ORIGINAL ARTICLE

New avenue in the treatment of temporal lobe epilepsy by classical anti-epileptics: A hypothetical establishment of executioner Caspase 3 inactivation by molecular modeling

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ABSTRACT

Patients with temporal lobe epilepsy (TLE) are prescribed first-line antiepileptic drugs and surgery to the management of this disorder. Unfortunately, the surgical treatment has been shown to be beneficial for the selected patients but fails to provide a seizure-free outcome in 20–30% of TLE patients. In our present study, we investigate the possibilities of marketed antiepileptic drugs in a different manner to improve the present situation in TLE. Molecular docking simulation study and various open source computational tools were used to perform the study. AutoDock 4.2 MGL tools, Pymol visualize tools, Patch dock server, and Swarm Dock servers (protein-protein docking) were used to perform the molecular modeling. FTsite and computed atlas of surface topography of protein open source server were used to understand the pocket and ligand binding information respectively. Toxtree application was used to determine the toxicity profile of the drug by Cramers rule. The obtained molecular docking models (Caspase 3, Procaspase 8, and Fas-associated death domain [FADD]) with selected compounds (Clonazepam, Clobazepam, and Retigabine) showed promising trio blocking event of FADD, Caspase 3, and Procaspase 8 (−6.66 kcal, −8.1 kcal, 6.46 kcal) by Clonazepam respectively. Protein-protein interaction study (Swarm Dock, Patch Dock server) indicated promising results that helped to establish our hypothesis. Toxtree showed a quantitative structure toxicity relationship report that helps to clarify the toxicity of the selected compounds. Clonazepam showed a trio inhibition property that may lead to develop a new era of the new generation benzodiazepine prototype drugs in the future. Filtered compounds will further process for higher *in vitro*, *in vivo* models for better understanding of the mechanism.

Key words: Caspase 3, Fas-associated death domain, Procaspase 8, quantitative structure toxicity relationship, temporal lobe epilepsy

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INTRODUCTION

Word Health Organization recorded data explored that epilepsy is a common chronic neurological disorder affecting about 50 million people worldwide. Interruption of normal function of the body is due to recurring seizures and harmonized discharges of large groups of neurons.[1] Epilepsy also characterized by so many other types, among them temporal lobe epilepsy (TLE) is the most important and frequent type in humans. Most of TLE affected patients are suffering from symptomatic focal epilepsies, which are generally caused by brain trauma, complicated febrile convulsions, prolonged seizures (status epilpeticus), ischemic lesions, and brain tumors.[2] At present,

carbamazepine, levetiracetam, and lamotrigine are the drugs of first choice in TLE. Apoptosis plays an important role in neurological disorder. Activation of the extrinsic death receptor pathway and the intrinsic mitochondrial pathway are major responsible for epilepsy.[3] The extrinsic pathway stimulates an initiator as Caspase. Activation of the cell surface death receptors (tumor necrosis factor receptor 1) causes the activation of ProCaspase-8 via Fas-associated death domain (FADD). Activation of the Caspase-8 also activates the executioner Caspase-3. It leads to cleavage of intracellular survival proteins and also responsible for the DNA fragmentation.[3,4] Several molecular studies have indicated that seizures can activate the both intrinsic and extrinsic pathway and promotes apoptosis. Recent study has shown that inactivation of Caspase (-3, -7, -8 and -9) promotes the cell survivability and prevent cell injury. Our present study depicts [Figure 1] a hypothetical establishment of Caspase 3, FADD, and Procaspase 8 inhibition through computational molecular modeling that indicates the possible remedy in TLE by the conventional anti-epileptics that has been already marketed.

MATERIALS AND METHODS

Molecular modelling and docking analysis study was carried out using AutoDock 4.2 MGL Tools (The Scripps Research Institute, USA) and Pymol Molecular Visualization package (Schrödinger).[5,6] Computed atlas of surface topography of protein (CastP) and FTsite open source server were used to determine the possible binding pockets and pocket capacity of the protein respectively.[7,8] Patch dock (http://bioinfo3d.cs.tau.ac.il/PatchDock/patchdock.html) and Swarm Dock server (Cancer Research UK, http://bmm.cancerresearchuk.org/~SwarmDock/) open source servers have been used for protein-protein docking and interpretation.[9,10] Chemdraw 8.0 and Chem 3D (Cambridge soft . Comp.)Pro were used to draw the structures of the compounds.[11] Toxtree 4.0 open source software package was used to predict the toxicity of the compounds.[12]

