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Original Article

VIRTUAL SCREENING AND ADMET ANALYSIS FOR IDENTIFICATION OF INHIBITORS AGAINST ACETYLCHOLINESTERASE ASSOCIATED WITH ALZHEIMER'S DISEASE

ANKIT SINGH, VINOD KUMAR JATAV, SUNITA SHARMA*

Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, M. P., India 474005.
Email: sunitasharma75@yahoo.co.in

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ABSTRACT

Objective: Alzheimer's disease a progressive neurodegenerative disorder characterized by oxidative stress, amyloid β deposition and due to low level of neurotransmitter acetylcholine in the brain. the reduction of acetylcholine in the brain is due to enhance activity of acetyl cholinesterase enzyme. This study is done to find out the possible inhibiters of acetyl cholinesterase. In lieu of that, the present study focus on to find possible analogs of known drug Rivastigmine.

Methods: Protein for study is retrieved through protein databank (PDB ID - 1B41) and constrains was removed using Swiss-pdbviewer. Analogs for docking were chosen from zinc database and docking was performed using Autodock 4.2, after docking ADME analysis and toxicity were done against the possible inhibitors.

Results: Out of fifty analogs chosen for docking only nine analogs showed minimum binding energy and good RMS value, out of that analogs two with id ZINC00004413 and ZINC967938 shows good results so they were chosen for ADME analysis and toxicity prediction.

Conclusion: The possible analogs obtained after study can be further used for study and preparation of novel drug against Alzheimer's disease.

Keywords: AD (Alzhimer's disease), Acetylcholinesterase, Rivastigmine's, AutoDock 4.2, ADMET.

INTRODUCTION

The neurodegenerative disorder characterized by the nerve cell dysfunctions and loss of neurons in the central nervous system was first discovered in 1907 by a German scientist, Alois Alzheimer, and was named as Alzheimer's disease (AD). Millions of people are reported to fall victims of this traumatic problem worldwide [1]. Recent epidemiological evidence suggests a worldwide prevalence of 24.3 million cases of dementia, with one new case developing every seven seconds [2]. In the United States, however, statistics show that Alzheimer's Disease is the leading cause of dementia affecting about four million of the U. S. population or 10% of Americans over the age of sixty five [3]. It was believed that Alzheimer's disease resulted from an increase in the production or accumulation of a specific protein (β - Amyloid protein) that leads to nerve cell death and decreased cholinergic activity in the brain [4]. At the early stages, the patient is faced with a decline in cognitive functions, exclusively short - term memory, which later result in the incapability to read, speak and/or think rationally [5].

Acetylcholine (ACh) is the almost abundant neurotransmitter in the body and the primary neurotransmitter in the brain that is responsible for cholinergic transmission. The enzyme AChE plays a key role in the hydrolysis of the neurotransmitter ACh. AChE tends to become deposited within the neurofibrillary tangles and amyloid plaques associated with Alzheimer's disease [6]. Recent reports on curative approaches to this ailing disease are based on the assumption of a cholinergic mechanism, with particular emphasis on acetylcholinesterase inhibition [5, 7]. Four cholinistrase inhibitor have been approved by FDA namely Donepezil hydrochloride (Aricept), Rivastigmine (Exelon), Galantamine (Razadyne - previously called Reminyl) and Tacrine (Cognex). Above three drugs are prescribed by physicians but not Tacrine because of their undesirable side effects. Rivastigmine appears to be beneficial for people with mild to moderate Alzheimer's diseases [8, 9].

It is also a reversible AChE inhibitor with high brain selectivity. Its use has been approved in at least 40 countries around the world. Plasmatic half-life is only 2 hr, however. Rivastigmine's adverse effects are gastrointestinal, including nausea, vomiting, anorexia, and weight loss. Thus, patients should take initially 1.5 mg/dose twice a day and then the dosage should be maintained via titration [10, 11]. Rivastigmine is an effective therapeutic agent for treating cognitive and behavioral symptoms in Alzheimer disease [12].

