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In Silico Screening of Molecular Interactions of Umbelliferone, A Type of Coumarin Compound with Multiple Therapeutic Targets of Epilepsy

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***Abstract*:** Epilepsy is one of the most common neurological disorders in all age groups of individuals. Still, the currently existing antiepileptic drugs are showing side effects and are resistant to various epileptic patients. Hence, there is a need to find out effective novel drug molecules without side effects. We have selected umbelliferone, a group of natural coumarin compounds, to study its binding efficacy and inhibitory activity against selected therapeutic targets of epilepsy using molecular docking studies. Further, we have evaluated its drug screening through Lipinski's rule and toxicity properties. The results demonstrate that umbelliferone is potentially and competitively inhibiting all the different therapeutic targets of epilepsy compared to the diazepam reference drug. Furthermore, the umbelliferone falls in with Lipinski's rule, which shows all standard drug parameters and is non-toxic to human beings. The docking results reveal that the umbelliferone could be a therapeutic drug to study its anticonvulsant activity using *in vitro* cellular and *in vivo* animal models.

Key words: Epilepsy, umbelliferone, diazepam, molecular docking, binding affinity.

I. INTRODUCTION

Epilepsy is the most common neurological and non-communicable brain disorder that affects almost all age groups of individuals. The current global prevalence of epilepsy is 50 million (Savage, 2014). WHO reported that nearly 80% of epilepsy people live in low- and middle-income countries (WHO, 2015). Treatment of epilepsy involves the usage of some classical antiepileptic drugs (AEDs), such as carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT) and valproate (VPA) etc (Zagaja, et al., 2015). however, the available medicines exhibit unfavorable side effects (Kwan and Brodie, 2000); Moreover, some epileptic patients show resistance against currently available AEDs (Elger and Schmidt, 2008); thus, there is an urgent need to develop a new class of active molecules with better antiseizure efficacy without side effects. Subsequently, natural compounds are the best choice to find efficient drugs against epilepsy because natural source compounds have no toxic side-effects and no drug resistance. Therefore, studies on natural compounds provide promising results to find the AEDs.

Coumarins (2*H*-1-benzopyran-2-one) consist of phenolic substances found in plants and are made of fused benzene and α-pyrone rings (Aoyama et al., 1992). They have been widely used as an alternative medicine due to their diverse pharmacological activities with low cost and few side effects (Gnonlonfin et al., 2012; Kontogiorgis et al., 2012; Venkata Sairam et al., 2016). Usually, coumarins are classified into six main types based on their chemical structure. Among the various types, umbelliferone is a simple coumarin with a benzopyrone in nature (Venugopala, K. N., Rashmi, V., & Odhav, B. 2013). It has multiple biological activities, including anti-hyperglycaemic, bronchodilating, anti-nociceptive, anti-edematogenic effects, etc. (Bryda et al., 2019). Furthermore, it has been reported that umbelliferone acts as neuroprotective agent (Subramaniam & Ellis, 2013).

In view of the above literature review, the present study aims to explore the anticonvulsant activity based on the molecular interactions of umbelliferone against various therapeutic targets of epilepsy using *in silico* molecular docking tools.

II. MATERIALS AND METHODS

2.1 Umbelliferone as ligand

We have selected umbelliferone as a ligand; it is a natural compound that belongs to coumarins (Subramaniam & Ellis (2013). Further, Diazepam was used as a reference drug to compare its binding affinity with umbelliferone. The 2D-structures for these compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) **Fig1.**

