A DISSERTATION ON

COMPUTER-AIDED DRUG DESIGN OF β-SECRETASE INHIBITORS FOR THE DISCOVERY OF NOVEL ALZHEIMER'S THERAPEUTICS

SUBMITTED TO

THE DEPARTMENT OF BIOSCIECES,

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT FOR THE

DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY

BY
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DECLARATION

I hereby declare that the present work on **"Computer-Aided Drug Design Of β-Secretase Inhibitors For The Discovery Of Novel Alzheimer's Therapeutics"** is a record of original work done by me under guidance of Dr. Mohammad Hayatul Islam, Assistant Professor, Department of Biosciences, Integral University,Lucknow during Feb, 2022 to June, 2022. I also declare not part of this thesis has previously been submitted to my Institution or any examining body for acquiring any diploma or degree.

***Place*:**

Date: **Ayesha Fatima**

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TO WHOM IT MAY CONCERN

This is to certify that Ms. Ayesha Fatima, a student of M.Sc. Microbiology (II Year, IV semester), Integral University has completed her four months dissertation work entitled **"Computer-Aided Drug Design Of β-Secretase Inhibitors For The Discovery Of Novel Alzheimer's Therapeutics "** successfully. She has completed this work from Department of Biosciences, Integral University, under the guidance of **Dr. Mohammad Hayatul Islam**

The dissertation was a compulsory part of her M.Sc. degree. I wish her good luck and bright future.

(Dr. Mir Snober Shabnam)

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by Ms. Ayesha Fatima during the four months training from February –June 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by candidate himself. The thesis entitled is **"Computer-Aided Drug Design Of β-Secretase Inhibitors For The Discovery Of Novel Alzheimer's Therapeutics"** Therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology, Department of Biosciences, Integral University, Lucknow (U.P)

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Ayesha Fatima

TABLE OF CONTENTS

1.	**Introduction**
2.	**Review of literature**
3.	**Materials and methods**
4.	**Results and Discussion**
5.	**Conclusion**
6.	**References**

INTRODUCTION

Neurodegenerative disease is an umbrella term for a range of conditions which primarily affect the neurons in the human brain. Neurodegenerative diseases represent a major threat to human health. These age-dependent disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years.

Neurodegenerative diseases are incurable and debilitating conditions that result in progressive degeneration and / or death of nerve cells. This causes problems with movement (called ataxias), mental functioning (called dementias) and affect a person's ability to move, speak and breathe (Gitler AD *et al*., 2020).

Examples of neurodegenerative diseases are:

- Alzheimer's disease (AD) and other dementias
- Parkinson's disease (PD) and Parkinsonism
- Prion disease
- Motor neurone diseases (MND)
- Huntington's disease (HD)
- Spinocerebellar ataxia (SCA)
- Spinal muscular atrophy (SMA).

As you are aware, Neurodegenerative disorders like Alzheimer's disease (AD) is an emergent condition worldwide over the last decade. Alzheimer's can be defined as a gradually progressive neurodegenerative disease characterized of memory impairment and subsequent disturbances in personality, mood, reasoning and perception. AD is a multifactorial disease, with no single cause known, and several modifiable and non-modifiable risk factors are associated with its development and progression. Age is the greatest risk factor for the development of AD.AD is a progressive neurodegenerative brain disorder that causes a significant disruption of normal brain structure and function. At the cellular level, AD is characterized by a progressive loss of cortical neurons, especially pyramidal cells that mediate higher

cognitive functions. (Mann *et al*., 1996; Norfray *et al*., 2004). Substantial evidence also suggests that AD causes synaptic dysfunction early in the disease process, disrupting communication within neural circuits important for memory and other cognitive functions (Selkoe *et al*., 2002). AD-related degeneration begins in the medial temporal lobe, specifically in the entorhinal cortex and hippocampus (jack *et al*., 1997). Our brain is made of approximately 100 billion nerve cells, called neurons. Neurons have the amazing ability to gather and transmit electrochemical signals.Different hypothesis for AD

The **amyloid cascade hypothesis** is the most widely discussed and researched hypothesis. Amyloid beta (A_β) deposits are the fundamental cause of the disease.

The **cholinergic hypothesis**, the oldest one on which most drug therapies are based. AD is caused by reduced synthesis of the neurotransmitter acetylcholine.

The **tau hypothesis** proposes that tau protein abnormalities also cause AD.

A **neurovascular hypothesis** has been proposed which states that poor functioning of the blood–brain barrier may be involved (Deane *et al*., 2007).

We know that the brain is made up of neurons and these are interconnected to form a vast network. These connections are known as synapsis. In AD two main lesions are form in the brain senile plaques composed of β amyloid protein and neurofibrillary tangles composed of tau protein. Damage to these brain structures results in memory and learning deficits that are classically observed with early clinical manifestations of AD. The degeneration then spreads throughout the temporal association cortex and to parietal areas. As the disease progresses, degeneration can be seen in the frontal cortex and eventually throughout most of the remaining neocortex.Senile plaque develops in brain,they initially absorbed in the cortex secondly in hippocampus and then the senile plaques developes in whole brain follow a centripetal movement. Neurofibrillary tangels first develops in the region called hippocampus which is essential to memory and learning than they reach the whole brain, follow in a centrifugal movement.(ISAO *et al*., 2013) . The chief component of the plaques is beta-amyloid, while the chief component of tangles is the tau protein.

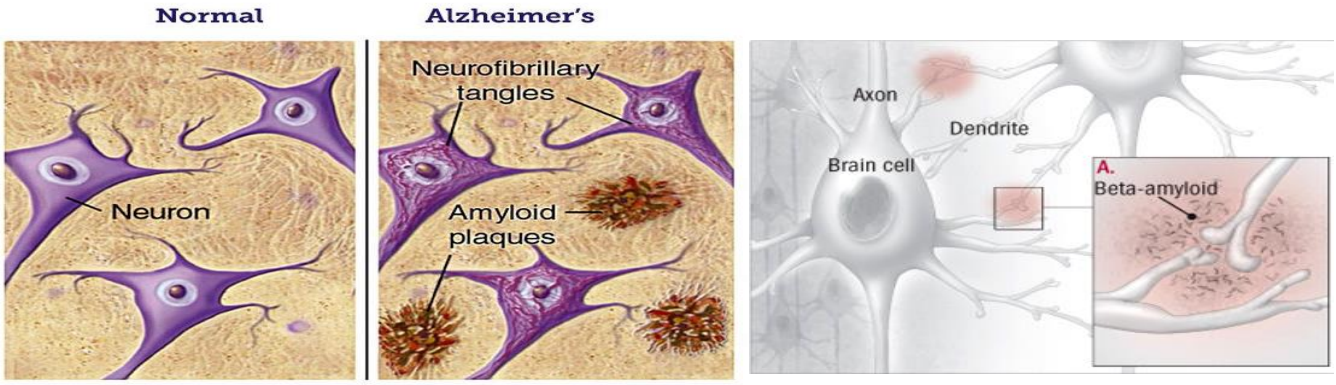


Fig 1: Formation of amyloid plaques in AD.

