COMPUTER AIDED DRUG DESIGNING: MOLECULAR DOCKING OF ACETYLCHOLINESTERASE INHIBITORS ON SELECTED DRUG TARGETS OF AD TO REVEAL NEW INSIGHTS IN ITS TREATMENT

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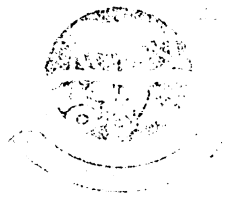

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This is to certify that thesis entitled **"Computer Aided Drug Designing: Molecular docking of acetylcholinesterase inhibitors on selected drug targets of ad to reveal new insights in its treatment"** submitted for award for the degree of **Master of Science** in the subject of bioinformatics embodies a faithful bonafied research work carried out by **Madhuparna Rath (Adm. No. 10BI/10)** under my guidance & supervision. No part of this thesis has been submitted by her for any other degree or diploma.

I further certify that any help or information received during the course of investigation have been duly acknowledged by her.

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CERTIFICATE –II

This is to certify that the dissertation entitled "**Computer Aided Drug Designing: Molecular docking of acetylcholinesterase inhibitors on selected drug targets of AD to reveal new insights in its treatment**" submitted by **Madhuparna Rath,** to the Orissa University of Agriculture & Technology, Bhubaneswar in the partial fulfillment of the requirements for the award of the degree of **Master of Science** in **Bioinformatics** has been approved by the students advisory committee after an oral examination of the same in collaboration with external examiner.

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ABSTRACT

Alzheimer's disease is the most common form of dementia among elderly people characterised by progressive and degenerative disorder of brain. An important characteristic of AD is the deposition of amyloid fibrils and neurofibrillary tangles in the brain of affected individuals mainly composed of β-amyloid protein and phosphorylated tauproteins respectively. There is also a loss of the presynaptic markers of the cholinergic system, such as acetylcholine. The important genes now targeted for treatment of early onset of AD are β–Amyloid Protein Precursor (APP), Apolipoprotein E (APOE) and Presenilin 1 (PS1). In our *in silico* study facilitated by molecular docking studies of four acetylcholinesterase inhibitors Tacrine, Donepezil, Rivastigmine and Galantamine on these three proteins revealed some important H-bond interaction with residues located in the active site pockets. This residue information can be used for further high throughput screening of large scale ligands, QSAR (2d and 3D), pharmacophore modeling and structure based drug designing.

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INTRODUCTION

1 INTRODUCTION

1. 1 Alzheimer's disease

Alzheimer's disease (AD) is an age-related, non-reversible brain disorder. Peoples suffering from it experience memory loss and confusion. The symptoms of AD actually seem to be due to old age of the patient but gradually it leads to change in behavior and personality of the patient. The ultimate result of AD is a severe loss of mental function and this loss is related to the worsening breakdown of the connections between certain neurons in the brain and their eventual death. AD is one of a group of disorders called dementias which is characterized by cognitive and behavioral problems. It is the most common cause of dementia among people age 65 and older.

1.1.1 Types of Alzheimer's disease

There are three types of Alzheimer's disease. They are:

Early-onset Alzheimer's disease

This is the rare form of Alzheimer's disease in which people are diagnosed with the disease before age 65. Less than 10% of all Alzheimer's disease patients have this type. Because they experience premature aging, people with Down syndrome are particularly at risk for a form of early onset Alzheimer's disease. Adults with Down syndrome are often in their mid- to late 40s or early 50s when symptoms first appear. Younger people who develop Alzheimer's disease have more of the brain abnormalities that are associated with it. Early-onset Alzheimer's appears to be linked with a genetic defect on chromosome 14. A condition called myoclonus a form of muscle twitching and spasm is also more commonly seen in early-onset Alzheimer's disease.

Late-onset Alzheimer's disease

This is the most common form of Alzheimer's disease, accounting for about 90% of cases and usually occurring after age 65. Late-onset Alzheimer's disease strikes almost half of all people over the age of 85 and may or may not be hereditary. Late-onset dementia is also called sporadic Alzheimer's disease.

Familial Alzheimer's disease (FAD)

This is a form of Alzheimer's disease that is known to be entirely inherited. In affected families, members of at least two generations have had Alzheimer's disease. FAD is extremely rare, accounting for less than 1% of all cases of Alzheimer's disease. It has a much earlier onset (often in the 40s) and can be clearly seen to run in families.

1.1.2 Cause

The cause for most cases of Alzheimer's disease is still unknown. Thus many competing hypotheses have been proposed to explain the cause of the disease. The oldest hypothesis, on which most currently available drug therapies are based, is the cholinergic hypothesis, which proposes that AD is caused by reduced synthesis of the neurotransmitter acetylcholine. The cholinergic hypothesis has not maintained widespread support, largely because medications intended to treat acetylcholine deficiency have not been very effective.

In 2009 the cholinergic hypothesis was updated, suggesting that a close relative of the beta-amyloid protein is the major culprit in the disease. The theory holds that an amyloid-related mechanism that prunes neuronal connections in the brain in the fast-growth phase of early life may be triggered by ageing-related processes in later life to cause the neuronal withering of Alzheimer's disease. N-APP, a fragment of APP from the peptide's N-terminus, is adjacent to beta-amyloid and is cleaved from APP by one of the same enzymes. N-APP triggers the self-destruct pathway by binding to a neuronal receptor called death receptor 6 (DR6, also known as TNFRSF21). DR6 is highly expressed in the human brain regions most affected by Alzheimer's, so it is possible that the N-APP/DR6 pathway might be hijacked in the ageing brain to cause damage. In this model, beta-amyloid plays a complementary role, by depressing synaptic function.

AD individuals show 70% loss of locus coeruleus cells that provide norepinephrine (in addition to its neurotransmitter role) that locally diffuses from "varicosities" as an endogenous anti-inflammatory agent in the microenvironment around the neurons, glial cells, and blood vessels in the neocortex and hippocampus. It has been shown that norepinephrine stimulates mouse microglia to suppress Aβ-induced production of cytokines and their phagocytosis of Aβ. This suggests that degeneration of the locus ceruleus might be responsible for increased Aβ deposition in AD brains.

1.1.3 Symptoms & Stages of Alzheimer's disease

Some common early symptoms of Alzheimer's disease include confusion, disturbances in short-term memory, problems with attention and spatial orientation, changes in personality, language difficulties and unexplained mood swings. Normally, these symptoms are very mild, and presence of the disease may not be apparent to the person experiencing the symptoms, loved ones or even health professionals. The three stages listed below represent the general progression of the disease. Although these symptoms will likely vary in severity and chronology, overlap and fluctuate, the overall progress of the disease is fairly predictable. On average, people live for 8 to 10 years after diagnosis, but this terminal disease can last for as long as 20 years. Alzheimer's generally leads to impairment of cognitive and memory function, communication problems, personality changes, erratic behavior, dependence and loss of control over bodily functions. Alzheimer's disease doesn't affect every person the same way, but symptoms normally progress in these stages.

This is of 3 types.

Stage 1 (Mild)

This stage can last from 2 to 4 years. Early in the illness, those with Alzheimer's tend to be less energetic and spontaneous.

Stage 2 (Moderate)

This is generally the longest stage and can last 2 to 10 years. In this stage, the person with Alzheimer's is clearly becoming disabled. Individuals can still perform simple tasks

Stage 3 (Severe)

This stage may last 1 to 3 years. During this final stage, people may lose the ability to feed themselves, speak, recognize people and control bodily functions

1.1.4. Management

There is no cure for Alzheimer's disease; available treatments offer relatively small symptomatic benefit but remain palliative in nature. Current treatments can be divided into pharmaceutical, psychosocial and care giving.

Pharmaceutical

Five medications are currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to treat the cognitive manifestations of AD: four are acetylcholinesterase

inhibitors (Tacrine, Rivastigmine, Galantamine and Donepezil) and the other (memantine) is an NMDA receptor antagonist. No drug has an indication for delaying or halting the progression of the disease.

