Original Research Article

AChE inhibition study for Alzheimer's disease treatment: An *In-silico* study

Abstract

Alzheimer's disease (AD) is the most common form of dementia associated with plaques and tangles in the brain. Several acetylcholinesterase inhibitors have been clinically used to delay or halt the progression of the disease. Solanadine (Snd) and gamma solamargine (Gsm) have been shown to inhibit acetylcholinesterase (AChE). The current study attempts to describe the molecular interactions between human brain AChE and inhibitors Snd and Gsm. The free energy of binding and estimated dissociation constant (Ki) for the 'Snd-AChE Catalytic Anionic site (CAS)-interaction' were determined to be –9.51 *kcal/mol* and 107.54 *nM*, respectively and the free energy of binding and estimated *Ki* for the 'Gsm -AChE CAS-interaction' were determined to be – 8.64 kcal/mol and 463.88 *nM*, respectively. Hydrophobic interactions, polar interactions, and hydrogen bonding play an important role in the correct positioning of Snd within the 'catalytic site' of AChE to permit docking, while hydrophobic interactions and polar interactions play a significant role in the correct positioning of Gsm within the 'catalytic site' of AChE to permit docking. It is hoped that the information provided in this study will help in the design of AChE-inhibitors as anti-Alzheimer agents.

Keywords: AChE; AutoDock Tools; Interactions; Binding energy.

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Introduction

Alzheimer's disease (AD) is classified as a neurodegenerative disorder (ND) of the brain that is characterized by dementia, brain atrophy, deposition of hyperphosphorylated tau protein and amyloid-beta (Aβ) peptide in the brain [1-3]. In AD, synaptic disturbance and decline in the capacity to recall current incidents, reasoning skills and actions have deteriorated [4-5]. The progression of AD can be divided into three stages. At the first stage, the patient has difficulty in thinking clearly, presenting a consequent decrease in performance in composite tasks. At the second stage, aphasia is evident, i.e. lack of ability to name the objects or to choose the right words to express an idea. In the third stage, there are prominent changes in the psychotic symptoms, and in the competency to walk, talk, and self-care [6].

Acetylcholinesterase (AChE) is a first neurotransmitter [7]. A large number of functions in diseases of high clinical significance including cancer and AD have been identified with AChE [8]. AChE is implicated in the abolition of quick hydrolysis of acetylcholine (ACh) impulse transmission in cholinergic pathways in the central and peripheral nervous systems [9]. As a result, most drug therapies are based on the cholinergic hypothesis, many inhibitors plays an essential role in the treatment of AD, in which AChE has greater capacity in the treatment process [10]. Medicinal plants are looking to play an important role in the treatment of diseases, especially in psychiatric and ND therapy. [11]. Therefore, natural products could be used in the management of ND [12].

AChE is usually located at neuromuscular junctions and cholinergic brain synapses. It plays an important role in the termination of impulse transmission at neurological synapses by rapid hydrolysis of the ACh to acetate and choline [13, 14]. AChE possesses a high 'turnover rate' of $2.5x10^4$ ACh molecules per second [15]. The patients with AD lose numerous forebrain cholinergic neurons as the disease progresses [16]. Computer-aided drug design (CADD) has emerged as an influential tool that plays a key role in the creation of new drug molecules [17].

Solanidine is a steroidal glycoalkaloid of potato (*Solanum tuberosum L.)*. It is an important precursor for some pharmacologically active compounds and for the synthesis of hormones [18]. Solanidine is responsible for the prevention of neuromuscular syndromes by cholinesterase [19-21]. The

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sugar component of these glycoalkaloids is hydrolyzed in the bloodstream, leaving the component of solanidine [22]. Solanidine exists in the blood serum of normal healthy individuals who consume potatoes, and the levels of solanidine decrease markedly as soon as the intake of potatoes stops [23]. Gamma-Solamargine is a type of steroid glycoalkaloid that is a type of nitrogenous secondary metabolite formed in the plant of the Solanaceae family [24].

