Chapter 1

(Introduction)

1.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurological disorder that can be recognized by symptoms including memory loss and cognitive impairment. In present era, the incidence of AD is a leading cause of death and disability and is expected to rise dramatically over the next ten years. An AD patient gradually develops permanent dementia and loses of all cognitive function, rendering them completely dependent on caregivers for basic daily requirements. Consequently, there may be severe financial, physical, and psychological burden on patients and caregivers [Association 2019]. In 1906, German physician Dr. Alois Alzheimer (1864–1915) developed the first scientific understanding of AD where he found several abnormal clusters and a few tangled bundles in the brain tissue of Augusta Deter, 1850-1906, a patient with an unusual mental illness and unpredictable behavior which are now known as amyloid plaques and tau tangles or neurofibrillary tangles, respectively [Hippius and Neundörfer 2003].

Dementia-like symptoms, which include memory loss and a decline in cognitive ability in those over 60, are the most common indications of AD [DeTure and Dickson 2019]. Based on prevalence, the disease can be divided into two categories: i) familial AD (FAD) appears as the onset of symptoms similar to AD before the age of 60; and ii) sporadic AD (SAD) appears in people over 65 and is often associated with a number of gene mutations, including those involving the amyloid precursor protein (APP) and presenilins (PSEN1 and PSEN2) [Li et al. 2022, Petit et al. 2022]. The latter is regarded to be the most common cause of SAD and accounts for less than 1% of instances of AD [Oumata et al. 2022].

AD progresses slowly and classified in three stages: mild, moderate, and severe where each stage corresponds to a distinct set of symptoms. Typically,. In the case of mild AD which persists for two to four years includes mood swings, linguistic difficulties,

depression, difficulty writing and driving, and lack of interest. Patients with moderate stage of AD could extend for two to ten years and reported to have worsening memory loss, which interferes with their everyday activities. In severe stage of AD, in addition to the symptoms of the earlier stages, it also includes significant side effects include weight loss, severe mood swings, seizures, hallucinations, skin infections, and motor dysfunctions. The patient may experience symptoms of the severe stage for one to three years and ultimately leads to their death [Braak and Braak 1997, Lyketsos et al. 2011, Reisberg et al. 1987].

The World Alzheimer's Report 2021, suggests that around 55 million people are suffering from the dementia and it is expected that this number could rise to 78 million by the year 2030 [Gauthier et al. 2021]. Due to a lack of awareness, an estimated 75% of dementia patients may not have a valid diagnosis, and in low- and middle-income countries, this number may reach to 90%. It is well reported that 60 to 70% of dementia survivors worldwide are anticipated to have AD, with 152 million survivors by the year 2050. There will likely be 14 million dementia patients in India by 2050 with 60–70% AD patients [Patterson 2018]. In India, this number may even be higher as the elderly population is growing rapidly, lack of knowledge, and the fact that the majority of the AD symptoms are similar to those of dementia in older peoples [Nandi et al. 2022].

1.2. AD-molecular mechanisms and major targets involved

Due to the complex etiology involved in the progression of disease, the precise cause and course of AD are yet unknown [Verma et al. 2022]. However, various pathophysiological hallmarks like ACh dysregulation in the synaptic cleft, neuro-inflammation, over-activation of *N*-methyl-D-aspartate receptor (NMDAR), Aβ and Tau aggregation, oxidative stress, CREB signaling modulation, biometal dysbalance, pathogenic contribution, and neuronal vascular malfunction are believed to be some of the key

players in AD progression (Figure 1.1) [Ashrafian et al. 2021, Elwishahy et al. 2021, Huang et al. 2016, Kisler et al. 2017, Li et al. 2017, Muralidar et al. 2020, Saura and Valero 2011, Su et al. 2018, Vecchio et al.].

