuries during childbirth, and infections affecting the body. Antiepileptic drugs are commonly used for treatment, but about one-third of individuals continue to experience seizures despite medication. These drugs effectively prevent seizures but do not address the underlying mechanisms of the disease. Inflammation, i.e. biological response of the body's immune system to harmful stimuli, can be the result or cause of various diseases and is recognized as one of the contributing factors to epilepsy. This study investigates the molecular-level connection between inflammation and epilepsy through mapping transcriptomic data to reconstructed protein-protein interaction (PPI) network, reveal important subnetworks and detect potential common drug targets for inflammation and epilepsy. The most connected hub proteins of the subnetwork related to inflammation, derived from the expression data mapping are AKT1, IL6, and TLR4. After conducting molecular docking studies of anti-inflammatory drugs with these targets, Resveratrol and Fentanyl were identified as potential drugs with antiinflammatory effects and suitable for epilepsy. Thus, we suggest further experimental studies for validation of using anti-inflammatory drugs Resveratrol and Fentanyl against epilepsy.

Epilepsy is a prevalent neurodegenerative disease that impacts approximately 46 million individuals worldwide, being the third most common chronic brain illness, causing seizures, convulsions, and altered consciousness (<u>Zhu et al., 2021</u>). The symptoms are seizures, convulsions, and altered consciousness, caused by unexpected discharges within neurons (<u>Beghi,</u> 2020). Epilepsy was first classified based on six criteria: etiology, age, seizure type, interictal and ictal features of electroencephalogram (EEG), and anatomical status (<u>Gastaut, 1970</u>). In this classification, the difference between epileptic syndrome and epileptic convulsions was also highlighted. Epilepsies are further classified into three subgroups based on etiology: idiopathic epilepsy is often associated with hereditary predisposition; in symptomatic epilepsy, a specific cause can be identified; and cryptogenic epilepsy is believed to be symptomatic (Shorvon, 2011). Additionally, seizures can be classified based on their onset type (Scheffer et al., 2017). Seizures starting from a specific cortical area are called focal seizures. Generalized seizures involve widespread and symmetrical involvement of both sides of the cortical region. The starting point is unclear in unknown onset seizures. These classifications help differentiate epilepsies based on their etiological and clinical characteristics, enabling more accurate diagnosis and treatment approaches (<u>Scheffer et al.,</u> 2017).

Epilepsy treatment is long-term and aims to control neuronal hyperexcitability. Surgery and antiepileptic drug (AED) therapies are the main treatments for epilepsy (Beghi, 2020). Treatment typically involves starting medication after the second seizure. Ideal characteristics of an antiepileptic drug (AED) include good oral bioavailability, no side effects, simple pharmacokinetics, no drug interactions, low cost, and the ability to be taken once or twice a day (Akdağ et al., 2016). A variety of AEDs are available, but approximately one-third of patients do not respond to medication and continue to experience seizures (Beghi, 2020). It is important to note that while AEDs can effectively prevent seizures in those who respond to treatment, they do not address the underlying cause of the disease (Beghi, 2020).

Epilepsy is frequently linked to a history of prior nervous system injuries where the activation of inflammatory cells is dysregulated and neuronal tissue is damaged, leading to epilepsy development (Rana & Musto, 2018). However, the specific mechanisms through which this imbalance in inflammation, i.e., the immune system response to injuries, contribute to epilepsy remain poorly understood. Inflammation is a mechanism that occurs when immune cells are activated and produce inflammatory mediators. Natural killer (NK) cells, granulocytes (neutrophils, eosinophils, basophils, mast cells), mononuclear cells (monocytes, macrophages, microglia), dendritic cells, and Toll-like receptors (TLRs) are cells that activate the innate immune response (Vezzani et al., 2011). Acute inflammation is short-term and rapidly developing inflammation, usually in response to infections, injuries, or toxic agents. Symptoms include redness, fever, swelling, pain, and difficulty moving. Some conditions are minor and can heal on their own, while others may require treatment. Chronic inflammation lasts for a long time and is usually the result of untreated acute inflammation or triggered by autoimmune diseases, infections, or environmental factors (Hannoodee & Nasuruddin, 2024). Inflammation can be one of the results as well as an underlying cause of epilepsy (Beghi, 2020) and thus, the relation between inflammation and epilepsy is not clear at the molecular level. Gaining insight into the neurobiology of inflammation related to epileptogenesis (complex biological processes leading to the development and progression of epilepsy) can aid in novel biomarker identification and new therapeutic target discovery for the prevention and treatment of epilepsy (Rana & Musto, 2018).

Transcriptomic and genetic analyses can help in finding drivers and offer new perspectives on the origin and persistence of seizures in epilepsy (<u>Guelfi et al.,</u> 2019). Moreover, these analyses can also help in revealing neuronal subtypes of epilepsy and gene expression underlying epileptogenesis (<u>Pfisterer et al.,</u> 2020). Integrating transcriptomic and genetic analysis

results with proteomic data is a comprehensive and valuable approach in many diseases (Uzuner et al., 2021; Maden & Acuner, 2021). Protein-protein interaction (PPI) networks related to the development of inflammation in the body can be a guide in understanding the effect of gene expression changes in disease conditions and analysis of important subnetworks can aid in the discovery of important drug targets in the PPI network (Maden & Acuner, 2021). These approaches typically include integrating transcriptomic data into PPI networks, functional enrichment of important subnetworks as well as topological analyses for discovering vital elements of the networks, such as hubs with a high number of interactions (Hollander et al., 2021; Maden & Acuner, 2021; Steinkellner et al., 2024).

