Deciphering molecular mechanism of amyloid inhibition through docking studies between amyloid beta peptide and selected small molecules

Thesis submitted in partial fulfilment of the requirements for the degree of

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By

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Certificate

This is to certify that the thesis entitled "Deciphering molecular mechanism of amyloid inhibition through docking studies between amyloid beta peptide and selected small molecules" by **Sangita Soren (109BM0018)** submitted to the National Institute of Technology , Rourkela, for the degree of Bachelor of Technology is a record of bonafide research work , carried out by her in the department of Biotechnology and Medical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university/ institute for the award of any degree or diploma.

Dr.Nandini Sarkar

Assistant Professor

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(Sangita Soren)

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ABSTRACT

The misfolding and aggregation of proteins into amyloids has been linked to a large group of variety amyloid-related diseases. This group consists of variety human diseases such as Alzheimers disease, Parkinson's disease, Hungtington's disease, atherosclerosis etc. Aggregation of proteins, such as aβ in Alzheimer's disease, appears to lead to the formation of toxic assemblies. These assemblies can be very small in size like oligomers(consisting of very few proteins) and can reach a maximum of thousands of proteins which leads to fibril formation. It has been found out that inhibiting the aggregation of these proteins could slow down the process of formation of these neurodegerative diseases. But still there is truancy of a possible therapy that could effectively prevent the formation of amyloid fibrils. Thus in present study we try to understand the mechanism by which the small compounds which we have selected inhibits the target protein or the amyloid beta peptide. Here we understand the type of interaction and whether the compounds binds near the amyloidogenic region. We have also tried to understand the binding between two amyloid protein. Here we have choosen an amyloidogenic protein beta amyloid (1-42), pdbid-1IYT, that is processed from the amyloid precursor protein(APP). While best known as the component of amyloid plaques in association with Alzheimer's disease, as A-beta is the main component of certain deposits found in the brain of patients with Alzheimer's disease. The small compounds selected here are mostly cholesterol reducing agents such as statins, wine related compounds, green tea related components, polyphenols, phenothiazines. These compounds are found to inhibit amyloid fibrillogenesis in vivo. So here we adduce a mechanism by which these small compounds inhibit the aggregation of amyloid beta(1-42)peptide.

KEYWORDS: Alzheimers disease, neurodegerative diseases, amyloid beta peptide, amyloidogenic region, amyloid precursor protein, amyloid fibrillogenesis.

CHAPTER 1:

INTRODUCTION

INTRODUCTION

In the last few decades, scientists have made substantial progress in the discovery of new medicines. These advances in medicine and technology are making our lives longer. Sadly as our life expectancy increases, the chances of getting a degenerative disease like Alzheimer's disease increase. The diseases are not caused by bacteria or virus but something which very elementary i.e. incorrect protein folding. Many proteins associated with many age-related diseases have dissimilar sequence, structure and function, but they can all form similar highly ordered amyloid fibrils. They have many common properties like, possession of cross beta structure, double refracts upon staining with dyes like congo red, protease resistance and insolubility in most solvents and also has a fibrillar morphology. Amyloid beta peptide accumulation within the brain is a hallmark of Alzheimer's disease pathology. Accumulation of aggregated abeta is hypothesized to initiate a pathological cascade that results in cognitive decline. Abeta species are generated from the amyloid beta protein precursor (APP) through cleavage by beta and gamma secretases. A 40 amino acid form of abeta (aβ 40) is the major secreted product of these proteolysis activity , but the minor 42-amino acid form of abeta (aβ42) has been found out to be the main initiating molecule in the pathogenesis of Alzheimer's disease. Aβ 42 aggregates very fast as compared to aβ 40. This type of aggregation leads to a large number of diseases such as Alzheimer's disease, Parkinson's disease, prion disease, and type 2 diabetes. No real cure is currently available towards treating the disease, the attenuation of amyloid fibrillation and capture of fibrillar species have been so far considered an effective approach. One approach is the use of small compounds that can inhibit the aggregation process. Several small compounds from various groups have been found out to inhibit the fibrillar assemblies in vivo and their associated cytotoxicity. By various observations related to this we can propose an effective mechanism about the interaction of such compounds with the peptide. This study of the mechanism may be highly essential for the treatment of amyloid related diseases.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Identification of the location of ligand binding sites on a protein is of fundamental importance for a range of applications including molecular docking, de-novo drug designing and structural identification and also helps in comparison of functional sites. Now-a-days the enhancement of bioinformatics and cheminformatics is paving the way for easy study of

protein-ligand interaction. Computational biology and bioinformatics have the potential to speed up this process and also it helps in reducing the cost. Docking is method which predicts the preferred orientation of one molecule to another molecule when they are bound together to form a stable complex molecule. Here two molecule bind to each other in a three-dimensional space. Docking can be referred to as “lock and key” model. Here the protein can be a lock and the ligand can be called as key. There are various tools used for docking studies with the help of bioinformatics. Using the bioinformatics tools time and cost of biological work reduces drastically.

CHAPTER 2:

LITERATURE REVIEW

LITERATURE REVIEW

PROTEINS

Proteins are biochemical compounds consisting of one or more polypeptides. These are generally folded into a globular or fibrous form which allows for a biological function. A polypeptide is a single linear polymer chain of amino acids. They bonding between them is made by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Proteins work together to achieve a particular function and they often associate to form stable protein complexes. Biochemistry is the science that studies the chemical processes in living organisms. Using different experimental models, biochemists demonstrated that most of the cell's chemical reaction and structural components are mediated or supplied by proteins. They concluded that proteins are very important for proper cell functioning. Each protein within the body has its special work. Some proteins are involved in structural support while others are involved in bodily movement. Some are also involved in defence against germs. The word protein comes from the greek word 'proteios' meaning 'first' or 'foremost' which itself indicates its importance. The proteins are basically long polymers which are made up of small units called amino acids.[1]

PROTEIN FOLDING

One important example of biological self assembly is folding of protein into its compact three dimensional structure. Understanding of this complex process is very important. Folding of protein is related to many biological processes such as molecular trafficking to particular cellular locations and regulation of cellular growth and differentiation. Failure of proteins to fold correctly is cause for a variety of diseases [1]. Almost half a century ago, Linus Pauling discovered two quite simple regular arrangements of amino acids, the alpha-helix and beta-sheet protein. In 1960's, Christian Anfinsen discovered that proteins actually tie themselves. He said that if proteins become unfolded, they immediately fold back to proper shape of their own and hence no shaper or folder is required [3]. Native states of proteins always correspond to structures that are most thermodynamically stable [4]. The change in conformation between the unfolded and incompletely folded polypeptide chain enable the

residues to come close to one another. The interactions in the native state are more stable than the non-native ones. Hence the native polypeptide chains find its lowest energy structure. (figure.1) shows the energy landscape. It shows the transition from a random coil to a native structure [4-5]. This is based on ideas of statistical mechanics and polymer physics than on those of classical dynamics. This is often referred as 'new view' of protein folding [6]. Folding in vitro of some of the proteins is in fact very easily achieved. Alpha-helices are able to form in less than 100ns, and beta-turns are able to form within 1 micro seconds. Folding of helical bundles is completed in less than 10 micro seconds. But beta –sheets can take longer time to fold [7]. The mechanism of protein folding involves the interaction of small number of residues to form a folding nucleus, about which the remainder of the structure rapidly condenses [8]. Fundamental mechanism of protein folding involves the interaction of small number of residues to form a folding nucleus. The figure (1) suggests that correct folding into minimum energy of the protein is finally achieved due to some properties. To reach the native point it has to cross a barrier which corresponds to the transition phase. The folding of proteins is achieved due to interaction between the amino acids. It follows a funnel like energy landscape which speeds up the folding process [3].

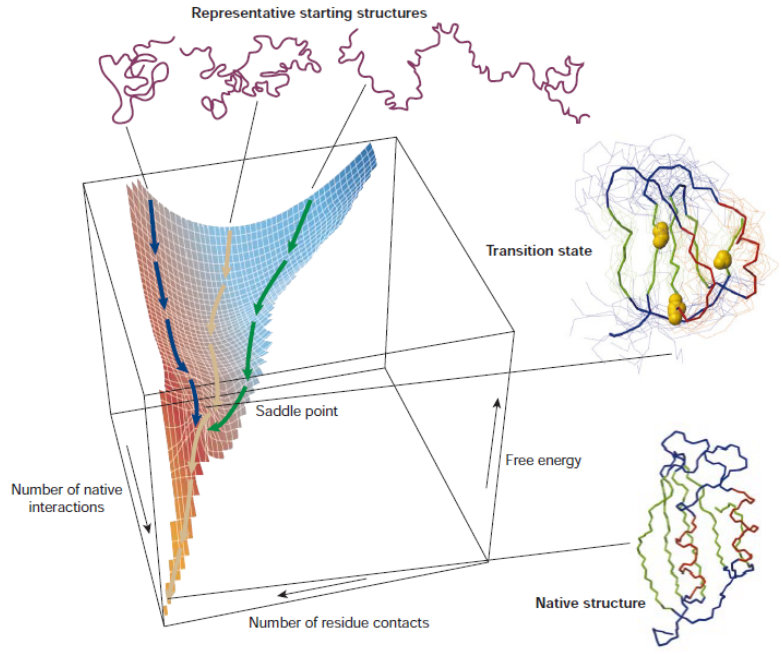


Figure 1: The energetic funnel.