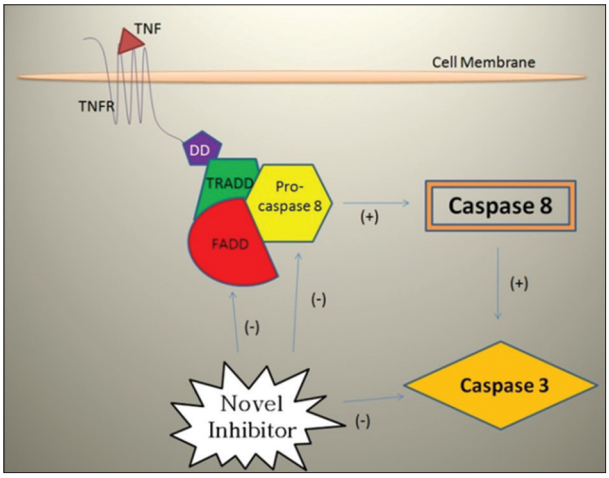


Figure 1: Schematic model of novel inhibitors in Fas-associated death domain-Procaspase 8 interaction and Caspase 3 activation via Caspase 8. Diagram shows a cell membrane bound death receptor tumor necrosis factor receptor activated by the binding of the ligand initiate the intrinsic apoptosis pathway. Activation of the pathway leads to the formation of death-inducing signaling complex, which initiate a cascade through Caspase 8

Experimental

Protein preparation

Caspase 3 high-resolution crystallographic structure was retrieved from RCSB Protein Data Bank (www.rcsb.org) identifier (PDB Id: 2XZT**)**. To understand the biologically activity of a drug molecule as prototype therapeutic agent, the knowledge of binding selectivity towards the protein environment is very essential. Docking study was performed to understand and correlate their biological efficacy toward the selected binding domain of the protein. We used the AutoDock 4.2-MGL Tools version 1.5.6 software packages for the molecular docking experiment and Pymol 1.3 Software package to analyze the results. Polar hydrogen atoms were added to the protein. The deletion of the both water molecule and the inorganic charges were done to avoid error. Gasteiger charge of the macromolecule was added. Ligand docking was carried out applying the Lamarckian genetic algorithm implemented in AutoDock 4.2. The grid size was set to 36, 36 and 36 along the X-, Y- and Z-axis to recognize the binding site. Spacing was set as 0.725 Å. The lowest binding energy conformers were selected out of 20 different conformers for each docking simulation and resultant data was further analyzed. Other miscellaneous parameters were assigned to the default values obtained from the AutoDock 4.2 program.

Ligand preparation

In our present study, commercially available classical, nonclassical anti-epileptic agents were taken as the test ligands for the study. Lipinski rule of thumb was applied to filter the test compounds, at last 20 best compounds has been selected for the further study. Chemdraw 8.0 and Chem 3D Pro were used to prepare all selected ligands. All ligands were converted to PDB extension file format (PDBQT). An extended PDB format, termed PDBQT, is used for coordinate files, which includes atomic partial charges and atom types. Torsion angles were calculated to assign the fixable and nonbonded rotations of the molecule.[13]

Pocket validation

All receptors are having their own active site or binding domain, where the ligands are supposed to fit. The active site contains a branch of amino acids. Grid generation is helped to recognize that binding region of the receptor. CastP server (http://sts.bioengr.uic.edu/castp/) has been used to validate the receptor pocket.[7]

Determination of ligand binding site

All possible binding sites Caspase 3 receptor were determined by the open source server called FTsite server (http://ftsite.bu.edu/cite). The PDB: 2XZT was uploaded into the server and also created the job name for identification of the result.[8]

Protein-protein docking study

Patch dock open source server (http://bioinfo3d.cs.tau.ac.il/PatchDock/) has been used to understand the protein-protein interaction between FADD and Procaspase 8 receptor, extracted from RCSB PDB (www.rcsb.org) identifier PDB id: 3OQ9 and PDB id: 2K7Z, respectively. Both PDB files were uploaded into the Patch dock server for protein-protein docking simulation.[9] Swarm Dock server has been used to understand the best docking poses by the hybrid particle swarm optimization (PSO)/local search, Minimizing, re-ranking and clustering the docked poses.[10]

Molecular docking of Fas-associated death domain and Procaspase-8

Fas-associated death domain and Procaspase-8 crystallographic structures were extracted from RCSB (PDB) identifier (PDB id: 3OQ9), and (PDB id: 2K7Z) respectively. Molecular docking simulation was carried out using a described method elsewhere.[14] Ligand binding sites were determined by CastP open server source.

