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COMPARATIVE ANALYSIS OF ALZHEIMER PROTEINS: STRUCTURE BASED DRUG DESIGNING STUDY

Shivani Patel[1], Venkateshwara Rao Joginipally[2], Vaishnavi Mallojala[1], Shravan Kumar Gunda[1]*

[1]Bioinformatics Division, PGRRCDE, Osmania University, Hyderabad – 500007, India

[2]Department of Zoology, University college of Science, Osmania University, Hyderabad – 500007, India

*Corresponding Author Email id: gunda14@gmail.com

Abstract

Neurodegenerative Disease refers to the condition of the brain cells that no longer have the ability to produce neurotransmitters which leads to unnecessary accumulation of proteins in the brain. The accumulation causes problems like memory retention and cognitive functions. Neurodegenerative Diseases are categorized by four major types such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotropic Lateral Sclerosis. Alzheimer's sickness (AD) is interminable neurodegenerative turmoil with characterized pathophysiological mechanisms which for the most part target the medial temporal lobe and associative neocortical structures. Pathological hallmarks of AD are neurotic plaques and neurofibrillary tangles. AD causes progressive and restricting declension of cognitive functions like memory, language, reasoning, attention, comprehension, judgment, psychosis, mood disturbance, agitation, and sleep abnormalities. The most common symptom seen in the early onset of the disease is selective short-term memory loss. The disease is invariably progressive, eventually leading to severe cognitive deterioration. The following molecular docking study is a comparative study with already existing Alzheimer's proteins. The proteins have been compared on the basis of their binding energies against five natural compounds which are selected because they are natural substances obtained from the plants. The results were compared with already existing drugs. The main purpose of using natural compounds is that their side effects are low to none when compared to synthetic compounds and the cost of their drug production will also be low.

Key Words: Alzheimer's, docking, natural compounds, comparative study.

Introduction

Dementia refers to the waning of cognitive abilities that are severe enough to disturb with the activities of daily life [1]. Alzheimer's disease (AD) is the most common reason for age-related dementia. It is denoted by cognitive impairment and severe neurodegeneration. It begins with subtle and poorly recognized failure of memory which slowly becomes more severe and eventually, debilitation. Collective studies incorporate disarray, misguided thinking, dialect unsettling influence, tumult, withdrawal, and mental trips [2]. Infrequent seizures, Parkinsonian highlights, expanded muscle tone, myoclonus, incontinence, and mutism happen. Passing as a

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rule results from general inanition, unhealthiness, and pneumonia. The regular clinical length of the ailment is eight to ten years, with a range from one to 25 years.

Abnormal deposition of neurotic plaques and neurofibrillary tangles are the pathological hallmarks of AD [3]. Plaques are globular microscopic lesions that have a core of extracellular amyloid beta peptide surrounded by inflamed axonal endings. Beta-amyloid peptide is acquired from a transmembrane protein called amyloid precursor protein (APP). The action of alpha, beta and gamma-secretase proteases cleaves the beta-amyloid peptide from APP. Generally, either alpha or beta-secretase cleaves APP into tiny fragments. These are almost nontoxic to the neurons. Although, consecutive cleavage of APP by beta followed by gamma-secretase results in 40 and 42 amino acid peptides (beta-amyloid 40 and beta-amyloid 42) [4]. Neuronal toxicity is caused by aggregation of amyloid in the brain due to elevation of beta-amyloid 42. Beta-amyloid 42 favors formation of aggregated fibrillary amyloid protein over regular APP degradation. *APP* gene is located on chromosome 21, one of the regions associated to the familial Alzheimer disease [5]. Deposition of amyloid occurs around the meningeal and cerebral vessels and the gray matter. Amyloid deposits on the gray matter are multifocal and they fuse to form structures called plaques [6]

Tau protein causes the formation of fibrillary intracytoplasmic structures called neurofibrillary tangles. The chief role of tau protein is to stabilize axonal microtubules. Microtubules play an important role in intercellular transport and they also run along the neuronal axons [7]. Their assembly is kept intact by tau protein. Aggregation of extracellular beta-amyloid that happens in AD causes hyper phosphorylation of tau which then causes the formation of tau aggregates. These aggregates form twisted coupled helical filaments called neurofibrillary tangles [8]. These tangles are initiated in the hippocampus and then may spread throughout the cerebral cortex. Another characteristic symptom of AD is granulovacuolar degeneration of hippocampal pyramidal cells caused by amyloid angiopathy cerebrovascular disease and fourfold with subcortical infarcts exaggerates the degree of dementia and its rate of progression in Alzheimer's disease [9].

The present study is a comparative report of five selected proteins for Alzheimer's disease. The proteins are compared on the basis of their protein-ligand interactions against selected natural compounds that have been known to work against Alzheimer's. The proteins selected for the study are: PDB id: *1EVE*, PDB id: *3IFO*, PDB id: *3ZLT*, PDB id: *4BTL* and PDB id: *5FOQ*.

Protein with the PDB id: 1EVE from Tetronarce californica produces an enzyme called acetylcholinterase which hydrolyses the neurotransmitter acetylcholine and is found at the synapse between nerve cells and muscle cells. Acetylcholine, a neurotransmitter vital for processing memory and learning, is reduced in both concentration and function in patients suffering from Alzheimer's disease [10]. It is also known to terminate signal transduction at the neuromuscular junction by quick hydrolysis of the neurotransmitter acetylcholine that is released in between the synaptic cleft. It is also presumed to take part in interactions between cells [11].

Protein with the PDB id: 3IFO from Mus musculus is known to produce amyloid beta peptides that are crucially involved in AD, a main component of amyloid plaques that causes the

shrinkage of the brain tissue of AD patients [12]. It functions as a cell surface receptor and it also performs physiological functions on neuronal surfaces which is relevant to neurite growth, adhesion of neuronal and axonogenesis. It is involved in mobility of cells and regulation of transcription through protein-protein interactions. It can also promote transcription activation by binding to APBB1-KAT5 and it is also known to inhibit the signaling of Notch by making interactions with Numb [13, 14, 15] It participates in the apoptosis-inducing pathways. It mediates the axonal transport of the enzymes beta-secretase and presenilin 1 by acting as a kinesin I membrane receptor. By copper ion reduction it controls the copper homeostasis/oxidative stress [16, 17].