(Source:Dobson , C.M. Protein Folding and Misfolding, Nature 426, 884-890(2003))

PROTEIN AGGREGATION

For many years scientists have been investigating on the causes of many types of diseases. They found out that many proteins associated with such diseases form similar highly ordered amyloid fibrils [2]. Amyloidoses is the condition when soluble proteins become insoluble and are deposited in tissues and organs and disrupting the normal function. The general cause for formation of amyloid aggregates is the special properties of the polypeptide chains. Aggregates of proteins not associated with disease can impair the ability of cells to function to a similar extent. It has also been found out that the mature amyloid fibrils are less toxic than the pre fibrillar aggregates. These pre fibrillar aggregates interacts with cellular membranes, causing oxidative stress and increase in free Ca^{2+} that leads to cell death. Use of chaperons can be very helpful in preventing such diseases [9]. The charge of molecule, secondary-structure and number of hydrophobic side surfaces influence the aggregation rates for a wide range of proteins [10]. The hydrophobic side chain and main polypeptide chain buries into the core region when proper folding occurs. After misfolding of the nascent protein it results in degradation or aggregation. The cellular environment, molecular chaperons are very vital for the transition between the different states of protein whose failure may cause misfolding diseases [11].

Table (1) shows a list of amyloidogenic diseases.

(Source: http://en.wikipedia.org/)

Disease	Protein featured	abbreviation
Alzheimer's disease	Beta- amyloid	aβ
Diabetes mellitus type 2	IAPP(amylin)	AIAPP
Parkinson's disease	Alpha - synnuclein	none
Huntington's disease	huntingtin	none

Medullary carcinoma of the thyroid	calcitonin	ACal
Cardiac arrhythmias, isolated atrial amyloidosis	Atrial matriuretic factor	AANF
Atherosclerosis	Apolipoprotein AI	AApoA1
Rheumatoid arthritis	Serum amyloid A	AA
Prolactinomas	Prolactin	APro

ABETA AMYLOIDOGENESIS

Deposits of large amount of single, insoluble proteins around the degenerating nerve cells gradually lead to Alzheimer's disease. Alzheimer's disease affects people over 65 years of age. Every year Alzheimer's disease not only kills 10,000 Americans but also costs billions for the cure of them. Brain regions involved in learning and memory process, including temporal and frontal lobe are reduced in size in AD patients. This is the result of death of neurons. In AD it has been found out that the brain of the patients contains "plaques and tangles". Plaques are insoluble deposits of fibrils and aggregates of amyloid beta peptide. Neurofibrillary tangles are fibrillar aggregates of the microtubule associated protein tau that exhibits hyperphosphorylation and oxidative modifications [12]. APP also known as amyloid precursor protein is the brain protein. It is produced in several different isoforms. The most abundant form in brain (APP695) is produced mainly by neurons. Cleavage of APP by the α–secretase releases a secreted APP (alpha) and leaves an 83-amino acid carboxy terminal APP fragment (c83). [12]. The APP is cleaved by two enzymes; β – secretase and forms aβ peptide. Another enzyme is γ secretase and forms aβ peptide and it aggregates in brain. The aβ amyloids are short sticky fragments [13].

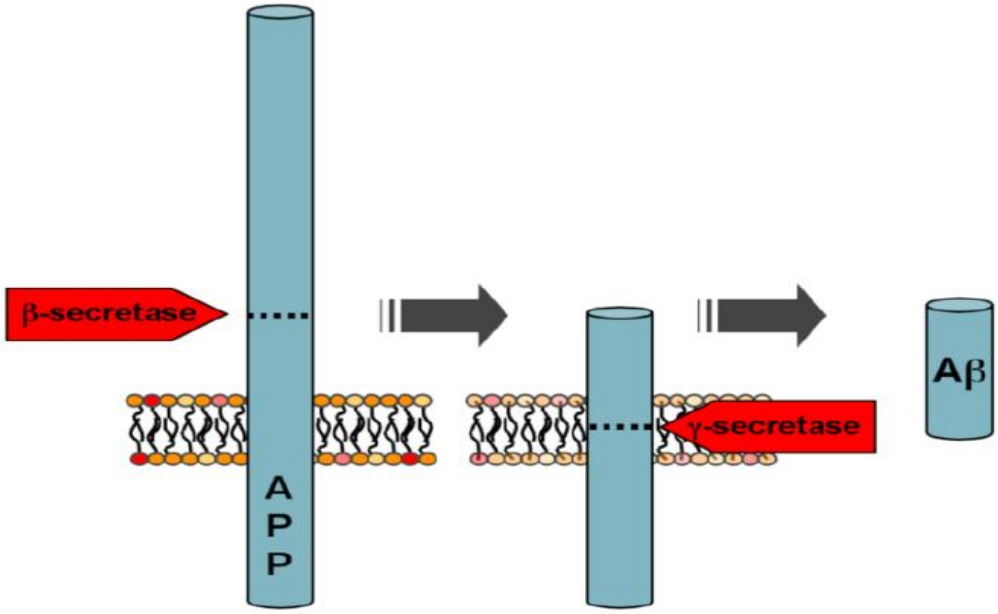


Figure 3: formation of amyloid beta peptide from APP.

(Source: *http://en.wikipedia.org/*)

INHIBITION OF AMYLOID BETA PEPTIDE BY SMALL COMPOUNDS

Compounds are reported which helps in reducing the deposition of amyloids in brain either by inhibiting the enzymes or by inhibiting aggregates of aβ peptide by the use of antibodies, small peptides, or small organic molecules, increasing amyloid degradation using amyloid vaccines. Earlier it was found out that congo red and thioflavin T shows fluorescence when acted on amyloids and also showed specific interaction and inhibited them upto some extent[14]. Here we have shown that few small compounds can be used to inhibit the amyloids in vivo. Several compounds which we have found out are curcumin, resveratrol, indomethacin, atorvastatin, simvastatin.

Curcumin: the phenolic yellow curry pigment has anti-inflammatory and anti oxidative activities and can also stop oxidative damage and cognitive impairments. It also acts as a promising inhibitor for amyloid aggregation. It has been found out that it particularly binds to the aβ binding site and also prevents toxicity and oligomer formation. In mice models when curcumin was used, preferential labelling of amyloid plaques was seen. In vivo studies say that curcumin injected into aged Tg mice crossed the blood brain barrier and also bound to

amyloid plaques. Hence it acts as a both as a blocking agent for aggregation and also stops fibril formation both invivo and invitro [15].

Atorvastatin: It belongs to the statin group. It has been found out that high cholesterol level in body can enhance the risk of development of Alzheimer's disease. Cholesterol metabolism and catabolism are seen to be affected by neurodegenerative disease. Literature say that APP and aβ production depend on cholesterol content and on isoprenoid intermediates in cholesterol biosynthesis pathway. Hence, reducing the cholesterol content may decrease the aβ formation. So this was found as evidence suggesting that inhibitors of cholesterol synthesis like statins could have a therapeutic role in AD and may inhibit the aggregation of abeta peptide [17].

Phenothiazines: Phenothiazines such as azure A and azure B have been shown to act as cholinesterase inhibitor when combined with rivastignine. They have been shown to slow down the process of aggregation of AD [18].

CHEMBL: It is a database of bioactive drug like molecule. It contains 2D structures and also calculated properties. It was used for drug discovery research problems. It contains 5.4 million bioactivity measurements for 1 million compounds and 5200 protein targets [19]. The access is available through web services at *http://www.ebi.ac.uk/chembldb*

CHAPTER 3:

MATERIALS AND METHODS

3. MATERIALS AND METHODS:

3.1 Tools and bioinformatics softwares used

3.1.1 Pubchem(pubchem.ncbi.nlm.nih.gov)

3.1.2 NCBI

3.1.3 Swissmodel.expacy.org

3.1.4 Pubmed

3.1.5 Chemical entities of biological interest(chebi)

3.1.6 PDB (pdb.org)

3.1.7 Tango

3.1.8 Waltz

3.1.9 Fold amyloid

3.1.10 Open babel software 2.3.1

3.1.11 Rasmol

3.1.12 Hex 6.3

3.1.13 Swissdock

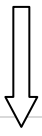
3.1.14 Swisspdb viewer

3.1.15 ligplot+ 1.3.6 and dimplot

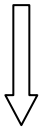
3.2 Protocol followed

3.2.1 Interaction between two proteins: i.e protein –protein interaction

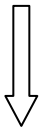
Retrieval of amino acid sequence of 1IYT protein from NCBI



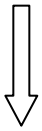
3-D structure of the protein model was retrieved from PDB (protein data bank)



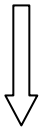
3-D structures of the small compounds was retrieved from Pubchem



Energy minimisation of the 3-D structures of protein was done by chimera



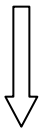
Docking of protein protein complex was done by hex 6.3



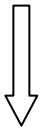
Retrieval of the 2-D structure of the protein protein complex by liplot+

3.2.2 Inhibition of amyloid beta peptide(1IYT)

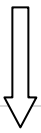
Retrieval of 3-D structure of 1IYT from PDB (protein data bank)



Collection of small ligand compounds from Pubchem database

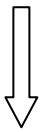


Conversion of all the ligand 3-D compounds from .sd file format to .mol2 file format



Docking of all the ligands compounds with the amyloid beta peptide by swissdock

Analysis of binding energy and site of binding obtained from docking by chimera



Saving of the output from chimera in.pdb file format

Energy minimisation of the result in swisspdb viewer

Analysis of 2D structure using ligplot+

3.3 **METHODOLOGY**

3.3.1 Retrieval of amino acid sequence of 1IYT protein from NCBI and PDB

NCBI stands for National Center for Biotechnology Information. It is a part of United States National Library of Medicine (NLM) and is a branch of National Institutes of Health (NIH). NCBI contains databases which are used in biotechnology and biomedicine. Major databases include GenBank for DNA sequences and PubMed . NCBI contains databases by the help of which we can examine and research on the structure and function of biologically important molecules. It can provide us with information about molecular biology, biochemistry and also genetics. Such databases are of great use in the field of research in biotechnology and biomedicine. URL is *http://www.ncbi.nlm.nih.gov*. PDB (protein data bank) provides us with

the 3-dimentional structural data of macromolecules such as proteins and nucleic acids. This was founded in Brookhaven National Laboratories (BNL). This is of much importance because the understanding of the structure of the compounds is the basic step.

a) The above URL was used for retrieval of amino acid sequence
b) From there, the PDB link was used or else directly *http://www.rcsb.org* was used
c) The amyloid beta peptide with the PDB ID 1IYT was selected
d) The FASTA sequence and the PDB file was downloaded.

