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The Discovery of Potential Acetyl Cholinesterase Inhibitors: A Combination of Virtual Screening and Molecular Docking Studies

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ABSTRACT

In present scenario there are different terminologies which are used for categorizing the branches of computer aided drug designing like molecular modeling, theoretical chemistry, bioinformatics, computational chemistry, chemo informatics, computational biology, etc fundamental scientific developments in this field has shaped up even before computational sciences emerged as a prominent field in engineering. Alzheimer disease is the most widespread origin of mental turn off, or dementia. But dementia too has various additional causes. The description of this disease for the first time was given by German psychiatrist as well as neuropathologist named Alois Alzheimer in year 1906 and so it was named after him.Rapid memory loss is familiar in public older than 60. The depositions of amyloid are known to be as "plaques" and it causes the brain cells to shrivel up and form "tangles", which will lead to changes in the brain structure and causes the brain cells to die. Treating medical circumstances which may contribute to perplexity or physical decline eg: anemia or lung disease, cheering social interface to help put off feelings of seclusion and depression, for tumbling misunderstanding normal habitual must be optimistic. Acetylcholinesterase (AChE) plays an significant function in Alzheimer's disease (AD).The extreme action of acetylcholinesterase causes a variety of neuronal troubles predominantly dementia as well as neuronal cell deaths.The tools and methodology which are used in this project are FASTA ,BLAST, Phyre2, Easy Modeller, AutoDock and ADMET. The main tools used are AutoDock for the docking of specific protein and ligands whereas ADMET is used for the toxicity check.The conclusion for this project is that β amyloid and abnormal tau protein is responsible for this disease and acetyl cholinesterase is a key inhibitor which is used for the treatment of this disease. The drugs used in this project may compensate for the death of cholinergic neurons and offer symptomatic relief by inhibiting acetylcholine turnover as it directly interact with amyloid β in a manner that increases the deposition of this peptide into insoluble plaques. After homology modeling we will get the final structure of our protein. The docking will be carried out with the best ligand. This drug will be helpful in the treatment of Alzheimer's disease.

Keywords: *computational biology, neuropathologist, Alzheimer, Easy Modeller, AutoDock*

1.1 AN INTRODUCTION TO COMPUTER AIDED DRUG DESIGN

One of the fundamental rational drug design method is Computer aided drug design (CADD). Discovery of medicines were very much superdipitous or done by blind experiments in earlier days of pharmaceutical researches. With the help of modern research methods, scientists are now able to identify the actual causes of the disease. With the help of that credible knowledge, research can be done for its cure (Kapetanovic and I M, 2008).

1.1.1 Branches of computer aided drug design

In present scenario there are different terminologies which are used for categorizing the branches of computer aided drug designing like molecular modeling, theoretical chemistry, bioinformatics, computational chemistry, chemo informatics, computational biology, etc fundamental scientific developments in this field has shaped up even before computational sciences emerged as a prominent field in engineering. Comparison with several other

branches of science, the enhancement in computer technology has been revolutionized the look (Kapetanovic and I M, 2008).

1.1.2. History of Computer Aided drug design

Although computer aided drug designing (CADD) itself doesn't have any history, its foundations of discipline was laid more than a 100s of years back (Khan et al., 2012). The experiment to apply mathematics in explaining and calculating chemical property has provided momentum for the earliest developments in computational chemistry. This branch, which can be refer to as theoretical chemistry, in CADD it is served as the foundations for progress. Once it was confirmed that svarious chemical phenomena can be explained by using several mathematical equations, scientists digged further into the mysteries of the chemical world using mathematics as the tool. Later, theories of quantum mechanics, molecular orbital's and electron provided a solid understanding of chemistry of the molecules (Khan et al., 2012).

1.1.3. Medicines as legal drugs

It is often seen that for any particular problem there is a medicine for it. Medicines are legal drugs, doctors are allowed to prescribe these legal drugs for patients. Stores can sell them, and people are allowed to buy those drugs. But it is neither legal nor safe, for people to use these medicines in any manner they want or to buy them from people who are selling them illegally (Kapetanovic and I M, 2008).

1.1.4. Illegal Drugs

There are some drugs which are sold in market which are illegal for example, LSD, marijuana, cocaine, ecstasy, crystal meth, and heroin. Illegal drugs are not good for any person, but these drugs are particularly bad for teens as well as for kids also whose body is still growing. These drugs can damage the brain, heart and several other important body organs (Kapetanovic and I M, 2008). The drug which can cause a heart attack in a teen or a kid is cocaine. With the use of these illegal drugs, people's performance starts decreasing in sports, schools and other activities. Not only this, but also using these drugs often creates difficulty in thinking clearly and also in making good decisions (Khan et al., 2012).

1.1.5. Types of Drug Designing

Basically there are four different methodologies which are commonly used in the drug designing.

- **Ligand based drug design(indirect drug design)**

  It relies on knowledge of molecules that binds with the biological target of interest, which may be used for deriving a pharmacophore model which will be helpful in defining the minimum necessary structural characteristics which a molecule must consists in order to bind to the target.

- **Structure based drug design(direct drug design)**

  It usually depends on knowledge of the three dimensional structure of a biological target which can be obtained through various methods such as X-ray crystallography and NMR spectroscopy. By using structure of a biological target molecule, candidate drugs can be predicted which will bind with high affinity and selectivity to the target. Interactive graphics and intuition of a medicinal chemist can be further used in this drug design process.

- **Rational drug design**

  It starts with a hypothesis which explains that modulation of a specific target molecule may have therapeutic values as compared to traditional design which depends on trial and error.

- **Computer assisted drug design**

  It basically uses computational chemistry for discovering, enhancing, or to study drugs and related biological active target molecules. The most fundamental goal of this concept is to predict the binding affinity of a given molecule to the target molecule and its associated binding kinetics.

Fig 1.1: Drug Design Process

(Rathinasabapathy, T et al., 2011)

1.1.6 Introduction of Alzheimer's disease

Alzheimer disease is the most widespread origin of mental turn off, or dementia. But dementia too has various additional causes. Alzheimer's disease damages the brain. It causes a sturdy loss of memory and how well it can verbalize, imagine, and do your on a daily basis work. It gets inferior over time, but how hastily this happens varies (Mehta et al., 2012).

1.1.6.1. Causes of Alzheimer's disease: Alzheimer's diseases occur due to changes occurring in the brain. Some of the deterioration may be related to loss of chemical messengers in the brain, called neurotransmitters that allow nerve cells in the brain to communicate properly (Hardy et al.,2002)The body attempt to stop this process by producing a protein known as amyloid. However, amyloid deposits assemble in the brain, which leads to further deterioration (Mejta et al.,2012). These depositions of amyloid are known to be as "plaques" and it causes the brain cells to shrivel up and form "tangles", which will lead to changes in the brain structure and causes the brain cells to die. There are several factors which may play an important role in the development of the condition. (Rosen et al., 1989)

- **Genetic factors,** such as the presence of, or changes to, certain genes
- **Environmental factors,** such as long term exposure to some environmental solvents or infection with certain viruses or bacteria
- **Lifestyle factors,** such as lack of exercise, poor quality sleep and a diet lacking fruit and vegetables
- Increasing age, Down syndrome, history of head injury, obesity, high blood pressure, high cholesterol, insulin resistance, family history of Alzheimer's disease, risk factors for blood vessel disease such as smoking (Rosen et al., 1989).

1.1.6.2. Symptoms of Alzheimer's disease

- Have trouble making decisions
- Be confused about what day and time it is
- Get lost in places you know well
- Have more trouble doing daily tasks like cooking a meal or paying bills
- Increasing short term memory loss
- Loss of appetite
- Troubles with conceptual judgment
- Extreme alteration in individuality
- Incomprehension to moment and position

Even though a person loses its many abilities when disease progresses, it is frequently helpful to edge on the abilities that continues, such as the sanity of touch, the ability to respond to emotion and audible range.