Toxicity estimation by Toxtree open source package

Toxtree package was used to estimate the carcinogenicity of the test ligands. Toxtree is a complete and flexible user-friendly open source application which is able to estimate toxic hazards of the compound by the decision tree approach. It also includes cramers rule, Verhaar scheme for predicting toxicity mode of actions, decision tree for estimating skin irritation and corrosion potential, decision tree for estimating eye irritation and corrosion potential, decision tree for estimating carcinogenicity and mutagenicity, and structural alerts for reactivity in Toxtree biodegradation and persistence.[12] Ligand PDB files were placed into the Toxtree application for toxicity prediction by carcinogenicity and mutagenicity decision tree.

RESULTS AND DISCUSSIONS

Lipinski rule of five analyses

In our present study, we listed out 20 classical and nonclassical anti-epileptic agents. We have considered five important physicochemical properties (hydrogen bond donor (not more than five), hydrogen bond acceptor (not more than five), Molecular weight (not more than 500 Dalton), 10 or fewer rotatable bonds, Log P (partition coefficient) not more than − 0.4 to + 5.6) for ranking the drugs.[15] Illustrated results are reported in Table 1. All the selected anti-epileptic agents satisfy the Lipinski rule of five gently.

Molecular docking analysis

Molecular docking analysis was done by the output file of the docking that was generated after the study. The binding energy, inhibition constant (K_i), intermolecular energy, torsional energy, unbound extended energy, reference RMS and the no. of hydrogen bonds were considered for the analysis. Initial study has been started with screening of all selected 19 antiepileptic drug with Caspase 3, identifier 2XZT. Among those established drugs, three of them showed promising binding energy and hydrogen bond interaction with the receptor. The final compounds were (Clobazepam, Clonazepam, and Rufinamide) further taken for next level of the study [Table 2]. Binding energy and bond distances of the final compounds (Clobazepam, Clonazepam, and Rufinamide) were −9.0 kcal, −8.1 kcal, and −7.9 kcal, respectively. The best three compounds (Clobazepam, Clonazepam, and Retigabine) have been taken for the next screening study [Figure 2] contains all clustering docking values for 20 ligands.

Pocket validation analysis

Computed atlas of surface topography of protein server (http://sts.bioengr.uic.edu/castp/) helped to recognize the binding pockets into the receptor. They generated several possible pockets by the computation based on the pocket algorithm of the alpha shape theory, and its core is the alpha shape. API is developed by Edelsbrunner's group and the NCSA. They depict the pocket information in respect to the volume and the area of the pocket. The annotated notations were mentioned to identify the pocket region precisely. Pocket information (ID: 125) for Caspase 3 was taken for the preparation of grid.

Ligand binding site analysis

FTsite determined the different binding sites of the protein. They have calculated the three different ligand binding domains into the protein. The residue set of the three different sites of the proteins was found. Site 1 annotation of

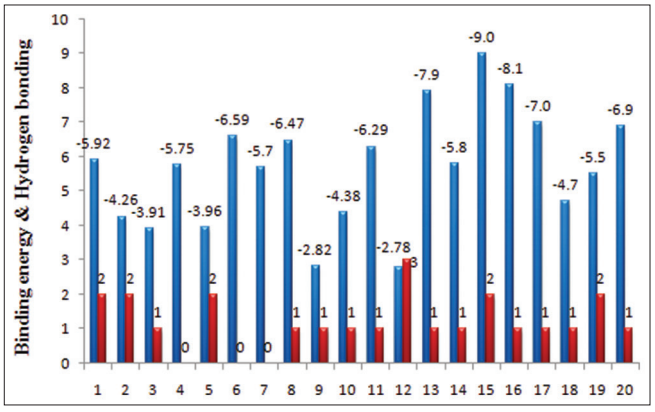
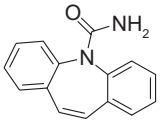
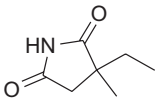
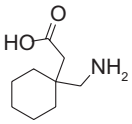
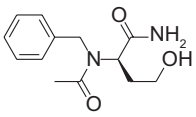
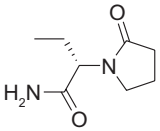
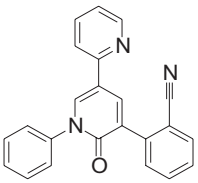
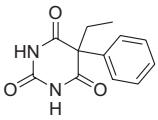
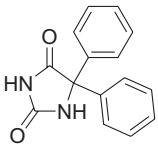
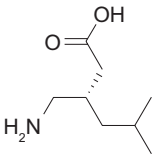
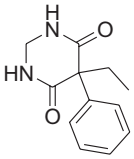
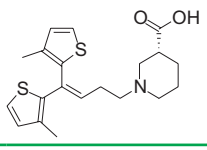