Figure (4): Homepage of PDB database from where the abeta peptide (PDB ID: 1IYT) was downloaded.

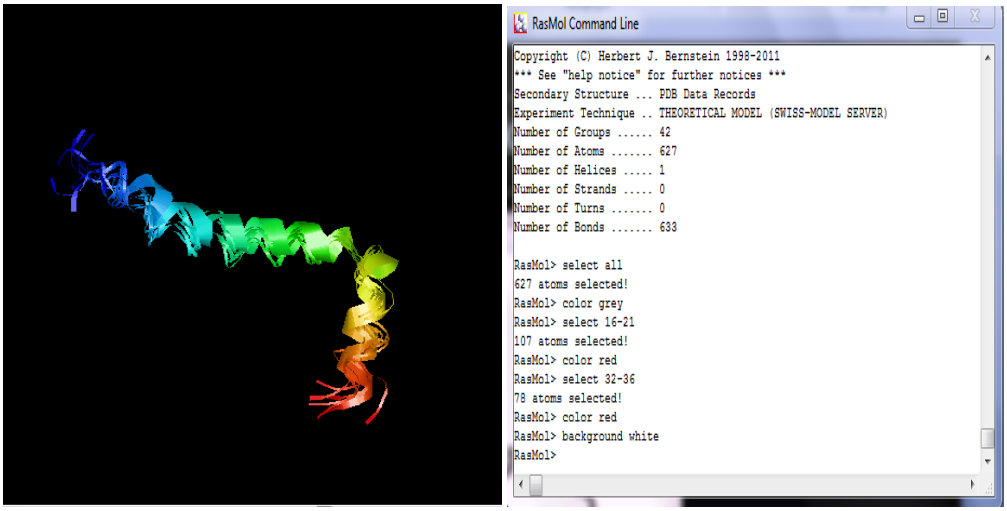


Figure5: 3D structure of 1IYT in rasmol(left) and rasmol command line (right)

3.3.1 Energy minimization of protein by chimera 1.6.1

Chimera 1.6.1 is used for visualizing or analyzing molecular structure and data which is related to it. The docking results can be visualized using this extensible programme. It speciality is that it can minimise energy of molecules providing them with high stability.

a) Chimera was opened and the structure of the amyloid beta peptide was retrieved.
b) Total residues were selected.
c) The tool option was selected. Then structure editing option was used and minimized structure option was clicked.
d) The minimized structure was saved in .pdb format for further use.

3.3.2 Prediction of the amyloidogenic region using various softwares.

Prediction of the amyloidic region in the polypeptide chains is very important because such regions are responsible for amyloid formation and aggregation. These softwares are useful to predict positions of amyloidogenic regions in protein chains.

a) Tango software

It is also a computer algorithm to predict aggregation nucleating regions in proteins as well as the effect of mutations and environmental conditions on the aggregation propensity of these regions. Tango correctly predicts the aggregation propensities of several disease related mutations in the Alzheimer's b-peptide. The server is available at *http://tango.crg.es/protected/correctlogin.jsp* using this URL, the protein sequence in FASTA format was submitted and the result was generated automatically. Here we got the amyloidogenic region between 16-21 and 32-36.

b) Waltz software

Waltz was developed with the main aim of prediction of amyloidogenic region. With the help of waltz scientists have been able to find out new unknown amyloid aggregates. The prediction of amyloid aggregates is the main starting point for research related to amyloid formation. The server is available at

http://waltzswitchlab.org. Here again the protein FASTA sequence was submitted online and the result was generated after sometime.

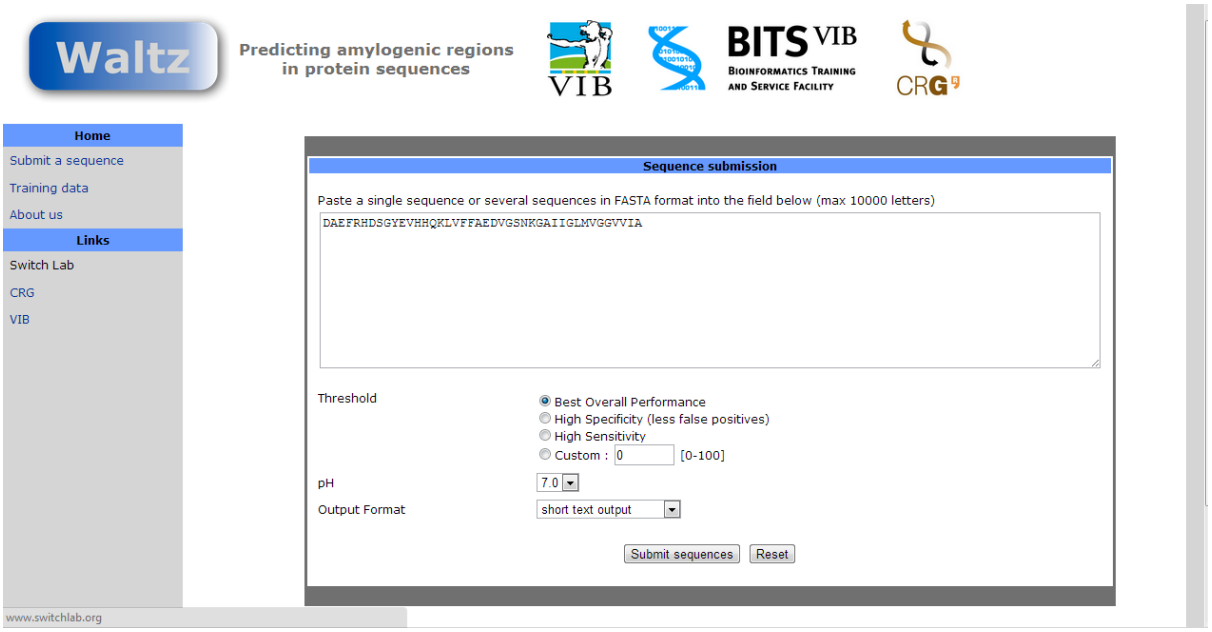


FIGURE 6: waltz page where input of the protein sequence is given in FASTA format.

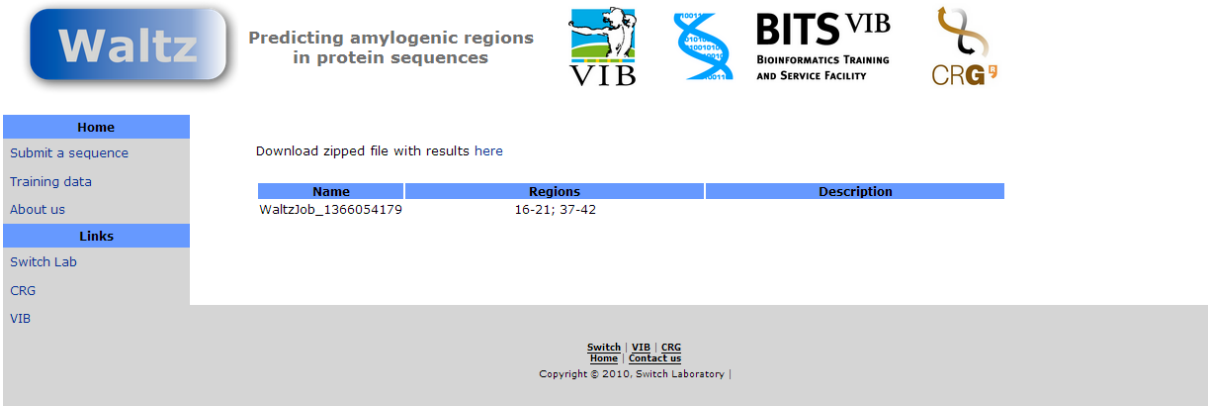


Figure 7: waltz prediction of the amyloidogenic region.

Again this software also gave us the same result. The amyloidic region is between 16-21 and 32-36.

c) <u>Fold amyloid software</u>

Here we take the pdbid of the abeta 42 peptide and copy and paste the sequence in the space provided. Here as well we use the fasta format. The result is generated

automatically the url for the website is *http://antares.protres.ru/fold-amyloid/*. Here also we get the same result i.e the amyloidic region between 16-21 and 32-36.

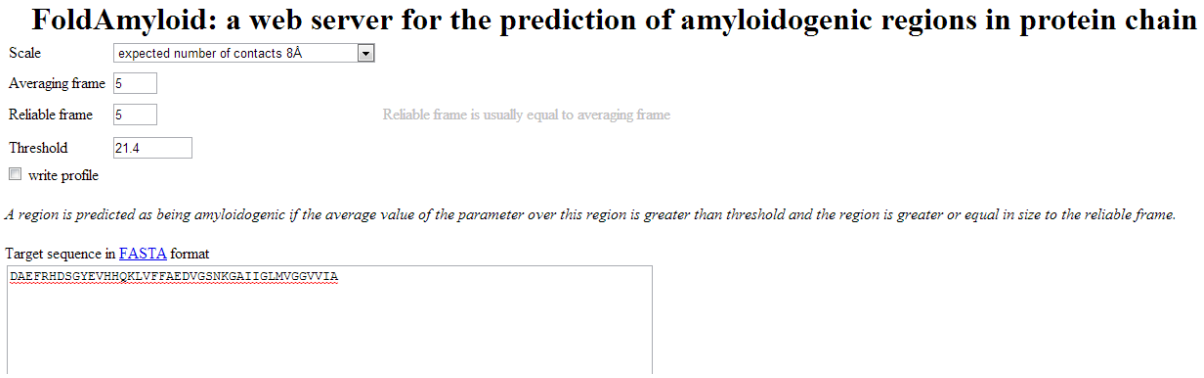


Figure 8: fold amyloid server where the input is given in the FASTA format.