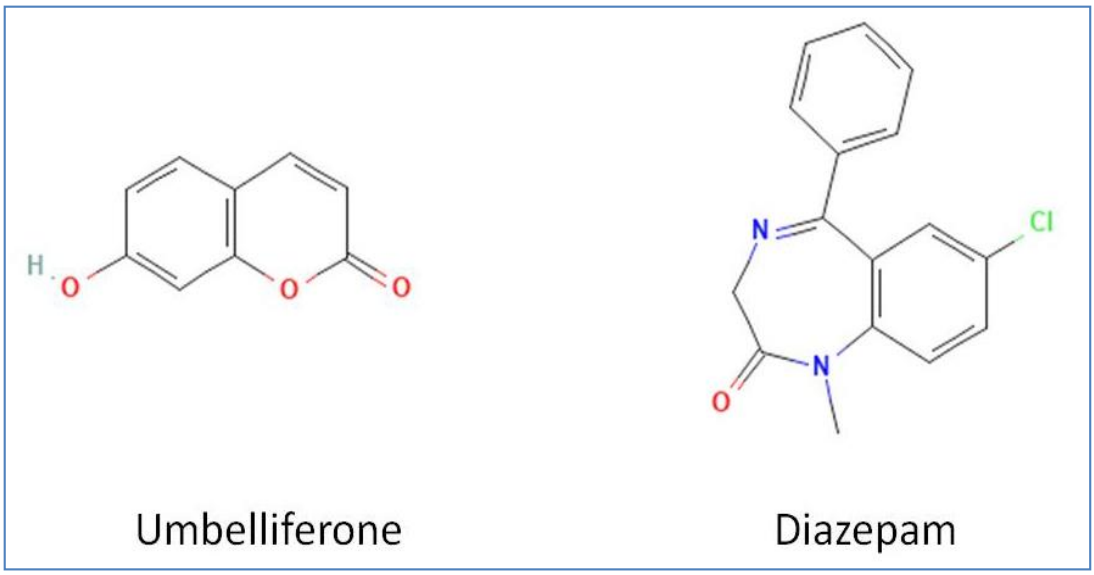

Figure 1. 2D structures of umbelliferone and diazepam

2.2 Drug targets of epilepsy:

The present study is mainly focused on various targets of epilepsy, Gama aminobutyric acid (A)-receptor-associated protein (PDB ID: 1KJT; Bavro et al., 2002), Crystal Structure of Ca2+/CaM-CaV2.1 IQ domain complex (PDB ID: 3DVM; Kim et al., 2008), Kainate receptor GluR5 (PDB ID: 1YCJ; Naur et al., 2005), GABA receptor (PDB ID: 4COF; Miller et al., 2014), Voltage-Gated Sodium Channel (PDB ID: 4F4L;), GABA(A) receptor-associated protein (PDB ID: 4XC2;), Kainate receptor GluR5 (PDB ID: 4JPZ; McCusker et al., 2012), NMDA Receptor (PDB ID: 5FXG; Tajimma et al., 2016). Aiming of the above protein targets is also gives potential drugs. Three-dimensional structures of all these drug targets were retrieved from the RCSB Protein Data Bank (www.rcsb.org). Before docking, the PDB structures were observed for amino acid sequence breakage using Pymol molecular visualization system (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.). The protein targets were refined for hetero-atoms and water molecules to demarcate active sites of proteins. Further, the Gasteiger charges and hydrogen atoms were added to each protein to maintain coordination between various interactions using UCSF Chimera-1.13.1 software.**Fig2.**

