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Development of Better Analogs of Valproic Acid for the Treatment of Epilepsy by CADD

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ABSTRACT
Epilepsy is one of the major neurological disorders occurring due to the abnormal functioning of the various receptors and enzymes in the central nervous system. Many potentials drugs were developed in recent times which act on ion channels like sodium (Na^+), calcium (Ca^{2+}), chloride (Cl^-), and receptors like GABA receptor and enzymes like GABA transaminase. Some drugs act as enzyme, ion channel inhibitors or blockers, and some drugs as receptor agonist like barbiturates, benzodiazepines acting on GABA receptors. In the present study performed computational techniques in order to develop better inhibitors for the enzyme GABA transaminase by modifying the terminal 'methyl' group of the Valproic acid structure with electrophilic, nucleophile and neutral pharmacophoric features. Molecular mechanics studies has been carried out for the analogs and protein – ligand interactions of these analogs was identified through docking studies using GOLD 4.1 software against the enzyme 4-aminobutyrate-aminotransferase(GABA transaminase). From the docking studies we found that replacement of methyl with amine, hydrogen and hydroxyl groups (hydrophilic groups), are showing better fitness than that of the valproic acid.

Keywords: Epilepsy, Valproic acid, GABA transaminase, molecular docking, Binding Affinity.

INTRODUCTION

As per the rate of the human evolution, we can observe the fastness in the new generations all because of the Neurons. Epilepsy or seizure disorder is a nervous disorder that is due to abnormal signaling by clusters of nerves in the brain, altering individual's consciousness, actions or movements i.e. the source of the disease is brain but may affect any part of the body later. [1] There are lots of reasons for the occurrence of the diseases. Mutations in some genes are linked to few types of epilepsy. [2] Several genes that code for protein subunits of voltage-gated and ligand-gated ion channels have been associated with forms of generalized epilepsy and infantile seizure syndromes. [3] It has been found that epilepsy is bit more common in men than in women and can occur in all age groups. African-Americans are mostly affected than Caucasians. [4] Individuals with Alzheimer, mental retardation or cerebral palsy, infection (meningitis) and stroke in their history are more prone to the disease, though hormone fluctuations, stress, sleep patterns and photosensitivity also are the effective factors. [5] Certain drugs and sometimes surgery to remove abnormal brain cells can also help to control epilepsy though cannot be cured and with correct treatment approach one can live fully functional and normal life afterwards. [6]

Epileptic malfunctioning of brain is due to abnormal signaling of nerves i.e. neurotransmitters. GABA (gamma amino butyric acid) is the most contributing neurotransmitter to seizures. [7] GABA is synthesized in brain by GABAergic neurons from amino acid glutamate in addition to vitamin B6 and acts by binding to certain plasma membrane's transmembrane receptors. [8] This binding causes opening of ion channels allowing negatively charged Cl ions and positively charged K ions in and out of the cell resulting action potential. Excitation in the brain must be balanced with inhibition and GABA regulates neuronal excitability throughout the nervous system i.e. inhibitory neurotransmission. [9] In general GABA after synthesis in GABAergic neurons releases in synapses and transported into presynaptic terminals or glia cells and metabolized by GABA transaminase enzyme into succinic semialdehyde and glutamate. Here 4-aminobutyrate-aminotransferase (GABA receptor) was taken as target in the present study as GABA is able to induce relaxation, analgesia, and sleep thus

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stimulation of GABA receptor can be helpful to induce relaxation and preventing seizures. [10]

Valproate (2-propylpentanoic acid) is a well known anticonvulsant drug used for antiepileptic and mood stabilizing effects, was taken for study. [11] It enhances the neurotransmission of GABA by inhibiting GABA transaminase activity. [12-13] It was first synthesized by B. S. Burton in 1882 as an analogue of valeric acid (pentanoic acid), found naturally in a herb 'Valerian' and named after it only. [14] Now numbers of pharmaceutical companies are producing this drug under different brand names as valcote, mylproin, sprinkle, epilex, deprioc etc. [15]

Present study is based on the optimization of Valproate drug activity against GABA transaminase enzyme using insilico methods, to predict more potent analogues in less time and expense as well. Structure based drug design and Analogues based drug design are the two well known insilico approaches used by the researchers. We performed our study using analogue based drug design approach. Thus five analogues have been designed by modification of Valproic acid at pharmacophoric region, satisfying Lipinski rule of five to analyze their binding site affinity towards GABA transaminase. Docking studies were performed to analyze binding affinity among analogues and target. Analogues with best binding affinity were considered as the plausible potent analogues, and can be taken for clinical studies further.