The need for a quick search for small molecules that may bind to targets of biological interest is of essential importance in the drug discovery process. Through structure based drug designing (SBDD) we can find out new drugs based on biological targets. Targets are biological molecules that are involved in particular disease condition; they are also involved in metabolic or signaling pathway of disease. SBDD is achieve through virtual screening in which compound which have to be virtually screened can be taken from corporate or commercial compound collection, or from virtual compound libraries. If structure of target is available then structure based virtual screening is done through 'Docking programs'. Through these program a small molecule can be docked to a particular target in different positions, conformations and orientations [13]. The present study was carried out to find out possible analogs for Rivastigmine to modulate acetylcholinestrase function through docking studies.

MATERIALS AND METHODS

Retrieval and preparation of protein

The three dimensional structure of Acetylcholinestrase for docking was Retrived from the protein data bank (PDB ID - 1B41). The protein structure was then refined using Swiss-PDB viewer and constrain of protein was gradually removed. The program Swiss-PDB viewer was designed to integrate functions for protein structure visualization, analysis and manipulation into a sequence-to-structure workbench with a user-friendly interface [14]. The ribbon structure of Acetylcholinestrase protein shown in Figure 1.

Active site prediction

The active sites were predicted by Q site finder. It is an energy based method for the prediction of protein ligand binding site, it uses the interaction energy between the protein and simple van der walls probe to locate energetically favorable binding site [15]. Energetically favorable probe sites were clustered according to their spatial proximity and cluster were ranked according to the sum of interaction energy.

Selection of Drug

The drug bank data base (http: //www. drugbank. ca) is a freely available, unique bioinformatics and cheminformatics resource that combines detailed drug (i. e. chemical, pharmacological and pharmaceuticals) data with the comprehensive drug target (i. e. sequence, structure and pathways) and drug action information. It was specifically designed to facilitate in silico drug target discovery, drug metabolism prediction, drug design, drug interaction prediction and general pharmaceutical education [16].

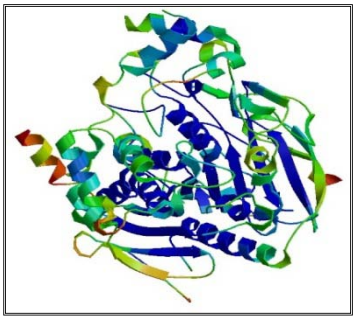


Fig. 1: Ribbon structure of Acetylcholinestrase protein

The database contains 7739 drug entries including 1584 FDA-approved small molecule drugs, 156 FDA-approved biotech (protein/peptide) drugs, 89 nutraceuticals and over 6000 experimental drugs. Additionally, 4283 non-redundant protein (i. e. drug target/enzyme/transporter/carrier) sequences are linked to these drug entries [17]. For docking purpose Rivastigmine has been searched using drug bank (Figure 2). The PDB file was then saved to computer using protein data bank which was then used for docking study.

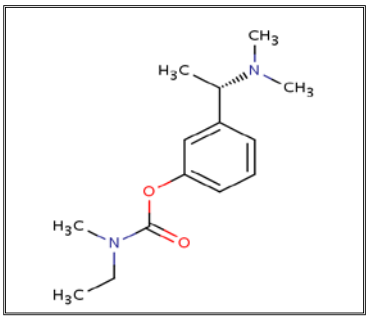


Fig. 2: Chemical structure of Rivastigmine drug.

Screening of Zinc Analogs

ZINC database contains over 13 million commercially available compounds in ready-to-dock, 3 D formats for structure based virtual screening. ZINC database was screened for analog with 70% similarity against Rivastigmine. A total of fifty compounds were selected for docking studies. These compounds were converted into PDB format from SDF by using Open Bable [18]. Their energy minimization was carried out with the GROMOS96, implemented in Swiss-PDB viewer software.

