4.1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disease involving impairment of cognitive function with both genetic and nongenetic causes, which is described by lost basal forebrain cholinergic neurons and diminished level of neurotransmitter acetylcholine (ACh) in hippocampal and cortical levels, prompting extreme memory and learning deficits (Butters et al., 1995). The overall incidence of AD is assessed at 35 million, a number anticipated that would quadruple by 2050, because of the expanding life expectancy of the total population (Brookmeyer et al., 2007). With an unfavorable prognosis and a life expectancy of roughly 8–10 years, AD is becoming one of the most costly diseases for society (Thies and Bleiler, 2013). Acetylcholinesterase (EC 3.1.1.7) is an enzyme that belongs to the superfamily of α/β-hydrolase fold proteins (Valle et al., 2008). AD is caused by a progressive and specific degeneration of neurons, with extracellular deposition of β-amyloid plaques, intracellular deposition of neuro fibrillary tangles, which lead to neurotoxicity and synaptic loss being hallmarks of the disease. Different research effort has been made to understand the molecular pathogenesis of AD from the most recent couple of decades. Inhibition of AChE enzyme is one of the major therapeutic strategies used for symptomatic treatment of AD (Greenblatt et al., 2003). It is the first adopted approach for the treatment of AD. This hypothesis is turned out to be fruitful today by the powerful utilization of cholinesterase inhibitors, for example, donepezil to enlarge surviving cholinergic action for the treatment of mild to moderate AD. This enzyme is responsible for the termination of cholinergic transmission, that is, the enzymatic breakdown of ACh (Radic et al., 1997). It is known as one of the fastest chemicals of our body (Nair et al., 1994), degrading, as a tetramer, around 25 000 particles of ACh every second. Right now five biomarkers for AD have been utilized for assessment of disease
Vincamine is a peripheral vasodilator that builds blood stream to the brain. Vincamine is a monoterpenoid indole alkaloid found in the leaves of Vinca minor (Indole Alkaloids, 1985). Vincamine is alkaloids known for their neuroprotective traits, improvement of cerebrovascular blood stream and antitumor impact of their subordinates (Fandy et al., 2016). Vincamine is generally utilized as a part of human medication to increase the regional blood flow in patients suffering from acute or subchronic cerebral ischemia. In present work, enzyme inhibition studies with the assistance of in silico molecular docking have been embraced as an endeavor to investigate the capacity of vincamine to go about as strong inhibitor of AChE and to elucidate the possible mechanism of action. Furthermore, the implications of conceivable hindrance by a compound may help in the improvement of new medications that display the counter Alzheimer's action.

4.2 Materials and methods

4.2.1 Protein preparation

The X-ray diffraction structure of AChE (PDB ID: 3LII) protein (Fig.4.1) was obtained from the Protein Data Bank at a resolution of 3.2Å (http://www.rcsb.org/pdb/home/home.do). Water molecules, ligands and other hetero atoms were removed from the protein molecule along with the chain B.

![Figure 4.1 3D structure of target protein](image)
4.2.2 Ligand preparation

The chemical structures of the selected ligand molecules Vincamine (Fig 4.2) was obtained from PubChem data base (http://pubchem.ncbi.nlm.nih.gov). The ligand was saved in PDB format.

![2D structure of ligand Vincamine.](image)

**Figure 4.2** 2D structure of ligand Vincamine.

4.2.3 Molecular properties and drug likeness

Drug likeness is a key consideration when selecting compounds during the early stages of drug discovery (Bickerton et al., 2012). The molecules which are having the hydrogen donors $\leq 5$, hydrogen bond acceptor $\leq 10$, molecular mass $\leq 500$ daltons, and $\log P \leq 5$ are likely to obey Lipinski's rule. The molecular property was calculated with the help of Molsoft software (http://molsoft.com/mprop/). According to this prediction, the values of hydrogen bond donor and hydrogen bond acceptor were 1 and 4 respectively while the molecular mass and $\log P$ of the compounds were 354.19 and 3.36 respectively.

