1.1 Alzheimer's disease

Alzheimer's disease (AD) was first described and named by German psychiatrist and pathologist Alois Alzheimer in 1906 (Berchtold et al., 1998). AD is the most typical cause of dementia in the aging people (Association, 2010). It is a progressive, neurodegenerative pathology that primarily affects the elderly population, and is estimated to account for 50 to 80% of dementia cases in persons over 65 years of age. But it is not just a disease of old age, since up to 5 percent of people with the disease have early onset Alzheimer's, which often appears when someone is in their 40s or 50s. Hauser and colleagues were the first to report that the incidence of seizures in patients with AD was 10-fold increase as compared to the reference population (Hauser et al., 1986). According to the World Health Organization assessment, about three-quarters of the 1.2 billion elders will be living in low- and middle-income countries by the year 2025. Dementia prevalence is high (above 5%) in most Asian and Latin American countries, according to the Age-adjusted estimates. However, prevalence rates of dementia seem to be lower (1-3%) in India and sub-Saharan Africa (Aprahamian et al., 2013). Cognitive dysfunction, primarily memory losses are the main symptoms associated with this disease. Other features associated with the later stages of AD include language deficits, depression, behavioral problems, including agitation, mood disturbances and psychosis. The etiology of AD is still not clear. A lot of researches have been undertaken in recent years and concluded with some strong hypotheses. Some of them are cholinergic hypothesis, Amyloid cascade hypothesis, Tau hypothesis, Oxidative stress hypothesis, Zinc dyshomeostasis hypothesis and Calpain-cathepsin hypothesis (Craig et al., 2011; Karran et al., 2011; Maccioni et al., 2010; Markesbery, 1997; Craddock et al., 2012; Yamashima, 2013). Current medication for AD includes
use of AChE inhibitors such as Donepezil (Aricept), rivastigmine (Exelon), and galantamine (Razadyne, formerly called Reminyl) (Bartus et al., 1982). There are many challenges related to the study of the neuropathologic correlates of cognitive impairment in the elderly. An autopsy is required for definitive AD diagnosis, yet autopsy rates are generally low and autopsy inevitably confers a selection bias. Furthermore, clinicopathologic correlation (CPC) studies rarely are a random sample of the population and clinic- and hospital-based CPCs are subject to other potential biases (Schneider et al., 2009). The existence of persons without dementia with some AD neuropathologic changes is therefore not problematic. It is expected that many persons die with brains that exhibit AD neuropathologic change in the preclinical phase of AD; indeed, the data support the modeled expectations (Braak et al., 2011; Nelson et al., 2009). Oxidative stress has been proposed as a pathogenic mechanism in Alzheimer's disease. One mechanism of oxidative damage is the nitration of tyrosine residues in proteins, mediated by peroxynitrite breakdown. Detailed proof of oxidative stress in AD incorporates expanded iron, changes in defensive catalysts, and markers of oxidative harm to proteins and lipids (Good et al., 1996). Accessible medications focusing on dementia are to a great extent characteristic towards brief advantages of these medications that essentially work on making acetylcholine accessible to its related receptor by repressing acetylcholinesterase. Few medications following this path include donepezil, galantamine or rivastigmine. Although many candidate drugs known to possess a distinct pharmacological mechanism failed to prove beneficial to humans in early clinical trials when tested in neurobiological models of AD delivering negative outcome. This review, recapitulates the available evidence on the new therapeutic
approaches that target amyloid and neurotransmitter in the AD focusing on pharmaceutical compounds that have been approved by FDA.