Protein with the PDB id: 3ZLT from Mus musculus also produces acetylcholinterase which hydrolyses the neurotransmitter acetylcholine thereby decreasing its concentration in the brain. Decreased quantity of acetylcholine that are seen in AD patients interferes with normal memory and learning functions hence making decreased cognitive activity a marker symptom for AD [18]. Tabun, Russian VX and cycosarin are nerve agents that inhibit the enzyme acetylcholineterase (AChE) by organophosphorylating the catalytic serine residue. Oximes are neucleophiles that are used as amtidotes as they have the capability to restore and reactivate the enzyme AChE. HI-6 is an oxime which shows a low activity on tabun adducts but can effectively reactivate both Russian VX and cyclosarin adducts [18].

Protein with the PDB id: 4BTL from Mus musculus is also known to produce acetylcholinterase that effects the production of acetylcholine in the brain [18]. Protein with the PDB id: 5FOQ from Mus musculus which produces acetylcholinterase that hydrolyses acetylcholine and causes intellectual deterioration in people with AD [19].

The compounds used for the study are: *Campesterol, Cudraflavone, Nimbin, Curcumin* and *Yanuthone*. These compounds are selected because they are natural substances obtained from the plants. The main purpose of using natural compounds is that their side effects are low to none when compared to synthetic compounds and the cost of their drug production will also be low. *Campesterol* is a plant sterol which, according to the recent studies, reduces the generation of amyloid beta generation. *Campesterol* being a phytosterol, is found in nuts, legumes, seeds and unrefined plant oils. Phytosterols decrease the absorption of cholesterol in the intestine and are able to cross the blood-brain barrier [20].

Cudraflavone B is a prenylated flavonoid that is found in large amounts in the roots of *Morus alba* and *Cudrania tricuspidata.* Various of studies on *cudraflavone B* have been conducted which has observed that the flavonoid shows mouse brain monoamine oxidase (MAO) inhibitory effects, anti-proliferative activity, hepato-protective activity and apoptotic actions in human gastric carcinoma cells and mouse melanoma cells. The study [21] demonstrated that C*udraflavone B* showed neuro-protective effects in neurodegenerative disorders. Glutamate is an essential excitatory neurotransmitter in the central nervous system [22]. *Cudraflavone B* had a potential application as a therapeutic agent for neurodegenerative disorders [23].

Nimbin is a triterpenoid that is isolated from neem and is also thought to be responsible for most of the biological activities of neem oil. A study by Heppner FL et.al [24] reports that new data from clinical and preclinical studies have discovered that immune system-mediated

actions do in fact contribute to and drive the pathogenesis of AD. Inflammation in AD primarily concerns with the innate immune system. Neuro-inflammation in Alzheimer's disease is primarily caused by the brain's intrinsic myeloid cells, also known as microglia, and it progresses with the advancement of the disease. *Nimbin* is reported to have antipyretic, anti-inflammatory, antihistamine and antiseptic properties.

Curcumin a compound extracted from *Curcuma longa* is a main curcuminoid and is considered to be the most active constituent of the plant. Nerve cell degradation in Alzheimer's is believed to show certain properties such as oxidative damage, inflammation, formation of beta-amyloid plaques and metal toxicity. Studies have shown that effects of *curcumin* like decreased Beta-amyloid plaques, metal-chelation, increased antioxidant effects, delayed degradation of neurons, anti-inflammatory effect, increase in hemoxygenase, decrease in free radical formation, decrease in phospholipase and cyclohexogenase, decreased microglia formation, and the overall improvement in memory have been observed in AD patients who have increased the uptake of the *curcumin* in the form of turmeric (*Curcuma longa*) [25].

Yanuthone E is a novel bioactive farnesylated epoxy cyclohexenone from the *Aspergillus niger* isolate obtained from the tissue homogenates of an orange *Aplidium* sp. Ascidian. Recent advancements in genetics and genomics have helped us study the metabolic pathways of fugal metabolites which has lead us to identify biologically interesting compounds Studies have shown that the metabolites obtained from *Aspergillus niger* can be sued as inhibitors for tau aggregation that is normally observed in the brains of the people suffering from AD. The study by Paranjape et.al. [26] was conducted on azaphilones which are secondary metabolites of *Aspergillus.* Many of the compounds that they had selected contained aromatic ring structures that were common to tau aggregation inhibitors. It is believed that the ring structures found in tau aggregation inhibitors interact with the β structure that characterizes aggregation prone tau conformations which thereby prevents the spread of filament formation. *Yanuthone E* being an *Aspergillus* metabolite is also believed to have such effects.

The activity of the docked ligand molecules were also compared to standard FDA approved drugs which were taken as control. The standard drugs that were used as controls are: *Rivastigmine, Memantine* and *Galantamine.*

Methodology

Sequence Selection

The structures used for the comparative study were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB)/Protein Data Bank (PDB). The ligand sites of the proteins were studied using PDBsum. It was also used to find the active sites of the selected proteins. The proteins that were narrowed down for the study have the following PDB ids: 1EVE, 3IFO, 3ZLT, 4BTL and 5FOQ. The crystal structures of the proteins were retrieved from protein database like PDB [11, 12, 18, 27, 19]. When choosing the protein, it is important to consider the resolution of the protein and its ligand sites. When both parameters are high the resulting model would be adequately good to allow structural and functional research.

Docking Methodology

Identification of the active site pockets: The active sites of the existing proteins were obtained from PDBsum. Protein with PDB id: 1EVE showed the following amino acids as active sites: Asn59 and Ser61. Protein with PDB id: 3IFO showed the following amino acids as active sites: His31, Ser32, Tyr54, Asp56, Asp58, Gly96, Ser97, Val99, Thr103 and Asp108. Protein with PDB id: 3ZLT showed the following amino acid as active site: Asn464. Protein with PDB id: 4BTL showed the following amino acids as active sites: Asp74, Trp86, Gly120, Gly121, Gly122, Tyr124, Glu202, Trp286, Ser293, Ile294, Phe295, Tyr337, Phe338, Tyr341 and His447. Protein with PDB id: 5FOQ showed the following amino acids as active sites: Gly345, Ser347, Asn350, Ser352 and Leu353.

In total five molecules were obtained from NCBIpubchem Compound. The structures were sketched using sybyl6.7 and were minimized by adding Gasteiger-Huckel charges and were saved in the .mol2 format. The natural compounds were docked against the entire five target proteins separately using AutoDock4.2 [28] program. Lamarckian Genetic Algorithm (LGA) was used and empirical free energy function was also implemented. The existing template proteins with PDB ids: 1EVE, 3IFO, 3ZLT, 4BTL and 5FOQ were first loaded in AutoDock4.2 individually and hydrogens were added and before they were saved in the PDBQT format. The ligand was loaded next and the confirmations were set after which it was also saved in the PDBQT format. AutoGrid was used to calculate the grid parameters after they were changed according to the protein's active site. For docking of all the molecules against all the target proteins, 0.375 Å was taken as the grid-point space and the grid box was given the dimensions of 60×60×60. X,Y,Z coordinates of each protein were added on the basis of the amino acids present in their respective active sites that were taken for all the target proteins from PDBsum. Autodock was then run.