The present study was accomplished to evaluate the ability of solanidine and gamma -solamargine to inhibit AChE by a molecular docking method with the aim of finding a potential therapeutic approach for the management of AD. Following the above hypothesis in the present study, we have analyzed that the selected compounds have potential to inhibit the AChE and could be promoted for the management of AD.

Materials and Methods

Preparation of enzyme structure

AChE's 3D structure was obtained from the Protein Data Bank (PDB ID: 3LII) (www.rcsb.org) to be used in the docking analysis. Heteroatoms and water were removed, and clean PDB was prepared for further study of the docking. The PDB structure is shown in figure 1.

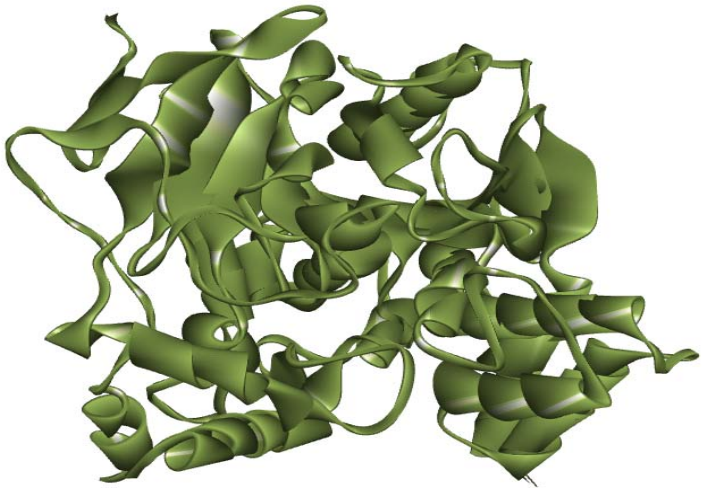

Figure 1: The 3-D structure of recombinant human acetylcholinesterase enzyme

Ligand Preparation

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The inhibitors SMILES notations were collected from the PubChem database. The 3-D structures were built using CORINA (http://www.molecular-networks.com/products/corina) for generating 3D structure of Snd and Gsm. The structures of the ligands are shown in figure 2.

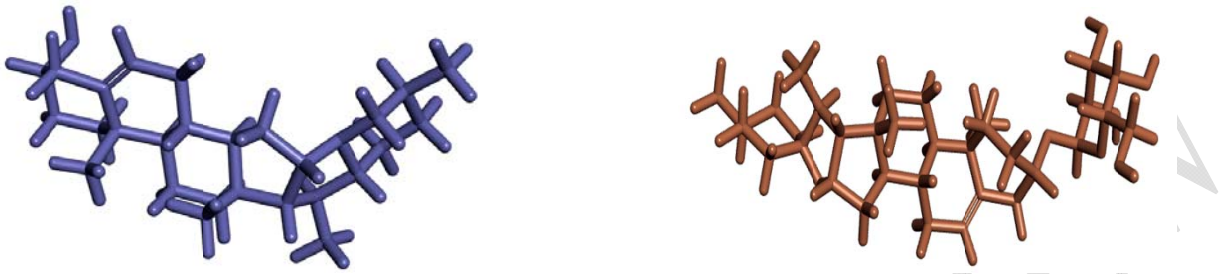

Figure 2: Chemical structure of Solanidine and Gamma-solamargine

Molecular interaction analysis

Thereafter, both the ligands were docked with AChE using 'Autodock4.2' software. Polar hydrogen atoms were included, and rotatable bonds were defined. Affinity (grid) of 60×60×60 Å were produced using the Autogrid tool to target grid coordinates close to the AChE catalytic site. The x, y, and z values used in docking calculations to target the catalytic site were 90.81, 83.98, and -8.04 for AChE, respectively [16]. Docking simulation has been conducted using the default parameters. With the aid of Discovery Studio Visualizer, the final figures were made [25].