1.2 Stages of Alzheimer’s

In people with AD, the increasing impairment of learning and memory eventually leads to a definitive diagnosis. In some cases, physical weaknesses are more noticeable than memory fatigue. These physical impairments include agnosia, apraxia and difficulty in language perception that includes reduction in word power and fluency. This results in common insolvency of verbal and written communication ( Förstl & Kurz, 1999; Taler & Phillips, 2008; Volicer et al., 2001). During the moderate phase, memory problems worsen, and the person may fail to recognize close relatives. Behavioural and neuropsychiatric changes become more prevalent. Basic indications are wandering irritability and labile effect, prompting crying, outbursts of unpremeditated aggression, or protection from mind giving ( Förstl and Kurz, 1999). In the last stages, the patient is absolutely dependent upon guardians. AD is of real concern everywhere throughout the world because of various factors, including (I) aging population (ii) expanding life expectancy and (iii) absence of powerful pharmacotherapy alternative. Individuals with AD will eventually not have the capacity to perform even the easiest undertakings autonomously; muscle mass and mobility disintegrate to the point where they are bedridden and unfit to sustain themselves. The reason for death is normally an outer factor, for example, such as infection of ulcers or pneumonia, not the simply disease itself ( Förstl & Kurz, 1999) (Fig 1.1).
1.3 Aggregation of beta amyloid

According to the amyloid hypothesis, extracellular amyloid beta (Aβ) deposits are the elementary ground of the AD disease & Allsop, 1991; Mudher & Lovestone, 2002), that can be clarified by the location of the gene for the amyloid precursor protein (APP) on chromosome 21, together with the fact that individuals with trisomy 21 (Down Syndrome) who have an additional gene copy almost collectively exhibit at least the earliest symptoms.
of AD by the age of 40 (Nistor et al., 2007; Lott & Head, 2005). Aβ signifies peptides of 36–43 amino acids, remarkably involved in Alzheimer's disease, as the main constituent of the amyloid plaques established in the brains of Alzheimer patients. Aβ, a peptide is usually formed by the cleavage of amyloid precursor protein (APP) in the presence of beta secretase and gamma secretase enzyme. The amyloid plaques are well known to produce toxicity in the nerve cells.

It is reported that misfolding of Aβ is also responsible for inducing misfolding in tau protein that forms prion-like misfolded oligomers responsible for persuading AD (Pulawski et al., 2012; Nagel-Steger et al., 2016). APP is a part of protein in mammals with two homologous proteins that is APP like protein 1 (APLP1) and APP like protein 2 (APLP2). APP is a transmembrane protein that includes both cytoplasmic and extracellular cleavage products that are applicable in AD. APP is firstly cleaved via α-secretase and β-secretase, which forms normal or pathological products respectively (fig 1.2). In this way, both α-secretase and β-secretase could act as Alzheimer therapeutics. Regardless of whether α-secretase or β-secretase cuts APP, there is an intracellular release of amyloid precursor protein intracellular domain (AiCD). Following α or β cleavage, γ-secretase cleavage yields extracellular Aβ and p3. β-Secretase 1 specifically has been found to have expanded action in Alzheimer’s patients and also those with quiet subjective demolition that went on to develop AD (Zetterberg et al., 2008).
Figure 1.2 Three-Dimensional structure of Alzheimer’s Amyloid beta [PDB ID: 2BEG].

The deposition of amyloid in affected brain tissue involves the development of intermediary species, with the lower molecular mass and an unexpected structure in comparison to develop amyloid fibrils, known as oligomers (Nagel-Steger et al., 2016). Aβ monomers are identified with the aggregation of insoluble fibrils typically recognized in solution by a nondestructive method known as small angle neutron scattering (Zhang-Haagen et al., 2016). Asparagine and glutamine rich amino acid sequences are disordered in monomeric structure which can accumulate in amyloids, as found in Q/N-rich prion domains. Amyloids fibrils are rich in β-sheet structures that can self-promulgate through protein-conformational chain reactions (Zhang et al., 2016). The general isoforms of Aβ are Aβ40 and Aβ42. The longer form is produced by the cleavage in the endoplasmic reticulum and the shorter form is formed by the cleavage in the trans-Golgi body (Hartmann et al., 1997).
1.4 Beta-site Amyloid Precursor Protein Cleaving Enzyme 1