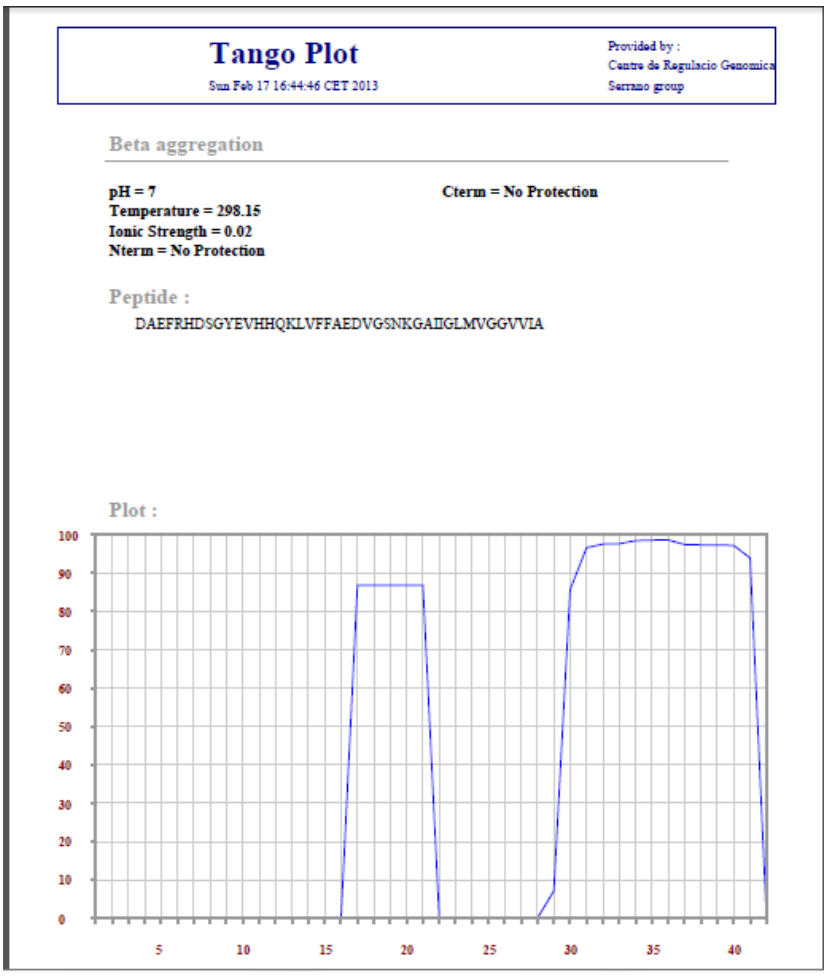


Figure 9: tango result of amyloidogenic region prediction.

3.3.3 Docking by Hex 6.3

Hex 6.3 is a molecular graphics program developed by Dave Ritchie. It is used for docking of protein with ligands and also docking between two proteins can also be done. It is mainly used for calculating intermolecular "energies". Energy is mainly related to all interactions between the molecules in terms of van der waal's, electrostatic.

a) Hex 6.3 was opened, then from the file the abeta peptide was input as the receptor and the same peptide was input as the ligand.
b) The option control was clicked and docking was selected and activated.
c) The docking result was obtained after 3
d) The binding energy(delta E) after the docking was complete was saved.
e) The docking complex was saved in .pdb format for future use.

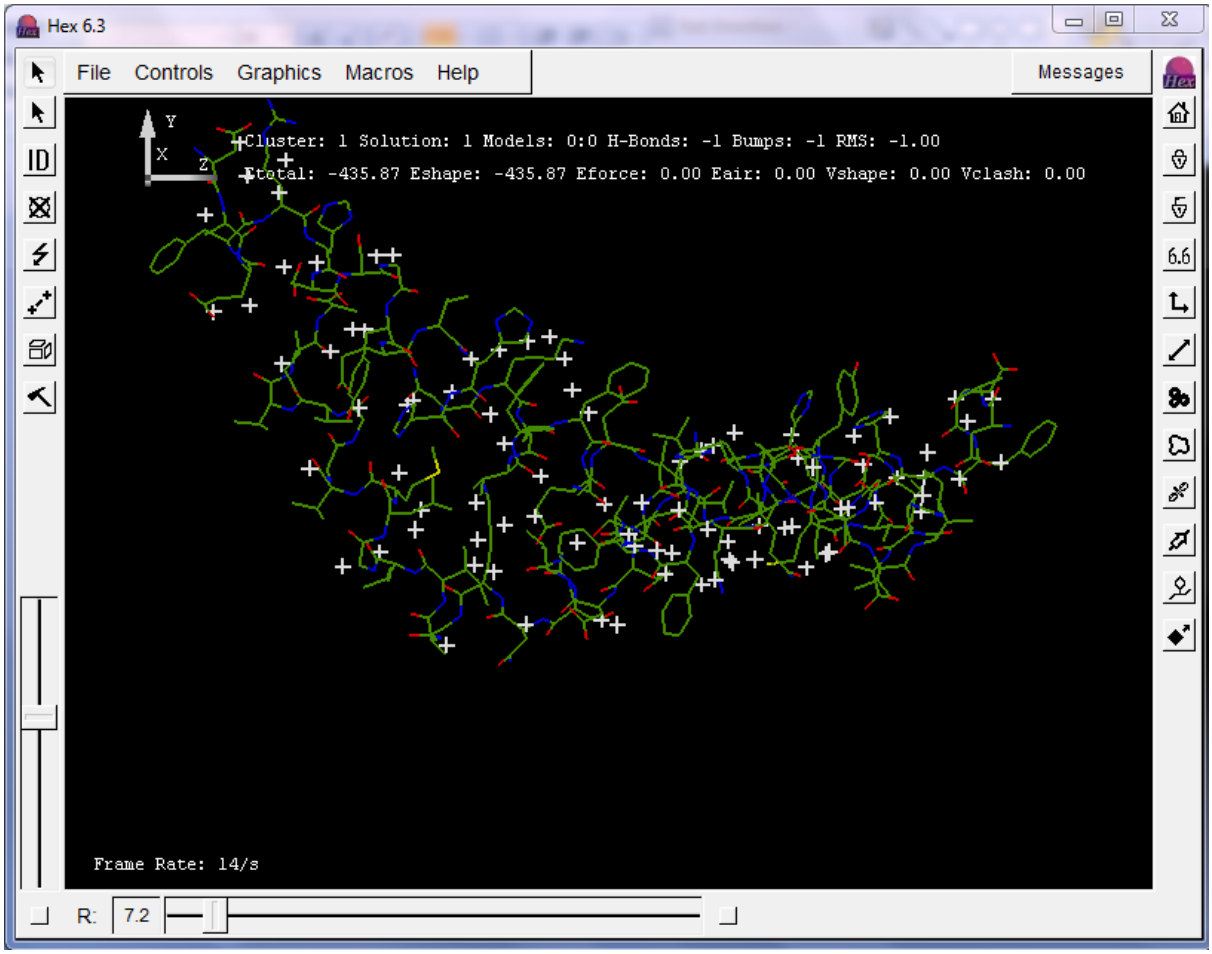


Figure 10: peptide peptide docking complex.

3.3.4 Inhibition of amyloid beta peptide by small compounds.

The already minimized protein structure was selected as the target for the inhibition study of it by various small compounds.

3.3.5 Retrieval of small compounds from pubchem

Pubchem is a database which provides us with structures of small organic molecules. It also contains information related to its origin and related literatures. It is updated by NCBI. Compounds are freely downloaded in .sd file format or chemical (CID) format.

a) The url for the site is pubchem.ncbi.nlm.nih.gov.
b) In the search box name of the inhibitors was typed.
c) The 3-D structures was retrieved in .sd file format.

3.3.6 Conversion of all the compounds from .sd file format to mol2 file format using OpenBabel software 2.3.1

It is used for searching, converting file format and analyzing chemical compounds. It is also used in molecular modelling and bioinformatics.

a)Open Babel 2.3.1 window was opened.

b) the input format was selected as .sd file format and then 3 dimensional structure was sleceted and then the output format was selected as mol2 file format.

c) the output was saved in a particular folder for future use.

3.3.7 Docking of abeta peptide and small ligand using swissdock.

Swissdock is a web server to predict the molecular interactions that may occur between a target protein and a small compound. It is based on EADock DSS engine , combined with setup scripts for curating common problems and for preparing both the target input and ligand input files[5]. The swissdock site is available online at *http://www.swissdock.vital-it.ch* . Here the structure of the target protein and as well as that of ligand, can be automatically prepared for docking. All calculations are performed on server side. The docking results or the docking predictions can be easily visualized in UCSF chimera molecular viewer, which can be downloaded directly from web server.

a) Firstly submit docking was clicked
b) In the target selection the minimized aβ peptide was uploaded.
c) Ligand selection was similarly used to upload the small molecules or inhibitors.
d) The job name was also given
e) Email address was given .
f) The result was automatically generated after 1 to 2 hours and it was downloaded as .chimerax file and was viewed in chimera.
g) The docked result was examined using chimera and the amyloidic region was highlighted and the least delta G value checked. The site of binding of the ligand in the protein was also studied.
h) The result was saved in .pdb file format

3.3.7 Ligplot/dimplot analyses to interprete the residues and bonds involved in binding at the binding site

a) ligplot window was opened

b) the protein ligand complex was opened in .pdb file

c) the ligplot analysis was done and for protein protein interaction dimplot analysis was done.

d) the figure was saved.

