Characterization of putative drugs for the clinical applications in Alzheimer's disease

A Major Project dissertation submitted

in partial fulfilment of the requirement for the degree of

Master of Technology

In

Biomedical Engineering

Submitted by

Noopur Kejriwal

(DTU/13/M.Tech/446)

Delhi Technological University, Delhi, India

Under the supervision of

Dr.Pravir Kumar

Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road,
Delhi-110042, India.

CERTIFICATE

This is to certify that the dissertation entitled "**Characterization of putative drugs for the clinical applications in Alzheimer's Disease"** submitted by **Noopur Kejriwal (DTU/13/M.Tech/446)** in the partial fulfilment of the requirements for the award the degree of Master of Technology (Biomedical Engineering), Delhi Technological University (Formerly Delhi College of Engineering), is a *bona fide* record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

Date: 22 July 2015

Dr. Pravir Kumar
(Project Mentor)
Department of Bio-Technology
Delhi Technological University

Prof. D. Kumar
(HOD)
Department of Biotechnology
Delhi Technological University

DECLARATION

I, **Noopur Kejriwal** do hereby declare that the dissertation entitled **Characterization of putative drugs for the clinical applications in Alzheimer's disease** has been undertaken by me for the award of Master of Technology in Biomedical Engineering. I have completed this study under the guidance of **Dr. Pravir Kumar**, Associate professor at Dept. of Biotechnology**,** Delhi Technological University, Delhi.

I also declare that this dissertation has not been submitted for the award of any Degree, Diploma or any other title in this university or any other university.

Date: 22 July 2015 Noopur Kejriwal

Registration No: DTU/13/M.Tech/446

ACKNOWLEDGEMENT

I Noopur Kejriwal, student of M.Tech-Biomedical Engineering, registration number-DTU/13/M.Tech/446 is presenting a project report on ”**Characterization of putative drugs for the clinical applications in Alzheimer's Disease**” under the supervision of Dr. Pravir Kumar (Associate Professor), Department of Biotechnology. He encouraged me to undertake this very interesting topic and gave me valuable suggestions and information which were mandatory for the completion of the project.

I would also like to express my gratitude towards respectable Head of Department and all other eminent faculty members whose teachings have always guided me towards brighter path and lastly, my friends who helped me in this project.

Noopur Kejriwal
2K13/BME/19

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ADL	Activities of Daily Living Scale
AICD	Amyloid precursor protein Intracellular Cytoplasmic/C-terminal domain
APP	Amyloid precursor protein
Aβ	Amyloid beta
BACE1	Beta site APP cleaving enzyme-1
BDS	Blessed Dementia Scale
E.C.	Enzyme classification
GABA	Gamma amino-butyric acid
IDE	Insulin degrading enzyme
kDa	Kilo Dalton
MCI	Mild cognitive impairment
NCBI	National Centre for Biotechnology Information
NEP	Neprilysin
NFTs	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate receptor
PCR	Polymerase chain reaction
PDB	Protein Databank
PHFs	Paired helical filaments
TEST	Toxicity Estimation Software Tool

Characterization of putative drugs for the clinical applications in Alzheimer's Disease

Noopur Kejriwal

Delhi Technological University, Delhi, India
E-mail ID: noopurkejriwal@gmail.com

ABSTRACT

Alzheimer's disease is remarked as one of the world's leading causes of dementia. In this proposal, we are trying to find out a suitable approach towards finding the cure for Alzheimer's disease by targeting the causative molecules or by targeting the signalling pathway leading to deposition of amyloid β in Alzheimer's disease, so that , a combinational therapy drug could be found. There are several drugs available in the market currently available for Alzheimer's disease, out of which, we are testing a novel combination that might result in better efficient treatment as compared to the conventional drugs solo treatment. The obtained combinatorial could be further characterised as putative drugs for Alzheimer's disease and could be scaled-up for clinical trials and applications. We are trying to find a more suitable drug which could either up-regulate the levels of insulin degrading enzyme/neprilysin, or act as inhibitors of Tau aggregation or as Acetyl cholinesterase modulators/ NMDA receptor modulator or enhance brain activity by being Neuro-protective compounds thereby reducing Oxidative stress & inflammation thus considerably reducing formation of amyloid β. Most of the available drugs show side-effects such as diarrhoea, nausea, mood changes, insomnia, fatigue, depression and loss of appetite etc. These drugs available as treatment options stabilize some of the symptoms of Alzheimer's disease for a limited period, say usually 6-12 months or longer. The drug treatments prescribed by the physicians can temporarily improve some symptoms or slow down the development of Alzheimer's disease. So, we are trying to find the least hazardous drugs with lesser side-effects and more effective proven results in reducing symptoms related to Alzheimer's disease.

Keywords: Alzheimer's disease, combinational therapy, insulin degrading enzyme, neprilysin, NMDA receptor, amyloid β.

2. INTRODUCTION

Alzheimer's disease is one of the most remarkably common causes leading to evolution of dementia. The expression 'dementia' is described as set of symptomatic diseases which includes memory loss, mood changes, lack of cognitive abilities and problems with reasoning and communication. Alzheimer's disease (AD) is a neurological turmoil that is marked by neuronal death in response to the deposition of amyloid beta plaques in brain which causes loss of memory and cognitive decline (Crews *et al*., 2010).The cognitive collapse in AD occurs due to the neuronal dysfunction that is contributed by the extracellular Aβ aggregates and the intra-neuronal aggregates of tau protein **(Figure 1)** which form the amyloid plaques and neurofibrillary tangles respectively (Kumar *et al*., 2015).

Apart from Aβ deposition and neurofibrillary tangles, other characteristic abnormalities include dystrophic neurites, impaired energy metabolism, chronic oxidative stress, mitochondrial dysfunction, DNA damage, elevated pro death genes and signaling pathways (Monte D. 2012; Moreira P. 2007). In order to elucidate the etiology of AD it is important to understand the Aβ production and degradation mechanisms. Alzheimer's disease is a progressive disorder, implicating that gradually, over time, more parts of the brain are seen damaged leading to worsening of symptoms over the time (Hemming *et al*., 2007).

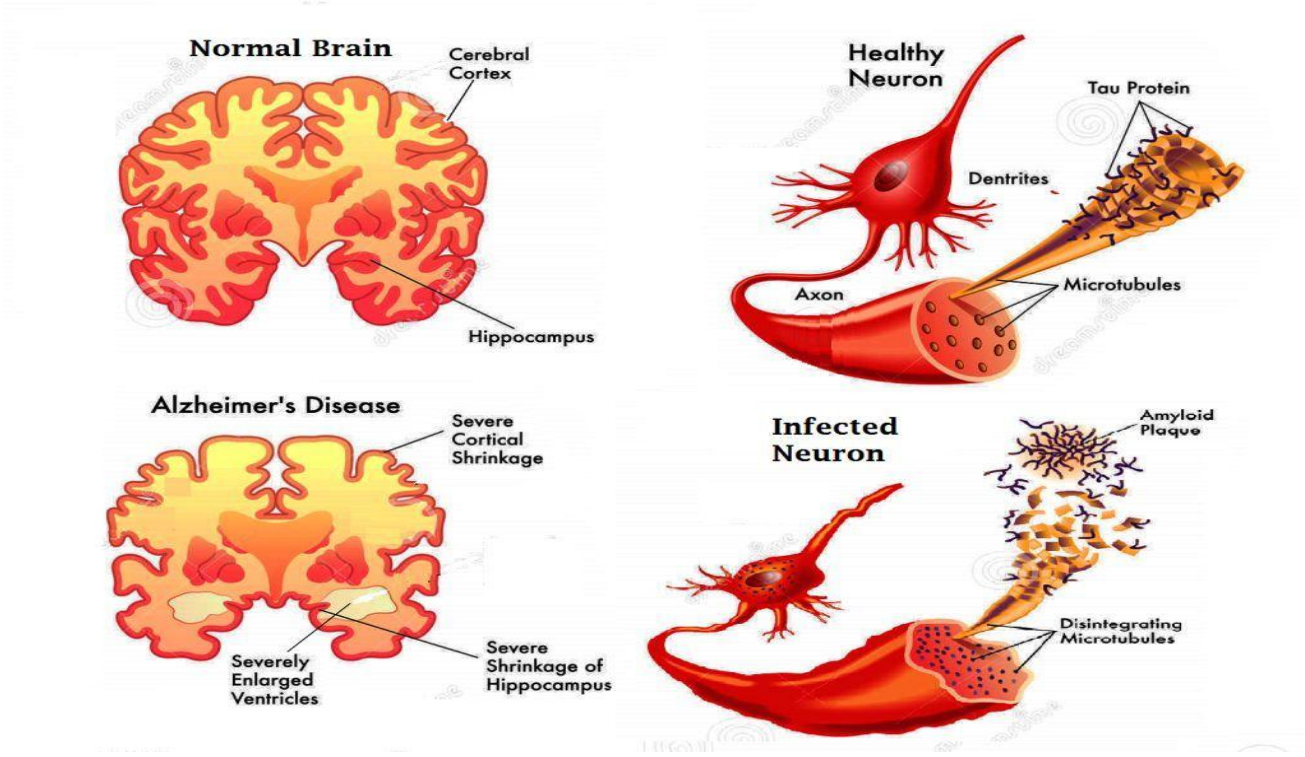


Figure 1: Differences between normal and AD brain

Image source: http://www.naturalhealthcommunity.org/

In Alzheimer's disease, there is amassing of toxic Aβ peptide that contains 40-42 residues. This is the basic and most commonly known neuropathology of AD. The Aβ peptide accumulation in brain depends on its rate of production by the cleavage of the precursor called the holo-APP (Amyloid Precursor Protein) which is 142 residues long, via the β- and γ- secretase (Lemere *et al.*, 2010). APP is proteolysed by two proteases; α-secretase and β-secretase where α-secretase generate sAPPα (soluble) and a 83 residue membrane-associated C-terminal fragment which is constitutively cleaved by γ-secretase to form p3 and APP **(Figure 2)** intracellular domain (AICD) (Hardy *et al.*, 2002). On the other hand β-secretase nicks 16 residues towards the N-terminal of the α-cleavage site and generates sAPPβ and C99 which is then cleaved by γ-secretase to give Aβ and AICD (Selkoe 2002). It is important to note down that products obtained after γ-cleavage of α-secretase generated fragments are soluble and non-amyloidogenic in nature while products obtained after γ-activity on the β-secretase generated fragments gives amyloidogenic Aβ and AICD (Vivian *et al.*, 2010).

In humans among two main types of Aβ; Aβ40 is produced predominantly under normal physiological conditions while Aβ42 in case of AD (Pauwels *et al.*, 2012). The Aβ undergoes polymerization to form fibrils that are pathological in nature and transported from presynaptic terminals to the extracellular matrix where, it facilitates the formation of Aβ plaque whereas AICD alters other cellular functions. Thus generated Aβ plaques are insoluble under normal physiological conditions and also resistant to proteolysis (Kurochkin 2001). Moreover, reasons and symptomatic features of AD along with its stages are shown in **Table 3** and **4**.

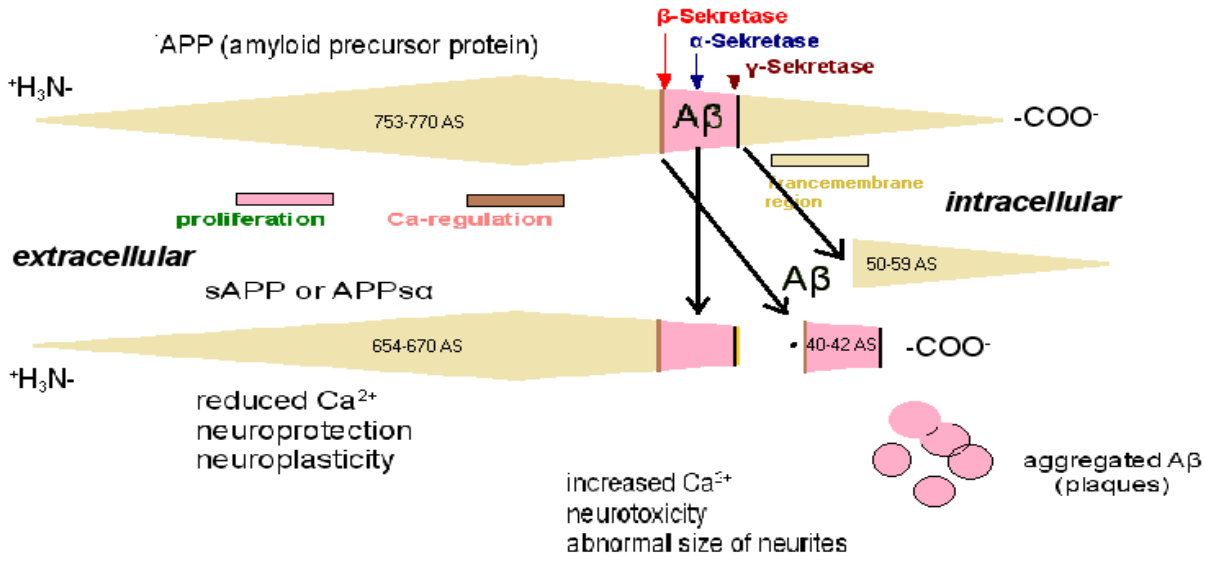


Figure 2: Working of amyloid precursor protein

Image source (https://en.wikipedia.org/wiki/Alpha_secretase)