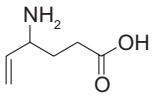
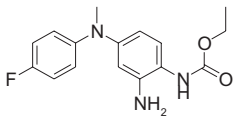
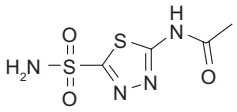
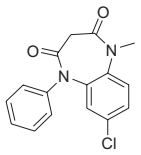
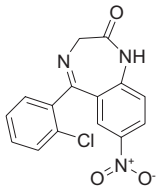
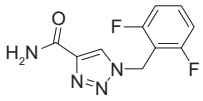
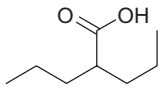
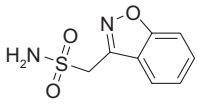
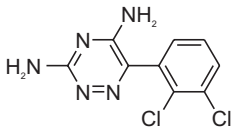
Figure 2: Clustering of binding energy and hydrogen bonding of the selected compounds

Table 1: Lipinski's rule of five analyses

Compounds	Structures	Hydrogen bond donor (not more than 5)	Hydrogen bond acceptor (not more than 5)	Molecular weight (not more than 500 Dalton)	10 or fewer rotatable bonds	Log P (partition coefficient) not more than −0.4 to +5.6
Carbamazepine		1	3	236.09	1	4.16
Ethosuximide		1	2	141.16	1	0.55
Gabapentin		2	6	171.23	3	−1.27
Lacosamide		2	3	250.29	6	−0.02
Levetiracetam		1	2	170.209	3	−0.59
Perampamel		0	3	349.3847	3	4.11
Phenobarbitone		2	3	232.2353	2	1.41
Phenytoin		2	2	252.268	2	2.15
Pregabalin		3	3	159.2261	5	−1.35
Primidone		2	2	218.2518	2	1.12
Tiagabine		1	2	375.548	6	2.60

Contd...

Table 1: Contd...

Compounds	Structures	Hydrogen bond donor (not more than 5)	Hydrogen bond acceptor (not more than 5)	Molecular weight (not more than 500 Dalton)	10 or fewer rotatable bonds	Log P (partition coefficient) not more than −0.4 to +5.6
Vigabatrin		3	3	129.157	4	−2.09
Retigabine		4	4	303.33	6	2.70
Acetazolamide		3	7	222.245	1	−0.26±0.30
Clobazepam		0	4	300.739	1	1.69±0.90
Clonazepam		1	6	315.71	2	2.34±0.56
Rufinamide		2	5	238.193	3	0.05±0.87
Valporic acid		1	2	144.211	5	2.8
Zonisamide		1	5	212.22	2	0.2
Lamotrigine		2	5	255.01	1	1.48

Caspase 3 contains ARG A 164, PRO B 201, GLY C 125, ASP C 135, LEU C 136, LYS C 137, THR C 140, ILE C 160, ARG C 164, TYR D 195, TYR D 197, and VAL D 266. Ligand binding site of FADD (site 1) contains ASP E 261, ALA E 263, GLU E 264, ASP J 127, GLU J 130, ASP J 131, GLY K 109, LYS K 110, ASP K 111, TRP K 112, ARG K 113, ARG K 114, LEU K 137, and ARG K 140. Site 1 of Procaspase 8 contains LYS A 253, LYS A 320, ILE A 357, GLN A 358, ALA A 359, GLN A 361, TYR A 365, THR A 390, MET A 403, ALA A 404, THR A 419, TRP A 420. Ligand binding site annotations helped to validate and also determine the possible binding regions of the protein.

Molecular docking analyses of Caspase 3 screened compounds (Clobazepam, Clonazepam, and Retigabine) with Fas-associated death domain target

Clobazepam-FADD docking job was undertaken to understand the binding details of the drug into the target. This will help to develop competitive inhibitor in future. The docking study was carried out using a described method elsewhere.[14]

The binding energy, inhibition constant (K_i), intermolecular energy, torsional energy, unbound extended energy, reference RMS, and the no. of hydrogen bonds

were considered for the analysis. Clobazepam has a benzodiazepine skeleton and the carboxylic functional group that has been participated for the molecular interaction with the target. Hydrogen bonding was found at (GLN 260) residue with (>C = O) group of Clobazepam. The bond distance and binding energy were found to be 2.118 Å and − 5.85 kcal, respectively. Docking data and ligand interaction image are reported in Table 3 and Figure 3b respectively.