SwissADME

The drug-likeness properties and pharmacokinetic profile of the ligand molecule were checked by SwissADME [24].

Results and Discussion

The structure of AChE could be contrasted with two hemispheres that compose the catalytic center between the omega and acyl loops. These loops serve as an active site with a dimension of approximately 300 $Å^3$. It is revealing that the ligands and protein were held flexible by the docking [15]. A significant feature of the oral drug design is human intestinal absorption (HIA), which tests the proportion of medications that can be consumed by the human body. HIA should be high enough for the effectiveness of medications. It depends on the molecular weight, the number of HB donors, the number of HB acceptors. HIA was calculated by the SwissADME prediction software, we calculated HIA for Snd and Gsm. This

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value of HIA for the compounds Snd and Gsm were 99.883852 and 82.727544 respectively. Among these selected compounds Snd has the ability to cross the blood-brain barrier [26].

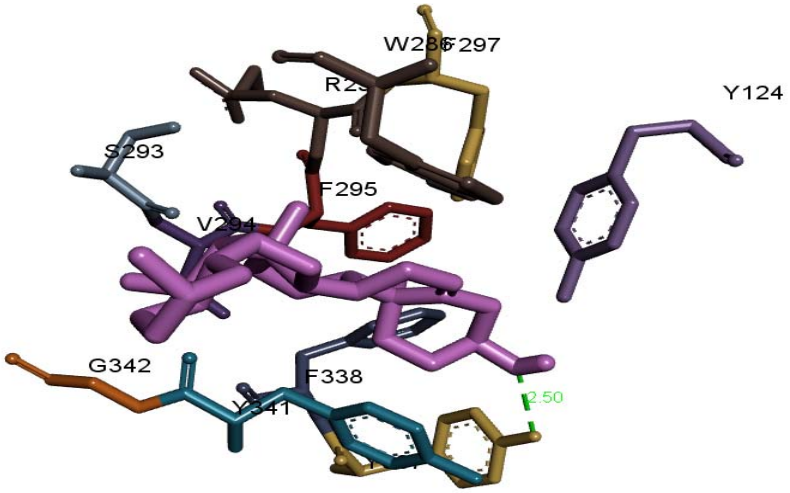

Figure 3: Interaction of solanidine docked to the "catalytic site'' of the AChE. The ligand has been shown in 'stick'(purple color) representation. The hydrogen bond is shown by green dotted lines.

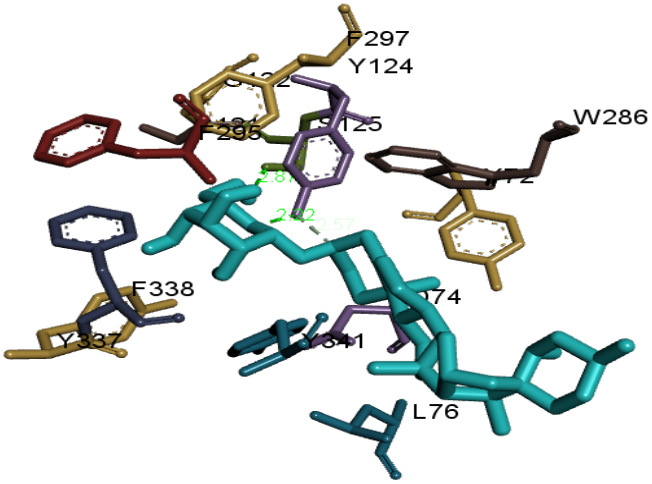

Figure 4: Interaction of Gamma-solamargine docked to the "catalytic site'' of the AChE. The ligand (Gsm) has been shown in 'stick'(sky blue color) representation. Hydrogen bonds are shown by green dotted lines.

The human brain AChE, CAS site was found to interact with Snd by 11 amino acid residues, namely, Y337, F338, G342, Y341, V294, S293, R296, F297, Y124, W286, and F295 (Figure **3**, Table **1**), and with

Gsm through 17 amino acid residues, namely F295, F297, W286, G122, Y124, S203, G121, G120, Y72, S125, G126, E202, D74, W86, Y337, H447 and L76 (Figure **4**, Table **1**).

