# *In Silico* Molecular Docking Approach Against Enzymes Causing Alzheimer's Disease Using *Borassus Flabellifer* Linn

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**Research Article**

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# Abstract

Alzheimer’s disease is a life-threatening neurodegenerative disorder. About 50 million people across the globe are affected by this disease. At final stages, this disease causes patients to lose cognitive ability, memory and brain cells to the point of being totally depend on other individuals for livelihood. The incidence of this disease is increasing across the world in the recent years, making the need of a better drug an urgency. Existing drugs show various side-effects and natural sources of medicinal drugs are being explored. In this study, we explore the activity of natural compounds isolated through GCMS analysis from the haustoria of palmyra palm against two major Alzheimer’s disease-causing enzymes, β-amyloid and Acetylcholinesterase. The binding affinity of these compounds against the target proteins and their pharmacokinetic properties were checked. Among the 37 compounds docked, 5 compounds showed good binding affinity and pharmacokinetic properties. These natural compounds showed a potential as a drug against Alzheimer’s disease. Further research is needed to study the synergistic activity of the compounds in live cells.


# Introduction

Alzheimer’s disease is a neurodegenerative disease, which gets worse over time. The pathology of the disease was first recognized by Dr. Alois Alzheimer, a German psychiatrist and neuropathologist, in a 51-year-old woman. He identifiedthe presence of miliary foci (plaques) and fibrils (tangles) in the brain of the patient.After more than a century of identification of the disease and research, a complete solution to this disease is not present[1]. Various theories have been proposed to explain the onset of the disease. Pathological lesions caused by two proteins are believed to be the cause of Alzheimer’s disease, namely β-amyloid and phosphorylated tau protein tangles. Alzheimer’s disease is a life-threatening disease[2]. Alzheimer’s disease increases suffering and pain in the life of individuals and their families. Daily self-care activities become an impossible task at later stages of Alzheimer’s disease, putting tremendous pressure on the caregivers. The Clinical symptoms of Alzheimer's disease include loss of memory, depreciation of the ability to speak and think[3].

The majority of the diseased individuals are above 65 years old. Recent studies show the possibility of incidence before 65 years of age. 50 million people worldwide are affected by Alzheimer's disease worldwide, out of which 4 million reside in India. Research suggests the APOE4 gene plays a major role in causing the disease. Cognitive impairment, loss of memory, difficulty in learning, brain cell damage progress with time to make living normal lives difficult with the progress of time.  The brain starts to shrink (atrophy), leading to a loss in memory, to the extent of forgetting important events in the life of patients. Incidence of psychopathic diseases may also occur[2].

Acetylcholinesterase is a major enzyme required in the breakdown of the neurotransmitter acetylcholine. The hydrolysis of acetylcholine to acetic acid and choline by acetylcholinesterase is necessary in healthy brain, but becomes an issue in Alzheimer’s disease[4]. This is because of the low concentrations of acetylcholine, which become even lower if acetylcholinesterase is left unchecked. The blocking of this

enzyme is done by action of various drugs which are available for patients. Drugs like Donepezil, rivastigmine, and galantamine block acetylcholinesterase to increase acetylcholine in synapses of neurons. These drugs have undesirable side-effects, and better drugs are needed.

