ISSN 2231-0541

PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 4 | Issue 2 | March-April 2013 | Pages 270-278

Original Research Article

IN SILICO DESIGN AND ADMET PREDICTION OF RIVASTIGMINE ANALOGUES FOR TREATMENT OF ALZHEIMER'S DISEASE

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Received: 06-04-2013 Revised: 11-04-2013

Accepted: 19-04-2013 Available online: 01-05-2013

ABSTRACT

Alzheimer's disease (AD) is the most prevalent, complex neurodegenerative disorder of the central nervous system, affecting over 20 million individuals worldwide. AD is a dementia- related disease which is characterized by t-protein aggregation, amyloid-ß deposits, oxidative stress and lowered levels of acetylcholine in the brain. Currently a few class of drugs such as Acetylcholinesterase (AChE) inhibitors, antioxidants, metal chelators, monoamine oxidase inhibitors, anti-inflammatory drugs and NMDA inhibitors are available and at best they offer some relief of symptoms. Presently, AChE inhibitors like Rivastigmine are the most effective therapy for AD. In this study, we analyzed seven Rivastigmine analogues de novo for pharmacokinetic profile (adsorption, distribution, metabolism and excretion) and toxicity by using *Pre-ADMET* tool which can predict physico-chemical, drug absorption and drug-like properties. The results showed that two Rivastigmine analogues (R=OH, R=Br) which showed good binding affinities for Acetyl cholinesterase (PDB ID: 1B41) exhibited more or similar ADME and toxicity properties in comparison to Rivastigmine. These designed analogues have the potential to become new lead compounds that might guide the design of drugs with optimized pharmacodynamic and pharmacokinetic properties in order to improve the treatment of Alzheimer's disease by creating new pharmacotherapeutic options.

Key words: Rivastigmine, Acetylcholinesterase, Alzheimer's disease, Drug design

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive disease of the brain that is characterized by impairment of memory (dementia) and eventually by disturbances in reasoning, planning, language and perception. 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 [1]. The reaction of a patient with Alzheimer's disease to the illness and his or her capacity to cope with it also vary, and may depend on such factors as lifelong personality patterns and the nature and severity of stress in the immediate environment. Depression, severe uneasiness, paranoia, or delusions may accompany or result from the disease, but these conditions can often be improved by appropriate treatments [2, 3]. Although there is no cure for Alzheimer's disease, treatments are available to alleviate many of the symptoms that cause suffering. Two different classes of drugs namely, Cholinesterase inhibitors and partial glutamate antagonists neither class of drugs has been proven to slow the rate of progression of Alzheimer's disease. Four ChEIs have been approved by the FDA, but only Donepezil hydrochloride (Aricept), Rivastigmine (Exelon), and Galantamine (Razadyne - previously called Reminyl) are used by most physicians because the fourth drug, tacrine (Cognex) has more undesirable side effects than the other three. Most experts in Alzheimer's disease do not believe there is an important difference in the effectiveness of these three drugs. Several studies suggest that the progression of symptoms of patients on these drugs seems to plateau for six to 12 months, but inevitably progression then begins again [4]. Rivastigmine appears to be beneficial for people with mild to moderate Alzheimer's diseases [5, 6]. Rivastigmine is an effective therapeutic agent for treating cognitive and behavioral symptoms in Alzheimer disease [7].