Beta-secretase 1 (BACE1), also known as beta-site amyloid precursor protein cleaving enzyme 1, beta- site APP cleaving enzyme 1, membrane-associated aspartic protease 2, memapsin-2, aspartyl protease 2, and ASP2 is an aspartic-acid protease important in the formation of myelin sheaths in peripheral nerve cells (Vassar et al., 1999; Willem et al., 2006). BACE (Fig 1.3) is the major protease of the amyloid precursor protein pathway which generates and accumulates Aβ in the brain. As high level of Aβ in the brain is directly associated with AD pathogenesis, BACE has been considered as a potential therapeutic target for AD for the development of novel inhibitor drugs for the reduction of the Aβ amount in the brain (Selkoe, 1999). Amyloid β (Aβ) plaques are one of the most prominent histological features found in the AD brain and supposed to play a crucial role in the pathology of AD (Gold et al., 2001; Williamson et al., 2009).

![Figure 1.3 Apo structure of Beta-secretase [PDB ID: 1W50]](image)

The endothelial cells of the blood-brain barrier form a structural and functional barrier maintaining brain homeostasis via paracellular tight junctions and specific transporters such
as P-glycoprotein. The blood-brain barrier is responsible for negligible bioavailability of many neuroprotective drugs. In Alzheimer's disease, current treatment approaches include inhibitors of BACE-1 (β-site of amyloid precursor protein cleaving enzyme), a proteinase generating neurotoxic β-amyloid. AD mouse model shows that BACE-1 is upregulated at the blood-brain barrier compared to healthy controls. We hence propose a critical part for BACE-1 at the blood-brain barrier in β-amyloid generation and in vascular aspects of Alzheimer, especially in the improvement of cerebral amyloid angiopathy (Devraj et al., 20016). BACE1 is fundamentally expressed in neurons in the cerebrum. β-Secretase inhibitors work to block the first cleavage of APP inside of the cell, at the endoplasmic reticulum while γ-Secretase inhibitors work to block the second cleavage of APP in the cell membrane and would then stop the subsequent formation of Aβ and its toxic fragments and selective Aβ42 lowering agents modulate γ-secretase to reduce Aβ42 creation in support of other Aβ versions. The β-secretase called BACE1 is a membrane-associated protease that initiates the generation of amyloid β-protein (Aβ), a key event in Alzheimer’s disease.

1.5 Amyloidogenic pathway in Alzheimer’s disease

Amyloidogenic pathway is the result of a mutation that replaces the normal pathway. Usually in normal pathway α-secretase work on the APP followed by γ-secretase to produce safe p-3 peptide but in case of the amyloidogenic pathway, breakdown of APP by β-secretase followed by γ-secretase to form the amyloid beta plaque (Sambamurti et al., 2002; Swerdlow, 2007). β-secretase was a promising target for creating new anti-Alzheimer drugs (Nisha et al., 2016). The amyloid-beta (Aβ) and tau proteins have been the two leading targets thought to be the causative agents leading to AD (Ittner & Götz,
2011; Götz et al., 2010; Blennow et al., 2006; Kang et al., 1987; Motter et al., 1995). The overall amyloidogenic pathway is depicted in (fig 1.4).

**Figure 1.4** Working of beta- secretase in the formation of Amyloid beta and Plaque formation.

**1.6 Role of RAGE in Alzheimer’s disease**

RAGE (Receptor for advanced glycation end products) protein has a place with the immunoglobulin superfamily, which was first recognized and described for its capacity to attach with advanced glycation end products (AGEs). AGEs and amyloid β-peptide are the
well known ligands that have been found to be recognized by RAGE (Schmidt et al., 1992; Neeper et al., 1992; Yan et al., 1997; Hori et al., 1995; Hofmann et al., 1999). Interaction of AGEs and Aβ to RAGE has been determined in the activation of transcription factor NF-κβ and the release of different cytokines such as IL-1, IL-6, TNF-α (Bierhaus et al., 2005; Bierhaus et al., 2001; Bierhaus et al., 2009). Activation of NF-κβ was observed to be involved in neuronal plasticity and the cellular reaction to neurodegeneration (Mattson & Camandola, 2001). The Aβ -RAGE interaction at the BBB not only results in neurovascular stress and expression of proinflammatory cytokines (TNF-α and IL-6) but also leads to decreased cerebral blood flow by enhancing the secretion of endothelin-1 to induce vasoconstriction (Deane et al., 2003).