Results and Discussion

Molecular docking is an important tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is the prediction of the predominant binding mode(s) of a ligand with a protein with an already existing three-dimensional structure [29]. The role of the present study was to compare the protein ligand interactions of natural compounds against target proteins for Alzheimer's disease. The plant secondary metabolites (ligands) were observed to show excellent inhibitory activity against the target templates. Genetic algorithm is used by AutoDock4.2 which takes into account binding free energy assessment to assign the best binding conformation. The activity of the docked ligand molecules were also compared to three standard FDA approved drugs: Rivastigmine, Memantime and Galantamine which were taken as control. In total, five natural compounds: Campesterol, Cudraflavone B, Nimbin, Curcumin and Yanuthone E were docked individually against the following already existing AD target proteins: PDB ids: 1EVE, 3IFO, 3ZLT, 4BTL and 5FOQ.

The role of docking is to place both, protein and ligand molecule in various orientations and conformational positions and the ones with lowest energy confirmations which are also energetically favorable are evaluated and analyzed to obtain interactions. Five molecules were

docked against five target proteins out of which most of them showed good interactions with lowest biding energies. The compound that showed the lowest free energy value is Yanuthone E. It showed three interactions (Leu29, Ser30 and Lys78) against the protein with PDB id: 3IFO with the binding energy of -11.57 KCal/mol and dissociation constant of 3.3nM.

The compound Campesterol exhibited good results against the proteins with PDB ids: 4BTL, 1EVE and 5FOQ. It exhibited one interaction (Trp286) against 4BTL with the binding energy of -11.55 KCal/mol and 3.41nM of dissociation constant. Against 1EVE, one interaction (Ser55) was observed with the binding energy of -8.40 KCal/mol and dissociation constant of 693.15nM. Campesterol interacted with one amino acid (Phe346) of 5FOQ and gave the binding energy of -8.04 KCal/mol and dissociation constant of 1.29μM.

Cudraflavone B exhibited two interacting amino acids (Asp74 and Ser293) with the protein with PDB id: 4BTL with the -10.26 KCal/mol binding energy and 30.1nM dissociation constant. The compound nimbin displayed one interaction (Thr75) with the protein with PDB id: 4BTL; the binding score was observed to be -8.75 KCal/mol with the dissociation constant 386.21nM.

Curcumin displayed four interactions (Asp74, Thr75, Leu76 and Arg296) with the protein with PDB id: 4BTL; the binding energy was shown to be -8.49 KCal/mol with the dissociation constant of 602.55nM. The natural compounds with their corresponding interactions and binding energies against all five target proteins are shown in table 1-5 and figures 1-5. The standard drugs that are used as a control are marked with an asterisk (*) and their interactions and binding energies are shown in the tables. The cartoon models of docking interactions with compounds that have high binding energy for each protein shown in the figure 1-5 viz., 1a, 5a, 1b, 1c, 1d for proteins with PDB ids: 1EVE, 3IFO, 3ZLT, 4BTL and 5FOQ respectively.

Table 1: Interactions and binding energies of the protein with PDB id: 1EVE with the corresponding natural compounds and standard drugs that are indicated by * (-17.528, 80.274, 46.241)

S.no	Compounds	Interacting Amino Acids	Binding Energy (ΔG) Kcal/mol	Dissociation Constant (KI)
1	Campesterol	Ser55	-08.40	693.15 nM
2	Cudraflavone B	Asp53, Val57, Asn59	-07.55	2.91 μM
3	Nimbin	Asn59, Asn65	-06.84	9.61 μM
4	Curcumin	Asn52, Asn59, Ser61, Thr62	-06.36	21.63 μM
5	Yanuthone E	-	-03.54	2.54 mM
6	Rivastigmine*	Asn59	-05.08	189.67 μM
7	Memantine*	Asn59, Gly32	-06.10	33.96 μM
8	Galantamine*	Asn59, Thr62	-06.80	10.3 μM

Table 2: Interactions and binding energies of the protein with PDB id: 3IFO with the corresponding natural compounds and standard drugs that are indicated by *

S.no	Compounds	Interacting Amino Acids	Binding Energy (ΔG) Kcal/mol	Dissociation Constant (KI)
1	Campesterol	Thr31	-04.36	633.81 µM
2	Cudraflavone B	Trp55, Asp56, Aps57, Lys73	-05.28	135.09 µM
3	Nimbin	Lys73, Thr75	-04.45	551.52 µM
4	Curcumin	Thr31, Ser32, Thr75, Asp74	-04.10	995.37 µM
5	Yanuthone E	Leu29, Ser30, Lys78	-11.57	3.3 nM
6	Rivastigmine*	Thr75, Lys73	-03.25	4.14 mM
7	Memantine*	-	-03.68	2.0 mM
8	Galantamine*	Lys73, Ser76, Asp74	-04.54	466.94 µM

Table 3: Interactions and binding energies of the protein with PDB id: 3ZLT with the corresponding natural compounds and standard drugs that are indicated by *

S.no	Compounds	Interacting Amino Acids	Binding Energy (ΔG) Kcal/mol	Dissociation Constant (KI)
1	Campesterol	Asn464	-07.47	3.33µM
2	Cudraflavone B	Asn464,Tyr465, Thr466	-05.27	137.83 µM
3	Nimbin	Thr467	-05.41	107.62 µM
4	Curcumin	Ser435, Glu452	-05.65	72.11 µM
5	Yanuthone E	-	-	-
6	Rivastigmine*	Leu459	-04.81	296.96 µM
7	Memantine*	Thr436	-06.11	33.25 µM
8	Galantamine*	-	-06.47	18.0 µM

Table 4: Interactions and binding energies of the protein with PDB id: 4BTL with the corresponding natural compounds and standard drugs that are indicated by *

S.no	Compounds	Interacting Amino Acids	Binding Energy (ΔG) Kcal/mol	Dissociation Constant (KI)
1	Campesterol	Trp286	-11.55	3.41nM

2	Cudraflavone B	Asp74, Ser293	-10.26	30.1nM
3	Nimbin	Thr75	-08.75	386.21nM
4	Curcumin	Asp74, Thr75,Leu76	-08.49	602.55nM
5	Yanuthone E	Ser293, Phe295, Arg296	-07.59	2.71 µM
6	Rivastigmine*	Phe295	-06.81	10.21 µM
7	Memantine*	-	-06.50	17.3 µM
8	Galantamine*	Tyr337	-08.60	496.33nM

