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# SIGNIFICANCE OF AROMATIC AMINO ACIDS IN STABILIZATION OF DIPLACONE AND HUMAN ACETYLCHOLINESTERASE COMPLEX

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**ABSTRACT**

Flavonoid induced inhibition of Acetylcholinesterase (AChE) is the standard therapy method for age related disorders. Simulated binding of natural inhibitors with human Ache was performed to study the mechanism of binding and inhibition.133 natural inhibitors were docked with the enzyme under high precision. Out of which top 5 inhibitors with highest docking score were studied. Active site having the highest score showed the highest affinity with Diplacone. Presence of Tyr341, Phe295, Arg296, Trp286 amino acids was most common in interaction with all five inhibitors. These aromatic amino acids were involved in direct interaction with the ligands making the ligand-human AChE complex stable. Study showed that aromatic amino acids provided the most potent binding sites for reversible yet stable interaction. The interaction of Diplacone-hu AChE complex leads to the stable inhibition of enzyme which is essential for the delaying of age related disorders.

***Keywords***: Human Acetylcholinesterse (huAChE), Natural inhibitor, Docking, Aromatic Amino Acids

## Introduction

Flavonoids are the class of plant secondary metabolites having a polyphenolic structure. They have miscellaneous favorable biochemical and antioxidant effects associated with various neurological diseases (Ovando *et al*., 2009; Burak *et al*., 1999; Lee *et al*., 2009). Acetyl cholinesterase is an essential enzyme in the central nervous system and inhibition and inhibition of it leads to increase of neural acetylcholine levels which is one of the therapies for symptomatic relief to moderate age related disorders (Perry *et al*., 1978). Inhibition of Ache by flavonoids is nowadays is in the centre of focus for the natural drug development for Alzheimer disease.

Docking studies of natural inhibitors from various plants with humanAChE shows that flavonoids act as a stable complexes with human Ache (Chaudhary *et al*., 2020). The polypeptide chain of the enzyme has 614 amino acid lengths. Human AChE has an ellipsoidal shape with dimensions ~45Å by ~60Å by ~65Å. It is a monomer having 12 stranded central mixed beta sheets surrounded by 14 alpha helix along with a remarkable feature which is a deep and narrow gauge of 20Å long penetrating into the enzyme (Manavalan *et al*., 1985).

The active site of AChE lies at the bottom of deep narrow gauge. The Active site is lines with aromatic amino acids that compose of various sub sites: The hydrophobic patch containing the cholin binding site and the hydrophobic site for the alkoxy leaving group of the substrate the peripheral site, and the acetyl pocket the narrowest part of the gorge, the bottleneck, is about halfway down the gorge and is composed of the aromatic amino acids Tyr121, Phe330 and Phe331 in *torpedo califernica* Ache (Sussman *et al*., 1991).

Reversible inhibitors are primarily responsible for the pharmacological manipulation of enzyme activity which is helpful in the treatment of neurological diseases (Mehta *et al*., 2012). So far only a few drugs are approved by Food and Drug Administration (FDA) for Alzheimer treatment such as tacrine, galanthamine, donepezil, rivastigmine (Stefanou *et al*., 2011; Camps *et al*., 2010). Several side effects have been observed caused by these drugs such as gastrointestinal disturbances, nausea, dizziness in the elderly because of low bioavailability.

Natural compounds having an inhibitory effect on AChE activity can serve as an alternative to these available drugs. With the availability of the bountiful amount of 3D data and structures computational tools such as virtual screening, docking and molecular dynamics are highly useful in discovery and development of new lead compound.

In this research we have focused on the role of active site aromatic amino acids in inhibition and binding pattern of human AChE with natural inhibitors. Binding and inhibitory patterns were analyzed using the Schrödinger software, its effectiveness can be useful in future drug development for age related disorders.

## Materials and Methods

### 3D structure retrieval of acetylcholinesterase (AChE)

Three Dimensional Structure of acetylcholinesterase (AChE) is retrieved from Protein Data Bank (PDB ID: 1B41) (Contestabile, 2009). It has 539 amino acids, the resolution of the structure obtain is 2.76 Å and it binds with three co-crystal ligands which were Alpha-L-Fucose,N-Acetyl-D-Glucosamine and 2-(Acetylamino)-2-Deoxy-A-D-Glucopyranose and utilized for further studies .