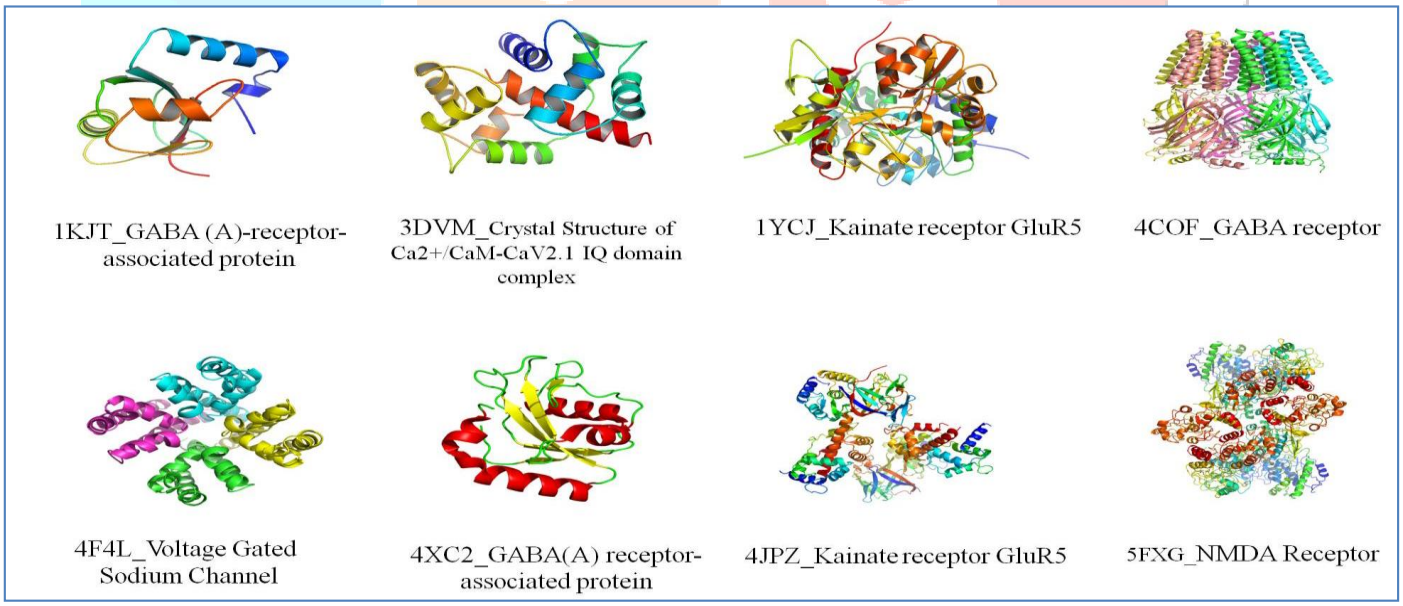

Table 2. 3D structures of various selected therapeutic targets of epilepsy (RCSB Protein DataBank).

2.3 Molecular docking

Molecular docking is an essential strategy in drug discovery. In our study, docking studies were performed for umbelliferone and diazepam to evaluate their binding affinity against various therapeutic targets of epilepsy using AutoDock vina (Version 1.1.2) (Trott, O., & Olson, A. J. 2010) AutoDock tools is a software package used in protein-ligand docking and calculated docking interaction energy. AutoDock Vina uses protein structure and ligand structure (Umbelliferone) in PDBQT format (it maintains the atom type and coordinates and partial charge). Another input that AutoDock Vina uses a rectangular box referred to as a docking region. The center of the square box and size of X-, Y- and Z- dimensions are provided in table 4. The Lamarckian Genetic Algorithm (LGA) was used for the best conformers of ligands. Docking was performed with the default parameters of AutoDock Vina, and it gives a maximum of nine conformers for each ligand and each protein. The docking outputs, such as interactions, energy values (binding affinity), and poses of the docked complexes, are described in the results section. Superimposing the output ligand pose file on the protein gives a docked protein-ligand complex.

2.4 Drug-likeness properties of the ligands

In our study, umbelliferone and diazepam were considered as ligands. The swissADME server was used to assess the ligand's drug likeliness, molecular properties and evaluated Lipinski's 'rule of five.'.(Lipinski, C. A. (2004).

2.5 Toxicological analysis

The toxicity properties of umbelliferone and diazepam were predicted using the ADMETlab server.(Xiong, G., et al. 2021) Toxic properties include AMES toxicity, hERG inhibition, hepatotoxicity, and skin sensitization.

III. RESULTS

In this study, umbelliferone is used to evaluate its binding affinity against various therapeutic targets of epilepsy by performing molecular docking and compared its binding potential with the reference drug diazepam. Our results demonstrate that the umbelliferone shows effective inhibition against all the drug targets employed in this study. The output results for each drug target are described as follows.