Plaques are 'sticky' proteins and can build up between nerve cells, and can cause significant problems to overall learning and cause memory loss. Tangles can disintegrate the main cell transport system, eventually killing the cell. (Armstrong *et al*., 1998)

One of the major hallmarks of Alzheimer's disease is the abnormal state of the Microtubule associated protein tau in neurons. It is both highly phosphorylated and aggregated into Neuro fibrillary tangles or paired helical filaments, and it is commonly assumed that the hyperphosphorylation of tau causes its detachment from microtubules and promotes its assembly into NFTs which will further lead into Alzheimer's disease (Nukala *et al*., 2017). AD is postulated to be characterized by intracellular neurofibrillary tangles, neuroinflammation, and neuronal dysfunction leading to death. Cumulatively, Abeta is considered the hallmark of AD responsible for triggering a complex pathological cascade leading to neurodegeneration (Golde *et al*., 2006). The β-secretase, widely known as β-site amyloid precursor protein cleaving enzyme 1 (BACE1), initiates the production of the toxic amyloid β (Aβ) that plays a crucial early part in AD pathogenesis. Due to its apparent rate limiting function, BACE1 appears to be a prime target to prevent and lowering the Aβ generation in AD. Further, b-site amyloid precursor protein cleaving enzyme 1 (BACE1) controls the rate limiting step in the production of Abeta responsible for the pathogenesis of AD, which has sought the researchers to target BACE1 for the mitigation of AD (Dash *et al*., 2014). Protein levels of BACE1 are significantly higher in patients having AD, which explains the high importance being given to BACE1 inhibition (Ahmed *et al*., 2010). Beta-site APP cleaving enzyme1 (BACE1) catalyzes the rate determining step in the generation of Aβ

peptide and is widely considered as a potential therapeutic drug target for AD. The cellular homeostasis of bio metals such as ionic copper, iron, and zinc is disrupted in AD. Most of autosomal dominant familial AD can be attributed to mutations in one of three genes: those encoding amyloid precursor protein (APP) and presenilins 1 and 2 (Archives of Neurology 2008). Most mutations in the APP and presenilin genes increase the production of a small protein called Aβ42, which is the main component of senile plaques. (Selkoe*et al*., 1999)

Acetylcholinesterase (acetylhydrolase or AChE) could be a serine protease that hydrolyzes acetylcholine that acts because the neurochemical in varied species. (Taylor *et al*., 1994; Quinn *et al*., 1987).AChE is found exuberant in brain, muscle, and blood corpuscle membrane. The loss of function of Ach is implicated to the development of AD(Perry *et al*., 1999). The acetylcholinesterase (AChE), an enzyme that breaks the neurotransmitter Ach into acetate and choline,hampers the normal neurotransmission. Cholinergic hypothesis of the disease states that the inhibition of AChE action may be one of the realistic approaches to the symptomatic management of AD (Weinstock, 1995). AChE acts as one of the most significant targets against AD (Giacobini, 2004). Some of the known inhibitors of AChE are donepezil, galantamine ,tacrine, huperzine, and 7-methoxytacrine (Colovic *et al*., 2013).

Symptoms and Risk factors of AD

Indications of the disease are difficulty in remembering recent events, problems with language, mood swings (change in thinking and behaviour), difficulty writing and speaking, poor judgment, loss of interest in daily activities, vision problems etc. About 70% of the risk is believed to be genetic with many genes usually involved (Ballard *et al*., 2013). Most cases of AD don’t exhibit autosomal-dominant inheritance and are termed sporadic AD, in which environmental and genetic differences may act as risk factors. The best known genetic risk factor is the inheritance of the ε4 allele of the apolipoprotein E (APOE) (Strittmatter *et al*., 1993; Mahley *et al*., 2006). Most common risk factors are Age(above 65-70years),Genetics (ApoE4 gene mostly involve in AD), Estrogens level (Women have a higher risk for AD than men), Diabetes, High blood pressure, Heart disease, Down’s syndrome, Smoking, Systemic markers of the innate immune system, air pollution, obesity, dyslipidemia, history of brain trauma,

cerebrovascular disease and vasculopathies etc.Other risk factors include a history of head injuries, depression, or hypertension(Burns *et al*., 2009).

Role of β -secretase in AD

The β -secretase is the enzyme that initiates the generation of amyloid beta. It is an attractive drug target for lowering cerebral levels of APP for the treatment of AD. APP is subjected to degradation via amyloidogenic pathway or via the non amyloidogenic pathway. APP is first cleaved either by α-secretase or β-secretase y-secretase enzymes, and the resultant membrane attached fragments are processed by g-secretase (Zhang *et al*., 2011). The products of a-cleavage followed by g-cleavage are highly soluble and nonamyloidogenic (De-Paula *et al*., 2012) whereas Aβ produced by β-secretase secretase mediated cleavage followed by g-cleavage is biochemically insoluble and prone to polymerization into pathological fibrils. Besides, amyloidogenic APP cleavage leads to the synthesis of a fragment named APP intracellular domain that alters diverse cellular functions (Saido *et al*., 2013). APP synthesized in the neuronal cell body, primarily undergoes axonal transport by being contained in transport vesicles is secreted from the presynaptic terminals into the extracellular matrix, and thus fibrillary Aβ deposits in AD are formed outside neurons. FAD mutations on the APP gene either enhance β -secretase -cleavage relative to a-cleavage or alter the activity of g-secretase to increase the ratio of amyloidogenic Ab β -secretase 2eA β -secretase 40, which forms fibrils less rapidly.4 This amyloid processing pathway makes beta-secretase (memapsin 2 or BACE1) an attractive target for the development of inhibitors against AD (Saido TC *et al*., 2013). BACE 1 is a type 1 transmembrane aspartyl protease and is predominantly located in the intracellular acidic compartments. Their expression is found to be highest in neurons. Interestingly, over-expression and knockdown of BACE1 increases and decreases the Ab production respectively.

BACE 1 has two aspartic acid residues in its active site (since it is an aspartyl protease) namely Asp32 and Asp228 present in the large hydrophobic cleft. Two conserved water molecules play an important role in maintaining the enzymatic stability and function (Vassar *et al*., 2013). The molecular docking based approach generated two first generation BACE1 inhibitors namely OM99-2 and OM00-3 which mimicked the natural substrate (Mancini *et al*., 2011). Some other reported inhibitors are the modified

molecules based on the parent structure of hydroxyethylene (HE), hydroxyethyleneamine (HEA), carbinamine, macrocyclic, acylguanidine, aminoimidazole, and aminoquinazoline(Ghosh *et al*., 2008). Synthetic coumarin derivatives were the first reported compounds which were computationally validated to be dual inhibitors of AChE and BACE1 (Ghosh *et al*., 2012; Piazzi *et al*., 2008) Using docking studies, some dual inhibitors of AChE and BACE1 have been generated using HE, HEA, and hydroxymethylcarbonyl as the scaffolds and two compounds even exhibited excellent activity in cell based assays (Zhu *et al*., 2009). In another computational study, flavonols and flavones namely quercetin, kaempferol, myricetin, morin, and apigenin have been validated to be potent BACE 1 inhibitors (Shimmyo *et al*., 2008). The most effective peptidomimetic BACE1 inhibitors have been the statine-based structures with great binding efficacy and IC50 values (Zuo *et al*., 2005).

Current status of ad in world worldwide:

Today, 47 million people live with dementia worldwide, more than the population of Spain. This number is projected to increase to more than 131 million by 2050, as populations' age. Dementia also has a huge economic impact. India houses more than 4 million people suffering from AD. Alzheimer's being the most common condition out of all of them affects around 1.6 million. Alarmingly, this number is set to triple by 2050 (Indian Times Report, 2017). In 2017, an estimated 700,000 Americans age ≥65 years will have AD when they die, and many of them will die because of the complications caused by AD (Alzheimer's 'Association Report, 2017).The total estimated worldwide cost of dementia is US$818 billion, and it will become a trillion dollar disease by 2018(Martin *et al*., 2013). Alzheimer disease (AD) accounts for nearly 60-70% of all dementia cases and is a major socio-economic health problem, affecting more than 36 million individuals worldwide (Thies *et al*., 2013). Alzheimer disease is the most frequent cause of dementia in Western societies.(Duthey *et al*., 2013). As the world population ages, the frequency is expected to double by 2030 and triple by 2050 (Blennow *et al*., 2006).The current estimates provide an indication of the numbers of people aged 60 years and over with dementia worldwide and in different world regions (Duthey *et al*., 2013).

Families have often to take care of a relative with Alzheimer disease, which is a challenging experience. With the ageing of the baby boomer generation, managing

dementia in elderly is one of the greatest challenges that Europe will have to face in the next 50 years. Incidence at age 80 was higher in North America (20.6/ per 1000 person years) and Europe (15.1) than in other countries (8.3). However, the doubling time was shorter in other countries (5.0 years) than in North America (6.0) or Europe (5.8). Incidence was slightly higher among women (13.7 per 1000 person years) than in men (10.6/1000 person years).The incidence of dementia appears to be higher in countries with high incomes.The financial costs of managing AD are enormous. The cost of illness is high in terms of both public and private resources. Families and caregivers who are required to provide care and patients affected by dementia also pay a high price in terms of their quality of life (Duthey *et al*., 2013). In high-income countries, informal care (45%) and formal social care (40%) account for the majority of costs, while the proportionate contribution of direct medical costs (15%) is much lower.