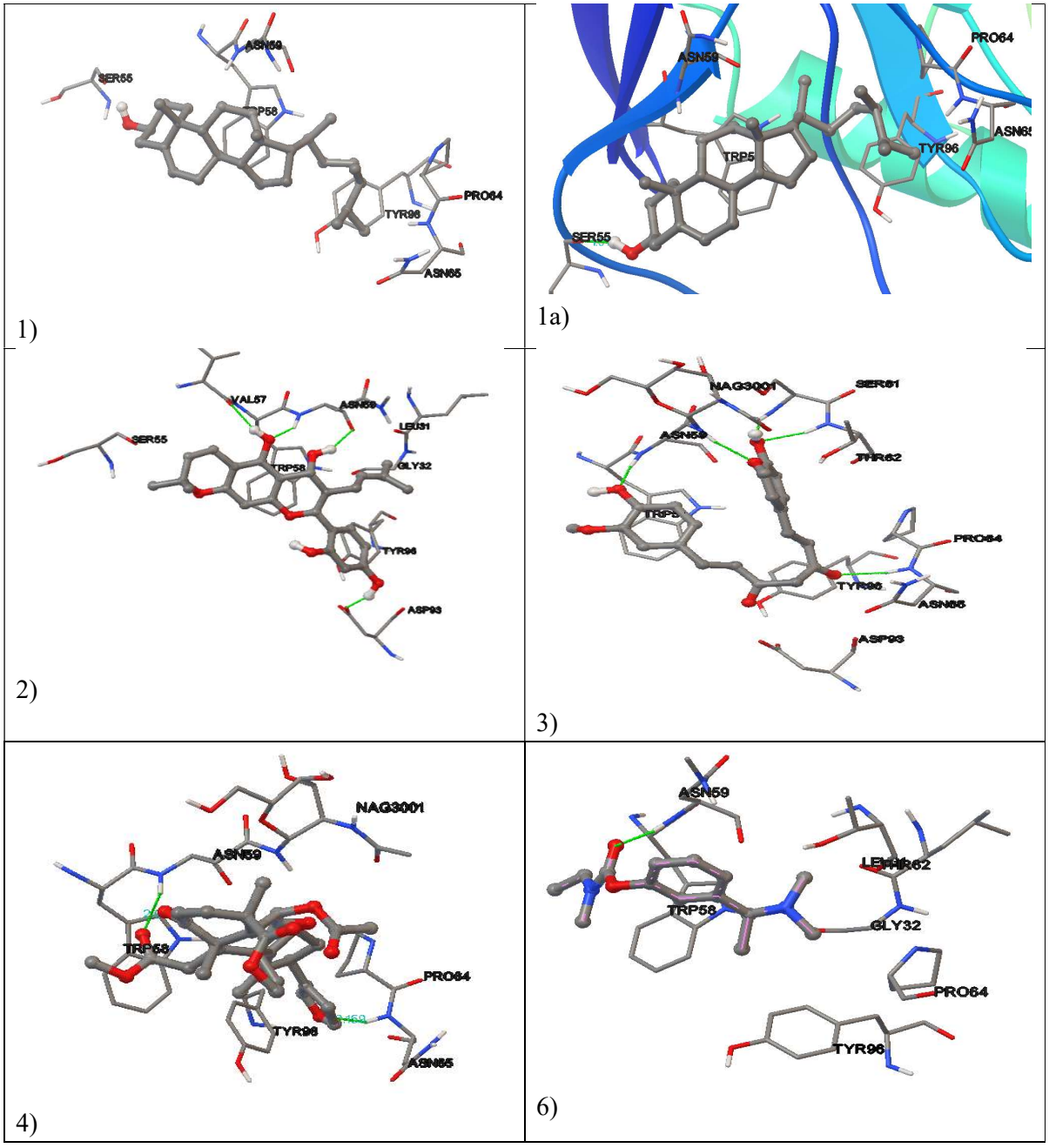
Table 5: Interactions and binding energies of the protein with PDB id: 5FOQ with the corresponding natural compounds and standard drugs that are indicated by *

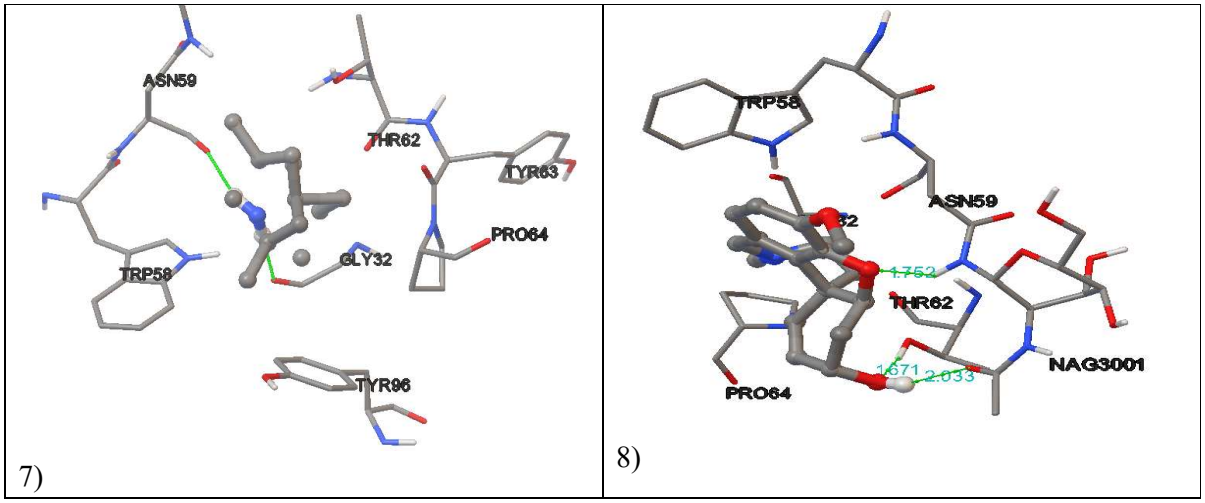
S.no	Compounds	Interacting Amino Acids	Binding Energy (ΔG) Kcal/mol	Dissociation Constant (KI)
1	Campesterol	Phe346	-08.04	1.29µM
2	Cudraflavone B	Gly345, Asn350	-06.26	25.69 µM
3	Nimbin	Tyr77, Gly345	-05.49	94.78 µM
4	Curcumin	Gly345	-05.16	163.69 µM
5	Yanuthone E	-	-	-
6	Rivastigmine*	Gly345	-04.10	988.35 µM
7	Memantine*	-	-05.20	154.27 µM
8	Galantamine*	-	-05.70	66.3 µM

"Figure Legends"

1. **Figure 1:** Interactions of the protein with PDB id: 1EVE with the corresponding natural compounds (1-4) and standard drugs (6-8)
2. **Figure 2:** Interactions of the protein with PDB id: 3IFO with the corresponding natural compounds (1-5) and standard drugs (6,8).
3. **Figure 3:** Interactions of the protein with PDB id: 3ZLT with the corresponding natural compounds (1-4) and standard drugs (6-8).
4. **Figure 4:** Interactions of the protein with PDB id: 4BTL with the corresponding natural compounds (1-5) and standard drugs (6,8)
5. **Figure 5:** Interactions of the protein with PDB id: 5FOQ with the corresponding natural compounds (1-4) and standard drugs (6).