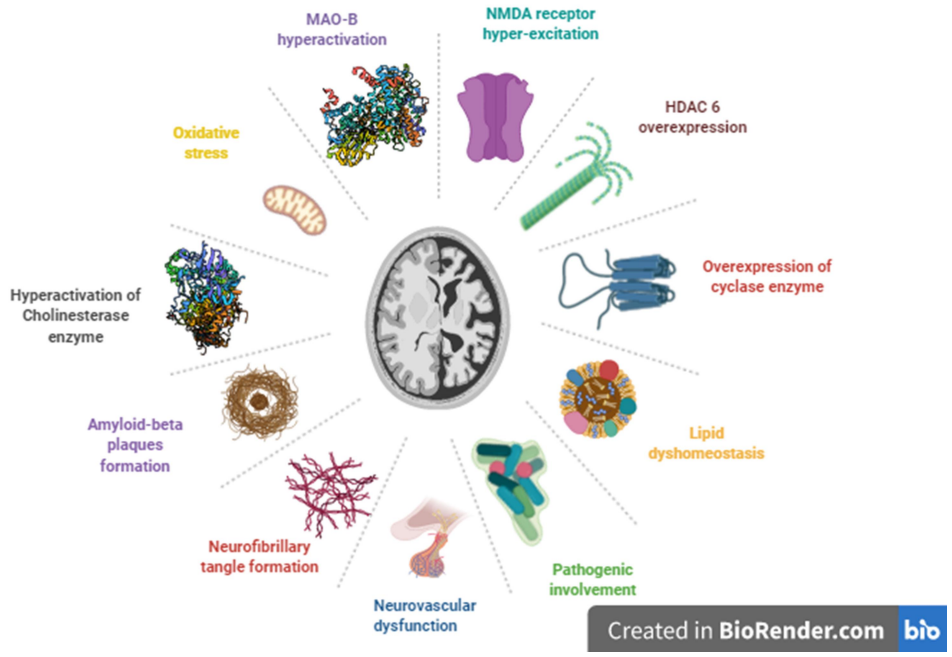

Figure 1.1. AD molecular mechanism and major targets involved [Verma et al. 2022].

1.2.1. Cholinergic hypothesis

The cholinergic hypothesis is one of the important and most acceptable hypotheses to understand the development of AD. In the mid-1970s, the post-mortem brain of AD patients was observed to have decreased level of choline acetyltransferase (CAT). The CAT is responsible for ACh production; ACh is a neurotransmitter that plays an important role in memory, learning, cognitive functions, etc. [Hasselmo 2006, Lombardo and Maskos 2015, Wallace and Bertrand 2013, Wilcock et al. 1982]. CAT was also observed to be declined drastically in neocortical and hippocampal areas, where neurodegeneration occurs during the AD (Figure 1.2). The imbalanced ACh release, choline transport, and muscarinic/nicotinic receptor malfunctioning may produce

behavioral alterations, cognitive impairment in AD patients [Winkler et al. 1995]. Hence, a cholinergic replacement could be an initial approach to overcome AD. Based on cholinergic theory, Cholinesterase (ChE) is also responsible for the hydrolysis of ACh in the basal forebrain's hippocampus region [Terry and Buccafusco 2003]. ChE is a serine hydrolase enzyme that causes hydrolytic cleavage of ACh into acetic acid and choline and classified into two types: (1) Acetylcholinesterase (AChE) (2) Butyrylcholinesterase (BuChE) [Pejchal et al. 2011]. BuChE is a pseudo-neuronal ChE enzyme that provokes catalysis of choline ester hydrolysis [Darvesh et al. 2003].

