1.1 Neurodegenerative disorders

The neurodegenerative disorder affects almost 30 million individuals, leading to the disability, cognitive disconnect, and death [Ransohoff 2016]. These disorders are characterized by pathological changes in the disease-specific area of the brain and degeneration of distinct neuron subsets [Heemels 2016]. Alzheimer's disease (AD) and Parkinson's disease (PD) are of utmost prevalence that leads to functional failure of neurons in the brain or peripheral nervous system over a period of time and creates a cognitive disturbance. Other neurodegenerative disorders include amyotrophic lateral sclerosis (ALS), Huntington's disease, fronto-temporal dementia, and spinocerebellar ataxia. These disorders lead to the distressing outcomes of miscommunications between nerve cells [Enciu et al. 2011]. Which affects an individual's locomotor functions, speech, muscle coordination, cognitive functions, intelligence, behavior, and much more [von Gunten et al. 2009]. These are the foremost threat to human health and the risk of being affected by a neurodegenerative disease upsurges intensely with age [Reddy 2008]. The current scenario accounts for an urgent need to expand our understanding to explore the exact causes of neurodegenerative disorders and discover novel methodologies for the treatment and prevention.

1.2 Alzheimer's disease

Several decades ago, scientists instigated to solve the mysteries of a dreadful neurodegenerative ailment known as AD. The disease was first diagnosed in 1906 and described by Dr. Alois Alzheimer in a 50-year-old patient suffering from dementia. The typical dementing condition was later named "AD" by Emil Kraepelin, in the 8th edition of his book "Psychiatrie" [Ramirez-Bermudez 2012]. The paranoid and pathological outcomes of characteristic plaques like neurofibrillary tangles in the autopsied brain was the first etiological symptoms of AD which is now affecting around

80% population of dementia [Sosa-Ortiz et al. 2012]. The worldwide burden of the population with AD was reported to be 44 million in 2015. This figure is projected to be double by 2030 and triple by 2050 if no efficacious treatment is placed by the researchers [WHO 2015].

This catastrophic brain disorder is affecting the lives of millions of people continuously. The silent creep of AD not only leads to the loss of memory but, in many cases, "the loss of life." The calamitous aspect of AD is dementia, which broadly covers numerous conditions manifested by the loss of mental capabilities. The disease progresses gradually and relentlessly causes the degeneration of brain cells, diminish memory, obstruction in the thinking, perception, and creates a financial and emotional burden in society, finally rip-off their dignity by draining their capacity to handle their normal life [Small et al. 1999]. Typically the AD originates in the hippocampal region of the brain and then advances to the other centers of the brain that control the rhetorical, physical, and judgmental activities of the patient [Iadecola 2004]. Substantial evidence has exposed the origin of AD from a combined array of neurochemical mechanisms causing cell death and tissue loss in the AD brain. The frightful disease is characterized by multifarious etiology in conjunction with its overlapping pathological mechanisms and clinical manifestations. The pathophysiological hallmarks of the disease include depleted levels of acetylcholine (ACh), a higher level of amyloid-beta (Aβ) plaques, that form *N*-methyl-D-aspartate receptor dysfunction, tau hyperphosphorylation of proteins that form neurofibrillary tangles, neuroinflammation, and increased reactive oxygen species production [Ravi and Reddy 2018].

The phenotype characteristic of AD is classified into mild, moderate, and severe, which are associated with diverse symptoms [Braak and Braak 1997]. The mild phase (2–4 years) is associated with symptoms like lack of interest, mood swings, problems in

writing and driving, language issues, depression, etc. These symptoms do not account for the confirmation of AD and may be associated with other disorders. The moderate phase (2–10 years) is, however, linked with a significant decline in memory with decreased capacity of the patients to live their normal life. While, the severe phase (1-3 years) of the disease represents the combination of symptoms of the previous phases along with other severe problems like hallucinations, disorientation, seizures, skin infections, dangerous mood swings, motor incoordination, loss of weight, etc. that finally lead to life-threatening consequences. At present, the available treatments may assist in relieving certain mental or physical symptoms related to the disease rather than a medication to cure and hinder the disease progression [Jagust and Mormino 2011].