1.7 Neurotransmitters in Alzheimer’s disease

1.7.1 Acetylcholinesterase

In 1991, J.L. Sussman determines the X-ray crystal structure of Acetylcholinesterase (AChE) (fig 1.5). It is 537 amino acid-long peptide monomer comprised of 12-stranded mixed beta sheet surrounded by 14 alpha helices in its tertiary structure is known to hydrolyze acetylcholine at the rate of 250000 molecules/second (Sussman, 1991). Its active site is positioned in a deep gorge containing 14 aromatic residues that widens halfway into the protein, thereby, stabilizing the quaternary ammonium ion of acetylcholine. Acetylcholinesterase (AChE, EC 3.1.1.7), is a serine hydrolase. The ‘structure-function relationship’ of AChE was explained by Silman and Sussman that the enzyme was derived from proteins that jointly, share a common α/β fold (Silman & Sussman, 2008). The amyloid plaques and the neuro fibrillary tangles (NFT) are the two major features that are reported to amplify the activity of AChE. Direct interaction of Aβ with AChE results in increased
deposition of plaque. AChE is also reported to interact with γ-secretase enzyme via its catalytic domain known as presenilin-1, thus influencing its expression level along with its biological activity in brain. AChE inhibitors usually bind to the active site of the protein or to the allosteric site known as peripheral anionic site (PAS). This allosteric site contains indole of Trp279 as a key component and helps in binding of ACh molecule to AChE (Darvesh et al., 2003; Silman & Sussman, 2005). Such proteins comprise enzymes that are esterases, lipases and proteases, along with non-enzymatic proteins that operate as adhesion molecules and pro-hormones.

![Structure of recombinant human acetylcholinesterase](image)

**Figure 1.5** Structure of recombinant human acetylcholinesterase [PDB ID: 3LII].

Neurotransmitters also known as chemical messengers are endogenous chemicals that enable neurotransmission. They transmit signals across a chemical synapse, such as a neuromuscular junction, from one neuron to another "target" neuron, muscle cell, or gland cell (Lodish et al., 2000). Neurotransmitters are produced from synaptic vesicles into the synaptic cleft; where they are interact with receptors on the target cells. Neurotransmitters play important role in everyday life and functions. The accurate numbers are unknown, but more than 100 chemical
messengers were identified (Kendra, 2014). ACh is an organic molecule that functions in the brain and different body parts in animals. Parts of the body that use or affected by acetylcholine are referred to as cholinergic (Goodsell, 2004).

The essential physiological role of AChE includes the termination of chemical transmission at cholinergic synapses and secretory organs by catalyzing the neurotransmitter acetylcholine, ACh, at a high turnover rate \(2.5 \times 10^4\) molecules per second. A significant number of roles have been attributed to AChE in diseases of high scientific concern e.g. cancer. Depending on their time-dependent concentration, mechanism of binding and use, inhibitors of AChE have been demonstrated to have efficacy (e.g., donepezil, rivastigmine and galantamine in AD; and pyridostigmine in myasthenia gravis) (Darreh-Shori & Soininen, 2010) as well as toxicity (e.g. organophosphate and carbamate pesticides in health) (Nunes, 2011).

1.7.2 Distribution of Acetylcholinesterase

AChE is identified in different types of conducting tissue like peripheral and central tissues, sensory and motor fibers, nerve and muscle, and cholinergic and noncholinergic fibers. The motor neurons have higher AChE action mechanism than in sensory neurons (Massoulie et al., 1993; Chacho & Cerf, 1960). AChE is also found on the red blood cell membranes, where different forms constitute the Yt blood group antigens (Koelle, 1954).