3.1 Molecular docking results

In this study, we have performed docking for umbelliferone and diazepam to compare their binding affinities with various therapeutic targets of epilepsy. Our results demonstrate that umbelliferone shows good inhibition against voltage-gated sodium channel- open channel conformation, GABA receptor, kainate receptor GluR5, NMDA Receptor, voltage-gated sodium channel – C terminal, GABA(A) receptor-associated protein, Crystal Structure of Ca2+/CaM-CaV2.1 IQ domain complex, gamma-aminobutyric acid (A)-receptor-associated protein with the binding affinities of -8.2, -7.8, -7.5, -7, -6.3, -6.3, -6, and -5.6 kcal/mole, respectively (Table 1).

Table 1. Binding affinity (Kcal/mole) and hydrogen bonding of umbelliferone against various therapeutic targets of epilepsy.

S. No	Protein PDB ID	Binding Affinity	No of 'H' Bonds	Hydrogen bond interactions		Bond Length
1	1KJT	-5.6	3	A/GLU`100/OE1 A/ASN`82/1HD2 A/MET`1/NH1	N/UNK`1/H N/UNK`1/O N/UNK`1/O	2.7 2.5 2.4
2	1YCJ	-7.5	6	B/VAL`685/H B/VAL`685/H B/SER`689/NH B/SER`689/NH B/SER`689/OG B/ARG`523/H	N/UNK`1/O N/UNK`1/O N/UNK`1/O N/UNK`1/O N/UNK`1/O N/UNK`1/O	2.0 3.7 2.2 2.1 3.1 2.6
3	3DVM	-6.0	1	A/ASN`111/H	N/UNK`1/O	2.6
4	4COF	-7.8	4	D/GLU`155/OE2 D/GLU`201/NH D/THR`202/NH D/THR`202/OG1	N/UNK`1/OH N/UNK`1/O N/UNK`1/O N/UNK`1/O	2.3 2.8 2.2 3.1
5	4F4L	-8.2	2	B/ALA`70/O B/SER`42/OG	N/UNK`1/O N/UNK`1/O	2.6 3.6
6	4JPZ	-6.3	3	A/SER`93/OG A/VAL`94/NG B/LYS`1836/H	N/UNK`1/O N/UNK`1/O N/UNK`1/O	3.0 2.2 2.2
7	4XC2	-6.3	3	A/GLN`59/O A/GLN`59/1HE2 A/GLN`59/2HE2	N/UNK`1/H N/UNK`1/O N/UNK`1/O	2.1 2.5 2.8
8	5FXG	-7.0	2	A/ARG`327/O A/VAL`107/O	N/UNK`1/H N/UNK`1/O	2.2 3.6

In contrast, the reference drug diazepam shows moderate inhibition against NMDA Receptor, GABA receptor, voltage-gated sodium channel-open channel confirmation, voltage-gated sodium channel- C-terminal, GABA(A) receptor-associated protein, kainate receptor GluR5, Crystal Structure of Ca2+/CaM-CaV2.1 IQ domain complex, gamma-aminobutyric acid (A)-receptor-associated protein with the binding affinities of -8.1, -7.9, -7.6, -7.4, -7,4, -6.6,-6.2, and -5.8 kcal/mole, respectively (Table 2).

Table 2. Binding affinity (Kcal/mole) and hydrogen bonding of diazepam against various therapeutic targets of epilepsy.

S. No.	Protein	Binding Affinity	No of 'H' Bonds	H Bond Interactions		Bond Length
1	1KJT	-5.8	0	-		-
2	1YCJ	-6.6	1	B/PHE`693/O	N/UNK`1/H	2.2
3	3DVM	-6.2	0	-		-
4	4COF	-7.9	1	D/TRP`241/O	N/UNK`1/H	2.6
5	4F4L	-7.6	0	-		-
6	4JPZ	-7.4	1	A/LYS`91/O	N/UNK`1/HZ1	2.6
7	4XC2	-7.4	2	B/TYR`61/O B/LEU`76/O	N/UNK`1/H N/UNK`1/H	2.6 2.3
8	5FXG	-8.1	1	C/ALA`536/O	N/UNK`1/H	2.1