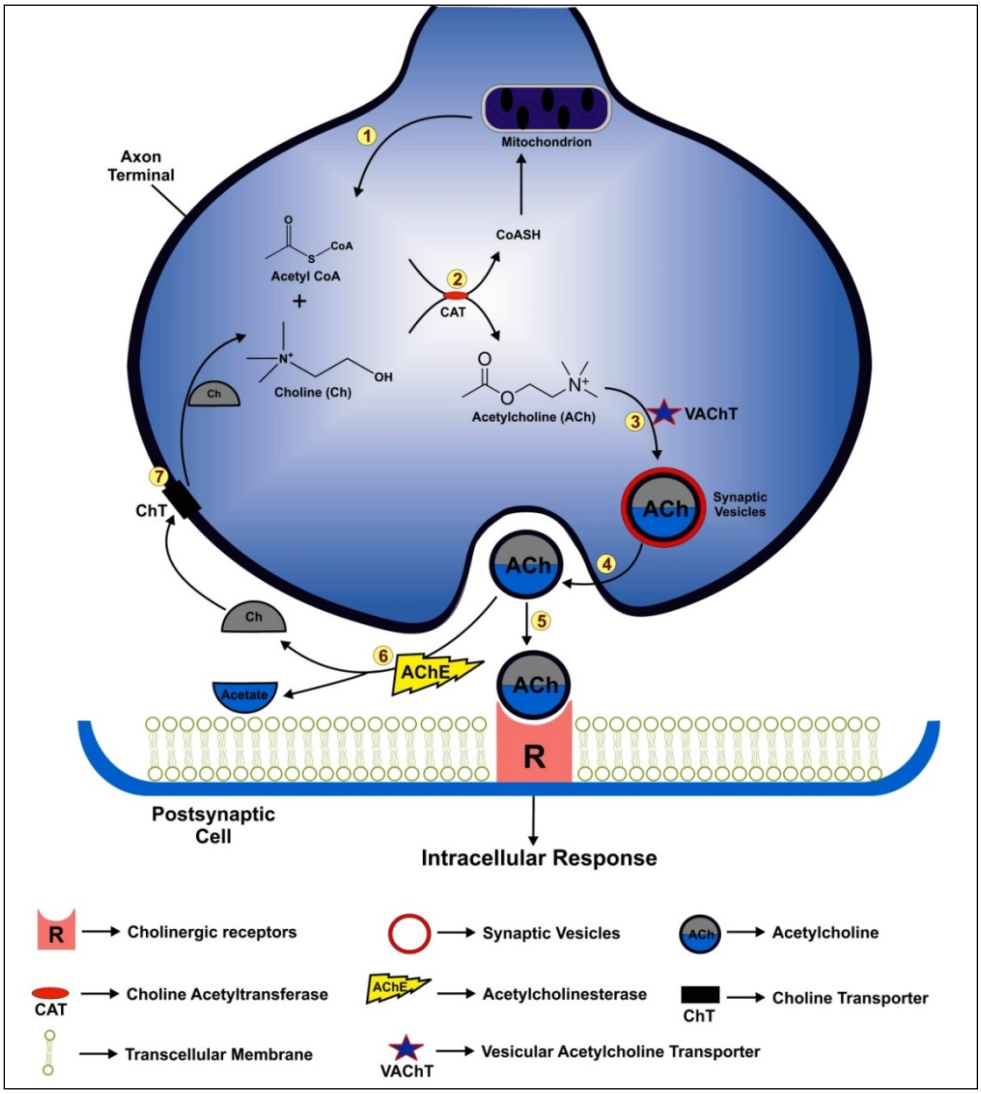

Figure 1.2. Synthesis of ACh and cholinergic neurotransmission [Sharma et al. 2019a].

AChE exists in two molecular forms like (1) Asymmetric form which are primarily located in the neuromuscular junctions (2) Globular forms which are present in the membrane of the hydrophobic domain in tetrameric G4 (preferentially) and monomeric G1. In AD condition, the ratio of G4 and G1 gets altered and leads to the reduced level of G4 form mainly in hippocampal region of the brain. The ChE inhibition is well considered as primary approach to design the inhibitors but have challenges to address the selectivity either in AChE or BuChE, as existing ChE-approved drugs only provide symptomatic relief without arresting the progression of the disease [Rosini et al. 2014]. Several other approaches directly or indirectly enhance the cognitive functions like activation of M1 and M4 muscarinic ACh receptors, nicotinic ACh receptor but they have failed at some level in the drug development process [Foster et al. 2014, Parri et al. 2011]. Therefore, there is an urgent requirement to optimize and develop novel cholinergic approach via implementing multi-targeted and drug repurposing strategies to develop novel candidates for AD management.

1.2.2. Amyloid beta (Aβ) hypothesis

The proteolytic breakdown of amyloid precursor protein (APP) produces small fragments of the protein called Aβ [Wilquet and De Strooper 2004]. APP is considered as a transmembrane protein having its C-terminal and N-terminal passing through the phospholipid bilayer of neuronal cells [Beyreuther et al. 1991]. When APP is activated, it gets fragmented and goes through a series of events that results in the production of oligomers, fibrils, and plaques where it causes abnormal molecular or cellular communication [Haass and Selkoe 2007]. Two secretase enzymes (α-secretase & β-secretase) are primarily accountable for the breakdown of APP into Aβ [Lichtenthaler 2011, Vassar et al. 2009]. α-secretase follows the non-amyloidogenic pathway by producing APPα subunit while β-secretase follows the amyloidogenic pathway by

producing APPβ subunit (Figure 1.3) [Nistor et al. 2007]. Further cleaved by γ-secretase where β subunit produces toxic $A\beta_{42}$ aggregates. Various factors such as increased Aβ aggregation, increased $A\beta_{42}/A\beta_{40}$ ratio, Aβ accumulation, and reduced Aβ clearance contribute to the formation of senile plaques in the brain. These plaques and fibrils are primarily responsible for the execution of the events like inflammatory responses, microglial activation, cytokine release which ultimately leads to the neuronal deficit and hence AD [De Strooper et al. 2010]. The senile plaque formation can be arrested by the application of clinical therapies such as specific secretase inhibitors. Moreover, genetic mutation of Presenilin (PSEN 1 and PSEN 2) is also responsible for elevated Aβ level. Both PSEN 1 and PSEN 2 genes encode for Presenilin 1 and 2 respectively. Presenilin is the catalytic part of the γ-secretase complex that executes the cleavage of proteins derived from APP [De Strooper 2007]. Therefore, to develop potential novel therapeutic drugs based on Aβ therapy requires a lot of patience and collaborations from different fields.