Clonazepam has been subjected for molecular simulation study with FADD target to finding out the possible binding interaction. Clonazepam showed three hydrogen bond interaction with HIS (256), TRP (112), ARG (113) and the bond distances were 2.089 Å, 1.983 Å, and 1.742 Å respectively. The (-NO_2) group of Clonazepam showed versatile interaction with HIS (256), TRP (112) and ARG (113). Nitrogen atom of benzodiazepine ring also showed an ionic interaction with (ASP 257). We investigated through this study that the nitro group of Clonazepam made potent interaction with the protein. Illustrated docking results are reported in Table 3.

Interaction of Retigabine with the FADD protein also showed three hydrogen-bonding ASP (257), LYS (110), ARG (135) and one ionic interaction with GLN (268). Secondary amino group (=NH_2) of Retigabine is responsible for interaction with both ASP 257 and ARG 135. The docked image of Retigabine with FADD and illustrated results are reported in [Figure 3e and Table 3] respectively.

Molecular docking analyses of Caspase 3 screened compounds (Clobazepam, Clonazepam, and Retigabine) with Procaspase 8 target

Clobazepam-Procaspase 8 complex depicts potent binding energy of −5.74 kcal but did not show up any possible

Table 2: Docking results of 20 best anti-epileptic agents

Compounds	Binding energy	K_i (inhibition constant) (μM)	Intermolecular energy	Torsional energy	Unbound extended energy	Number of hydrogen bonding
Carbamazepine	−5.92	45.98	−5.92	0.0	−0.07	2
Ethosuximide	−4.26	758.35	−4.55	0.3	−0.07	2
Gabapentin	−3.91	1.36	−5.4	1.49	−0.13	1
Lacosamide	−5.75	61.14	−6.64	0.89	−0.51	-
Levetiracetam	−3.96	1250.0	−4.85	0.89	−0.38	2
Perampamel	−6.59	14.89	−7.48	0.89	−0.99	-
Phenobarbitone	−5.7	66.24	−6.3	0.6	−0.39	-
Phenytoin	−6.47	17.99	−7.07	0.6	−0.48	1
Pregablin	−2.82	8590.0	−4.91	2.09	−1.81	1
Primidione	−4.38	611.43	−4.98	0.6	−0.04	1
Tiagabine	−6.29	24.55	−9.27	2.98	−1.8	1
Vigabatrin	−2.78	9160.0	−4.57	1.79	−0.33	3
Retigabine	−7.9	20.25	−7.2	0.6	−0.7	1
Acetazolamide	−5.8	8.55	−5.1	0.6	−0.7	1
Clobazepam	−9.0	14.25	−4.56	0.6	−4.44	2
Clonazepam	−8.1	9.13	0.5	0.6	0	1
Rufinamide	−7.0	59.26	−2.6	1.79	−0.5	1
Valporic acid	−4.7	522.06	−4.7	0.0	−0.09	1
Zonsimide	−5.5	69.12	−5.1	−0.4	−0.4	2
Lamotrigine	−6.9	26.35	−6.0	0.3	−0.9	1

Table 3: Molecular docking results of FADD/Procaspase 8

Target	Drugs	Binding energy (kcal)	K_i (inhibition constant) (μm)	Intermolecular energy	Unbound energy	Torsional energy	Hydrogen bonding
FADD	Clobazepam	−5.85	51.93	−5.85	0.0	0.0	1
	Clonazepam	−6.66	13.23	−7.25	−0.28	0.6	3
	Retigabine	−5.21	152.92	−6.99	−0.76	1.79	3
Procaspase 8	Clobazepam	−5.74	61.78	−6.04	0.0	0.3	-
	Clonazepam	−6.47	18.18	−7.06	−0.29	0.6	1
	Retigabine	−4.6	426.41	−6.39	−0.63	1.79	2

FADD: Fas-associated death domain

hydrogen bonding interaction with the target [Figure 3d]. Clonazepam showed single hydrogen bonding with the Procaspase 8, identifier residue LYS (320). Binding energy and bond distances were found as − 6.47 kcal and 1.897 Å, respectively [Figure 3c].

Interaction towards the Procaspase 8 and Retigabine also left promising results. Retigabine showed two hydrogen bond interactions with the target. The interaction sites were GLN (361) and TYR (365), respectively. Secondary amino group of Retigabine is responsible for the hydrogen bonding with the target. The summary of results are reported in Table 3 and Figures 3a-f,h.