Failure of AD drugs

Alzheimer's disease clinical trials have had an excessively high failure rate over the past 15 years, with 99.6% of drugs failing in 2002-2012 (Cummings 2014). Five drugs, viz. Tacrine, Donepezil, Rivastigmine, Galantamine and Memantine approved by FDA are available in market in order to treat AD (Hansen *et al*., 2008). Four of them are acetylcholinesterase inhibitors which act on symptoms of AD and only slow down the progression of disease. Although many of these drugs were likely inactive, a 2-sided 0.05 alpha should result in a 2.5% success rate on the primary endpoint by chance alone. Other addressable factors such as patient heterogeneity, variable outcomes and variable measurement processes contribute to this particularly high failure rate. Successful Alzheimer's clinical trials require an active compound and successful study design demonstrated by narrow confidence intervals. Phase 2 studies should use different standards for success than phase 3 studies, and statistical and psychometric issues should be fully considered. Accurately identifying compounds with small effect sizes is the first step toward developing better treatments with larger effect sizes. Narrow confidence intervals indicate more precision in treatment effect size estimates leading to failing ineffective treatments and success for effective treatments (Hendrix *et al*., 2017). The amyloid hypothesis indicated that amyloid is the initial cause of AD disease contributing to plaques accumulation; one of AD hallmarks is an aggregation of

amyloid (Aβ) leading to deposition of β-amyloid in the brain (Querfurth *et al*., 1994). In Aβ reducing approaches, numerous studies demonstratethat amyloid vaccine can remove the amyloid plaques fromthe brains of the mice and reverse cognitive impairment (Morgan *et al*., 2000;Wilcock *et al*., 2004), but in human clinical trials, the immunotherapy has side effects during the process of treatment, including autoimumunity (Wisniewski *et al*., 2008) and high incidence of meningoencephalitis (Rasool *et al*., 2012);clearance of Aβ deposition still has problems for developing AD therapy.Four drugs are approved and currently used in AD: donepezil (*Aricept*) 1997, rivastigmine (*Exelon*) 2000, galantamine (*Reminyl*) 2001, and memantine (*Namenda*) 2003. Currently, many new compounds are in clinical testing or will shortly enter clinical testing for AD and MCI (Giacobini*et al*., 2007). Concerns are that methodological factors, relatively widely reported in the literature as barriers to CT successes, will interfere with investigators providing a fair test for these new AD drug candidates. (Becker *et al*., 2008) (Becker *et al*., 2007) Becker and Greig and Becker questioned whether or not drug development and CTs failed the drugs they tested because methodological deficiencies increased the probability of Type II errors. The low rates of attention we found to methodological issues that could invalidate drug development investigations, such as unreliability that leads to variance, reduced power, large numbers of subjects to meet power requirements, large numbers of sites to provide subjects, heterogeneous samples, inadequate monitoring and re-training of site personnel during studies, and so forth, sustain the concern that current AD. CT methods and practices may lead to rejection of compounds that could be efficacious in AD or indicative of mechanisms of drug action efficacious in AD. Added to these problems is that dementia has become a graveyard for a large number of promising drugs. The researcher's findings paint a gloomy picture. Of those 244 compounds, only one was approved. The researchers report that this gives Alzheimer's disease drug candidates one of the highest failures rates of any disease area 99.6%, compared with 81% for cancer.

Mechanism of ad drugs:

Our brain is made of approximately 100 billion nerve cells, called neurons. Neurons have the amazing ability to gather and transmit electrochemical signals. We know that

the brain is made up of neurons and these are interconnected to form a vast network. These connections are known as synapsis. In AD two main lesions are form in the brain senile plaques composed of β amyloid protein and neurofibrillary tangles composed of tau protein. Alzheimer's disease has been identified as a protein misfolding disease (proteopathy) caused by plaque accumulation of abnormally folded amyloid beta protein and tau protein in the brain (Hashimoto *et al*., 2003)

Importance of *in silico* approach

In silico approach in bioinformatics hold a lot of prospective in target identification (generally proteins/enzymes), target validation, understanding the protein, evolution and phylogeny and protein modeling (Arthur M Lesk -2014) In silico analysis can not only accelerate drug target identification and drug candidate screening and refinement, but also facilitate characterization of side effects and predict drug resistance. One of the major thrusts of current bioinformatics approaches is the prediction and identification of biologically active candidates, and mining and storage of related information. It also provides strategies and algorithm to predict new drug targets and to store and manage available drug target information (Rao and K. Srinivas-2014)In molecular docking: Docking is an automated computer algorithm that attempts to find the best matching between two molecules which is a computational determination of binding affinity between molecules. This includes determining the orientation of the compound, its conformational geometry, and the scoring. The scoring may be a binding energy, free energy, or a qualitative numerical measure. In some way, every docking algorithm automatically tries to put the compound in many different orientations and conformations in the active site, and then computes a score for each. Some bioinformatics programs store the data for all of the tested orientations, but most only keep a number of those with the best scores .Docking can be done using bioinformatics 13 tools which are able to search a database containing molecular structures and retrieve the molecules that can interact with the query structure .It also aids in the building up chemical and biological information databases about ligands and targets/proteins to identify and optimize novel drugs .It is involved in devising in silico filters to calculate drug likeness or pharmacokinetic properties for the chemical compounds prior to screening to enable early detection of the compounds which are

more likely to fail in clinical stages and further to enhance detection of promising entities. In silico study tools help in the identification of homologs of functional proteins such as motif, protein families or domains. It helps in the identification of targets by cross species examination by the use of pairwise or multiple alignments. The tools help in the visualization of molecular models. It allows identifying drug candidates from a large collection of compound libraries by means of virtual high-throughput screening (VHTS).Homology modeling is extensively used for active site prediction of candidate drugs (Rao and K. Srinivas-2011).

Druglikeness of compound

Drug like ness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features influence the behavior of molecule in a living organism, including 14 bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolicstabilityandmanyothers.(www.molinspiration.com/docu/miscreen/druglikeness.html).

ADMET

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of your molecules are of vital importance. The ability to quickly and accurately predict these properties simply from the 2D structure of the molecule is extremely helpful in making decisions that can determine the success of your project. ADMET Predictor is state-of-the-art ADMET property prediction software. ADMET Predictor is a machine learning software tool that quickly and accurately predicts over 175 properties including solubility, logP, pKa, sites of CYP metabolism, and Ames mutagenicity. ADMET Predictor allows one to rapidly and easily create high-quality QSAR/QSPR models based on your own data. The newest module offers advanced data mining, clustering, and matched molecular pair analysis. The program has an intuitive user interface that allows one to easily manipulate and visualize data (https://www.simulationsplus.com/software/admetpredictor).

AutoDock tool

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders.

Molecular Docking

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear. This chapter discusses the background and theory of molecular docking software, and covers the usage of some of the most-cited docking software.

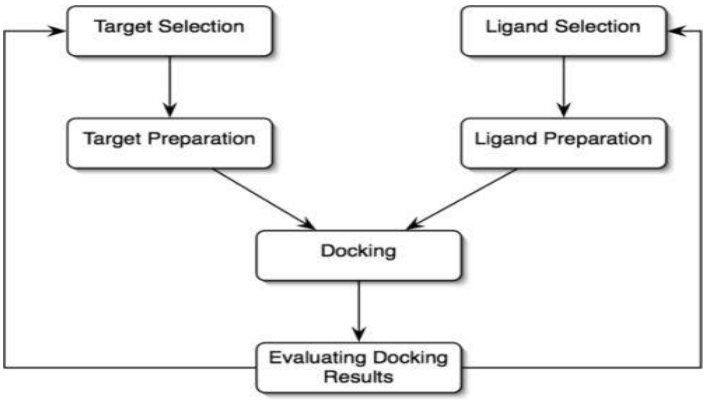


Fig:2 flowchart of Molecular Docking Method.