In this work, to understand how inflammation contributes to epilepsy at a molecular level, we reconstructed the protein-protein interaction network related to inflammation and epilepsy, and detected and analyzed the subnetworks by mapping the transcriptomic data related to epilepsy onto the reconstructed network. As such, we have combined transcriptomic and proteomic data to reveal important subnetworks simultaneously contributing to inflammation and epilepsy. Finally, we investigated the anti-inflammatory drugs targeting key proteins in the network to indicate anti-inflammatory drugs potentially suitable for epilepsy treatment.

Materials and Methods

Here we describe the computational methods used in the reconstruction of a protein-protein interaction (PPI) network for inflammation- and epilepsy-related genes, mapping transcriptomic data for epilepsy on the PPI network for detection of important subnetworks, and analyzing them for inflammation- and epilepsyrelated important pathways and hubs targetable by anti-inflammatory drugs.

Reconstruction of the PPI network

In the first stage, the inflammation- and epilepsyrelated PPI network was reconstructed and visualized based on the protein/gene data related to inflammation and epilepsy. Proteins related to inflammation and epilepsy were retrieved (on 04.08.2022) from the Uniprot database (Wang et al., 2021) and some proteins encoded by genes related to both, namely TNF, IL11, and IL15, were manually added after a literature review (Medina-Ceja et al., 2019; Vezzani et al., 2011; Vezzani et al., 2016). The PPI network was reconstructed using these seed proteins and extended further via the STRING database (Szklarczyk et al., 2023). The resulting network was uploaded to Cytoscape (Shannon et al., 2003). Visualization, topological analysis, and functional enrichment of the network were also performed in Cytoscape.

Determination of differentially expressed genes

The second step is the identification of genes with significant changes related to the epilepsy condition (differentially expressed genes, DEGs). To find data on epilepsy gene expression, we conducted a search on the Gene Expression Omnibus (GEO) database (Barrett et al., 2013). From the available datasets, we selected the most suitable ones, which are identified as the GSE143272 and GSE20977. GSE143272 dataset contains 142 samples, comprising 34 recently diagnosed epilepsy patients (including idiopathic, cryptogenic, and symptomatic epilepsy) who are not on medication (drug-naïve), 57 patients who are undergoing treatment with antiepileptic drugs, and 51 healthy individuals (Rawat et al., 2020). We included 34 drug-naïve epilepsy patients in the "patient" group, and the healthy control group comprised 51 "control" samples. The GSE20977 dataset includes seven epilepsy patients with heterozygous chr16p13.11 deletion and eight controls with no deletion at this locus to evaluate the deletion as a risk factor in epilepsy patients (Heinzen et al., 2010). Thus, the "patient" group had 7 individuals with epilepsy who had a deletion, while the "control" group had 8 healthy samples without the deletion.

We analyzed the comparative gene expression levels after acquiring the suitable datasets and groups using the interactive web tool GEO2R in GEO. DEGs are decided based on the p-value, such that those with a value less than 0.05 are considered as a gene whose expression changes significantly in the disease state. The false discovery rate (FDR) p-value threshold (p-value < 0.05) was applied to elucidate DEGs (<u>Thiese et al., 2016</u>) such that 0.05 indicates the amount of possible errors we will make when we decide whether there is a statistically significant difference. Moreover, Log2-fold change (logFC) values calculated between two experimental conditions were used to find the up- and down-regulated genes with the threshold of the absolute value of logFC being greater than or equal to 1. Lastly, heatmaps were created for the top 20 DEGs of each dataset based on the logFC values, using Python's Heatmap function in the Seaborn library.

Discovery and topological analysis of PPI subnetworks related to inflammation and epilepsy

The third step was the discovery of the important subnetwork(s) in the reconstructed PPI network for inflammation and epilepsy by mapping expression data (DEGs) to the PPI network and analysis of subnetwork(s) to find enriched pathways and hub proteins as potential antiepileptic drug targets through the use of Cytoscape's KPM and ClueGO plugins as well as topological analysis options.

KPM (KeyPathwayMiner) is a highly preferred subnet discovery algorithm due to its easy-to-use for parameter changes and module display and better results compared to other subnet discovery algorithms (Alcaraz et al., 2012). The KPM algorithm utilizes pvalues as '1' and '0'. In the KPM input, a p-value less than 0.05 is set to '1' for significantly expressed genes, while a p-value greater than 0.05 is set as '0' for genes whose expression is not significant. In KPM, the K parameter value is a crucial factor representing the maximum allowable number of unchanged genes in a discovered subnetwork. The optimal K value was chosen as 5 because it was observed in a study that no new significant genes were added when it was above 5 (Alcaraz et al., 2016). To create a KPM input file for epilepsy, specific conditions were applied to the transcriptome data: data other than the gene name and p-value were deleted, and the p-values less than 0.05 were recorded as 1, or else as 0. In Cytoscape, subnet discovery was performed using the method of mapping the protein-protein interactions of inflammation and epilepsy and the expression data obtained for epilepsy. The combined PPI networks opened in the Cytoscape application, and transcriptome data from the GSE143272 and GSE20977 datasets were integrated through KPM. As a result, epilepsy-related proteinprotein interaction subnetworks were determined. The hub proteins (genes with the highest number of interactions, that is, the degree) in the subnetwork we obtained were determined through topological analysis, and the discovered subnetwork was functionally enriched by using the ClueGO plugin of Cytoscape to reveal the related pathways to this critical subnetwork.

Evaluation of potential antiepileptic usage of antiinflammatory drugs through docking

The final step was finding suitable antiinflammatory drugs targeting the subnetwork through analyzing drug-gene interactions and applying molecular docking for molecular details. The hub proteins in the subnet were searched in the drug-gene interactions section of DGIdb (Freshour et al., 2021). Based on these drug-gene interactions results, we selected anti-inflammatory drugs with multiple targets in the subnet; namely, Resveratrol and Fentanyl, as our candidate drugs. Molecular docking studies were the final step in understanding drug-protein interactions at the atomic detail.