Recently Structure Based Drug Design (SBDD) plays an important role in drug design and discovery. SBDD is the approach of finding new drugs based on their biological targets. Typically, a drug target is a key molecule involved in a particular metabolic or signalling pathway that is specific to a disease condition or pathology. This approach to SBDD optimizes the fit of a Ligand in receptor site. A promising tool for SBDD is virtual (in-silico) screening, where small molecules docked into a drug target and the binding affinities are estimated using simplified free energy calculation methods. However, optimum fit in a target site does not guarantee that the desired activity of the drug will be enhanced or that undesired side effects will be diminished. Moreover, this approach does not consider the pharmaco-kinetics of the drug [8]. Computer Aided Drug Design (CADD) methods and Bioinformatics tools offer significant benefits for drug designing programs like Cost Saving, Time-to-Market, the deep insight that researchers acquire about drug-receptor interactions, they often come up with new ideas on how to modify the drug compounds for improved fit [9]. The present study was carried out to investigate the pharmacokinetic (ADME) and toxicity of new Rivastigmine analogues in order to produce new lead molecule for the

treatment of Alzheimer's disease (AD) using virtual (in-silico) screening.

MATERIALS AND METHODS

Software's used:

The commercial software's like HyperChem Release 7.5, OPENEYE and *Pre*-ADMET were used for in-silico ADMET prediction of Rivastigmine and its seven analogues. HyperChem is a versatile molecular modeller and editor and a powerful computational package. It performs many actions like building and displaying molecules, optimizing the structures of molecules, investigating the reactivity of molecules and functional groups, generating and viewing orbital and electronic plots, evaluating chemical pathways, mechanisms studying the dynamic behavior of molecules and molecular and quantum mechanics calculations. OPENEYE software was utilized to identify the intermolecular energy of all molecules in both complex and solvated system. *Pre*-ADMET commercial software also used to predict the Pharmaco-kinetics and toxicity properties (ADMET) of drug candidates in silico.

Protein Identification and Preparation: The three dimensional structure of Acetylcholinestrase was obtained from the protein data bank (PDB ID - 1B41). The protein structure was subjected to a refinement protocol using HYPERCHEM in which the constraints on the enzyme were gradually removed. Figure 1. Acetyl cholinesterase is a serine hydrolase, which is responsible for regulation of nerve signal transmission. It rapidly cleaves acetylcholine and is one of the fastest enzymes known. It belongs to the enzyme classification Hydrolase/ Toxin. It has four chains, 4642 atoms and 4840 bonds.

Analogues of Rivastigmine: The structural analogs of Rivastigmine were developed with structural modifications in hydrophilic region of Rivastigmine with different substituents. The structures were built and the energy minimizations were done with HYPERCHEM 7.2 version software.

Identification and Optimization of Solvent: Solvation plays an important role in many aspects of medicinal chemistry in particular. For instance, most compounds of relevance for medicinal chemistry are flexible and very often have polar functional groups. As the most important solvent in medicinal chemistry is water, conformational properties in this highly polar solvent may be drastically different from the properties in vacuo and only calculations including the solvent may yield meaningful results. Calculations on various aspects of ligand–enzyme/receptor interactions and partitioning between phases require the consideration of solvation effects. Thus the accurate estimation of solvent effects is a key problem in computational medicinal chemistry.

The molecules were solvated and then it was optimized in four stages**.** It starts from hydrogen atoms, water molecules, part of the molecule, and then entire system was selected and optimized. In each time the optimization has done by two steps. Steepest descent, Conjugate Gradient (Polak ribiere) each stage was carried out using the previous stage output as its input. The optimized structure was used to calculate the intramolecular energy such as bond angle, bond length, and torsion angle of ligand in solvated system using HYPERCHEM. Intermolecular energy of ligand was

calculated in solvated system using OPENEYE.