Principles of molecular docking

Molecular docking is a structure-based drug design method that predicts the binding mode and affinity by studying the interaction of organic small molecule ligands with biological macromolecular receptors. Molecular docking methods have a wide range of applications in the fields of enzymology research and drug design. Since the Kuntz team at California State University, San Francisco developed the first molecular docking software DOCK in 1982, scientists have developed a variety of theoretical models and docking algorithms. The most important theoretical models and corresponding docking methods are:

1. Lock-and-key model, rigid docking;
2. Induced fit model (induced-fit), flexible docking (flexible docking) and semi-flexible docking (semi-flexible docking);
3. Conformation ensemble, ensemble docking.

The essence of molecular docking is the recognition process between two or more molecules, involving spatial matching and energy matching between molecules. The docking software places small ligand molecules at the active site of the receptor target, and searches for ligands by continuously optimizing the position, conformation, dihedral angle of the rotatable bond, and the side chain and skeleton of the receptor amino acid residues. The best conformation for binding of small molecules to the receptor target, prediction of its binding mode and affinity.

REVIEW OF LITERATURE

Alzheimer's disease (AD), one of the most common neurodegenerative disorders, accounts for about 60–80% of all cases of dementia (Wiemann, J.*et al*., 2017, Mattila, J *et al*., 2012). According to the statistics, nearly 50 million people worldwide have AD or a related dementia, and the number of AD patients is expected to triple by 2050. AD has now been the third leading cause of death, outpaced only by cardiovascular diseases and cancer (Yu, Y.F.; Huang, *et al*., 2017). However, there is still no successful therapy or drug to reverse or even slow the course of this disease (Lu, X.; Yang, H *et al*., 2019) Although the pathogenesis of AD is complex and not fully understood, several important clinical hallmarks, such as low level of acetylcholine (ACh), beta-amyloid (Aβ) protein aggregation, and tau (τ)protein phosphorylation,are involved in the occurrence and development of AD(Scarpini *et al*., 2003).

In recent years, therapies for anti-AD primarily focused on Aβ and tau have received more attention (Congdon *et al*., 2018) however, various Aβ- and tau-targeting agents have failed in clinical trials (Kodamullil *et al* 2017). Based on cholinergic dysfunction hypothesis, increasing the level of ACh in the brain to improve cholinergic neurotransmission is still the most effective therapy for AD treatment. Alzheimer's disease is named after Dr. Alois Alzheimer. Dr. Alzheimer detected certain abnormalities in a woman's brain tissue after she died of an obscure and unique mental disease in 1906. She exhibits symptoms such as memory loss, communication difficulties, and erratic conduct. Dr. Alzheimer examined her brain after she died and discovered many aberrant aggregated masses and twisted fibres. These aberrant clumps in the brain tissues, now known as plaques and tangles, are thought to be one of the key symptoms of Alzheimer's disease. The breakdown of connections between neuronal cells in the brain is another hallmark of Alzheimer's disease. The specific cause and pathophysiology of Alzheimer's disease are unknown, and there is no known cure. (Mattson, 2004).

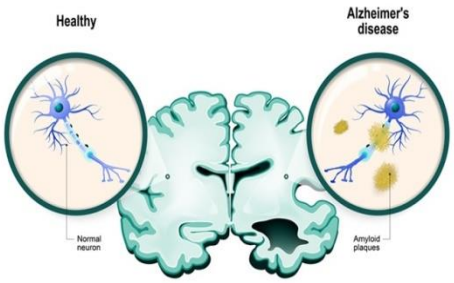


Fig:3 Alzheimer's disease

Beta Secretase a key target of AD

The β secretase, referred to as β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), is the enzyme that initiates Aβ production by cleaving the extracellular domain of APP. Inhibitors of BACE1 are being considered at present for their potential to lower cerebral Aβ concentrations and to treat and prevent Alzheimer's disease. Although several promising BACE1 inhibitors are being tested in human clinical trials, many questions remain about the safety of these drugs, the optimum level of BACE1 inhibition to achieve efficacy without unacceptable side-effects, and the stage of disease at which to treat for greatest therapeutic gain. Here, we review the potential of therapeutic BACE1 inhibition for Alzheimer's disease at a crucial time in the search for effective approaches to treatment and prevention.

Discovery of BACE-1

A number of indirect studies were carried out on this enzyme, as it was the Holy Grail in AD research from its discovery in 1992 to its definitive identification in 1999. Several attempts were made to identify β-secretase including the suggestion that it was actually cathepsin-D, which cleaves APP-derived peptide substrates with specificity similar to β-secretase (Chevallier N *et al*., 1999, Brown AM *et al*., 1996). However, knockout mice lacking cathepsin-D demonstrated that it was not the major β-secretase (Saftig P *et al*., 1996). Additional studies suggested that β-secretase cleaves wild type APP in an intracellular compartment after endocytosis, but FAD mutant APP in the secretory pathway (probably in the same compartment as α-secretase), and also that 4-(2-Aminoethyl) benzenesulfonyl fluoride, a serine protease inhibitor, reduces the yield of β-secretase-cleavage products (Steinhilb ML *et al*., 2001, . Citron M etal., 1996).

Moreover, substrate mutation studies demonstrated that unlike α-secretase, β-secretase is sequence specific (Citron M *et al*., 1995). Additionally, most sAPPβ is secrete in the basolateral membranes of polarized MDCK cells expressing APPwt, but sAPPβ from the APP670NL mutant is shifted by ~20% to the apical surface (De Strooper *et al*., 1995,Capell A 2002).

Despite their importance in understanding the cleavage process, none of these studies on the cell biology could aid in the identification of the elusive β-secretase.

In 1999, five years after the discovery of β-secretase cleavage, five groups simultaneously reported the discovery of β-secretase as a novel integral membrane aspartyl protease, the first of its kind reported in vertebrates (Lin X *et al*., 2000, Hussain I *et al*., 2000, Yan R *et al* 1999, Sinha S *et al*., 1999). Three of these groups used the evidence that one of the secretases is an aspartyl protease to identify novel mammalian aspartyl proteases from the human genome databases. Two groups called the enzyme Asp-2 to denote the second novel aspartyl protease detected in their bioinformatics screens (Hussain I *et al*., 2000, Yan R *et al*., 1999). A third group termed the enzyme memapsin-2 for “membrane-anchored protease” as per the convention for aspartyl proteases to end with “in” as in cathepsin, pepsin, gastricin, renin and napsin (Lin X *et al*., 2000). The fourth group isolated β-secretase cDNA in an expression screen for cDNAs that increase Aβ and termed the enzyme BACE for Beta Site APP-Cleaving Enzyme (Vassar R *et al*., 1999), which has been adopted by most scientists in the field. However, the immediate recognition of the presence of a homologue of BACE, named BACE-2, led to the former being named BACE-1.

The fifth group used conventional biochemistry to isolate and purify the active enzyme from brain membranes and preferred to continue calling it β-secretase to avoid the confusion generated by changing nomenclature (Sinha S *et al*., 1999). These findings generated a lot of excitement and have initiated a large body of studies aimed at understanding BACE-1 structure, function, localization, regulation and changes in AD. The availability of the pure enzyme has also allowed the discovery of specific inhibitors that lower Aβ and might prevent or treat AD.

Structure

BACE-1 is a type-I integral membrane glycoprotein with a 21-residue cleavable signal sequence, a large ectodomain of ~434 aa, a single transmembrane domain of ~22 aa and a short cytoplasmic tail of 24 residues based on predictions using PSORT (www.psort.org)andSig-Phre(www.bioinformatics.leeds.ac.uk/prot_analysis/Signal.html). However, the prediction of the transmembrane domains from the primary sequence analysis is not very precise as palmitate residues, which also bind the sequence to the membrane, modify C-terminal cysteine residues in the cytoplasmic domain and can act as additional membrane anchors. A signal for intracellular transport motif, mapped to the C-terminal region of BACE-1 is the DDISLL sequence, also termed the acid cluster dileucine (ACDL) sequence, was found to interact with GGA proteins and facilitate intracellular transport and recycling [He X *et al*., 2003, Zhu G *et al*., 2000]. BACE-1 is a compact globular protein, which is formed by two domains: 1) residues 47–146; and 2) residues 146–385 (Hong L *et al*., 2000).