Firstly, the structures of the drugs were downloaded from DrugBank, and target proteins from the PDB (Berman et al., 2000; Wishart et al., 2018). In the Chimera application, water molecules in the target protein structures were removed, and other molecules were excluded if present. Drug molecules were also prepared in Chimera for binding. Blind docking studies were conducted without selecting a specific interaction region and considering the entire protein for both the prepared protein and drug molecules. These procedures were repeated separately for each drug and protein pair. The docking score is a scoring function that predicts the binding affinities of a ligand and target after docking. Chimera version 1.16 was used to prepare the structures and perform molecular docking via Autodock Vina (Trott <u>& Olson, 2010</u>). We performed docking studies with four target proteins for two drugs, resulting in eight different drug-protein complex structure models. The obtained docking models were analyzed and visualized using Discovery Studio (Gao & Huang, 2011) and binding regions were compared with the known binding sites of the target proteins using the PDBsum (Pictorial database of 3D structures in the Protein Data Bank) web server (Laskowski et al., 2018).

Results and Discussion

Information about the PPI network

To obtain a Protein-Protein Interaction (PPI) network for inflammation and epilepsy, we scanned the literature for relevant proteins and added them to the lists of 'Inflammation' and 'Epilepsy' proteins downloaded from the UniProt database. The number of inflammation proteins obtained from the UniProt database is 539, and the number of epilepsy proteins is 532. Gaps and duplicates in the obtained protein lists were removed. Additionally, three proteins associated with inflammation found in the literature (TNF, IL11, IL15) were added, bringing the total number of proteins to 953. By increasing the interaction count in the STRING database, an interaction network was obtained with 10,463 interactions and 910 nodes. The reconstructed inflammation-epilepsy PPI interaction network was downloaded, visualized, and topologically analyzed via Cytoscape. The top 5 hub proteins according to their number of degrees within this network were found to be TNF (243), IL1B (211), IL6 (206), AKT1 (187), and GAPDH (187). The top three hubs with the highest number of interactions were previously shown to be chemical mediators relevant to neuro-inflammation that may contribute to epileptogenesis (Rana & Musto, <u>2018</u>).

Identification of DEGs in epilepsy patients

DEG analysis resulted in the identification of 1,682 and 3,279 DEGs in the GSE20977 and GSE143272 datasets, respectively. The common DEGs between these two datasets (151) are shown in <u>Table 1</u>, and the top 20 DEGs for each dataset are represented as heat maps in <u>Figure 1</u>. Although we could not find any up- or E143272 dataset, in tl

21

down-regulated genes for the GSE143272 dataset, in the GSE20977 dataset; as an impact of the deletion of the chr16p13.11 on epilepsy patients' gene expression, we observed 1 up-regulated (RNA28S5 with logFC 1.35) and 2 down-regulated genes, namely NDE1 (with logFC - 1.12, Figure 1A) and CXCL8/IL8 (with logFC -1.09).

Nuclear distribution factor E (NDE1) was shown to be an epilepsy-causing candidate gene located at 16p13.11, and the protein encoded by NDE1 is important for cortical development (Heinzen et al., 2010; Liu et al., 2012; Pei et al., 2014). Moreover, it was reported that the 16p13.11 deletion led to the reduction of NDE1 expression levels (Heinzen et al., 2010). Thus, these results support our observation of the downregulation of NDE1 in epilepsy patients with heterozygous chr16p13.11 deletions. Moreover, the other down-regulated gene, CXCL8/IL8, is a chemokine having a significant role in inflammation. Evidence indicates that CXCL1-IL-8 signaling is activated in epilepsy patients, leading to acute and chronic seizures, and this pathway can be targeted for anti-ictogenic effects, i.e., to prevent or reduce seizure activity by targeting mechanisms that trigger seizures (Di Sapia et al., 2021). Although a direct relation could not be found in the literature, we observe that chr16p13.11 deletions also cause a down-regulation of CXCL8 gene expression, indicating a possible relation between this gene and/or interaction partners with chr16p13.11.

Subnetwork analysis

In the first part of this study, we identified proteinprotein interactions related to inflammation and epilepsy. In the second part, we analyzed differential gene expression data for epilepsy. We then combined these findings using Cystoscape's KPM implementation, which allowed us to map the expression data onto the established PPI network. Through this process, we discovered a significant subnetwork that contained common elements between inflammation and epilepsy (Figure 2). This subnet consists of 200 nodes and 856 edges, and the top 5 hub proteins within this subnet based on their number of degrees, namely AKT1 (56), IL6

Tahla 1	Common	DEGs	hotwoon	GEO	datasets	GSE20977	and	GSF1/13272
I able I.	COMMON	DEGS	Detween	GEO	ualasels	G3E209//	anu	G3E143Z/Z

			CLS OSEE05	77 and COLT	19272				
ABLIM1	ESYT2	LAT	POMT1	STAMBP	BCCIP	FBXO18	LUC7L	PRRG4	TAF15
ACOT7	EXOSC10	LAT2	PPP6R2	STK24	BLVRB	FBXO30	MAX	PSME1	TALDO1
AGFG1	EYA3	LOC105377348	PRKAB1	SUGP2	BTBD10	FEZ1	METTL17	QPCT	TATDN3
ARF3	EZH2	LRFN3	PRKCB	SUMO1P1	BTG1	FGGY	MPRIP	QSOX1	TBC1D9B
ARRB1	FAR2	LSMEM1	PRR5	SVIL	CAPN5	FGL2	MPZL2	RAB11FIP1	TFRC
CASP4	GPAT3	MSRB3	RAC1	TM9SF1	CLEC4A	HIST2H2BE	NME3	RNF125	TSPO
CASP8	GTF2H4	MUT	RAD51D	TMED4	CLIP1	HM13	NOL12	RNF145	TSPOAP1
CD47	GTF3C6	NAB1	RBM4	TNFSF14	CPSF4	HMGB2	NPEPPS	RNF146	UBA3
CDC14A	HACD3	NAE1	RBM43	TRMT11	CREBBP	HSD17B11	NRBP2	RNFT1	URGCP
СНКА	HIST2H2AA3	NDUFAF7	RIT1	TROVE2	CRELD2	HSH2D	NT5C3A	RORA	UROS
CRIPT	IKBIP	NUP210	RPIA	USP15	DYNLRB1	KIFC2	PDPK1	SMARCA4	ZDHHC8
CUL4A	IL7R	NUP214	RPS15	VMA21	DYNLT1	KLRC3	PELO	SMIM12	ZMAT3
CXCL8	ISCU	ORM1	RPS19	VPS13B	ECHDC2	KLRG1	PFDN6	SNHG8	ZMYND19
CYFIP2	ITPR3	PANK2	RTN3	WTAP	EIF4G1	KMT2A	PGAM4	ST13P4	ZNF200
DDIAS	KIAA0430	PDIA3P1	SIAH1	ZBTB44	ELF2	LAS1L	PILRA	ST3GAL6	ZNF337
ZNF791									