Table 1: Interacting amino acid of AChE with inhibitors

Target	Compound	Binding Energy (kcal/mol)	Ki(nM)	Interacting amino acid	H- Bond Interaction	Hydrogen Bond length (Å)
AChE	Solanidine	-9.51	107.54	Y337, F338, G342, Y341, V294, S293, R296, F297, Y124, W286 and F295	TYR337:OH - UNK1:O26	2.50
	Gamma-solamargine	-8.64	463.88	F295, F297, W286, G122, Y124, S203, G121, G120, Y72, S125, G126, E202, D74, W86, Y337, H447 and L76.	TYR124:OH - UNK1:O25 TYR124:OH - UNK1:O31	2.22 2.87

We measure the free binding energy for the protein-ligand complex of the 3D structure. The role takes into account, for example, H-bonds, ion interactions, protein-ligand touch surface, and the number of rotatable bonds in the ligand for complex characterization [27]. The free energy of binding and estimated *Ki* for the 'Snd-AChE CAS-interaction' were determined to be –9.51 *kcal/mol* and 107.54 *nM*, respectively, and the free energy of binding and estimated *Ki* for the 'Gsm-AChE CAS-interaction' were determined to be –8.64 *kcal/mol* and 463.88 *nM*, respectively. Y337 is involved in the H-bonding interaction, in which OH of Y337 binds with O26 of Snd. Y124, W286, S293, V294, F295 G342 are interacting through hydrophobic interaction. In the interacted complex, OH is acting as a donor atom while O26 is working as an acceptor atom. The H-bond distance in the complex is 2.50 Å. Two atoms of TYR124 are involved in the interaction of H-bonding. OH group of TYR124 binds with O25 and O31 of Gsm, respectively. Y72, L76, D74, G120, G121, G122, and G126 are interacting through hydrophobic interaction. Both the OH of TYR124 acting as donor atoms while O25 and O31 are acting as an acceptor atom in the Gsm complex. The hydrophobic interaction helps to elucidate the potency of the compounds

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to inhibit the enzyme [28]. The distance of both the H-bonds are 2.22 and 2.87 Å respectively. The amino acid residues of target Y337, F297, Y124, W286 and F295 were commonly interacting with Snd and Gsm ligands respectively.

It was found that Snd and Gsm can inhibit AChE. Figures (3 and 4) show Snd and Gsm docked to the 'CAS' sites of the human brain AChE enzyme. In table 1, most residues are known to be highly conserved and have been assigned functional roles. Higher (negative) free binding energy is a measure of successful activity between the enzyme and the inhibitor [29]. The free binding energy for complexes was found to be-9.51 kcal /mol and-8.64 kcal/mol, respectively, meaning that Snd and Gsm are successful inhibitors of human brain AChE. The present research is supposed to assist in the potential creation of more complex pharmacological compounds. It is worth observing that the free energy ΔG and K*i* values obtained can only indicate binding efficiency for the enzyme-ligand pair. The present research is aimed to serve as the basis for possible AD therapy.

Conclusions

This study investigates chemical associations between AChE and ligands (Snd and Gsm). Hydrophobic interactions, polar interactions and H-bonding play an essential role in the correct placing of Snd within the 'catalytic site' of AChE to allow docking, whereas only hydrophobic interactions and polar interactions play a major role in the correct positioning of Gsm within the 'catalytic site' of AChE to allow docking. This data confirms that both the ligands are effective inhibitors of AChE based on their *Ki* and ΔG values.

Disclaimer regarding Consent and Ethical Approval:

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

List of Abbreviations

AChE: Acetylcholinesterase
ACh: Acetylcholine
ND: Neurodegenerative disorder
CAS: Catalytic Anionic site

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Snd: Solanidine
Gsm: Gamma-solamargine
AD: Alzheimer disease

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