Figure 1:





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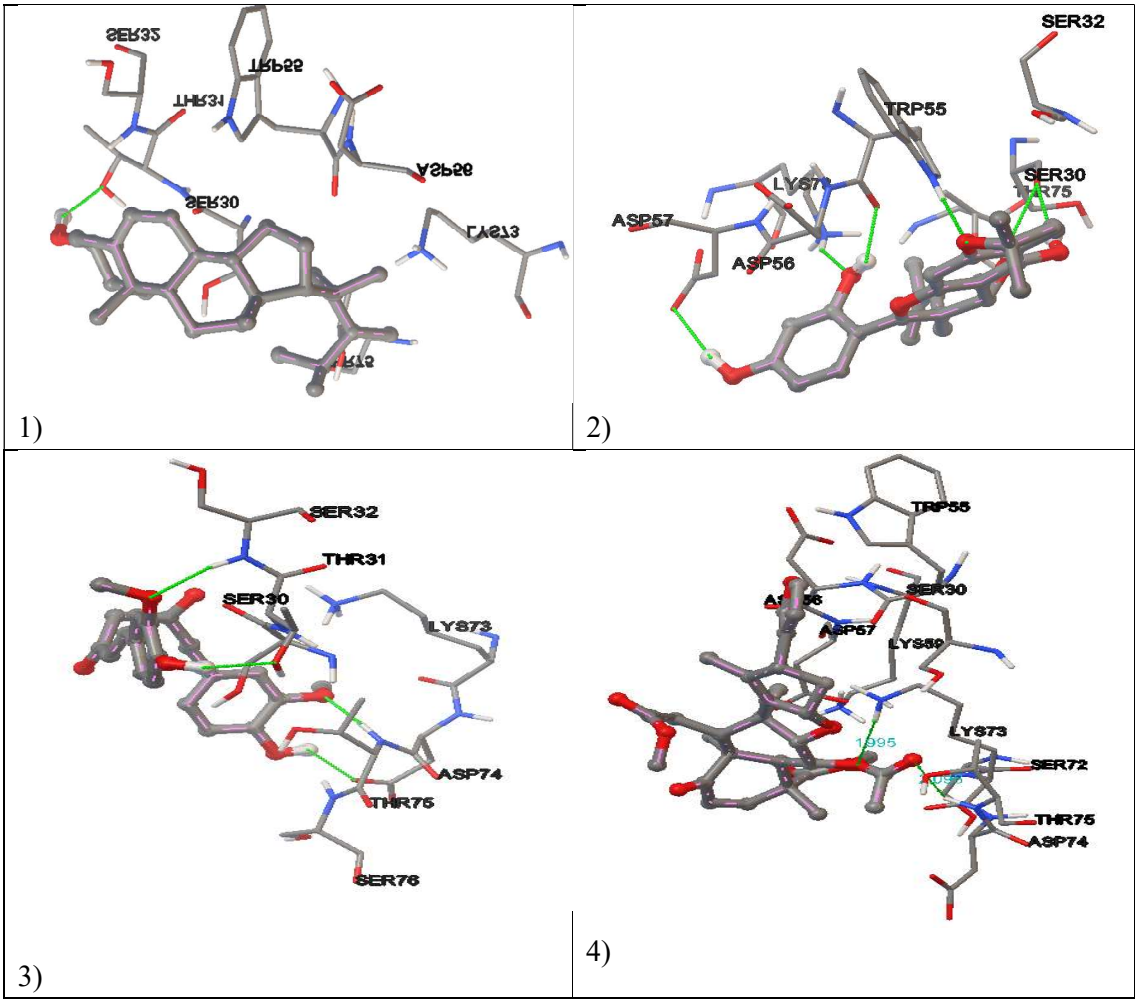