1.3 Pathogenesis of AD

The cerebral cortex deterioration, synaptic damage, and neuronal death are the key features of the AD. Several hypotheses have been proposed to explain the AD pathogenesis, where extracellular Aβ plaques, hyperphosphorylation of tau protein, and formation neurofibrillary tangles (NFT) leading to cognitive declining are the hallmarks of pathological symptoms of AD. AD is associated with a noticeable scarcity of acetylcholine owing to atrophy and degeneration of subcortical cholinergic neurons of the basal forebrain. Other pathophysiological mechanisms that emerged over the past few years include activation of *N*-methyl-D-aspartate receptor (NMDAR), oxidative stress, apolipoprotein E4 (APOε4) gene transcription, cyclic-AMP-response element-binding protein (CREB) signaling pathways, etc. to explain the complexity of the disease [Sharma et al. 2019].

1.3.1. Cholinergic hypothesis

The cholinergic hypothesis was the first hypothesis that defines the pathogenesis of AD. The shortfall of acetylcholine in the brain, especially in the hippocampus, cortex,

amygdala damages cholinergic neurons [Perry 1986]. These specific brain regions are crucial in serving cognitive functions, especially attention, learning, and memory [Beninger et al. 1989]. The depletion and scarcity of acetylcholine (ACh) in the brain occur either due to the declined production of neurotransmitter or augmented activity of acetylcholinesterase (AChE). ACh plays an essential part in the regulation of cognitive functions. The complete cholinergic transmission is conferred in Figure 1.1 [Sharma et al. 2019]. It was investigated that, intense loss of choline acetyltransferase (CAT) takes place in the brain of AD patients. CAT basically plays an active role in the synthesis of ACh and significant reduction of this enzyme in hippocampal and neocortical areas of the brain leads to neurodegeneration at a crucial site in AD. In other way around ACh is hydrolyzed by the action of acetylcholinesterase (AChE) and form choline and acetic acid. Clinical findings has revealed the substantial role of BChE in the regulation of ACh and upholding usual cholinergic functions, which makes BChE an additional promising target against AD. The low levels of ACh in cholinergic synapse grounds for memory loss diminished attention and cognitive dysfunction. This hypothesis suggests that the cholinergic amplification will recover the cognition in AD [Sarter et al. 1990].