1.7.3 Mechanism of AChE in Alzheimer’s disease

During transmission of signals from nerves, ACh is released from the nerve into the synaptic cleft that binds to the ACh receptors on the post-synaptic membrane. AChE located on the post-synaptic membrane, ceases the signal transmission by hydrolyzing ACh. The discharged choline is taken up again by the pre-synaptic nerve and ACh is synthesized by adding with
acetyl-CoA through the action of choline acetyltransferase (Bartels et al., 1993; Whittaker, 1990).

Cholinergic hypothesis is the oldest one and it is still promising in understanding AD for designing drugs. This hypothesis proposed that degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex and other areas contributed significantly to the deterioration in cognitive function seen in patients with AD. As it has been reported that AD is caused by multiple risk factors, hence, this hypothesis is modified by certain authors. AChE inhibitors are employed to reduce the rate at which ACh is broken down, thereby increasing the concentration of ACh in the brain and combating the loss of ACh caused by the death of cholinergic neurons (Purves et al., 2008). The mechanism of AChE is well illustrated in fig 1.6.

**Figure 1.6** AChE mechanism of action.
1.8 Treatment of AD

At present, the mainstays of AD therapy are drugs that target neurotransmitter systems in the brain. AD primarily damages glutamate and acetylcholine-producing neurons and their associated synapses, and this damage correlates well with early cognitive symptoms of AD (Hay et al., 2010). Acetylcholinesterase inhibitors help improve memory function and attention in AD patients by interfering with the breakdown of acetylcholine, thereby increasing the levels of the neurotransmitter at the synapse. Drugs that act to decrease the amount of Aβ protein in the brain have received the most attention due to the prominent pathogenic role ascribed to Aβ in the AD literature (Selkoe, 2002; De Strooper et al., 2010). Another strategy that has been attempted is by using drugs that promote the clearance of Aβ through active or passive immunization (Schenk et al., 2012). Five medications are currently used to treat the cognitive problems of AD: four are acetylcholinesterase inhibitors (tacrine, rivastigmine, galantamine and donepezil) and the other (memantine) is a N-Methyl-D-aspartate receptor (NMDA) antagonist (Pohanka, 2011). Advanced medical imaging with computed tomography (CT) or magnetic resonance imaging (MRI), and with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used to help exclude other cerebral pathology or subtypes of dementia (HIH, 2006). The U.S. Food and Drug Administration (FDA) have approved the following drugs to treat the symptoms of Alzheimer’s disease. The detailed information about the drugs is summarized in Table 1 and the 2D chemical structure is shown in fig 1.7.
Table 1.1 List of FDA approved drugs for the cure of Alzheimer’s disease.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug Name</th>
<th>Approved Year</th>
<th>Brand Name</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Memantine</td>
<td>2014</td>
<td>Namzaric</td>
<td>Headache, constipation, confusion etc.</td>
</tr>
<tr>
<td>2</td>
<td>Rivastigmine</td>
<td>2000</td>
<td>Exelon</td>
<td>Nausea, Vomiting, Loss of Appetite etc.</td>
</tr>
<tr>
<td>3</td>
<td>Galantamine</td>
<td>2001</td>
<td>Razadyne</td>
<td>Nausea, Vomiting, Loss of Appetite etc.</td>
</tr>
<tr>
<td>4</td>
<td>Donepezil</td>
<td>1996</td>
<td>Aricept</td>
<td>Nausea, Vomiting, Loss of Appetite etc.</td>
</tr>
<tr>
<td>5</td>
<td>Tacrine</td>
<td>1993</td>
<td>Cognex</td>
<td>Liver damage, Nausea, Vomiting etc.</td>
</tr>
</tbody>
</table>

Figure 1.7 Structure of FDA approved drugs for the cure of Alzheimer’s disease

1.9 New therapeutic candidates for the treatment of Alzheimer’s disease

1.9.1 Resveratrol

Resveratrol (3, 4, 5-trihydroxystilbene) is a naturally occurring phytochemical possessing antioxidant, anti-inflammatory, anti-viral, and anti-cancer properties with a potential remedial character in reducing the threat of neurodegeneration with AD in particular. The red
wine, grapes, berries, chocolate and peanuts are the natural source of resveratrol. Several in vitro and in vivo studies conducted reveals the neuroprotective effects of resveratrol by facilitating non-amyloidogenic breakdown of the amyloid precursor protein (APP), and endorse elimination of neurotoxic amyloid beta (Aβ) peptides, a decisive step in averting and slowing down AD pathology (Braidy et al., 2016).