The amyloid precursor proteins (APP) are responsible for the growth and repair of neurons[3]. The APP is cleaved by enzymes and convert APP into soluble protein fibers it will be broken and recycled. Three types of secretase enzymes are known to play a role in the cutting of APP alpha-secretase, beta-secretase, gamma-secretase. But in the case of the Beta-secretase enzyme, it cleaves the Amyloid precursor protein (APP) into insoluble monomer β-amyloid protein. The insoluble monomer protein fibers are sticky and adhere between the neuron cells causing beta-amyloid plaques and tacky inside the neuron cells creates tangles (Figure 1). These plaques interrupt signals between neuron to neuron, block the blood vessels in the brain, cause amyloid angiopathy, haemorrhage, and rupture of cells. The tau protein present between the microtubules prevents it from damage to the cytoskeleton (Figure 2). Insulin resistance could have a role in Alzheimer's disease. The exact mechanisms are unknown. the β-amyloid plaques initiate a cascade of reactions inside the microtubules by activating phosphate kinase andtransfer phosphate group to tau protein. Tau protein structuresare modified and stop supporting microtubules causing neurofibrillary tangles. This leads to neuron having non-functional microtubules, which then sends signals for apoptosis, leading to neuron death i.e., atrophy. This atrophy causesthe shrinking of the brain. This atrophy of brain cells leads to short-term memory, loss of motor skills and language, long-term memory loss, disorientation all of which lead ultimately to the person becoming bedridden and cause death[3]. Diagnosis is still a challenge and one definite way to diagnose the disease is not present to date.

With no ultimate remedy for the disease, researchers are still searching for a solution. Natural sources of compounds like plants are also being explored due to the rich diversity of medicinal compounds present in plants. India is known for its rich flora and abundance of medicinal plants across the world. Earlier studies with the natural compounds show results that indicated that plant compounds are potential drug candidates against various diseases, including Alzheimer's disease[5][6]. Natural compounds from medicinal plants are studied as they are less toxic and have lower side effects to synthetically prepared compounds. Here in our study, we have identified potential compound in the haustoria of Palmyra palm plant by GCMS analysis. These compounds were then studied for docking affinities against two protein targets, namely Acetylcholinesterase and β-amyloid. The compounds showed good binding scores and had shown good ADME properties, indicating that these compounds have potential as drugs against Alzheimer's disease.

# Materials And Methods

### Retrieval of ligands:

36 potential ligands were identified from palmyra palm ethanolic extract. PubChem and Chemspider databases were used for the retrieval of ligands. These ligands were chosen to be docked against target

proteins. 3D structures of these ligands were downloaded for docking and ADME studies.

### ADME and toxicity analysis:

Failures in clinical trials of potential compounds could cause loss of time, money, and other resources. The possibility of such failures is significantly reduced when the ligands are checked for pharmacological and pharmacokinetic properties. The computational studies on the structure of ligand could reveal how successful it would be In vivo.

Swiss ADME (http://www.swissadme.ch) was used to screen the ligands for the best pharmacokinetically relevant compounds. ADME stands for absorption, distribution, metabolism, and excretion. Other factors like oral bioavailability, blood-brain-barrier permeability, lead likeness are also considered. Lipinski's rule of five was used to analyze the drug-likeness of the chosen ligands.

### Retrieval and preparation of protein:

The target proteins were Acetylcholinesterase and beta-secretase. The proteins were retrieved from PDB Database (http://www.rcsb.org/pdb/home/home.do). The retrieval ID of Acetylcholinesterase is 2ACE (10.2210/pdb2ACE/pdb). Beta-secretase was retrieved from the PDB database with ID 1SGZ (10.2210/pdb1SGZ/pdb). The proteins were prepared separately on the Discovery studio visualization tool. Polar Hydrogens were added and Gasteiger charges were added.

### Virtual screening and Visualization:

### Acetylcholinesterase:

Acetylcholinesterase is an enzyme that breaks down acetylcholine through hydrolysis to yield reusable derivatives which are recycled as transmitters[4]. The ligands were docked against the target protein using PyRx[7]. PyRx is a virtual screening software which can test for the binding affinity of multiple ligands against a target protein. PyRx is a useful, user-friendly, quick virtual screening tool.

AutoDock Vina plugin tool was used to dock the potential compounds retrieved from PubChem against Acetylcholinesterase target protein. Entire protein was covered under grid box and docking was done.The scoring function of the virtual screening tool have a prominent place in predicting the degree of successful interaction between ligands against the target protein. The Scoring function used is the AutoDock Vina tool in PyRx 0.8. The Discovery Studio visualization tool was used to visualize the docked compounds[8].