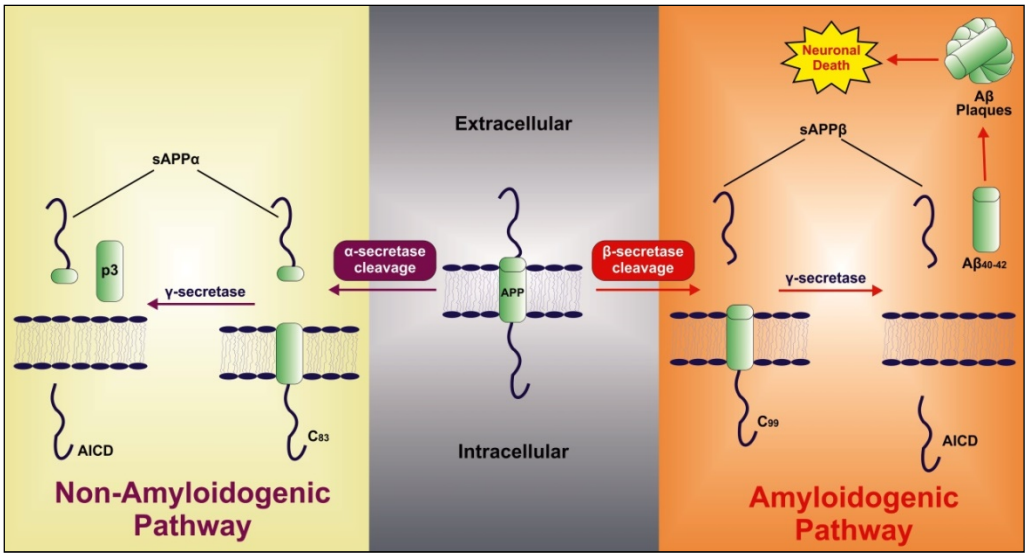

Figure 1.3. Amyloidogenic and non-amyloidogenic pathways [Sharma et al. 2019a].

1.2.3. Tau hypothesis

Tau protein performs an important function in microtubule stability and maintains the cell integrity. It exists in six isoforms with three main binding sites: N-terminal projection, C-

terminal microtubule binding domain, and shorter tailing sequence [Lee et al. 1989]. In a normal situation, it exists in the phosphorylated form in the axonal membrane. Hyper-phosphorylation of tau proteins causes microtubule instability that leads to NFT generation (Figure 1.4). Factors involving hyper-phosphorylation of tau protein in AD include the release of nuclear factor-kB (NF-kB), tumor necrosis factor-alpha (TNF-α), and interleukins (ILs), which ultimately cause neuritic injury in the brain. Inflammatory mediator overproduction also activates the microtubule-associated protein (MAP) kinase, which then activates the cyclin-dependent kinase-5 (CDK-5) [Alawdi et al. 2017, Churcher 2006, Ghosh et al. 2013, Maccioni et al. 2010, Xia and Hyman 2002]. Hyper-activation of various kinases like tau protein kinase-1 (TPK-1), microtubule-affinity regulating kinase (MARK), mitogen-activated protein kinase (MAPK), glycogen synthase kinase-3-beta (GSK-3β), AMP-activated protein kinase (AMPK), and dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) may create a series of events which results into excessive phosphorylation of tau [Ferrer et al. 2005].

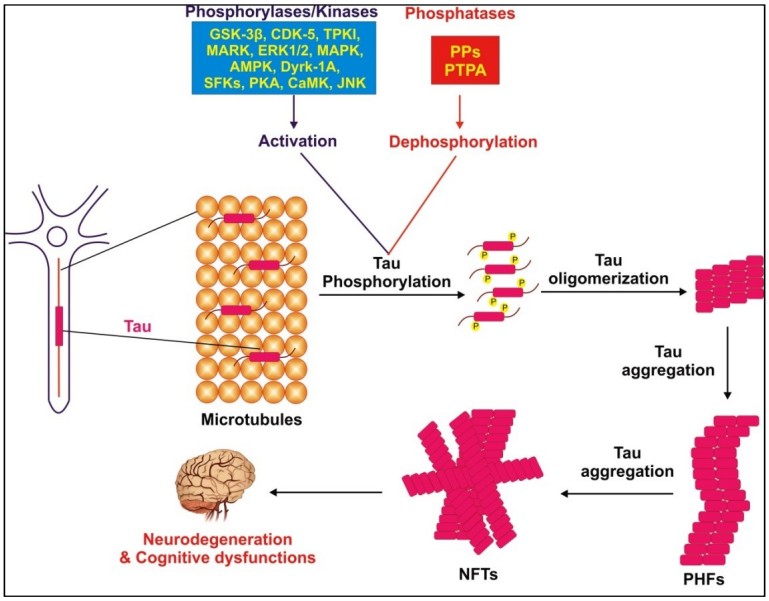

Figure 1.4. Tau hyper-phosphorylation and NFTs generation [Sharma et al. 2019a].