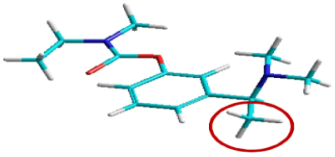
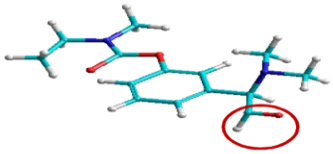
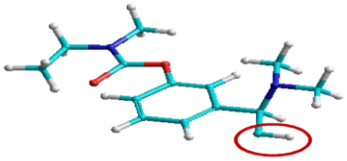
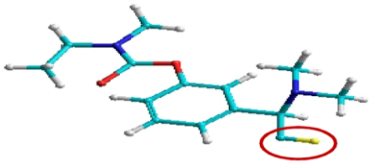
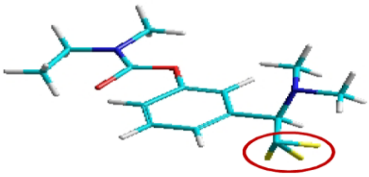
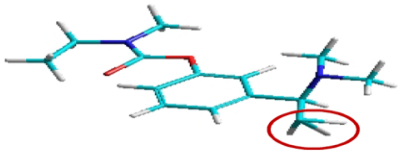
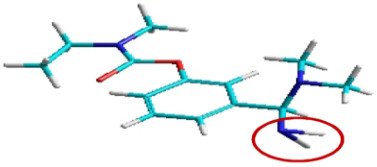
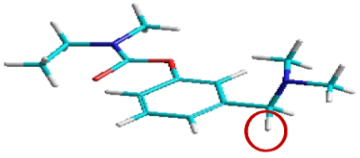
ADMET Prediction: To avoid the failure due to deficiencies in ADME or toxicological properties during the development, a set of *in vitro* ADME screening has been implemented in most pharmaceutical companies for predicting drug-likeness with the aim of discarding compounds in the discovery phase that are likely to fail further down the lane. Pre-ADMET is a program designed to predict or calculate many physical properties (non polar & polar surface area, water solubility, logP, etc.), the absorption properties of compounds, human Intestinal absorption (both passive and active), permeability for Caco-2 cell, MDCK cell, Blood-Brain-Barrier (BBB) penetration, skin permeability and plasma protein binding. Pre-ADMET is useful for high throughput screening and combinatorial chemistry library design considering the Lipinski's rule or lead-like rule, drug absorption and water solubility.

RESULTS AND DISCUSSION

Analogues of Rivastigmine: Rivastigmine was the drug selected and modified. Earlier study by Preeth *et al.* replaced the hydrophilic region on the target molecule with other created seven analogs of the drug functional groups (considered at random) [10]. The Rivastigmine and the seven analogs were studied by performing various energy, simulation as well as QSAR calculations. Rivastigmine has been considered along with the seven analogues for various studies as a kind of "Blank", so as to enable the comparative study of the analogs and to help us analyze relative superiority of the analogs to the initial drug itself. The Rivastigmine and analogs 2D structure was converted into 3D form by clicking. The Rivastigmine and the seven analogs listed in **Table No 1.**

Identification and Optimization of Solvent: First Rivastigmine has selected and it was optimized gradually like, hydrogen, H_2O, part of the molecule and entire molecule. Here we are solvating the Rivastigmine, if we are considering only the docking energy of molecule, solvent may also interact and interfere with ADMET prediction. In order to reduce that interaction, we solvated the Rivastigmine molecules first. Finally we optimized the entire solvated system using OPENEYE. Also all the Rivastigmine analogues were processed in same way for solvation process. Solvated molecules **Figure 1** has given below.

Table.1.Rivastigmine and its analogues with their minimized structures

S.NO	Ligand	R- Group	
1	Rivastigmine		CH3
2	Mol- I		OH
3	Mol - II		Cl
4	Mol- III		Br
5	Mol- IV		CF_3
6	Mol- V		CCl_3
7	Mol- VI		NH_2
8	Mol- VII		H