### Beta-Secretase:

Beta-Secretase is an enzyme that is responsible for the incorrect cleaving of the Amyloid precursor protein to give beta- amyloid fragments which cause plaques and disrupt brain signalling[9]. The ligands were docked against the protein through PyRx virtual screening tool[7].

AutoDock Vina plugin tool was used to dock the potential compounds retrieved from PubChem against Beta-Secretase target protein. Complete protein was covered under grid box and docking was done. The visualization of docked ligands was done using Discovery Studio visualization tool.

# Result And Discussion:

### Prediction of drug likeness through ADMET analysis:

Computational methods have been used to predict the drug likeness and pharmacokinetic properties of the compounds. The selected compounds were screened for the ADME analysis and the best compounds were studied further for binding scores. ADME stands for absorption, distribution, metabolism and excretion. Pharmacokinetic properties are to be studied before the clinical trials for potential compounds to decrease failure in vivo studies. Parameters like molecular weight, number of hydrogen bond donors, hydrogen bond acceptors, rotatable bonds, Log P value are checked for the estimation of drug likeness of the compounds. Lipinski's rule of five is used to study the drug likeness and predict the possibility of successful trial in vivo[10]. Other factors like gastrointestinal absorption, blood brain barrier permeability, bioavailability was checked.

Log P values are a major player in the success of a potential compound. Best scores for Log P are below 5. Higher Log P values indicate the compounds have high metabolic turnover, low solubility and oral absorption, which are not optimal for potential drug candidates. Bioavailability scores of 0.55 are considered best. Bioavailability scores are a combination of Lipinski's rule of five, TPSA and molecular charge. Verber's rule scores also indicate the oral availability of a potential compound.

All the compounds showed good pharmacokinetic properties and showed to be promising compounds for further research. Particularly 4 compounds showed excellent properties, Thiazolo[3.2-a]benzimidazol-3(2H)-one, 2-(4-acetoxybenzylideno)-, Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl-, 1(2H)-Naphthalenone, 3,4-dihydro-5,7-dimethyl- and Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- are those compounds. These are non-mutagenic, high gastrointestinal absorption, blood-brain-barrier permeants which are excellent candidates for further research.

### Virtual screening of ligands:

The ligands were docked against two protein targets, acetylcholinesterase and beta-secretase. Acetylcholinesterase is an enzyme that breaks down acetylcholine into reusable derivatives through hydrolysis. This action of enzyme is necessary to recycle the acetylcholine in normal conditions after transmission of signal. But, in anAlzheimer's patient, the synapses of the neurons are blocked by plaques which cause delay or loss of signalling. The inhibition of acetylcholinesterase can improve signalling by decreasing the rate of hydrolysis of acetylcholine to choline moiety.

Beta-Secretase is known to be responsible for the cleaving of beta amyloid protein to beta-amyloid plaques, which are the hallmark of Alzheimer's and dementia. The inhibition of the action of beta-

secretase can ensure that the amyloid precursor protein is not cut into insoluble beta-amyloid.