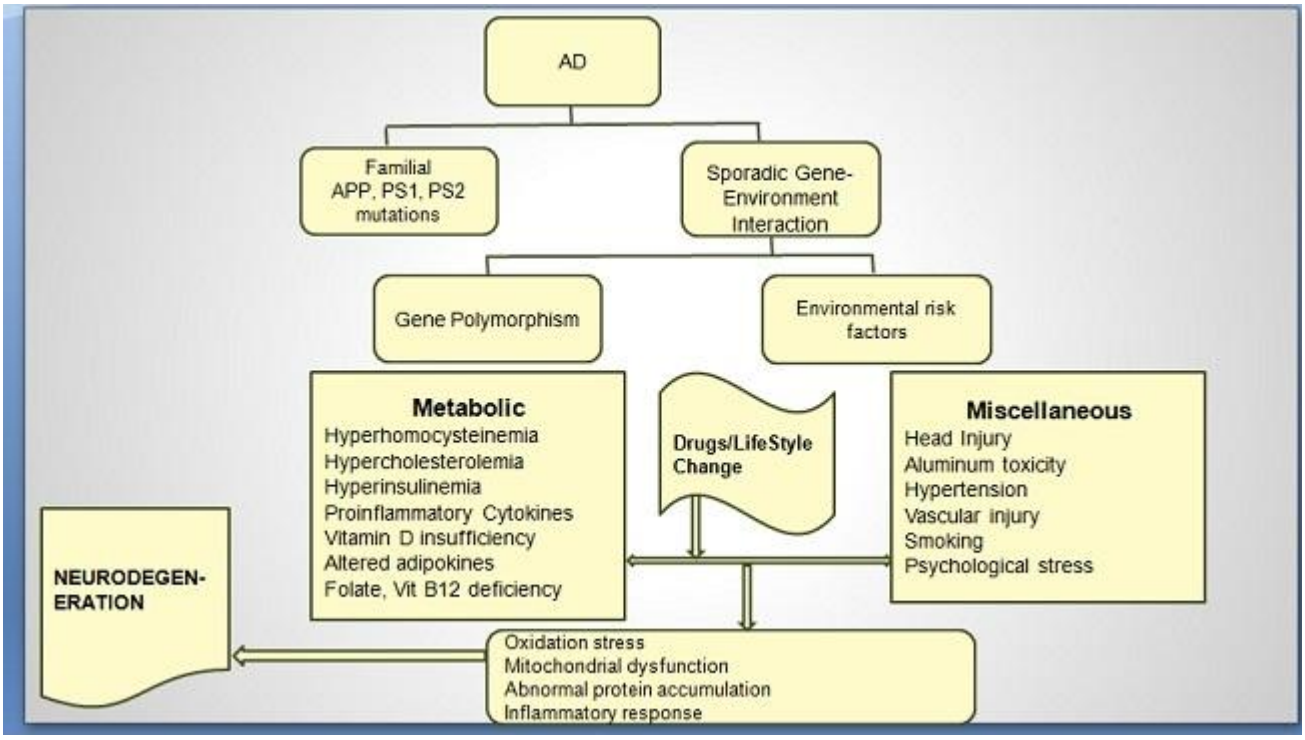


Figure 3: Types of AD followed by reasons responsible for AD

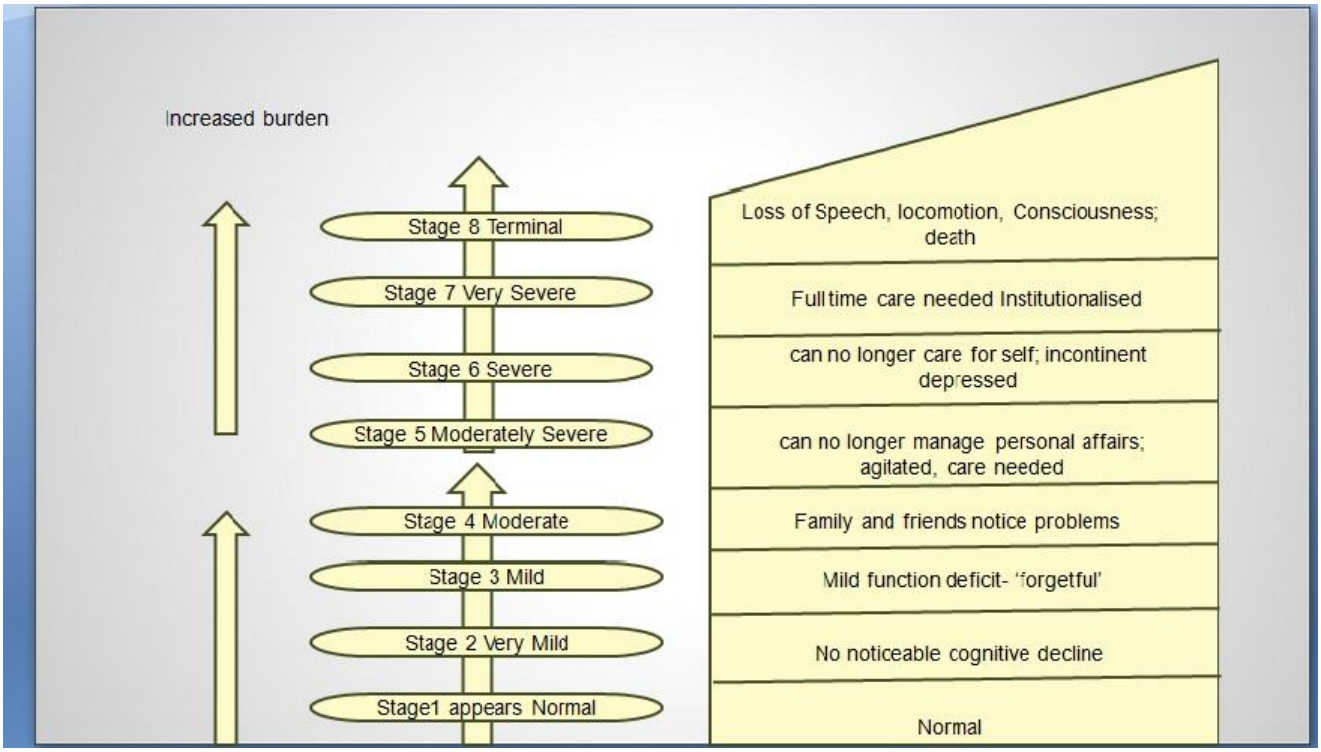


Figure 4: Symptoms sidelined with stages of AD

Getting a diagnosis

There is no as such profound signature test for Alzheimer's disease or for any other causes of dementia. The doctor needs to examine and check out conditions such as infections, anxiety, vitamin deficiency, depression, thyroid problems, memory impairment and the other doable side-effects of medication. A diagnosis beforehand is always beneficial giving the opportunity to safe-plan for the upcoming future and right use of treatment, advice and support on time. The individual under diagnosis is prearranged a blood test and a full physical examination to check medical troubles. The person's memory is testified, to begin with questions about current events and then precedent memories. Their memory and thinking skills is afterward also analyzed in depth by a psychologist. A brain scan is taken out to get some clues regarding the changes taking place in that person's brain (Steve I. 2012).

Mild cognitive impairment

Mild cognitive impairment (MCI) phrase is used when a person has trouble memorizing about things or unable to have clear thought process but in some way, the symptoms are not rigorous enough to necessitate a diagnosis of Alzheimer's disease, however, fresh researches has shown that folks showing symptoms of MCI have an elevated risk of budding Alzheimer's disease (Hemming *et al*., 2007).

Treatment

There is at present no fixed cure for Alzheimer's disease. Drug treatments which are found at this time in the market can temporarily improve few symptoms or slow down the evolution in some populace. Alzheimer's disease brains show shortage of a chemical known as acetylcholine. The drugs namely Aricept, Exelon and Reminyl which are trade names for the drugs donepezil hydrochloride, rivastigmine and galantamine respectively, works on the mechanism of gratifying the existing stores of acetylcholine. These drugs are suggested as treatment medication option for patients in the mild-to-moderate stages of Alzheimer's disease. Side-effects are commonly minor but it may differ person to person. It may cause side-effects such as diarrhea, depression, nausea, mood swings, insomnia, fatigue and loss of appetite etc. A drug named Ebixa universally called trade name for the drug memantine announced in year 2002, works in a slight dissimilar way compared to other drugs and is recommended as treatment profile for people in both the moderate and severe stages of Alzheimer's disease. Side-effects are also numerous and often it includes dizziness, headaches and fatigue, nausea and rarely hallucinations or confusion. Such drugs do not provide an ideal cure, but they stabilize some of the symptoms of Alzheimer's disease for a period of say around 6-12 months or may be longer (Steve I. 2012).

3. LITERATURE REVIEW

This research study is directed towards finding compounds that show sensitivity for APP cleavage, predominantly that inhibit the creation of the aggregating form, Aβ (42) and accumulation of tau protein. Compounds/drugs that target the docking site of substrate rather than their active site of enzyme are also being screened as an alternative strategy (Evin *et al.,* 2006).

3.1 Insulin Degrading Enzyme

It is an intracellular protease that degrades the amyloid forming proteins in the cell and thus their accumulation is being prevented. It is also known as insulysin or insulinase. IDE is situated on chromosome 10q, which is near to a region that has previously been associated with late-onset AD (Williamson *et al*., 2009). The subcellular locations of IDE include cytosol, endosomes, peroxisomes and cell surface. It is an 110kDa single polypeptide chain and is present as a dimer or a trimer. Zinc is required for its proteolytic activity. The active site of the enzyme consists of (His-X-X-Glu-His) sequence required for catalytic activity of the enzyme. It is inverted as compared to the sequence at the active site of NEP which is, (His-Glu-X-x-His) (Tullio *et al*., 2008). Its activity is regulated by a 14kDa inhibitor .This enzyme is unique because it has substrate specificity but yet it can recognize only secondary or tertiary structure. Based upon these observations and various other different studies indicate that IDE recognizes and cleaves the peptides that have the capability to form amyloid-fibrils under specific physiological conditions. It can undergo proteolytic degradation of both soluble and insoluble forms of Aβ unlike NEP that can clear only the insoluble form (Farris *et al*., 2004). Molecular structure of IDE obtained through protein databank server is shown in **Figure 5**.

Role of Insulin Degrading Enzyme (IDE)

IDE is expressed ubiquitously with its maximum expression seen in brain, testes, liver and muscles. Genetic studies have found that the gene responsible for AD due to Aβ42 accumulation and the IDE gene are both located on the same chromosome 10q. IDE is secreted at higher levels by the microglial cells under specific physiological. IDE is the principal enzyme in degradation of insulin in the body and because of its ability to degrade the amyloid deposits that are formed during type 2 diabetes. IDE-knockout mice shows considerably increased levels of Aβ40 and Aβ42 in the soluble fractions and in the brain membrane obtained from IDE deficient mice and also from the cultured neurons obtained from IDE-knockout mice as compared to normal mice models. IDE is not only responsible for reducing the levels of Aβ and insulin but also of unphosphorylated AICD which otherwise enters nucleus and acts as a transcriptional regulator (Farris *et al*., 2003).

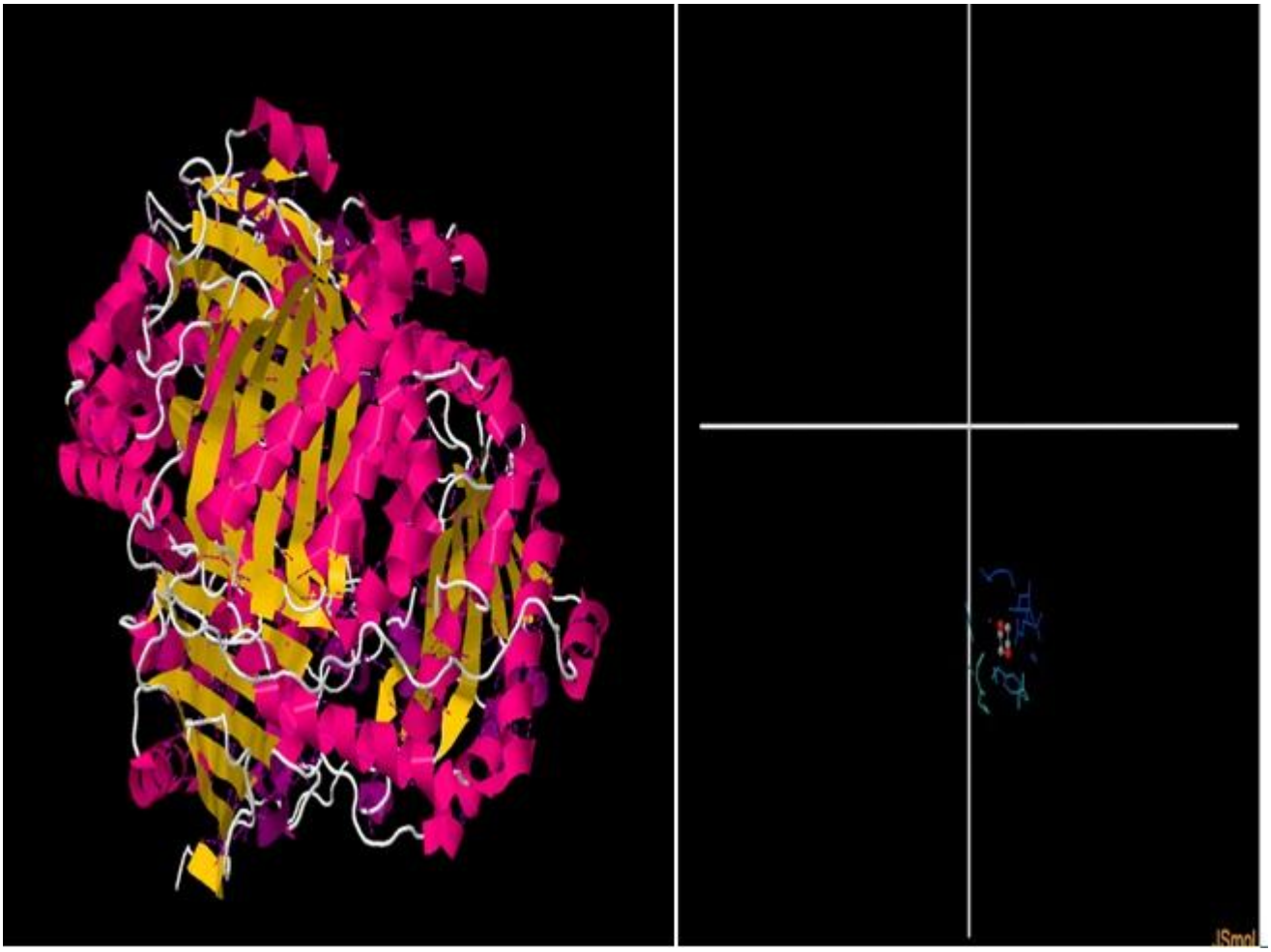


Figure 5: Molecular structure a) 3-D structure of IDE; b) and its ligands shown around symmetry

3.2 Neprilysin (NEP)

Neprilysin (EC 3.4.24.11) is a 97kDa weighing plasma membrane glycoprotein which has N-terminal cytosolic domain which is a membrane-spanning segment and a C-terminal extracellular with the catalytic domain. Another name for this enzyme is neural endopeptidase. This ectoenzyme degrades oligopeptides which are (<5kDa) on the amino side of hydrophobic residues. So it is further appropriate for the breakdown of smaller 40-42 hydrophobic Aβ peptides that are formed in AD (Hashi *et al.,* 2005). Molecular representation of 3-dimensional structure of Neprilysin obtained through protein databank server is shown in **Figure 6**. The profound levels of Aβ-peptide are determined by bringing the balance between its rate of synthesis from its precursor and its rate of clearance. The clearance of these toxic peptides of amyloid beta is generally due to two types of protease enzymes: Neprilysin (NEP) and Insulin-Degrading Enzyme (IDE). NEP's ecto-domain is towards the extracellular space at the pre-synaptic site. It can possibly degrade both monomeric and polymeric forms of Aβ peptide. The level of neprilysin mRNA is found

lower in the hippocampal and temporal cortex region in AD patients as compared to the normal controls that are of the same age (Lindahl *et al.*, 2008).