1.9.2 Bisnorcymserine

Bisnorcymserine is a derivative of another drug Cymserine, which is associated to physostigmine. It is highly selective for butyrylcholinesterase (Shaikh et al., 2014). It has potential to improve the symptoms of patients with severe AD (Shaikh et al., 2014). It lowers the amyloid plaque-associated protein, amyloid-beta peptide (Kamal et al., 2006).

1.9.3 Huperzine A

Huperzine A (HupA) is a Lycopodium alkaloid isolated from the Chinese medicinal herb Huperzia serrata. It is used in memory deficit condition, and is really fascinating as it acts as a exceptionally selective, reversible and an effective AChE inhibitor. It is of 2 types – HupA and Hup B. Hup B is a natural homologue of HupA (Shaikh et al., 2014). Huperzine B has been established as an effective and reversible inhibitor of AChE. HupB is less potent and selective as compared to HupA, but it has higher therapeutic index and other positive benefits (Alam et al., 2014).

1.9.4 Tolserine

Tolserine is a fourth generation Acetylcholinesterase inhibitor known for the treatment of AD. It is rapidly engrossed through the membranes and can also be applied topically to the conjunctiva. It is basically used to treat severe anticholinergic toxicity as it holds blood-brain barrier (BBB) crossing properties (Alam et al., 2014).
1.9.5 Galangin

Galangin is a dietary flavonoid that works as an inhibitor of AChE. It is accounted to inhibit the catabolic breakdown of 7, 1, 2-dimethylbenz [a] anthracene (DMBA), as measured by thin-layer chromatography (TLC), in a dose-dependent manner (Shaikh et al., 2014).

1.9.6 Phenserine

Phenserine, a phenylcarbamate derivative of physostig-only inhibits ACh but also modulates the amount of beta-mine. It is non-competitive inhibitor of AChE (Shaikh et al., 2014). The phenserine is a novel cholinesterase inhibitor reported to inhibit the action of AChE in human erythrocyte at a concentration of 0.025-0.40 µM, in a concentration-dependent manner with IC50 estimation of 0.0453 µM (Al-Jafari et al., 1998).

1.9.7 Naringenin

Naringenin: The positive outcome of naringenin in the development of learning and memory was appraised in an AD rat model. Rats injected with Aβ and pretreated with naringenin are reported to lower the malondialdehyde (MDA) concentration in hippocampus without any noticeable outcome on nitrite and superoxide dismutase (SOD) activity with reduction in apoptosis. These are experimental outcomes proposes that naringenin pretreatment attenuates Aβ-induced impairment of learning and memory through alleviation of lipid peroxidation and apoptosis and its advantageous consequence is somewhat mediated by means of estrogenic pathway (Ghofrani et al., 2015).

1.9.8 Cymserine

Cymserine is a reversible BuChE inhibitor and helpful for treating AD without creating side effects like tremor, lacrimation and salivation. Its various derivatives have been identified and several of its analogues have been tested in animals. These were found to increase brain
ACh levels, and did not produce tropic effects. Furthermore, these were found to decrease the levels of amyloid precursor protein and amyloid beta, the known biomarkers for the development of AD (Greig et al., 2001; Kamal et al., 2006; Kamal et al., 2008).