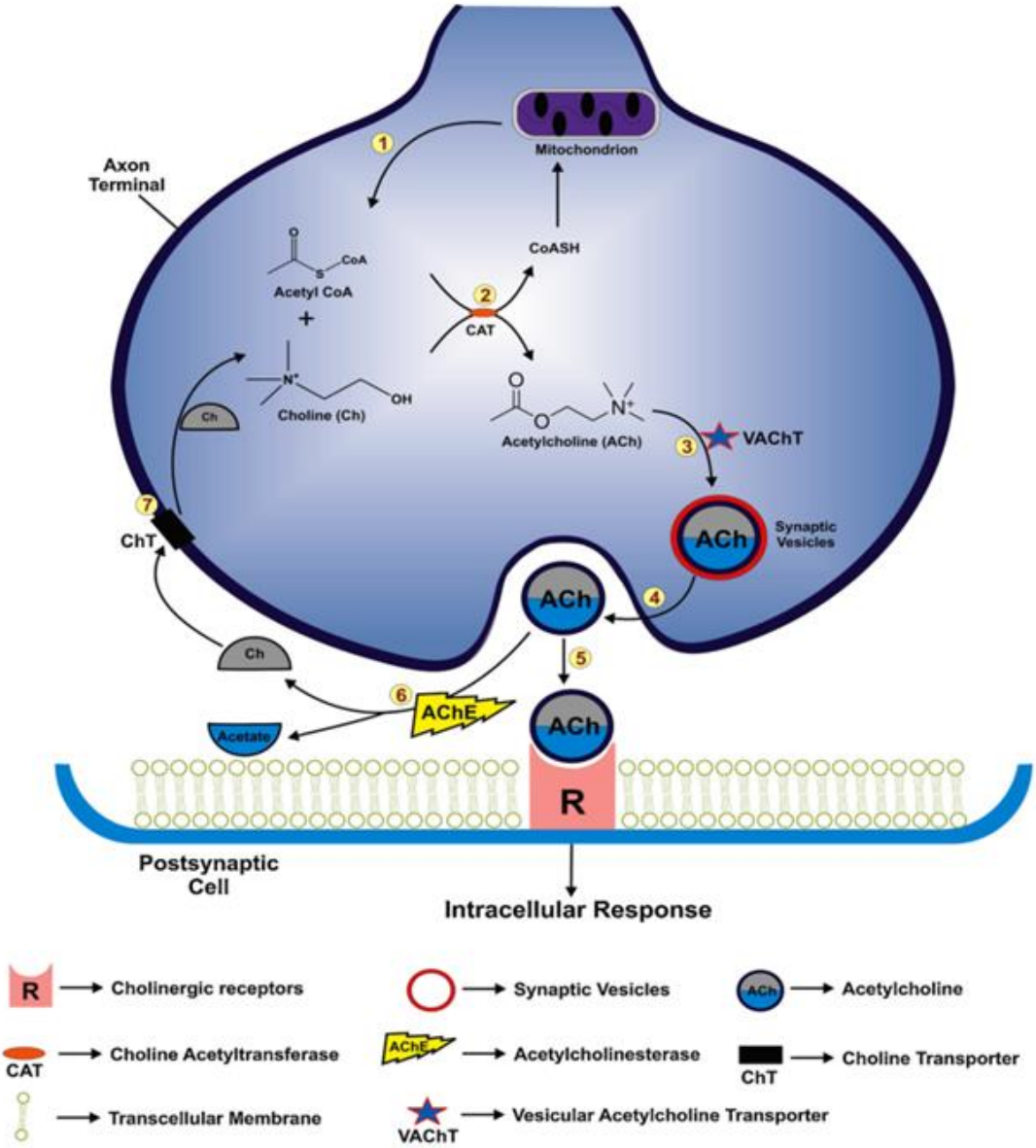


Figure 1.1. Synthesis of ACh and cholinergic neurotransmission. [Sharma et al. 2019].

1.3.2. Aβ hypothesis

The amyloid cascade theory of AD states that the disease is investigated as a sequence of deviations in the process and secretion of the amyloid precursor protein (APP). The hypothesis is based on an imbalance between the synthesis and clearance of Aβ (Figure 1.2). The formation & deposition of toxic Aβ aggregates in the form of senile plaques in nerve cells is considered to be the major pathological feature of AD for the last two decades [Hardy and Higgins 1992]. Typically, Aβ is small soluble peptides generated

due to breakdown of APP by the activity of proteolytic enzyme activity of α-secretase, β-secretase and γ-secretase [Plaschke et al. 1997].