The active site contains the two conserved aspartic acid residues, Asp32 and Asp228 within conserved motifs of eukaryotic aspartic proteinases. The residues responsible for catalytic activity are completely encoded within the ectodomain, and the molecule resembles cathepsin D with a membrane anchor [Hong L *et al*., 2000]. In addition to the substrate-binding site, BACE-1 also contains an exosite that was mapped using bacteriophage display libraries (Kornacker MG *et al*., 2005). According to crystal structures of BACE-1, the catalytic region is loctated between the N- and C-terminal lobes, within the substrate binding site in the cleft (Hong L *et al*., 2000, Hong L *et al*., 2004, . Hong L, Turner RT *et al*., 2002). Crystal structures of BACE-1/inhibitor complexes have served to further elucidate subsite positions in the protease. When comparing the structures of other mammalian aspartyl proteases, BACE-1 seems to have an extra loop, which could facilitate the addition of more subsites and increase the size of the target recognition site. There are currently eleven such sites on BACE-1 that recognize the sequence from P7 through P4' as discussed further above (BACE-1 substrate specificity subsection) (Turner RT *et al*., 2001).

Using the OM99-2 inhibitor, the structure of the BACE-1 catalytic unit was revealed along with the features of the active site, including eight subsites that bind the eight

substrate-like residues on OM99-2 (P4 to P4' as described under substrate specificity) (Hong L *et al*., 2002). Subsites S5 through S7 that bound the substrate were then mapped using a larger P10-P4'StatVal inhibitor (Sinha S *et al*., 1999) which extends the structure of OM99-2. These three new subsites, P7 through P5 (Turner RT *et al*., 2005) are found in the active site cleft in a region that extends from the previous eight residues (aa 158–167) in an insertion helix (Hong L *et al*., 2000). This region is thought to be unique for BACE-1, as it is not seen in the structure of other aspartyl proteases and creates the extendend substrate requirement for BACE-1. The side chains for P7 through P5 have a preference for hydrophobic residues, particularly tryptophan [Turner RT *et al*., 2005]. The impact on cellular activity of these subsites has not been fully characterized by mutagenesis of the enzyme and the substrate.

BACE: the β-secretase in Alzheimer's disease

Although the etiology of Alzheimer's disease (AD) is not completely understood, the study of disease genes that cause AD has revealed important clues about the pathogenesis of this disorder. Familial AD (FAD) cases are caused by autosomal dominant mutations in the genes for amyloid precursor protein (APP) and the presenilins (PS1 and PS2) (Sisodia and St George-Hyslop, 2002). These mutations increase production of the 42-aa-long, fibrillogenic form of Aβ ($A\beta_{42}$), relative to $A\beta_{40}$. In addition, patients with APP gene duplications or individuals with Down's syndrome (trisomy 21), who have increased dosage of the APP gene (located on chromosome 21), develop early-onset AD and overproduce $A\beta_{42}$ (Hardy, 2006). These findings, along with a large body of evidence from other sources (Selkoe, 2008), strongly suggest that $A\beta_{42}$ plays a central, early role in AD pathogenesis. Thus, therapeutic strategies to lower cerebral $A\beta_{42}$ levels are expected to be beneficial for the treatment or prevention of AD.

Aβ is produced through the endoproteolysis of APP, a large type 1 transmembrane protein. Cleavage of APP by two proteases, the β- and γ-secretases, is required to liberate Aβ from APP (Tanzi and Bertram, 2005). The β-secretase cuts APP first to generate the N terminus of Aβ, thus producing a membrane bound C-terminal fragment called C99. Then, γ-secretase cleaves C99 to release the mature Aβ peptide. A third protease, α-secretase, cuts APP within the Aβ domain, thus precluding Aβ formation.

γ -secretase processing produces several Aβ peptides with heterogeneous C termini ranging from 38 to 43 residues in length. However, β-secretase cleavage occurs precisely at Asp+1 and Glu+11 of Aβ, indicating that β-secretase is a site-specific protease. Importantly, therapeutic inhibition of β-secretase would decrease production of all forms of Aβ, including the pathogenic Aβ42.

The identity of the β-secretase had long been sought because of its prime status as a drug target for AD. Before the enzyme's discovery, the properties of β-secretase activity in cells and tissues had been extensively characterized, the knowledge of which was instrumental in its identification. In 1999, five groups reported the molecular cloning of the β-secretase, variously naming the enzyme BACE (Vassar *et al*., 1999), β-secretase (Sinha *et al*., 1999), Asp2 (Hussain *et al*., 1999; Yan *et al*., 1999), or memapsin 2 (Lin *et al*., 2000) (here, β-secretase will be referred to primarily as BACE). The groups used very different isolation methods (i.e., expression cloning, protein purification, genomics), y*et al*l identified the same enzyme and concurred that it possessed all the known characteristics of β-secretase (Cole and Vassar, 2008).

BACE is a novel 501 aa type 1 transmembrane aspartic protease related to the pepsin and retroviral aspartic protease families. BACE activity has a low pH optimum, and the enzyme is predominantly localized in acidic intracellular compartments (e.g., endosomes, *trans*-Golgi) with its active site in the lumen of the vesicles. The highest expression levels of BACE are found in neurons of the brain, as expected for β-secretase. Importantly, BACE cDNA transfection or BACE antisense oligonucleotide treatment of APP-overexpressing cells increases or decreases production of Aβ and β-secretase-cleaved APP fragments, respectively. In addition, the specific activity of recombinant BACE on wild-type and mutant APP substrates is consistent with β-secretase. For example, BACE cleaves APP with the Swedish FAD-causing mutation (APPswe) ~10- to 100-fold more efficiently than wild-type APP, as expected for β-secretase. Soon after BACE was discovered, a homolog was identified, BACE2. BACE1 and BACE2 share 64% amino acid sequence similarity, which raised the possibility that BACE2 was also a β-secretase. However, BACE2 is expressed at low levels in neurons of the brain and it does not have the same cleavage activity on APP as β-secretase, thus indicating that it was a poor β-secretase candidate.

To unequivocally exclude BACE2 and validate BACE1 as the β-secretase in vivo, BACE1−/− mice were generated by several groups (Cai *et al*., 2001; Luo *et al*., 2001; Roberds *et al*., 2001).

Initial reports indicated that BACE1−/− mice were viable and fertile, suggesting that therapeutic inhibition of BACE1 might produce few mechanism-based side effects. However, recent studies have shown that BACE1−/− mice are not completely normal .It is not yet known whether therapeutic inhibition of BACE1 would produce these abnormalities in humans and cause untoward side effects.

Importantly, Aβ generation, amyloid pathology, electrophysiological dysfunction, and cognitive deficits are abrogated when BACE1−/− mice are bred to APP transgenics (Luo *et al*., 2001, 2003; Ohno *et al*., 2004, 2007; Laird *et al*., 2005). BACE1−/− mice are devoid of cerebral Aβ production, demonstrating that BACE1 is the major if not only β-secretase enzyme in the brain. This notion is further supported by reports of lentiviral delivery of BACE1 RNA interference (RNAi) that can attenuate Aβ amyloidosis and cognitive deficits in APP transgenic mice (Laird *et al*., 2005; Singer *et al*., 2005). In addition, the rescue of memory deficits in BACE1−/−; APP bigenic mice suggests that therapeutic BACE1 inhibition should improve Aβ- dependent cognitive impairment in humans with AD. Together, the BACE1 characterization and validation studies have unequivocally demonstrated that BACE1 is the authentic β-secretase in the brain and that it is a promising therapeutic target for lowering cerebral Aβ levels in AD.