Virtual screening and molecular docking

Structure based drug designing (SBDD) is essential to find out new drugs by virtual screening,carried out through molecular docking. Docking is the process by which two molecules fit together in 3D space. Here we use Autodock 4.2 for molecular docking. Molecular docking fits two molecules in favorable configuration using their topographical features. Practically molecular docking has been an important technique for the modeling protein-ligand interactions and has been used in studies of the structural basis of biological functions. For the inhibitor, charges of the Gasteiger type were assigned and maximum six number of active torsion are given to the lead compounds using Autodock Tool [19]. Essential parameters like hydrogen atoms, solvation and kollman charges were added to the modeled protein structure using Autodock tool. Grid box was then generated using Autogrid program so that it cover entire protein catalytic sites and make ligand to moved freely in that site. The Autogrid parameters are assigned values in X, Y and Z plane. Lamarckian genetic search algorithm was employed and thirty search attempts were performed for ligand with a population size of 25000. Other docking parameters were set to the software's default values. After docking completion the docked model was ranked according to their docked energy as implemented in the AutoDock program.

ADME Analysis

A significant bottleneck remains in the drug discovery procedure, particularly in the later stages of lead discovery, is analysis of the ADME and over toxicity properties of drug candidates. Over 50% of the candidates failed due to ADMET deficiencies during development. To avoid this failure at the development,a set of *in vitro* ADME screens has been implemented in most pharmaceutical companies with the aim of discarding compounds in the discovery phase that are likely to fail further down the line. Even though the early stage *in vitro* ADME reduces the probability of the failure at the development stage, it is still time-consuming and resource-intensive. This program calculates the human intestinal absorption, in vitro Caco-2 cell permeability, Maden Darby Canine Kidney (MDCK) cell permeability, skin permeability, plasma protein binding, blood brain barrier penetration, and carcinogenicity. The prediction system is composed of MLR and Artificial Neural Network and is trained with experimental data. The absorption properties were described with the descriptors that were selected with Genetic Algorithm. PreADME is useful for high throughput screening and combinatorial chemistry library design considering the Linpinski's rule or lead-like rule, drug absorption and water solubility [20].

RESULT AND DISCUSSION

Active site prediction

Prediction of Active site was done through Q-site finder. The server gives best binding site location along with their respective site volume and involved residue. Binding site of AChE was constituted by amino acid GLN71, TYR72, VAL73, ASP74, GLY82, THR83, GLU84, TRP86, ASN87, PRO88, TYR119, GLY120, GLY121, GLY122, TYR124, SER125, GLY126, ALA127, LEU130, TYR133, GLU202, SER203, PHE295, PHE297, TYR337, PHE338, TYR341, TRP439, HIS447, GLY448, TYR449 and ILE451 shown in Figure 3.

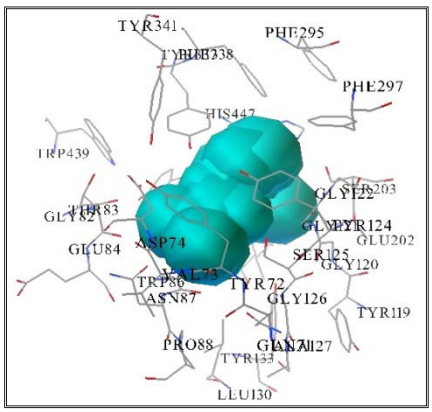


Fig. 3: Predicted pocket (cyan color) of AChE and involved amino acid.

Virtual screening

Virtual screening was done through Autodock tool version 4.2 which was used to prepare, run and analyze docking simulation. Total of

fifty compounds were generated through Autodock 4.2 program. Out of fifty compounds we got nine best compounds with minimum docking energy and good RMS value shown in Table 1, 2. The best conformations analyzed through python molecular viewer for interaction study (Figure 4a, 4b).