CHAPTER 4:

RESULT AND DISCUSSION

Alzheimer's disease (AD) pathogenesis is widely believed to be driven by the production and deposition of the amyloid beta peptide(Aβ) [6].In the current study mechanism of inhibition of the small compounds on the target protein was studied. Attempt was made to study the potential binding sites between amyloid inhibitor compounds and aβ peptide. Till now no such drugs are approved that can the formation of amyloid deposit. Hence in silico approach was used to study the mechanism of binding between them. Abeta is a short peptide with 42 residues. It consists of two alpha helical regions. Main characteristics of the amyloids are it has a cross beta structure irrespective of the source of protein, it is highly ordered , it aggregates very easily and is stable and can't be reversed. It also shows binding to specific dyes like congo red and thioflavin –T. The fold amyloid server successfully predicted the amyloidogenic region in the peptide. Amino acid portin from16-21 and (GYEVH) and 32-36(VFFAEDVGSN) were found to be amyloidogenic as shown in fig(11).

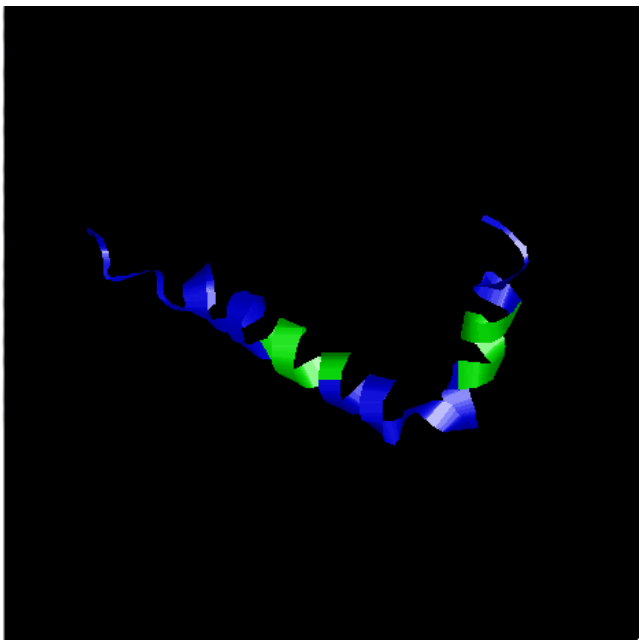


Figure 11: solution structure of amyloid beta peptide (1-42) with pdb id :1IYT. The amyloidogenic region (16-21 and 32-36) is highlighted in green colour.

Inhibitory compounds were selected which were already reported to inhibit the amyloid protein in vivo. The compounds selected were curcumin, atorvastatin, azure A, azure B, epigallocatechin gallate, CHEMBL205907, CHEMBL208350, CHEMBL208504, CHEMBL236182, CHEMBL236187, CHEMBL 236189, CHEMBL236192, indomethacin, vitamin e.

To identify the binding sites between aβ peptide and the compounds molecular docking was done using swissdock. Figure(12-left) shows the docking result between aβ peptide and curcumin. It was observed that the ligand binds in the vicinity of the amyloidogenic region VFFAEDVGSN formed by 32-36 residues. Later ligplus software was used to identify the interaction between the both which showed a special hydrophobic interaction in fig(12-right). The peptide residues which were involved in hydrophobic interaction were Ala21, glu22, Gly25,Lys28, Val24, Phe20.

This suggests that inhibitor binds near the amyloidogenic region and prevents aggregation by masking the amyloidogenic region.

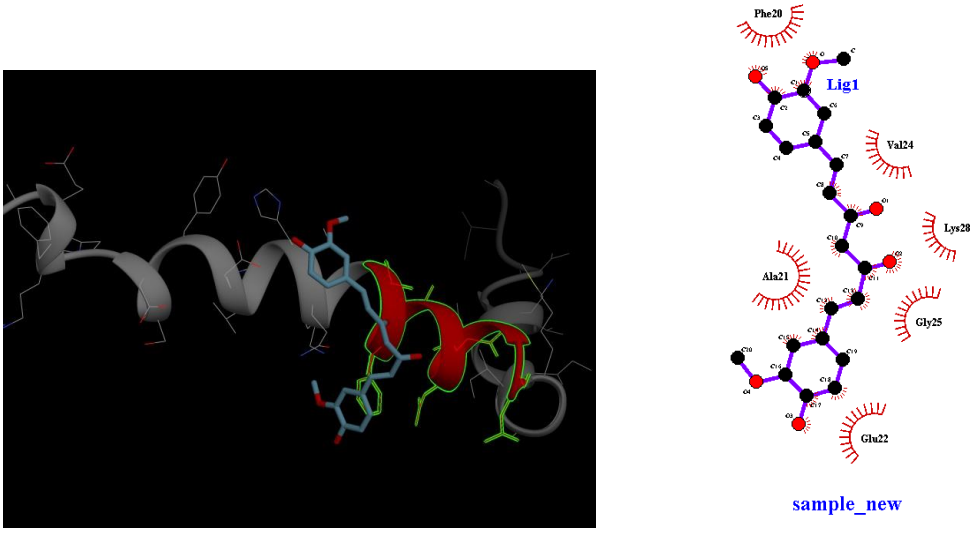
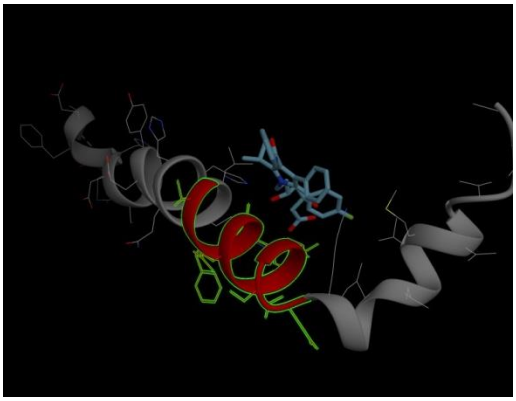


Figure 12: docking complex of abeta peptide with curcumin using swissdock(left) Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Figure (13) shows the docking result between the abeta peptide and atorvastatin. Here the ligand was found to interact with the 32-36 amyloidogenic region composed of VFFAEDVGSN . Ligplus shows the peptide residue which were involved in hydrogen bonding and also hydrophobic interaction. The residue involved in hydrogen bonding was Lys16 and the residues involved in hydrophobic interaction were Leu17, Ala21, Gly25, Val24, Leu34, Phe20.



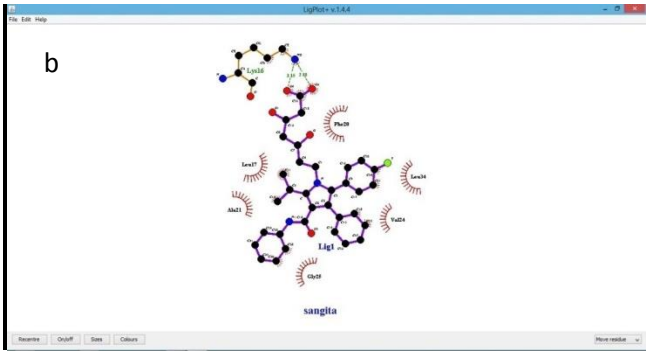


Figure 13: docking complex of abeta peptide with atorvastatin using swissdock(above).Corresponding Ligplot image (below) of the docking complex showing specific interactions.

Figure (14) shows the docking result between aβ peptide and azure A. Here the compound was found to bind in the vicinity of the amyloidogenic 32-36 region. Ligplus showed the hydrophobic interaction .The major residues involved were Phe20, Leu17, Ala21.

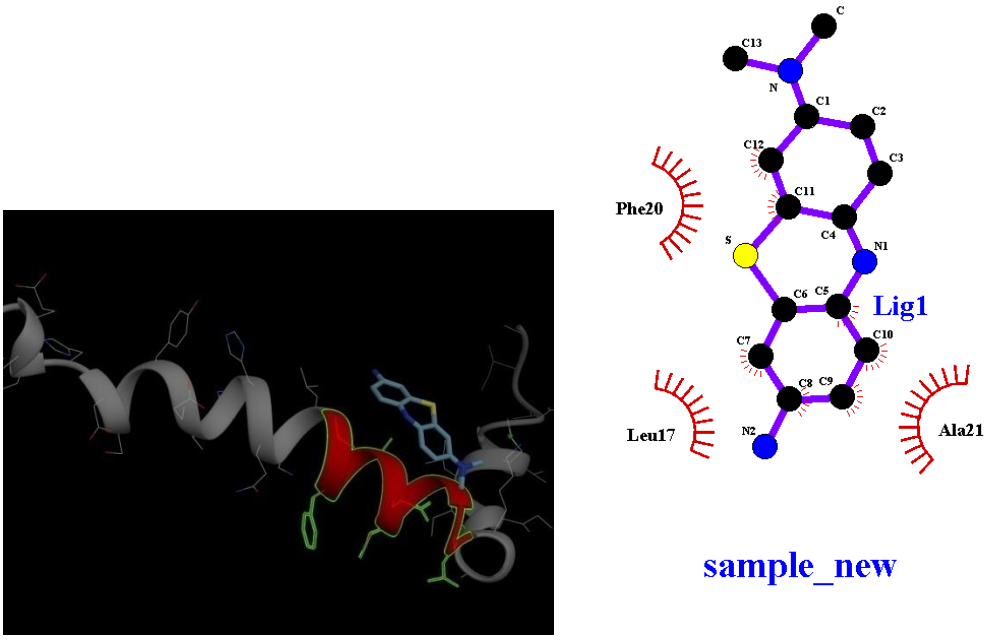


Figure 14: docking complex of abeta peptide with azure A using swissdock(left) Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Figure (15) shows the docking result between aβ peptide and azure B. Here again the compound was found to bind in the vicinity of the amyloidogenic 32-36 region. ligplus showed the major residues Ala2,Glu22,Ser26,Gly25,Val24 involved in hydrophobic interaction.