Kinase-induced tau hyper-phosphorylation is upregulated by the silencing of phosphatases. After hyper-phosphorylation, tau gets converted into insoluble intracellular neurofibrillary tangles (NFTs) and ultimately leads to neurodegeneration. Due to its insolubility, it shows reduced clearance and thus results in cognitive impairment. Therefore, researchers and scientists are trying to design and develop novel strategies to either inhibit the formation of NFT's or to elevate their clearance.

1.2.4. Oxidative stress hypothesis

Due to excessive consumption of oxygen in the brain, there is always a possibility of the generation of free radicals. Excessive free radical generation and decreased levels of anti-oxidant enzymes cause neurodegeneration [Gandhi and Abramov 2012]. The presence of nucleic acid oxidase, abnormal energy metabolism, lipid peroxidation, availability of trace elements like iron, zinc, etc., are also responsible for causing oxidative stress [Swerdlow 2018]. During initial studies on AD, it is concluded that Aβ disrupts the mechanism of the electron transport chain (ETC) after entering into mitochondria, and leads to free radical generation, oxidative stress and ultimately causes neurodegeneration and also causes cell death by the mitochondrial permeability transition (MPT) pore opening [Butterfield and Boyd-Kimball 2004] [Du and Yan 2010, Shevtzova et al. 2001]. This hypothesis and MPT as a target for the potential drug is important and is actively used in the creation of multi-target drugs in combination with AChE and BuChE inhibition properties [Makhaeva et al. 2019, Shevtsova et al. 2021]. Several environmental factors may directly or indirectly influence the free radical generation and oxidative stress like UV radiation, reactive oxygen species (ROS), reactive nitrogen species (RNS), etc. Several enzymes such as NADPH oxidase, cytochrome p450, xanthine oxidase (XO), nitric oxide oxidase, lipoxygenase (LOX), cyclooxygenase (COX) etc. are also responsible for generation of free radicals [Brown 1997, Federico et

al. 2012, Halliwell 2006, Kleinschnitz et al. 2010, Nunomura et al. 2012, Phillis et al. 2006, Praticò et al. 2004, Singh et al. 2004]. Therefore, there is a need to explore and to elaborate on the mechanism of oxidative stress and its correlation with AD.

1.2.5. MAO inhibition

Monoamine oxidases (MAO), present on the outer membrane of mitochondria, are flavin-containing enzymes that catalyze the oxidation of monoamines by clipping off the amine group with oxygen. MAO enzymatic activity involves catalytic deamination of several exogenous amines (tyramine) as well as neurotransmitters (noradrenaline, dopamine, and serotonin) which lead to the production of hydrogen peroxide, which is possibly the primary source of oxidative stress [Melo et al. 2011]. MAO can also be classified into two types based on coding at the genetic level, tissue distribution, and inhibitor specificity into MAO-A and MAOB [Kennedy et al. 2003]. According to the inhibitor specificity, MAO-A plays a significant role in the deamination of serotonin and is considered to be sensitive for MAO-A inhibitors like clorgyline whereas MAO-B has a tendency to deaminate β-phenylethylamine and is sensitive to inhibit MAO-B enzyme like selegiline [Carreiras and Marco 2004]. MAO-A inhibition is primarily helpful in the development of antidepressant therapy [Meyer et al. 2009]. Whereas MAO-B inhibitors are utilized for the cure and mitigation of various neurodegenerative disorders like AD as well as Parkinson's disease [Ucar et al. 2005]. Hence, researchers and scientists are trying to explore more about the MAO pathophysiology and its correlation with the AD so that they can design and develop specific inhibitors against MAO-B.

1.2.6. NMDA receptor inhibition

The hippocampal and cortex region of the brain contains an excitatory neurotransmitter i.e. glutamate, which after binding to its specific receptor, helps in maintaining the

sodium and calcium influx in theneuronal cell [Green and LaFerla 2008]. Hyper-activation of glutamate can cause excitotoxicity and ultimately neuronal cell death [Zott et al. 2019]. There are two types of glutamate receptor present: (1) Ion channel (ionotropic) receptor, (2) Gprotein coupled (GPCR) receptor [Barnes and Slevin 2003]. NMDA is considered an ionotropic receptor that tends to maintain the level of calcium and sodium influx in the neuronal cell after binding with glutamate neurotransmitters. In the case of AD patients, there is either hyper-activation of the NMDA receptor or overproduction of glutamate which leads to the sudden and frequent influx of calcium and sodium ions causing inhibition of neuronal signaling, neurodegeneration and ultimately cellular death [Liu et al. 2019]. Therefore, several researchers and scientists are trying to design and develop appropriate drugs which can act as competitive inhibitors against glutamate receptors and may useful in the treatment of AD.

