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Establishing an in-silico medication towards treatment of Petit mal Epilepsy

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Abstract- Epilepsy is a neurological disorder. About 0.5%-1% of world population is affected by epilepsy. Petit mal epilepsy is the main seizure type in 15-20% of epileptic children. The mainstream intervention for petit mal epilepsy is T-type Ca ++ channel blocker like ethosuximide. However, there is a need for safer and effective drug. The protein-ligand interaction plays a significant role in structure based drug designing. In the present work we have taken t-type calcium channel as the target and identified natural compounds that can be used against petit mal epilepsy. Here the drug ethosuximide is used as standard (E value = -132.35). Twelve natural compounds are retrieved from PubMed and are checked for drug likeness with bioinformatics tool ADMETox to satisfy Lipinski's rule of five. Ten natural drugs qualify this step and are docked with receptor using HEX docking software. All the compounds taken for docking were found to be better than the conventional drug, Ethosuximide.
Keywords- Docking, HEX, Ethosuximide, Lipinski, Petitmal

Introduction

Epilepsy is a chronic neurological disorder. It results from episodic neuronal discharges in the brain. Epilepsy affects about 0.5%-1% of world population. Two common forms of epilepsy are the tonic-clonic fit (grand mal) and the absence seizure (petit mal). Absence seizures occur in children. The drugs used specifically to treat absence seizures act mainly by blocking T-type Ca ++ channels [1]. The structure of T-type Ca++ channel is given in "Fig. (1)". Current antiepileptic drugs are effective in controlling seizures in about 70% cases, but their use is often limited by side effects. Many new antiepileptic drugs have been developed in the past 15-20 years – one of the most active areas of drug development – in attempts to improve their efficacy and side effects profile. Improvements have been steady rather than spectacular and epilepsy remains a difficult problem [1]. The need for ongoing development of new drugs needs emphasis in light of the current global situation of health and disease. The shortcoming of traditional drug discovery, as well as the allure of a more deterministic approach to combating disease has led to the concept of rational drug design [2]. Rational drug design (RDD) plays significant role to speed up the drug designing process. These processes are used in pharmaceutical industry to discover and develop new drugs. RDD uses a variety of computational method to identify novel compounds. One of those methods is docking of drug molecules with receptor [3]. So in the present study efforts are made in non-conventional methods of drug designing to identify novel natural compounds possessing antiepileptic activity using various bioinformatics tools.

Tools & Materials Used

For our present study we used various bioinformatics tools, biological database like PubMed, Drug bank, PDB (Protein Data Bank), ADMETox and software like modeller 9v5, Arguslab, Hex. Arguslab4.0.1 is molecular design software. It is used for molecular modeling. In contrast to useful molecular modeling programs such as molecular dynamics and quantum chemistry programs, this software directly supports aspects related to construction of molecular models [4]. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of Protein and DNA molecules. Hex can also calculate protein-ligand docking, assuming the ligand is rigid and it can superpose pairs of molecule using only knowledge of their 3D-shapes [5]. The PDB is a single worldwide archive of structural data of biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971 [6]. It contains structural information of the macromolecules determined by X-ray crystallographic, NMR method etc. PubMed is a free digital archive of biomedical and life sciences journal literature at the U.S. National Centre for Biotechnology Information (NCBI) in National Library of medicine. PubMed is a search engine for accessing the MEDLINE database [7]. Homology or comparative modeling of protein three-dimensional structures is done by MODELLER. The alignment of a sequence to be modeled with known related structures is provided by the user and MODELLER automatically calculates a model containing all non-hydrogen atoms. It implements comparative protein structure modeling by satisfaction of spatial restraints and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases,

comparison of protein structures, etc.[8]. ADMETox is maintained by RPBS (Ressource Parisienne en Bioinformatique Structurale) is a resource dedicated primarily to structural bioinformatics. It is the result of a joint effort by several teams to set up an interface that offers original and powerful methods in the field [9]. The receptor or target responsible for petit mal epilepsy i.e. T- type calcium channel and compounds isolated from the plants like Retusin, Chrysin, Apigenin, Pincocembrin, shanzhiside, Geissoschizine, santonin, huperzine-A, Acteoside, Piperine, Hirsutine and Pinocembrin are used in this study. Here the antiepileptic drug Ethosuximide is taken as standard. All the natural molecules selected are the active constituents of plants possessing anti-epileptic activity as per their traditional claims. Before docking, the molecules under study are checked to satisfy Lipinski's rule of five by using ADMETox. The drugs which do not satisfy above rule (have more than 10 hydrogen bond donors) are discarded from further study. The docking analysis of ethosuximide and the above natural compounds with t-type calcium channel was carried by Hex docking Software. The collection of Ethosuximide and natural compound receptor complexes was identified via docking; their relative stabilities were evaluated using molecular dynamics and their binding affinities using free energy simulation.

Result

The molecules which satisfy Lipinski's rule were identified and which do not satisfy is ruled out. The results are given in table-1. Acteoside and Shanziside are discarded from further study as they do not satisfy above rule (have more than 10 hydrogen bond donors). Docking results between T-type calcium channel and the conventional drug Ethosuximide as well as with natural compounds are shown in table-2. All the natural compounds have lower energy value than ethosuximide. The drug curcumin is having the lowest energy value.