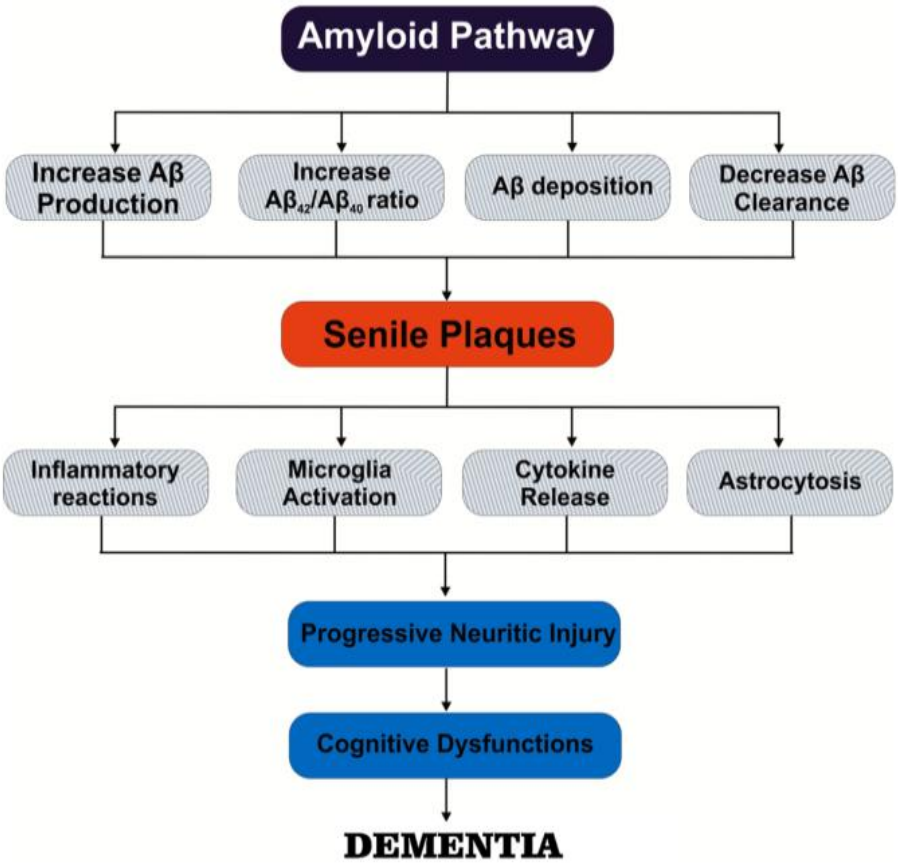


Figure 1.2. The amyloidogenic pathway and its manifestations are leading to dementia. [Sharma et al. 2019].

The amyloidogenic and non-amyloidogenic pathways are involved in the splitting APP, where as α- secretase produces APP-α precursor and C83 α-subunit via the non-amyloidogenic pathway and considered to exert neuroprotective activity [Naslund et al. 1994]. However, the activity of β-secretase enzyme produces APP-β along with C99 β-subunit via an amyloidogenic mechanism which was further processed into the β-subunit form ($Aβ_{42}$) by the action of γ-secretase. This β-subunit form accumulates and forms toxic senile plaques in the AD brain that leads to inflammation, microglial activation, cytokine release, and astrocytosis [Yates et al. 2000]. Overall these interaction causes progressive nerve cell injury, neuronal deficiencies, and cognitive

abnormalities. This hypothesis has the uppermost acceptance rate; though, present investigations revealed that apart from Aβ, additional fragments generated from the splitting of APP, like C83 or AICD, also contribute to the pathogenesis of AD questioning the exclusiveness of this hypothesis [Hoang S et al. 2015].

1.3.3. The Tau (τ) protein theory

The τ-protein hypothesis corresponds to the hyperphosphorylation of the τ protein that consequently results in neurodegeneration [Goedert 2004]. The stability of microtubules is governed by an unfolded & extremely soluble protein known as "Tau", which remains overload in the neurons of the central nervous system. However, the τ protein does not form aggregates or tangles under normal physiological conditions. The literature also revealed that excess stimulation of kinases and diminished activity of phosphatases considerably induces tau hyperphosphorylation [Maccioni et al. 2010]. Some study of neuronal cell lines and cultures that the Aβ persuade τ-alterations that includes enhanced phosphorylation followed by a cytoplasmic and dendritic translocation that leads to neurodegeneration [Borroni et al. 2018]. This protein's atypical self-assembly in AD contributes to the formation of neurofibrillary tangles (NFT) composed of paired helical filaments that disrupt neuronal plasticity and trigger neurodegeneration [Avila et al. 2004]. Therefore, the cognitive anomalies can be recovered by synthesizing novel compounds that stabilize the microtubule and prevents the hyperphosphorylation of τ protein.