1.2.7. HDAC inhibition

Histone deacetylase (HDACs) comes under the category of enzymes that eliminates the acetyl group from ε-N-acetyl-lysine amino acid and hence, termed as lysine deacetylase [Van Dyke 2014]. It can be grouped into four major categories based upon their homology sequence of yeast and comprises eleven HDAC subtypes [Ruijter et al. 2003]. HDAC-6 is prominently expressed in the cytoplasm of hippocampal neuronal cells and also has distinctive properties from other HDACs. A non-histone HDAC-6 is utilized to deacetylase α-acetyl-tubulin (α-Ac-tubulin) to maintain microtubule stability. Therefore, overexpression of HDAC-6 leads to microtubule dysfunction via decreased α-Ac-tubulin level. It also influences the level of phosphorylated tau protein [Hubbert et al. 2002]. A clear correlation is also yet to develop between up-regulated HDAC-6 and neuronal damage (Fig. 1). In AD patients there is a significant increase in the level of HDAC-6 [Zhang et al. 2013]. However, non-specific or partial selective type HDAC-6 inhibitors

can causes unwanted responses including cardiotoxicity. Hence, continuous efforts are being made by the scientists to design and develop more specific inhibitors which could be utilized in microtubule stability to cure and manage AD.

1.2.8. Cyclase inhibition

According to the recent findings, it has been observed that persons suffering from AD have elevated level of pyroglutamate (PE) $A\beta_{3\text{-}40/42}$ and (PE) $A\beta_{11\text{-}40/42}$. Truncated Aβ with glutamate gets cyclized in presence of cyclase enzyme and forms pyroglutamate (PE) Aβ. There are several N-truncated Aβ associated with pyroglutamate (Glu-3 and Glu11) amino acid residues [Schlenzig et al. 2009]. These modified Aβ contributes it's major role in Aβ plaques formation in AD. They also uplift Aβ aggregation, increase the hydrophobicity of Aβ plaques and thus makes them inactive against several proteolytic degrading enzymes. It is also observed that this pyroglutamatization process is enhanced or catalyzed by glutaminyl cyclase (QC) enzyme (also known as QPCT, EC 2.5.2.3) which is predominantly present in the hippocampal and cortex region of the brain. Hence, overexpression of QC directly proportionates the level of PE $A\beta_{3\text{-}42}$ and other PE Aβ in AD (Fig. 1). It is reported that specific inhibition of QC significantly reduces the PE $A\beta_{3\text{-}42}$ level [Morawski et al. 2014]. Increasing the proteolytic degradation of PE $A\beta_{3\text{-}42}$ as well as its clearance could be a promising approach to design and develop novel lead candidates in AD therapy.

1.2.9. Exopeptidase inhibition

Cathepsin B (CTSB) is a protease enzyme that plays a key role in the pathogenesis of variety of disorders, including AD and cancer. In AD, it is associated with the formation and deposition of Aβ plaques. CTSB is present in endosomes and lysosomes where Aβ is formed and it is also demonstrated that it cleaves APP at β-sites more efficiently than

BACE-1. The study in CTSB gene knockout mice models for AD suggested that treatment with a specific CTSB inhibitor reduced the production of pGluA, an N-terminally truncated species of Aβ involved in the aggregation of Aβ oligomers and showed improved memory deficits and reduced Aβ plaque load [Hook et al. 2020]. As discussed earlier in the cyclase inhibition section that pE3-Aβ is toxic and prone to form Aβ aggregation faster. QC is responsible for the cyclisation of glutamate residues, and its pharmacological and genetic inhibition significantly reduces AD-related morphological lesions and cognitive impairments in animal models. To allow further biotransformation, the Aβ N-terminal aspartyl residue is removed prior to cyclisation of the glutamate in position 3. Aminopeptidase A is the main exopeptidase implicated in the N-terminal truncation of Aβ and plays an important role in AD related morphological and behavioral abnormalities. Potentially targeting aminopeptidase A by specific inhibitor could interfere the proteolytic activity and hence influence AD pathology.

1.2.10. Lipid dyshomeostasis in AD

Another hallmark of AD includes lipid dyshomeostasis, which indicates the significant connection between lipid metabolism and AD through the involvement of apolipoprotein E (APOE) genetic factors (Fig. 1). APOE serves as a critical part of the cholesterol metabolism of the whole body including CNS. Free APOE tends to aggregate Aβ while lapidated-APOE inhibits Aβ aggregation [Kim et al. 2009]. The Cholesterol extracted in CNS through BBB via *de-novo* synthesis pathway as well as periphery HDLs is metabolized or reduced by the HMG-CoA reductase. Inhibition of the HMG-CoA enzyme tends to lower the production of Aβ aggregates [Vance et al. 2005]. Free cholesterol is transformed into a cholesterol-ester in the presence of sterol O-acetyltransferase 2 (ACAT). Specific inhibition of ACAT by its specific inhibitor also influences the Aβ assemblage by preventing their aggregation [Bhattacharyya and Kovacs 2010]. Hence,

selectively targeting the HMG-CoA reductase and ACAT enzyme may improve AD therapy [Di Paolo and Kim 2011].