Discussion

Petit mal or absence seizure is the main seizure type in 15-20% of epileptic children. The low threshold Ca ++ currents carried by T-type Ca ++ channels play a major role in the generation of petit mal epilepsy. Ethosuximide is a major drug used for the treatment of absence seizures [10]. In silico drug screening is an effective alternative for identification of lead compounds. Lead compounds are identified and tested using molecular docking for their effectiveness against major molecules of interest for different diseases [11]. This in silico work makes use of Hex in computer aided drug designing. Lipinski's rule of five is a rule of thumb to evaluate drug likeness. This rule is formulated by Christopher A. Lipinski in 1997. This rule describes molecular properties important for drug pharmacokinetics in human body including their Absorption, distribution, metabolism and Excretion. Lipinski's rule says that in general an orally active drug has no more than one violation of following criteria i.e. has no more than 5 hydrogen bond donors,not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient Log P less than 5 [12]. Out of 12 natural compounds, 2 compounds (Acteoside and Shanziside) are discarded as they do not have necessary ADME and toxicity profile. Docking is the process of fitting together of two molecules in 3-dimensional space. Docking allows the scientist to virtually screen a database of compounds and predicts the strongest binders based on various scoring function. It explores ways in which two molecule such as drug and t-type calcium channel fit together and dock to each other well, like pieces of a three dimensional jigsaw puzzle. The molecules binding to a receptor, inhibits function, and acts as a drug [13]. When a drug binds to a target in molecular modeling and molecular design softwares, the lower the energy value the higher is the affinity of the drug [14]. The drug Ethosuximide binds to the target (T-type calcium channel) and produces an energy value of -132.35. The used natural molecules also decrease the energy-value. The energy value for all the natural compounds is lower than Ethosuximide. The drug curcumin is having the lowest energy value. So it is clear from the result that these natural compounds can take an important part in curing epilepsy with relatively less toxic effect. This achievement may prove to be a boon towards Epilepsy research if similar revelations come out of animal and clinical studies.

Conclusion

The protein- ligand interaction plays a significant role in structure based drug designing. In the present study we have taken T-type calcium channel as the target and identified the natural compounds that can be used as antiepileptic drug. From the docking result it is clear that all the natural compounds docked can be better candidates for epilepsy as compared to Ethosuximide. Out of the 12 natural compounds studied, Curcumin (-258.67) produces the best score. These compounds in future can be used for in vitro study.

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Table 1-Result of ADME and toxicity property of natural compounds

Compound	Mol.wt	Mol. form.	Log p	H-B	H-B Acceptor	Remark
				Donor		
Retusin	358.34	$C_{19}H_{18}O_7$	2.9	1	7	P
Santonin	246.3	$C_{15}H_{18}O_3$	2	0	3	P
Chrysin	254.23	$C_{15}H_{10}O_4$	2.8	2	4	P
Acteoside	624.58	$C_{29}H_{36}O_{15}$	0.3	9	15	F
Apigenin	270.23	$C_{15}H_{10}O_5$	1.6	3	5	P
Curcumin	368.37	$C_{21}H_{20}O_6$	3.1	2	6	P
Huperzine-A	242.31	$C_{15}H_{18}N_{2O}$	0.2	2	2	P
Shanzhiside	392.35	$C_{16}H_{24}O_{11}$	-2.8	7	11	F
Piperine	285.33	$C_{17}H_{19}NO_3$	3.2	0	3	P
Hirsutine	368.46	$C_{22}H_{28}N_2O_3$	3.3	1	5	P
Geissoschizine	352.42	$C_{21}H_{24}N_2O_3$	2.7	1	5	P
Pinocembrin	224.2	$C_{15}H_{12}O_2$	3.2	0	2	P

H-B = Hydrogen bond, Mol.wt = Molecular weight, P= Pass, F = Fail.

Table 2-Docking Result of T-Type Calcium Channel with natural compounds

Ligand	E-Value
Ethosuximide	-132.35
Retusin	-249.8
Santonin	-167.78
Chrysin	-215.68
Apigenin	-217.99
Curcumin	-258.67
Huperzine-A	-172.31
Piperine	-213.85
Hirsutine	-232.48
Geissoschizine	-213.87
Pinocembrin	-183.8

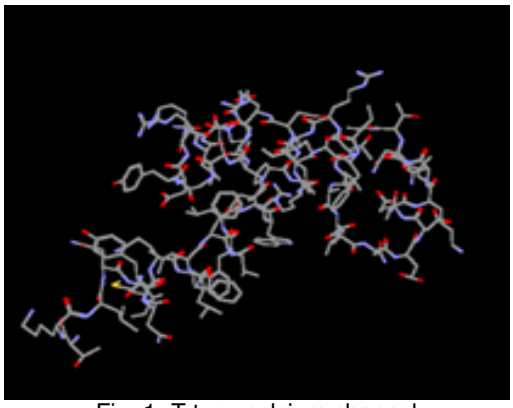

Fig. 1- T-type calcium channel