Docking validation study

Docking validation study establishes the modeling hypothesis. The nonparametric statistic, sum of the sum of log-rank statistic has been employed to validate our docking scores. SSRL statistics is not only depicts the early detection of active compounds but also supports for correct ordering of the active compounds by their known inhibitory constants. The weight vector $w = \{n, n - 1 \ldots 1\}$ has been used to make the data set more specific. The data sets have been ordered by their inhibitory constants, that is, r_1 being the rank of the most active compound and r_n being the rank of the least active compound. The following SSRL equation has been used for the calculation.[16]

$$\mathrm{SSLR} = n\log(r_1) + (n-1)\log(r_2) + \ldots + \log(r_n)$$

$$= \sum_{i=1}^{n} (n - i + 1)\log(r_i) = \sum_{i=1}^{n}\sum_{j=1}^{i} \log(r_j)$$

Where r_j is the rank of the j^{th} active compound among all n.

Protein-protein interaction analysis

Protein-protein interaction study is widely accepted method to understand the biological phenomenon and it also a challenge for the proteomics community at present. The ultimate aim is to understand and simulate the biological environment in-silico. Our present study depicts multiple protein interaction causes for certain biological phenomenon that has been managed by the small drug molecules essential for a certain activity. FADD and Procaspase 8 interaction promotes the cleavage of Procaspase 8 and activation of Caspase 3 via Caspase 8, promotes apoptosis. Patch dock server has been used to understand the FADD-Procaspase interaction. Patch dock is an algorithm for molecular modeling of various proteins, DNA, antibody, etc., Molecular shape representation, surface matching, and filtering-scoring process are used to investigate the interaction site of the target. FADD-Procaspase 8 docking study showed some hydrogen bonding between FADD (GLN, SER, ASN, THR, and GLU) and Procaspase 8 (VAL, LYS, TYR, GLN, ALA, THR), respectively. Reported data's are summarized in Table 4.

Swarm Dock server predicted 10 best docking poses by filtering solutions with low maximum equilibrium population. Docking process of Swarm Dock server based on optimization of a search vector. The search vector consists of the position (Cartesian center of mass) and orientation (quaternion) of the ligand, as well as normal mode coefficients to model the conformation of the receptor and the ligand. The objective function being minimized is given by the D complex distance-dependent atomic pair potential. The optimization is performed by a hybrid algorithm consisting PSO and a local search. Finished docking poses were minimized again using CHARMM.[14] The minimized docking structure has been re-ranked by centroid potential. Clustered molecules were again filtered by the server, and final eight protein complex were reported along with their binding energy. Maximum occupancy of filtered structure (24a.PDB, Max occupancy: 2.419850) was reported. Calculated results are tabulated in Tables 5 and 6. Pictorial data [Figure 3g and i] depicts protein-protein interaction of best complex model. The study also enlightens some possibilities of a competitive inhibitor of both FADD, and Procaspase 8. Novel inhibitor would bind competitively to the FADD or Procaspase 8 before their interaction and could stop the cleavage of Procaspase 8. Best interaction residues are mentioned in Figure 4.

Toxicity analysis of clonazepam by Toxtree 4.0

Molecular docking study revealed the possible selectivity of clonazpam toward the Caspase 3, FADD, and Procaspase 8 that may lead to toxic towards the body. Toxtree open source application helps to analyze the

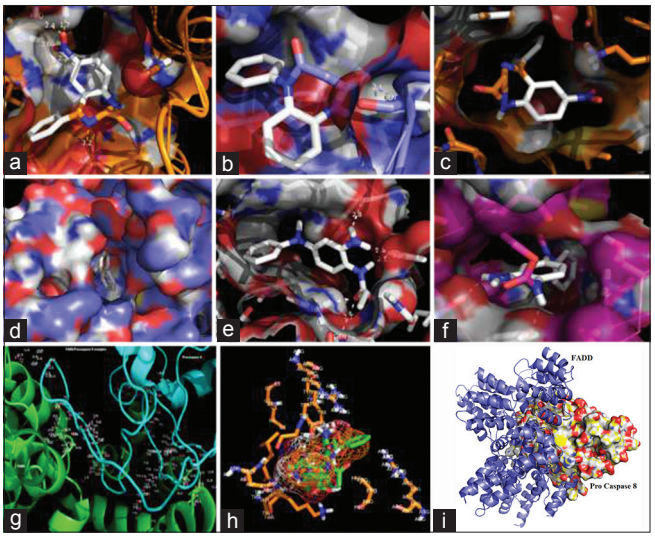


Figure 3: Best docking poses of the selected compounds. (a) Fas-associated death domain (FADD)-Clonazepam docking poses (b) FADD-Clobazepam docking poses (c) Procaspase 8-clonazepam docking poses (d) Procaspase 8-Clobazepam docking poses (e) FADD-Retigabine docking poses (f) Procaspase 8- Retigabine docking poses (g) FADD-Procaspase 8 docking interaction (h) ligand binding pocket of Caspase 3 annotated by FTsite (i) Pictorial representation of FADD-Procaspase 8 interaction