MATERIALS AND METHODS

The structure of the target 4-aminobutyrate-aminotransferase was retrieved from http://www.rcsb.org/pdb server with PDB ID 1OHV possessing 4 identical chains, resolution 2.30Å and consists of 472 residues on which 461 residues were identified in domain region. Stability of the structure is due to Alpha helices which comprises 43 % (22 helices; 204 residues) and beta sheets comprising 14 % (22 strands; 68 residues) of the protein. Structure of drug Valproic acid was obtained from Drugbank and its analogs were developed by using Accelrys Symex Draw4.0, then prepared for docking by Hyperchem 8.0 and docked against the target using GOLD 4.1 tools.

Analogue preparation

The drug Valproic acid was sketched and converted into 3D model using Accelrys Symex Draw 4.0. All the five analogs were then prepared by modification in the functional group region of the drug considering the Lipinski rule of five in mind. We have taken the Eclectrophile, Nucleophile and Neutral R-groups for the replacement of the functional group that are –NH2, -CH2CH3, -OH, -Cl & -H. All these analogues were then typed with CHARMm forcefield and energy minimization was done with Polak Ribiere algorithm, RMS gradient 0.1 and Max Steps 2000 parameter values in Hyperchem 8.0. Structural modifications to the valproic acid can be visualized in the Fig. 1.

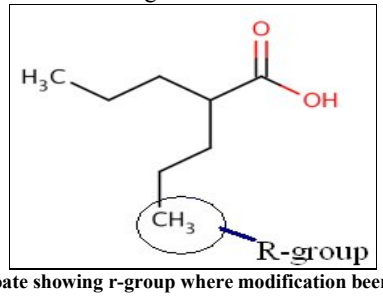

Fig. 1: Valproate showing r-group where modification been done

Protein preparation

The protein 4-aminobutyrate-aminotransferase (PDB Id: 1OHV) was retrieved from PDB website and structure was shown in the Fig. 2. Geometry optimization of the retrieved protein was performed by removing water molecules, ions, hetero atoms and ligands. Hydrogens were added to fulfill the valency of the molecule. Energy minimization was performed, after typing the molecule with CHARMm27 molecular mechanics force field, by using Polak-Ribiere (conjugate gradient) geometry optimizer of Hyperchem 8.0 at RMS gradient 0.1 and Max Steps 2000 parameter values. Active site with ASN 140, PHE 189, ARG 192, Glu270, Asp 298, Val 300, Tyr348, Gly440 and few more residues, was identified by online resource PDBsum, which can be seen in Fig. 3.

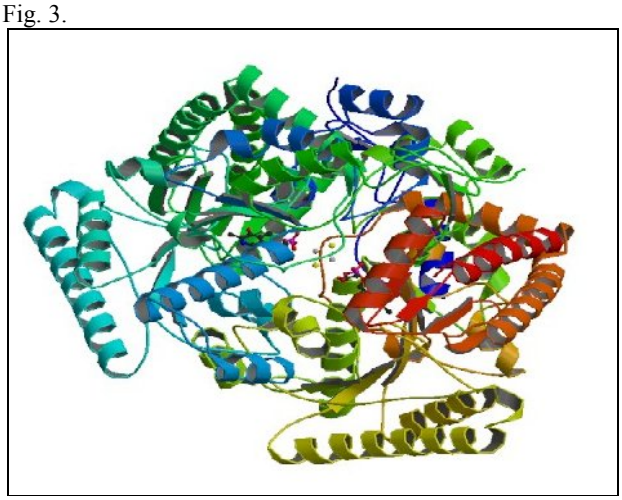

Fig. 2: structure of target (pdb id: 1ohv)