Figure 1. Heatmaps based on logFC values of top 20 differentially expressed genes (DEGs) in analyzed datasets.

(52), TLR4 (50), CTNNB1 (41), and RELA (38), are considered as potential drug targets.



Figure 2. Inflammation- and epilepsy-related PPI subnetwork obtained by Cytoscape-KPM. Round-shaped white nodes indicate mapped but inactive genes in both expression datasets. Round and light green colors indicate genes mapped in two datasets; one active and the other inactive. Rounded and dark green colors are active genes in both datasets. The hexagon and white shape means that the gene is in one dataset but not in the other and is not active. Genes with hexagons and greens are present and active in one dataset but absent in the other.

Our research findings have revealed proteins associated with inflammation and epilepsy, and these proteins, referred to as hub proteins, are supported by the literature. *AKT1*, one of three closely related serine/threonine-protein kinases (*AKT1*, *AKT2*, and *AKT3*), is termed *AKT* kinase and regulates various

processes such as metabolism, proliferation, cell survival, growth, and angiogenesis (Rönnstrand, 2004). Bittigau et al. proposed a potential mechanism on rats to explain cognitive impairment and decreased brain mass associated with prenatal or postnatal exposure to antiepileptic drugs (AEDs). They tested whether common AEDs induced neurodegeneration in the developing rat brain. Rats aged 3-30 days were administered phenytoin, phenobarbital, diazepam, clonazepam, vigabatrin, or valproic acid. Histological examination of the brains revealed widespread and dose-dependent apoptotic neurodegeneration during the brain growth spurt period in developing rat brains. The findings suggested that the depression of MAPK and PI3-AKT pathways by AEDs contributed to the induction of neuronal apoptosis in the developing brain (Bittigau et al., 2003). Chouchi et al. extensively reviewed numerous research studies that used pharmacogenetics in interventions aiming to improve therapeutic responses based on personalized treatment for epilepsy (Chouchi et al., 2019). They found that ABCB1 polymorphism increased the risk of developing AED resistance (Chouchi et al., 2019). Uddin et al. stated that CCL2, CXCL8, and CXCL10 are chemokines playing a significant role in inflammation in the central nervous system (Uddin et al., 2005). Octavio Fabián Mercado-Gómez et al. (2018) measured the gene expression of inflammatory-related and nitric oxide synthase genes in olfactory bulb (OB) tissue from patients with frontal lobe epilepsy (FLE). They conducted qPCR for IL-18, IL-6, TNF- α , RELA, and Toll-like receptor 4 (TLR4) and found a significant increase in downstream transcription factor NFκB p65 (Mercado-Gómez et al., 2018). The proinflammatory cytokine IL6 and Toll-like receptor 4 (TLR4) involved in signaling related to ictogenesis are examples of neuro-inflammation-related chemical mediators and receptors contributing to epileptogenesis (Rana & Musto, 2018). Moreover, tumor-suppressor CTNNB1



Figure 3. Functional enrichment of the inflammation- and epilepsy-related PPI subnetwork.

was found to be involved in neurodevelopmental disorders, such as epilepsy (<u>Zhuang et al., 2023</u>).

Enrichment analysis reveals that the subnetwork is significantly enriched with amino or fatty acid catabolism (Figure 3). In line with this finding, it was recently observed that disorders in organic acid metabolism, specifically catabolism, have been associated with the occurrence of epileptic seizures due to the toxic accumulation of metabolites (Shi et al., 2024; Yu & Pearly, 2013).

Anti-inflammatory drugs and molecular docking

In the final section, the hub proteins obtained (51 proteins with a degree \geq 10) were used as input for the drug-gene interaction in DGldb. The drugs targeting these proteins with the highest number of interactions are shown in <u>Table 2</u>. Each of the top five drugs (Resveratrol, Fentanyl, Nelfinavir, Lauroguadin, and Paclitaxel) interacts with four hub proteins in the subnetwork. Cenisertib, on the other hand, interacts with five proteins, making it the drug targeting the highest number of hubs simultaneously. However, further structural analyses were not conducted as it is a drug primarily designed for cancer, and it does not align with our research objectives.