Role of plant derived metabolites in AD

Plants remain an important source of new drugs, new drug leads and new chemical entities. The plant based drug discovery resulted mainly in the development of anticancer and anti-infectious agents and continues to contribute to the new leads in clinical trials. Natural flavonoids are well known anti-oxidants. In addition, numerous studies have reported the protective effects of natural polyphenol, including flavonols and flavones, against various insults, such as Aβ. It is hypothesized that natural flavonoids may counter the progress of dementia pathogenesis through the activities of its constituent flavonoids (Commenges *et al*., 2000; Kilduff *et al*., 2005). Moreover, many compounds, including natural plant extracts and flavonoids, have been analyzed

for their ability to decrease Aβ-induced neuronal cell death (Levites *et al*., 2003). Previously, we reported that the natural flavonol myricetin showed a neuroprotective effect against Aβ- induced neuronal cell injury (Shimmyo *et al*., 2007). A total of 91 plant-derived compounds in clinical trials as of September 2007 are described in this review. A summary of the plant-based drugs launched during 2000–2006 is given. A total of 26 plant-based drugs were approved/launched during 2000–2006, which also include novel molecule-based drugs like Galanthamine HBr (Reminyl1), Miglustat (Zavesca1) and Nitisinone (Orfadin).Plant-derived natural products in clinical trials For many centuries plants have been the main source of crude drugs used to cure or alleviate human sickness. In today's era of medicine engineering also, plants play an equally important role in drug discovery and development. The plant-derived compounds presently in clinical trials are discussed below for important therapeutic category.Pain and neurological disease applications In the modern world, neurological disorders, such as Alzheimer's disease, Parkinsonism, migraine, epilepsy, multiple sclerosis, and so on, are highly prevalent. It is estimated that in 2000, mental and neurological disorders accounted for 12% of the total disabilityadjusted life years (DALYs) lost because of all diseases and injuries. By 2020, it is projected that the burden of these disorders will have increased by 15% [http://www.who.int/whr/2001/]. Some of the earliest drugs used for this category include opiate alkaloids from Papaver somniferum, tropane alkaloids like cocaine from Erythroxylon coca, galanthamine from Galanthusnivalis and the anticholinestrase agent physostigmine from Physostigmavenenosum, and so on. The plant-derived drugs presently in clinical trials for this category are discussed below. DA-5018 in Phase II: It is a synthetic capsaicin analogue that is being developed by the Korean company Dong-A Pharmaceuticals as a non-narcotic analgesic. Capsaicin causes the burning sensation associated with eating chillies by binding to the ion channel receptor transient receptor potential vanilloid (TRPV1) formerly vanilloid receptor subtype1 (VR1). Dexanabinol in Phase III & II: It is being developed by Pharmos as a neuroprotective product. Dexanabinol is a non-psychotropic dextrocannabinoid, currently undergoing Phase III clinical trials as a treatment for traumatic brain injury and Phase II testing as a preventative agent against post-surgical (CABG) cognitive impairment. Dexanabinol is an antioxidant, anti-inflammatory and a weak and safe N-methyl-D-aspartate receptor antagonist

Ganstigmine (CHF2819) in Phase II: It is a novel AChE inhibitor derived from genserine, for which animal models suggest significant neuroprotection independent from its cholinergic activity. ChiesiFarmaceutici had been testing ganstigminehydrochloride in Phase II for the treatment of AD; however, the company discontinued development of the drug candidate in order to focus resources on other therapeutic area. IP-751 (Ajulemic acid, CT-3) in Phase II: It is a synthetic analogue of the THC metabolite, THC-11-oic acid developed by Atlantic Technology Ventures, U.S.A. and is currently at Indevus in Phase II clinical trials for the treatment of neuropathic pain. It is also undergoing clinical trials for treatment of tremor and spasticity in multiple sclerosis. IP-751 appears to inhibit COX-2 and other inflammatory cytokines, particularly interleukin-1b, TNF-a and also the Peroxisomes Proliferating Activated Receptor-g (PPAR-g) and is partial cannabinoid (CB) receptor agonist. LLL-2011 (Amigra) in Phase III: It is a botanical drug being developed by Lupin as a nasal spray for prophylaxis of migraine. Lupin has received regulatory approval in India to conduct clinical trials in 10 centres. In Phase II clinical trial it was found to be safe and well tolerated with good efficacy data [37]; [http://www.lupinworld.com/]. Lobeline in Phase I: It is a pyridine alkaloid isolated from Lobelia inflata (Campanulaceae), which has been used for centuries as an emetic and respiratory stimulant and, more recently, as a smoking cessation agent. Yaupon Therapeutics and NIH are evaluating Lobeline for methamphetamine addiction. Preclinical studies have suggested that lobeline has utility in helping to treat attention deficit hyperactivity disorder (ADHD) .M6G in Phase III: It is morphine 6-glucuronide a metabolite of morphine, the naturally occurring alkaloid in the opium poppy (Papaver somniferum). M6G is being developed by CeNeS Pharmaceuticals plc for post-operative pain following surgical procedure and has shown promising results comparable to morphine. It has superior side effect profile in terms of reduced liability to induce nausea, vomiting and respiratory depression. It has higher efficacy and low affinity on m-opioid receptor than morphine. The U.S. FDA has approved the IND application for the clinical development of M6G. CeNeS is currently completing the protocol design of the first U.S. Phase III trial [38]; [http://www.cenes.com/index.htm]. NGX-4010 in Phase III & II: It is an application of a pure, highconcentration of synthetic trans-capsaicin developed by NeurogesX and is directly applied via a rapid-delivery dermal application system. Currently it is being studied in Phase III trials in post-herpetic

neuralgia (PHN) and neuropathic pain related to HIV-associated neuropathy. Phase II trials are also underway for neuropathic pain related to peripheral diabetic neuropathy. The local anaesthetic effect results from continuous activation of the TRPV1 receptor, a ligand-gated ion channel activated by agonists such as capsaicin. NeurogesX plans to complete a confirmatory Phase III trial in PHN in the second half of 2007. P58 (PYM-50028, CoganeTM) in Phase II: It is a plant-derived compound obtained from a traditional Asian 'tonic' that has been found beneficial to those with dementia. This novel nonpeptide is being developed by Phytopharm for the treatment of Parkinson's disease and AD type dementia. CoganeTM reverses the changes in area of the brain involved in Parkinson's disease by inducing the production of neurotrophic factors. These growth factors promote the growth and connectivity of neurones and reverse the atrophy of this area of the brain. In addition, this restores the learning and memory ability in Alzheimer's disease models and thereby offers the potential to reverse the symptoms of Alzheimer's disease. Phenserine (Phenserine tartrate, PosiphenTM) in Phase III/Phase I: It is a third generation derivative of phytostigmine isolated from Physostigmavenenosum (Leguminosae) being developed by Axonyx to treat mild-to-moderate Alzheimer's disease (AD). It is a reversible acetylcholinestrase (AChE) inhibitor and also reduces the production of beta amyloid precursor protein (bAPP). Because of this dual mechanism of action, Phenserine has the potential to improve memory as well as to slow AD progression. Posiphen is an antiamyloidogenic agent in Phase I clinical trials at TorreyPines for the treatment of AD and was recently acquired through a reverse merger with Axonyx. RU 47213 in Phase II: It is a pro-drug based on arecoline, an alkaloid found in Areca catechu L. (Palmae) under development for treatment of AD by Sanofi-aventis, whose carbamate function is hydrolyzed in vivo to form the tetrahydropyridine oxime RU 35963, a muscarinic M1 agonist. After oral administration, RU 47213 seems superior to arecoline in terms of potency, central selectivity and duration of action, and is also active in animal models of cognition, without eliciting significant cholinergic side effects [39]. THC-CBD (Dronabinol/cannabidiol, GW-1000-02, Sativex1) in Phase III: It is a cannabis (Cannabis sativa)-based pharmaceutical product containing delta 9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a 1:1 ratio and is being developed by GW Pharmaceuticals. It has been approved as adjunctive treatment for neuropathic pain and cancer pain with

multiple sclerosis (MS) in U.K. and Canada. It is being investigated for the management of other MS symptoms, such as spasticity. They act on CB receptors that are involved in the control of spasticity where there is neurological damage. The most common adverse events (AEs) reported in trials were dizziness, sleepiness, fatigue, feeling of intoxication and a bad taste. ZT-1 (DEBIO-9902) in Phase II: It is a pro-drug of huperzine isolated from the club moss, Huperziaserrata (Lycopodiaceae). ZT-1 was originally synthesized by Zhu and co-workers at Shanghai Institute of Material Medica. It is being evaluated by Debiopharm for the treatment of AD.