1.1.7. Diagnosis of Alzheimer's disease

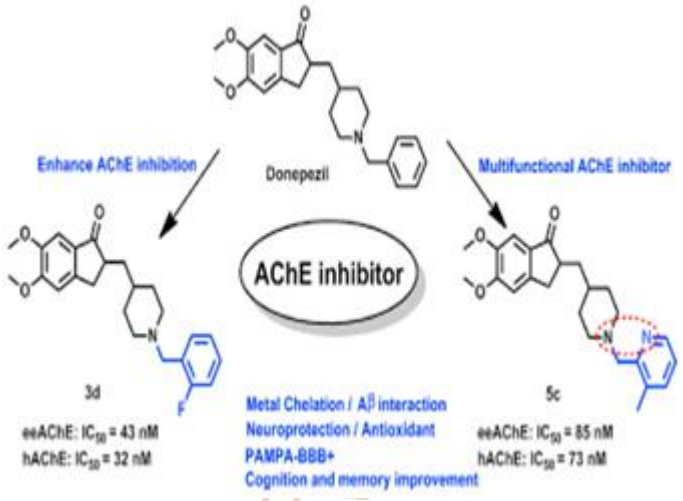
For the diagnosis of Alzheimer's disease there is no single test. For the other possible causes there is a full assessment of medical and psychological history. For obtaining a conclusive diagnosis it requires a variety of tests which are:

- Brain scans
- Mental status assessment for determining the level of mental deterioration

- Physical and neurological examination
- Caregiver interview for determining the level of dependency
- Blood and urine tests

For checking brain structure and its function scans may be recommended. Different types of scans which may include:-

- CT scanning(computerized tomography)
- MRI (magnetic resonance imaging)
- PET(positron emission tomography)



Mechanism of action of Acetylcholinesterase inhibitor

Fig 1.2: Mechanism of action of Acetylcholinesterase inhibitor

1.1.8. Epidemiology

Rates after age 65

Age	New affected per thousand person- years
65-69	**3**
70-74	**6**
75-79	**9**
80-84	**23**
85-89	**40**
90-above	**69**

Table 1: Epidemiology rates after age 65

There are two main measures which are used in epidemiological studies. Incidence is the number of new cases per unit of person-time at risk (usually number of new cases /1000 person- years). Prevalence is the total number of cases of the disease in the population at any given time (Mehta et al., 2012)

1.1.9. Treatment and Medications

Treating medical circumstances which may contribute to perplexity or physical decline e.g., anemia or lung disease, cheering social interface to help put off feelings of seclusion and depression, for tumbling misunderstanding normal habitual must be optimistic (Hardy et al., 2002).

Medications such as sleeping tablets and tranquillizers may help to control to control symptoms such as sleeplessness and agitation. A cluster of medications known as cholinesterase inhibitors have revealed some efficacy in slowing the succession of the stipulation in some people. More recently, another drug named memantine is available in New Zealand (Mehta et al.,2012, Rosen et al., 1989).

1.1.10 Drugs widely used for Alzheimer's disease

- Aricept is the merely cure which is permitted through the FDA for all stages of Alzheimer's disease: gentle, sensible, as well as brutal. This drug can be taken as a pill which gulp down or dissolves in our mouth.

- Razadyne (previously known Reminyl) is as well for gentle to modest Alzheimer's. It can obtain it as a capsule which works accurately, a tablet that gives off the tablets gradually, and in liquid forms.

- Exelon is for public who contain gentle to reasonable Alzheimer's. We can spot a skin scrap which has the drug, or get it in capsules as well as in liquid form.

- Memantine (Namenda) treats modest to brutal Alzheimer's ailment. It works by means of altering the quantity of an intellect chemical known glutamate, which act as a task in erudition as well as recall. Brain cells in public through Alzheimer's disease provide inedible too much glutamate. Namenda keeps stages of that chemical in test. It might perk up how well the brain performsas well as how finely several people can do daily errands. The remedy may effort even superior when we obtain it through Aricept, Razadyne or Exelon. Namenda's side effects comprise of fatigue, faintness, puzzlement, constipation, as well as annoyance (Jackson and G.A., 2014).

- **Namzaric .** This medicine is a blend of Namenda as well as Aricept. It's most excellent for public with

modest to brutal Alzheimer's who by now acquire the two drugs discretely (Jackson and G.A., 2014).

1.2. Review of literature

In the present scenario the most sensitive global issue is the Alzheimer's disease and the reason behind this is the rise in social impact and increase in its economic cost (Wischik et al., 2014).The description of this disease for the first time was given by German psychiatrist as well as neuropathologist named Alois Alzheimer in year 1906 and so it was named after him (Ahmad and J, 2013, Samant et al) . Dementia which is a problem of memory loss it is related with old age, but in case of Alzheimer's disease it is problem of neurodegeneration (Rasool et al.,2015). The primary event in the Alzheimer's disease is oxidative stress so for this neurodegenerative disorder the most promising step could be the therapy which could based on antioxidant (Nisha et al.,2016). The plant which was took for the study of Alzheimer's disease *Moringa oleifera*. It is estimated over 50-60% cases of dementia in person occur over 65 years of age. Moringa oleifera is inhabitant to sub-tropical areas of countries like India, Pakistan, Bangladesh and Afghanistan and it is also consumed in the form of food (Samant et al., , Lagunin et al., 2014). The special class of molecules of AD with its quality and quantity are expected that they can grow at very faster rate in future, but we really need to thanks to rapid advancement in technologies of biophysics, medicinal chemistry, biochemistry and pharmacology (Ahmad and J, 2013). There are various databases which have been reported for exploring the molecular mechanism of Alzheimer's disease namely AlzGene and Alzpathway (Rasool et al., 2015). AD described its association with low density lipoprotein receptor related protein (LRP) in biochemical and genetic studies. Due to the unfavorable pharmacokinetics and pharmacodynamics of drugs the frequent failure of therapy occurs (Liu et al., 2012). Acetylcholinesterase (AChE) plays an significant function in Alzheimer's disease (AD).The extreme action of acetylcholinesterase causes a variety of neuronal troubles predominantly dementia as well as neuronal cell deaths. Normally, anti-AChE drugs tempt various solemn neuronal side effects in humans. Consequently, this study required to recognize substitute drug molecules as of natural goods by revenue of smaller side effects than those of predictable drugs for curing AD (Subramaniyan et al., 2017). A variety of approaches comprised to augment cholinergic transmission in Alzheimer's disease (Tsolaki et al., 2001).

1.2.1. Approaches in the treatment of Alzheimer disease

There are various pathogenic mechanism used for AD which includes oxidative stress (antioxidant therapy), amyloid cascade (Aβ) vaccine, loss of cholinergic functions (neutropins and cholinergic replacement therapy), γ secretase inhibitors, role of dietary factors (Gupta et al., 2010).

1.2.2. Alzheimer disease pathophysiology

The major neuronal death and synaptic loss which is observed in the brain regions responsible for cognitive fuctions of AD is histopathologically characterized , includes the entorhinal cortex, ventral striatum, the cerebral cortex and hippocampus (Gupta et al., 2010). There are two hypothesis which have been proposed for pathophysiology and etiology studies of Alzheimer disease: the first hypothesis refers to the neurodegeneration of amyloid cascade whereas the second hypothesis refers to the malfunction of the cholinergic system which contains metal- mediated toxicity, tau aggregation and inflammation (Singh et al.,2014, Gupta et al., 2010, Nisha et al., 2016).

According to the neurodegeneration of amyloid cascade hypothesis, Alzheimer disease attars to begin with the proteolytic cleavage of the amyloid precursor protein (APP) and it also results in the production, aggregation, and the deposition of amyloid plaques and β-amyloid (Aβ) (Singh et al., 2014, Cummings et al., 2016).

Cholinergic hypothesis, the malfunction of the cholinergic system which is enough for producing a memory deficit in animal models which is similar to Alzheimer disease. The activities of choline acetyltransferase (ChAT) and (AChE) were reduced in the cerebral cortexes of patients with Alzheimer disease (Cummings et al., 2016).

The enzyme which works for the conversion of phosphatidylcholine to choline and synthesis of chemical mediators of inflammation is Phospholipase A2 (PA2).

1.2.3. Role of AChE in AD

AChE plays a very important role in cholinergic transmission at the both peripheral nervous system as well as central nervous system (Gupta et al., 2010).

Various inhibitors of AChE can work through cholinergic pathway. Inhibitors of AChE can target any step which consists of glycosylation, trafficking and phosphorylation (Gupta et al., 2010, Shineman et al., 2011).