4.2.4 Molecular docking and visualization

Docking studies with inhibitory compounds against acetylcholinesterase were done by AutoDock. Protein and ligands were analyzed and adjusted for docking reason by utilizing AutoDock Tools which is incorporated in the MGL devices (http://mgltools.scripps.edu). The examination of the binding conformation of protein-ligand complex were performed utilizing
a scoring capacity in view of the free energy of binding (Huey et al., 2007). Polar hydrogen atoms were incorporated, and rotatable bonds were characterized. Docking was performed on the protein model. Fundamental hydrogen atoms, Kollman united atoms type charges, and solvation parameters were included with the guide of AutoDock tool. Affinity (grid) maps of 40×40×40 Å matrix were created utilizing the Autogrid program expected to target lattice co-ordinates with the catalytic site of AChE. 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 (Shaikh et al., 2014). AutoDock parameter set and the separation dependent dielectric functions were utilized in calculation of the Vander Waals and the electrostatic terms, separately. Docking simulation was performed utilizing the ‘Lamarckian genetic algorithm’. Each docking test was derived from 10 unique runs that were set to end after a greatest of 2,500,000 energy assessments. After total execution of AutoDock ten conformations of ligand in complex with the receptor were obtained, which were at last positioned on the premise of interaction energies. The subsequent adaptations were visualized by discovery studio visualizer.

4.3 Results and Discussion

4.3.1 Blood–brain barrier

The Blood–brain barrier (BBB) is a physical obstruction in the circulatory framework that ligands must cross to go into the central nervous system (Garg & Verma, 2006). AD drug candidate requirement to pass this barrier. The crossing capacity through the BBB is measured by the logarithm base 10 of the proportion of the compound concentration in the brain, \( C_{\text{brain}} \) to that in the blood \( C_{\text{blood}} \). BB is likely related to local hydrophobicity, molecular size, lipophilicity and molecular flexibility (Crivori et al., 1998). In this study,
BBB is computed using the PreADMET prediction software (Clark, 1999). The efficient drug should be able to cross the BBB to interfere their activity. Using the PreADMET prediction method, we have calculated the values of BBB of the compounds vincamine was 0.871568.

4.3.2 Human intestinal absorption

Human intestinal absorption (HIA) is the another critical part of the oral medication plan (Wessel et al., 1998) which measures drug percentage that can be absorbed by the human body. HIA should be high enough for drug efficacy. The HIA value of vincamine was obtained 95.95%.

AD is the most common type of dementia. In the absence of a current cure, effective therapeutic strategies are still needed. Since available drugs are not efficient to treat AD, the searches for new lead compounds are of great interest (Son & Mai, 2013).

4.3.3 Molecular Interaction Study

The present study reveals the inhibitor property of a natural compound vincamine against human AChE. Since, human AChE is very well known for its role in AD where its inhibition is one of the strategies to cure AD. Docking has turned out to be one of the essential strategies for improving the productivity in lead compound improvement. This method has been found very effective in digging most suited and highly potent compound which can interact with protein with maximum binding energy. The advantage of docking is to recognize the binding site and mode of interaction of ligand with proteins to determine the best preferred binding between protein and ligands (Klebe, 2006). The interaction studies were performed by using verified 3D structure (crystal structure) of AChE at predetermined region with vincamine ligand (Oprea and Matter, 2004; Mizutani and Itai, 2004). It is worth specifying that the "ligand" and "protein" were held adaptable by the docking programming.
through the study. The CAS site of human brain AChE was identified to interact with vincamine through 18 amino acid residues, specifically Gln71, Tyr72, Asp74, Trp86, Asn87, Gly120, Gly121, Gly122, Tyr124, Ser125, Gly126, Tyr133, Glu202, Ser203, Phe297, Tyr337, Phe338 and His447 (Fig 4.3). The free vitality of binding and evaluated Ki for the 'Vincamine - AChE CAS-interaction' was determined to be - 10.77 kcal/mol and 31.91nM respectively. Vincamine was seen to be engaged with four hydrogen bonding as UNK0:H45 - TYR124:OH, TYR124:OH - UNK0:O1, TYR124:OH - UNK0:O2 and TYR337:OH - UNK0:O1 at the active site of AChE. In a current report the – OH of Tyr124 likewise demonstrated a hydrogen bond. The CAS containing Ser-His-Glu catalytic triad, has a unique orientation along the active-site gorge, extending from the CAS, at the base close Trp86. (Jiansong et al., 2014).