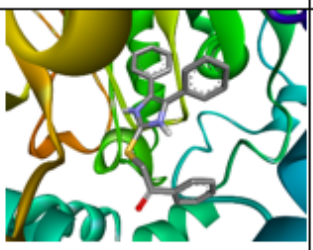
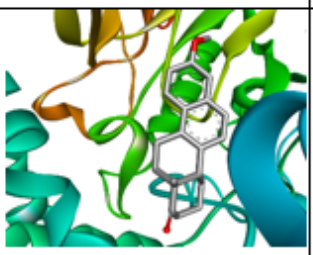
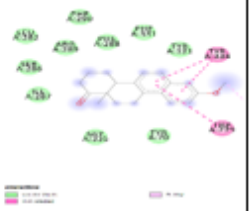
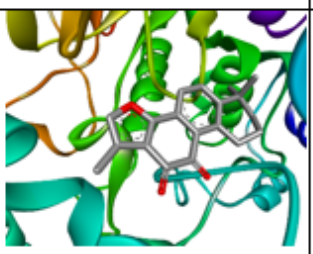
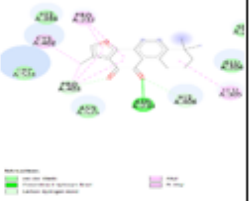
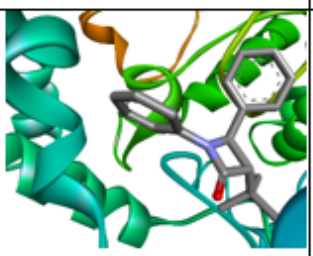
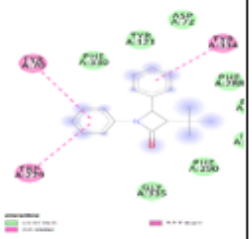
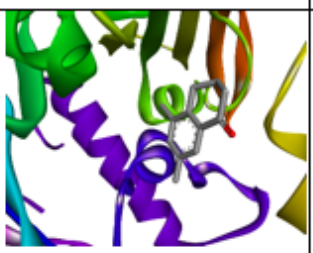
The results for acetylcholinesterase were between -3.9 to -11.4, which are considered good represented in table 1. The results for beta-secretase were between -3.3 to -9.7 kcal/molrepresented in table 2. The results for both the proteins indicated that these compounds are excellent candidates for further research. Only compounds which scored above -8 kcal/mol were considered as best ligands among the 36 docked compounds.

Among these, the best binding affinity was shown by Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- with a score of -11.4 kcal/mol. Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- is a 4,5-diarylimidazole-2-thione derivative, which is a subclass of Thione. Derivatives are known for various biological activities[11].

Thiazolo[3.2-a]benzimidazol-3(2H)-one, 2-(4-acetoxybenzylideno)- also showed a good binding affinity of -10.6 kcal/mol with acetylcholinesterase. It shows excellent pharmacokinetic properties. It is a Thiazolo[3,2-a]benzimidazole derivative, which is known to be biologically active[12].

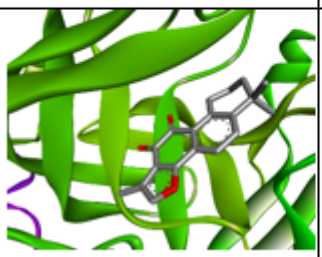
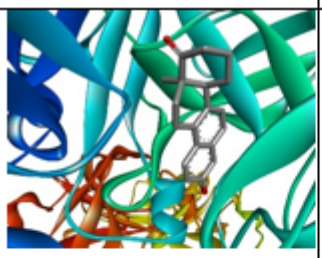
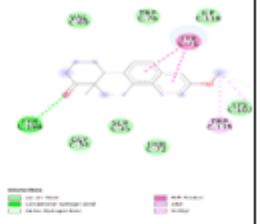
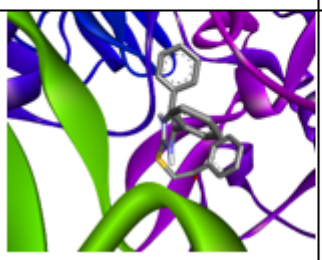
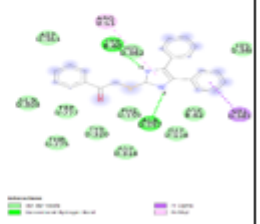
Phenanthro[1,2-b] furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- is another compound which showed good binding scores of -9.9 kcal/mol. It is a medically valuable compound because of its various biological actions[13][14][15]. 2-Azetidinone, 3-(1,1-dimethylethyl)-1,4-diphenyl-, trans- also shows good binding score of -8.5 kcal/mol. 1(2H)-Naphthalenone, 3,4-dihydro-5,7-dimethyl- is a tetralone derivative and has the binding affinity of -8.3 kcal/mol. Tetralone derivatives are also known for action on serotonin and dopamine[16].