Materials and Methods

Software Required

Offline Software

- AutoDock 4.2.6.
- Cygwin.
- Discovery Studio 2020 Client.
- Pymol.

Online Servers

- Pubchem (https://pubchem.ncbi.nlm.nih.gov).
- RCSB PDB (https://www.rcsb.org).
- Molinspiration (https://molinspiration.com/cgi-bin/properties).
- PreADMET Online Prediction Tool (https://preadmet.bmdrc.kr/adme/).

Preparation of ligands

Over 176 plant derived metabolites were compiled from available literature. Chemical properties and mol files of the compounds were retrieved from the NCBI–PubChem Compound database (http//pubchem.ncbi.nlm.nih.gov/). In addition commercially available drugs for AD i.e. Donepezil and Galantamine were considered as controls for the study.

Pharmacokinetic Analyses

- **Drug likeness calculations**

Drug scans were carried out to determine whether the plant metabolites fulfil the drug-likeness conditions. Lipinski's filters using Molinspiration (http://www.molinspiration.com/) were applied for examining drug likeness attributes as including quantity of hydrogen acceptors (should not be more than 10), quantity of hydrogen donors (should not be more than 5), molecular weight (mass should be more than 500 daltons) and partition coefficient log P (should not be less than 5). The smiles

format of each of the compound was uploaded for the analysis (Singh *et al*., 2017; Molinspiration, 2016).

- **ADME/Tox properties**

The ADME/Tox properties (Absorption, Distribution, Metabolism, Excretion/ Toxicology) of all compounds were calculated using the online server PreADMET (http://preadmet.bmdrc.org/). This server calculates pharmacokinetic properties as: Human Intestinal Absorption (HIA), cell permeability Caco-2 in vitro (Pcaco-2), cell permeability of Maden Darby Canine Kidney (PMDCK), skin permeability (PSkin), Plasma Protein Binding (PPB) and the penetration of the blood brain barrier (C_{Brain}/C_{Blood}), and toxicological properties such as: mutagenicity and carcinogenicity (Yashmita *et al*., 2000).

Molecular Docking Analysis

The docking was done with the help of AutoDock Tool 4.2.6 in order to find a suitable binding conformation of the target and ligand. The analysis of Binding conformation of the target-ligand complex was done and they were ranked according to the scoring function of the free energy of binding and inhibition constant (Cosconati, 2010). Four coordinate files are created ligand.pdbqt, receptor.pdbqt, grid.gpf and dock.dpf. The “Lamarckian genetic algorithm” was applied to determine the binding affinity of the complex. The torsion of the ligand was set random. With the assistance of docking polar hydrogen atoms, atomic solvation parameters, Kollman charges and fragmental volume were allocated to the protein. The grid spacing was 0.375Å between the two connecting grid points. Every grid point in x, y and z-axis was set to 90 x 90 x 90Å. While for docking test, 10 runs with a population size 150 and maximum number of evaluations was 25, 00,000 was set. The rest of the parameters were set to default with 0.02 rate of gene mutation, 0.8 rate of cross over with maximum number of generation was 27,000. The results were generated in.glg and .dlg file were further studied for the ligand and protein interaction.

The final outcomes of docking were compiled from free energy (Binding energy) and inhibition constant (Ki). The best docked structures were developed using the Accelry's Discovery Studio Visualizer 2020.

Step to step protocol of molecular Docking

- Click on file option. Then select read molecule option. Then select your target file which is in pdb format and click on open option.
- Then click on edit option. Select hydrogen and then select add option. Then click on option polar only and select option okay.
- Click again on edit option and then select charges. Then click on option Add kollman charges and select okay.
- Again, click on option Edit and then select option Atoms. Then select option Assign AD4type and click on Okay.
- Click on option Grid and then select option macromolecules. Then select choose. Click on option Target and then select your target. Then a dialogue box will appear and click on okay option on it. Then another dialogue box will appear. There save your target in pdbqt file format.
- Now select option ligand and click on input option. Select open. A dialogue box will appear where we have to select the file format as all files. Then select your ligand and click on option open.
- Now again select option ligand and then select option torsion tree and click on choose torsion tree.
- Again, click on option torsion tree and select option Detect root.
- Again, click on option torsion tree and select option Show/Hide root marker.
- Again, click on option torsion tree and select option Choose torsion. A dialogue box will appear. Click on option done.
- Again, click on option torsion tree and select option Set number of torsions. A dialogue box will appear. If the number of torsions here is 6 or below 6 then click on dismiss. If it is more than 6, then set it to 6 and then click on dismiss option.
- Now select option Ligand. Click on option output. Save your ligand file in pdbqt file format.

- Now click on option Grid. Select option Select map types. Then select option Choose ligand and select your ligand from there.
- Then select option Grid and click on option Grid box. A dialogue box will appear and s*et al*l parameter to 60 and click on okay. Then in the same dialogue box, click on option File and then select option Close saving current.
- Then again select option Grid. Select option Output. Save file by the name grid in the gpf file format.
- Then select option Docking. Select Macromolecule. Then select option Set rigid filename. Open your target file saved in pdbqt file format.
- Now again select option Docking. Click on option Ligand and then select your ligand and click on option Accept.
- Now again click on option Docking. Then select option Search parameters and then click on option Genetic Algorithm and click on Accept option.
- Click on option Docking and then select on option Docking parameters and click on Accept.
- At last click again on option Docking and select option Output. Then Lamarkian and then save your file with the name dock.dpf.
- Now for visualizing the complex formed, open Cygwin. Type “cd ..” and press enter.
- Now type “ls” and enter. All your files will come.
- Now type “autogrid4.exe -p grid.gpf -l grid.glg&” and press enter.
- Now type “tail -f grid.glg&” and press enter.
- Then a line will appear. Press enter.
- Now type “autodock4.exe -p dock.dpf -l dock.dlg&”and press enter.
- Now type “tail -f dock.dlg&” and press enter. Then it will load and once it loads completely, press enter.
- Now type “grep '^DOCKED' dock.dlg | cut -c9- >a.pdbqt” and press enter.
- Now type “cut -c-66 a.pdbqt> a.pdb” and press enter.
- Now type “cat Target.pdb a.pdb | grep -v '^END ' | grep -v '^END$' > complex.pdb” where replace “target” with the name of your target file and then press enter.

RESULTS AND DISCUSSION

Study of drug likeness and pharmacokinetic analyses

Pharmacokinetic study of over 176 plant derived compounds revealed that only four compounds satisfy all the descriptors of ADME/Tox analysis. Furthermore these compounds were subjected to Lipinski's analysis using Molinspiration software, which is essential for rational drug design and also to determine their bioactivity score (Molinspiration, 2016). It was found that the selected four compounds showed no violation of all the five rules i.e. not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight of compounds less than 500, partition coefficient (log P) less than 5, rotatable bonds less than 10, topological polar surface area (TPSA) of not greater than 140 .