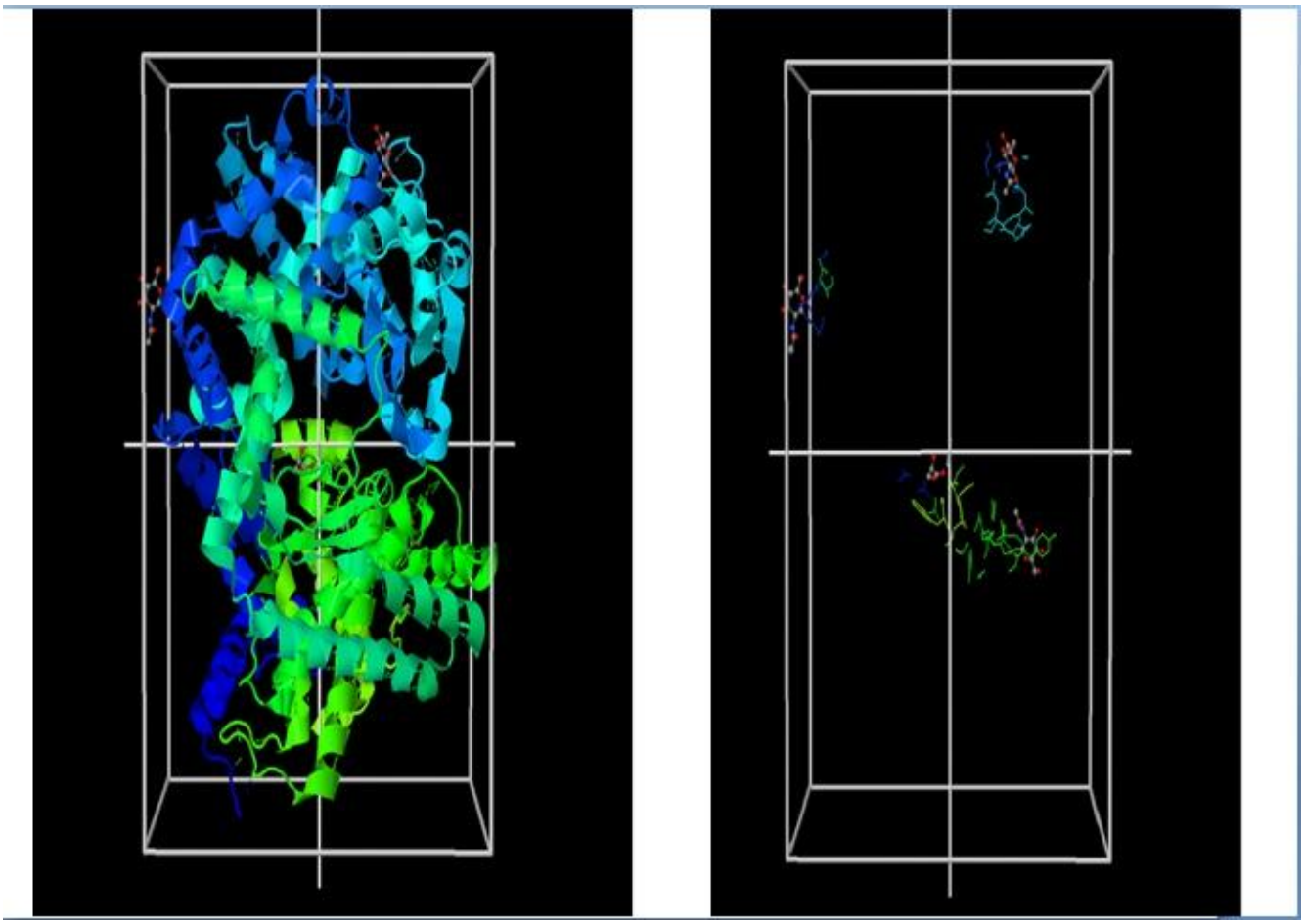


Figure 6: Molecular structure a) 3-D structure of Neprilysin; b) and its Ligands shown around symmetry

Role of NEP

The degradation of Aβ peptides can be further enhanced by over-expression of NEP in the plaque prone areas of the brain **(Figure 7)**. This brings upon degradation of the Aβ peptides thus lowering the progression of the disease. This can also be achieved through lentiviral mediated over-expression of NEP. Thus by over-expressing NEP through gene therapy in the areas of brain those are susceptible to Aβ aggregation in AD can protect neurons from toxicity and can alter the development of AD (El-Amouri *et al.*, 2007).

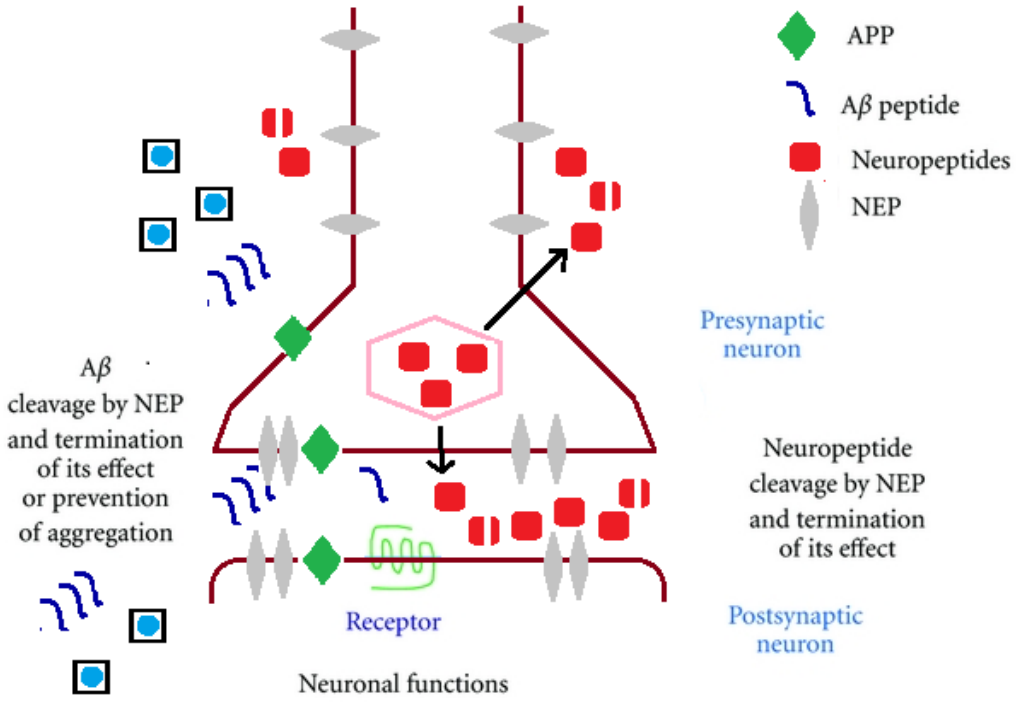


Figure 7: Role of NEP in AD pathology: Diagrammatic arrangement of NEP localization and its purposeful movement in the brain. In this picture, NEP undergoes pre-localisation and post-synaptical alterations in neuronal cells to cleave its neuropeptide substrates terminating their properties

(Image modified and adapted from: http://www.hindawi.com/journals/ijad/2012/383796/fig1/)

Effect of NEP inhibitor

Its role in degrading Aβ and preventing its deposition can also be seen from the experiments where NEP is artificially inhibited by using an inhibitor such as, thiorphan and phosphoramidon, specific for NEP which caused accumulation of Aβ in the NEP inhibited cells. This study was performed in transgenic mice models of AD. It also showed decrease in Aβ degradation in NEP-knockout mice and an elevated level of Aβ40 and Aβ42 as compared to the normal controls (Eckman *et al*., 2003).

A differential tabular representation to sketch out general characteristics of IDE and NEP has been made and shown as **Table 1** derived through protein databank server (PDB). Furthermore, a list of agonists which possibly enhance action of IDE and NEP on amyloid beta clearance has been shown in **Table 2**.

Table 1: Differences between IDE and NEP based on physical and biological characteristics

Differences based on characteristics	Insulin degrading enzyme	Neprilysin
Classification	Hydrolase	Hydrolase
Structure weight	337557.58	80673.64
Molecule	Insulin degrading enzyme	Neprilysin
Polymer	1	1
Chains	A,B	A
Enzyme Classification	3.4.24.56	3.4.24.11
Organism	Homosapiens	Homosapiens
Type	Protein	protein
Length	990 aa	696 aa
Function	It is shown playing a role in the cellular breakdown of several compounds such as insulin, IAPP, Glucagon, bradykinin, kallidin and other peptides, and thereby plays a role in intercellular peptide signalling. May be playing a role in the degradation and clearance of naturally found amyloid beta-protein by neurons and microglia. Degrades amyloid which are formed by APP and IAPP	It has Thermolysin-like specificity but is confined on acting on polypeptides made of up to 30 amino acids. It has a biological importance in the destruction of opioid peptides such as Met and Leuenkephalins by cleaving of a Gly-Phe bond .it has ability to cleave angiotensin 1-9. It is involved in the degradation of atrial natriuretic factor (ANF).
Catalytic activity	It Degrades insulin, glucagon and other polypeptides but no similar action on proteins.	It does preferential cleavage of polypeptides in between hydrophobic residues, particularly with Phe or Tyr at P1.
Subunit structure	Homodimer can form higher oligomers forming interactions with varicella-zoster virus (VZV) envelope glycoprotein E (via N-terminus).	N/A
Domain	The SlyX motif may be seen involved in non-conventional secretion of the protein moiety.	N/A

Table 2: List of drug-able compounds regulating IDE/NEP expressions

S.No.	Compounds/Drugs	Targets	Molecular Functions	References
1	DHA (Docosahexaenoic acid)	IDE	It is the most important fatty acid in the brain especially rich in the neurons and synaptosomes of the cerebral cortex. DHA significantly up regulates the expression of IDE in neural cells.	(Du *et al*., 2010)
2	Valproic acid	NEP	Valproic acid attenuates the prenatal hypoxia-induced Aβ neuropathology and deficits in learning and memory via inhibiting the activation of histone deacetylase 1 (HDAC1), preventing the decrease in H3-Ace in the NEP promoter regions and reducing the down-regulation of NEP.	(Nalivaeva *et al*., 2012; Wang *et al*., 2014)
3	Propranolol	IDE	It enhances the expression of IDE. Moreover, the expression of Akt, BDNF and Tau hyperphosphorylation is decreased by propranolol treatment as shown by Tg2576 mice.	(Dobarro *et al*., 2013)
4	Neuropep-1	IDE & NEP	It increases the level of IDE and NEP. However, Neuropep-1 treatment does not alter the expression of full-length APP, α-, β-, or γ-secretase.	(Shin *et al*., 2014)
5	Somatostatin (octreotide)	IDE	Somatostatin directly interacts with IDE. It binds to the active site of one IDE subunit induces an enhancement of IDE proteolytic activity toward fluorogenic Aβ by another subunit.	(Ciaccio *et al*., 2009; Tundo *et al*., 2012)
6	Imidazole-derived 2-[N-carbamoylmethyl-alkylamino] acetic acids	IDE	It acts as substrate-dependent modulators of IDE in Aβ hydrolysis.	(Charton *et al*., 2014)
7	Apomorphine (APO)	IDE	Apomorphine, a kind of dopamine receptor agonists, to promote the intracellular Aβ degradation via activating IDE.	(Ohyagi 2011)
8	Leptin	IDE	Leptin enhances the expression levels of IDE putatively by activating the Akt pathway.	(Marwarha *et al*., 2010, 2012)
9	Suramin	IDE	Suramin increases the activity of the enzyme IDE by changing the turnover rate of the enzyme for its substrate.	(Leissring *et al*., 2003; Perez *et al*., 2000)
10	Humanin (HN)	NEP	Humanin (HN) is a 24-residue peptide, acting as a neuroprotective factor by increasing the expression of	(Niikura *et al*., 2011)

			NEP and shows anti-cell death activity against a wide spectrum of Alzheimer's disease.	
11	Trichostatin (TSA)	NEP	TSA treatment significantly enhances NEP expression by elevating the acetylation of histone H3 on NEP promoter.	(Deng *et al*., 2014)
12	5-aza-deoxycytidine (5-Aza-dc)	NEP	5-Aza-dc prompts the demethylation of NEP gene and significantly increases NEP expression in a dose-dependent manner.	(Deng *et al*., 2014)
13	Ginsenoside Rg1	IDE	It increases the IDE expression in the hippocampus by upregulating PPARγ, thereby decreasing Aβ burden.	(Quan *et al*., 2013)
14	Imatinib (Gleevec)	NEP	It is a known tyrosine kinase inhibitor elevates AICD in H4 human neuroglioma cells and increases expression of NEP protein.	(Bauer *et al*., 2011)
15	GW742	IDE & NEP both	APPARdelta agonist reduces amyloid burden by enhancing the expression of IDE and NEP.	(Kalinin *et al*., 2009)
16	EGCG	NEP	EGCG strongly increases the NEP activity leads to Aβ degradation.	(Melzig *et al*., 2003)
17	Curcumin	IDE & NEP both	It increases Aβ clearance by increasing IDE and NEP activity and also prevents Aβ production by inhibiting PS2 a catalytic component of γ-secretase.	(Wang *et al*., 2014)
18	Resveratrol	NEP	RSV significantly increases both the estradiol level and NEP level that decrease Aβ deposition; by upregulation of estradiol level this consequently leads to increase in the level of NEP level thus leads to Aβ degradation.	(El-Sayed *et al*., 2015)
19	Norepinephrine	IDE	Norepinephrine incites microglia to partake and Degrade Amyloid β Peptides via Up-regulation of IDE.	(Kong *et al*., 2010)

3.3 Tau phosphorylation

Alzheimer's disease (AD) shows characteristics such as dementia, cognitive dysfunctionalities, along with tauopathy. Tau also mostly known as microtubule associated protein (MAP) is also essential in maintaining the neuronal network. The change in phosphorylation state of tau protein brings about disruption of the microtubular network while dephosphorylation permits reconstitution of the microtubule network **(Figure 8).** Several accredited kinases, e.g., MAPK, protein kinase C and MARK are set up to hyperphosphorylate tau, further causing disruption of the network of microtubules and development of neurofibrillary tangles (NFTs), which brings about glycosylation, glycation, and adducts of lipid peroxide impairing transport system of neurons in turn affecting memory formation and retention (Kumar *et al.,* 2015). The burden on protein amyloid initiates activation of AMP-activated protein kinase that heightens phosphorylation of tau at positions Ser262/Ser356 and Ser396. Several phosphatases present at lower levels in AD patient brains and down regulation of them results in abnormal hyperphosphorylation of tau. The evidences strengthen a likely probable link between phosphorylation of tau and chaperone mediated metabolism of tau having crucial role in tauopathy and tau clearance (Kumar *et al*., 2015). A tabular representation of general characteristics of TAU protein has been shown in **Table 3** taken from PDB.