Psychosocial intervention

Psychological interventions are used as an adjunct to pharmaceutical treatment and can be classified within behaviour-, emotion-, cognition- or stimulation-oriented approaches. Research on efficacy is unavailable and rarely specific to AD, focusing instead on dementia in general

1.2 MOLECULAR DOCKING

1.2.1 Docking

Molecular docking, predicts the structures (or structures) of the intermolecular complex formed between two or more molecules. Docking is widely used to suggest the binding modes of protein inhibitors. Most docking algorithms are able to generate a large number of possible structures, and so they also require a means to score each structure to identify those of most interest. The docking problem is thus concerned with the generation and evaluation of plausible structures of intermoleculer complexes.

The docking problem involves many degrees of freedom. There are six degress of translational and rotational freedom of one molecule relative to the other as well as the conformational degrees of freedom of each molecule. The docking problem can be tackled manually, using interactive computer graphics. This 'hands on' approach can be very effective if we have a good idea of the expected binding mode, for example because we already know the binding mode of a closely related ligand. However, even in such cases one must be wary; X-ray crystallographic experiments have revealed that even very similar inhibitors may adopt quite different binding modes. Automatic docking algorithms can be less biased than human modellers and usually consider many more possibilities.

Various algorithms have been developed to tackle the docking problem. These can be charactrised according to the number of degrees of freedom that they ignore. Thus, the simpleast algorithms treat the two molecules as rigid bodies and explore only the six degrees of translational and rotational freedom. The earliest algorithms for docking small molecule ligands into the binding sites of proteins and DNA used this approximation. A well known example of such an algorithm is the DOCK program of Kuntz and co-workers. DOCK is designed to find molecules with a high degree of shape complementarity to the binding site.

The Program first derives a 'negative image' of the binding site from the molecular surface of the macromolecule. This negative image consist of a collection of overlapping spheres of varing radii, each of which touches the molecular surface at just two points as shown in the figure-1.

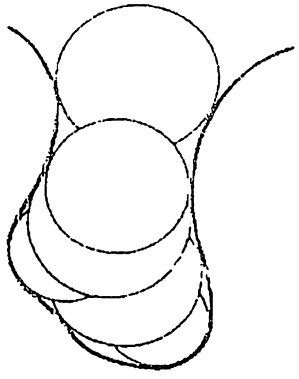

Figure-1: A binding site represented as a collection of spheres

Ligand atoms are then matched to the sphere centres to find matching sets (cliques) in which all the distances between the ligand atoms in the set are equal to the corresponding sphere centre-sphere centre distances (within same user-specified tolerance). The ligand can be oriented within the site by performing a least-square fot of the atoms to the sphere centres, as shown in figure-2.

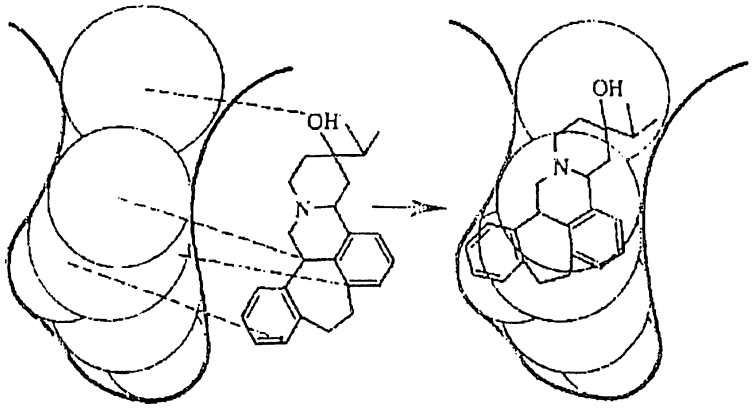

Figure-2: The DOCK algorithm atoms are mateched to the sphere centres and then the molecule is positioned within the binding site.

The orientation is checked to ensure there are no unacceptable steric interaction between the ligand and the receptor. If the orientation is acceptable then an interaction energy is computed to give the 'score' for that binding mode. New orientations are generated by matching different sets of atoms and sphere centres. The top-scoring orientations are retained for subsequent analysis.

To perform conformationally flexible docking the conformational degrees of freedom need to be taken into account. Most of the methods that attempt to include the conformational degrees of freedom only consider the conformational space of the ligand; the receptor is in variably assumed to be rigid. All of the common methos for searching conformational space have been incorporated at some stage into a docking algorithm. For example, Monte Carlo methods have been used to perform molecular docking, often in conjugation with simulated annealing. At each iteration of the Monte Carlo procedure the internal conformation of the ligand is changed (by rotating about a bond) or the entire molecule is randomly translated or rotated. The energy of the ligand within the binding site is calculated using molecular mechanics and the move is then accepted or rejected using the standard metropolis criterion. An interesting variant on the basic Monte carlo approach is the tabu search. This maintains a record of those regions of the search space that have already been visited, so ensuring that the method is encouraged to explore more of the binding site.

Genetic algorithms can also be used to perform molecular docking. Each chromosome codes not only for the internal conformation of the ligand but also for the orientation of the ligand within the receptor site. Both the orientation and the internal conformation will thus vary as the populations eveolve. The score of each docked structure within the site acts as the fitness function used to select the indivisuals for the next iteration.

Distance geometry can be used to perform molecular docking. The major problem to be addressed with this method is to find a way to generate conformations of the ligand within the binding site. One way to achive this is by using a modified penalty function that forces the ligand conformation to remain within the binding site. For example, an additional penalty term can be added which has the effect of forcing the ligand to lie in the DOCK-derived cluster of spheres that represetns the binding site.

An approach that is used by a number of programs involves the incremental construction of the ligand. The main difference, of course , is that in docking the conformational search is performed within the binding site. Atypical incremental construction algorithm first identifies one or more 'base fragments' within the ligand. These base fragments are often chosen to be a reasonably significant, fairly rigid part of the molecule such as a ring system. The base fragments are docked into the binding site and may be clustered to remove similar orientations. Each docked orienation of the base fragments then represents the starting point for the the conformational analysis of the rest of the ligand. One might anticipate that such an approach would be very time-consuming, as it is in effect necessary to perform the

conformational analysis for each orientation of the base fragmanets. However, it is often found thet the protein provides a particularly useful constriant, enabling the search tree to be pruned very effictively.

The ideal docking method would allow both ligand and receptor to explore their conformational degrees of freedom. Perhaps the most 'natural' way to incorporate the flexibility of the binding site is via a molecular dynamics simulation of the ligand-receptor complex. However, such calculations are computationally very demanding and are in practice only useful for refining structures produced using other docking methods; molecular dynamics doesnot explore the range of binding modes very well expect for very small, mobile ligands. For many systems, the energy barriers that separate one binding mode from another are often too large to be overcome. Some other attempts have been made to incorporate protein flexibility (at least at the level of the side chains but these methosd are generally in their infancy and take much longer than rigid-protein docking.

When the first docking methods were developed the speed of the typical computer was such that only rigid-body docking of single molecules was feasible. As computational performance increased it was recognised that rigid-body docking could be used to examine large numbers of molecules form a datbase. At approxmately the same time, algorithms that addressed the conformational flexibility of the ligand were devised. With the passage of time, it is now possible to search databases using a flexible-ligand algorithm. However, there is still a clear distinction between the use of docking to predict the binding mode of a single active molecule, where one can afford to use a perticularly thorough search, and the use of docking for searching databses for possible lead compounds.

1.2.2 Scoring functions for moleculer docking

Most docking algorithms are capable of generating a large number of potential solutions. Some of these can be rejected immediately because they have a high-energy clash with the protein. The remainder must be assesses using some scoring function. When we are only interested in how a single ligand binds to the protein then the scoring function need only be able to identify the docked orientation that most closely corresponds to the 'true' structure of the intermolecular complex. However, when docking a database of molecules then not only should the scoring function be able to identify the 'true' docking mode of a given ligand but it also needs to be able to rank one ligand relative to another. Moreover, the large number of orientations that may be generated during a docking run means that it must be possible to calculate the scoring function rapidly.