1.2.4. Achetylcholinesterase Enzyme

AChE contains two types of polypeptide chains and each are present twice with molecular weight of 32 kDa. It has very high catalytic activity and its every compound degrades about 5000 compounds of ACh per second (Gupta et al., 2010). Inhibition of AChE was measured to be pragmatic as a counteractive objective because of recognized inhibition of secondary AChE as a heal for myasthenia gravis (MG) proving that the advance was achievable (Mehta et al.,2012).

1.2.5. Neuroinflammation: The main causes of neuroinflammation in AD which is very well known are both NFT and Aβ fibrils. Inflammatory gene expression are activated inneurons in and around senile Aβ plaques and in microglia that are mainly regulated by Mitogen-activated protein kinase (MAPK) cascades (p38 module) (Singh et al., 2014).

1.2.6. Small molecules as inhibitors

Small molecules which efficiently and specifically inhibit Aβ aggregation, it can be used as therapeutic agents for Alzheimer,s disease (Shineman et al.,2011). Berberineand its analogues, Congo red as well as β-sheet breaker peptide are well-known inhibitors of Aβ aggregation (Singh et al.,2014, Gupta et al.,2010). The change in absorbance spectrum of the dye upon binding to Aβ fibrilshas been proven by Binding of CR to Aβ fibrils. The potential therapeutic agent which has been suggested against Alzheimer's disease is CR, as it can bind to critical intermediate structural forms of Aβ. The inhibitor which has shown to be useful in the treatment of AD is Dipeptide-derived inhibitors of BACE1 enzyme (Singh et al., 2014).

1.2.7. B-site APP cleaving enzyme (BACE)

Aβ results from APP via proteolytic cleavage by two enzymes: BACE and γ-secretase. The large type 1transmembrane protein which is acting as a precursor for amyloid formation is APP (Gupta et al., 2010).Because of the important role of BACE1 in Aβ formation, BACE1 appears as a primary target which helps in preventing Aβ formation in brain. The new aspartic protease which helps in initiating Aβ formation from APP is BACE1 (Gupta et al.,2010). Other transmembraneaspartic protease which is homologous to BACE1 is BACE2. BACE1 has been reported as the key enzyme as it initiates the formation of Aβ, and it also exhibits the functional characteristics of β-secretase (Cummings et al., 2016).. In the knockout mice it has been observed that complete disruption of BACE1 may result in some physiological as well as behavioral changes (Nisha et al., 2016). Earlier results of familial Alzheimer disease occurs because of mutation in the following three genes (APP, PSEN1, PSEN2), which results in the formation of Aβ, mainly composed of 42 amino acids (Ahmad and J, 2013).The mechanism of BACE mRNA recognition can be inhibited utilizing antisense action of small inhibitory nucleic acids (siNAs). This strategy can includesmall interfering RNAs (siRNAs), catalytic nucleic acids andantisense oligonucleotides (Shineman et al., 2011).

1.2.7.1. γ-secretase

The γ-secretase enzyme may be a potentially attractive drug target because it has an important role in the production of Aβ fragments by creating peptides of various lengths, namely Aβ40 and Aβ42 (Singh et al., 2014). It comprises of a molecular complex which has four integral membrane proteins namely:nicastrin, presenilin (PSEN), PEN-2 and APH-1 (Singh et al., 2014, Ahmad and J, 2013).It has been observed that NSAID analogues preferentially inhibit Aβ formation and it does not affect the Notch processing and other developments; so these NSAID analogues may be serve as a lead for optimizing the biological activity or starting point for future drug development against AD (Singh et al.,2014).

1.2.7.2. Aβ and Aβ fibrils

Alzheimer's disease is discriminated by immense extracellular collection of Aβ plaques which is preferably composed of Aβ peptide aggregates, as well as intracellular accumulation of hyperphosphorylated tau protein in the cerebellar region.α- and β-secretase are bthe two competing enzymes which are used to cleave APP, these are specific in their site of cleavageand siteof proteolysis. α-cleavage is catalysed by several enzymes followed by γ-secretase in normal individuals and in case of Alzheimers disease it causes cleavage of APP so that it can generate small non-amyloidogenic fragments, however, the BACE1pathway involves sequential cleavages of APP by the BACE1 and γ-secretase

complexes and generates Aβ (Singh et al., 2014, Gupta et al., 2010). Secretion of Aβ from the cell into the extracellular space there it undergoes aggregation. The major source of Aβ production in the central nervous system (CNS) is neurons. For the formation of Aβ fibrils these Aβ peptides have the capability to self-aggregate. The C-terminal residues of Aβ are highly hydrophobic and clump together due to hydrophobic-hydrophobic interactions (Shineman et al., 2011, Lagunin et al., 2014). For the stability to the structure of Aβ fibrilsthis hydrophobic contact is providedare the major source of Aβ production. Deposition of these Aβ fibrils in the brain causes inflammation, apoptosis and problems in impulse conduction resulting in memory loss and recognition problems.Aβ is used as a potential disease-modifying, therapeutic target for Alzheimer disease, and drugs directed against Aβ were found to speed up the elimination of Aβ from the brain (Lagunin et al.,2014) . The advanced monoclonal antibody which is directly targeting Aβ is Bapineuzumab, as it appears capable of reducing the cerebral Aβ peptide burden in AD patients; however, after phase II trialsits ability to slow disease progression remains uncertain and potentially severe adverse effects may limit its applicability (Singh et al.,2014, Samant et al.,).

1.2.8. Aβ immunotherapy

An Aβ vaccine with the name AN1792 consists of synthetic aggregated Aβ1-42 peptide and the surfaceactive saponin adjuvant, QS-21. It was found thatthe extent of Aβ removal is strongly correlated with mean anti-Aβ antibody titers as Aβ antibodies reduce the Aβ load. The vaccines which activate T helper cells (TH1) are Aβ vaccines as it promote humoral immunity and suppress autoimmune encephalitis T-cell epitopes which have been reported on the C-terminal of the peptide (15-42 amino acids). It is believed that the C-terminus of the peptide is responsible for triggering meningoencephalitis in the AN1792 trial. Active vaccination of AN-1792 was developed but it has immunological sideeffects.

1.2.9. Pharmacokinetics/Pharmacodynamics, ADME-Toxicology

Studies should include pharmacokinetics (PK) and pharma co dynamics (PD) assessments to determine whether the compound exposure is sufficient and whether it is interacting with the target of interest. Depending on whether a study is exploratory or therapeutics, the degree to which absorption, distribution, metabolism, excretion, and toxicity (ADMET) are profiled should be considered as part of the prospective study design (Fonseca-Santos et al., 2015).

1.2.10. Repurposed drugs

Drugs that have been approved for other indication are repurposed agents but they may havesome pharmacological effects which are relevant to the treatment of Alzheimer's disease (Cummings et al., 2016). In AD repurposed agents have some possible effects included, but they are not limited to, anti-hypertensives, statins, cancer treatment agents, and anticonvulsants (Shineman et al., 2011). For the AD drug development timeline repurposed agents have the potential of accelerating. They have already been through preclinical toxicology assessments;

- Phase 1 human safety, tolerability and pharmacokinetic assessments;
- Phase 2 safety and efficacy studies for the original indication;
- Phase 3 studies for the original indication, and regulatory review for the original indication.

Development of a repurposed agent for use in the AD field could begin with a Phase 2 proof-of-concept and dosing study for AD, thus avoiding the time and expense of preclinical development and Phase 1 (Cummings et al., 2016 , Langunin et al., 2014).

1.2.11. Diagnosis and treatment of AD

AD is a clinical diagnosis; however, the diagnosis of Alzheimer's disease is depend on the depressiondiagnosis, which occurs in approximately 30%–50% of patients with AD (Lu et al., 2012). Motivational disturbancesare more often features in patients committed with ADis depression, such aspsychomotor slowing, fatigue, and apathy, whereas depression in geriatric patients without cognitive impairment tends to feature mood symptoms, such as, suicidality, depressed mood, anxiety, and disturbances in sleep and appetite (Singh et al.,2014, Muthusamy et al., 2016). Instruments which are commonly used for assessing depression were designed for use in other patient populations and may be less reliable in patients with AD.

For the diagnosis of a AD techniques are ancillary imaging studies, such as computed tomography (CT) or magnetic resonance imaging (MRI) and, in selectedcases, single-photon emission CT (SPECT) or

positron emission tomography (PET). Brain MRI or CT scanning has indicated the use of structural neuroimaging to detect lesions that may result in cognitive impairment (Singh et al., 2014, Langunin et al., 2014).

