Chapter 1

Chapter 1

Introduction and Literature Review

1.1. Alzheimer's Disease: A Progressive Neurodegenerative Disease

Neurodegenerative diseases represent a diverse group of disorders primarily associated with age-related factors and are typically characterized by the gradual loss of neurons or progressive neurodegeneration within the central nervous system or peripheral nervous system, having an exceptionally high fatality rate, and are majorly linked to various genetic factors and increasing age (1). The prevalence of such neurodegenerative diseases has seen an exponential rise around the globe, corresponding to the increasing ageing population, imposing significant economic and societal burdens on healthcare systems. Dementia, in particular, has emerged as a critical global health challenge (2).

Alzheimer's disease (AD) is one such progressive neurodegenerative disorder and is most commonly associated with around 60-70% of dementia cases, and is associated with progressive deterioration and gradual degradation of cognition and intelligence (3). Although, such symptoms seem to appear at much later stages of the progression, as such neurodegenerative changes begins as early as 20 years or more before the actual symptoms appear and only evident when severity progressed to the extent where it starts to interfere with the person's ability to perform day-to-day tasks, a person is then diagnosed to have AD (4). About 5% of people between the ages of 65-74 are affected with AD and the number rises to 33.4% for people above the age of 85 suffering from AD. The World Health Organization (WHO) estimates that at present scenario above 48.6 million people are affected worldwide and the number is expected to triple by 2050 if no effective treatments are found (5).

Various underlying factors contribute to the neurodegenerative processes and considered quite complex and diverse in nature and can vary for each patient. The pathophysiological changes in the AD brain begin many years before the actual symptoms become apparent and gradually

develop with time, leading to prominent symptoms (**Figure 1.1**) In the preclinical stage amyloid plaques and Tau tangles start accumulating in the brain leading to subtle pathological changes in the brain displaying no significant signs. Further, advancing to mild cognitive impairment a person may often experience very minute changes in their cognitive ability which may not interfere significantly in their day-to-day activities. But further progression of AD can ultimately lead to severe memory and cognitive impairment moving from mild dementia to moderate dementia due to AD making day-to-day activities a challenging task, confusion deepens, hindrance in communication skills and personality changes occur. Finally, leading to the progression of dementia due to severe AD where there is significant loss of cognitive abilities, loss of motor skills leading to the need for full-time caregiver for the patient (6).

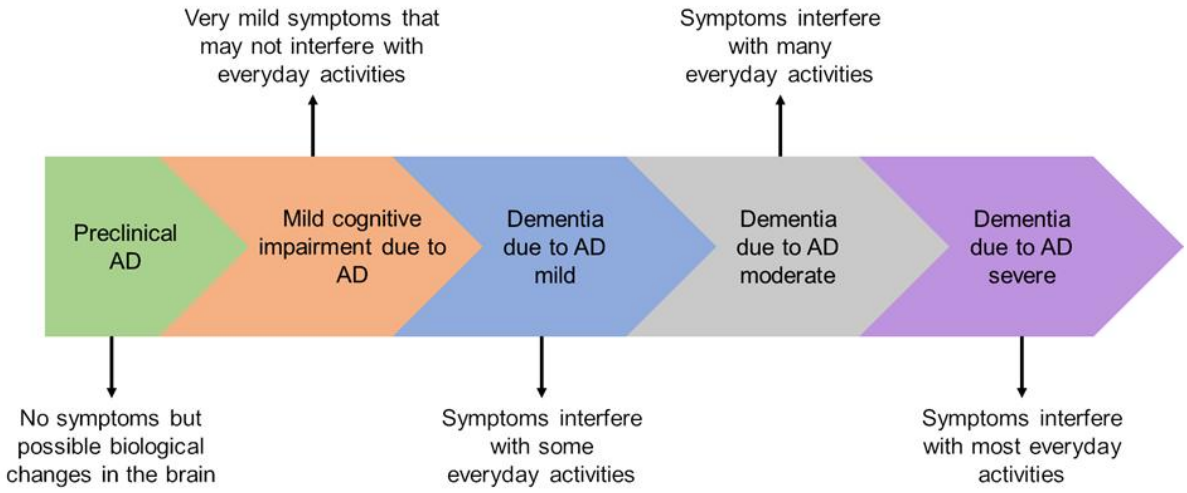


Figure 1.1. Alzheimer's Disease Continuum. The progression of AD from brain changes that are unnoticed by the affected person to the brain changes that leads to the memory impairment and eventually physical disability.

At present only symptomatic treatments are available to manage or halt the progression, which indicates a major shortcoming in the current therapeutics, not addressing the major underlying factor that leads to the progression of such disease. Recent advances in the field of neuroscience have paved a path for researchers by providing crucial insights into the pathogenesis of the neurodegenerative disorders (7). As research progressions are made in elucidating the critical

aspects of the progression of such diseases and its related pathophysiological changes that lead to such condition, therapeutic approach to such condition is gradually evolving and new treatments are being evaluated for targeting the underlying cause of the disease.