Many of the scoring functions in common use attempt to approximate the binding free energy for the ligand binding to the receptor. We have previously encountered a number of ways in which simulation techniques can be used to predict (relative) free energies of binding, but these are far too slow to be of value in docking calculations. Faster, more approximate methods tend to be consider that the free energy of binding can be written as an additive equation of various components to reflect the various contributions to binding. A complete equation of this kind would have the following contributions.

$$\Delta G_{bind} = \Delta G_{solvent} + \Delta G_{conf} + \Delta G_{int} + \Delta G_{rot} + \Delta G_{t/r} + \Delta G_{vib} \quad \text{(Equation-1)}$$

Where $\Delta G_{solvent}$ is the contribution due to solvent effects, arising from the balance of interactins between the solvent and the ligand, protein and intermolecular complex. Various methods can be used to determine these contributions. ΔG_{conf} arises from conformational changes in the protein and in the ligand. In many cases, the protein doesnot change much on binding (which is fortunate, given that most docking methods assume a rigid receptor). By contrast, the ligand changes from an ensemble of conformations in solution to what is often assumed to be a single dominant conformation in the bound state. Various analyses have been performed to try to determine the size of this energetic penalty for the ligand. When measured relative to the most significant conformation in solution, an average penalty of 3Kcal/mol was found [103] .ΔG_{int} is the free energy due to specific protein-ligand interactions, ΔG_{rot} is the free energy loss associated with freezing internal rotaions of the protein and ligand. This is mostly due to the entropic contribution. The simpleast wat to calculate this penalty is to assume that there are three states per rotatable bond (trans and ± gauche) of equal energy, thus leading to a free energy loss of RTln3 (~0.7Kcal/mol) per rotatable bond, $\Delta G_{t/r}$ is the loss in translational and rotational free energy caused by the association of two bodies (the ligand and the receptor) to give a single body (the intermolecular complex). This is often assumed to be constant for all ligands and so is ignored when one is interested in the relative binding strengths of different ligands. ΔG_{vib} is the free energy due to changes in vibrationsl modes. This contribution is difficult to calculate and is usually ignored.

Each of the terms in the equation 1 has been the subject of considerable discussion in the literature, and for some of them there may be a number of different approches to their estimation. However, many of these methods are unsuitable for docking, due to the calculation time required. Some very simple functions have been employed for docking, such

as that originally used in the DOCK program (illustrated in figure-3 together with another similar form, the piecewise linear potential)

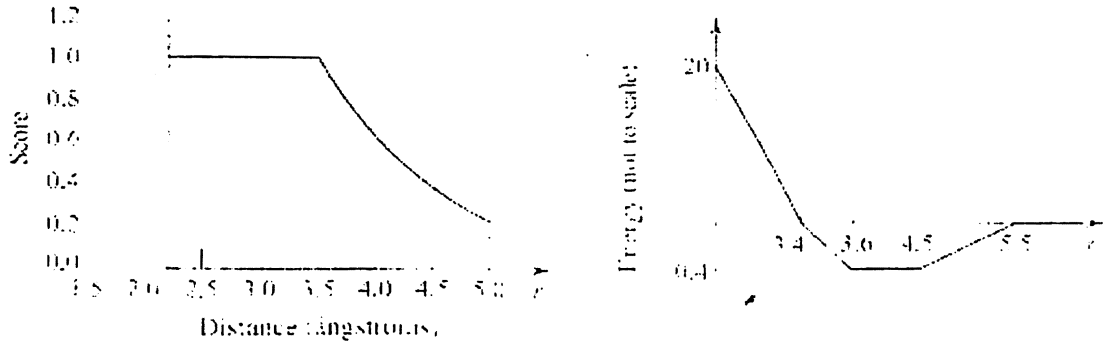

Figure-3: Two simple scoring functions used in docking. On the left side the basic scoring function used by DOCK program and on the right side the scoring function used by piecewise linear potential.

Despite their apperent simplicity, such functions continue to rate well in comparisions of different functional forms. Molecular mechanics is also widely used to calculate the energy of ineraction; one way in which such a calculation can be speeded up is to pre-calculate electrostatic and van der Waals 'potentials' on a regular grid which covers the binding site [106]. The computational effort required to calculate the energy of interaction between n the ligand and protein is then linear in the number of atoms in the ligand, rather than being proportional to the product of the number of ligand atoms multiplied by the number of protein atoms.

Simple molecular mechanics scoring functions are popular, but we can see from the equation1 that they provide only part of the overall free energy of binding. Thus whilst they have proved successful in some cases (such as a study of HIV-protease inhibitors), one should not be surpriesd if they do not always work. An interesting approach to this problem was suggested by Bohm. He tried to find a simple linear re;ationship between the free energy of binding and a variety of parameters which it was anticipated would be relevent to the overall free energy of binding and which could also be calculated rapidly. The terms in this original formulation related to hydrogen bonding, ionic interactions, lipophilic interactions and loss of inernal degrees of freedom of the ligand.

$$\Delta G_{bind} = \Delta G_0 + \Delta G_{hb} \sum_{h\text{-bonds}} f(\Delta R, \Delta \alpha) + \Delta G_{ionic} \sum_{ionic\ interaction} f(\Delta R, \Delta \alpha) + \Delta G_{lipo} |A_{lipo}| + \Delta G_{rot}\ NROT \quad \text{(Equation-2)}$$

Where ΔG_0 is aconstant term, independent of the system, which was inte rpreted to correspond to the overall change in translational /rotatioal free energy ($\Delta G_{t/r}$ in equation1).

ΔG_{hb} corresponds to the contribution from an ideal hydrogen bond. The contribution is multiplied by a penalty function $f(\Delta R, \Delta\alpha)$ which accounts for large deviations of the hydrogen bond from the ideal geometry; ΔR is the deviation of the hydrogen-bond distance from its ideal value of 1.9Å, and $\Delta\alpha$ is the deviation from the ideal angel of 180. The same gemotric dependency is applied to the ionic interactions. ΔG_{lipo} is a contribution from lipophilic interactions, which are assumed to be proportional to the lipophilic contact surface (i.e. involving non-polar atoms) between the protein and ligand, A_{lipo}. ΔG_{rot} is the loss of free energy due to freezing a rotatable bond in the ligand upon binding. It is thus multiplied by the number of rotatable bonds in the ligand, NROT.

Experimental binding data on 45 protein-ligand complexes was extracted from the literature and then a multiple linear regression analysis was performed to derive the parameters in the equation (i.e. the various ΔG values). The values of the parameters obtained from this analysis (ΔG_{hb} = -1.2kcal/mole, ΔG_{ionic} = -2.0kcal/mole, ΔG_{lipo} = -0.04kcal/mol Å^2, ΔG_{rot} = +0.3 kcal/mol, ΔG_0 = +1.3kcal/mol) mostly correspond resonably closely to values estimated from other approaches, with the expection of the constant term, ΔG_0, for which a value between 7 and 11 kcal/mol is generally agreed. The model reproduced the exponential relationship between the binding free energy and the equilibrium constant means that a change of just 1.4kcal/mol in the free energy corresponds to a ten-fold change in affinity. This work has spawned a number of related studies, which differ in the terms included. For example, the surface area is commonly divided into polar and non-polar regions, with different parameters for polar/polar, polar/non polar and non-polar/non-polar interactions. Various statistical techniques have been used to derive the equation and various sources of data used to derive the function. One possible problem with such functions is that they are typically derived from ligands that bind very tightly to their receptor, whereas docking is increasingly used to identify ligands of only modest potency from a large database. For this particular problem, combining the results from more than one scoring function has been shown to give better results than just using individual scoring functions on their own, an approach referred to as 'consensus scoring'.

1.2.3 Applications of docking

There are a number of published studies that demonstrate the utility of 3D database searching in drug design, using both docking and pharmacophore searching. Kuntz's group has used the DOCK program against a number of targets, including HIV protease, DNA, thymidylase synthase and haemagglutinin. In each case the first generation was then used to perform more exhaustive database searches to identify yet more potent compounds. The structures of some of the 'hits' were determined by X-ray crystallography, revealing that not all of the ligands bound in the same way as predicted by the docking algorithm. A degree of serendipity is still important even with automated docking methods. For this reason, it is important to assess the performance of any new docking methods against as many experimentally determined protein-ligand complexes as possible. The much larger number of X-ray structures now available means that it is possible to choose at least one hundred ligands that vary in size, shape, flexibility and functionality (charged, polar, hydrophobic) and which dock into many different proteins. Two good examples of the kind of analysis that is now possible are those evaluating the GOLD program and the FlexX program. Gold uses a genetic algorithm, whereas FlexX uses an incremental construction method.There were some differences between the way in which each program was assessed, the most obvious approach being to calculate the RMS deviation between the theoretical and experimental structures, although this can sometimes be rather simplistic and sometimes misleading metric. However, the best docking programs are able to get 'close' to the correct result for approximately 70% of the ligands.