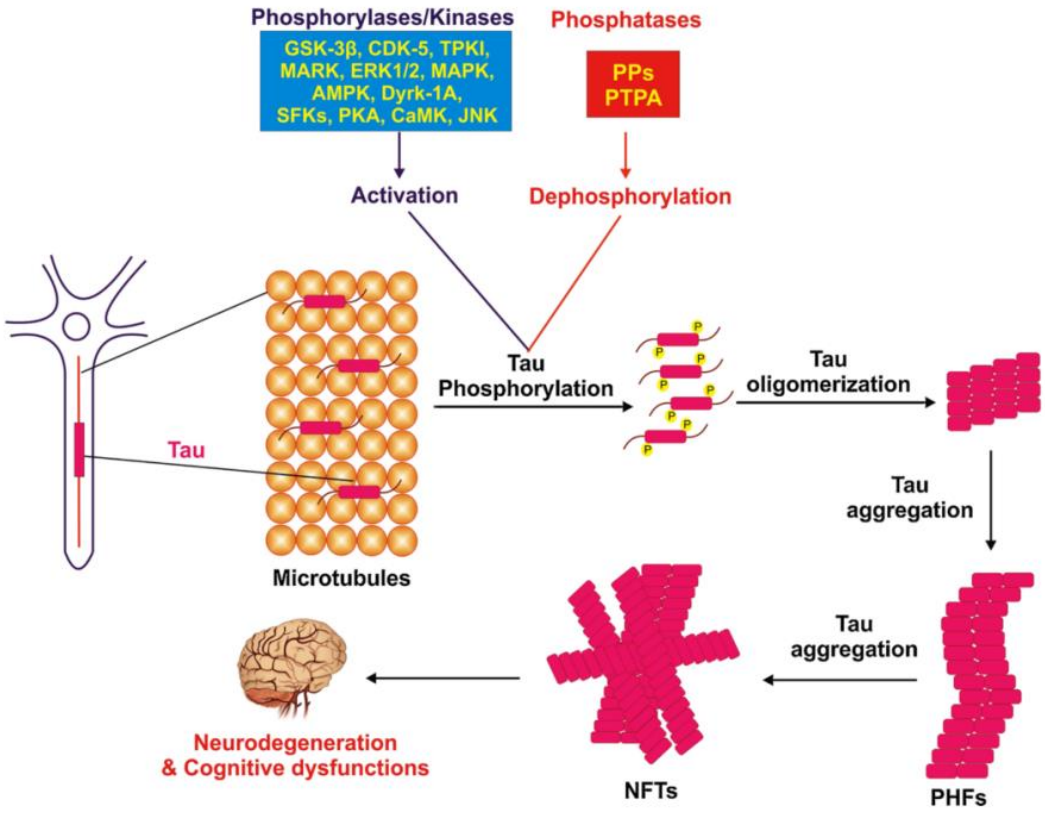


Figure 1.3. Schematic illustration of tau aggregation and formation of NFTs [Sharma et al. 2019].

1.3.4. Oxidative stress hypothesis

Neurodegeneration condition of AD linked with oxidative stress and cell apoptosis. Whereas the imbalance between free radicals and antioxidants defense tremendously fluctuates. Reactive oxygen species (ROS) and nitric oxide sometimes time identified as the main or subsidiary component of neurodegeneration [Mittler 2002]. Along with the well-known pathology of amyloid plaques and neurofibrillary tangles, the existence of widespread OS is a distinguished property of AD brains. The build-up of free radicals induced injury, changes in the activities of antioxidant enzymes like superoxide dismutase and catalase also contribute to the pathogenesis of AD [Gsell et al. 1995]. The inconsistent discharge of free radicals stimulates protein oxidation and lipid peroxidation that consequently results in impaired cognitive functions. The oxidative destruction in the brain is reported to be commenced by several reasons like altered

mitochondrial energy metabolism, accumulation of Aβ peptides, DNA oxidation, lipid peroxidation, and occurrence of disproportionate trace elements and transition metals (aluminum, mercury, copper, zinc, and iron) [Smith et al. 2000, Zhu et al. 2007].