1.9.9 Aptiom

Aptiom is permitted by the FDA as a medication to facilitate the treatment of epileptic seizures (Elger et al., 2009). It is absorbed to a minimum 90% within the gut and is quickly metabolised to eslicarbazepine, in order that the initial stuff cannot be detected within the circulation system. Plasma protein binding of Aptiom is less than 40% and is known to reach the peaks after 2–3 (1–4) hours. Its half-life is anticipated to be 10 to 20 hours, and steady-state concentrations are inwards in four to five days after beginning of the treatment (Austria-Codex, 2015).

1.9.10 Fetzima

Fetzima is chemically known as levomilnacipran. It might act as a potent inhibitor of BACE-1 and expected to form the basis of a future therapy against AD. It is approved by Food and Drug Administration in July 2013. Levomilnacipran was recently used to act as an inhibitor of BACE-1, which is liable for β-amylloid plaque development, and therefore may be a potentially useful drug in the cure of AD (Rizvi et al., 2014).

The detailed information about the above mentioned compounds is summarized in table 1.2 and their respective 2D chemical structure is shown in fig 1.8.
Table 1. List of novel therapeutic candidates against Alzheimer’s disease.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compounds Name</th>
<th>IUPAC Name</th>
<th>PubChem CID</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Resveratrol</td>
<td>5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol</td>
<td>445154</td>
<td>C_{14}H_{12}O_{3}</td>
</tr>
<tr>
<td>2.</td>
<td>Bisnorcymserine</td>
<td>[(3aR,8bS)-8b-methyl-2,3,3a,4-tetrahydro-1H-pyrrolo[2,3-b]indol-7-yl] N-(4-propan-2-ylphenyl)carbamate</td>
<td>71587645</td>
<td>C_{21}H_{25}N_{3}O_{2}</td>
</tr>
<tr>
<td>3.</td>
<td>Huperzine A</td>
<td>(1R,9S,13E)-1-Amino-13-ethyldiene-11-methyl-6-azatricyclo[7.3.1.0^{2,7}]trideca-2(7),3,10-trien-5-one</td>
<td>5912039</td>
<td>C_{15}H_{16}N_{2}O</td>
</tr>
<tr>
<td>4.</td>
<td>Tolserine</td>
<td>[(3aR,8bS)-3,4,8b-trimethyl-2,3a-dihydro-1H-pyrrolo[2,3-b]indol-7-yl] N-(2-methylphenyl)carbamate</td>
<td>9928397</td>
<td>C_{21}H_{25}N_{3}O_{2}</td>
</tr>
<tr>
<td>5.</td>
<td>Galangin</td>
<td>3,5,7-trihydroxy-2-phenylchromen-4-one</td>
<td>5281616</td>
<td>C_{15}H_{10}O_{5}</td>
</tr>
<tr>
<td>6.</td>
<td>Phenserine</td>
<td>[(3aR,8bS)-3,4,8b-trimethyl-2,3a-dihydro-1H-pyrrolo[2,3-b]indol-7-yl] N-phenylcarbamate</td>
<td>192706</td>
<td>C_{20}H_{23}N_{2}O_{2}</td>
</tr>
<tr>
<td>7.</td>
<td>Naringenin</td>
<td>5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one</td>
<td>932</td>
<td>C_{14}H_{12}O_{5}</td>
</tr>
<tr>
<td>8.</td>
<td>Cymserine</td>
<td>[(3aR,8bS)-3,4,8b-trimethyl-2,3a-dihydro-1H-pyrrolo[2,3-b]indol-7-yl] N-(4-propan-2-ylphenyl)carbamate</td>
<td>9907847</td>
<td>C_{23}H_{25}N_{2}O_{2}</td>
</tr>
<tr>
<td>9.</td>
<td>Aptiom</td>
<td>[(5S)-11-carbamoyl-5,6-dihydrobenzo[b][1]benzazepin-5-yl]acetate</td>
<td>179344</td>
<td>C_{17}H_{16}N_{3}O_{3}</td>
</tr>
<tr>
<td>10.</td>
<td>Fetzima</td>
<td>(1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide</td>
<td>6917779</td>
<td>C_{15}H_{22}N_{2}O</td>
</tr>
</tbody>
</table>
Figure 1.8 Structure of novel therapeutic candidates for the treatment of Alzheimer’s disease.
1.10 New therapeutic antibody for the treatment of Alzheimer’s disease