For the detection of amyloid peptides (Aβ), nanotechnology is used as a diagnostic tool depends on which are used as targets in the development of biological markers for the diagnosis of AD (Muthusamy et al., 2016).

Materials and methodologies: Tools used are:

- NCBI (National Center for Biotechnological Information)
- FASTA (Fast Alignment)
- BLAST (Basic Local Alignment Search Tool)
- PDB (Protein Data Bank)
- Phyre2
- Easy Modeller
- ModLoop
- Swiss PDB Viewer
- Drug Bank
- AutoDock
- AutoDock Vina

2.1. TOOLS

2.1.1. NCBI

The National Centre for Biotechnological Information (NCBI) a multi-disciplinary research group which is served as a resource for molecular biology information. It was first time formed in 1988 as a complement to the activities of the National Institute of Health (NIH) and the National Library of Medicine (NLM). It is basically located in Bethesda, Maryland, USA. NCBI's creation was intended so that we aid in understanding the various molecular mechanisms which affect human health and diseases with the several goals: to create and maintain public databases, build up software to analyze genomic statistics, and to accomplish research in computational ecology. In time, and with broad exploit of Internet, NCBI has turn out to be progressively more conscious with the function of unadulterated biological research. Molecular biology has turn out to be as significant as biomedical research. This was evidently seen as a variety of expert databases were being formed by NCBI, so that we can balance those categories which dealt openly with human health and disease. NCBI in progress of compliance services also:

They started increasing novel methodologies so that they can compact with capacity as well as complexicity of individuals information that are researching with methods which assist in analyzing the construction as well as purpose of macromolecules

For storing and analyzing data of molecular biology they started creating computerized systems.

They are providing access for analysis as well as computing tools (which can facilitate the use of various biological databases and softwares) for researchers and public.

In the development of databases, NCBI forms standard of its databases such as database nomenclature which can also be use by several other non-NCBI databases. GenBank is one of NCBI database, which is a nucleic acid sequence database which consists of sequence information from over 200,000 diverse organisms. It is perhaps one of the most popular database which is in use, and it essentially predates NCBI.

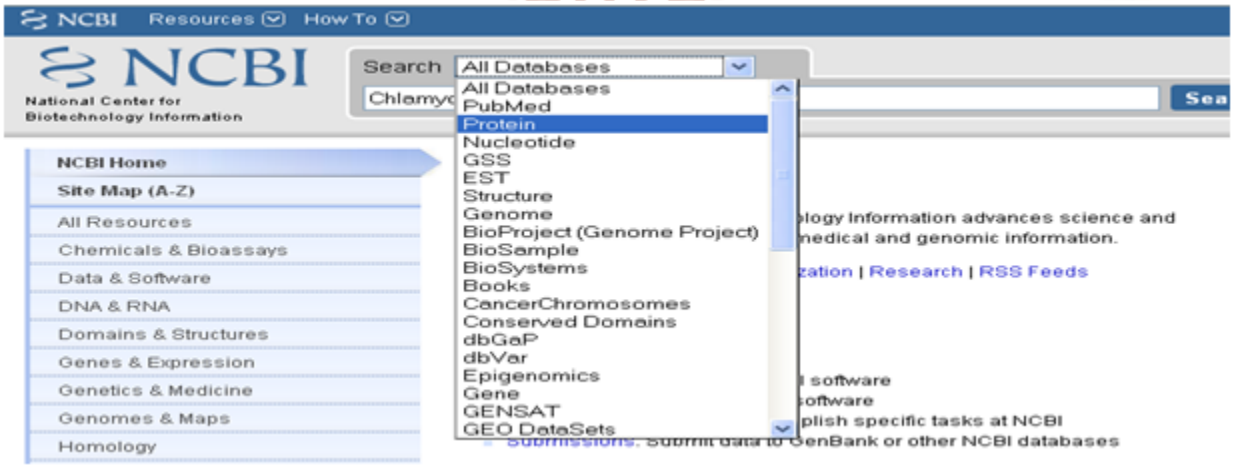


Fig: 2.1 NCBI homepage

2.1.2. FASTA Sequence database similarity search

FASTA is a similarity search program for rapid alignment of protein pairs and DNA sequences. Instead of comparing individual residues in the two sequences, it searches for similar sequence pattern or expressions, called k-tuples. These patterns contain k repeated matches of letters in both sequences. The process then attempts to make a local alignment which is based on these word matches. Because of the capacity of the algorithm to search for matching sequences in a sequence database with elevated speed, FASTA is functional for usual database search of this type.

Method which is comparable with FASTA program is the BLAST program, which works faster than FASTA, and is comparable compassion for protein sequence or queries, and also DNA searches, and programs which use Smith Waterman dynamic programming algorithm for protein and DNA searches, which are slower but more susceptible when full-length of protein sequences are used as queries.

2.1.3. BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST)

BLAST is a local alignment search software tool in which two sequences are aligned in order to decide whether there is sequence similarity exists between two sequences. These sequences can be either two protein sequences or two nucleotide sequences. From the sequence similarity of protein or nucleotide sequence, homology can be inferred, although there is the discrete difference between the two sequences. Homology indicates that the sequences studied came from a common ancestral sequence. There are different statistical models which exist for protein sequences. NCBI offers a diversity of BLAST-based tools so that we can analyze different data types. Instead of using BLAST to sustain a conclusion about homology between two sequences, it is possible that we can BLAST a nucleotide or protein query sequence against human genome or the mouse genome to appear for homologous sequences.

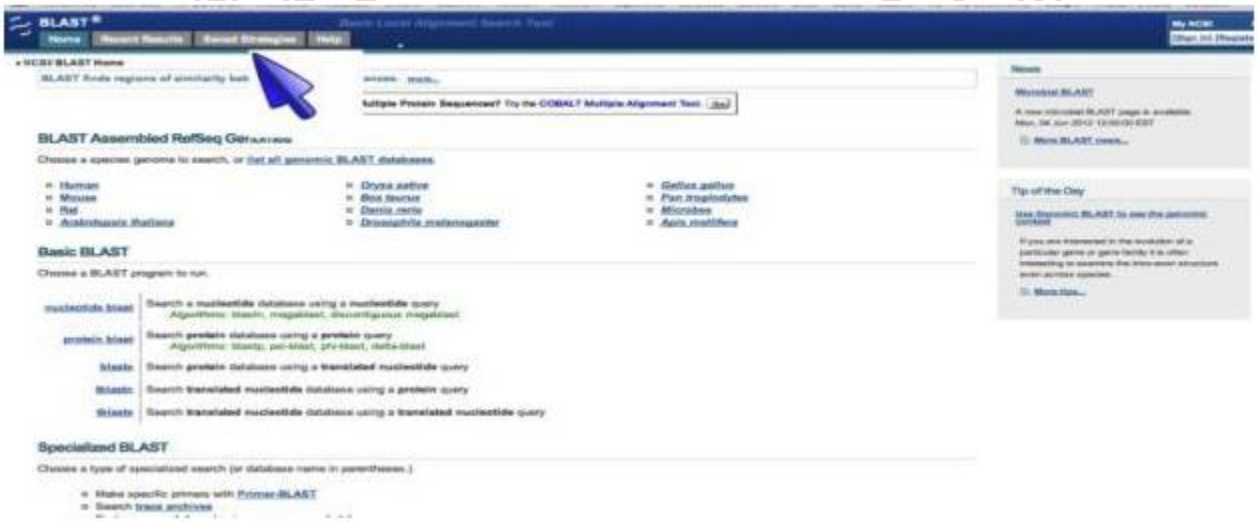


Fig: 2.2 BLAST homepage

2.1.4. PROTEIN DATA BANK

Protein Data Bank files information about proteins, nucleic acids, and difficult assemblies 3 dimensional shapes which help students and researchers for recognizing all aspects of biomedicine and agriculture, from synthesis of protein to health and disease.

The RCSB PDB collects the data by developing tools for students and research in field like structural biology, molecular biology, computational biology, and further than.