Figure 4.3 Interaction of Vincamine docked to acetylcholinesterase (AChE). The ligand Vincamine has been shown in ‘green color stick’ representation.
Two carbon atoms of vincamine, namely C11 and C12 were found to be involved in hydrophobic interactions with CE2 and CZ of amino acid residues Tyr124 and Tyr72 of the enzyme respectively. C18 of vincamine was observed in pi-pi interactions with CE2 and CZ of the amino acid residue Tyr72. Van der Waals, Hydrogen Bond and Desolvation energy components for vincamine interaction with AChE were found to be -11.22 kcal/mol, while the Electrostatic energy component was found to be -0.74 Kcal/mol. Total interacting surface area for vincamine-AChE complex was found to be 890.952 Å². It is seen that an ideal AChE inhibitor should bind to the catalytic sites which could disturb the interactions between the enzyme and the Aβ peptide, and hence, slowdown the progression of the disease (Bartolini et al., 2003). Further insight into the nature and number of bonds formed revealed that hydrogen bonds, hydrophobic interactions and pi-pi play an important role in vincamine–AChE interaction.

Substrate Acetylcholine iodide (AChI) was docked into the same site of AChE. It was observed that the amino acid residues, Trp86, Gly120, Gly121, Gly122, Tyr133, Glu202, Ser203, Phe295, Phe297, His447, Gly448 and Ile451 played a major role in the binding of AChI. Interestingly, amino acid residues, Trp86, Gly120, Gly121, Gly122, Tyr133, Glu202, Ser203, Phe297 and His447 of AChE were also found to be involved in the interaction with Vincamine. The free energy of binding and estimated Ki for the ‘AChI -AChE CAS-interaction’ was determined to be -3.94 kcal/mol and 1.3mM respectively. ‘Van der Waals’, ‘Hydrogen Bond’ and ‘Desolvation’ energy components for AChI interaction with AChE were -4.23 kcal/mol, while the ‘Electrostatic’ energy component was found to be -0.03kcal/mol. Total interacting surface area for AChI-AChE complex was found to be 652.511 Å². For the better understanding as to how vincamine and the substrate influence the
binding mode with AChE, the free energy of binding of two complexes were examined. The values of free energy of binding (ΔG) for ‘substrate–AChE’ and ‘vincamine–AChE’ interaction were found to be -3.94 kcal/mol and -10.77 kcal/mol respectively. This approves the competitive nature of binding of vincamine to the active site of AChE. It is appropriate to mention that ΔG value obtained through computational analysis can only suggest efficiency of binding for an enzyme-ligand complex (Aftab et al., 2014). The selected natural compound vincamine has the highest drug likeness score 1.18 in comparison to FDA approved drug tacrine which has drug likeness value 0.97. The overall drug likeliness score for drug molecules constitute a distribution that is skewed to right and peaks in the range of 0.8 to 1.2 in (Rezaul Islam et al., 2013) fig 4.4.

![Graph representing the Drug-likeness model score](image.png)

**Figure 4.4** Graph representing the Drug-likeness model score

It has been reported that hydrogen bonds formed between compound and protein usually contribute to the stability of the protein-ligand complexes, and a large number of hydrogen bonds form more stable complex (Steiner & Koellner, 2001; Weiss et al., 2001). Thus by
considering the above obtained results and observations we can expect the vincamine as potent AChE inhibitor which have the power to act as anti-Alzheimer’s agents.

4.4 Conclusion

In this study, several natural compounds were tested against AChE to fetch best natural inhibitor by using computational approaches like docking. Finally vincamine was selected based on several adopted parameters during computational studies. Further the free energy of binding during docking for ‘AChI–AChE’ and ‘vincamine–AChE’ interaction was found to be -3.94 kcal/mol and -10.77 kcal/mol respectively. Vincamine and substrate formed the complex with the AChE enzyme at the same locus. Interestingly, amino acid residues, Trp86, Gly120, Gly121, Gly122, Tyr133, Glu202, Ser203, Phe297 and His447 of AChE enzyme were found common to its interaction with the two studied ligands i.e. vincamine and AChI. These discoveries partly explain the useful impacts of vincamine against AD and provide a basis for the designing and development of AChE inhibitor. The computational investigation could serve as an important step towards the development of more potent AChE inhibitor for the treatment of AD.