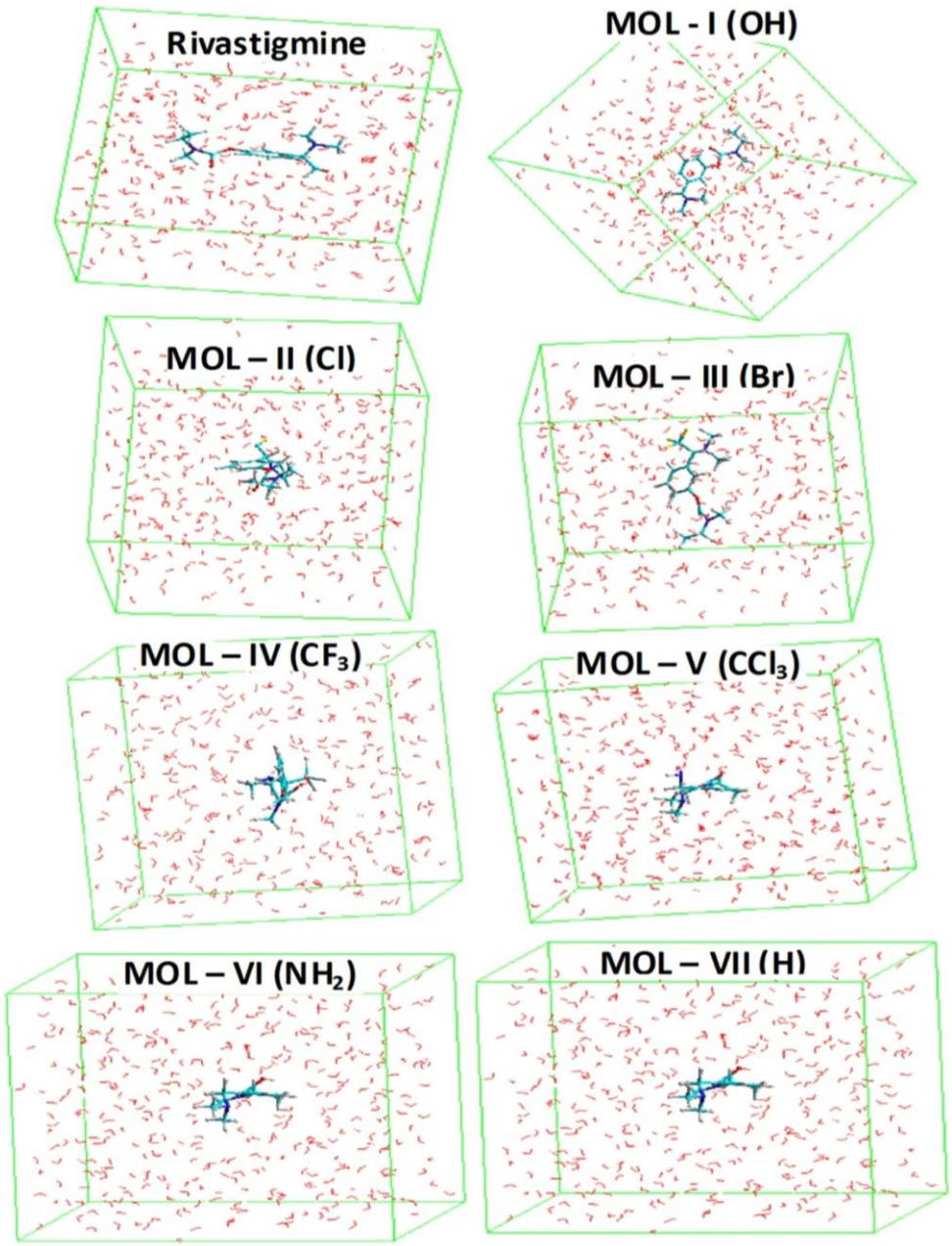


Fig.1.Energy optimization of solvated Rivastigmine and its seven analogues

Protein Active site and Analogue selection: Investigation of the active site of AChE allows the identification of one peripheral anionic site (PAS) and at least five major binding sites: the oxyanion hole (OH), the esteratic site (ES), the anionic substrate binding site (AS), the active site-selective aromatic binding site (AACS), and the acyl binding site (ACS) [11,12]. These studies show that these inhibitors of AChE bind to the active site or to the peripheral anionic site (PAS), an allosteric site located at the active site center gorge

entrance, or they span the two sites there by occupy much of the active center gorge. Mutagenesis and structural studies have revealed the functional role of the residues Tyr72, Asp74, Tyr124, Trp286 and Tyr341 at the PAS [13-16]. The three dimensional structure of Acetylcholinestrase complexed with Rivastigmine (PDB ID: 1GQR), its binding pocket with a molecule of Rivastigmine and active site of AChE was shown in **Figure 2**.