**Table 1.** Showing docking images of best ligands against acetylcholinesterase

| S. no | Best scoring compound | PubChem ID | Binding scores(kcal/mol) | Ligand-protein interactions | 2D interactions |
|---|---|---|---|---|---|
| | Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- | 622124 | -11.4 |  |  |
| | Thiazolo[3.2-a]benzimidazol-3(2H)-one, 2-(4-acetoxybenzylideno)- | 626328 | -10.6 |  |  |
| | Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl | 164676 | -9.9 |  |  |
| | 2-Azetidinone, 3-(1,1-dimethylethyl)-1,4-diphenyl-, trans- | 11076811 | -8.5 |  |  |
| | 1(2H)-Naphthalenone, 3,4-dihydro-5,7-dimethyl- | 83619 | -8.3 |  |  |

These ligands also showed good binding scores for beta-secretase. Among the docked ligands, Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- showed the highest docking score of -9.7kcal/mol. Thiazolo[3.2-a]benzimidazol-3(2H)-one, 2-(4-acetoxybenzylideno)- showed a score of -9 kcal/mol. Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- showed a score of -8.6 kcal/mol.

**Table 2.** Docking images of best ligands against Beta- secretase

| S. no | Best scoring compounds | PubChem ID | Binding Scores(kcal/mol) | Ligand-protein interactions | 2D interactions |
|---|---|---|---|---|---|
| | Phenanthro[1,2-b]furan-10,11-dione, 6,7,8,9-tetrahydro-1,6,6-trimethyl- | 164676 | -9.7 |  |  |
| | Thiazolo[3.2-a]benzimidazol-3(2H)-one, 2-(4-acetoxybenzylideno)- | 626328 | -9 |  |  |
| | Ethanone, 1-phenyl-2-(4,5-diphenyl-2-imidazolylthio)- | 622124 | -8.6 |  |  |

# Conclusion

The docking results showed that the potential compounds of *Borassus flabellifer* may be potential compounds for further studies against Alzheimer disease. Among the 36 ligands which were docked against beta- secretase and acetylcholinesterase, the best results against both proteins were showed by 3 compounds. Binding scores above -8 kcal/mol were considered as top ligands. Acetylcholinesterase showed for 5 best compounds, and beta-secretase showed 3 best compounds. These compounds also showed excellent pharmacokinetic properties. The potential use of these compounds against both acetylcholinesterase and beta-secretase could be highly valuable in treatment. Further studies could show valuable insight to the effectiveness of these compounds in vivo. Studies could also indicate the synergistic action of the natural compounds against Alzheimer's disease.

# Declarations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**Conflict of Interest** The authors declare no competing interests

# Author Contributions

Jason Abraham: Investigation, Conceptualization, Writing- Original draft preparation, Reviewing and Editing, Formal analysisand Art work. H. Noorul Samsoon Maharifa: Methodology, Investigation, Conceptualization, Writing- Original draft preparation, Resources, Reviewing and Editing, Visualization, Formal analysis and Art work. S. Hemalatha: Conceptualization, Resources, Supervision, Data Curation, Validationand Project Administration.