1.3.5 Excitotoxic hypothesis

The excitotoxic mechanism some time plays an important role in neurodegeneration, such as Huntington's disease, PD, ALS, and AD. In these illnesses, the slowly evolving neuronal death is unlikely to be due to a sudden release of glutamate [Beal M 1992] and cortex and acts via glutamate receptors: (1) Ionotropic and (2) Metabotropic receptors [Willis et al. 1996]. The glutamate mostly binds to ionotropic receptor subtypes, i.e., NMDAR, and promotes depolarization, followed by magnesium ions mediated closing of the cationic channel to prevent the entry of calcium ions in the resting stage. In AD, there is excessive activation of the NMDA type glutamate receptor in neuronal cells that causes the release of bound magnesium ions and allows the entry of calcium ions into the neuronal cells. The excessive influx of calcium ions hampers the neuronal transmission, damages the nerve cells, and is responsible for neurodegeneration and cell death [Olney et al. 1997].

1.3.6 Apolipoprotein E (ApoE) hypothesis

The advanced stage of AD is generally associated with the genetic risk factor ApoE ε4 allele observed in various studies. The ε4 promotes the Aβ aggregation and lowers the Aβ clearance. A ε4 allele is also associated with the pathogenesis of AD, such as neuro-inflammation, tauopathy, and reduced rate of glucose metabolism [Galpern and Lang 2006, Michaelson 2014].

1.3.7 CREB signaling pathways

The CREB regulates several downstream genes such as somatostatin [Gonzalez and Montminy 1989], enkephalin [Mayr and Montminy 2001], corticoliberin [Kageyama et

al. 2007], and period circadian protein homolog 1/2 [Dibner et al. 2010]. CREB is abundantly expressed in the hippocampal and neocortex region in AD patients [Moore et al. 1996]. CREB dependent genes are regulated by phosphorylation, which is significantly controlled by protein kinases [Bitner 2012]. CREB expression is found to be considerably downregulated in several mouse models of the AD [Pugazhenthi et al. 2011]. It is also evident that the effect of Aβ induced memory and synaptic loss mediate CREB signaling pathway [Saura and Valero 2011].

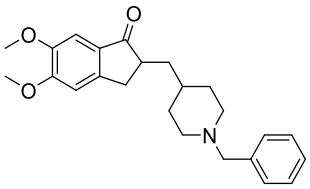
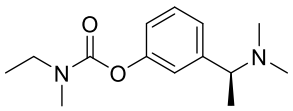
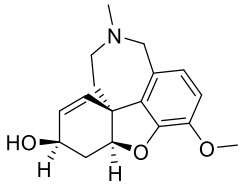
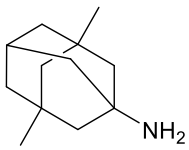
1.3.8 The chronic inflammation hypothesis

The Aβ plaque deposition stimulates the inflammation of microglia, which is sensitive to neuronal injury and neurodegenerative disorders. During AD, it stimulates, and releases the inflammatory molecules such as cytokines, ROSs, and proteinases, and subsequently causes neuronal cell death [Wyss-Coray and Mucke 2002].

1.4 Available neurotherapeutics for the treatment of AD

Currently, there are four drugs approved for the treatment of AD. Among them, three are cholinesterase inhibitors: Donepezil, Rivastigmine, and Galantamine, and one is an NMDA receptor antagonist, memantine. The structures, mechanism, and adverse effects of available therapeutics are tabulated in Table 1.1. All the currently available therapeutics only provide symptomatic relief and are unable to halt the progression of AD.

Table 1.1. Chemical structures, mechanisms, and adverse effects of USFDA approved drugs for the treatment of AD.