**Protien preparation**

The protein structure was subjected to the Protein Preparation Wizard of workflow implemented in Maestro 9.5 Schrodinger software (Sastry *et al*., 2013). It includes the biological unit and zero-order bonds to metals, assigned bond orders, deleted water molecules beyond 5 Å from hetero groups, generated metal-binding states, completed any missing side chains and loops, formed disulfide bonds, added missing hydrogens, and energy minimization using the OPLS_2005 force field (Jorgensen *et al*.,1988).

**Active site analysis and grid generation:**

The binding site study has an essential role in drug discovery research because of binding site residues of the protein bind to the ligand residues with its hydrogen bond donors and acceptors (Anderson, 2003). The Active site of AChE protein was analyzed by SiteMap (Schrodinger) (Anderson, 2003). SiteMap assigns numerical descriptors to calculate predicted binding sites by a series of physical parameters such as tightness, size, hydrophobic/hydrophilic character, degree of enclosure/exposure and hydrogen bonding possibilities. A weighted average of these measurements is then assigned to rank possible binding sites. In this analysis, the AChE protein have shown five major sites with site scores and their volume. Among the binding sites, we choose one binding site used for grid generation, which was determined by their site scores and volume of the sites. Afterwards, the suitable site was used to grid generation. The grid generation panel of the Glide module Maestro v9.5 software tool was placed on the drug target site in the target molecule(Schrodinger Release,2014). In the receptor grid generation panel, The grid box assisted to fix the drug binding site in the centroid of the target.

**Ligand selection and preparation:**

133 Natural AChE ligand Inhibitors listed were collected from literature and their structures were downloaded from different publicly available databases of ligand such as Pubchem, Zinc database, Chem Spider and some ligands were drawn by Chemsketch (Dos Santos *et al*., 2018; Murray *et al*., 2013; Ahmed, 2013; Colovic *et al*., 2013). All ligands can be retrieved in .sdf format. The set of molecules were prepared using the Ligprep tool available in Schrodinger suite (Schrodinger Release, 2014). It serves the purpose of converting molecules to 3D structures from 2D .sdf files, steric isomers and geometry minimization of ligands and probing for tautomers. All the molecules were geometrically optimized through Optimized Potentials Liquid Simulations 2005 (OPLS, 2005) force field. The partial atomic charges were also computed by OPLS2005 force field.

**High throughput virtual screening (HTVS)**

The High **t**hroughput virtual screening workflow of Maestro v9.5was applied to screen the 133 Natural ligand Inhibitors against the binding site of the AChE protein target. The virtual screening workflow screening of ligand libraries is a fast and accurate technique to discover novel drugs in the drug discovery process. preliminary virtual screening steps, we skipped the ligand preparation step due to their previous preparation with Ligprep. The further step of virtual screening involves the assessments of drug-likeliness of ligands. Drug-like molecules show positive parameters of absorption, distribution, metabolism and excretion (ADME). If any ligand to be considered a probable drug candidate, it must satisfy these ADME properties profile (Halgren *et al*., 2009). The Qikprop (Schrodinger Release, 2014) tool was used to examine these parameters. After Qikprop analysis, the screening of ligands initialized using the receptor grid file. Virtual screening of Natural ligand Inhibitors was carried out by HTVS, SP (Standard Precession) and XP (Xtra Precession) modes of filtering to results lead molecules. The final lead molecules were prioritized based on their glide score, glide energy and ADME properties.

**Binding free energy calculations**

The protein-ligand complex of binding free energies is calculated using the Prime MM-GBSA approach (C.2014-2: QikProp, version 4.0). AChE protein with top lead molecules binding free energies was calculated using Prime-MM/GBSA method with OPLS_2005 force field applied in Schrodinger suite. Binding free energy of complex (Protein to ligand) calculated using the following equation :

$$\Delta Gbind = G\ (ligand\text{-}receptor) - (Gprotein + Gligand)$$

## Results and Discussion

**Binding site Prediction**

The active site was predicted on the target AChE protein. In this prediction, the target site displayed five suitable binding sites (Table-1). Out of 5 different sites, site with the highest Sitescore was selected (Figure-1). The active site was include by 34 residues, namely Tyr 72,Val 73,Asp 74,Thr 83,Trp 86,Asn 87,Tyr 119,Gly 120,Gly 121,Gly 122,Tyr 124,Ser 125,Gly 126,Leu 130,Tyr133,Glu 202,Ser 203,Trp 286,His 287,Leu 289,Gln 291,Glu 292,Ser 293,Val 294,Phe 295,Arg 296,Phe 297,Tyr 337,Phe 338,Tyr 341,Trp 439,His 447,Gly 448 And Tyr 449 and used for generation of docking grid.