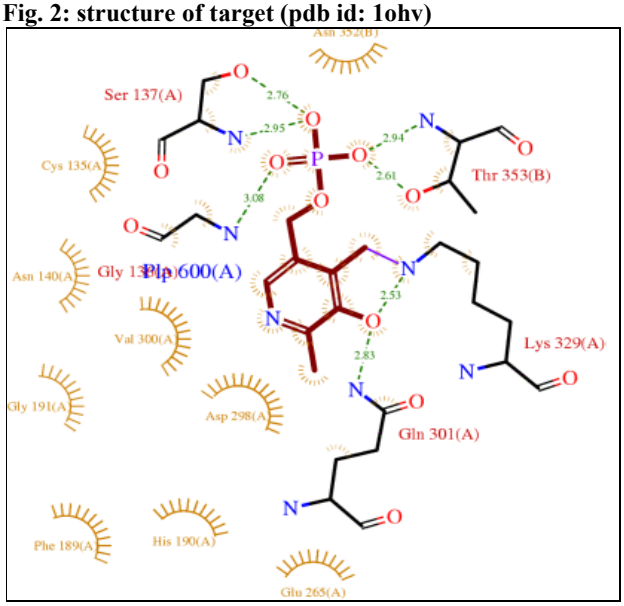

Fig. 3: ligplot showing active site interaction map using PDBSum

Docking using GOLD

Prepared protein and all analogues were then subjected to docking using Genetic Optimization for Ligand Docking (GOLD) 4.1 software to carry out the Protein analogues interaction studies. It uses genetic algorithm parameters for docking, which involves a conformational search to find out the best complementary analogue binding pose with target active site. Active site atom number is identified to perform the docking.

The interaction between protein and analogs can be visualized and total number of hydrogen bond and its distances can be calculated. Hydrogen bond and vander waals forces determine the fitness of docked analogues. Result are in the format of Gold fitness which is comprise of four terms, two inter atomic hydrogen bond, vander waals interactions and two intra atomic hydrogen, vander waals interactions respectively. Depicted as:

GOLD Fitness = Shb_ext + 1. 3750 × Svdw_ext + Shb_int + Svdw_int

RESULTS & DISCUSSION

Docking performed for analysis of protein ligand interactions using Gold 4.01 software resulted the following fitness scores:

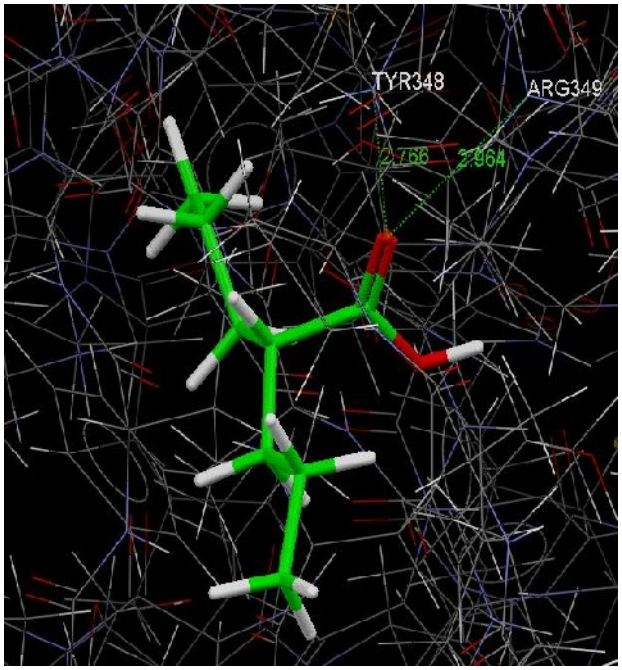

Fig. 4: Valproic acid docked with target

Table 1: Docking Results

Ligand	Fitness	Shb_ext	Svdw_ext	Shb_int	Svdw_int
R=CH_3(Valproate)	24.8	0.00	10.51	0.00	-14.69
R = NH_2	36.86	6.00	29.78	0.00	-10.09
R = CH_2CH_3	4.48	0.12	31.35	0.00	-38.75
R = OH	25.07	0.28	29.60	0.00	-15.91
R = CL	14.96	2.38	28.44	0.00	-26.53
R = H	28.53	0.57	28.31	0.00	-10.97

Fitness function was calculated for the prediction of binding affinities, and the analogues with higher fitness score i.e. –NH2/36.86, –H/28.53 and -OH/25.07 have been considered as the potent analogues, where the drug Valproate has shown the fitness score 24.8 and GOLD Fitness score of other ligands was shown in the Table 1. As per GOLD fitness score components external hydrogen bond energies and external vander waals energies are having higher scores than Valproate drug and internal vander waals energy scores as well are having variations thus found as the determining factors of the drug-protein complex fitness. Binding interactions of the Valproic acid to the enzyme GABA transaminase was shown in Fig. 4.

The results obtained above indicate that before synthesis, biological activity testing and clinical trials of new analogs, one can use molecular mechanics and analogue based drug designing methods for qualitative assessment of binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. The analogues with substituents –NH_2, -H and –OH have been found as the most suitable analogues in the study and may have chances to show the better results than Valproate in laboratory as well.

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