Name	Structure	Mechanism	Adverse effects	Ref.
Donepezil		Cholinesterase inhibitor	Nausea, vomiting, loss of appetite/weight loss, diarrhea, weakness, dizziness, drowsiness, tremor, slow/irregular heartbeat, fainting, black stools, vomit that looks like coffee grounds, severe stomach/abdominal pain, seizures, trouble urinating.	[Wilkinson 1999]
Rivastigmine		Cholinesterase inhibitor		[Polinsky 1998]
Galantamine		Cholinesterase inhibitor		[Marco-Contelles et al. 2006]
Memantine		NMDA receptor antagonist	Body aches, dizziness, constipation, headache, and trouble breathing	[Mobius et al. 2004]

1.5 Description of a problem

After the discovery of this eponymous disease, researchers have made astonishing strides in developing effective therapies and understanding the underlying pathophysiological mechanism involved. Irrespective of the extensive expansion in the high-throughput screening procedures and the colossal efforts for the discovery of novel molecules for AD, their numbers have not increased significantly over the last few decades. Only a handful of drugs, including AChE inhibitors like donepezil, rivastigmine, and galantamine, are available to treat AD. These drugs enhance the compromised levels of acetylcholine in AD by inhibiting its hydrolytic degradation mediated by cholinesterases, including AChE and BChE. Considerable efforts were also exercised to design novel molecules that restrict the deposition of Aβ plaques and tangles that lead to a deficit in the normal cognitive functionalities of the brain. However, these drugs typically enhance cognitive functions and ameliorate the

symptoms of AD, but none of them prohibit or halt the progression of the disease or offer a cure.

Several promising compounds have been tested against individual targets. But failed in regulatory review, and further declined in phase trial studies. The researchers are now well convinced that the targeting of a single node of the Alzheimer's classical pathway by conventional therapeutics only exerts a little effect on the multifactorial AD network. The findings suggest an immense requirement of novel drugs with a strong potential of treatment that modulates multiple targets and decelerates the AD progression rather than a diminution of symptoms. Recent investigations are shifted towards the development of multitargeted and multifunctional agents to treat AD an extremely promising strategy. It corresponds to the "one compound multiple targets" to treat multifaceted disorders. Therefore the concept of molecular hybridization was successfully utilized in this research to design & synthesize novel molecules for multiple targets.

1.6 Design hypothesis in the present study

The elevation of ACh through AChE inhibition and prevention of Aβ aggregation are the two most promising approaches to decelerate the progression of the disease. The reduction of oxidative stress is another factor that is responsible for memory impairment.

The designing of the present work is based on bioisosteric replacement and molecular hybridization approach to developing promising lead candidates. Bioisosterism is one of the important strategies of analog design that modify pharmacological activity, where compounds or groups possess near-equal molecular shapes and volumes, approximately the same distribution of electrons that exhibit similar physical properties [Mannhold R 2012]. Bioisosteric compounds affect the same biochemically associated systems (agonists or antagonists) and thereby produce biological properties [Burger 1991]. There

are two classes of Bioisosteres: (a) classical bioisosteres, i.e., monovalent, divalent, trivalent atoms, tetrasubstituted atoms, and ring equivalents, (b) nonclassical bioisosteres, i.e., rings vs. noncyclic, structures and exchangeable groups. These replacements are carried out in designing new drugs to improve the physiochemical properties that attenuate the toxicity and improve pharmacological activity, with increased selectivity towards targets [Patani and LaVoie 1996].

The molecular hybridization approach is a novel concept in drug design and discovery, which involves the hybridization of two or more pharmacophores in a single molecule with enhanced biological activity compared to individual parent molecules. The concept of molecular hybridization approach is now well adopted by medicinal and biological chemists to design novel compounds to improve affinity and efficacy toward biological targets, and reduced toxic effects [Viegas-Junior et al. 2007].

The AD is multifactorial disease involves several pathophysiological mechanisms, and very few commercial therapeutics are available for the treatment, that extended only symptomatic relief rather affecting the disease progression. Therefore, pharmacophoric moieties of available therapeutics could be hybridized to improve the biological activity toward multiple targets, reduce toxicity, and improve pharmacokinetic profile compared to the parent molecule.