The results demonstrate that umbelliferone displays a potential inhibition against different therapeutic targets of epilepsy with binding affinity ranges from -5.6 to -8.2 kcal/mole. As a result, similar to the diazepam, umbelliferone potentially inhibits all selected targets of epilepsy. These results suggest that the umbelliferone could act as a potential inhibitor for all the targets of epilepsy used in this study. The molecular interactions of umbelliferone and diazepam with different drug targets of epilepsy are displayed in **fig3a, 3b, 4a,** and **4b.**

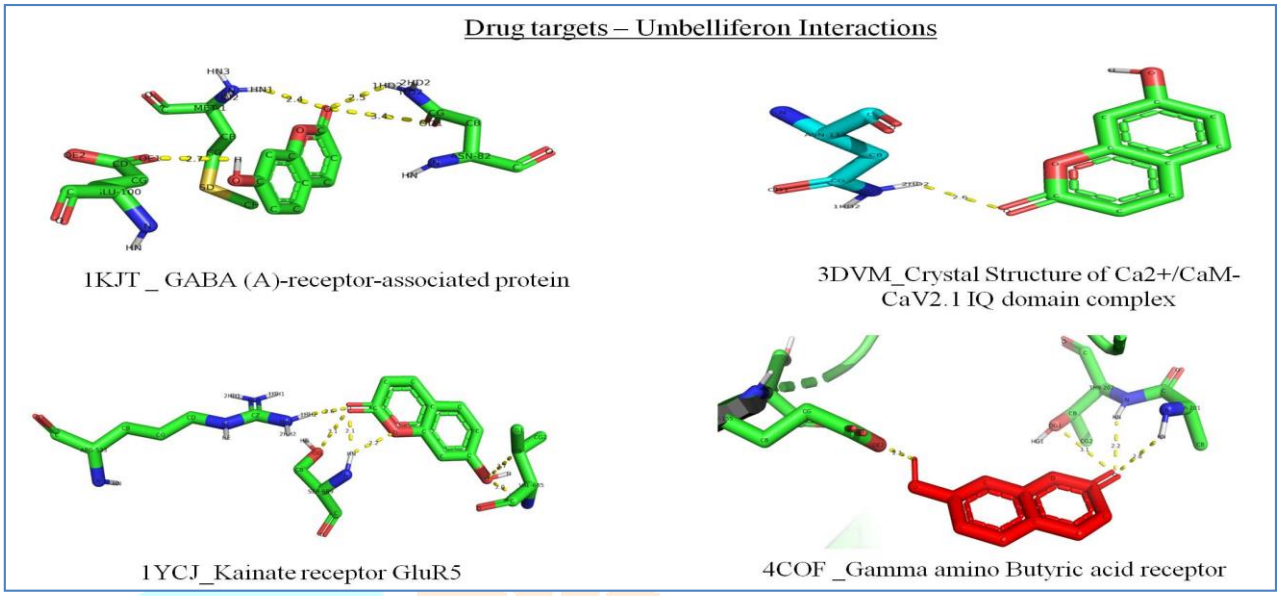

Figure 3a. Umbelliferone is showing molecular interactions with different therapeutic targets of epilepsy