Table 1: ADME/Tox properties of selected compounds

		Toxicity		Absorption				Distribution		Metabolism
S.NO.	**Compound Name**	**Mutagenicity (Ames test)**	**Carcinogenicity**	**HIA**	**Caco2**	**MDCK**	**Skin Permeability**	**PPB (Plasma Protein Binding)**	**BBB (Blood Brain Barrier)**	**CYP2D6 Inhibition**
1.	Alpha-Asarone	M	NC	100.0	58.09	324.9	-1.680	93.39	1.229	Non-Inhibitor
2.	Anaferine	M	NC	90.02	39.96	20.31	-3.434	10.00	1.030	Non-Inhibitor
3.	Beta-Asarone	M	NC	100.0	58.09	324.9	-1.680	93.39	1.229	Non-Inhibitor
4.	Glabrene	NM	NC	93.87	29.10	0.109	-2.419	100.0	1.844	Non-Inhibitor
5.	Donepezil*	M	NC	97.95	55.51	0.138	-3.041	84.61	0.187	Inhibitor
6.	Galantamine*	M	NC	95.40	20.93	78.09	-4.176	25.77	0.578	Inhibitor

Mutagenicity (NM=Non-mutagenic, M Mutagenic), bCarcinogenicity (NC=Non-Carcinogenic), cHIA=Percentage of human intestinal absorption, dPCaco-2=Cell permeability (Caco-2 in nm/sec), ePMDCK= Cell permeability Maden Darby Canine Kidney in nm/sec, fPSkin=Skin permeability (nm/sec), gPPB=Percentage of plasma protein binding, hBBB =Blood Brain Barrier (CBrain/CBlood), iCYP2D6= Cytochrome P450 2D6 binding (Non-inhibitor).

Table 2: Lipinski's parameters for ADMET screened compounds

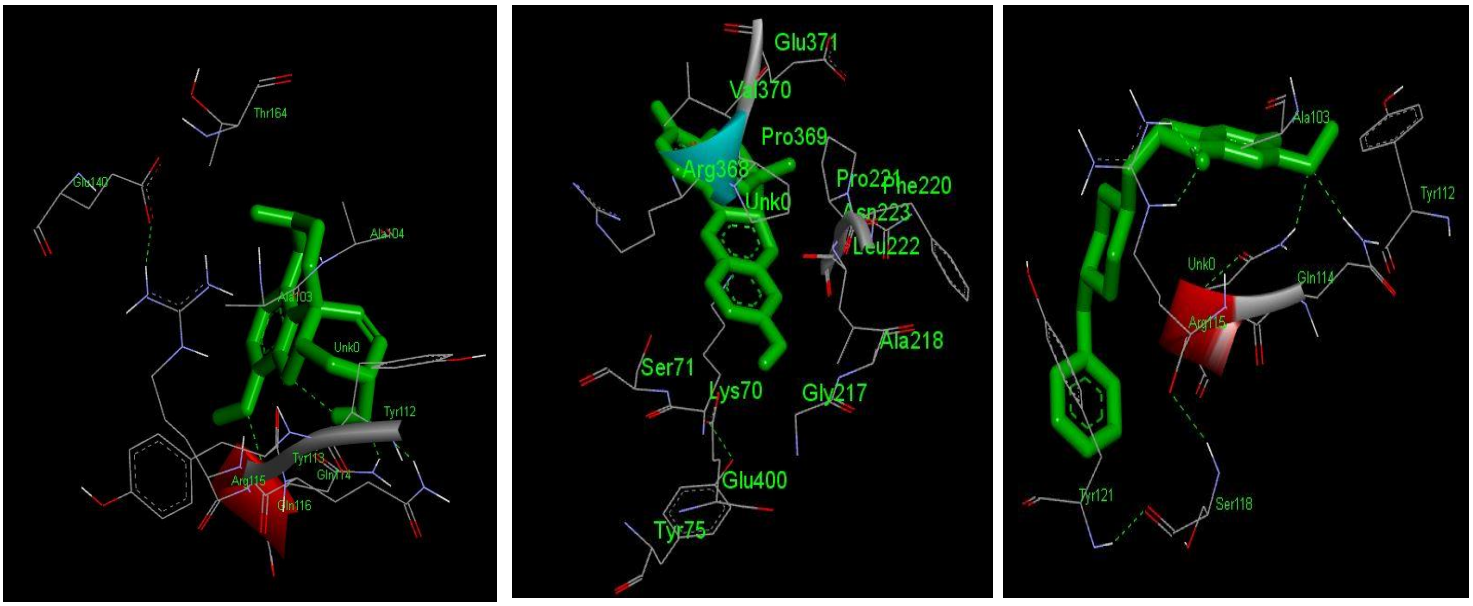
S. No.	Compounds	miLogP	TPSA	natoms	MW	nON	nOHNH	NV	nrotb	volume
1.	Alpha-Asarone	2.49	27.70	15	208.26	3	0	0	4	204.66
2.	Anaferine	1.38	41.12	16	224.35	3	2	0	4	236.41
3.	Beta-Asarone	2.49	27.70	15	208.26	3	0	0	4	204.66
4.	Glabrene	4.31	58.92	24	322.36	4	2	0	1	289.04
5.	Donepezil*	4.10	38.78	28	379.50	4	0	0	6	367.89
6.	Galantamine*	1.54	41.93	21	287.36	4	1	0	-1	268.19

Molecular Docking study

Pharmacokinetically screened compounds subjected to molecular docking analysis along with the donepezil and galantamine as standard control. All the tested compounds were showed the binding affinity with selected target of AD (Table 3). Among the all tested compounds glabrene showed highest binding affinity with target (BE -8.43Kcal/Mol).

Table 3: Molecular docking results of selected compounds with β-secretase.

S.No.	Compounds	BE (Kcal/Mol)	Ki	Interacting amino acids
1.	Alpha-Asarone	-6.74	11.39 µM	GLY72,SER71,TYR75,CYS216,TYR75,GLU400
2.	Anaferine	-6.95	8.04 µM	GLY95,ASP93,GLY95,ASP289,SER96,ASP93, TYR132,LYS168,GLY291,ASP289,THR292, ASP289
3.	Beta-Asarone	-6.40	20.20 µM	GLY72,SER71,TYR75,GLU400
4.	Glabrene	-8.43	666.42 nM	GLU371,VAL370,PRO221,PHE220,LEU222, ASN223,ALA218,GLY217,GLU400,LYS70, SER71,TYR75
5.	Donepezil*	-8.27	264.93 nM	GLY74,LSY70,SER96,ASP93,TRP176,GLN73 GLY181,ASN98,THR292,ASP289,THR293,SER71
6.	Galantamine*	-7.14	5.85 nM	GLY95,ASP93,GLY95, ASP289,SER96, ASP93,TYR132,LYS168, GLY291,ASP289



A **B** **C**

Figure 4: Interactive amino acids of β-secretase with glabrene [A], Donepezil [B] and Glantamine [C].

Molecular docking results exhibited that among the all tested compounds glabrene showed best binding affinity in the active site of BACE 1 protein. Amino acids of target protein namely GLU371, VAL370, PRO221, PHE220, LEU222, ASN223, ALA218, GLY217, GLU400, SER71, TYR75 and LYS70 were found to interact with glabrene.

Glabrene is an isoflavonoid that is found in Glycyrrhiza glabra (licorice). It has estrogenic activity, showing estrogenic effects on breast, vascular, and bone tissue, and hence is a phytoestrogen. It has also been found to act as a tyrosinase inhibitor and to inhibit the formation of melanin in melanocytes, and for these reasons, has been suggested as a potential skin-lightening agent. Glabrene is widely considered to be a phytoestrogen and has been associated with numerous biological properties ranging from antioxidant, anti-inflammatory, neuroprotective, anti-atherogenic effects, to the regulation of energy metabolism, but also including anti-tumorigenic, anti-nephritic, antibacterial and skin-whitening activities (EFSA Journal 2011).

CONCLUSION

Alzheimer’s is a neurodegenerative disease characterized by progressive neuronal death/loss and synapses loss in human brain. β-site APP cleaving enzyme I (β secretase) initiates the production of the toxic amyloid β (Aβ) that plays a crucial early part in Alzheimer's disease pathogenesis. Computational structure-based design and ligand-based design including molecular docking analyses are widely using by the scientific community for the development of small molecule inhibitors. In this view Plant derived metabolites were screened and analyzed for the binding affinity with β-secretase protein. Glabrene (an isoflavonoid of *Glycyrriza glabra*) showed lowest binding energy among the all tested compounds against β secretase. Thus Glabrene is expected to be used in the treatment of Alzheimer's disease in future. Further in vitro studies are required to study the exact mechanism involved their neuroprotective effect.

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