Commercial 3D database systems for performing pharmacophore searches were available from the early 1990s, but it took several years for real applications to be reported in the literature, largely due to the confidential nature of many of the results. One example of a fairly typical study is that of Marriott and colleagues, who were looking for new lead molecules active against the muscarinic M3 receptor. Antagonists of this particular receptor have potential therapeutic value in condition such as irritable bowel syndrome, chronic obstructive airway disease and urinary incontinence. Three acive molecules were used to define a series of 3d pharmacophores (using the clique detection method). The initial list of five pharmacophores was pruned follwing visual examination to give two similar pharmacophores containing a positively charged amine, a hydrogen-bond acceptor atom and two hydrogen-bond donor sites. Searching a 3D database and combining the selected

molecules gave 172, which were tested. Three compounds were found to have significant activity in the assay, one of which proved to be of particular interest, being a simple molecule particularly amenable to lead optimisation.

Objective:

1. Identification of proteins responsible for Alzheimer's disease.
2. Identification and preparation of ligands responsible for Alzheimer's disease.
3. Docking studies of ligands (four acetyl cholinesterase inhibitors) on the protein molecules.
4. Docked value obtained and tabulated.

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

Alzheimer's disease is characterized by selective neuronal cell death, the presence of extracellular amyloid deposits in the core of neuritic plaques and the formation of intraneuronal neurofibrillary tangles in the brain of afflicted individuals. Neurochemically, these deficits are associated with dramatic losses of cortically projecting cholinergic neurons and by a reduction in the presynaptic markers of the cholinergic system, particularly in the areas of the brain related to memory and learning. Alzheimer's disease does more than rob people of their memories; people with Alzheimer's actually experience two different kinds of symptoms. The first, which are referred to as cognitive symptoms, disrupt memory, language and thinking. The second, known as behavioral and psychiatric symptoms, can cause personality changes and agitation.

Alzheimer's disease is believed to result from a series of steps in pathogenic pathways leading to amyloid deposition and neurodegeneration in key areas of the brain involved in cognition and memory. The emerging picture is that AD is a genetically complex, heterogeneous disorder. Mutations and or polymorphisms in several genes located in at least four different chromosomes 1, 14, 19 and 21 are involved in AD. Besides APP, other gene products are known to be involved in AD. Recent studies have identified point mutations in at least seven genes that contribute to or cause the disease. Mutations in three of these genes, APP on chromosome 21, PS1 on chromosome 14, PS2 on chromosome 1, are dominant and lead to AD with virtually 100% penetrance, while inheritance of certain alleles of the APOE gene (e4) strongly increases the risk for developing AD at an earlier age. There are recent reports of a susceptibility locus for AD on chromosome 10 and a genetic linkage of AD to chromosome 10q. A linkage of plasma Ab42 to a quantitative locus on chromosome 10 in a late onset AD pedigree has also been identified. Alternative theories about AD, such as considering the AD process as similar to cancer due to a loss ofcell-cycle control or viewing AD as a result of a dysfunctional signaling pathway mediated by APP have also beenproposed. Other approaches, such as nutritional factors and genetic and environmentalfactors in AD are also being studied.In addition, there are recent reviews of the roles of glialcells CD40 signaling neuronal ERK, JNK/SAPK, and p38 pathways in AD. Roles for oxidation of Abpeptide and of oxidative damage in AD have also been documented. Indeed, our current understanding ofthe role of oxidative stress in AD has

resulted in developing the beneficial use of antioxidants, such asvitamin E,in preventing or delaying disease onset.Depressive symptoms and sleep disturbances inpatients with AD have been investigated and characterized. The striking association of AD with vascular dementia and the cardiovascular illnesses has been reviewed and has led to coining ofthe popular phrase, ''what is bad for the heart is bad forthe mind'' .Many other lines of ongoing research may alsolead to a deeper understanding of the processes thatoccur in the AD brain. Promising areas include: (1)neurotransmitter deficiencies and dysfunction in braincell communication, (2) beta amyloid protein andsenile plaques and their role in the disease process, (3)tau protein and neurofibrillary tangles and their role inthe disease process, (4) the role of estrogen in thebrain, (5) inflammation and its effect on brain cellactivity, (6) oxidative stress and its effect on brain cellprocess, and (7) genetic factors related to onset of thedisease (source: Alzheimer's Association, Chicago). We will briefly review the current state of knowledge inAlzheimer's research, and the potential basis for newdiagnostic and treatment strategies for AD. The present review will focus mainly on advances related to testing and development of three current hypothesesof AD, ''amyloid,'' ''tangles,''and ''cholinergic,'' andhow they lead to the development of new potential drug targets for AD treatment.

The term "behavioral and psychiatric symptoms" refers to a large group of symptoms that occur in many — but not all — individuals with Alzheimer's. In early stages of the disease, people may experience irritability, anxiety or depression. In later stages, other symptoms may occur, including:

- Sleep disturbances
- Physical or verbal outbursts
- Emotional distress
- Restlessness, pacing, shredding paper or tissues and yelling
- Delusions (firmly held belief in things that are not real)
- Hallucinations (seeing, hearing or feeling things that are not there)

The chief cause of behavioral and psychiatric symptoms is the progressive deterioration of brain cells. However, medication, environmental influences and some medical conditions can also cause symptoms or make them worse.

For example, behavioral symptoms can sometimes be traced to an underlying medical condition. Anyone experiencing behavioral symptoms should receive a thorough medical evaluation, especially when symptoms appear suddenly. Examples of treatable conditions that

can trigger behavioral symptoms include infections of the ear, sinuses, urinary or respiratory tracts; constipation; and uncorrected problems with hearing or vision.
Side effects of prescription medication are another common contributing factor to behavioral symptoms. Side effects are especially likely to occur when individuals are taking multiple medications for several health conditions, as that creates the potential for drug interactions.

Many risk factors are under investigation for association with AD. For example, certain genes make some families vulnerable, head injuries may increase risk, and high blood pressure is a new suspect. But the biggest risk for AD is age: cases double with every 5 years as patients age between 65 and 85. Up to now, there are several risk factors that are known to lead to an earlier onset of AD. One of these is, in some rare familial form, a mutation in the APP gene, the second is an additional copy of this gene in individuals with Down's syndrome, and the third is a mutation in a gene on chromosome 14. However, the mechanism by which these genetic alterations influence beta-amyloid formation remains to be determined. Additionally, the E4 allele of APOE constitutes a major susceptibility factor for the development of the familial and sporadic forms of late-onset AD. As only a few cases of AD can be explained in this way and since the prevalence of AD exceeds 20% among people over 80 years of age, still other risk factors must have to exist. As an example, the transcriptional control of the APP gene has not yet been fully explored.

Research in AD is rapidly expanding and it currently encompasses various cellular, molecular, genetic, clinical, and therapeutic aspects. Reviewing all these diverse areas is beyond the scope of the present work. However, we will briefly mention the salient features of the definitive review work of other investigators in different AD fields. The molecular genetics of AD and its relationship to other primary neurodegenerative disease have recently been reviewed. There are also recent reviews of the role of key protein molecules that are believed to participate in AD pathogenesis. For example, the cell biology of AD, particularly the roles of secretases, presenilin and notch have also been reviewed. Similarly, the role of tau gene mutations and neurodegeneration on AD pathology has recently been summarized. In addition to APP and PS-1, there is a recent spotlight on BACE as a target for treatment in AD. Lately, APP has been proposed to link kinesin-I to a different, also unknown, class of axonal vesicles. This finding of a possible functional interaction between kinesin-I and APP may implicate kinesin-I based transport in the development of AD.

Our current literature review reveals that AD is identified both in young and adult stages but the people identified with AD in early stage are at risk. Three genes APOE, APP and PS1 are responsible for early onset of AD. Here we represent an *in silico* approach facilitated by molecular docking studies of important acetylcholinesterase inhibitors on these three receptors to reveal novel insights in AD treatment.