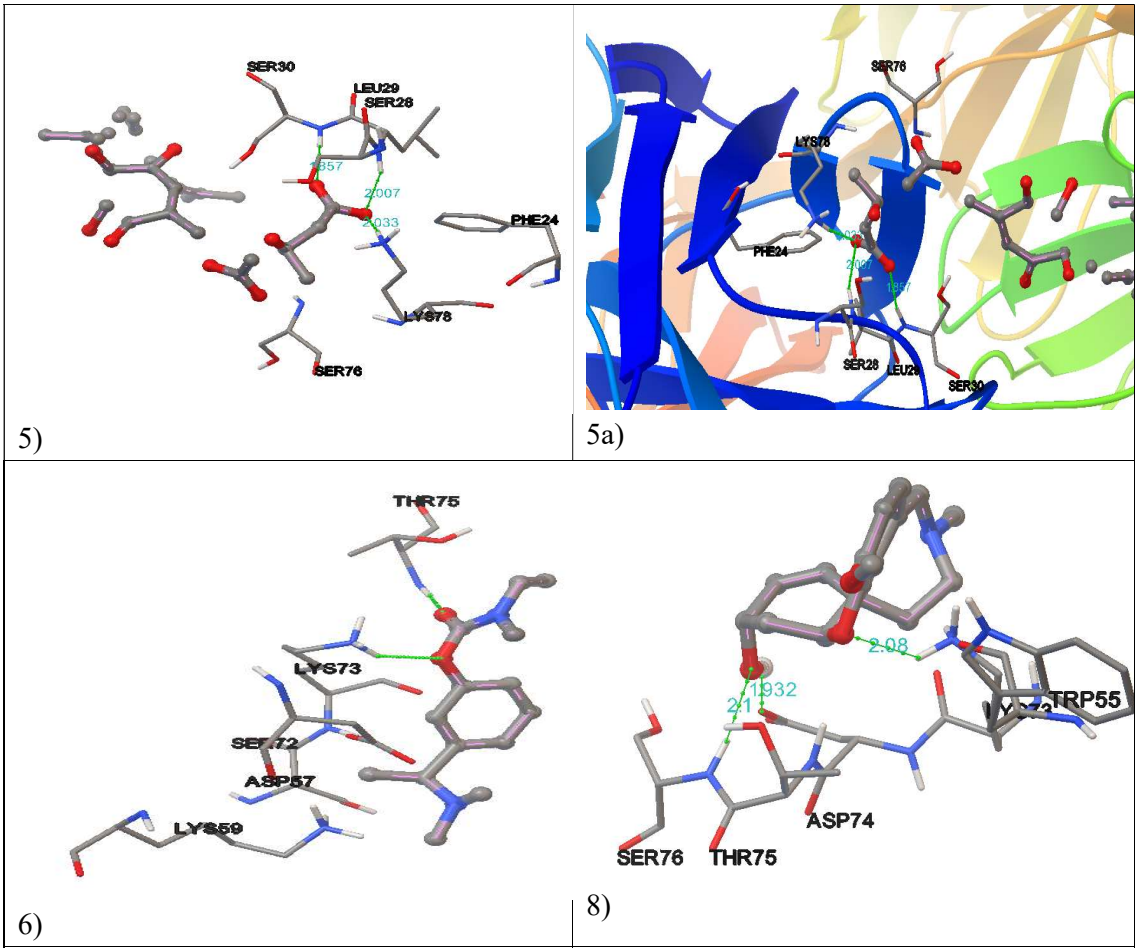

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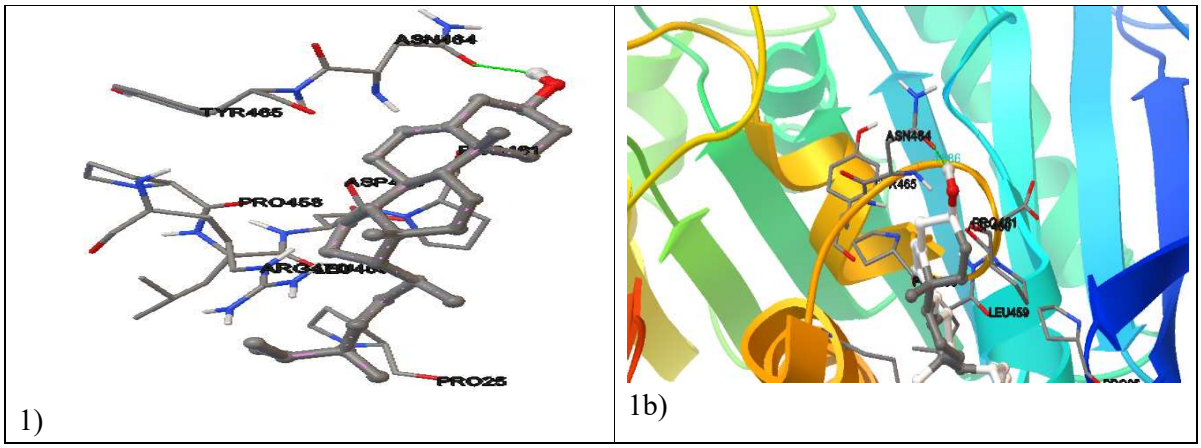

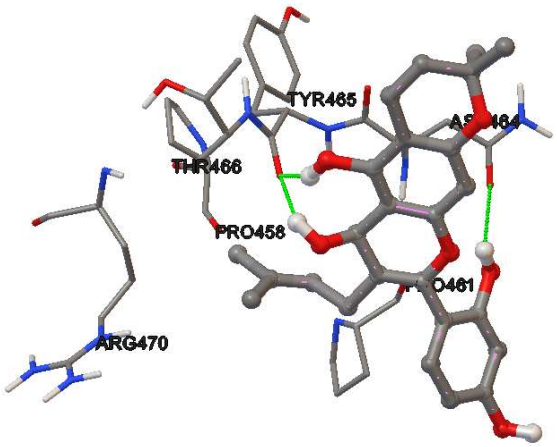

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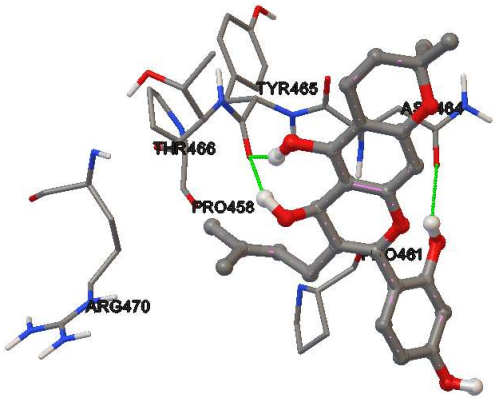

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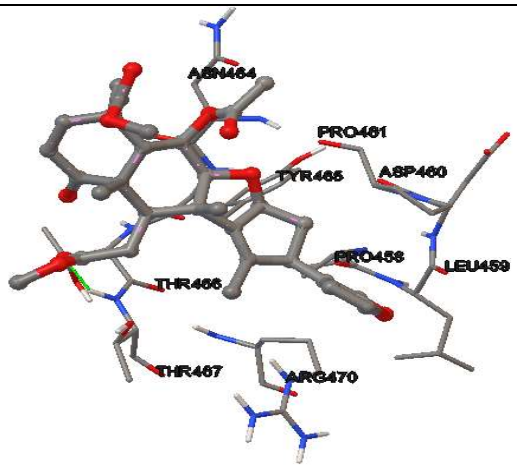

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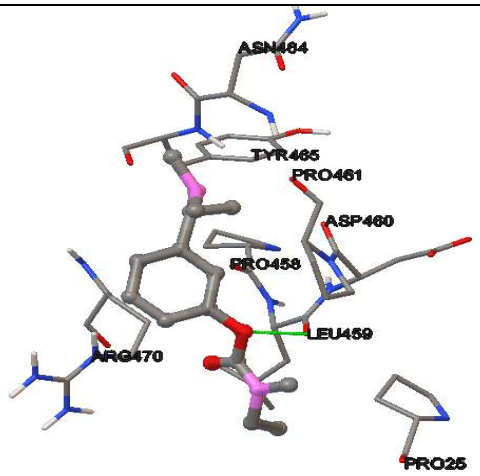