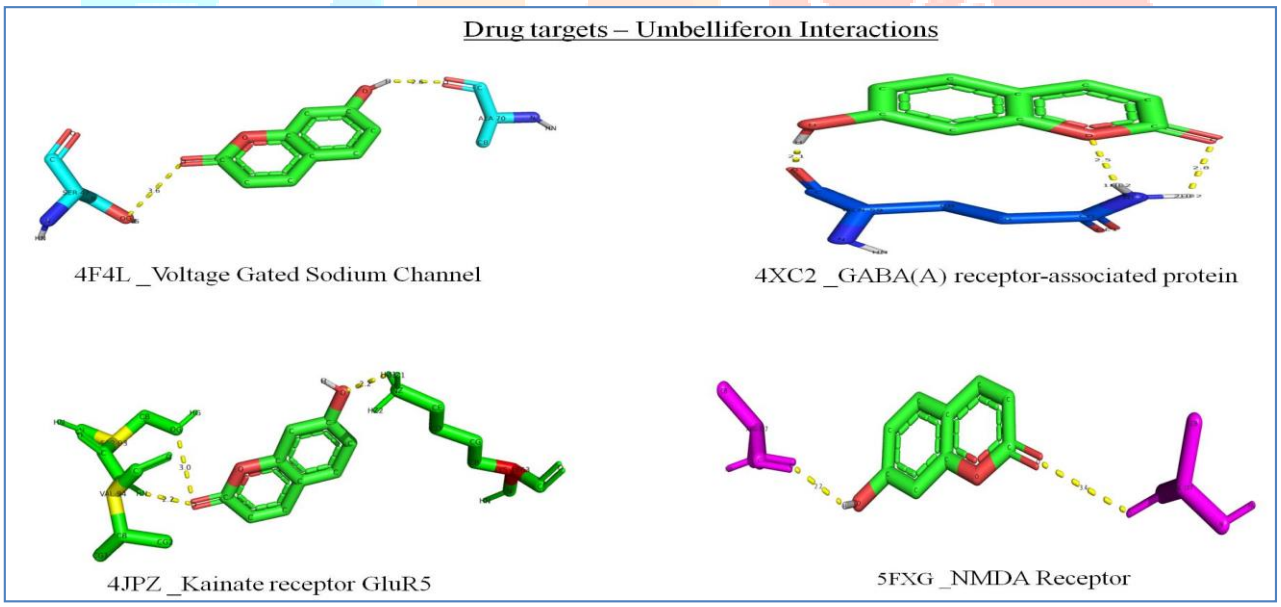

Figure 3b. Umbelliferone is showing molecular interactions with different therapeutic targets of epilepsy.

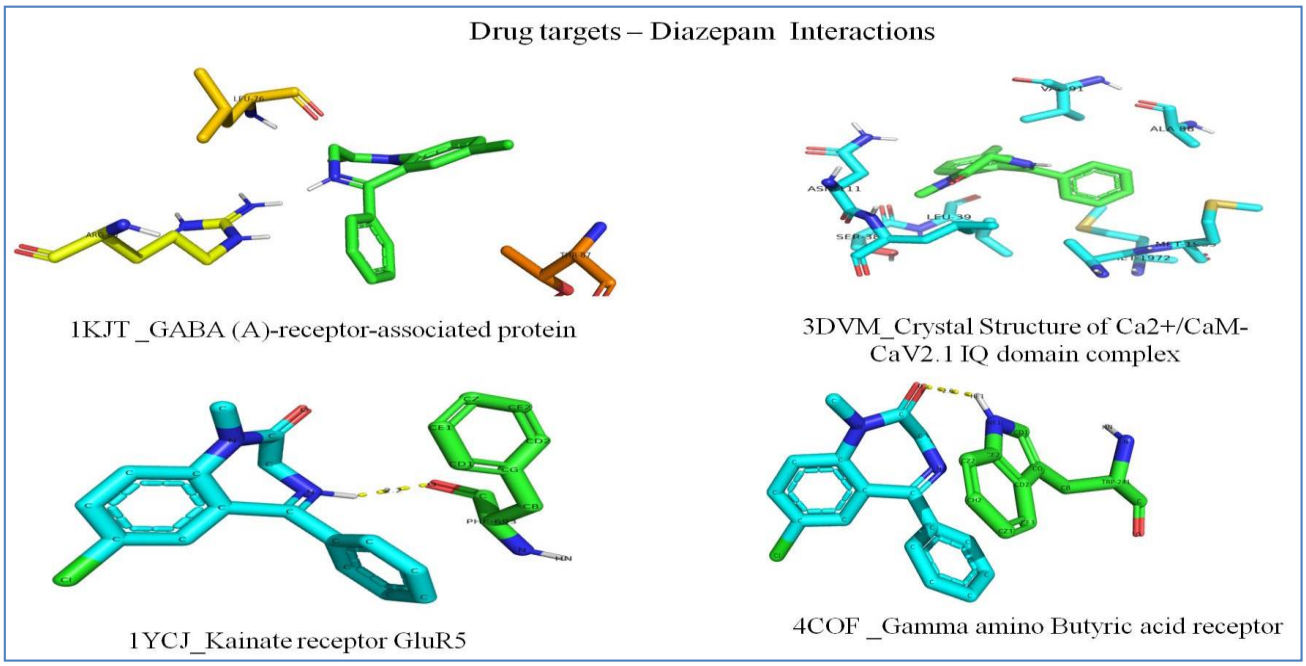

Figure 4a. Diazepam is showing molecular interactions with different therapeutic targets of epilepsy.