Table 4: Ten best protein-protein docking (FADD-Procaspase 8) parameter values generated by PatchDock server

Solubility no.	Score	Area	ACE	Transformation
1	18,006	2661.50	279.29	1.34 −0.63 0.74 41.63 −12.44 85.60
2	17,392	2697.10	324.99	−1.56 0.35 1.48 20.94 −13.96 −12.05
3	17,314	2488.80	354.36	−2.10 0.46 −0.99 47.44 6.61 −19.52
4	16,986	2589.20	303.73	0.10 −0.76 −2.25 29.51 −29.80 48.79
5	16,644	2339.80	247.73	−1.99 0.06 −0.68 44.38 9.35 −15.39
6	16,256	2872.00	436.08	2.63 0.83 −1.90 20.33 48.09 57.40
7	16,056	2365.00	477.06	−1.08 −0.33 −2.64 48.76 −14.80 1.47
8	15,944	2047.10	238.61	1.76 0.37 0.48 38.48 −12.73 72.47
9	15,938	2167.30	288.99	1.53 −0.00 −1.35 −2.07 53.22 66.25
10	15,934	2084.40	426.62	−2.79 −1.15 −0.23 34.50 −5.66 −2.85

FADD: Fas-associated death domain, ACE: Atomic contact energy

Table 5: Cluster after filtering the protein files by SwarmDock server

Structure subsets of (PPC)*	Energy (kcal)	Number of members	Total contacts	User receptor's residues contacts	User ligand's residues contacts	Mean energy	Standard deviation of the mean energy
48c.pdb	−31.79	3	[48c.pdb|48a.pdb|49c.pdb]	721	0	−25.070	7.069
24a.pdb	−21.47	2	[24a.pdb|25d.pdb]	421	0	−20.300	1.170
104a.pdb	−18.74	5	[104a.pdb|105b.pdb|104d.pdb|105d.pdb|105c.pdb]	771	0	−11.382	5.413
48d.pdb	−18.46	2	[48d.pdb|48b.pdb]	770	0	−14.485	3.975
24b.pdb	−17.06	2	[24b.pdb|25b.pdb]	297	0	−16.215	0.845
105a.pdb	−14.21	1	[105a.pdb]	652	0	−14.210	0.0
77d.pdb	−12.58	4	[77d.pdb|94a.pdb|76b.pdb|77b.pdb]	751	0	−8.607	3.019
24c.pdb	−11.38	1	[24c.pdb]	580	0	−6.650	4.290

PPC: Polarized protein-specific charge

Table 6: Occupancy after filtering the structures by SwarmDock server

PDB structure	Occupancy	Maximum occupancy assigned
24a.pdb	0.077936	2.419850
24b.pdb	2.341914	2.419850
24c.pdb	2.419850	2.419850
25b.pdb	0.160300	2.419850
25d.pdb	0.000000	2.419850
48a.pdb	0.000001	2.306729
48b.pdb	2.306729	2.306729
48c.pdb	0.386539	2.306729
49c.pdb	0.000001	2.306729
76b.pdb	0.000226	2.999531
77b.pdb	2.999531	2.999531
77d.pdb	0.000449	2.999531
93b.pdb	2.999531	2.999531
94a.pdb	0.000262	2.999531
94b.pdb	0.000000	2.999531
104a.pdb	3.000000	3.000000
104d.pdb	0.000000	3.000000
105a.pdb	0.000000	3.000000
105b.pdb	3.000000	3.000000
105c.pdb	0.000000	3.000000
105d.pdb	0.000000	3.000000

PDB: Protein data bank

toxicity of Clonazepam by Cramers rule. A decision tree has been made to understand the queries of quantitative structure toxicity relationship for Clonazepam. Cramers rule estimates the toxicity by three different classes. Class I ments for low toxicity, Class II depicts intermediate, and Class III shows high toxicity. The classification results are shown in Figure 5 that suggests green highlight for Class I, yellow highlight for Class II, and red highlight for Class III, respectively. Clonazepam contains substituted pyrimidine-2 (1H)-one that may lead Class I (low) toxicity. Open chain chlorine substitution of clonazepam also showed Class III (high) toxicity towards the body. Nitrobenzene group of clonazepam could show Class II (Intermediate) toxicity.