Fig: 2.3 PDB homepage

2.1.5. PHYRE2

Phyre2 is a tool accessible on the net to calculate and evaluate protein structure, function and mutations occurring on it. The center of Phyre2 is to build accessible biologists with a simple as well as intuitive boundary to state-of-the-art protein bioinformatics tools. Phyre2 replaces Phyre, which is the new description of the server for which we prior published a paper in Nature Protocol. In this restructured protocol, we demonstrate Phyre2, which uses vastly developed remote homology detection methods to construct 3D models, which predict ligand binding sites and analyze the effect of amino acid variants (e.g., nonsynonymous SNPs (nsSNPs)) for a user's protein sequence. Users are guided through results by a simple interface at a level of detail they determine. This procedure will direct users from submitting a protein sequence to interpreting the secondary as well as tertiary structure of their models, their field composition in addition to model superiority.

The compilation of obtainable tools is described which is worn for finding the construction of protein available in a genome, so that we can submit huge quantity of sequences at once and for manually running weekly searches for proteins which are complicated to model. The server for Phyre2 is available at http://www.sbg.bio.ic.ac.uk/phyre2. A distinctive configuration prediction will be returned between 2 hour and 30 min after its submission.

Fig: 2.4 Phyre 2 homepage

2.1.6. Easy Modeller

MODELLER is a program which is run so that we can produce protein's tertiary as well as quaternary structures (rarer) homology models. Modeller uses a technique which can be stimulated by nuclear magnetic resonance which is referred as contentment of spatial restraints, with which a cluster of geometrical criteria are used to generate a probability density function for the location of each atom in protein. The method relies on an input sequence alignment between the target amino acid sequence to be modeled and a template protein whose structure has been solved.The prospectus also incorporates fractional functionality for ab initio configuration prediction of loop regions of proteins, which are frequently vastly variable even amongst homologous proteins and therefore complex to calculate by homology modeling.

MODELLER be formerly written and is at present maintained by Adrez Sali at the University of

California, San Francisco. Even though it is liberally accessible for intellectual use, graphical user interfaces as well as viable versions are circulated by Accelrys. A web server named ModWeb virtual protein arrangement modeling web server is a server which is based on MODELLER as well as additional tools for modeling a protein configuration automatically with an alternative to locate down the resultant models into Mod Base. **Easy Modeller is** a generously accessible GUI to MODELLER which is urbanized by Kuntal Kumar Bhusan at University of Hyderabad, India.

2.1.7. MOD LOOP

ModLoop is a web server for computerized modeling of loops in protein structure. The contribution is the atomic coordinates of the protein structure in the Protein Data Bank format, and the requirement of the first and finale residues of one or more segments to be modeled, containing no more than 20 residues in total. The coordinates of the non- hydrogen atoms in the modeled segments is the end product. A user provides the input to the server via simple web interface, and receives the output by email. The server basically depends on the loop modeling practice in MODELLER which predicts the loop confirmations by contentment of spatial restrains, devoid of relying on a database of identified protein structures. For a quick retort ModLoop runs on a group of linux PC computer.

Fig: 2.5 ModLoop : Modelling of loops in protein homepage

2.1.8. SWISS PDB VIEWER

Swiss-Pdb Viewer is a purpose which provides a user friendly edge allowing to examine numerous proteins at the equal moment. The proteins can be superimposed in order to infer structural alignments and estimate their energetic sites or any other applicable parts. Amino acid mutations, angles, H-bonds and space flanked by atoms are uncomplicated to attain gratitude to the perceptive graphic and menu edge.

Swiss –Pdb Viewer (aka DEEP VIEW) has been developed since 1994 by Nicolas Guex. Swiss-Pdb Viewer is tightly linked to SWISS-MODEL, an automated homology modeling server developed within the Swiss Institute of Bioinformatics (SIB) at the structural bioinformatics group at the Biozentrum is Basel.

Functioning with both the programs deeply decreases the quantity of work essential to produce models, because it is potential to thread primary sequence of protein onto a 3D pattern and obtain an instantaneous response of how nicely the threaded protein will be established by the orientation construction earlier than submitting a appeal to construct lost loops and treat side chain space filler.

Swiss-Pdb Viewer can also examine electron density maps, and can provide a variety of tools to construct into the density. In addition, a variety of modeling tools are incorporated and residues can be mutated.

At last, as a unique additional benefit, POV-RAY scenes can be constructed from the present observation in order to construct dazzling ray-traced quality descriptions.

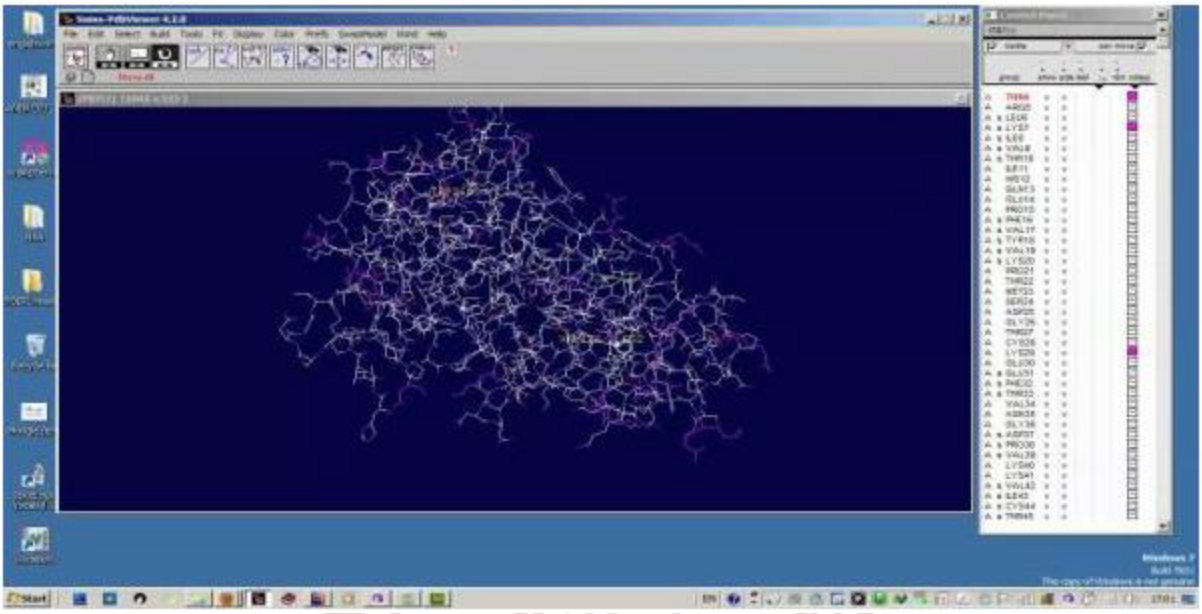


Fig: 2.6 SWISS –PDB Viewer

2.1.9. DRUG BANK

Drug bank is a type of database which is an exclusive bioinformatics and cheminformatics source that forms a cluster of detailed drug(i.e. pharmacological, chemical, and pharmaceuticals) data with entire drug target (i.e. structure, sequence, and pathway) information . The database consists of 7759 drug entries which contains 1602 FDA- agreed tiny particle drugs, 161 FDA- established biotech (peptide/ protein) drugs,89 nutraceuticals and additional 6000 trial drugs. In addition, 4300 non –redundant protein (i.e. enzyme/ drug target/ carrier/ transporter) sequences are associated to these drug entries. An entire elucidation of all the DrugCard fields and sources is given at this point. The PharmaBrowse key allows users to look through drugs clustered by their suggestion. This is particularly useful for pharmacists and physicians, but also for pharmaceutical researcher looking for potential drug leads. The ChemQuery buttons allows user to draw (using MarvinSketch applet or ChemSketch applet) or write (SMILES strings) a chemical compound and to search DrugBank for chemicals similar or identical to the query compound. The TextQuery key supports a added complicated text search (fractional word matches, misspelling, case sensitive, etc) of the text segment of DrugBank . The SeqSearch key allows users to carry out BLASTP (protein) sequence searches of the 18000 sequences controlled in DrugBank. In cooperation single and multiple sequences (i.e. whole proteome) BLAST queries are supported. The Advanced Search button opens an easy-to-use relational query search tool that allows users to select or search over various combinations of subfields. The Data Extractor is the most sophisticated search tool for DrugBank.

Fig: 2.7 Drug Bank

2.1.10. AUTODOCK

AutoDock is a collection of computerized docking tools. It is constructed to forecast how tiny molecules, such as drug candidates or substrates, which attach to a receptor of well-known 3D configuration.AutoDock 4 is open and is accessible beneath the GNU General Public License.