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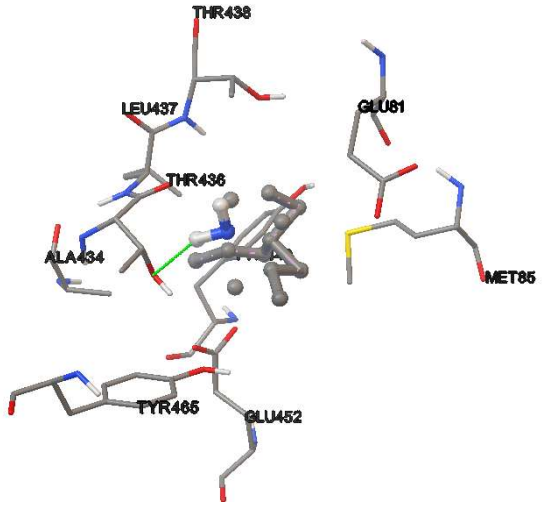

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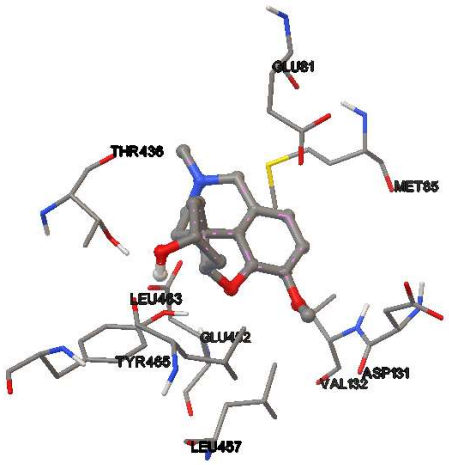

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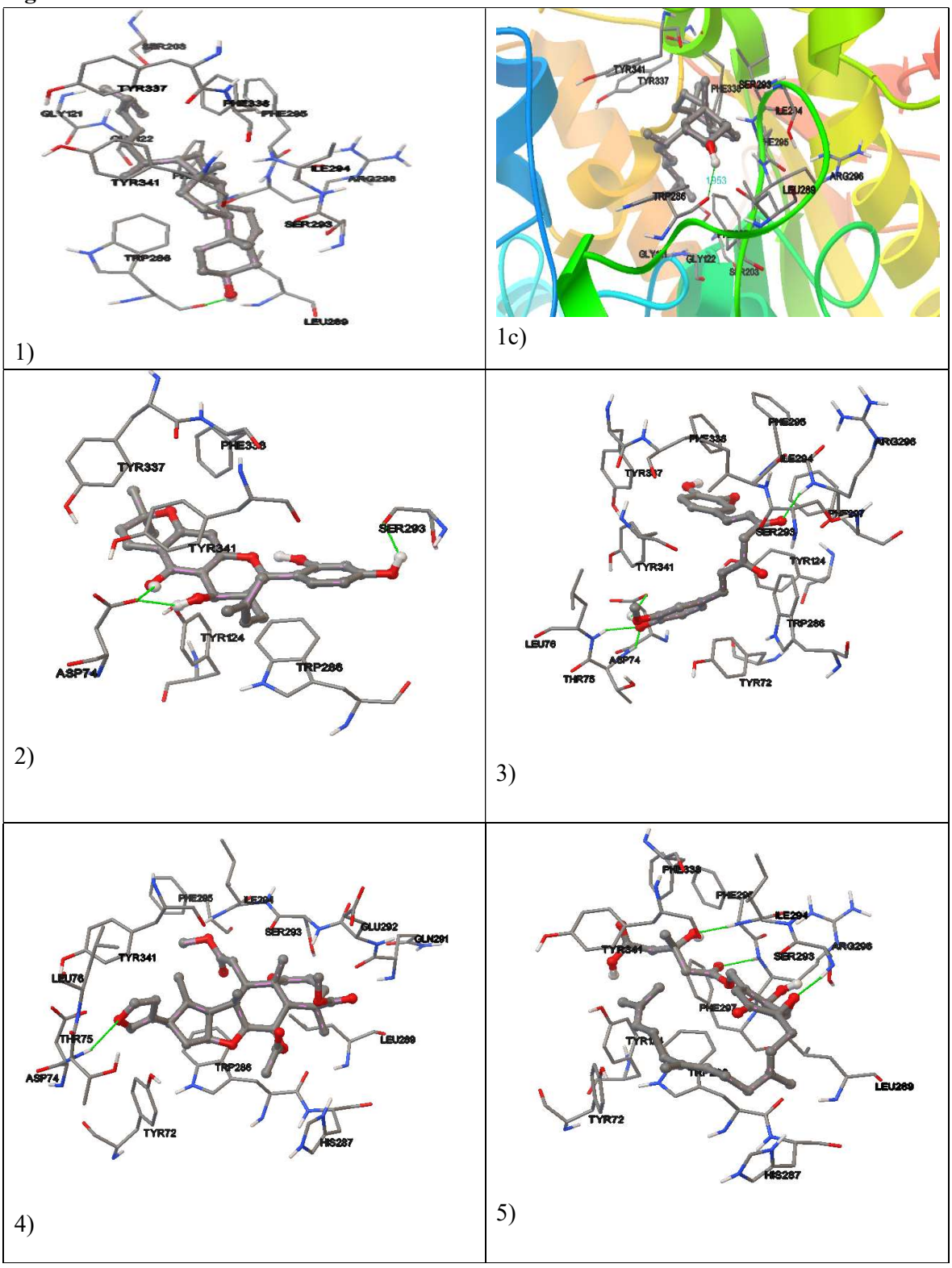