ADME analysis

ADME analysis of 9 compounds predicted about Adsorption, Distribution, Metabolism, and Excretion through Pre-ADMET software.

Out of these compounds ZINC00004413 and ZINC 00967938 shows suitable result with respect to Rivastigmine (Table 3, 4). These results may used for generation of new drugs against acetylcolinistrase.

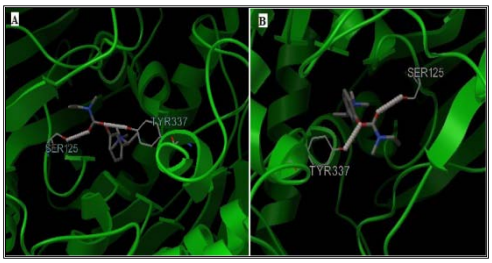
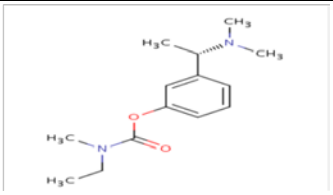
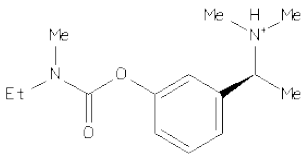
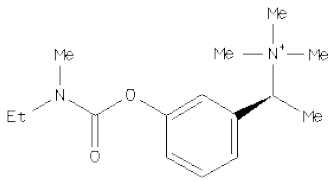
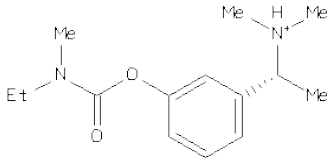
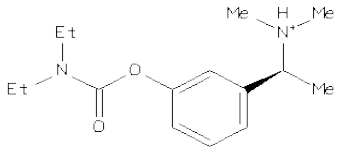
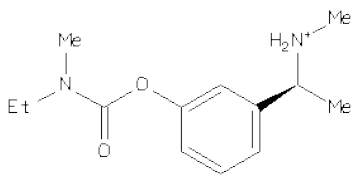


Fig. 4: Docking conformation of: a) ZINC00004413 b) ZINC967938 analyzed by Python Molecular Viewer (docked ligand shown by balls and sticks while hydrogen bonds shown by white spheres).

Table 1: Chemical properties and structures of Rivastigime and its ZINC analogs

Compound	Structure	MOL wt(g/mol)	xLogp	H donar	H acceptor	Rotable bonds
Rivastigmine		250.33	2.3	0	3	5
ZINC00004413		251.35	2.28	1	4	5
ZINC77303349		265.377	-1.7	0	4	5
ZINC00967938		251.35	2.28	1	4	5
ZINC33969568		265.377	2.65	1	4	6
ZINC90411665		237.323	2.03	2	4	5

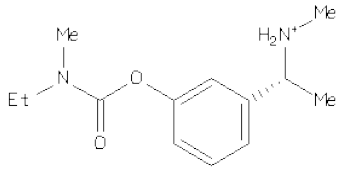
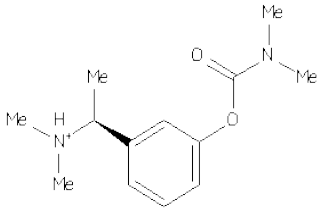
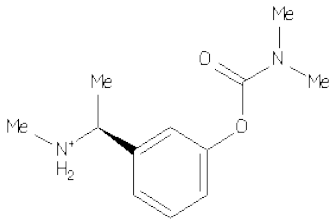
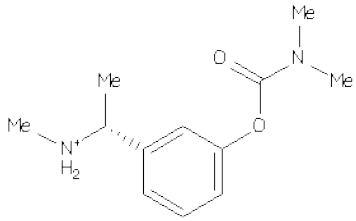
ZINC90411664		237.323	2.03	2	4	5
ZINC13492195		237.323	1.9	1	4	4
ZINC90411518		223.296	1.66	2	4	4
ZINC90411521		223.296	1.66	2	4	4