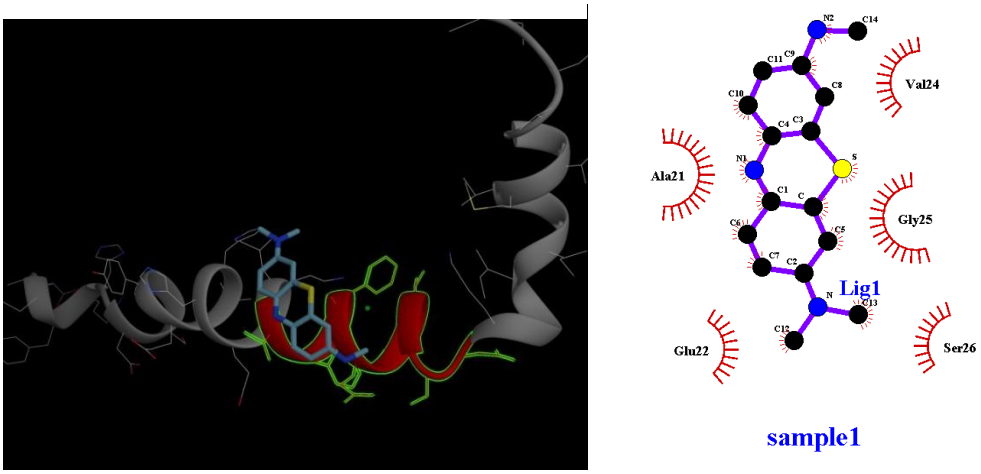


Figure 15: docking complex of abeta peptide with azure B using swissdock(left) Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig(16) shows the docking result between abeta peptide and epigallocatechin gallate. The compound was shown to inhibit in the amyloidogenic region 32-36. Ligplus showed hydrophobic interactions between the Glu22,Gly25,Ala21,Vall8 residues of the peptide and the atoms of the inhibitor molecuole.

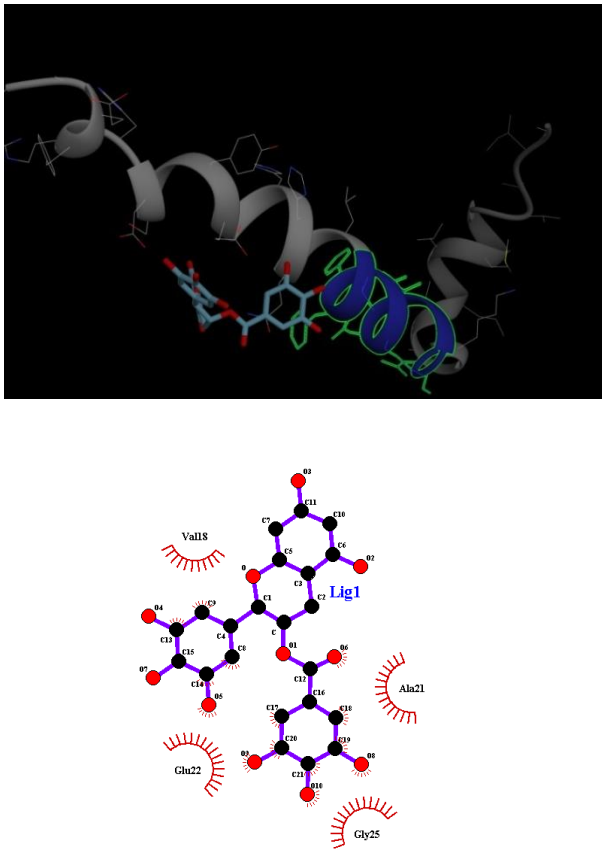


Figure 16: Docking complex of abeta peptide with epigallocatechin gallate using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig(17) shows the docking result between abeta peptide and CHEMBL205907. Here also the ligand bound to the amyloidogenic region highlighted in red colour. The ligplot showed hydrogen bonding between the Asp23,Glu22, Ser21 residues of the peptide and the ligand and hydrophobic interaction between the Asn27 residue of peptide with the ligand.

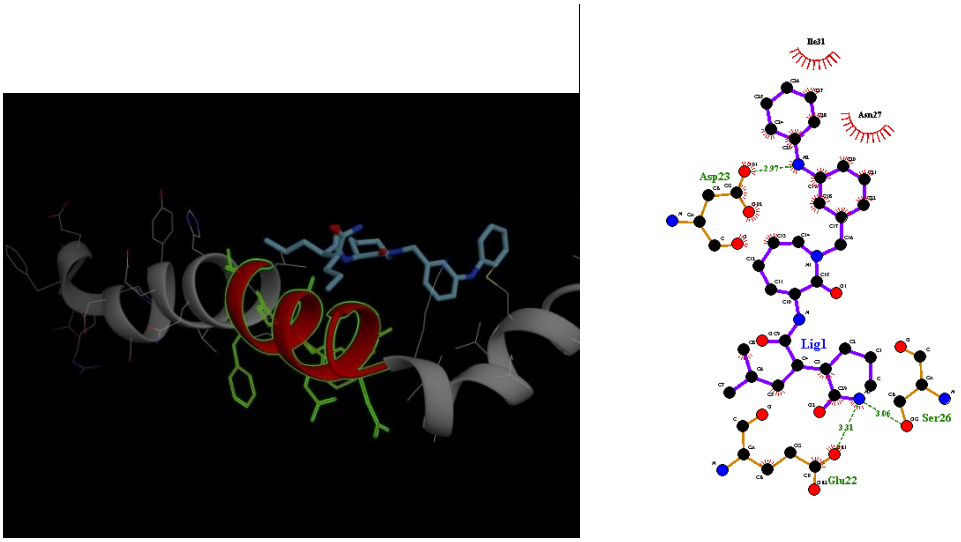


Figure 17: docking complex of abeta peptide with CHEMBL205907 using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig(18) shows the docking result between abeta peptide and CHEMBL208350. The ligand binds near amyloidogenic region. Ligplot showed hydrophobic interaction mainly involving the residues Ala21, Lys28, Leu34, Phe20, Val24, Met35 of the peptide .

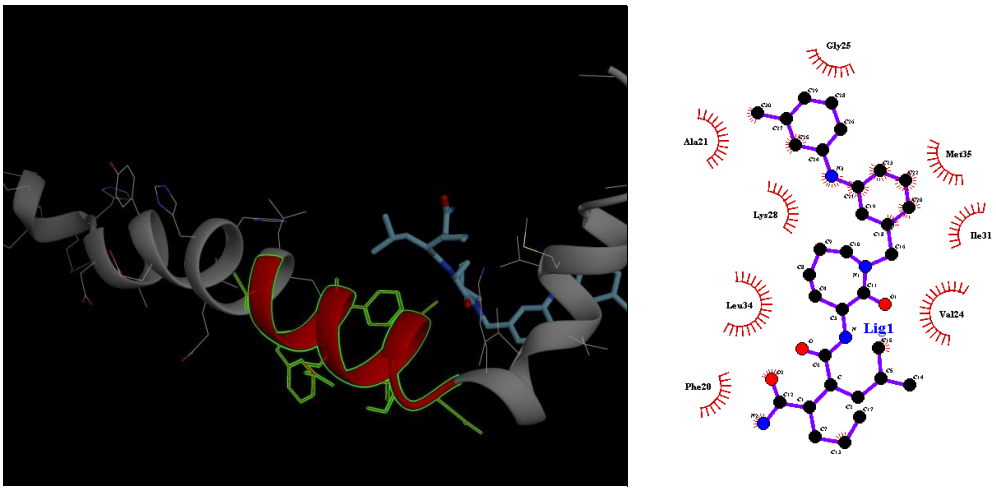


Figure 18: docking complex of abeta peptide with CHEMBL208350 using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig (19) shows docking result between abeta peptide and CHEMBL208504. Here also the ligand binds near peptide sequence of 32-36 and ligplot showed hydrogen interaction between Leu17 and hydrophobic interaction between Ala21, Leu34, Phe20 residues and the ligand.

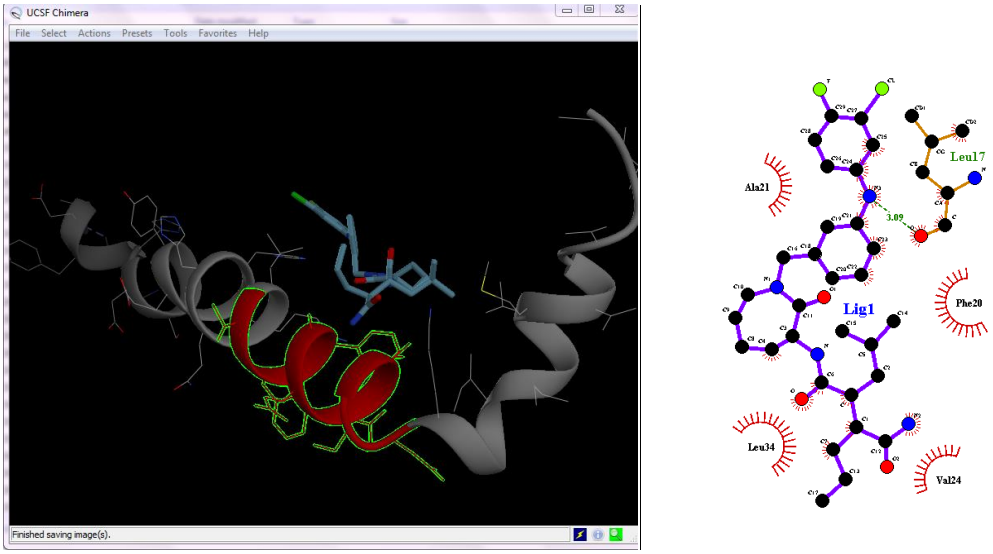


Figure 19: docking complex of abeta peptide with CHEMBL208504 using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig(20) shows docking result between abeta peptide and CHEMBL236182. Ligplot shows hydrophobic interaction between Met35,Ala42, Gly38,Leu34,Val24,Phe20 residues and ligand.