AutoDock has been extensively used and there are several examples of its flourishing function in the journal in 2006, it was the most cited docking software. It is very rapid, and provides elevated value predictions of ligand confirmations, and high-quality correlations among predicted inhibition constants and investigational ones. AutoDock has also been revealed to be functional in visor docking in which the position of binding spot is not recognized. Plus, AutoDock is open software and edition 4 is circulated beneath the GNU General Public License; it is simple to acquire too.

2.1.11. AUTODOCK VINA

AutoDock Vina is a novel program for finding a new drug, molecular docking and virtual screening, contribute fractional receptor litheness, multi-core potential, increased performance and improved precision and its simplicity to make use of. AutoDock Vina achieves roughly two guidelines of enormous speed compared by means of the molecular docking software. AutoDock 4, while also extensively civilizing the precision of the binding manner predictions. On a classic present workspace, Vina should frequently be capable to consistently create the predicted binding modes and affinities of a ligand in seconds to minutes, which basically depends on the ligand intricacy.

RESULTS AND DISCUSSION

3.1 FASTA SEQUENCE OF ACETYLCHOLINESTERASE

>AAA68151.1 acetylcholinesterase [Homo sapiens]
MRPPQCLLHTPSLASPLLLLLLWLLGGGVGAEG
REDAELLVTVRGGRLRGIRLKTPGGPVSAFLGIP
FAE
PPMGPRRFLPPEPKQPWSGVVDATTFQSVCYQY
VDTLYPGFEGTEMWNPNRELSEDCLYLNVWTP
YPRPT
SPTPVLVWIYGGGFYSGASSLDVYDGRFLVQAE
RTVLVSMNYRVGAFGFLALPGSREAPGNVGLL
DQRLA
LQWVQENVAAFGGDPTSVTLFGESAGAASVGM
HLLSPPSRGLFHRAVLQSGAPNGPWATVGMGE
ARRRAT
QLAHLVGCPPGGTGGNDTELVACLRTRPAQVL
VNHEWHVLPQESVFRFSFVPVVDGDFLSDTPEA
LINAG
DFHGLQVLVGVVKDEGSYFLVYGAPGFSKDNE
SLISRAEFLAGVRVGVPQVSDLAAEAVVLHYTD
WLHPE
DPARLREALSDVVGDHNVVCPVAQLAGRLAAQ
GARVYAYVFEHRASTLSWPLWMGVPHGYEIEF
IFGIPL
DPSRNYTAEEKIFAQRLMRYWANFARTGDPNE
PRDPKAPQWPPYTAGAQQYVSLDLRPLEVRRG
LRAQAC
AFWNRFLPKLLSATDTLDEAERQWKAEFHRWS
SYMVHWKNQFDHYSKQDRCSDL

3.2 BLAST RESULTS

3.2.1Graphic summary

Fig :3.1 Graphic summary

3.2.2Sequence producing significant alignments

Fig: 3.2 significant alignmen

3.2.3.Alignments

Fig: 3.3 Alignments

3.3 PHYRE2 RESULTS

21	c2qgdA	Alignment	not modelled	100.0	38	**PDBTitle:** crystal structure of the geobacillus stearothermophilus2 carboxylesterase est55 at ph 6.2
22	c5a2g8	Alignment	not modelled	100.0	28	**PDB header:**hydrolase **Chain:** 8: **PDB Molecule:**carboxylic ester hydrolase; **PDBTitle:** an esterase from anaerobic clostridium hathewayi can2 hydrolyze aliphatic aromatic polyesters
23	c4bc4A	Alignment	not modelled	100.0	28	**PDB header:**hydrolase **Chain:** A: **PDB Molecule:**sterol esterase; **PDBTitle:** closed conformation of o. picease sterol esterase
24	d1thga_	Alignment	not modelled	100.0	29	**Fold:**alpha/beta-Hydrolases **Superfamily:**alpha/beta-Hydrolases **Family:**Fungal lipases
25	d1tfa_	Alignment	not modelled	100.0	29	**Fold:**alpha/beta-Hydrolases **Superfamily:**alpha/beta-Hydrolases **Family:**Fungal lipases
26	d1ac5a_	Alignment	not modelled	100.0	34	**Fold:**alpha/beta-Hydrolases **Superfamily:**alpha/beta-Hydrolases **Family:**Acetylcholinesterase-like
27	d1gz7a_	Alignment	not modelled	100.0	30	**Fold:**alpha/beta-Hydrolases **Superfamily:**alpha/beta-Hydrolases