Active and passive immunotherapies are being developed to target AD. The neurotoxic species like mature Aβ fibrils are considered as an important centre for development of antibody that identifies the ‘aggregation epitopes’ and thus, helps in inhibition protein aggregation responsible for the onset of AD. Some of the major antibodies are discussed below:

1.10.1 Bapineuzumab

Bapineuzumab, a humanized monoclonal antibody currently in Phase III clinical trial is designed to decrease plaque formation and encourage clearance of Aβ being specific to the N-terminus of the amyloid β (Aβ) protein. Bapineuzumab binds to the soluble and fibrillar forms of Aβ and is well-tolerated at doses less than 2mg/Kg (Black et al., 2010) decreasing total brain Aβ load (Salloway et al., 2014).

1.10.2 Aducanumab

Aducanumab (BIIB037) is a human recombinant monoclonal antibody presently in phase III clinical trial. It is well reported to target Aβ soluble oligomers along with insoluble fibrils that settle down into the Aβ plaque in the brain of AD patients. Aducanumab treatment has been seen to slow down the clinical impairment in patients with mild AD in a considerable way (Westwood & Lawson, 2015). It is well reported that one year of monthly intravenous infusions of aducanumab results in reduction of Aβ in brain of patients suffering from prodromal or placid AD (Sevigny et al., 2016).
1.10.3 Solanezumab and Crenezumab

Solanezumab and crenezumab developed by Eli Lilly and Genentech respectively are the chief clinical antibodies targeting Aβ. It has been tested in multiple Phase III clinical trials for the prevention of AD in susceptible individuals. These antibodies generally capture Aβ in the mid domain region forming Aβ-anti- Aβ complex crystal structure. The major exciting feature of solanezumab and crenezumab is that they differ in their immunoglobulin backbone i.e. IgG1 and IgG4 respectively (Crespi et al., 2015; Liu-Seiferta et al., 2015).

1.10.4 BAN2401

BAN2401 is the humanized monoclonal antibody developed by immunization of mouse with protofibrils having arctic Aβ42 mutation thus remains in a prefibrillar setting rather than fibrillar. Due to its ability to selectively bind, neutralize and eliminate protofibrils, BAN/mAb158 is successfully being assessed in clinical Phase II trial (Moreth et al., 2013).

1.10.5 Gantenerumab

Gantenerumab is an untried fully human anti- amyloid beta monoclonal antibody with a elevated capacity to bind and confiscate beta-amyloid plaques in the brain. It generally targets the N-terminus and central portion of Aβ with high binding affinity to cerebral amyloid plaques. It is presently undertaken for Phase II and III clinical trials as a potent antibody bearing disease transforming prospective in AD (Novakovic et al., 2013).
1.11 Hypothesis

AD is a progressive neurodegenerative disease. Currently, there is no specific treatment strategy for exact cure of Alzheimer's. There are several prominent targets viz neurotransmitters, amyloid beta and tau- proteins which play a major role in the development of this disease and have been frequently targeted in pharmaceutical industries. In this study we have selected two most crucial therapeutic targets BACE1 and AChE for being actively participating into the development of disease at protein –ligand as well as protein – protein interaction level. We have tried to propose novel natural leads effective enough to inhibit BACE1 and AChE in the cure of Alzheimer disease using *in- silico* and *in vitro* platforms.

1.12 Objectives

1. Screening of natural compounds to observe their potential against amyloid beta production and aggregation using molecular docking approach.

2. *In silico* screening of natural compounds to observe their amyloid beta clearance potential.

3. To observe the potency of selected natural compounds against neurotransmitter system using enzoinformatics study.

4. To validate the anti- Alzheimer potential of selected natural compounds in *in-vitro* system.