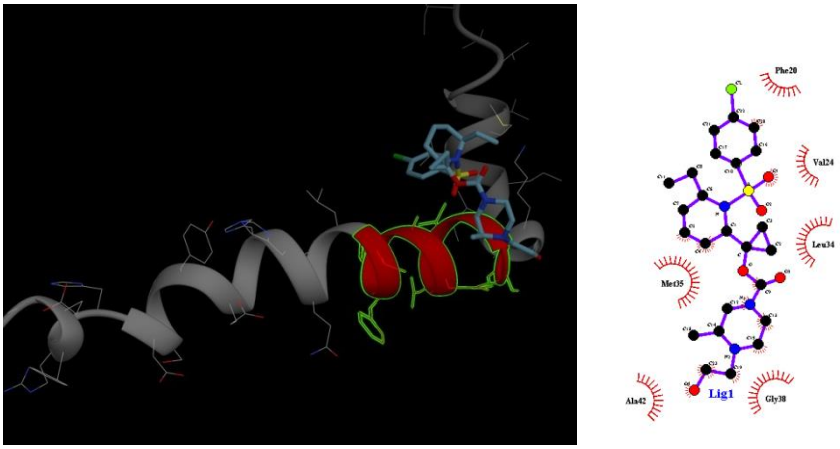


Figure 20: docking complex of aβ peptide with CHEMBL236182 swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig(21) shows docking result between aβ peptide and CHEMBL 236187.ligplot shows hydrophobic interaction between Val24,Leu34,Lys28,Leu17,Met35 residues and ligand.

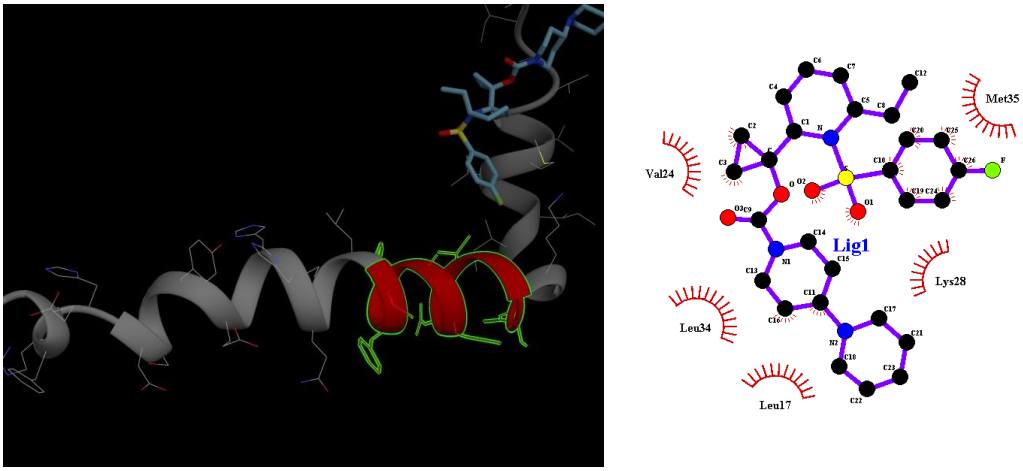


Figure 21: docking complex of aβ peptide with CHEMBL 236187 using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Figure 22: shows the docking result between aβ peptide and CHEMBL236192 . The key residues involved in hydrophobic interaction between the both are Val24, Gly25, Glu22, Ala21, Met35.

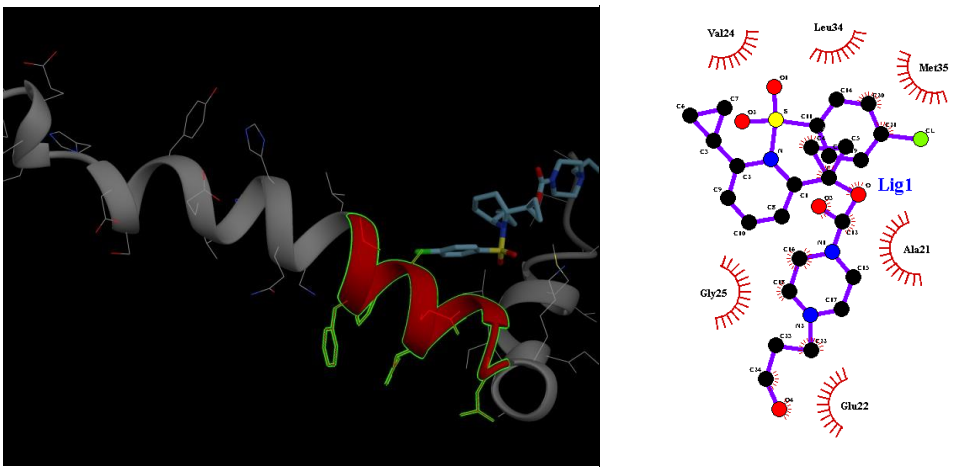


Figure 22: docking complex of aβ peptide with CHEMBL 236192 using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

FIG 23 shows docking result between aβ peptide and indomethacin. Ligplot shows hydrogen bonding between Lys28 and hydrophobic interaction between Val24, Gly38, Val39, Met35, Leu34 and the ligand.

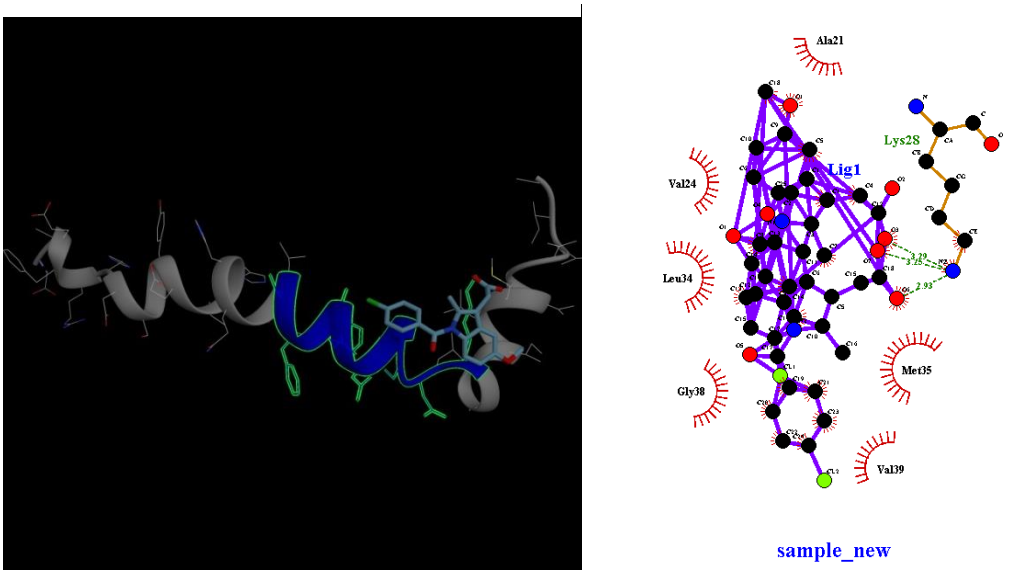
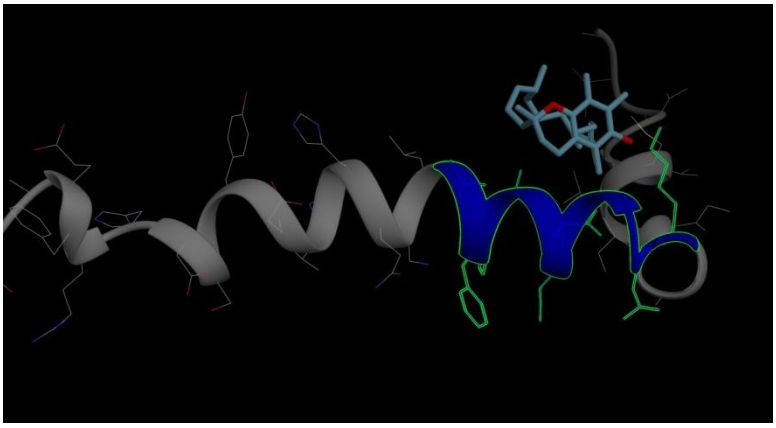
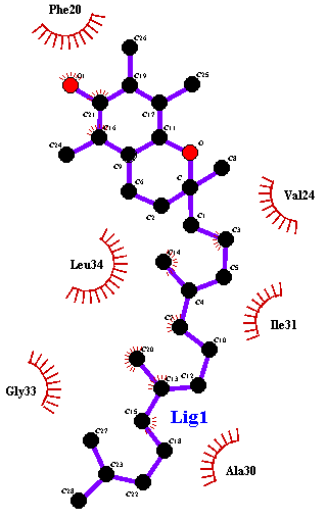


Figure 23: docking complex of aβ peptide with indomethacin using swissdock(left).Corresponding Ligplot image (right) of the docking complex showing specific interactions.

Fig 24 shows docking result between aβ peptide and vitamin e(alpha tocopherol). Ligplot shows hydrophobic interaction between the residues Phe20, Leu34, Gly35, Ala30, Val24 and the ligand.