MATERIALS

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METHOD

3 MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 The Alzheimer's disease Targets

During literature review we found out seven reported genes that are currently treated as drug targets for Alzheimer's disease. (Table-1) Out of these seven genes we have selected β–Amyloid Protein Precursor (APP), Apolipoprotein E (APOE) and Presenilin 1 (PS1) for our *in silico* analysis as these genes were responsible for early onset of AD. These genes were searched in Gene Sorter program of UCSC Human Gene Sorter to find related genes and their relationships including protein-level homology, and similarity of gene expression profiles. The protein structures for each three genes were downloaded in PDB format by visiting the protein structure page. The PDB files of the receptors have three main fields they are structure annotation filed, amino acid field and cofactor field. We are only interested in the amino acid field as it contains atomic coordinates of the main chain residues. We have deleted the waters, hetero atoms and metals present in the receptor structure and saved for docking studies. From the receptor structures the residues included in the active site are noted for receptor grid generation.

Tabel-1: Reported genes implicated in Alzheimer's disease

Sl. No.	Gene	Chromosomal Location	On Set of AD
1	APP	21q21	Early (B50s)
2	APOE	19q13	Late (E4 earlier than E3)
3	S182/PS1	14q24	Early (B40s and 50s)
4	STM2/PS2	1q31-42	Early (B50s)
5	α-2 Macroglobulin	12	Late-onset
6	IDE (Insulin Degrading Enzyme)	10 (locus unknown)	Late-onset AD
7	UPA	10 (locus unknown)	Late-onset AD

3.1.2 Alzheimer's disease Inhibitors

The four currently available drugs for treating AD belong to the same drug category, cholinesterase inhibitors. All four have been approved by the FDA to primarily treat the symptoms of AD. (Table-2) Tacrine (Cognexs) is available for treatment since 1993, Donepezil (Aricepts) is available for treatment since 1996, Rivastigmine (Exelon) is available for treatment since 2000 and Galantamine (Reminyls) is available for treatment since 2001.

All these four drugs work by increasing the supply of acetylcholine to the brain which is deficient in AD.

Tabel-2: FDA Approved Drug for Alzheimer's disease

Sl. No.	Drug	Brand Name	Company	Type
1	Tacrine	Cognex	Park-Davis	acetylcholinesterase inhibitor
2	Donepezil	Aricept	Eisai	acetylcholinesterase inhibitor
3	Rivastigmine	Exelon	Novartis	acetylcholinesterase inhibitor
4	Galantamine	Reminyl	Janssen	acetylcholinesterase inhibitor

We have searched the DrugBank to gather useful information regarding all four approved drugs for the treatment of AD. Each individual drug was searched by putting respective name in the search box of DrugBank database and their structures were downloaded in SDF format for docking studies. SDF (Structure Data File) is a chemical-data file format intended especially for structural information of ligands. This format is most significant as it is able to include associated information with structural information. The 2D representation of four inhibitors is shown in figure-4 and few important experimental and predicted properties are listed in table-3.

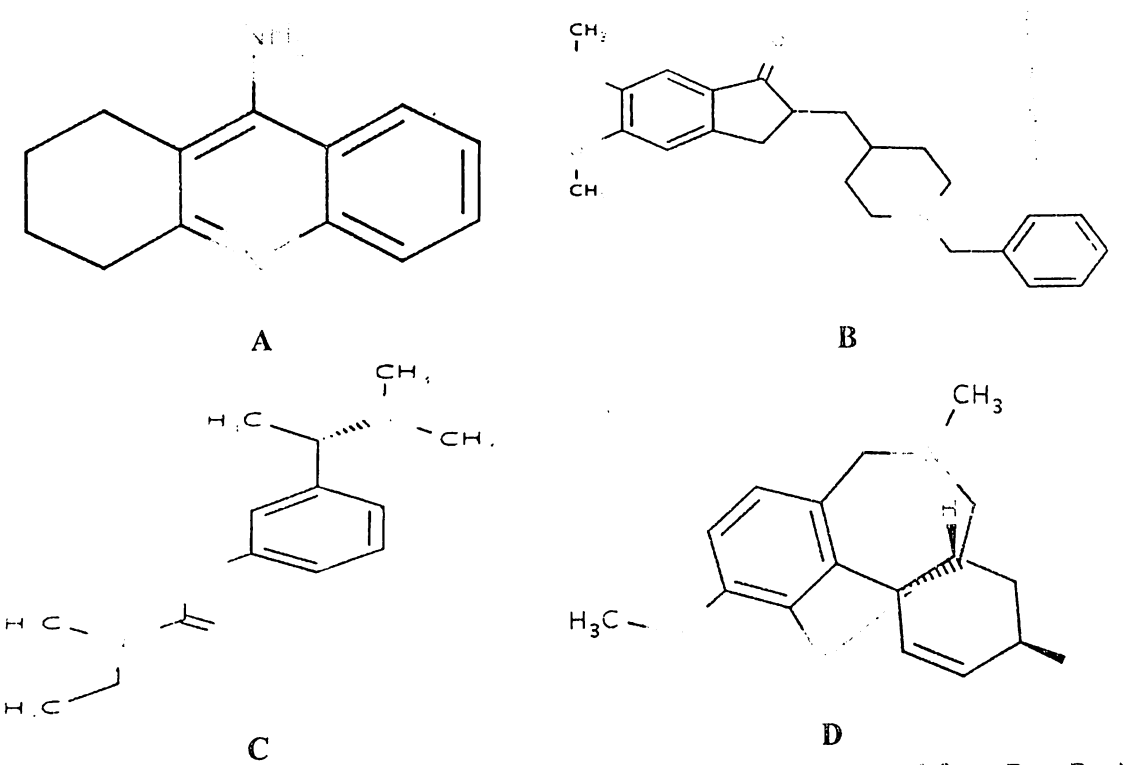


Figure-4: The 2D representation of acetylcholinesterase inhibitors reported from DrugBank. A. Tacrine B. Donepezil C. Rivastigmine D. Galantamine

Table-3: Important experimental and predicted properties of some acetylcholinesterase inhibitors.

Sl. No.	Name		
1	Tacrine	Synonym	Tetrahydroaminacrine
		Brand names	Romotal
		Chemical Formula	C13H14N2
		Weight	Average: 198.2637
		State	Solid
		Type	small molecule
		Groups	Approved
		Water solubility	1.36e-01 g/l
		LogP	3.13
		Logs	-3.2
		PKa	0
		Hydrogen acceptor count	2
		Hydrogen donor count	1
2	Donepezil	Synonym	
		Brand names	Aricept
		Chemical Formula	C24H29NO3
		Weight	Average: 379.492
		State	Solid
		Type	small molecule
		Groups	Approved
		Water solubility	4.50e-03 g/l
		LogP	4.14
		Logs	4.9
		PKa	0
		Hydrogen acceptor count	4
		Hydrogen donor count	0

3	Rivastigmine	Synonym	Ena 713 Free Base
		Brand names	Exelon
		Chemical Formula	C14H22N2O2
		Weight	Average: 250.3367
		State	Solid
		Type	small molecule
		Groups	Approved
		Water solubility	2.04e+00 g/l
		LogP	2.41
		Logs	-2.1
		PKa	0
		Hydrogen acceptor count	2
		Hydrogen donor count	0
4	Galantamine	Synonym	Galanthamine hydrobromide
		Brand names	Lycoremin
		Chemical Formula	C17H21NO3
		Weight	Average: 287.3535
		State	Solid
		Type	small molecule
		Groups	Approved
		Water solubility	10 mg/mL (HBr salt)
		LogP	1.16
		Logs	-2.2
		PKa	0
		Hydrogen acceptor count	4
		Hydrogen donor count	1

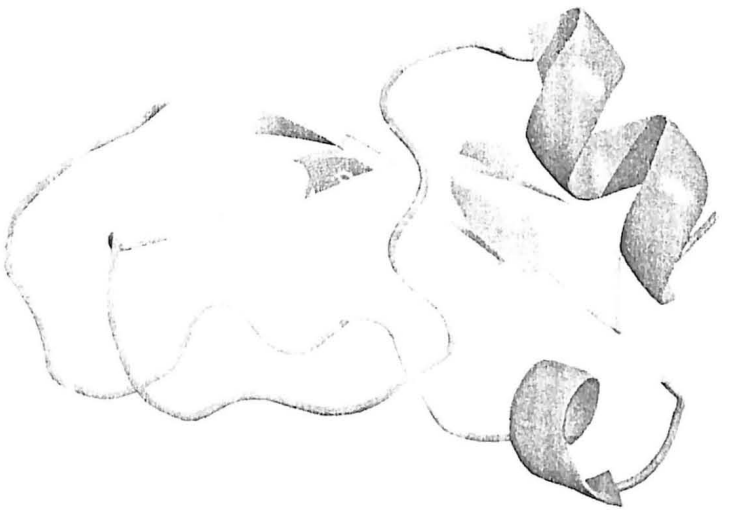
3.2 METHODS

3.2.1 Flexible docking of ligands on proteins by Schrodinger

Preparation of receptor

Prior to docking three proteins (APOE, APP and PS1) were imported to the Maestro workspace and preprocessed by assigning bond orders, adding polar hydrogens. Then the assignments of H-bond were optimized at neutral pH. Finally the imperfect minimization of hydrogens was achieved in OPLS-2005 (Optimized Potentials for Liquid Simulations) force field. The 3D structures of three proteins are shown in figure-xx and the calculated energies after minimization are represented in table-4.