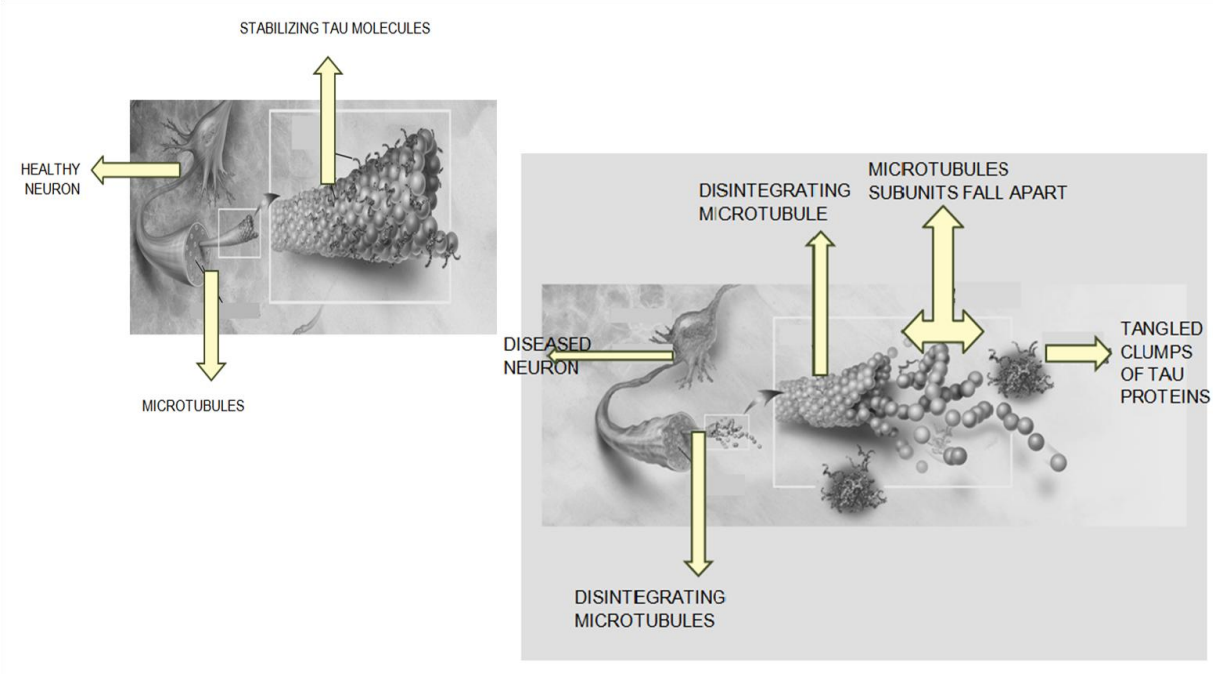


Figure 8: Tau and microtubules phosphorylation and destabilisation

Image modified and adapted from- http://www.nia.nih.gov/nr/rdonlyres/a01d12ce-17e3-4d3d-bcef-9abc4ff91900/0/tangles_high.jpg

The amassing of phosphorylated tau protein and its exclusion from the milieu shows that the chaperone is interacting with phosphorylated tau and promoting its degradation. For example, Hsp90 and cdc37 regulates the tau stability and phosphorylation dynamics but Hsp27 modifies neuronal plasticity, whilst 14-3-3 is seen being involved with interactions of tau with smaller HSPs. In therapeutics interventions of AD, Hsp70 ATPase acts as a modulator whereas Hsc70 hastily engages tau after destabilization of microtubules (Kumar *et al*., 2015).

Various researches have shown that tau (MAP) is the main element constituting paired helical filaments in short called as PHFs that constitute the neurofibrillary tangles (NFTs) (Grundke-Iqbal *et al*., 1986; Lee *et al*., 1991). The tau in PHFs and NFTs in case of AD are abnormally hyperphosphorylated. Regulation of tau phosphorylation is vital in to maintain the equilibrium amid microtubule agility and stability in development of axons. In a normal human brain, phosphorylated and dephosphorylated tau maintains equilibrium (Matsuo *et al*., 1994). The disorder of this stability shows results of dysfunctioning tau which plays a crucial role in neurodegeneration (Mandelkow *et al*., 1995). Hyperphosphorylation of tau brings dissociation from microtubular network which forms Paired Helical Filaments that in due course comprehend to form NFTs. The NFTs are attribute features in an AD brain. The normal tau has soluble propensity as seen in nature. Nevertheless the NFTs fashioned due to abnormal hyperphosphorylation are highly insoluble in nature. This brings upon a major disorder at the level of neuronic cytoskeleton, as a consequence distressing the job of axon transport causing synaptic dysfunction (Masters *et al*., 1985; Selkoe D. 1994). The exact cause-effect relationship linking tau and Aβ plaques is not well understood. The limitations in the in vitro and in vivo studies taken so far to this date in AD research is primarily cause of this concern.(Ballatore *et al*., 2007).

As mentioned previously in this literature, the balance between phosphorylated state of tau and dephosphorylated tau requires to be maintained in the axons. Every disturbance brought in this equilibrium will cause tau dysfunction resulting in neurodegeneration (Johnson *et al*., 2004). When tau hyperphosphorylated occurs by activity of kinases, the echelon of free tau increases which in turn lowers the affinity for the microtubules. So, more tau is dissociated from the microtubules increasing level of intracellular free tau. There are various stages of development from free tau to NFTs. These three stages of development of neurofibrillary tangles are: pre-Neuro-Fibrillary Tangles, intraneuronal Neuro-Fibrillary Tangles, and extraneuronal Neurofibrillary Tangles. So, loss of neurons is brought about by hyperphosphorylation in tau causing gigantic detachment and faster deterioration in the structure of the microtubules. (Maltsev *et al*., 2014). The heat shock proteins (HSPs) are type of proteins whose expression are seen when cell undergoes intense stress conditions .The main function of HSP is to act as molecular chaperons and they play important role during the progression of the disease. (Dickey *et al*., 2007). The HSPs recognize the misfolded or phosphorylated tau and employ protein phosphates to dephosphorylate. On the other hand if these HSPs not succeed to do so, CHIP comes into scenario. It ubiquinates the protein and results in degradation (Johnson *et al*., 2009).

Hsp70-CHIP composite plays a very vital part in pathology related to tau. The recognition sites for Hsp70 are the phosphorylated sites on tau protein that act as markers for the protein showing ubiquitination and subsequent degradation of protein by E3 ligase. This shows that Hsp70 binds specifically to the phosphorylated sites on tau. Simultaneously CHIP also particularly ubiquinates these phosphorylated tau for degradation. The gathering of hyperphosphorylated tau is unsolvable and toxic leading to cell death. Henceforth the ubiquitination removes the insoluble aggregates of tau by degradation and also protects the cell from its cytotoxic effects and death (Zou *et al*., 1998). CHIP (a molecular co-chaperon and E3 ligase) also has significant role in suppressing inflammatory reaction in neurodegeneration and hold cytoprotectant activity. So, the therapeutics should be designed as such to inhibit the causes that encourage tau hyperphosphorylation and enhances its cytotoxic effects (Kumar *et al*., 2012). Metformin and its derivative phenformin (anti type II diabetic drugs) is capable to activate PP2A, which is a important phosphatase that dephosphorylates tau in vivo thus protein phosphatases play an vital task in the ruling of tau phosphorylation (Kickstein *et al*., 2010; Liu *et al*., 2005). Drugs showing Tau modifications are shown as a tabular chart in **Table 4**.

Recent researches have shown that acetylation of the middle domain of Hsp90 inhibits its ATPase activity and further block ATP from binding toHsp90 (Scroggins *et al*., 2007; Bali *et al*., 2005). Curcumin is a bioflavonoid which can correct tau-dependent behavioural and memory deficits by reducing soluble tau and prominent HSPs involved in tau clearance (Ma *et al*., 2013). In addition, one of the plants extracted compounds Luteolin reduces zinc-induced tau hyperphosphorylation and is wonderful example representing therapeutics in the case of AD (Zhou *et al*., 2012).

Table 3: General characteristics of Tau protein

Characteristics	Description
Name of protein-	Tau protein, Microtubule-associated protein tau, Neurofibrillary tangle protein, Paired helical filament-tau (PHF-tau)
Structure weight	4892.73
Molecule name	Microtubule-associated protein tau
Polymer	1
Chains	A
Organism	Homosapiens
Type	Protein
Length	758 aa

Function	Regulates microtubule assembly and its stability, and may be involved in the establishment and maintenance of neuronal polarity. The C-terminus binds axonal microtubules while the N-terminus binds neural plasma membrane components that suggest tau functions as a linker protein between both. Axonal polarity is pre-determined by TAU/MAPT localization in the area of the cell body.
Subunit structure	Interaction with PSMC2 through SQSTM1 when polyubiquitinated. Interacts with FKBP4, SGK1. Binds to CSNK1D. Interacts with EPM2A; the interaction dephosphorylates MAPT at Ser-396.
Domain	The tau/MAP repeat shows binding to tubulin. Type I isoforms contain three repeats while type II isoforms contains four repeats.

Table 4: Drugs under research for tautopathy

S.No.	Name of drug molecule	Name of target molecule	Mode of action	References
1	Lithium	Tau	Lithium specifically protect against Alzheimer's by protecting against pathology of tau proteins through the enzyme glycogen synthase kinase-3 beta. In one of the clinical trial, it has reported that the phosphorylation of tau proteins is reduced by one year of treatment with a low pharmaceutical dose of lithium.	Noble *et al*., 2005; Forlenza *et al*., 2011
2	Resveratrol	Tau, amyloid beta	In a mouse model of Alzheimer's disease, daily doses of resveratrol reduced brain levels of beta-amyloid plaques, which are being regulated through increased activation of the Sirtuin 1 protein. It has been found that less Sirtuin1 in the brain indirectly correlates with greater levels of beta-amyloid plaques and tau protein tangles.	Julien *et al.*, 2009
3	Epithilone D	Tau	This drug in actual fact hits a tau target by correcting tau loss of function, thereby stabilizing microtubules and offsetting the failure of tau due to its development into neurofibrillary tangles in animal	Brunden *et al.*, 2010

			models, which suggests that this could be an significant choice to mediate tau role in Alzheimer's and other tau-based neurodegenerative diseases.	
4	Edaravone	Tau,amyloid beta	Edaravone dose-dependently inhibited the increase in phosphorylation of Tau at Ser396 site induced by Aβ	Shu-Sheng *et al*., 2015
5	Gracilins	Tau, BACE1, ERK	Assays done in-vitro has shown that gracilins are able to inhibit BACE1, reduce tau hyperphosphorylation and inhibit ERK. Gracilins are sponge-derived diterpenoid compounds that can act as antioxidants in the course of mitochondrial targeting and through the induction of Nrf2 translocation.	Leirós *et al*., 2015
6	Valproate	Tau	Valproate reduces tau phosphorylation via cyclin-dependent kinase-5 and glycogen synthase kinase-3 signaling pathways.	Hu *et al*., 2011
7	L-3-n-butylphthalide (L-NBP)	Tau	The expressions of cyclin-dependent kinase and glycogen synthase kinase 3β, the chief kinases involved in tau phosphorylation, were markedly reduced by L-NBP treatment.	Peng *et al*., 2012
8	Propranolol	Tau	Tau hyperphosphorylation is decreased by propranolol treatment as shown by Tg2576 mice as well as enhances the expression of IDE.	Dobarro *et al*., 2013
9	Berberine	Tau	It significantly reduces the levels of C-terminal remains of APP and the hyperphosphorylation of APP and tau via the Akt/glycogen synthase kinase 3 signaling pathway in N2a mouse neuroblastoma cells.	Durairajan *et al*., 2012
10	Magnesium sulphate	Tau	Magnesium sulfate treatment reduces tau hyperphosphorylation by escalating the inhibitory phosphorylation of GSK-3β at serine9, thus increasing the action of Akt at Ser473 and PI3K at Tyr458/199, and recuperating insulin sensitivity.	Xu *et al*., 2014

3.4 Combination studies of drug molecules

Combination therapeutics slows down cognitive and functional decline in AD patients as compared to CI-monotherapy or no treatment. The benefits were small-to-medium effect sizes that increased further with time on treatment and were continued for years (Alireza *et al*., 2008). Current FDA (Food and Drug Administration) and EMA (European Agency for the Evaluation of Medical Products) has approved cholinesterase-inhibitors (CI) and Memantine (MEM), which have self-effacing symptomatic but not curative effects (Cummings JL 2004). Subjects diagnosed with AD which participated in short-term clinical trials and longer-term open-label extensions profiles show transient improvement and stabilization or reduced deterioration on measures of cognition, behaviour and daily living routine follow-ups (Winblad *et al*., 2007). The data of these trials might rigorously demonstrate the clinical safety and efficacy of drug versus placebo treatment in highly selected cohorts of subjects for shorter durations (usually 24–28 weeks in randomized, double-blinded, and placebo-controlled fashion, and in additional open-label extension studies), still, long-term, real-world clinical effectiveness of such data for monotherapy or combination therapy is lacking. A fewer short duration studies have assessed the effects of CI treatment in a "real-world" clinical setting (Aguglia *et al.,* 2004) and a fresh study from Japan has assessed effects on patients clinically treated with donepezil for up to two years duration.