### Availability of data and material

Data will be available on request

### Code availability

Not Applicabl

### Ethics approval

Not Applicable

### Consent to participate

Not Applicable

### Consent for publication

All authors read and approved the manuscript for publication

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# Figures

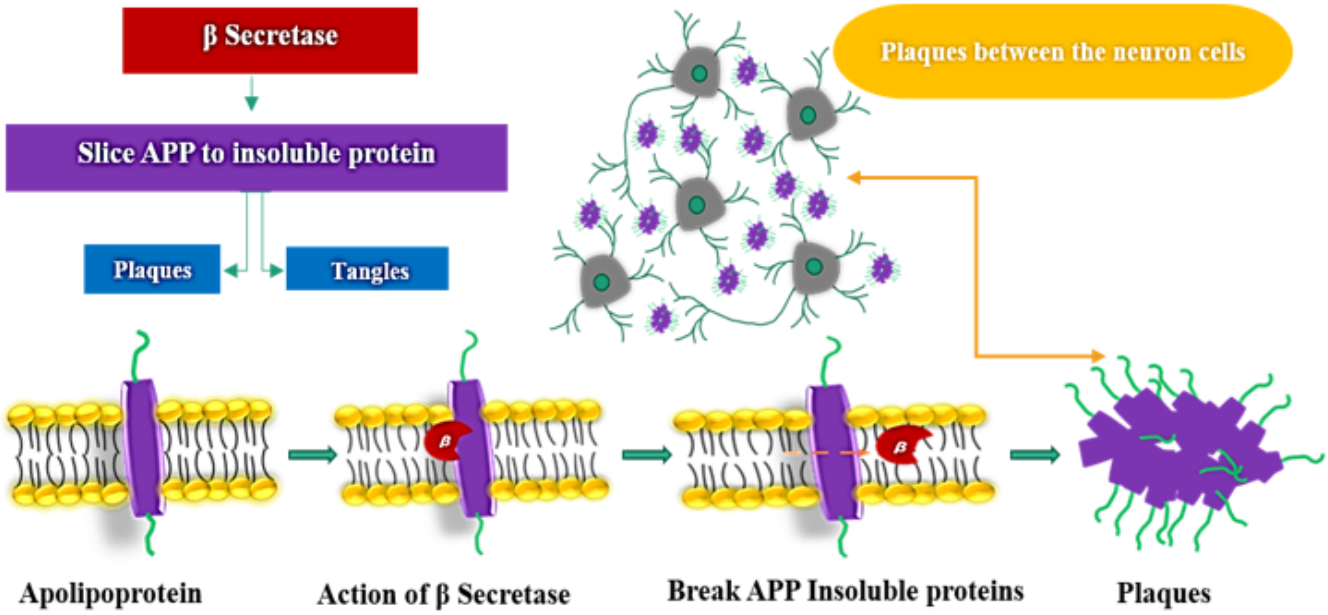
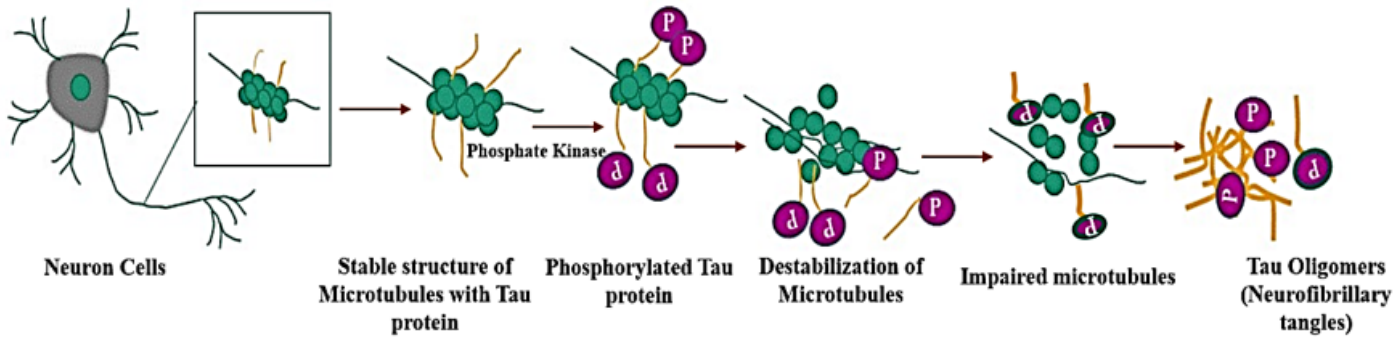