A



B

C

Figure-5: **A**: 3D representation of APOE (3A7Q), **B**: 3D representation of APP (1AAP), **C**: 3D representation of PS1 (2KR6).

Table-4: Optimized parameters of proteins in OPLS2005 force field

Sl. No.	Protein Name	Features after preparation	Values
1	APOE	Potential Energy	-18224.15039
		RMS Derivative	0.047777
		Max Derivative	1.540459
		Stretch Energy	30.929555
		Bend Energy	528.181631
		LJ-14 Energy	1023.312002
		El-14 Energy	-750.857151
		Van der Waals Energy	-1178.980081
		Electrostatic Energy	-18208.30805
		Dihedral Energy	331.571588
		target temperature	298
2	APP	Potential Energy	-1079.289185
		RMS Derivative	0.052917
		Max Derivative	0.568019
		Stretch Energy	0.678325
		Bend Energy	38.4869
		LJ-14 Energy	75.399173
		El-14 Energy	-78.684244
		Van der Waals Energy	-95.892964
		Electrostatic Energy	-1042.59816
		Dihedral Energy	23.321788
		target temperature	298
3	PS1	Potential Energy	-2220.867676
		RMS Derivative	0.048387
		Max Derivative	0.986591
		Stretch Energy	4.098793
		Bend Energy	118.145965
		LJ-14 Energy	293.41002
		El-14 Energy	-155.518595
		Van der Waals Energy	-110.975027
		Electrostatic Energy	-2553.118714
		Dihedral Energy	183.089864
		target temperature	298

Generation of receptor grid

There were three preprocessed, optimized and minimized receptors (APOE, APP and PS1) with us prior to receptor grid generation. Then suitable receptors grids were generated of the prepared proteins around the centroid of selected residues by selecting only those residues which fall in the active site proximity of proteins. In creating and adding the receptor grids to the docking program we instruct the program to dock ligands in a specific area of the protein.

Preparation of ligands

All the four ligands were imported to Maestro workspace and prepared in OPLS2005 force field by generating possible steps at target pH 7.0 +/- 2.0 using Epik. The program was instructed to generate at most 32 stereoisomers per ligand. After preparation the program produced about 32 conformers for Galantamine, 32 conformers for Donepzil, 80 conformers for Revastigmine and 32 conformers of Tacrine respectively. The generated conformers were saved for prediction of ADME properties if needed in future.

Glide Docking

The docking of four ligands on the generated receptor grids were achieved using Glide with XP (extra pricision), flexible docking (sample nitrogen inversions, sample ring conformations) and adding Epik state penalties to the docking score. The docking results for each ligand were exported in CSV (Comma Separate Values) format. The results were analyzed for each docking and only the best poses for each ligand on its respective receptor were represented in table-xx. Here the dock score was GScore which is, the Glide Score. The GScore is the total sum of rewards and penalties for the generated XP terms of each pose of docking.

The flexible docking of Galantamine, Donepezil, Revastigmine and Tacrine on the active pocket of APOE, APP and PS1 illustrated many docking poses with a total of 11 docking types from which the best poses of 11 different docking processes are listed in Table-5. The docking results were obtained in form of GScore. It also includes the rewards scores for Lipophilic EvdW, H-bond, electrostatic, sitemap, Low MW and penalties like phobic penalty and rotational penalty.

Table-5: Docking result of best poses of 4 ligands (Galantamine, Donepezil, Revastigmine, Tacrine) on 3 receptors (APOE, APP, PS1)

Sl. No.	Receptors	Ligands	GScore	LipophilicEvdW	PhobEn	HBond	Electro	LowMW	PhobicPenal	RotPenal
1	APOE	Galantamine	-6.06	-2.79	-1.20	-0.98	-0.58	-0.50	0.00	0.00
2		Donepezil	-8.42	-5.79	-0.80	-1.08	-0.78	-0.18	0.00	0.22
3		Revastigmine	-6.09	-3.91	-0.58	-0.96	-0.59	-0.50	0.00	0.45
4		Tacrine	-5.52	-3.11	-1.27	0.00	-0.64	-0.50	0.00	0.00
5	APP	Galantamine	-3.15	-1.17	0.00	-1.25	-0.60	-0.50	0.36	0.00
6		Revastigmine	-2.92	-1.07	0.00	-2.02	-0.62	-0.50	0.84	0.45
7		Tacrine	-2.61	-0.54	0.00	-1.20	-0.44	-0.50	0.07	0.00
8	PS1	Galantamine	-4.00	-1.00	0.00	-0.70	-2.10	-0.50	0.30	0.00
9		Donepezil	-5.02	-2.20	0.00	-0.70	-2.31	-0.18	0.16	0.22
10		Revastigmine	-5.54	-0.49	0.00	-1.86	-4.05	-0.50	0.91	0.45
11		Tacrine	-5.84	-0.24	0.00	-2.00	-3.94	-0.50	0.84	0.00

GScore	Total Glide Score, sum of XP terms
LipophilicEvdW	ChemScore lipophilic pair term and fraction of the total protein-ligand vdW energy
PhobEn	Hydrophobic enclosure reward
HBond	ChemScore H-Bond pair term
Electro	Electrostatic rewards
LowMW	Rewards for ligands with low Molecular weights
PhobicPenal	Penalty for exposed hydrophobic ligand groups
RotPenal	Rotatable bond penalty

RESULTS

&

DISCUSSION

4 RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Docking of ligands on APOE receptor

The docking of Galantamine on APOE shows h-bond interactions with residues ARG2640 and ILE2605. The docking of Donepezil on APOE shows h-bond interactions with residues GLN2643 and ARG2561. The docking of Rivastigmine on APOE shows h-bond interactions with residues CYS2559 and PRO2644. The docking of Tacrine on APOE shows h-bond interaction with THR2443. (Table-6)

Table-6: Hydrogen bond interaction parameters for each compound and APOE residues

Ligands	Residue atom involved	Ligand atom involved	H-bond distance (Å)
Galantamine	ARG2640: HH11	O1	1.7
	ILE2605: O	HO	2.1
Donepezil	GLN2643: O	HO	1.8
	ARG2561: H	O1	2.2
Rivastigmine	CYS2559: O	N4	3.3
	PRO2644: O	HO	1.9
Tacrine	THR2443: O	H1	2.3

4.1.2 Docking of ligands on APP receptor

The docking of Galantamine on APP shows h-bond interactions with residues ARG20, ASN44 and ARG20. We could not found a suitable docking of Donepezil on APP. The docking of Rivastigmine on APP shows h-bond interactions with residues ASP46 and ASN44. The docking of Tacrine on APP shows h-bond interaction with ASP46. (Table-7)

Table-7: Hydrogen bond interaction parameters for each compound and APP residues

Ligands	Residue atom involved	Ligand atom involved	H-bond distance (Å)
Galantamine	ARG20: HH12	O1	2.1
	ASN44: O	HO	2.1
	ARG20: HH22	O3	2.2
Rivastigmine	ASP46: OD2	H1	2.2
	ASN44: O	HO	1.9
Tacrine	ASP46: OD1	HN	2.5
	ASP46: OD2	HN	3.1