Significantly different results were obtained when in subjects with AD receiving clinical care at a memory disorders unit were compared across a four-year span which included a group never treated with cholinesterase-inhibitor or memantine (NO-RX group), a group clinically treated with cholinesterase-inhibitor monotherapy (CI group) and a group clinically treated with combination therapy consisting of memantine added onto a cholinesterase-inhibitor (COMBO group) on the basis of their cognitive and functional deterioration. Considerable incremental decreases in the rate of development of cognitive impairment going from the NO-RX to the CI to the COMBO groups were reflected by the annual rate of increase in the number of errors made on the BDS (Blessed Dementia Scale) when adjusting for baseline differences between the three groups on BDS and activities of daily living scores, age, education, duration of illness, and interactions of baseline scores with years. So, it was found in study that measure of cognition, CI was superior to NO-RX, and COMBO was superior to both CI and NO-RX (Iwasaki *et al*., 2007).

It was found that the COMBO group had appreciably lower rate of decline on the ADL than the NO-RX and the CI groups, which did not remarkably differ from each other. This study results supports the current use of drugs in treatment of AD patients in both COMBO and CI produced outcomes than NO-RX. These results are also dependable with a new study that compared CI (donepezil)-treated and untreated patients in an outpatient clinical setting in Japan and found lesser cognitive deterioration on the MMSE (Mini Mental State Examination) with treatment, and benefits that lasted at least two years (Iwasaki *et al*., 2007). Combination therapy is usually prescribed for patients with moderate or severe stages of AD with memantine add-on therapy to a cholinesterase-inhibitor. One of the strongest

substantiation that supported the advantage of COMBO Rx therapy over CI-monotherapy was found from a 24-week pivotal phase III clinical trial of memantine add-on therapy to chronically stable donepezil treatment in highly selected subjects with moderate to severe AD. On a account of approximately 149 patient, years of completer's data, this study showed considerably better results for memantine-add-on combination therapy than placebo-add-on donepezil monotherapy with respect to measures of behaviour, global outcome, cognition and activities of daily routine (Rockwood *et al*., 2004).

The results obtained thus go a long way in findings from short-term clinical trials and provide substantial indication that combination therapy with cholinesterase-inhibitor and memantine has real-world clinical efficacy in the treatment of patients with AD. The findings suggest that COMBO Rx is superior to NO- RX and CI monotherapy sustained for at least two years. The mild to moderate effect size estimates for this dominance are reliable with those found in meta-analyses of short-term clinical trials of CI monotherapy (Locascio *et al*., 1995). On average, CI monotherapy decreases the deterioration and cognitive decline by about one error per year and that COMBO Rx decreases it by about two errors per year. Such findings support conclusions from other clinical trials as well presenting that COMBO Rx is drastically superior to placebo and CI monotherapy: COMBO decreased the rate of functional decline on the ADL scale compared to NO-RX and CI. On another finding, CI alone did not affect the slope of decline on the ADL, while the slope was just about halved during COMBO treatment. The benefits are more evident at transitional impairment levels (Reisberg *et al*., 2006).

All these studies has proven that COMBO is superior to NO-RX and CI monotherapy in slowing developmental evolution of cognitive and functional decline in this well-characterized and prospectively assessed cohort of patients with AD. It was also found that with respect to cognition, CI monotherapy was more superior to NO-RX comparison. The benefits of COMBO therapy were remarkably significant, with small-to-medium effect sizes that were sustained for longer duration of years. The results also lift the intriguing likelihood that COMBO therapy discreetly modifies the long-term clinical course of AD, although sustained symptomatic pharmacologic effects that vary in strength with time are similarly probable (Tariot *et al*., 2004). In AD, the regions of brain concerning cognitive functions implicate a pool of dysfunctionalities counting developmental loss of neurons as well as synapses often accompanied with neuro-inflammation, added with neuronal degeneration, atypical blood-brain barrier (BBB) permeability (Zlokovic 2008).

The progressive studies of therapeutic interventions at the stages of cellular processing and proceedings of these peptides haven't been a huge success so far (Berk *et al*., 2013). The etiological factors leading to development of symptomatic AD are not fully understood yet. Such noteworthy co-morbidity flanked by AD and cerebrovascular deregulation generates idea that these deficits are imperative features of AD which has acted synergistically with amplifying feed-back loops thus, implying these peptides and other toxic metabolites make the disease conditional challenging to mono-therapeutic interventions (Pimentel-Coelho *et al.,* 2012). For acting on different elements contribute to this intricate disorder, a new

combinational approach is being proposed for the equilibrium between GABA, glutamate, and glycine systems involving progress of pathological features outstanding in Alzheimer's Disease that would accentuate roles in degeneration of structures of brain. Glutamate scheme of the brain is essential for signal transmission and plasticity as well as also involved in regulation of endurance or stimulation of apoptosis of cells in the brain. The system is furthermore oppositely balanced by GABA inhibitory action and signalling of glycine in normal functions of brain secured by the fine balance among these inhibitory and excitatory (E/I) activities. A tabular representation of combination drugs is shown in **Table 5**.

One of the mechanisms responsible for cause of AD is deregulation of glutamate excitatory neuronal signalling (Paula-Lima *et al*., 2013). Although GABAergic neurons are measured as relatively preserved in subjects of AD, a more recent studycase has shown insightful remodelling of molecular machinery of GABAergic neurotransmission with intricate changes in the relative concentrations of its diverse molecular constituents, which themselves could negotiate the overall balance of E/I signalling (Limon *et al*., 2012). The toxic oligomers of Aβ could possibly modify the balance, in so doing impairing cognitive functions and driving the pro-apoptotic activities both in neuronal and endothelial cells. So, it is important to select several drug candidates, each of them having potential for acting on multiple targets in these pathways. The ultimate goal is to progress rapidly towards the treatment options of curing AD, so, the drugs has to meet two primary criterions:

1) Already tested for a longer durations in treatment of other disorders, and

2) Assuring a good quality safety profile.

Here, in this study, the first drug taken is acamprosate calcium (ACP), which is an anticraving agent that manipulates glutamatergic transmission and brings changes in the E/I balance twisted in ethanol addiction. ACP is a potentially less harmful drug until being consumed at exceptionally high doses (Appleby *et al*., 2013). The ACP levels in the human brain are brought down by glutamate controls for the period of ethanol withdrawal and opposites the resulting hyperexcitability (Dahcour *et al*., 1998). Glutamate receptors are very essential in balancing between cell death and its survival however their function is substantially tailored in AD (Revett *et al*., 2013). Probable targets for ACP comprise of the iono/metabotropic glutamate receptors and also inhibitory glycine gated ion channels (Witte *et al*., 2005; Chau *et al*., 2010).

GABA is a drug having noteworthy changes in AD subjects which also resist the likely probable feedback changes at some stage in chronic treatment acts on brain major inhibitory system. A second drug, baclofen (BCL), used for the dealing in spasticity for more than a few decades which activates inhibitory metabotropic GABAB receptors is also focused here in this study. BCL has favourable safety profile results even when used at much higher doses and is the only agonist of GABAB receptors that is till date authorised for human use. One important reflection of this study should be taken that GABA, glutamate and glycine

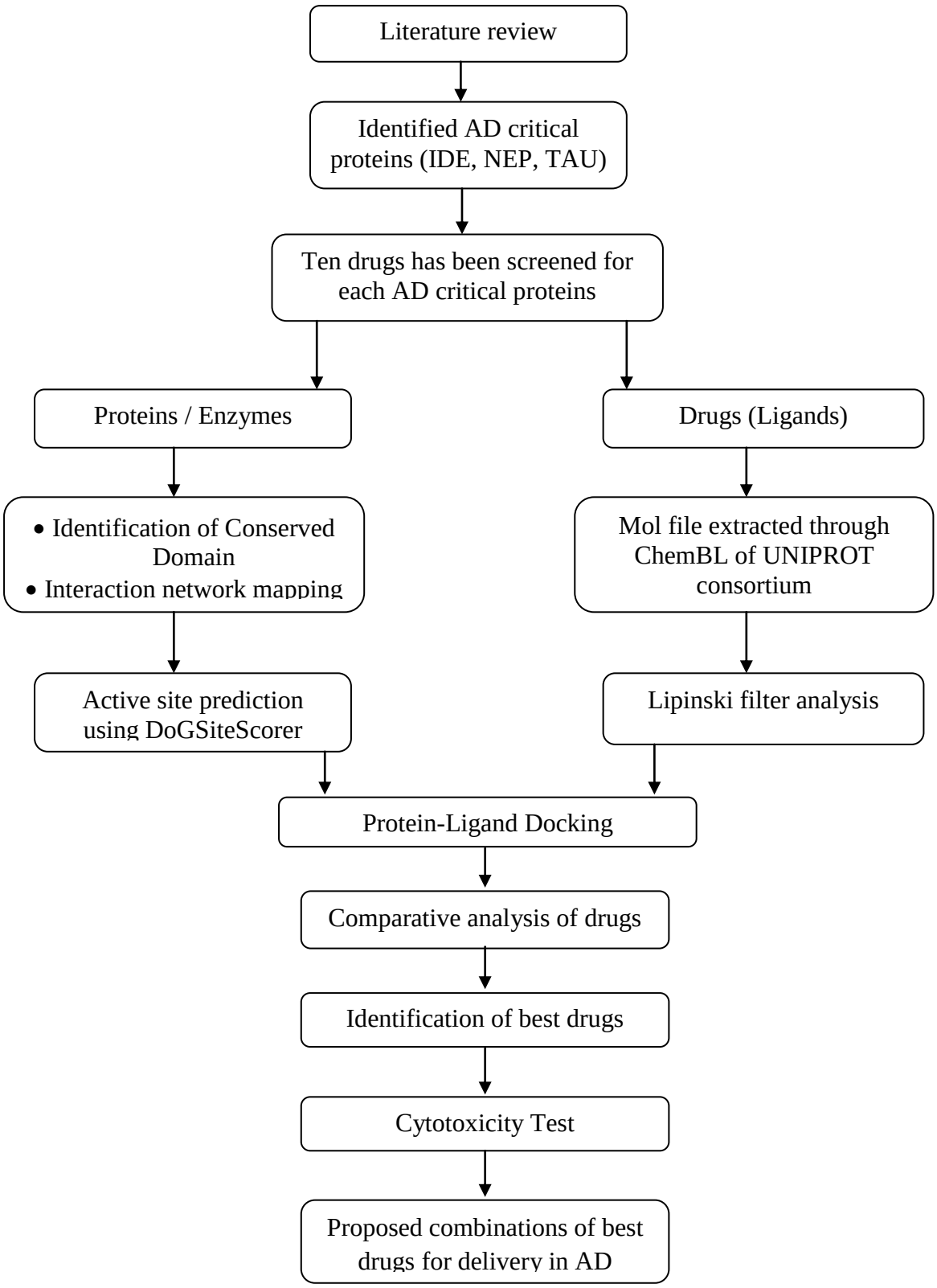
receptors targeted by ACP and BCL are also significant for the role of the endothelial BBB. (Collard *et al.*, 2002; Eynden *et al.*, 2009; Shastry *et al*., 2006)

Table 5: Combinational drugs under trial in clinical applications

S.No	Name of Combination Drug	Pharmacotheography	Results	References
1	Memantine and donepzil	Excitotoxicity	Significant improvement in cognitive function compared with patients treated with donepezil plus placebo, and showed significantly less decline in daily function Patients received donepezil for the immediate preceding throughout the study period and were randomised to receive memantine or placebo.	Tariot et al., 2004
2	Tacrine and estrogen	Neurotrophic factors	Tacrine treatment as the randomisation/nonrandomised	Schneider et al., 1997
3	Rivastigmine and HRT	Excitotoxicity	No significant changes in favour of HRT were found on any efficacy parameters when compared by ITT analysis of HRT vs placebo in menopausal women treated with rivastigmine. Patients were receiving rivastigmine and were randomised to receive HRT or placebo	Rigaud et al., 2003
4	α-Tocopherol and selegiline	Oxidative stress	Combination therapy with α-tocopherol plus selegiline did not show any additional benefit as compared with either treatment alone of them.	Sano et al., 1997
5	Donepezil and tocopherol	Oxidative stress	Average cumulative change found over 1 and 3 years.	Klatte et al., 2003
6	Selegiline and tacrine, physostigmine	Oxidative stress	First period effect for selegiline compared with placebo gave nonsignificant Wilcoxon test small number of patients; change scores were analysed for the comparison of the groups between the first that received selegiline with all groups which first received placebo	Schneider et al., 1993
7	AChE inhibitor and α-lipoic acid	Oxidative stress	Stabilisation of cognitive functions for at least 337 days (α-Lipoic acid was added to patients' existing standard) treatment with AChE inhibitors; small sample size, short	Hager et al., 2001

			duration, lack of placebo control group, unblinded dosage	
8	Donepezil and d-cycloserine	Oxidative stress	No statistically significant effect found with any outcome measures D-cycloserine was added to patients' existing treatment with donepezil; small sample size, short duration, lack of placebo control group, unblended dosage	Falk et al., 2002
9	Pravastatin and tocopherol	Oxidative stress	No significant changes occurred in either quality of life or cognition measures after 6 or 12months of therapy with pravastatin, tocopherol or their combination.	Carlsson et al., 2002

4. MATERIALS AND METHODS



Steps1: A large pool of data have been read and investigated for the appropriate information needed to include in the research findings of this project through the NCBI archives as well as other reputed journals.

Step2: In the research, it was found that three major proteins are involved in epigenetic modification known as Insulin Degrading Enzyme (IDE), Neprilysin (NEP), Tau protein which can play a key role in treating AD by alteration in their synergistic and control mechanisms.

Step3: In the literature survey, it was also found that there are many bio-molecules and drug compounds which act on these domains and maintain the action of IDE, NEP, and Tau by playing as neuro-protective and lowering oxidative stress simultaneously thereby delaying the neuronal degeneration on the path of dementia leading to AD.

Step4: After that, ten drugs/bio-flavanoids for each essential category has been selected through the journals on which undergoing trials are being performed towards finding a better cure in elimination of AD.

Step5: From here, there goes two ways for the protein taken as receptor molecules and drugs chosen as Ligands and their further study.