Fig: 3.4 Phyre 2 results

3.4 RAMPAGE RESULTS (Best Model Generated)

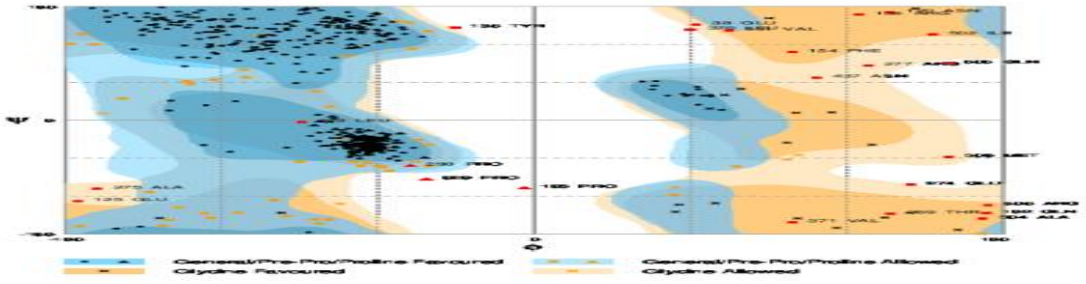


Fig: 3.5 Rampage result

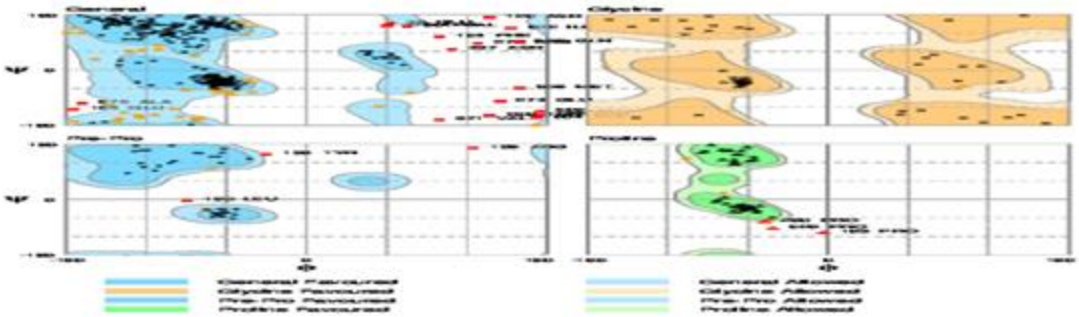


Fig:3.6 Generated models

Evaluation of residues

```
Residue [    34 :ARG] (-140.23,-157.74) in Allowed region
Residue [    35 :GLU] ( -99.66,-126.25) in Allowed region
Residue [    57 :GLY] (  54.26,-180.00) in Allowed region
Residue [    72 :PRO] ( -78.16,   22.18) in Allowed region
Residue [    87 :TRP] (-142.25,-166.71) in Allowed region
Residue [   110 :GLY] (-165.54,   10.90) in Allowed region
Residue [   119 :PRO] ( -65.68,  -64.75) in Allowed region
Residue [   124 :SER] ( -66.54,  113.36) in Allowed region
Residue [   127 :CYS] ( -78.78,-157.87) in Allowed region
Residue [   133 :TRP] (-110.34,   67.89) in Allowed region
Residue [   134 :THR] ( -75.62,  -29.58) in Allowed region
Residue [   141 :SER] ( -71.64,  100.87) in Allowed region
Residue [   155 :TYR] (-136.43,-137.66) in Allowed region
Residue [   189 :PHE] (-133.26,   61.52) in Allowed region
Residue [   234 :SER] (  53.95,-118.06) in Allowed region
Residue [   251 :GLY] (  101.62,  -54.96) in Allowed region
Residue [   259 :GLN] ( -82.58,-163.37) in Allowed region
Residue [   266 :PRO] (-105.12,  134.71) in Allowed region
Residue [   293 :THR] ( -94.79,-151.46) in Allowed region
Residue [   297 :ASP] ( -69.28,  -65.53) in Allowed region
Residue [   300 :LEU] ( -83.79,  -45.74) in Allowed region
Residue [   301 :VAL] (-158.11,   34.38) in Allowed region
Residue [   303 :CYS] (-147.66,-113.63) in Allowed region
Residue [   304 :LEU] ( -68.54,  -72.25) in Allowed region
Residue [   305 :ARG] (-113.61,-146.20) in Allowed region
Residue [   310 :GLN] (-174.28,-167.67) in Allowed region
Residue [   313 :VAL] ( -84.85,-156.63) in Allowed region
Residue [   314 :ASN] ( -63.96,  -67.86) in Allowed region
Residue [   366 :GLY] ( -74.60,-132.13) in Allowed region
Residue [   367 :SER] ( -58.85,  -73.96) in Allowed region
Residue [   368 :TYR] (-178.16,  120.85) in Allowed region
Residue [   381 :ASN] (-122.70,   64.10) in Allowed region
Residue [   386 :SER] ( -59.81,  -72.23) in Allowed region
Residue [   387 :ARG] (-168.71,  119.98) in Allowed region
Residue [   390 :PHE] (-126.65,-147.83) in Allowed region
Residue [   393 :GLY] (   97.15,  -62.29) in Allowed region
Residue [   395 :ARG] (   77.68,  -72.88) in Allowed region
Residue [   435 :ASP] (-131.81,   57.28) in Allowed region
Residue [   436 :HIS] ( -67.78,  154.59) in Allowed region
Residue [   450 :ALA] ( -68.55,  140.93) in Allowed region
Residue [   466 :SER] ( -55.25,  -80.12) in Allowed region
Residue [   469 :SER] ( -90.88,  -65.72) in Allowed region
Residue [   494 :ARG] (-111.42,   80.17) in Allowed region
Residue [   497 :THR] ( -68.55,  -39.19) in Allowed region
Residue [   498 :ALA] ( -66.58,  -72.16) in Allowed region
Residue [   501 :LYS] ( -64.84,  -31.13) in Allowed region
Residue [   509 :ARG] ( -52.21,  156.38) in Allowed region
Residue [   527 :LYS] (-124.14,-154.58) in Allowed region
Residue [   556 :ARG] (  171.78,-179.36) in Allowed region
Residue [   578 :ASP] (-173.51,  136.23) in Allowed region
Residue [    38 :GLU] (   62.39,  151.21) in Outlier region
Residue [   102 :GLN] (  173.66,-146.66) in Outlier region
Residue [   120 :ASN] (  136.51,  170.58) in Outlier region
Residue [   125 :GLU] (-175.11,-127.81) in Outlier region
Residue [   136 :TYR] ( -38.38,  146.42) in Outlier region
Residue [   138 :ARG] (  124.73,  167.19) in Outlier region
Residue [   154 :PHE] (   99.19,  107.77) in Outlier region
Residue [   192 :LEU] ( -89.28,   -2.96) in Outlier region
Residue [   193 :PRO] (   -3.52,-107.87) in Outlier region
Residue [   269 :THR] (  136.96,-147.91) in Outlier region
Residue [   274 :GLU] (  164.65,-101.77) in Outlier region
Residue [   275 :ALA] (-167.72,-108.24) in Outlier region
Residue [   277 :ARG] (  128.38,   86.59) in Outlier region
Residue [   290 :PRO] ( -47.42,  -71.37) in Outlier region
Residue [   370 :LEU] (   68.29,  143.21) in Outlier region
Residue [   371 :VAL] (   99.24,-161.48) in Outlier region
Residue [   437 :ASN] (  108.46,   67.29) in Outlier region
Residue [   502 :ILE] (  153.86,  135.71) in Outlier region
Residue [   504 :ALA] (  172.21,-155.48) in Outlier region
Residue [   505 :GLN] (  158.98,   98.17) in Outlier region
Residue [   506 :ARG] (  174.44,-136.28) in Outlier region
Residue [   508 :MET] (  159.28,  -58.15) in Outlier region
Residue [   529 :PRO] ( -61.27,  -93.83) in Outlier region
Residue [   551 :VAL] (   74.88,  141.86) in Outlier region
Number of residues in favoured region     (~98.0% expected)  :   538 ( 87.9%)
Number of residues in allowed region      ( ~2.0% expected)  :    50 (  8.2%)
Number of residues in outlier region                         :    24 (  3.9%)
```

Fig: 3.7 Evaluation of residues

3.5 DRUG LIST

Donepezil	Dimebon	Zaleplon	Ergoloid mesylates	Promazine	Citalopram	Rebaxetine
Galantamine	PBT2	Trazodone	Nutr-E-sol	Moclobemide	Doxepin	Sertraline
Memantine	Carbamazepine (tegretol)	Namenda XR	Nilotinil	Quetiapine	Fluoxetine	Venlaflaxine
Rivastigmine	Valproate	Vit-e	Amisulpride	Sulpiride	Fluvoxamine	Lithium carbonate
Insulin	Risperidone	Hydergine	Chlorpromazine	Trifluoperazine	Imipramine	Alprazolam
CSP-1103	Olanzapine	Aqua-E	Esitalopram	Zuclopenthizol	Mirtazipine	Buspirone
Inteperidine	Temazepam	Aquasol E	Fluphenazine	Amitriptyline	Nortriptyline	Diazepam
Tacrine	Zolgidem	E600	Haloperidol	Dothiepin	Paroxetine	Lorazepam
Oxazepam	Flurozepam	Nitrozepam	Zopiclone	Huperzine A	Dopamine Agonists	Levodopa
Chloral hydrate	Amantadine	Cognetin	COMT inhibitor	Bromocriptine	Apomorphine HCL	Neupro
Chlomethiazole	Mirapex	Ropinirole	Benzatropine Mesylate	Trihexy Phenidyl	Piracetam	Tolteradine
Oxybutynin	Solifenacin	Darifenacin	N-methyl D-aspartate	Amoxapine	Clomipramine	Desipramine
Protriptyline	Trimipramine	Fesoterodine	Flavoxate	Trospium	Clozapine	Loxapine
Perphenazine	Thioridazine	Carbamate	Edrophonium	Distigmine	Ambenonium	Neostigmine
HI-6	K027	C203	Methoxine	Obidoxime	Physostigmine	Pralidoxime
Propinerine	Trimedoxime					

Table 2: Drug lists

3.6 LIGAND LIBRARY

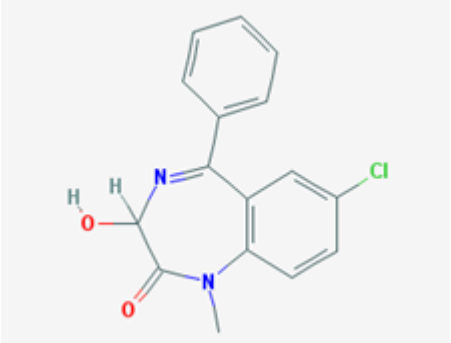
S.No	ID	Name	Structure
1.	CID_5391	Temazepam	

Table 3: Ligand library

3.7 DOCKING RESULTS

S.No.	Ligand	Affinity(kcal/mol)
1.	CID_5391	-46.68

Table 4: Docking results

Inference: ligand with least binding affinity -46.68. On the basis of 3D structure when we search for its synonyms its result was not better than that from the ligand **CID_5391** It also follows Lipinski rule of 5. So we concluded, that **CID_5391** is best for Alzheimer's disease.