4.1.3 Docking of ligands on PS1 receptor

The docking of Galantamine on PS1 shows h-bond interactions with residues ALA461, TYR466. The docking of Donepezil on PS1 shows h-bond interactions with residues LYS395, ALA398 and THR399. The docking of Rivastigmine on PS1 shows h-bond interactions with residues SER397 and ALA398. The docking of Tacrine on PS1 shows h-bond interaction with residues GLU341 and GLU356. (Table-8)

Table-8: Hydrogen bond interaction parameters for each compound and PS1 residues

Ligands	Residue atom involved	Ligand atom involved	H-bond distance (Å)
Galantamine	ALA461: O	H1	2.0
	TYR466: O	HO	1.8
Donepezil	LYS395: 2HZ	O1	2.6
	ALA398: OG1	HO	2.0
	THR399: O	H1	1.6
Rivastigmine	SER397: O	H2	1.9
	ALA398: O	H2	2.3
	ALA398: O	HO	1.8
	GLU341: OE1	H1	1.7
Tacrine	GLU341:OE1	H1	2.1
	GLU341:OE1	H2	2.2
	GLU356:OE1	HN2	1.9

Ligand interaction figures

The best docking result of each docking were imported to PYMOL that had the ligand and receptor residues those were within 6Å area of the ligand. The ligand was selected and polar contacts were detected to the acive site residues. The ligand interaction figures for docking of Galantamine, Donepezil, Rivastigmine and Tacrine on APOE active site is represented in figure-6. The ligand interaction figures for docking of Galantamine, Donepezil, Rivastigmine and Tacrine on APP active site is represented in figure-7. The ligand interaction figures for docking of Galantamine, Donepezil, Rivastigmine and Tacrine on APOE active site is represented in figure-8.

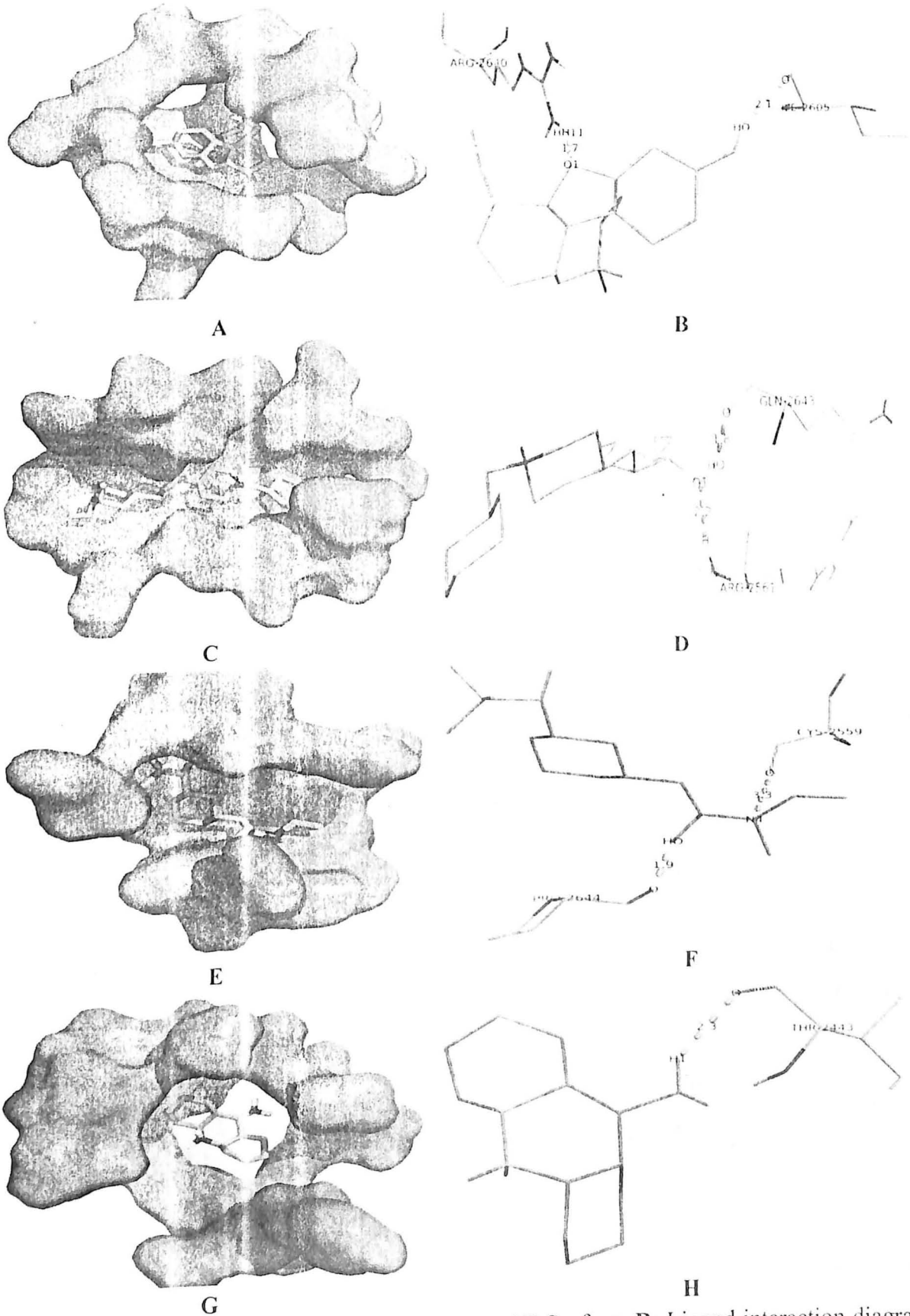


Figure-6: **A**: The docking of Galantamine on APOE Surface, **B**: Ligand interaction diagram for docking of Galantamine on APOE, **C**: The docking of Donepezil on APOE surface, **D**: Ligand interaction diagram for docking of Donepezil on APOE. **E**: The docking of

Rivastigmine on APOE surface, **F**: Ligand interaction diagram for Rivastigmine on APOE. **G**: The docking of Tacrine on APOE surface, **H**: Ligand interaction diagram for Tacrine on APOE.