1.2.11. Pathogenic involvement of Porphyromonas gingivalis in AD

Novel robust evidence indicated pathogenic involvement in neurodegeneration like AD. In chronic periodontitis (CP) condition, *Porphyromonas gingivalis* (virulent infectious agent for CP development) gets translocated and accumulated in extraneuronal spaces [Kamer et al. 2015]. These aggregated pathogens colonized themselves and resulted in excretion of gingipain (toxic cysteine protease comprising lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB)) in extraneuronal space that causes neurotoxicity (Figure 1.1). Accumulation and colonization of P. gingivalis cause activation of various immunological processes such as microglial activation, aberrant cytokines and ultimately result in neuro-inflammation [Gui et al. 2016]. In-depth characterization and observation suggested that these pathogens performed a key part in the enhanced level of Aβ forming amyloid plaques and tau hyper-phosphorylation in neuronal damage. Therefore, the development and optimization of specific gingipain inhibitors may provide a good approach in pathogenic associated AD treatment [Dominy et al. 2019].

1.2.12. Neurovascular dysfunction in AD

Blood vessels provide essential nutrients and oxygen to neurons and extract other toxicological metabolic residues from the interstitial fluid of the brain. In neurodegenerative diseases, including AD is connected with neurovascular malfunctioning, neurovascular disintegration, and BBB malfunctioning (Figure 1.1). All these linked neurovascular deficiencies deteriorate cerebral blood flow which consecutively inhibits brain oxygen supply, nutrients as well as energy supplements.

Additionally, the excretion of various neurotoxic metabolites gets reduced, which tends to accumulate and cause neurotoxicity, cerebral β-amyloidosis, cerebral amyloid angiopathy, etc. [Kalaria 2010, Zipser et al. 2007, Zlokovic 2005]. By considering the specific pathophysiology linked with BBB malfunction in AD, researchers can develop a novel therapeutic strategy to counter these neuro vasculature dysfunctions [Zlokovic 2011].

1.3. Modern strategies to develop novel compounds for AD treatment

Many attempts have been undertaken in the last few decades to create innovative therapies for the efficient treatment of AD. Several novel medication candidates were discovered to treat AD; however, due to their toxicity at higher doses and lack of clinical efficacy, they were ultimately forced to be removed from the clinical studies i.e. Metifonate, tesofensine, velnacrine, eptastigmine, and huperzine [Imbimbo et al. 2000, López-Arrieta and Schneider 2006, Murphy et al. 1991, Yaari and Hake 2015]. Furthermore, a number of disease-modifying candidates, including bapineuzumab (Pfizer), solanezumab (Eli Lilly), gantenerumab, and crenezumab (Roche), have been reported as failed clinical candidates [Mehta et al. 2017]. Therefore, there is an urgent need to create novel treatment strategies for the development of neurotherapeutics against the progression of AD.

1.3.1. Multi-targeting ligand treatment strategy

In AD, pathophysiological changes such as decreased ACh levels, increased Aβ aggregation and its deposition, BACE-1 activation, tau related hyper-phosphorylation, formation of NFTs, oxidative stress, raised MAO-B expression, and presence of excessive metal ions considered as a multifaceted disorder. By multi-targeting ligand approach, a single chemical entity may target multiple enzymes associated with AD. Thus multi-

targeting strategy is new hope in addressing the true cause of neuronal damage than symptomatic therapy [Hughes et al. 2016]. Because of the multiple targets involved in AD, the researchers chose to shift their attention from single targets to MTLs. For example, Ligands that bind to both the PAS and the CAS of AChE may be able to inhibit BACE-1 from developing Aβ aggregation [Chen et al. 2014]. Three AChEIs (donepezil, rivastigmine, and galantamine) and one NMDA receptor antagonist (memantine) are among the currently available commercial medications that have been explored and found to have great potential in addressing several AD pathways [Sarno et al. 2017]. Hybridizing two FDA approved medications, galantamine (AChEI) and memantine (NMDA receptor antagonist), to exert both effects simultaneously has also been attempted [Simoni et al. 2012]. Researchers are still looking at the possibility of reducing the early development of cognitive impairment. It is therefore necessary to understand the underlying mechanism of all target associated approaches in identifying a successful clinical candidate through a multi-targeted approach.

1.3.2. Computer-aided drug design approach

Drug discovery divisions of top pharmaceutical and biotechnological corporations are heavily experimenting with the computer-aided drug design (CADD) technique. In identifying the most promising drug candidate inside the databases' library and offer superiority and reliability than other methods of drug discovery [Nadendla 2004]. The initial screening from these computational approaches can minimize the total time and expense. These computational techniques can be separated into two main categories including structure- and ligand-based drug design (SBDD and LBDD), which are sometimes referred to as direct and indirect approaches.