Among these drugs, Resveratrol and Fentanyl, which have anti-inflammatory effects and exhibit strong interactions with important proteins in the epilepsy subnetwork, were selected as potential drugs for further structural analysis via molecular docking. Four hub proteins interacting with these two drugs and the three-dimensional structures of the drugs were obtained from PDB and DrugBank (Table 3). Docked models shown in Figures 4 and 5 correspond to the best poses obtained based on their scores (Table 3). The interactions between drugs and proteins, including hydrogen (H) bonds, van der Waals forces, π -anion, π - π stacking, π -cation, alkali, and π -sigma interactions, are also

illustrated in Figures 4 and 5. It is expected that the complexes with higher numbers of H bonds and/or nonbonded interactions have better prediction scores, i.e., lower negative values (Table 3). These docking models are obtained through blind docking (ligands target the whole structures of proteins without prior specification of binding sites), therefore, we also checked the literature to validate predicted binding sites (Table 3, Figures 4 and 5). Resveratrol drug is docked with the following hub targets, and there are common binding sites between predicted and experimental binding sites for AKT1, RIPK1, and PRKCA (Table 3, red residues). Binding site information for Fentanyl hub targets found in the literature is in line with the predicted residues of ABCB1 (Mora Lagares et al., 2020; Cheema et al., 2024), CXCL8, and IL6 (Nada et al., 2023; Tran et al., 2023) (Table 3). Although we do not have exact residue matches for RELA and ARRB1 models, the residues are not very far away from the experimental counterparts (Table 3).

|--|

Drugs	Target Genes
Resveratrol	AKT1, RELA, RIPK1, PRKCA
Fentanyl	ABCB1, ARRB1, CXCL8, IL6
Nelfinavir	ABCB1, AKT1, IL6, TLR4
Lauroguadin	AKT1, CHUK, PAK1, PRKCZ
Paklitaksel	ABCB1, AKT1, CXCL8, SYK
Cenisertib	AKT1, LYN, PAK1, PRKCZ, SYK

Targeting these hub proteins, Resveratrol and Fentanyl, which demonstrated the highest interactions, can be considered as candidates for epilepsy treatment in line with the project objectives. The connection between these drugs and epilepsy is also supported in the literature. Resveratrol is considered a potential therapeutic agent for the treatment of various diseases such as inflammation, pain, tissue damage, diabetes, and cancer (<u>Acungil & Özşahin Delibaş, 2022</u>). Resveratrol possesses anti-inflammatory, anti-aging, and anti-cancer properties. Shetty highlighted its ability to enter the brain without any adverse effects on the brain or body after peripheral administration, making it an interesting feature for use as a therapy in brain damage or neurodegenerative diseases (<u>Shetty, 2011</u>). The article discussed the application of Resveratrol to treat acute seizures, prevent chronic epilepsy resulting from acute seizures or status epilepticus (SE), and alleviate chronic epilepsy characterized by spontaneous recurring motor seizures (<u>Shetty, 2011</u>). Resveratrol was also proposed as a promising drug candidate for epilepsy treatment in another study (<u>Li et al., 2017</u>).



Figure 4. Complex structure models of Resveratrol with target proteins (PDB:Chain IDs) A) AKT1 (3096:A), B) RELA (3GUT:A), C) RIPK1 (4NEU:A), and D) PRKCA (3IW4:A).

To confirm this, they tested the protective effect of Resveratrol on a kainic acid (KA)-induced epilepsy model in rats and investigated the underlying mechanism (<u>Li et</u> <u>al., 2017</u>). Fentanyl is an opioid, and there are studies showing high doses of pain-relievers can induce epilepsy seizures, however, there is also evidence that using opioids can modulate anti-inflammatory microglial activation, leading to a reduction in the frequency and intensity of seizures (Lankhuijzen & Ridler, 2024). It was also recently mentioned that Fentanyl usage could extend epilepsy treatment (<u>Ramos-Matos et al., 2023</u>).



Figure 5. Complex structure models of Fentanyl with target proteins (PDB:Chain IDs) A) ABCB1 (6QEX:A), B) ARRB1 (7SRS:C), C) CXCL8 (5D14:A), and D) IL6 (1ALU:A).

Conclusion

Overall, this study investigated the link between epilepsy and inflammation, and some of the genes important for both of the conditions were found to be AKT1, IL6, TLR4, CTNNB1, and RELA, and significantly enriched pathways are related to amino or fatty acid catabolism. We computationally investigated the connection between inflammation and epilepsy at the molecular level and demonstrated that the antioxidant Resveratrol and the opioid Fentanyl drugs, carrying antiinflammatory properties, could also be candidate antiepileptic drugs targeting epilepsy-related proteins and pathways. However, further experimental research (*in vitro* and *in vivo* experiments) is needed to confirm the efficiency of these drugs in epilepsy therapy.

Table 5. Docking details of Resveration and rentary drugs	Table 3	 Docking 	details o	of Resveratrol	and I	Fentanyl drugs
---	---------	-----------------------------	-----------	----------------	-------	----------------