Protein analysis

The protein molecules are selected and there detailed analysis have been done through NCBI and protein databank PDB portals exploring their underlying mechanisms and functionality, their 3-dimesional structures, their agonists and antagonists, molecular profiling. The FASTA sequences of each protein moiety of selective Homo sapiens model have been found and restored.

a) Conserved domain analysis

Conserved domains of these proteins have identified though CDD database of NCBI server. CDD database is a central repository for protein domains. Careful observation of conserved domains on these profiles has been studied according to their FASTA sequences as to find common sub-domain to enhance catalytic activities of the Ligands (Bauer *et al*., 2011).

b) Interaction network builder

Interaction network of IDE, NEP and Tau has been made using STRING database to see their interactions with APP and amongst themselves (Franceschini *et al*., 2013).

c) Active site prediction using DoGsitescorer

Active sites of these proteins have been extracted through DoGSiteScorer website. DogSiteScorer is automated pocket discovery and scrutiny tool which can be used for protein druggability assessment. Predictions with DoGSiteScorer are based on calculation of size,

shape and various other chemical features of automatically predicted pockets, integrated into a support vector machine for druggability evaluation (Volkamer *et al.*, 2012).

Ligand analysis

a) Structural file extraction

The molecular structures of drugs are searched through the ChemBL database which is a central repository of collection of data of drug compounds and bio-molecules. The .mol file extension is derived for their further procedural investigation. The ChemBL database belongs comes under to the UNIPROT consortium (Gaulton *et al*., 2012).

b) Lipinski filter analysis

The drugs with .mol extension file were converted to .pdb extension using software Open Babel (O'Boyle NM et al., 2011).The drugs were passed through a filter called as Lipinski to check there druggability (Lipinski 2004).

Lipinski filter for validation of selected molecules to act as drugs under certain criteria of Lipinski Rule of Five: The Lipinski rule of 5 is made to help characterize between drug like and non drug like molecules. It predicts the likelihood of success or failure due to drug likeness for molecules following with 2 or more of these rules-

1. Molecules whose Molecular mass are lesser than 500 Dalton.

2. Their lipophilicity should be high (expressed as LogP less than 5).

3. They should have lesser than 5 hydrogen bond donors.

4. They should contain less than 10 hydrogen bond acceptors.

5. Molar refractivity should be in between 40- 130.

Applying these filters in drug analysis aids in early preclinical development and may perhaps help let alone costly late-stage preclinical and clinical failures.

Docking analysis

The protein ligand docking has been performed using HEX software application. (Macindoe *et al.*, 2010). Hex is a software application which allows interactive molecular graphics program to calculate and display practicable docking mode of protein and DNA molecules pairs. Hex also helps in evaluation of protein-ligand docking, assuming the rigidness of ligand, and it would also superimpose molecular pairs using only knowledge of their 3D shapes. In Hex's docking assessments, each molecule is modelled by means of 3D expansions of orthogonal real polar spherical basis algorithms to encode together potential distributions, electrostatic charge and surface shape. Fundamentally, this eases the use of

making every property represented as vector of coefficients (which are parts of the fundamental functions).

In Hex the surface shapes of proteins are shown using a two-term surface skin plus vander Walls steric density model, while the electrostatic model is imitated from classical electrostatic theory. By in scripting expressions for the overlie of pairs of parametric functions, we can obtain an on the whole docking score as a function of the six degrees of freedom in a rigid body docking investigation. The interaction energy should be minimised which the docking score is obtained by suitable scaling. To control Docking calculations, docking control panel is being used. Normally, the docking exploration proceeds by rotation of the receptor and ligand about their centroids at a piece of a range of intermolecular distances. Each of the receptor and ligand are assigned two Euler rotation angles, and the final rotation is obtained by twist of the ligand with reference to the intermolecular axis. The default manner is then to carry out a complete six-dimensional rummage around the full rotational ranges.

Cyto-toxicity test

The Ligands have been tested for their toxic levels by performing the cyto-toxicity tests of selected drugs through TEST software by EPA i.e. Environment Protection Agency of United States. This automated tool evaluates the toxicity and physical properties of compounds on basis of their molecular structures. To discover potential risks near the beginning in the design stage, this software is used to rapidly assess the hazard of chemicals, focusing on the locale of chemical manufacturing design. It combines hierarchical clustering with a genetic algorithm approach to obtain the predictive models.

Based on these analysis and tests, best drugs have been proposed which are currently under trials and further, suitable combinations of drugs have been suggested for more efficient treatment mechanisms for Alzheimer’s disease.

5. RESULTS

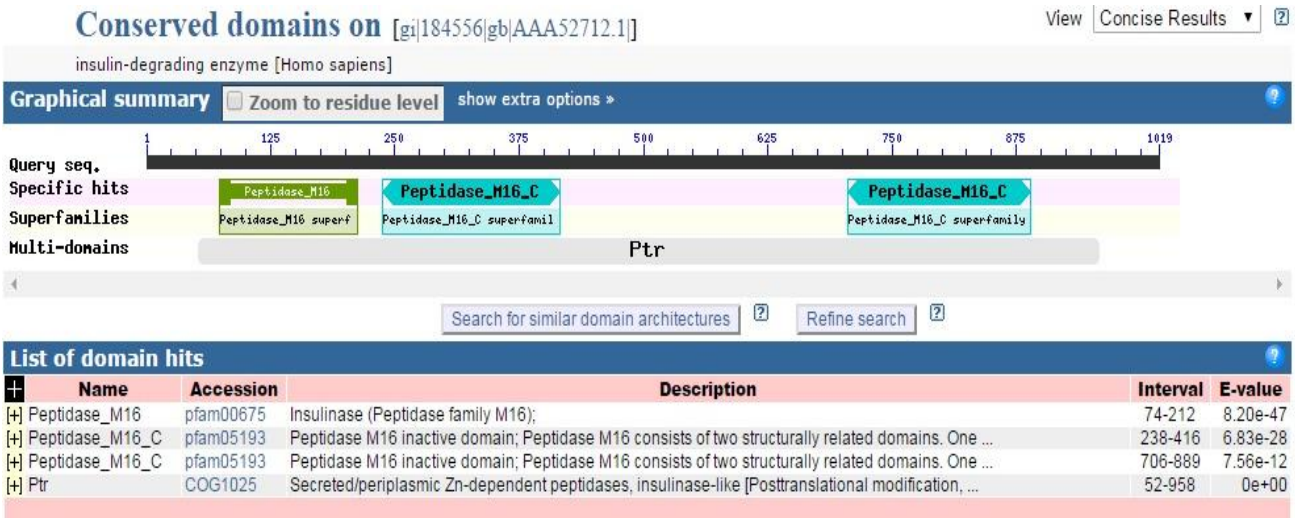
5.1 Ten drugs identified for each class of critical protein of AD obtained through literature survey has been summarised in **Table 6**.

Table 6: Drugs under trial written against their receptor molecule

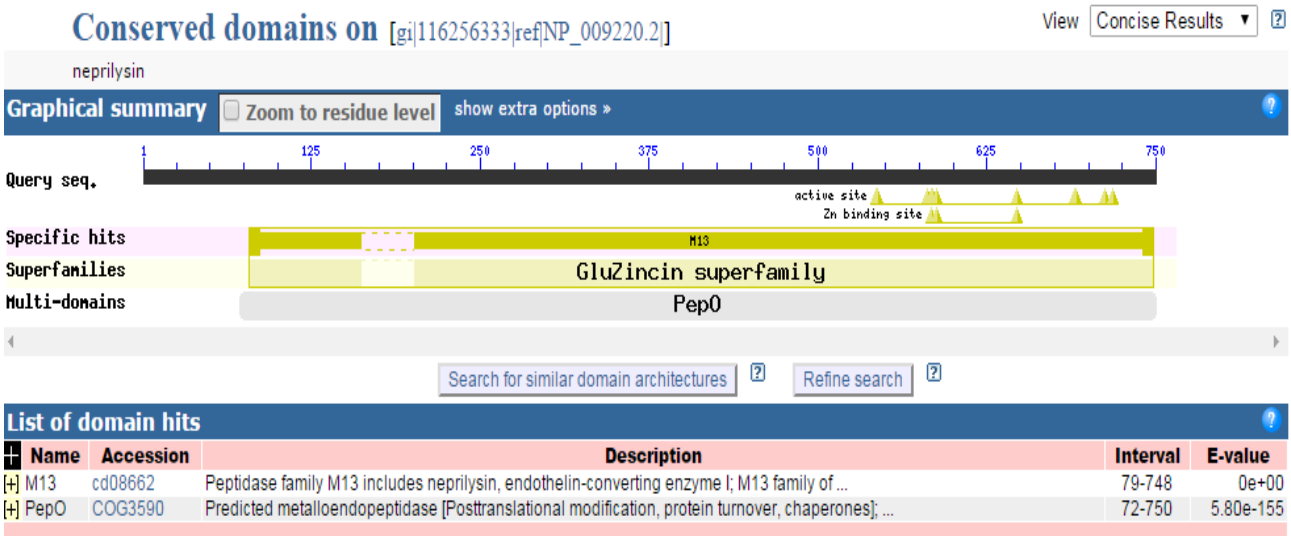
S.No.	IDE	NEP	Tau
1	Aniracetam	Abilify	Cinnamon
2	Bacoside a	Apomorphine	Epothilone D
3	Berberine	Azadiracta	Edaravone
4	Curcumin	Huperzine a	Indolethylbenzamides
5	Daunorubicin	Methylphenidate	Methylene blue
6	Nicotine	Modafinil	L-3-n-butylphthalide (L-NBP)
7	Pentostatin	Tianeptine	Propranolol
8	Plicamycin	Vinpocetine	Paclitaxel
9	Reserpine	Vitamin E	Resveratrol
10	Vitamin E	Xanthine-caffiene	Valproate

5.2 Conserved domains

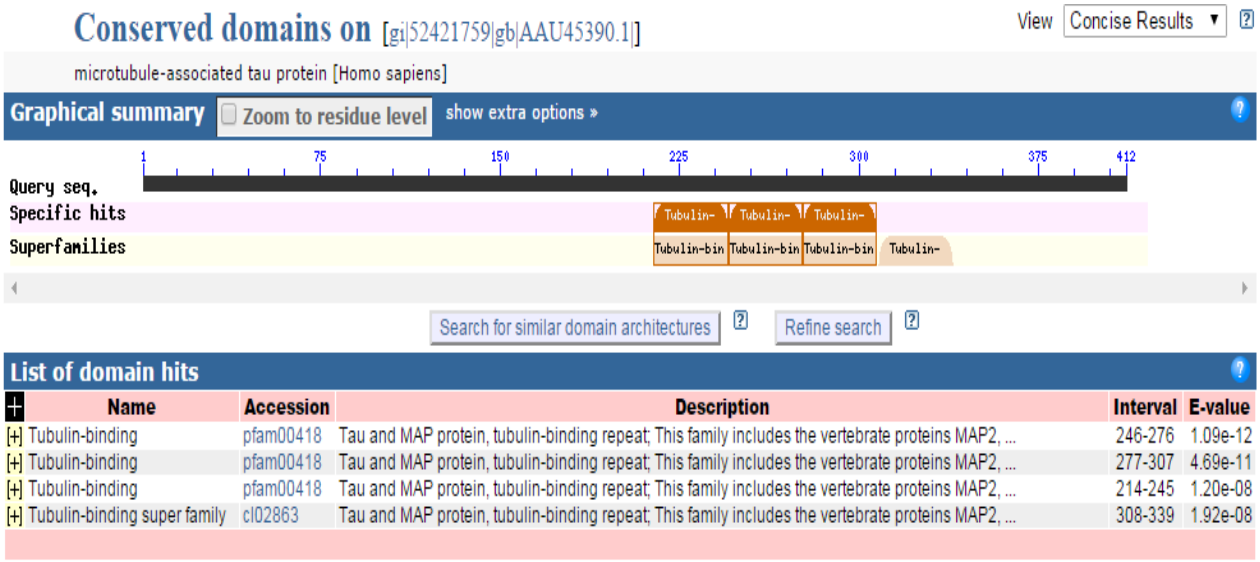
5.2.1 Insulin degrading enzyme



5.2.2 Neprilysin

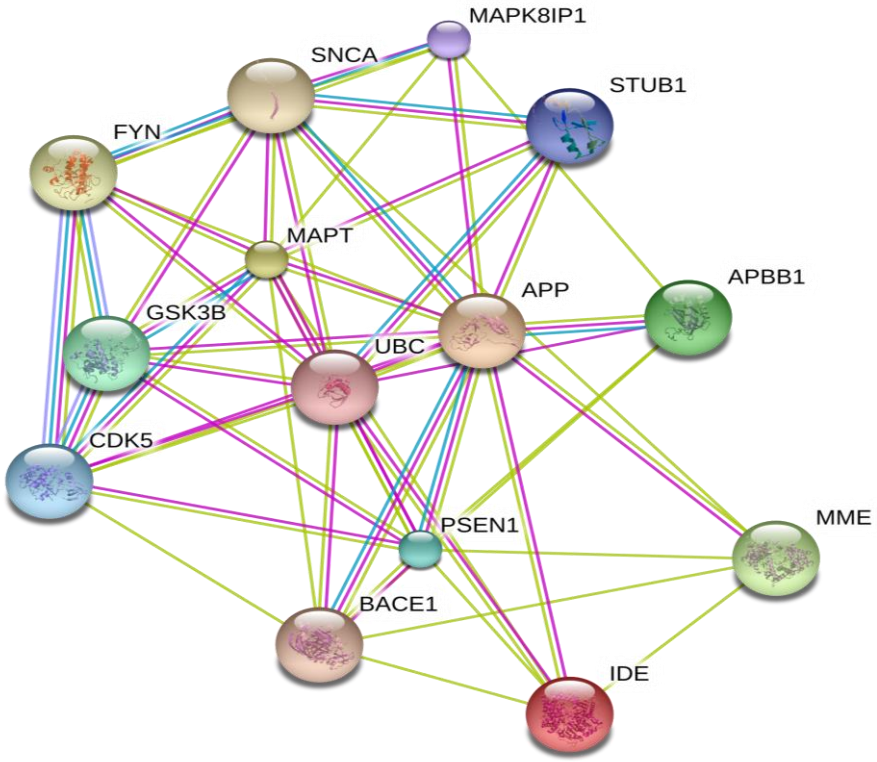


5.2.3 Tau



On the basis of obtained results through conserved domain server, it was found that the family of IDE and NEP is same i.e. they belong to peptidase family but different class of it which is M16 and M13 respectively. The Tau belongs to tubulin binding family having tubulin-binding super family as well.