**Table 1 :** Active binding sites of Ache.

| AChE receptor | Site scores |
|---|---|
| *Site 1 | 1.069 |
| Site 2 | 1.014 |
| Site 3 | 0.948 |
| Site 4 | 0.874 |
| Site 5 | 0.849 |

*Highest site score binding site

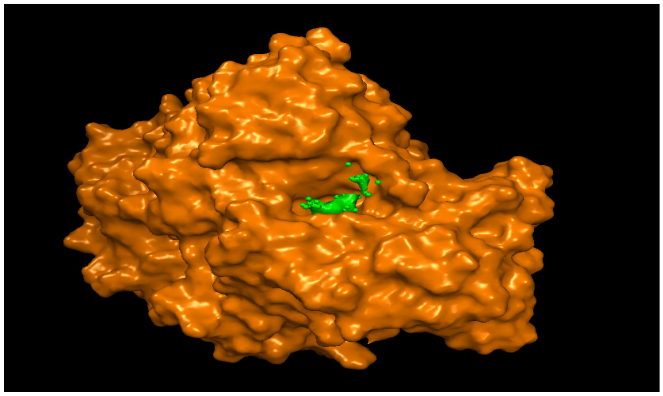

**Fig. 1:** Top Sitemap (site-1) position in protein surface area.

**Virtual high throughput screening**

For drug discovery research, virtual screening is a broadly used approach that effectively affords new lead ligand compounds. In the current study, virtual screening was done to design effective inhibitors by screening of AChE

ligand Inhibitors on the basis of their affinity for the AChE protein. 133 Qikprop filtered compounds subjected to the virtual screening docking. 116 ligands were short listed from HTVS ligand docking. This was more followed by SP and XP docking, which filtered the number of remaining compounds to 58 and 29, respectively. Table-2 illustrates the molecular docking output for the XP docked compounds against AChE (only the top 50% compounds with higher dock scores).

**Table 2:** Docking results of top file lead molecules(Ligands)

| Ligands | Gscore | Glide energy (kcal/mol) |
|---|---|---|
| ***Diplacone** | **-11.49** | **-55.92** |
| Kuwanon E | -11.11 | -46.60 |
| Macluraxanthone | -11.00 | -43.44 |
| Lycorine | -10.65 | -39.55 |
| 11alpha-Hydroxy Galantamine | -10.54 | -34.30 |