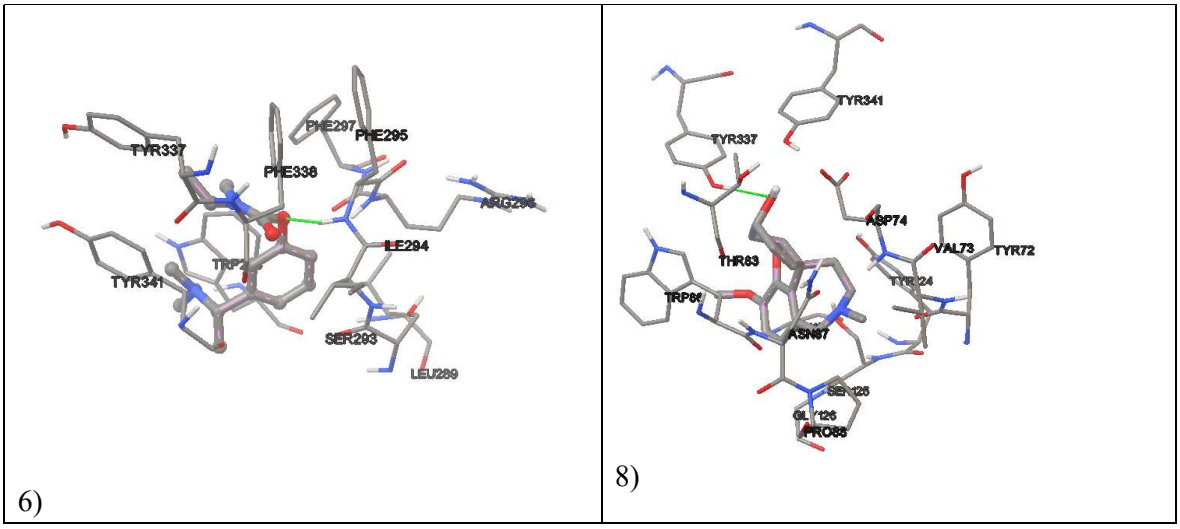

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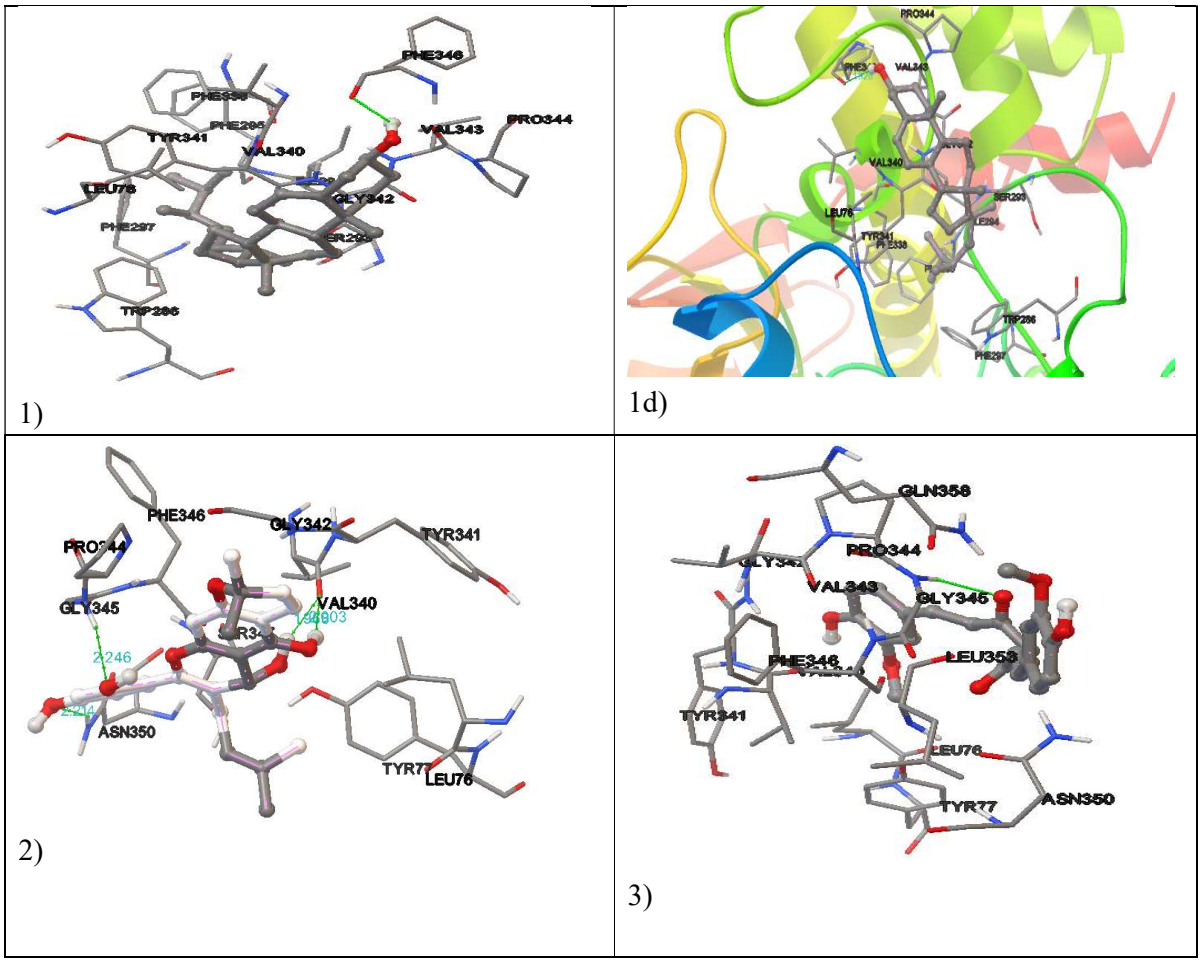

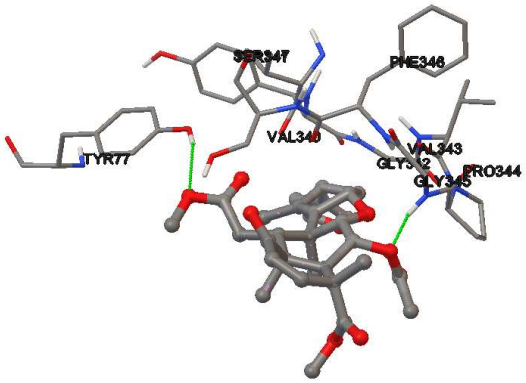

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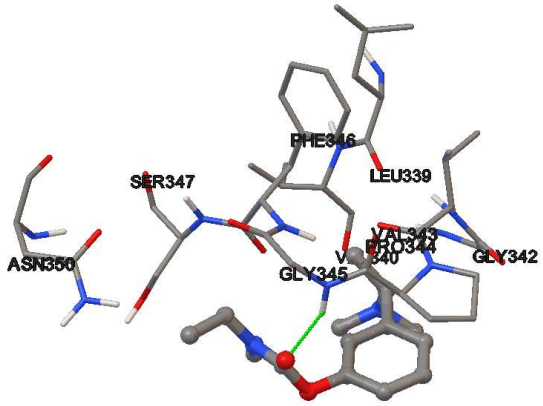

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Conclusion

The comparative docking study were performed using the existing target proteins for Alzheimer's disease. The crystal structures of the proteins were taken from PDB database. The proteins taken for the study had the PDB ids: 1EVE, 3IFO, 3ZLT, 4BTL and 5FOQ. The proteins were docked against five natural compounds and three FDA approved drugs were taken as controls. The natural compounds were noted to show better binding energies than already existing drugs. Campesterol exhibited the highest bonding energy of -11.55 Kcal/mol and showed interaction with Trp286. The study proves that naturally existing compounds are more effective than already existing drugs for Alzheimer's disease.

Conflict of interest: None

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