Drug	Target	PDB:Chain IDs	Partners of Targets in PDB	PDBsum Binding Site Information on Targets	Docking score (kcal/mol)	Predicted Binding Residues
	AKT1	3096:A	Allosteric inhibitor (IQO)	Hbond: SER205, Nonbonded: 80, 83- 85, 210, 211, 264, 268, 272, 273, 274, 190, 292, 296	-8.4	Hbonds:THR81, THR82, ASP292 Nonbonded: 79, 179, 211, 227, 270-272, 290, 291
Resveratrol	RELA (TF p65 of HIV-1 LTR region	3GUT:A	NF-kappa-B p105	Hbonds: ASN200, CYS216, ASP243, HIS245, ARG246 Salt Bridges: ARG198, ASP217, ASP243, ARG246 Nonbonded: 197- 200, 213, 215-217, 243, 245, 246, 248, 251	-5.6	Hbonds: GLU49, ALA192 Nonbonded: 28-30, 50-52, 191, 194, 274, 279
	RIPK1	4NEU:A	1- aminoisoquinoline inhibitor (Q1A)	Hbonds: GLU63, MET95, ASP156 Nonbonded: 31, 43, 63, 66, 67, 70, 75, 76, 92-95, 145, 155-157	-7.6	Hbonds: MET95, LEU157 Nonbonded: 23, 25, 31, 43, 45, 63, 67, 92, 94, 98, 145, 155, 156
	PRKCA	3IW4:A	NVP-AEB071 inhibitor (LW4)	Hbonds: GLU418, VAL420 Nonbonded: 345, 350, 353, 366, 368, 387, 401, 417-420, 467, 468, 470, 480, 481	-7.5	Hbonds: GLU387, GLU418 Nonbonded: 345, 350, 353, 368, 387, 391, 401, 415, 417, 419, 420, 470, 480, 481
	ABCB1	6QEX:A	UIC2 fab and taxol (TA1)	Hbonds: TYR307, TYR310, GLN725 Nonbonded: 69, 232, 307, 310, 336, 339, 340, 343, 344, 347, 725, 946, 953, 983, 986, 990 PHE303, ILE306, TYR310, LEU339, PHE343, GLN990 (<u>Mora Lagares et al.,</u> 2020) TRP232, PHE303, GLN990 (Cheema et al., 2024)	-8.6	Nonbonded: 69, 228, 229, 302, 303, 306, 307, 310, 336, 339, 340, 725, 728, 983, 990
<u>Fentany</u>	ARRB1	7SRS:C	5-HT2B receptor with LSD (7LD) (7SRS:R)	Hbonds: VAL8, LYS11, ARG25, TYR63 Nonbonded: 6-11, 25, 63, 65, 67, 70, 72-74, 76, 107, 241, 244, 245, 249, 285 ASP67, LYS160, ARG165, VAL167, LYS292 (Kushwah, 2024)	-7.2	Nonbonded: 26, 171-173, 296-299, 352, 354-356
	CXCL8	5D14:A (Binding info from 8IC0:F)	None (CXCR1 partner from 8IC0:A)	Hbonds: GLU2, ARG4, LYS9, PRO30, ARG45, GLU46 Salt Bridges: GLU2, ARG4, LYS9, Nonbonded: 1-6, 9, 11, 13, 26, 28-30, 32, 38, 45-48	-6.4	Nonbonded: 11, 14-17, 19, 41, 47, 59
	IL6	1ALU:A	None	LEU 57, TRP157 (<u>Nada et al., 2023</u>) SER47, GLU106, GLN156, TRP157, ASP160 (<u>Tran et al., 2023</u>)	-6.1	Nonbonded: 42, 43, 46, 104-107, 157, 160, 163

Funding Information

This study was funded by TÜBİTAK (Turkish Scientific and Technological Research Council) 2209-A University Students Research Projects Support Program with project number 1919B012105124. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

BNM: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft, review and editing; SEA: Supervision, Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft, review and editing.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or

personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the Scientific and Technological Research Council of Turkey (TÜBİTAK) for supporting our research through the 2209-A program under project number 1919B012105124.

References

- Acungil Z, Özşahin Delibaş EA. (2022). Resveratrol, Oxinflammation and Epilepsy. J TOGU Heal Sci., 2(1), 72-87.
- Akdağ G, Algın D, Erdinç O. EPİLEPSİ / EPILEPSY. Osmangazi Tıp Dergisi. 2016;38(1).
- Alcaraz, N., Friedrich, T., Kötzing, T., Krohmer, A., Müller, J., Pauling, J., & Baumbach, J. (2012). Efficient key pathway mining: combining networks and OMICS data. *Integrative biology: quantitative biosciences from nano* to macro, 4(7), 756–764. https://doi.org/10.1039/c2ib00133k

- Alcaraz, N., List, M., Dissing-Hansen, M., Rehmsmeier, M., Tan, Q., Mollenhauer, J., Ditzel, H. J., & Baumbach, J. (2016).
 Robust de novo pathway enrichment with KeyPathwayMiner 5. *F1000Research*, *5*, 1531. <u>https://doi.org/10.12688/f1000research.9054.1</u>
- Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2013). NCBI GEO: archive for functional genomics data sets--update. *Nucleic acids research*, *41*(Database issue), D991–D995.

https://doi.org/10.1093/nar/gks1193

- Beghi E. (2020). The Epidemiology of Epilepsy. Neuroepidemiology, 54(2), 185–191. https://doi.org/10.1159/000503831
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000). The Protein Data Bank. *Nucleic acids research*, 28(1), 235– 242.

https://doi.org/10.1093/nar/28.1.235

Bittigau, P., Sifringer, M., & Ikonomidou, C. (2003). Antiepileptic drugs and apoptosis in the developing brain. Annals of the New York Academy of Sciences, 993, 103–124.

https://doi.org/10.1111/j.1749-6632.2003.tb07517.x

- Cheema, Y., Linton, K. J., & Jabeen, I. (2024). Molecular Modeling Studies to Probe the Binding Hypothesis of Novel Lead Compounds against Multidrug Resistance Protein ABCB1. *Biomolecules*, 14(1), 114. <u>https://doi.org/10.3390/biom14010114</u>
- Chouchi, M., Klaa, H., Ben-Youssef Turki, I., & Hila, L. (2019). ABCB1 Polymorphisms and Drug-Resistant Epilepsy in a Tunisian Population. *Disease markers*, 2019, 1343650. https://doi.org/10.1155/2019/1343650
- Di Sapia, R., Zimmer, T. S., Kebede, V., Balosso, S., Ravizza, T., Sorrentino, D., Castillo, M. A. M., Porcu, L., Cattani, F., Ruocco, A., Aronica, E., Allegretti, M., Brandolini, L., & Vezzani, A. (2021). CXCL1-CXCR1/2 signaling is induced in human temporal lobe epilepsy and contributes to seizures in a murine model of acquired epilepsy. *Neurobiology of disease*, *158*, 105468. https://doi.org/10.1016/j.nbd.2021.105468
- Freshour, S. L., Kiwala, S., Cotto, K. C., Coffman, A. C., McMichael, J. F., Song, J. J., Griffith, M., Griffith, O. L., & Wagner, A. H. (2021). Integration of the Drug-Gene Interaction Database (DGldb 4.0) with open crowdsource efforts. *Nucleic acids research*, 49(D1), D1144–D1151.