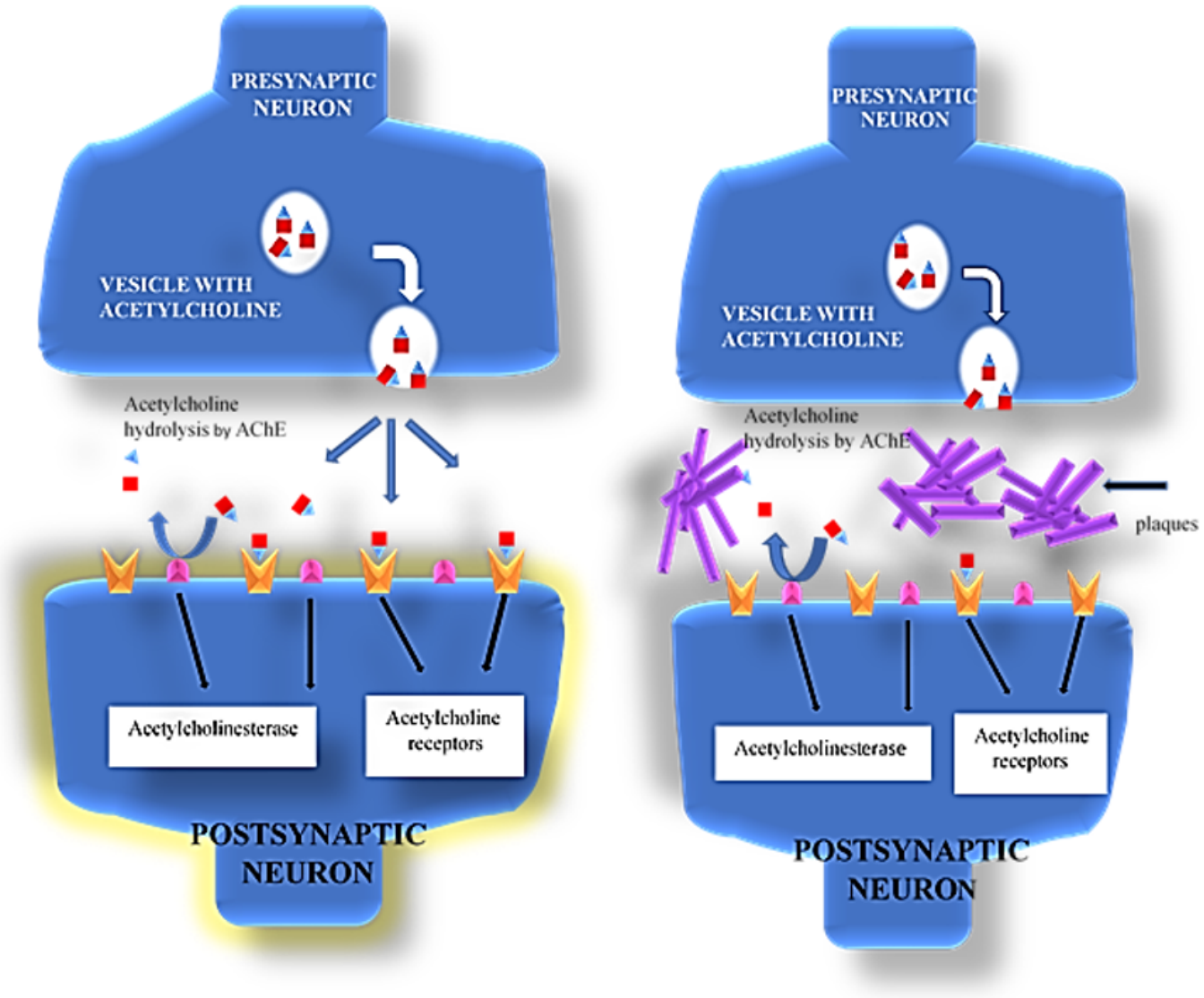

**Figure 1**

Image showing the involvement of β secretase in causing plaques and tangles. Presence of plaques in between neuron cells and the action of β secretase on Amyloid precursor protein is also shown.




## Figure 2

Image showing formation of Tau oligomers in the neuron cells.




## Figure 3

Diagrammatic representation Synaptic action of AChE (Acetylcholinesterase) in healthy, diseased cells.