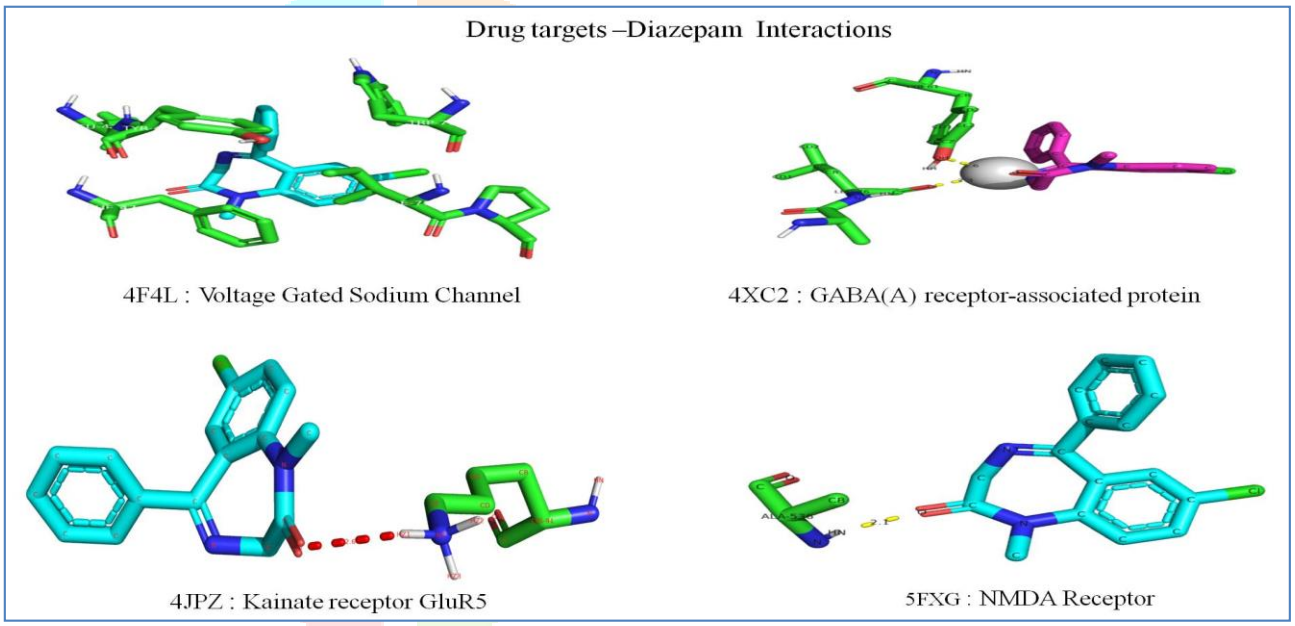

Figure 4b. Diazepam is showing molecular interactions with different therapeutic targets of epilepsy.

3.2 Drug-likeness properties of umbelliferone:

In the present work, we have also assessed the drug-likeness properties for umbelliferone using "Lipinski's rule of five." Umbelliferone fallows Lipinski's rule of five (Molecular weight <500, hydrogen acceptor <10, hydrogen donor <5, and LogP<5). These results reveal that umbelliferone abide by the "Rule of Five" that exhibit potential pharmacological activities through their absorption, distribution, metabolism, and excretion (Table 3)

Table 3. Drug-likeness properties of Umbelliferone and Diazepam predicted in swissADME web tool.