*Top Docked Score Ligand Complex

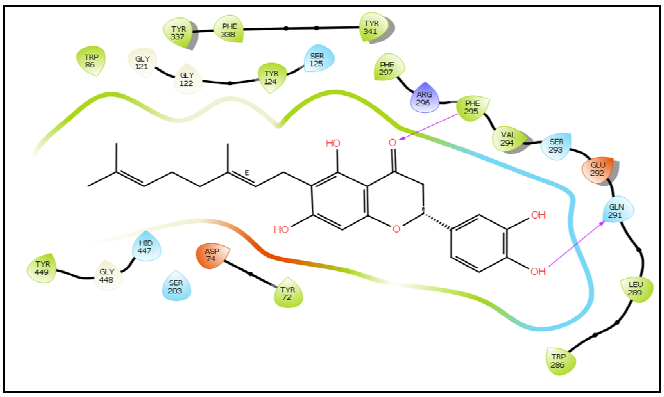

**Fig. 2A:** Ligand interaction diagram (2D) of the diplacone with AChE

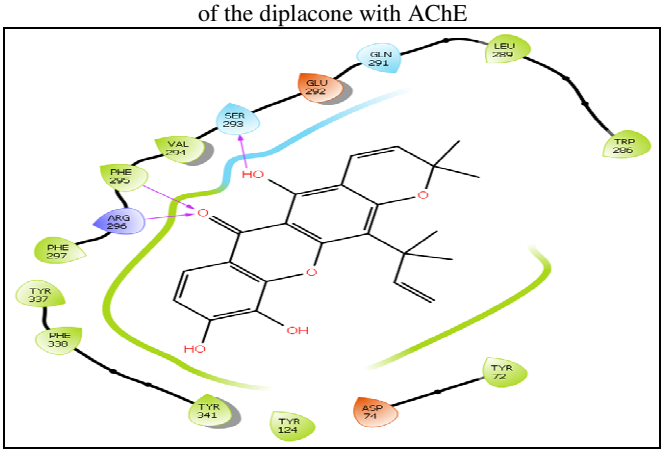

**Fig. 2B:** Ligand interaction diagram (2D) of the kuwanon E with AChE

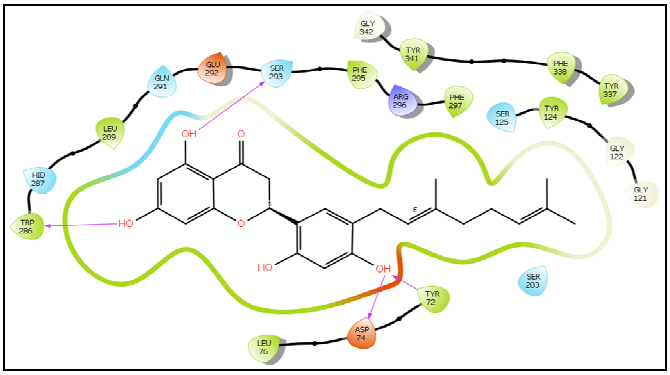

**Fig. 2C:** Ligand interaction diagram (2D) of the Macluraxanthone with AChe

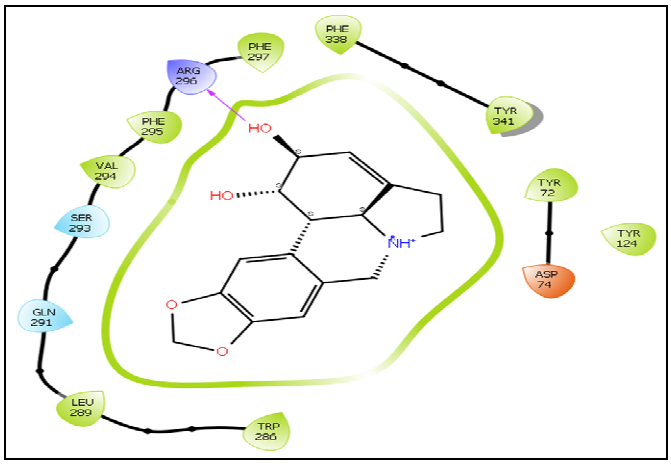

**Fig. 2D:** Ligand interaction diagram (2D) of the lycorine with AChE

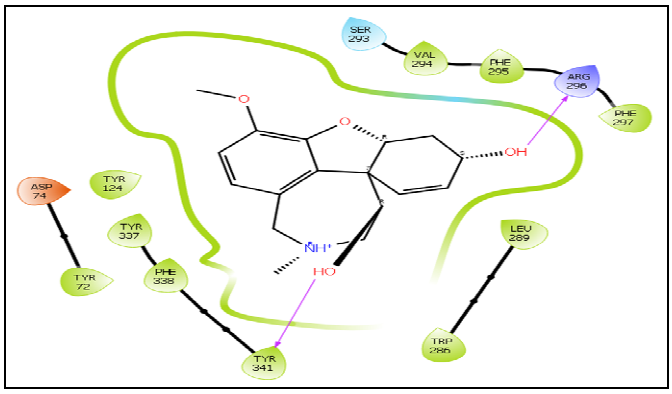

**Fig. 2E:** Ligand interaction diagram (2D) of the 11alpha-Hydroxy Galantamine with AChE

From the docking interaction profiles as explained in figures 2A-2E and in Table-2, diplacone scored a high dock score of -11.49 kcal/mol and shared hydrogen bond interaction with Phe 295 and Gln2 91. kuwanon E scored a high dock score of -11.11 kcal/mol and shared hydrogen bond interaction with Tyr72, Tyr74, Trp286 and Ser293. Macluraxanthone scored a high dock score of -11.00 kcal/mol and shared hydrogen bond interaction with Ser293, Phe295 and Arg296. Lycorine Scored a high dock score of -10.65 kcal/mol and shared hydrogen bond interaction with Arg296. 11alpha-Hydroxy Galantamine scored a high dock score of -10.54 kcal/mol and shared hydrogen bond interaction with Arg296 and Tyr341. The ADME properties of identified lead molecules are calculated using Qikprop in Schrodinger suite to estimate drug like activity.