The direct method, or SBDD, relies on identifying the compounds that meet specific geometric requirements based on the known three-dimensional structure of the biological

targets. The tools that can be employed in SBDD include molecular docking and dynamics, X-ray diffraction (XRD), nuclear magnetic resonance (NMR), homology modeling, and molecular mechanics generalized Born surface area (MM-GBSA).

The indirect drug design method or LBDD creates a lead molecule by comparing different structural traits of known active and inactive compounds. Numerous methods are used in LBDD, including pharmacophore modeling, high throughput virtual screening (HTVS), combinatorial chemistry, comparative molecular field analysis (CoMFA), comparative molecular similarity indices analysis (CoMSIA), and quantitative structure-activity relationship (QSAR) [Bacilieri and Moro 2006].

In fact, molecular modeling facilitates the understanding of the underlying molecular mechanisms behind a given biological activity as well as the identification of moieties engaged in the interaction with a certain protein. CADD speeds up the process of creating novel active compounds that could become effective treatment candidates. Nevertheless, as simulation accuracy is limited to the precision of the constructed models, computational simulations must be compared to the results of *in-vitro* and *in-vivo* experiments to verify the model's correctness and provide more accurate system representations.

1.3.3. Molecular hybridization

A recent idea in drug design and discovery is the molecular hybridization technique, which combines two or more pharmacophores to create a single molecule with more biological activity than the sum of its separate parent molecules. Nowadays, medicinal and biological chemists are making good use of the molecular hybridization technique to create new molecules that have better affinity and efficacy for biological targets and less harmful consequences [Viegas-Junior et al. 2007]. There is a complex mechanism underlying AD, and the few commercial medications that are available for treatment may

simply address symptoms rather than slowing the disease's progression. In order to increase biological activity toward various targets, decrease toxicity, and improve the pharmacokinetic profile relative to parent compounds, the pharmacophoric moieties of already available medicines may be hybridized.

1.4. Design hypothesis in the present study

The multitarget directed ligand (MTDL) approach has demonstrated efficacy in slowing the advancement of Alzheimer's disease (AD). In this work, a candidate molecule that could interact with the many targets primarily linked to AD was designed using an MTDL technique. The majority of AD patients' prescription regimens consist of many medications combined, which only relieves symptoms temporarily and also has negative effects on metabolism, bioavailability, drug-drug interactions, and, most importantly, patient noncompliance.

It was assumed that a single agent could inhibit both cholinesterase (AChE & BChE) and tau aggregation, reducing the neuro-inflammation that developed in response to $A\beta_{1\text{-}42}$ and NFTs deposition. Beta secretase-1 (BACE-1) is an enzyme that induces the formation of insoluble $A\beta_{1\text{-}42}$ aggregates through catalytic proteolysis of amyloid precursor protein (APP). These $A\beta_{1\text{-}42}$ aggregates are partially responsible for neuritic injury and neuronal cell death. Therefore, simultaneous inhibition of BACE-1 and ChEs through the MTDL approach may improve learning and memory impairments and retard the progression of AD.

In the first part of thesis, to find out the most promising multi-targeting ligands for AD therapy, the structure-based drug design technique was employed by developing e-pharmacophore models of protein structures (hAChE and hBACE-1). The co-crystallized ligands of both the enzymes (hAChE: donepezil and hBACE-1: F1M) were selected for e-pharmacophore model generation. The ZINC15 database undergoes filtrations processes

including various computational methods like phase screening, virtual screening (VS), docking-post processing (DPP), and Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) estimation. The pharmacophoric features of the identified hit (ZINC000015441499) and most potent compound **AV-2** were also analysed. A series of compounds (**AV-1 to AV-21**) with better binding properties at the aspartate dyad of hBACE-1, catalytic active site (CAS), and PAS of hAChE was rationally designed using the identified potential hit.

In the second part of thesis, considering the MTDL properties of the compound **AV-2** to be optimized further for better inhibitory activity against various targets associated with AD progression. The rigorous literature survey of compounds containing quinazoline moiety revealed that quinazoline derivatives played a significant role in inhibiting various targets responsible for the progression of AD. A series of quinazoline derivatives (**AK-1** to **AK-14**) was rationally designed based on the lead optimization technique by tethering substituted piperazines and phenyl rings at the C2 and C4 positions of the quinazoline nucleus, respectively. The substituted piperazines at the C4 position of quinazoline were introduced according to bioisosteric replacement of benzylpiperidine ring (pharmacophoric feature of FDA-approved drug donepezil). Further, to improve its binding towards the PAS region of AChE, the methoxy (-OCH_3) groups were introduced at the C7 and C8 positions of the quinazoline ring.