https://doi.org/10.1093/nar/gkaa1084

Gao, Y. D., & Huang, J. F. (2011). An extension strategy of Discovery Studio 2.0 for non-bonded interaction energy automatic calculation at the residue level. *Zoological research*, 32(3), 262–266.

https://doi.org/10.3724/SP.J.1141.2011.03262

Gastaut H. (1970). Clinical and electroencephalographical classification of epileptic seizures. *Epilepsia*, *11*(1), 102–113.

https://doi.org/10.1111/j.1528-1157.1970.tb03871.x

Guelfi, S., Botia, J. A., Thom, M., Ramasamy, A., Perona, M., Stanyer, L., Martinian, L., Trabzuni, D., Smith, C., Walker, R., Ryten, M., Reimers, M., Weale, M. E., Hardy, J., & Matarin, M. (2019). Transcriptomic and genetic analyses reveal potential causal drivers for intractable partial epilepsy. Brain : a journal of neurology, 142(6), 1616–1630.

https://doi.org/10.1093/brain/awz074

- Hannoodee, S., & Nasuruddin, D. N. (2024). Acute Inflammatory Response. In *StatPearls*. StatPearls Publishing. PMID: 32310543.
- Heinzen, E. L., Radtke, R. A., Urban, T. J., Cavalleri, G. L., Depondt, C., Need, A. C., Walley, N. M., Nicoletti, P., Ge, D., Catarino, C. B., Duncan, J. S., Kasperaviciūte, D., Tate, S. K., Caboclo, L. O., Sander, J. W., Clayton, L., Linney, K. N., Shianna, K. V., Gumbs, C. E., Smith, J., ... Goldstein, D. B. (2010). Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *American journal of human genetics*, 86(5), 707–718. https://doi.org/10.1016/j.ajhg.2010.03.018
- Hollander, M., Do, T., Will, T., & Helms, V. (2021). Detecting Rewiring Events in Protein-Protein Interaction Networks Based on Transcriptomic Data. *Frontiers in bioinformatics*, 1, 724297. <u>https://doi.org/10.3389/fbinf.2021.724297</u>
- Kushwah RBS. Exploring withanolide a as a potential therapeutic agent against oral cancer: insights from molecular docking and dft calculations. *IJBTCM*. 2024; Volume 3 (Issue 2): Page 38-45.
- Lankhuijzen, L. M., & Ridler, T. (2024). Opioids, microglia, and temporal lobe epilepsy. *Frontiers in neurology*, 14, 1298489.

https://doi.org/10.3389/fneur.2023.1298489

- Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., & Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein science : a publication of the Protein Society*, 27(1), 129–134. <u>https://doi.org/10.1002/pro.3289</u>
- Li, Z., You, Z., Li, M., Pang, L., Cheng, J., & Wang, L. (2017). Protective Effect of Resveratrol on the Brain in a Rat Model of Epilepsy. *Neuroscience bulletin*, 33(3), 273– 280.

https://doi.org/10.1007/s12264-017-0097-2

Liu JYW, Kasperavičiūtė D, Martinian L, Thom M, Sisodiya SM (2012) Neuropathology of 16p13.11 Deletion in Epilepsy. *PLoS ONE* 7(4): e34813.

https://doi.org/10.1371/journal.pone.0034813

- Maden, S. F., & Acuner, S. E. (2021). Mapping Transcriptome Data to Protein-Protein Interaction Networks of Inflammatory Bowel Diseases Reveals Disease-Specific Subnetworks. *Frontiers in genetics*, 12, 688447. <u>https://doi.org/10.3389/fgene.2021</u>.
- Medina-Ceja, L., Salazar-Sánchez, J. C., Ortega-Ibarra, J., & Morales-Villagrán, A. (2019). Connexins-Based Hemichannels/Channels and Their Relationship with Inflammation, Seizures and Epilepsy. International journal of molecular sciences, 20(23), 5976. https://doi.org/10.3390/ijms20235976
- Mercado-Gómez, O. F., Córdova-Dávalos, L., García-Betanzo, D., Rocha, L., Alonso-Vanegas, M. A., Cienfuegos, J., & Guevara-Guzmán, R. (2018). Overexpression of inflammatory-related and nitric oxide synthase genes in olfactory bulbs from frontal lobe epilepsy patients. *Epilepsy research*, 148, 37–43.

https://doi.org/10.1016/j.eplepsyres.2018.09.012

Mora Lagares, L., Minovski, N., Caballero Alfonso, A. Y., Benfenati, E., Wellens, S., Culot, M., Gosselet, F., & Novič, M. (2020). Homology Modeling of the Human Pglycoprotein (ABCB1) and Insights into Ligand Binding through Molecular Docking Studies. *International* journal of molecular sciences, 21(11), 4058. https://doi.org/10.3390/ijms21114058

Nada, H., Sivaraman, A., Lu, Q., Min, K., Kim, S., Goo, J. I., Choi, Y., & Lee, K. (2023). Perspective for Discovery of Small Molecule IL-6 Inhibitors through Study of Structure-Activity Relationships and Molecular Docking. Journal of medicinal chemistry, 66(7), 4417–4433. https://doi.org/10.1021/acs.jmedchem.2c01957

- Pei, Z., Lang, B., Fragoso, Y. D., Shearer, K. D., Zhao, L., Mccaffery, P. J., Shen, S., Ding, Y. Q., McCaig, C. D., & Collinson, J. M. (2014). The expression and roles of Nde1 and Ndel1 in the adult mammalian central nervous system. Neuroscience, 271(100), 119-136. https://doi.org/10.1016/j.neuroscience.2014.04.031
- Pfisterer, U., Petukhov, V., Demharter, S. et al. Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis. Nat Commun 11, 5038 (2020).