Table 2: Molecular docking results of Rivastigmine analogs with AChE

S. No.	Compound	Ref RMS	Docking energy(Kcal/mol)	H -bond	Binding residue
	Rivastigmine	**198.34**	**-09.23**	**1**	**SER125**
1.	ZINC00004413	206.3	-13.79	2	SER125, TYR 337
2.	ZINC90411518	211.92	-15.22	2	GLY121,GLU202
3.	ZINC90411521	210.89	-15.92	2	ASP74, TYR337
4.	ZINC967938	208.11	-13.8	2	SER125, TYR337
5.	ZINC77303349	205.35	-10.87	3	SER125,TYR124,TYR337
6.	ZINC90411664	209.34	-15.81	2	SER125, TYR337
7.	ZINC90411665	207.55	-14.88	2	TYR125, TYR337
8.	ZINC33969568	205.45	-14.44	1	SER125
9.	ZINC13492195	205.52	-13.57	1	PHE295

Table 3: ADME prediction of compounds using Pre-ADMET tool

Compound	Human Intestinal Absorption	Caco2 Cell Permeability	MDCK Cell Permeability	Plasma protein biding	Blood Brain Barrier Penetration
Rivastigmine	99.4152	47.5911	43.566	26.3876	1.1621
ZINC00004413	99.9864	47.8943	47.486	32.1069	1.53129
ZINC77303349	100.000	46.2033	20.7631	14.9917	0.5924
ZINC00967938	89.9864	37.8943	17.486	12.1069	1.53129
ZINC33969568	90.4056	43.7116	46.7195	25.4870	2.0434
ZINC90411665	89.7372	27.1706	15.0099	2.0957	0.4261
ZINC90411664	89.7372	27.1706	15.0099	2.0957	0.4261
ZINC13492195	89.5285	24.7335	10.6688	8.1281	2.0228
ZINC90411518	89.4308	17.6427	9.3100	0.000	0.8707
ZINC90411521	89.4308	17.6427	9.3100	0.000	0.8707

Table 4: Toxicity prediction of selected compounds by Pre-ADMET tool

Compound	AMES Test							Carcinogenicity	
	TA100 +s9	TA100 -s9	TA1535 +s9	TA1535 -s9	TA98 +s9	TA98 -s9	Result	Mouse	Rat
Rivastigmine	**-**	**+**	**-**	**-**	**+**	**+**	**M**	**-**	**-**
ZINC00004413	**-**	**+**	**-**	**-**	**+**	**+**	M	**-**	**-**
ZINC77303349	**-**	**-**	**-**	**-**	**+**	**-**	M	**-**	**-**
ZINC00967938	**-**	**+**	**-**	**-**	**+**	**+**	M	**-**	**-**
ZINC33969568	**-**	**+**	**-**	**-**	**+**	**+**	M	**-**	**-**
ZINC90411665	**-**	**+**	**-**	**-**	**+**	**-**	M	**-**	**-**
ZINC90411664	**-**	**+**	**-**	**-**	**+**	**-**	M	**-**	**-**
ZINC13492195	**-**	**+**	**-**	**-**	**+**	**+**	M	**-**	**+**
ZINC90411518	**-**	**+**	**-**	**-**	**+**	**-**	M	**-**	**+**
ZINC90411521	**-**	**+**	**-**	**-**	**+**	**-**	M	**-**	**+**

CONCLUSION

Acetylcolinestrase increased activity is an important factor for Alzheimer's disease. In the present study two analogs of Rivastigmine. with ZINC ID, ZINC00004413 (-13.79 Kcal/mol) and ZINC97938 (-13.8 Kcal/mol) shows minimum Docking energy and positive ADME result. hence, they may be considered as a suitable drug for the treatment of Alzheimer's disease and can also be used for further studies.

CONFLICT OF INTERESTS

Authors declare that there is no conflict of interest.

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