S. No	Ligand Name	Bioavailability Score	M.W (150-500g/mol)	H-acceptors(≤10)	H-donors (≤5)	Log P (0.7-5.0)	No of Violations (Rule of 5)	TPSA (20-130 Å²)	Rotatable Bonds	LogS(>-6)
1	Umbelliferone	0.55	162.14	3	1	1.44	0	50.44	0	-2.46
2	Diazepam	0.5567	284.74	2	0	2.68	0	32.67	1	-3.87

3.3 Toxicity analysis

In our study, umbelliferone was subjected for prediction of toxicity properties (hERG inhibition, hepatotoxicity, AMES toxicity, and skin sensitization) using the ADMETlab server **(Table 4).**

Table 4. Toxicity Properties of Umbelliferone and reference Drug Diazepam

S.No	Compound	hERG	H-HT	DILI	Ames	ROA	FDAMDD	SkinSen	Carcinogenicity	Eye Ccorrosion	Eye Irritation	Respiratory
1	Umbelliferone	0.101	0.121	0.723	0.047	0.378	0.191	0.428	0.841	0.575	0.99	0.17
2	Diazipaam	0.223	0.117	0.826	0.009	0.24	0.986	0.155	0.039	0.003	0.02	0.024

Umbelliferone shows none of the above toxicity properties. Hence, umbelliferone is a non-toxic compound and there is a possibility to study more about its therapeutic efficacy for various diseases.

IV. Discussion:

In the present study, we have performed molecular docking on umbelliferone and diazepam against various therapeutic targets of epilepsy which include voltage-gated sodium channel- open channel conformation, GABA receptor, kainate receptor GluR5, NMDA Receptor, voltage-gated sodium channel – C terminal, GABA(A) receptor-associated protein, acetylcholinesterase, gamma-aminobutyric acid (A)-receptor-associated protein.

The docking results of both umbelliferone and diazepam have been compared with each other against various targets of epilepsy. Comparatively, the results on both umbelliferone and diazepam have shown more or similar binding affinities against each therapeutic target used in this study, respectively. The binding affinity of umbelliferone with voltage-gated sodium channel-open channel conformation is -8.2 kcal/mole, whereas diazepam has shown -8.1 kcal/mole. Similarly, binding affinities against GABA receptors are -7.8 and -7.9 kcal/mole by umbelliferone and diazepam, respectively. Both umbelliferone and diazepam have shown relatively same binding affinities against kainate receptor GluR5 are -7.5 and -7.6 kcal/mole. Likewise, NMDA receptor has shown binding affinities by umbelliferone (-7 kcal/mole) and diazepam (-7.4 kcal/mole). In contrast to the above docking results, diazepam has shown the highest binding affinity (-7.4 kcal/mole) against voltage-gated sodium channel – C terminal. In comparison, the umbelliferone has shown a comparatively low binding affinity (-6.3 kcal/mole). The remaining targets, GABA(A) receptor-associated protein, acetylcholinesterase, gamma-aminobutyric acid (A)-receptor-associated protein, have shown comparatively similar binding affinities with umbelliferone and diazepam are -6.3 & -6.6, -6, &-6.2 and -5.6 & -5.8 kcal/mole, respectively. The results clearly demonstrate that umbelliferone has shown potential inhibitory activity and more molecular interactions, including hydrogen, hydrophobic, Vander wall, electrostatic interactions with each therapeutic target of epilepsy compared to diazepam.

The umbelliferone has shown effective antiepileptic properties in the combination of antiepileptic drugs (carbamazepine, phenytoin, Phenobarbital, and valproate) treatment of maximal electroshock-induced seizure (MES) mouse model (Zagaja et al., 2015). It has been recently reported that umbelliferone has shown neuroprotective activity in the ischemic injury model by inhibiting the neuroinflammatory pathways (Liang et al., 2020). Our study provides basic *in silico* research findings that umbelliferone interacts with multiple drug targets of epilepsy. Moreover, limited literature is available related to the research findings of umbelliferone and epilepsy; here, our study directs the researchers to initiate and explore antiepileptic properties of umbelliferone using cellular and animal models.

Acknowledgment

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