5.3 Interactions network of IDE, NEP, and TAU proteins



IDE	insulin-degrading enzyme; Plays a role in the cellular breakdown of insulin, IAPP, glucagon, bradykinin, kallidin and other peptides, and thereby plays a role in intercellular peptide signaling. Degrades amyloid formed by APP and IAPP. May play a role in the degradation and clearance of naturally secreted amyloid beta-protein by neurons and microglia (1019 aa)
APP	amyloid beta (A4) precursor protein (770 aa)
MAPT	microtubule-associated protein tau (776 aa)
MME	membrane metallo-endopeptidase; Thermolysin-like specificity, but is almost confined on acting on polypeptides of up to 30 amino acids. Biologically important in the destruction of opioid peptides such as Met- and Leu-enkephalins by cleavage of a Gly-Phe bond. Able to cleave angiotensin-1, angiotensin-2 and angiotensin 1-9. Involved in the degradation of atrial natriuretic factor (ANF). Displays UV- inducible elastase activity toward skin preelastic and elastic fibers (750 aa)

(Homo sapiens)

Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
APBB1	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65); Transcription coregulat [...] (708 aa)					•	•	•		0.999
GSK3B	glycogen synthase kinase 3 beta; Constitutively active protein kinase that acts as a negative r [...] (433 aa)					•	•	•		0.999
PSEN1	presenilin 1; Probable catalytic subunit of the gamma-secretase complex, an endoprotease comple [...] (467 aa)					•	•	•		0.999
CDK5	cyclin-dependent kinase 5; Proline-directed serine/threonine-protein kinase essential for neuro [...] (292 aa)					•	•	•		0.999
STUB1	STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase; E3 ubiquitin-protei [...] (303 aa)					•				0.999
MAPK8IP1	mitogen-activated protein kinase 8 interacting protein 1; The JNK-interacting protein (JIP) gro [...] (711 aa)					•		•		0.998
UBC	ubiquitin C (685 aa)					•				0.999
BACE1	beta-site APP-cleaving enzyme 1; Responsible for the proteolytic processing of the amyloid prec [...] (501 aa)					•	•	•		0.998
SNCA	synuclein, alpha (non A4 component of amyloid precursor); May be involved in the regulation of [...] (140 aa)					•	•	•		0.998
FYN	FYN oncogene related to SRC, FGR, YES; Non-receptor tyrosine-protein kinase that plays a role i [...] (537 aa)					•		•		0.997

According to the network map obtained, it has been found that IDE has interactions with APP, NEP, ubiquitin C, presenilin-1, and BACE1 (beta site APP cleaving enzyme 1). NEP has interactions with IDE, BACE1, presenilin-1, APP, and synuclein. Tau (MAPT) has interactions with synuclein, APP, BACE1, STUB1, CDK5 (cyclin dependent kinase 5), ubiquitin C, FYN oncogene, GSK3B (glycogen synthase kinase 3 beta), MAPK8IP1 (mitogen activated protein kinase 8 interacting protein 1).

5.4 Active site using DOGSITESCORER

Insulin Degrading Enzyme (IDE)

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	2610.02	2565.54	1761.37	38.02	0.81
P1	1104.85	1100.04	647.24	24.35	0.81
P2	934.93	799.21	442.69	22.63	0.82
P3	869.77	942.09	592.69	24.26	0.84
P4	814.69	977.62	650.54	14.75	0.75
P5	766.09	699.15	435.88	29.78	0.87
P6	668.53	805.49	539.13	17.45	0.81
P7	657.73	1015.90	579.68	17.15	0.79
P8	650.89	940.02	631.92	22.74	0.84
P9	613.08	645.61	384.65	21.74	0.84
P10	473.40	772.02	411.43	16.28	0.71
P11	455.40	591.83	301.88	18.44	0.76
P12	421.92	614.34	424.45	21.04	0.82
P13	421.20	636.95	301.58	13.08	0.61
P14	415.08	620.28	381.71	15.60	0.67
P15	408.24	620.00	273.62	18.25	0.74
P16	364.68	500.10	248.44	9.78	0.47
P17	364.32	460.68	220.72	9.28	0.43
P18	349.20	495.20	317.80	15.22	0.66
P19	343.08	571.87	327.50	14.73	0.63
P20	326.16	479.69	352.61	21.41	0.80
P21	325.80	521.69	343.01	20.87	0.79
P22	324.36	383.60	179.92	13.53	0.54
P23	318.60	488.21	254.69	16.67	0.65
P24	312.84	500.04	303.49	13.76	0.56
P25	292.32	458.83	265.81	13.74	0.58

Descriptor information for pocket P5 of target XPRO

Size and shape descriptors

Descriptor	Value
volume [Å³]	766.09
surface [Å²]	699.15
lipophilic surface [Å²]	435.88
depth [Å]	29.78
ellipsoid main axis ratio c/a	0.09
ellipsoid main axis ratio b/a	0.15
enclosure	0.20

Element descriptors

Descriptor	Value
# pocket atoms	181
# carbons (C)	130
# nitrogens (N)	23
# oxygens	27
# sulfors (S)	1
# other elements	0

Amino acid descriptors

Descriptor	Value
# ALA	1
# ARG	3
# ASN	3
# ASP	0
# CYS	1
# GLN	1
# GLU	4
# GLY	2
# HIS	0
# ILE	1
# LEU	3
# LYS	1
# MET	0
# PHE	6
# PRO	1
# SER	6
# THR	2
# TRP	0
# TYR	3
# VAL	1
# special amino acids	0

Functional group descriptors

Descriptor	Value
# hydrogen bond donors	18
# hydrogen bond acceptors	54
# metals	0
# hydrophobic interactions	36
hydrophobicity ratio	0.33

Amino acid composition

Descriptor	Value
apolar amino acid ratio	0.33
polar amino acid ratio	0.46
positive amino acid ratio	0.10
negative amino acid ratio	0.10

Pocket 5 of IDE has been found as most suitable active pocket having 6 serine and phenylalanine each, 4 glutamic acid and 3 tyrosine, arginine, asparagines, leucine each residues along with few others as well. The other suitable parameters of this active site are given in the image above.

Neprilysin

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	1322.30	1427.88	824.39	24.03	0.80
P1	526.73	817.80	524.37	18.30	0.77
P2	341.11	517.66	259.88	17.04	0.72
P3	303.29	483.38	251.18	12.75	0.53
P4	296.74	509.36	237.66	18.48	0.72
P5	269.78	301.77	155.17	12.70	0.52
P6	255.17	396.67	235.36	11.40	0.50
P7	249.74	472.51	296.97	10.30	0.38
P8	198.54	288.32	168.83	10.01	0.37
P9	186.19	258.45	137.00	11.58	0.35
P10	175.70	316.25	190.62	9.78	0.35
P11	173.45	318.76	119.29	7.53	0.18
P12	173.08	305.36	209.73	9.13	0.28
P13	146.21	319.09	169.12	10.16	0.30
P14	141.16	209.08	130.88	8.27	0.27
P15	136.85	235.34	147.96	9.27	0.29
P16	134.61	230.44	121.92	6.13	0.14
P17	129.93	177.47	129.85	8.97	0.28
P18	118.69	253.86	136.96	7.19	0.19
P19	117.85	106.20	53.43	9.79	0.31
P20	108.58	179.42	142.88	6.93	0.14

Descriptor information for pocket P0 of target XPRO

Size and shape descriptors

Descriptor	Value
volume [Å³]	1322.30
surface [Å²]	1427.88
lipophilic surface [Å²]	824.39
depth [Å]	24.03
ellipsoid main axis ratio c/a	0.08
ellipsoid main axis ratio b/a	0.44
enclosure	0.13

Element descriptors

Descriptor	Value
# pocket atoms	300
# carbons (C)	202
# nitrogens (N)	48
# oxygens	48
# sulfors (S)	1
# other elements	1

Amino acid descriptors

Descriptor	Value
# ALA	1
# ARG	3
# ASN	8
# ASP	6
# CYS	1
# GLN	0
# GLU	3
# GLY	7
# HIS	3
# ILE	3
# LEU	4
# LYS	1
# MET	1
# PHE	5
# PRO	2
# SER	4
# THR	3
# TRP	2
# TYR	3
# VAL	3
# special amino acids	1

Functional group descriptors

Descriptor	Value
# hydrogen bond donors	37
# hydrogen bond acceptors	91
# metals	1
# hydrophobic interactions	74
hydrophobicity ratio	0.36

Amino acid composition

Descriptor	Value
apolar amino acid ratio	0.33
polar amino acid ratio	0.41
positive amino acid ratio	0.11
negative amino acid ratio	0.14

The pocket P0 obtained in most suitable active site predicted for Neprilysin which has maximum of 8 residues of asparagine, 7 glycine, 6 aspartic acid, 5 phenylalanine are present i.e. important for activity. Further information regarding pocket P0 has been described in the image above.

TAU

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	206.46	591.01	342.62	8.77	0.30
P1	196.93	629.70	455.98	16.27	0.60

Descriptor information for pocket P1 of target XPRO

Size and shape descriptors

Descriptor	Value
volume [Å³]	196.93
surface [Å²]	629.70
lipophilic surface [Å²]	455.98
depth [Å]	16.27
ellipsoid main axis ratio c/a	0.10
ellipsoid main axis ratio b/a	0.12
enclosure	0.16

Element descriptors

Descriptor	Value
# pocket atoms	57
# carbons (C)	39
# nitrogens (N)	8
# oxygens	10
# sulfors (S)	0
# other elements	0

Amino acid descriptors

Descriptor	Value
# ALA	0
# ARG	0
# ASN	0
# ASP	1
# CYS	1
# GLN	1
# GLU	0
# GLY	1
# HIS	0
# ILE	1
# LEU	2
# LYS	2
# MET	0
# PHE	0
# PRO	0
# SER	2
# THR	0
# TRP	0
# TYR	0
# VAL	1
# special amino acids	0

Functional group descriptors

Descriptor	Value
# hydrogen bond donors	10
# hydrogen bond acceptors	20
# metals	0
# hydrophobic interactions	35
hydrophobicity ratio	0.54

Amino acid composition

Descriptor	Value
apolar amino acid ratio	0.33
polar amino acid ratio	0.42
positive amino acid ratio	0.17
negative amino acid ratio	0.08

The P1 pocket is most active site found in Tau protein having various amino acid residues as shown in image above, also describing various other elementary descriptions about that pocket.

5.5 Docking results

Insulin Degrading Enzyme

Table 7: Drugs against Insulin Degrading Enzyme and their Enthalpy values

Test Drugs

S.No.	Name of drug	Lipinski filter	E-value / Docking result	References
1	Aniracetam	passed	-181.27	Lee CR *et al*., 1994
2	Bacoside a	failed	NA	Sebastian A *et al*., 2013
3	Berberine	passed	-241.13	Durairajan SS *et al*., 2012
4	Curcumin	passed	-234.56	Lim GP *et al.*, 2001
5	Daunorubicin	failed	NA	Howlett DR *et al*., 1999
6	Nicotine	passed	-161.51	Dwayne B *et al*., 2013
7	Pentostatin	passed	-119.54	Von L *et al*., 2012
8	Plicamycin	failed	NA	Michelle AC *et al*., 2004
9	Reserpine	failed	NA	Saharia K *et al.*, 2012
10	Vitamin E	passed	-261.92	Li FJ et al., 2012

Conventional Drugs

1	Aricept	passed	-233.01	Tinklenberg JR *et al*., 2015
2	Memantine	passed	-183.90	Gao L *et al*., 2015
3	Rivastigmine	passed	-186.09	Maria LO *et al*., 2007
4	Tacrine	passed	-160.62	El-Malah A *et al*., 2014

The lowest energy values have obtained from docking of Vitamin E, Berberine against Insulin degrading enzyme.

Neprilysin

Table 8: Drugs against Neprilysin and their Enthalpy values

Test Drugs

S.No.	Name of drug	Lipinski filter	E-value/ Docking result	References
1	Abilify	passed	-265.18	Izchak K *et al*., 2010
2	Apomorphine	passed	-178.63	John WS *et al*., 2011
3	Azadiracta	failed	NA	Raghavendra M *et al*., 2013
4	Huperzine a	passed	-173.62	Huang XT *et al*., 2014
5	Methylphenidate	passed	-245.75	Paul BR *et al.*, 2013
6	Modafinil	passed	-196.16	Minzenberg MJ *et al*., 2008
7	Tianeptine	passed	-223.90	McEwen BS *et al.*, 2010
8	Vinpocetine	passed	-230.74	Alexandre EM 2010
9	Vitamin E	passed	-258.55	Li FJ et al., 2012
10	Xanthine-caffiene	passed	-140.22	Cimini A *et al*., 2013

Conventional Drugs

1	Aricept	passed	-244.20	Tinklenberg JR *et al*., 2015
2	Memantine	passed	-211.99	Gao L *et al*., 2015
3	Rivastigmine	passed	-182.67	Maria LO *et al*., 2007
4	Tacrine	passed	-160.37	El-Malah A *et al*., 2014

The lowest energy values have been obtained from Abilify and Vitamin E against docking with Neprilysin. These values are greater than conventional drugs present in the market.