According to earlier study, out of seven Rivastigmine analogues only two analogues; MOL- I (R=OH) and MOL-III (R=Br) showed good binding energies, low relative free binding energy and high affinities towards AChE in comparison to Rivastigmine [10]. So, these two Rivastigmine analogues were selected for further ADMET prediction studies.

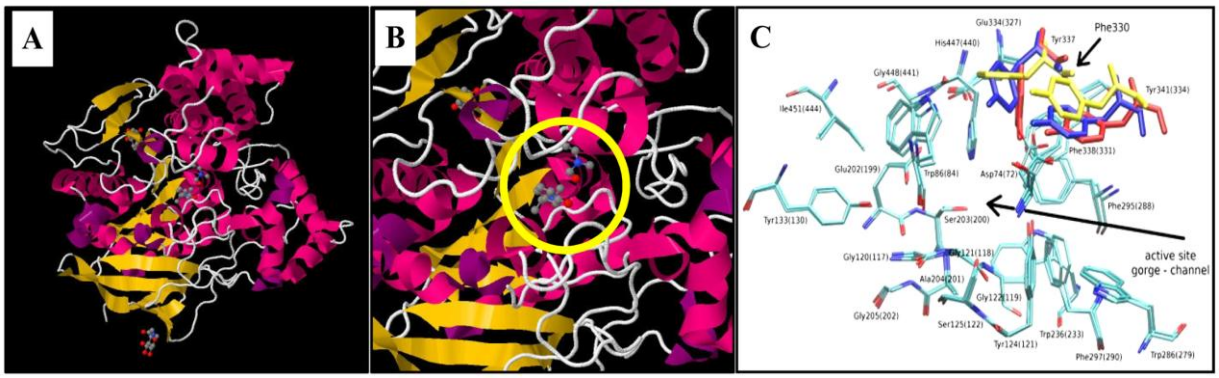


Fig.2.Binding of Acetyl cholinesterase (AChE) with Rivastigmine and its active site. A) Crystal structure of Acetyl cholinesterase complexed with Rivastigmine (PDB ID- 1GQR) B) Rivastigmine molecule fitted in binding pocket of AChE. C) Detailed view of the active site after alignment of Acetylcholinesterase from *Homo sapiens, Musculus and Torpedo California*.

ADMET Prediction:

A significant bottleneck remains in the drug discovery procedure, in particular in the later stages of lead discovery, is analysis of the ADME and overt toxicity properties of drug candidates. Over 50% of the candidates failed due to ADME/Tox 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. Pre-ADME is a program designed to predict physico-chemical, drug absorption and drug-like properties. It calculates many physical properties and some mathematical descriptions, descriptors, for example, non polar & polar surface area, water solubility, logP, etc... The absorption properties of compounds, Caco-2 cell, MDCK cell, Blood-Brain-Barrier (BBB) penetration, and human Intestinal absorption (both passive and active) can be predicted by Pre-ADME. 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. The Pharmaco Kinetics (ADME) and toxicity properties of Rivastigmine, Molecule I and III were predicted using Pre-ADMET commercial software and results were shown in **Table 2** and **Table 3** respectively. Here the absorption and distribution were similar like Rivastigmine. Molecule I have shown positive results for carcinogenicity in rats like R*ivastigmine* and but molecule III shown the out of range carcinogenicity.