### Binding free energy calculations

Binding free energies of Top five docked ligands are calculated using the in Schrodinger suite prime-MM/GBSA tool. Binding free energies of ligands with AChE protein are calculated using OPLS(2005) force field and GBSA continuum solvent model. Lead molecules with AChE receptors Binding free energies were shown in Table-3.

**Table 3 :** Binding energy calculations of file lead molecules

| S. No | Ligands | $\Delta G_{bind}$ (kJ/mol) |
|---|---|---|
| 1 | **Diplacone** | **-57.1722092** |
| 2 | Kuwanon E | -59.01897498 |
| 3 | Macluraxanthone | -53.29212808 |
| 4 | Lycorine | -58.32483151 |
| 5 | 11alpha-Hydroxy Galantamine | -49.78192344 |

**Comparative analysis of amino acidsof flavonoid with Standard Drugs Binding sites**

The previous experimental studies reported, Ser-203, His-447, and Glu-334 residues were the most catalytic site residues in the structure of AChE. From the studies it was known that Currently available Ache standard drugs and Previous reported best inhibitors were Donepezil, rivastigmine, galantamine, tacrine, huperzine A, TV-3326 and anseculin which all bind with Asp74, Thr75, Leu76, Gly82, Thr83, Trp84, Trp86, Asn87, Ser200, Glu202, Ser203, Trp279, Trp286, Phe295, Phe297, Glu327, Phe330, Glu334, Tyr334, Tyr337, Phe338, Tyr341, Trp439, His440, His447, Gly448, Tyr449 (Da Silva *et al*., 2007; Sharma *et al*., 2011). From the comparative analysis, we observed that catalytic site residues and previously reported AChE inhibitors binding residues were present in our site map. Approximately more than 95% of the residues were present in our top sitemap (site 1) area.

**Bond interaction analysis at active site:**

Analysing the figure2(A-E), It was find out that the amino acids of enzyme which interacted with the ligands are Tyr341,Arg296,Phe295,Gln291,Ser293,Trp286,Asp74. They formed stable hydrogen bonds with the flavonoids. Their interaction was reversible with the stable bond length.

Frequency of bonds compared to the amino acids were shown in the Figure3.

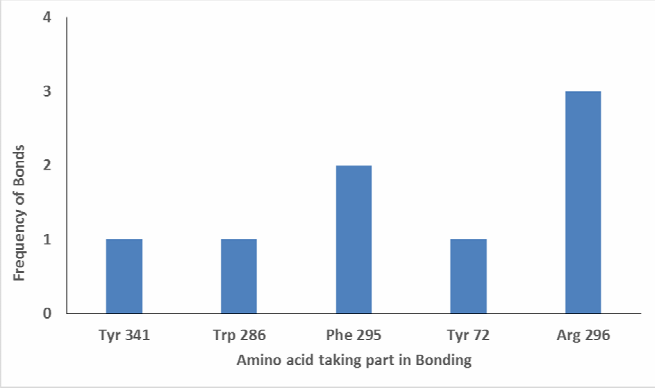


**Fig. 3 :** Amino acids taking part in bond interactions

## Conclusion

Flavonoids were interacted individually in In-sillico with human AChE enzyme on defined binding site. Analyzing all individual complexes, it seems convincing that flavonoids prefer Binding site which is lined with aromatic amino acids and form stable yet reversible interaction with the enzyme. These amino acids are actively stabilizing the interaction with their anionic surface. In this study we successfully employed in-sillico screening and docking to identify the stabilizing factor in flavonoid-HumanAChe complex, which will further be helpful in in vitro and in sillico studies of natural drug development of age related disorders.

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