https://doi.org/10.1038/s41467-020-18752-7

- Ramos-Matos CF, Bistas KG, Lopez-Ojeda W. (2023). Fentanyl. National Library of Medicine. Treasure Island (FL): StatPearls Publishing. PMID: 29083586. https://www.ncbi.nlm.nih.gov/books/NBK459275/
- Rana, A., & Musto, A. E. (2018). The role of inflammation in the development of epilepsy. Journal of neuroinflammation, 15(1), 144.

https://doi.org/10.1186/s12974-018-1192-7

- Rawat, C., Kutum, R., Kukal, S., Srivastava, A., Dahiya, U. R., Kushwaha, S., Sharma, S., Dash, D., Saso, L., Srivastava, A. K., & Kukreti, R. (2020). Downregulation of peripheral PTGS2/COX-2 in response to valproate treatment in patients with epilepsy. Scientific reports, 10(1), 2546. https://doi.org/10.1038/s41598-020-59259-x
- Rönnstrand L. (2004). Signal transduction via the stem cell factor receptor/c-Kit. Cellular and molecular life sciences: CMLS, 61(19-20), 2535-2548. https://doi.org/10.1007/s00018-004-4189-6
- Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G. W., Moshé, S. L., Nordli, D. R., Perucca, E., Tomson, T., Wiebe, S., Zhang, Y. H., & Zuberi, S. M. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia, 58(4), 512-521. https://doi.org/10.1111/epi.13709
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research, 13(11), 2498-2504. https://doi.org/10.1101/gr.1239303
- Shetty A. K. (2011). Promise of resveratrol for easing status epilepticus and epilepsy. Pharmacology & therapeutics, 131(3), 269-286.

https://doi.org/10.1016/j.pharmthera.2011.04.008

Shi, Y., Wei, Z., Feng, Y. et al. Disorders of organic acid metabolism and epilepsy. Acta Epileptologica 6, 24 (2024).

https://doi.org/10.1186/s42494-024-00167-2

Shorvon S. D. (2011). The etiologic classification of epilepsy. Epilepsia, 52(6), 1052-1057.

https://doi.org/10.1111/j.1528-1167.2011.03041.x

Steinkellner, H., Madritsch, S., Kluge, M., Seipel, T., Sarne, V., Huber, A., Schosserer, M., Oberle, R., Neuhaus, W., Beribisky, A. V., & Laccone, F. (2024). RNA Sequencing

and Weighted Gene Co-Expression Network Analysis Highlight DNA Replication and Key Genes in Nucleolin-Depleted Hepatoblastoma Cells. Genes, 15(12), 1514. https://doi.org/10.3390/genes15121514

- Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic acids research, 51(D1), D638-D646. https://doi.org/10.1093/nar/gkac1000
- Tran, Q. H., Cao, H. N., Nguyen, D. N., Tran, T. T. N., Le, M. T., Nguyen, Q. T., ... Thai, K. M. (2023). Targeting Olokizumab-Interleukin 6 interaction interface to discover novel IL-6 inhibitors. Journal of Biomolecular Structure and Dynamics, 41(23), 14003–14015. https://doi.org/10.1080/07391102.2023.2193990
- Thiese, M. S., Ronna, B., & Ott, U. (2016). P value interpretations and considerations. Journal of thoracic disease, 8(9), E928–E931.

https://doi.org/10.21037/jtd.2016.08.16

- Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461. https://doi.org/10.1002/jcc.21334
- Uddin, J., Garcia, H. H., Gilman, R. H., Gonzalez, A. E., & Friedland, J. S. (2005). Monocyte-astrocyte networks and the regulation of chemokine secretion in neurocysticercosis. Journal of immunology (Baltimore, Md.: 1950), 175(5), 3273-3281.

https://doi.org/10.4049/jimmunol.175.5.3273

Uzuner, D., Akkoç, Y., Peker, N. et al. Transcriptional landscape of cellular networks reveal interactions driving the dormancy mechanisms in cancer. Sci Rep 11, 15806 (2021).

https://doi.org/10.1038/s41598-021-94005-x

Vezzani, A., French, J., Bartfai, T., & Baram, T. Z. (2011). The role of inflammation in epilepsy. Nature reviews. Neurology, 7(1), 31-40.

https://doi.org/10.1038/nrneurol.2010.178

Vezzani, A., Fujinami, R. S., White, H. S., Preux, P. M., Blümcke, I., Sander, J. W., & Löscher, W. (2016). Infections, inflammation and epilepsy. Acta neuropathologica, 131(2), 211-234.

https://doi.org/10.1007/s00401-015-1481-5

- Wang, Y., Wang, Q., Huang, H., Huang, W., Chen, Y., McGarvey, P. B., Wu, C. H., Arighi, C. N., & UniProt Consortium (2021). A crowdsourcing open platform for literature curation in UniProt. PLoS biology, 19(12), e3001464. https://doi.org/10.1371/journal.pbio.3001464
- Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., lynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., Pon, A., ... Wilson, M. (2018). DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic acids research, 46(D1), D1074–D1082. https://doi.org/10.1093/nar/gkx1037
- Yu, J. Y., & Pearl, P. L. (2013). Metabolic causes of epileptic encephalopathy. Epilepsy research and treatment, 2013, 124934.

https://doi.org/10.1155/2013/124934

- Zhu, Y., Huang, D., Zhao, Z., & Lu, C. (2021). Bioinformatic analysis identifies potential key genes of epilepsy. *PloS* one, 16(9), e0254326. <u>https://doi.org/10.1371/journal.pone.0254326n</u>
- Zhuang, W., Ye, T., Wang, W., Song, W., & Tan, T. (2023). CTNNB1 in neurodevelopmental disorders. *Frontiers in psychiatry*, *14*, 1143328. <u>https://doi.org/10.3389/fpsyt.2023.1143328</u>