Table.2.Predication of Absorption and Distribution of Rivastigmine, Mol-I and Mol-III

Molecule	ADME	Parameters	Values
Rivastigmine	Absorption (4 Items)	Human intestinal absorption (HIA, %)	100.000000
		Invitro Caco-2 cell permeability (nm/sec)	22.2027*
		Invitro MDCK cell permeability(nm/sec)	0.0434155*
		Invitro skin permeability (logKp, cm/hr)	-0.721767*
	Distribution (2 Items)	*Invitro* plasma protein binding (%)	100.000000
		Invivo blood-brain barrier penetration (C.brain/C.blood)	28.1352*
MOL- I (R=OH)	Absorption (4 Items)	Human intestinal absorption (HIA, %)	100.000000
		In vitro Caco-2 cell permeability (nm/sec)	22.2027*
		Invitro MDCK cell permeability(nm/sec)	0.0434531*
		Invitro skin permeability (logKp, cm/hr)	-0.719455*
	Distribution (2 Items)	*Invitro* plasma protein binding (%)	100.000000
		Invivo blood-brain barrier penetration (C.brain/C.blood)	28.3904*
MOL-III (R=Br)	Absorption (4 Items)	Human intestinal absorption (HIA, %)	100.000000
		In vitro Caco-2 cell permeability(nm/sec)	58.1936**
		Invitro MDCK cell permeability(nm/sec)	0.0434155*
		Invitro skin permeability (logKp, cm/hr)	-0.795268**
	Distribution (2 Items)	*Invitro* plasma protein binding (%)	97.448797**
		Invivo blood-brain barrier penetration (C.brain/C.blood)	22.1507**

Table.3.Predication of Toxicity of Rivastigmine, Mol-I and Mol-III

Molecule	Toxicity	Parameters	Values
Rivastigmine	Ames test(7 Items)	Ames TA100 (+S9)	Negative
		Ames TA100 (-S9)	negative
		Ames TA1535 (+S9)	negative
		Ames TA1535 (-S9)	negative
		Ames TA98 (+S9)	negative
		Ames TA98 (-S9)	negative
		Ames test	non-mutagen
	Carcinogenicity	Carcinogenicity (Mouse)	Negative
		Carcinogenicity (Rat)	positive
MOL-I (R=OH)	Ames test (7 Items)	Ames TA100 (+S9)	Negative
		Ames TA100 (-S9)	negative
		Ames TA1535 (+S9)	negative
		Ames TA1535 (-S9)	negative
		Ames TA98 (+S9)	negative
		Ames TA98 (-S9)	negative
		Ames test	Non- mutagen
	Carcinogenicity	Carcinogenicity (Mouse)	Negative
		Carcinogenicity (Rat)	positive
MOL-III (R=Br)	Ames test (7 Items)	Ames TA100 (+S9)	Negative
		Ames TA100 (-S9)	negative
		Ames TA1535 (+S9)	negative
		Ames TA1535 (-S9)	negative
		Ames TA98 (+S9)	negative
		Ames TA98 (-S9)	negative
		Ames test	non-mutagen
	Carcinogenicity	Carcinogenicity (Mouse)	Out of range
		Carcinogenicity (Rat)	Out of range

These results has clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis.

Rivastigmine have 27.50. Binding energy, for

newly designed molecules I and III have a similar binding energy, followed by we calculated the relative free binding energy for all molecule in that also molecules I and III have a less relative free binding energy. Therefore, we are suggesting that molecule I and III may have a biological activity like *Rivastigmine* or more than *Rivastigmine*.

In Second stage of our study is ADMET prediction. Molecule I (OH) have shown positive result in carcinogenicity in rats like rivastigmine, but molecule III (Br) showed out of range result in carcinogenicity. The results obtained so far will be useful for development of novel second generation AChE inhibitor candidates structurally related to the known standard drugs. Here we are suggesting for the further depth toxicity prediction like hepato toxicity, cardiotoxicity, and nephrotoxicity. Also, synthesis in wet lab and NMR, IR studies with these molecules may result in future leads for effective clinical use in Alzheimer's disease.

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