4.7.1 Properties of best drug

PROPERTIES	CID_5391
Mass	360.742
Hydrogen bond Donor (HBDC)	1
Hydrogen bond Acceptor (HBAC)	3
LOGP	2.2

Table 5: Properties of Best Drug

3.8 ADME RESULTS

3.8.1 ADME

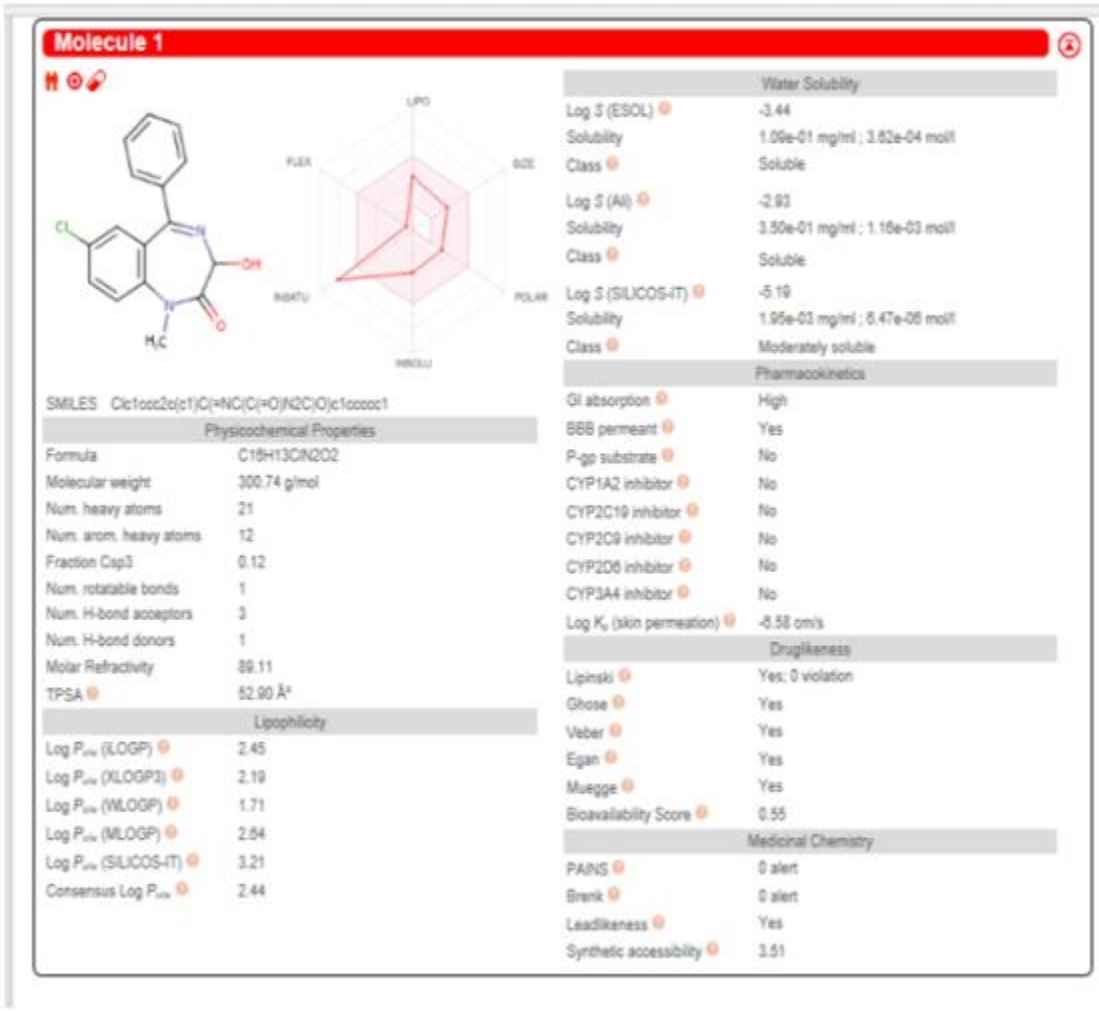


Fig: 3.8 ADME result

3.8.2 Target prediction report

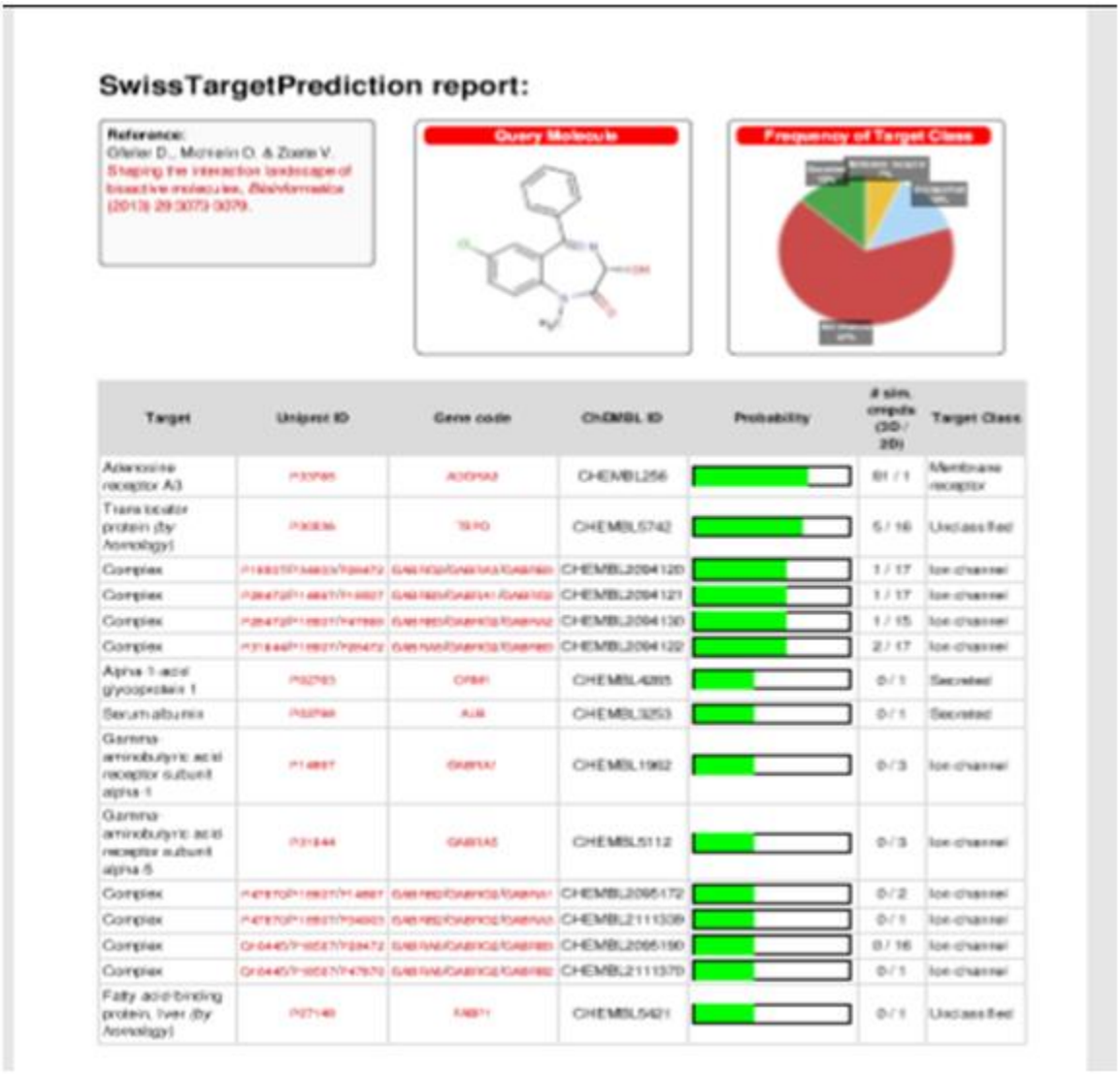


Fig: 3.9 Target prediction report

CONCLUSION

In this project, from review of literature we got the information that the abnormal protein clumps causes the death of brain cells which is a dangerous form of dementia called Alzheimer's disease.β amyloid and abnormal tau protein is responsible for this disease and acetyl cholinesterase is a key inhibitor which is used for the treatment of this disease. The Temazepam drug used in this task may reimburse for the fatality of cholinergic neurons as well as offer suggestive respite by inhibiting acetylcholine revenue as it straightforwardly interrelate by means of amyloid β in a way that increases the deposition of this peptide into insoluble plaques. The working of temazepam is when benzodiazepine combines in a wide range to benzodiazepine receptors, which affects muscle repose, motor organization, anticonvulsant action as well as recall. As this receptor is in contemplation to be united to GABA (γ aminobutyric acid-A) receptors, this enhances the conclusion of GABA by rising of inhibitory neurotransmitter GABA to the arrangement that opens chloride channel consequential in a hyperpolarized cell membrane that prevents additional excitation of cell. After homology modeling we got the final structure of our protein. The docking was carried out with the best ligand. This drug will be helpful in the treatment of Alzheimer's disease.

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