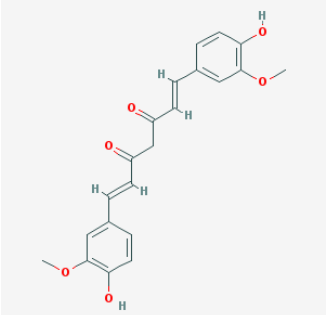


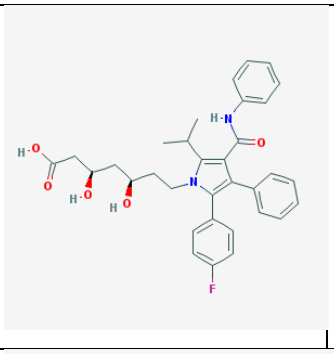
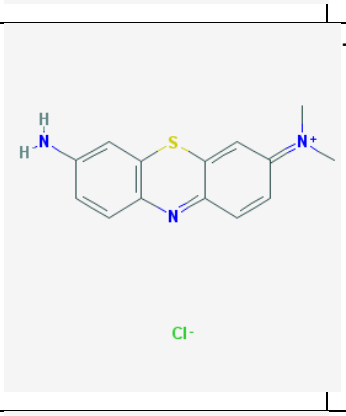
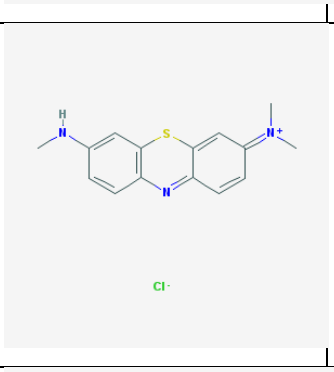
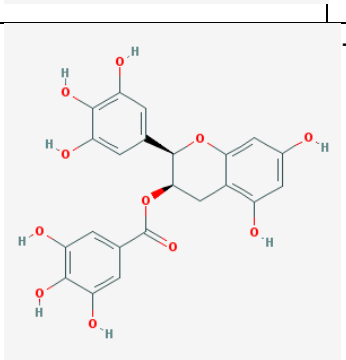
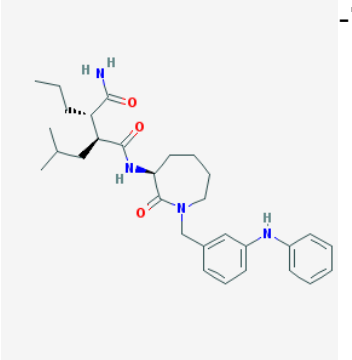
sample_new

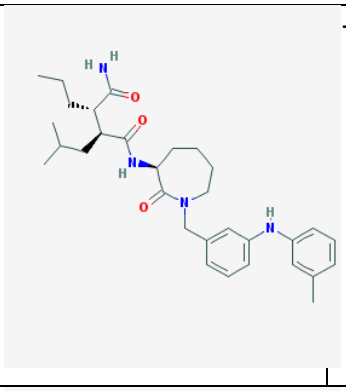
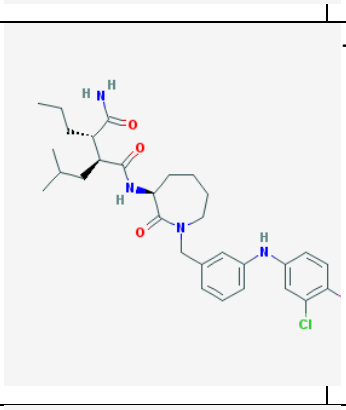
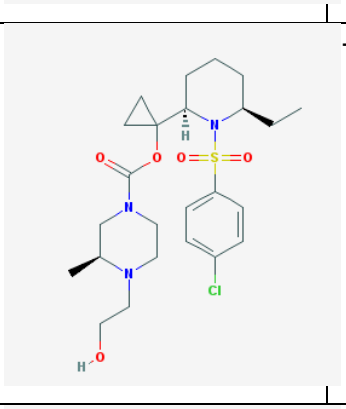
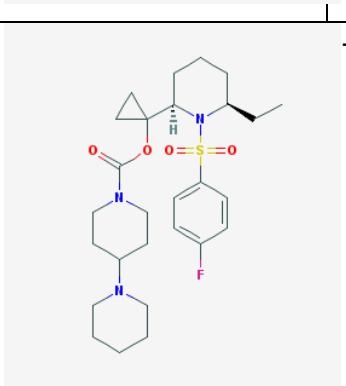
Figure 24: docking complex of aβ peptide with vitamin e(alpha tocopherol)using swissdock(above).Corresponding Ligplot image (below) of the docking complex showing specific interactions.

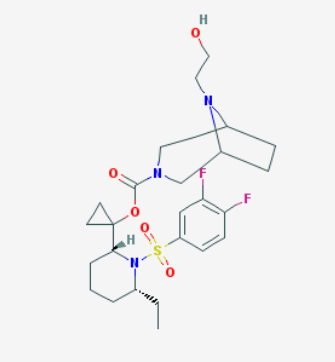
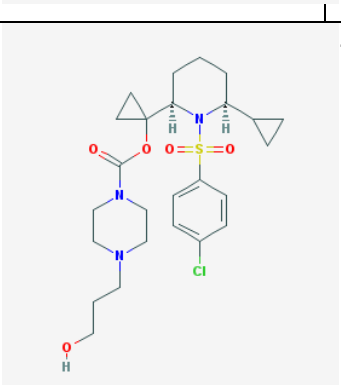
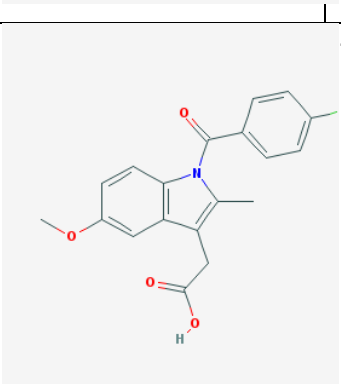
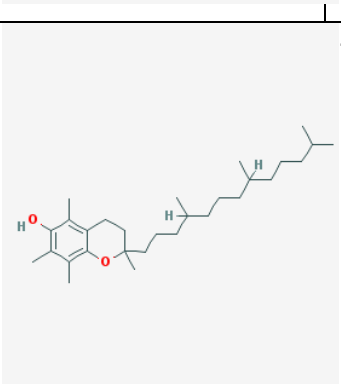
Thus the docking result showed that all the inhibitor compounds bound near the vicinity of the amyloidogenic region of the aβ peptide as predicted by fold amyloid software.

Table(2): list of selected inhibitor molecule with delta G value of docking with aβ peptide.

Sl.no	Inhibitor name	Pubchem id/chembl id	2D structure	Delta G value
1.	curcumin	CID 969516		-7.16

2.	Atorvastatin	CID 60823		-8.09
3.	Azure A	CID 13735		-8.26
4.	Azure B	CID 68275		-7.09
5.	Epigallocatechin gallate	CID 65064		-7.13
6.	CHEMBL 205907	CID11962185		-7.3

7.	CHEMBL 208350	CID44410 629		-7.57
8.	CHEMBL 208504	CID44410 506		-7.41
9.	CHEMBL 236182	CID44435 381		-8.07
10.	CHEMBL 236187	CID44435 387		-8.36

11.	CHEMBL 236189	CID44435389		-8.07
12.	CHEMBL 236192	CID44435393		-8.14
13.	Indomethacin	CID3715		-7.91
15.	Vitamin e(alpha tocopherol)	CID2116		-7.00

Docking study was also done between two aβ peptide molecules to understand the mechanism of binding and type of interactions. Fig(27) shows the docking result of protein protein interaction. It has been noticed that there is a strong binding between the two aβ molecules which has a binding energy of -435.87 KJ/mol.

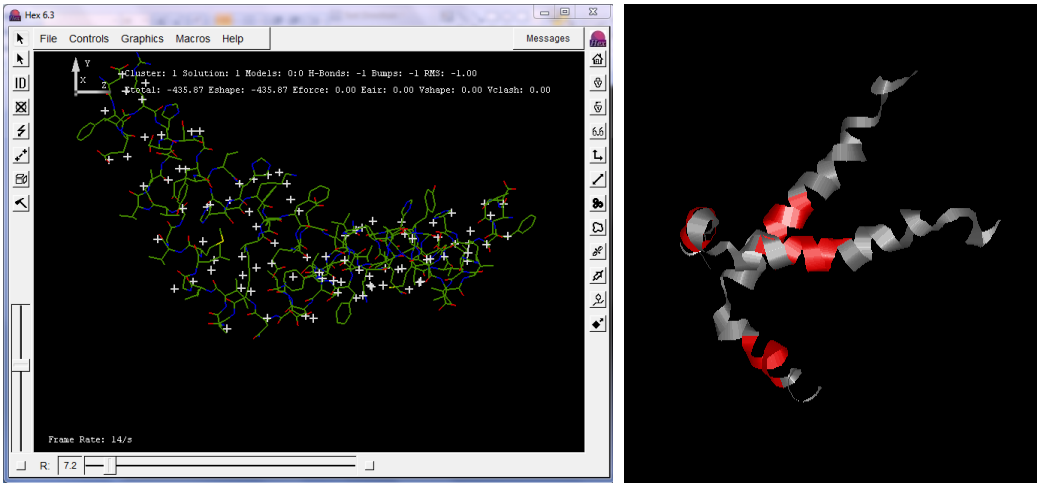


Figure 25: peptide peptide docking result.

CONCLUSION

In the current study we found out that the small compounds which we found out based on the literature reviews bound to the aβ peptide in the vicinity of the amyloidogenic region. Amyloid beta peptide is the main component which is responsible for the onset of Alzheimer's disease. Based on the docking study between amyloid inhibitor small compounds and aβ peptide we gained knowledge and by the help of which we could prevent further aggregation. The amyloidogenic region in the aβ peptide was predicted by the fold amyloid software. It was found out that the inhibitor compounds bound in the vicinity of the amyloidogenic region suggesting their mechanism of action by masking the amyloidogenic region and further preventing aggregation of the peptide. By the use of ligplot software we could see the detailed interaction between the inhibitors and the peptide. It was concluded that the major interaction between them was due to the hydrophobic and hydrogen bonding which made the complex more stable which might play a role in preventing further aggregation.

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