CONCLUSION

To our knowledge, this study represents the first reported molecular modeling study of marketed antiepileptic agents as an inhibitor of Caspase 3, FADD, Procaspase 8, which may lead to a promising remedy in TLE. The interactions between the target (Caspase 3, FADD, Procaspase 8) and molecule proposed in this study could help the future research, for better understanding the potential binding site and novel mechanism of Clonazepam as an anti-apoptotic agent. Docking studies

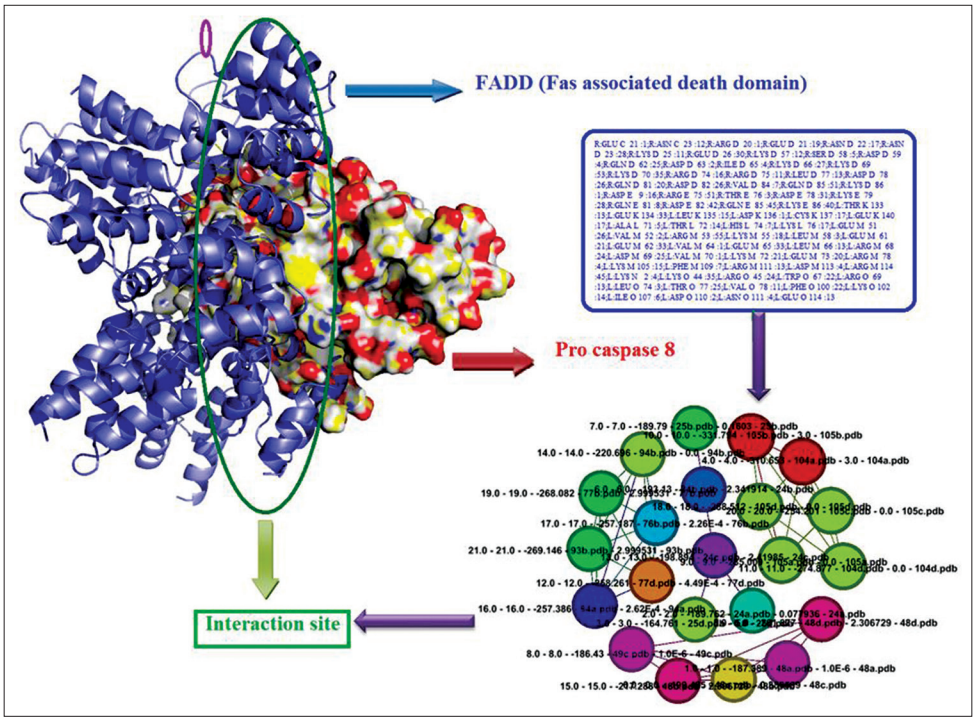


Figure 4: Protein-Protein interaction with gephi generated site map. Best clustered protein 48c.pdb total interaction and contacts generated by SwarmDock server. List of contacts (R-Receptor, L-Ligand, UR-User Receptor, UL-User ligand)

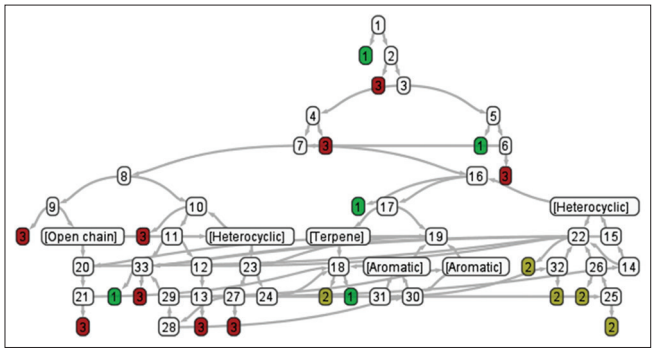


Figure 5: Cramers rule decision tree generated by Toxtree application

were conducted on Caspase 3, FADD, Procaspase 8, it can be concluded that the predicted binding poses and the score help to understand the future in silico drug design approaches. Clonazepam showed promising binding energy for both FADD and Procaspase 8 receptors as well. Clonazepam also scored immaculate binding energy and hydrogen bond interaction for Caspase 3. Benzodiazepine ring depicts possible selectivity for the proposed targets. FADD-Procaspase 8 interaction study by Patch dock server also helped to understand the molecular mechanism of Procaspase 8 cleavage and activation of Caspase 3 via caspase 8. Prediction of toxicity in Toxtree application by cramers rule enhances the drug profile for future study. Therefore, the present investigation can be further evaluated by *in vitro* and *in vivo* models for the development of future cognitive research.

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