TAU

Table 9: Drugs against Tau and their Enthalpy values

Test Drugs

S.No.	Name of drug	Lipinski filter	E-value/ Docking result	References
1	Cinnamon	failed	NA	Peterson DW *et al*., 2009
2	Edaravone	passed	-143.60	Shu-Sheng J *et al*., 2015
3	Epothilone D	passed	-237.57	Brunden, KR et al., 2010
4	Indolethylbenzamides	passed	-229.34	Gerard Rosse 2012
5	L-3-n-butylphthalide (L-NBP)	passed	-148.00	Peng Y *et al*., 2012
6	Methylene blue	passed	-184.62	Hochgräfe K *et al*., 2015
7	Paclitaxel	failed	NA	Erez H et al., 2014
8	Propranolol	passed	-171.03	Dobarro *et al*., 2013
9	Resveratrol	passed	-164.75	Julien, C *et al*., 2009
10	Valproate	passed	-142.57	Hu JP *et al*., 2011

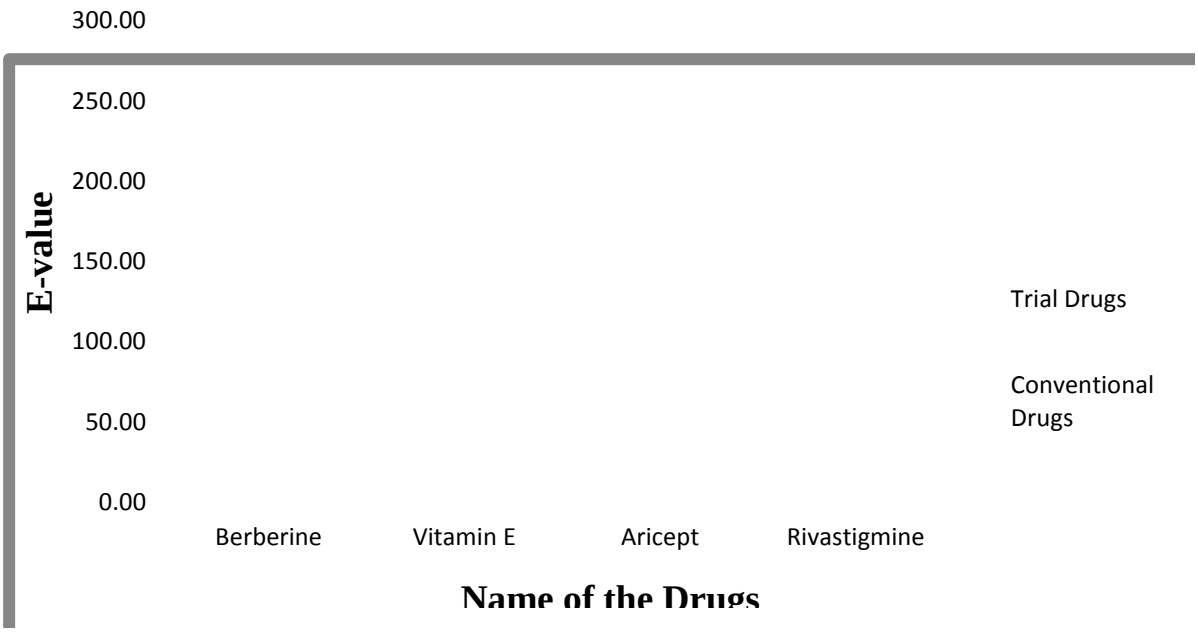
Conventional Drugs

1	Aricept	passed	-216.08	Tinklenberg JR *et al*., 2015
2	Memantine	passed	-163.29	Gao L *et al*., 2015
3	Rivastigmine	passed	-187.44	Maria LO *et al*., 2007
4	Tacrine	passed	-147.57	El-Malah A *et al*., 2014

The minimum energy values are obtained from Epothilone D and Indolethylbenzamides against Tau protein docking. These values obtained are greater than conventional drugs present already in the market as prescribed Alzheimer's disease treatment.

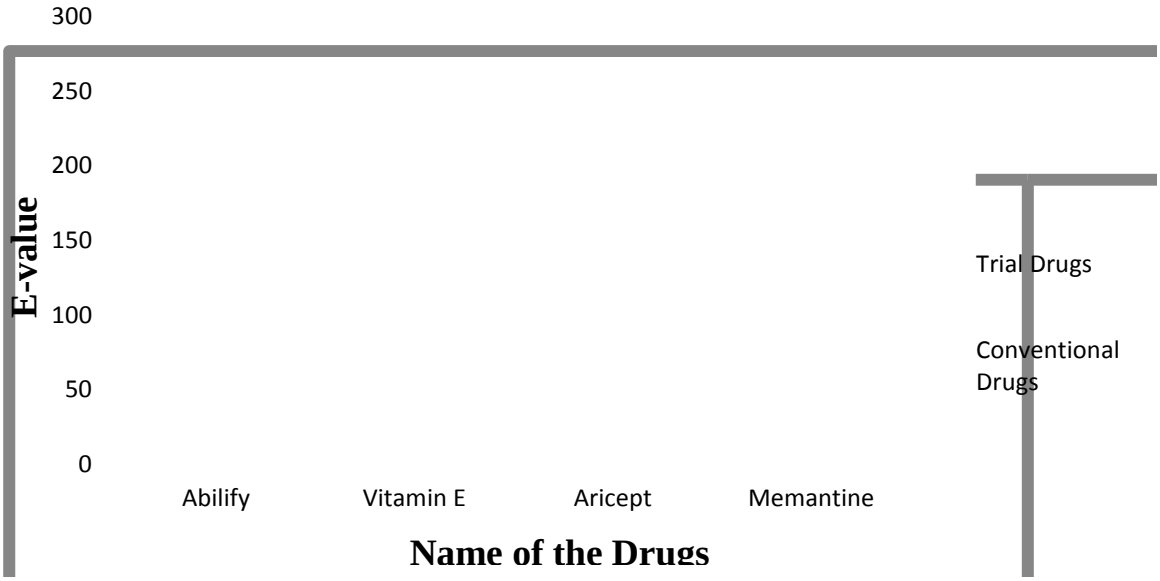
5.6 Comparative graphs between proposed and conventional drugs

Insulin Degrading Enzyme



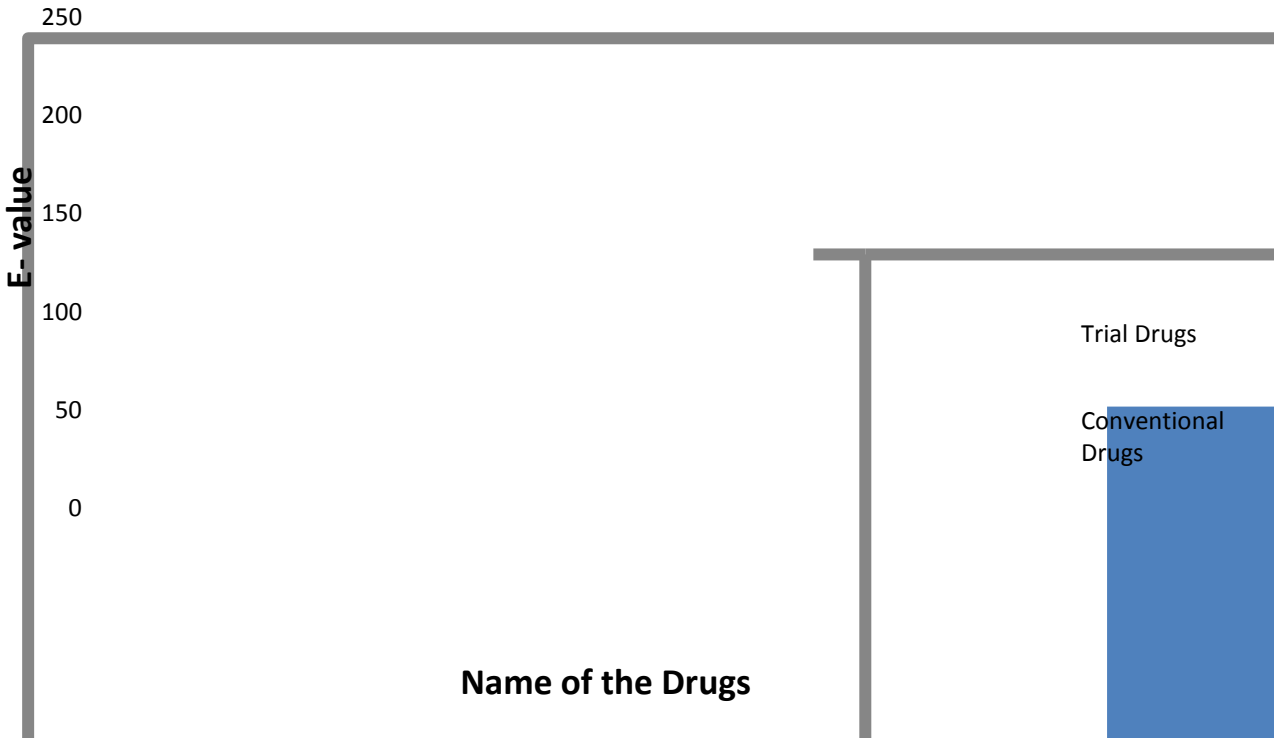
Graph is drawn on the basis of E values as obtained by docking studies done between drugs and IDE. E-values of these drugs are – Aricept -233.01 kJ/mol; Vitamin E -261.92 kJ/mol; Rivastigmine -186.09 kJ/mol; Berberine -241.13 kJ/mol.

NEP



Graph is drawn on the basis of E values as obtained by docking studies done between drugs and NEP. E-value of these drugs is – Aricept -244.20 kJ/mol; Abilify -265.18 kJ/mol; Memantine -211.99 kJ/mol; Vitamin E -258.55 kJ/mol.

Tau



Graph is drawn on the basis of E values as obtained by docking studies done between drugs and Tau. E-value of these drugs is – Epothilone D -237.57 kJ/mol; Indolethylbenzamides -229.34 kJ/mol; Aricept -216.08 kJ/mol; Rivastigmine -187.44 kJ/mol.

5.7 Cyto-toxicity Tests of Drugs Using TEST

Table 10: Various drugs and their TEST scores

S.No.	Name of the drug under trials	Toxicity value (Predicted and FDA) respectively using TEST software by EPA	
1	Abilify	0.70	0.34
2	Berberine	0.89	0.98
3	Epothilone D	0.65	0.63
4	Indolethylbenzamides	0.88	0.80
5	Vitamin E	0.88	0.95
Conventional Drugs			
1	Aricept	0.79	0.97
2	Memantine	0.82	1.05

3	Rivastigmine	1.15	1.74

The least toxic drugs found are Abilify and Epothilone D which have toxicity levels even lower than conventional drugs present already in the market. These values are obtained from TEST software available by EPA i.e. Environment Protection Agency of United States.

5.8 Proposed possible combinations of best obtained results

Table 11: Drugs and their combinations made on various filters and docking values

S.No	Drug name	Molecule Name	E value	Possible Combinations 1		Possible combinations 2	
1	Vitamin E	IDE	-261.92	Vit. e	Epothilone D	Vit. e	Indolethylbenzamides
2	Berberine	IDE	-241.13	berberine	Epithilone D	berberine	Indolethylbenzamides
3	Abilify	NEP	-265.18	Abilify	Epithilone D	Abilify	Indolethylbenzamides
4	Vitamin E	NEP	-258.55	NA	NA	NA	NA
5	Epothilone D	TAU	-237.57	NA	NA	NA	NA
6	Indolethylbenzamides	TAU	-229.34	NA	NA	NA	NA

The best combination obtained from least toxic and most stable energy conformations are jotted down in this table as possible predicted combinations.

6. DISCUSSIONS

AD is a multifactorial disease. Therefore, it is indispensable to target multiple factors collectively in order to lessen the sternness of the disease to a larger degree. It is probably expected that any combination therapy will consist of two classes of agents of drugs or bio-molecules in order to relate their efficacy and/or tolerability of the combinatorial to the corresponding monotherapy in designing of a treatment regimen. Few things can be kept in mind while designing a combination for treatment of dementia related disorders are 1) Pharmacological targets- where the combination regimen should act upon most distinctive pathophysiological cascades, 2) Timings of the intervention i.e. at particular stage of AD is very important and 3) The symptoms we need to target which include Cognitive symptoms and Behavioural symptoms. Herein we targeted IDE, NEP and Tau proteins with the biomolecules whose modulation can bring changes in AD metabolism. Various drugs have been identified and studied which can alter the functioning of these protein moieties. Based on the Lipinski filter analysis, 6 out of 10 for IDE, 9 out of 10 for NEP, and 8 out of 10 for Tau has passed and chosen further to pass through other parameters to see their efficacy in accordance with these proteins. After passing through these filters, these drugs as Ligands are docked against their respective receptor molecules to confirm their energy stabilisations. The resultant E values as obtained by docking studies done between drugs and IDE. E-values of test drugs are Berberine -241.13 kJ/mol and Vitamin E -261.92 kJ/mol vs conventional drugs Aricept -233.01 kJ/mol and Rivastigmine -186.09 kJ/mol. Similarly, the E values obtained by docking studies between drugs and NEP as follows. E-value of test drugs are Abilify -265.18 kJ/mol and Vitamin E -258.55 kJ/mol vs conventional drugs Aricept -244.20 kJ/mol and Memantine -211.99 kJ/mol. Further E values as obtained by docking studies between test drugs and Tau are Epothilone D -237.57 kJ/mol and Indolethylbenzamides -229.34 kJ/mol vs conventional drugs Aricept -216.08 kJ/mol and Rivastigmine -187.44 kJ/mol. The significance of E-value means docking scores expressed as binding enthalpy. This means, the amount of energy that the system consumes to perform such a binding action is calculated. So, we are looking for the most negative number (often kcal/mol or kJ/mol). Most scoring functions are physics-based molecular mechanics force fields that estimate the energy of the pose; a low (negative) energy indicates a stable system and thus a likely binding interaction.

On the basis of obtained results against IDE, it was found that the best scoring drugs are Vitamin E and Berberine while as the Abilify and Vitamin E gave best scores against NEP. Epothilone D and Indolethylbenzamides gave best scores against Tau protein. Further, they were tested against their cyto-toxicity tests to see the level of their harmfulness upon consumption by human bodies. It was found that their toxic levels are lower than the conventional drugs which are present in the market for the treatment of AD. The best scoring results on all the parameters are grouped together to make combinations predicted as packaged drugs for delivery to AD subjects which are Vitamin E - Epothilone D; Vitamin E - Indolethylbenzamides; Berberine - Epothilone D; Berberine - Indolethylbenzamides; Abilify - Epothilone D; Abilify - Indolethylbenzamides. A lot of scope and improvements can be

made in this area via significant trials and testing before any procedural development steps of drug production on any commercial level. A suitable approach towards the cure of Alzheimer's disease can be found through these suggestive methods and practical application in terms of commercialized production of applied therapy/drugs which would bring a breakthrough in treating the world's leading cause of dementia that is Alzheimer's disease. Further elucidation of AD metabolism will be important for identifying new potential therapies to reduce Aβ accumulation and combat the severity associated with Alzheimer's disease.

7. FUTURE PERSPECTIVES

The results can be validated through lab trials and clinical trials of the drugs as one of future perspective options. One can also find the aberrant unknown side effects associated with these drugs that could arise upon combination with other potentiating drug molecules. Moreover, differential expression of the genes can be analysed that would be knocked down or perturbed due to ingestion of these drug combination or changes brought in any signalling pathway leading to faulty mechanisms inside body. Further their association studies could be done to explore the metabolic pathways that might get affected with the implication of these drugs. Furthermore mutational analysis could be done to find the potential threat to the crucial proteins upon drug treatment. Drug processing and development should need to be addressed so as to minimize the drug diffusion in order to deliver it at the target site. Finally, co-linking all the results through various cross-checks and thus proclaiming about finding a new possibility to cure Alzheimer's disease can be made.

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9. APPENDIX