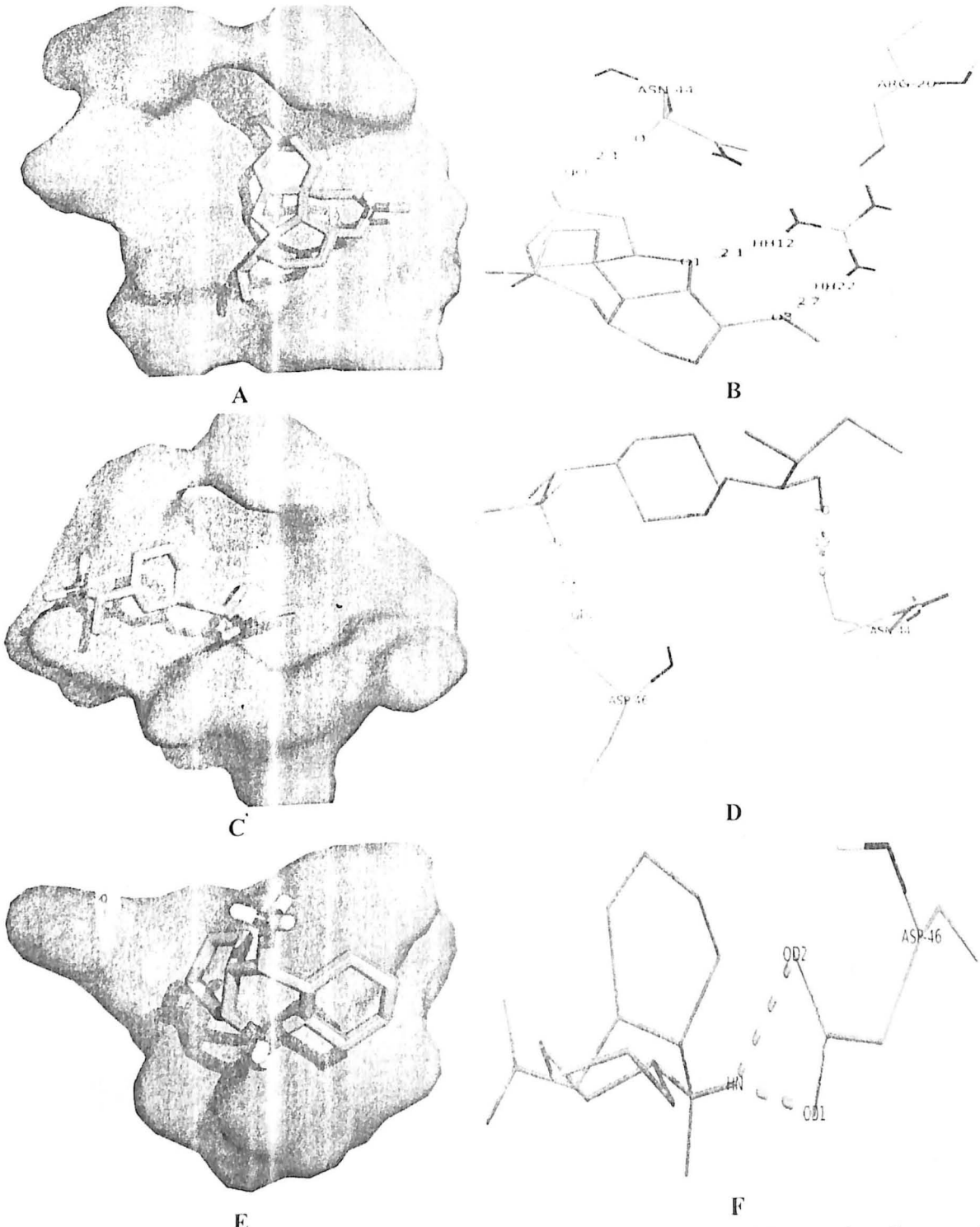


Figure-7: **A**: The docking of Galantamine on APP Surface, **B**: Ligand interaction diagram for docking of Galantamine on APP, **C**: The docking of Rivastigmine on APP surface, **D**: Ligand interaction diagram for Rivastigmine on APP, **E**: The docking of Tacrine on APP surface, **F**: Ligand interaction diagram for Tacrine on APP.

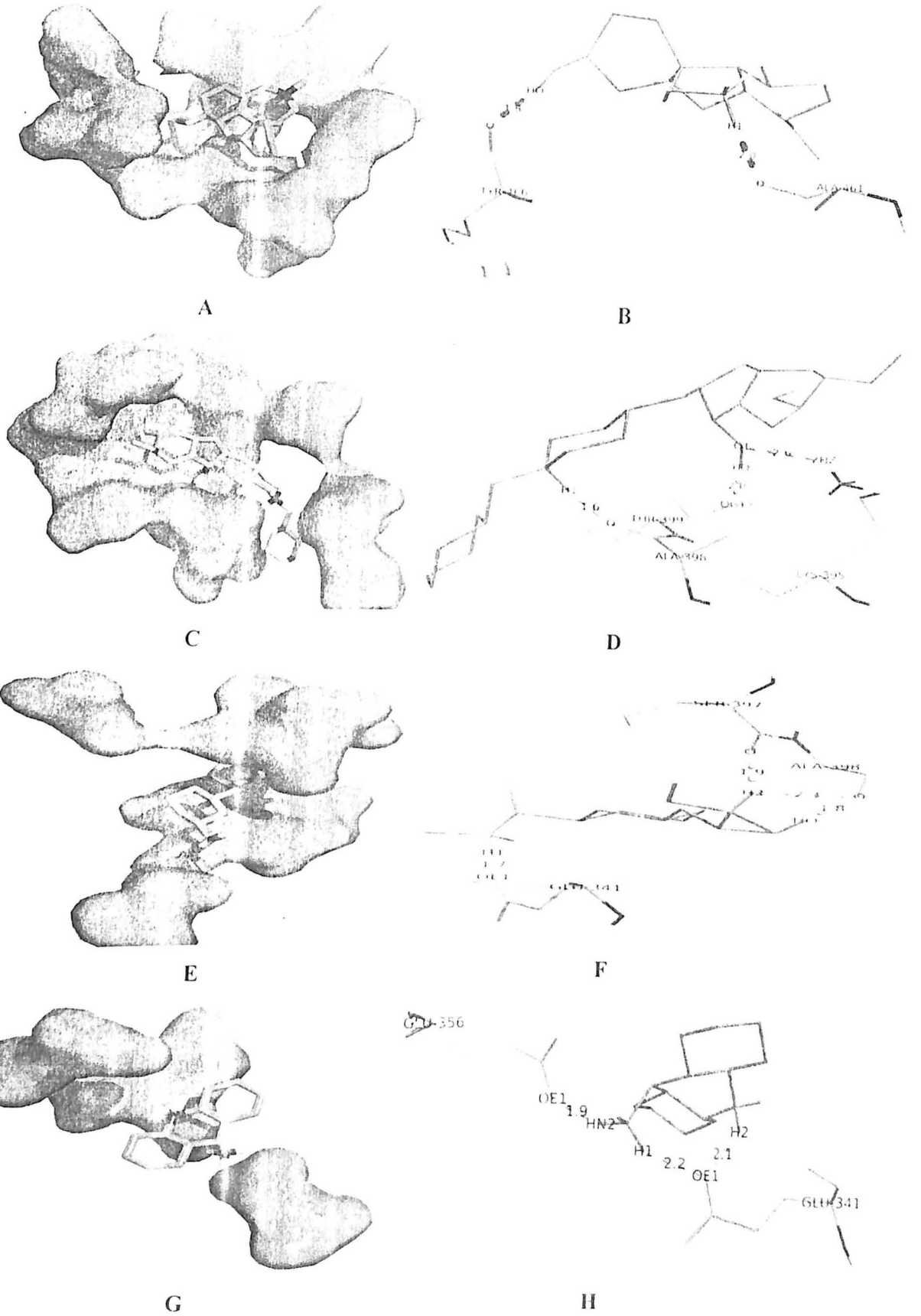


Figure-8: **A**: The docking of Galantamine on PS1 Surface, **B**: Ligand interaction diagram for docking of Galantamine on PS1, **C**: The docking of Donepezil on PS1 surface, **D**: Ligand interaction diagram for docking of Donepezil on PS1. **E**: The docking of Rivastigmine on

PS1 surface, **F**: Ligand interaction diagram for Rivastigmine on PS1. **G**: The docking of Tacrine on PS1 surface, **H**: Ligand interaction diagram for Tacrine on PS1.

4.2 DISCUSSION

In the molecular complex binding process, both the complementarily receptor-ligand binding site and the steric of ligand in the complex are the key factors. The electrostatic, Van der Waals, hydrogen bonding, hydrophobic interactions are the driving force behind the formation of molecular complex. The docking of four acetyl cholinesterase inhibitors revealed some interesting results. The residues ARG2640, ILE2605, GLN2643, ARG2561, CYS2559, PRO2644 and THR2443 located in the active site pocket of AOPE receptor were important as many atoms from them were involved in h-bond formation with the ligand atoms. The residues ARG20, ASN44, ASP46 located in the active site pocket of APP receptor were important as many atoms from them were involved in h-bond interaction with the ligand atoms. Similarly the residues ALA461, TYR466, LYS395, ALA398, THR399, SER397, GLU341 and GLU356 located in the active site pocket of PS1 receptor were important as many atoms from them were involved in h-bond formation with the ligand atoms.

During our docking studies we have selected the model of receptors from the PDB. We have also used the active site information present it the structure file for ligand interaction studies. The overall result were quite interesting as all four reported acetyl cholinesterase inhibitors (Galantamine, Donepezil, Rivastigmine and Tacrine) revealed some good interaction with the active site residues of three receptors (APOE, APP and PS1). Again we could not found a suitable docking of Donepezil on APP. This may be due to the failing of h-bond donor and h-bond accepter atoms of both ligand and receptor to come a near proximity to build the hydrogen bond.

We also found out some new residues in the active site pockets of the receptors other tan the predicted ones as reported from the receptor structure. This residue information can be used for further high throughput screening of large scale ligands, QSAR (2d and 3D), pharmacophore modeling and structure based drug designing.

SUMMARY

5 SUMMARY

In our current *in silico* this study, the docking of four acetyl cholinesterase inhibitors (Galantamine, Donepezil, Rivastigmine and Tacrine) on 3D structure of APOE, APP and PS1 revealed some important h-bond interaction with both reported active site residues with some new residues. These docking models bounded with four important inhibitors revealed novel insights in the mode of inhibitory action and may provide basis for the design of new acetyl cholinesterase inhibitors for treatment of AD.