1.2. Prevalence of AD

AD possesses a major health burden to the world-wide population in the 21st century, due to the increased life expectancy along with reduced fertility rates as suggested to be the reason for the ever-increasing age structure of the world population, where there is a significant rise in number of elderly people than what it was in the past few decades. Due to the development and upliftment of the healthcare sector these demographic trends can be expected to be carried on with a significant rise in elderly population thus leading to the rise in number of incidences of dementia and AD around the globe (8).

According to the Global Burden of Disease Study (GBDS) 2019, the number of incidences of dementia cases will undergo an exponential rise of 166%, impacting the lives of nearly ~152·8 million individual around the globe; these estimates were similar to the predictions made by WHO. The evolving geographical distribution is an important aspect for the AD demographics. As 58% of the individuals affected with dementia are from low and middle-income countries. However, as we look ahead to 2050, this proportion is predicted to rise to 68%. Due to the increasing numbers of elderly population, the individuals most affected by dementia are increasing in countries like China, India and their counterparts in South Asia and the Western Pacific (9).

The Indian scenario is no more different, the elevation in the number of dementia cases is projected to be one of the highest up to 330%, as it is ranked low on the Socio-demographic index (SDI). Where, SDI is a measurement of the summation of average years of education in individuals aged >15 years, overall fertility rate, and lag-distributed per capita income. India was the 4th largest contributor of the global burden of dementia in the year 2019 and is expected

to surpass Japan and USA to become the 2nd largest contributor to the number of dementia cases next only to China by the year 2050 (10).

The **Figure 1.2A** represents the estimated lifetime risk for AD at the age of 45 is about 1 out of 5 (19.5%) for women and 1 out of 10 (10.3%) for men. Further, the risk factor was slightly higher in both women and men by the age of 65. Based on the recorded deaths of the patients from AD in between 2000-2021, as indicated on the death certificates, there seems to be a surge of 140.9%. When compared to the deaths occurred due to cardiovascular diseases seems to be reduced by 2.1%. Likewise, it can be observed that it kills more than breast cancer (increased by 1.2%) and prostate cancer (increased by 4.8%) combined (**Figure 1.2B**) (5).

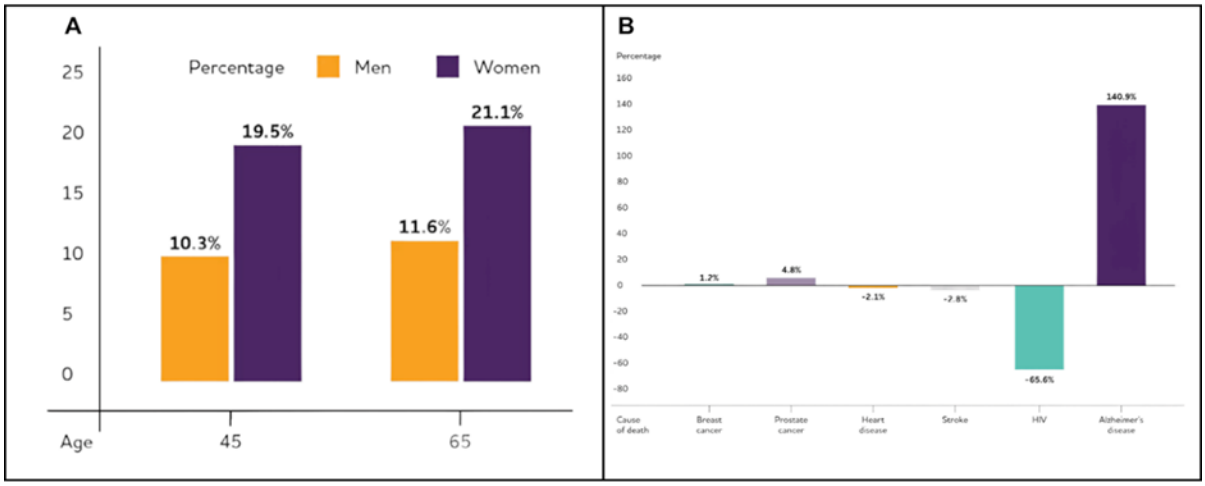


Figure 1.2. Alzheimer's Disease risk in numbers. (A) Estimated lifetime risk for AD, by sex, at the age of 45 and 65 years. (B) The percentage changes in selected causes of death between 2000 and 2024. Reprinted under the Creative Commons Attribution-Non-Commercial-No Derivs License from Reference 2024 Alzheimer's disease facts and figures, Alzheimer's Dement. 2024 (11)

As of the current scenario there is no definitive approach to prevent the progression of AD, and only symptomatic treatment is available which does not cure the disease from its root cause. However, given the large number of populations living with AD and other related dementias worldwide with the continuous growing number of individuals affected by it, the overwhelming

effects of it on the patient and their families, communities and health care systems, have put a huge economic burden (12, 13). Thus, discovery of newer and better drugs that can efficiently prevent, manage and cure AD and other related dementias has become a top priority for researchers all around the world.

1.3. Pathophysiology of AD

AD is classified as a multifactorial disorder and since there are no definitive pathway which leads to the its incidence, likely due to complexity of the disease. Various hypotheses have been proposed that may unravel the pathogenesis of AD. It can be broadly classified into two categories familial form which accounts for about 1-5% of the total AD cases and sporadic form accounting for over 95% of such cases (14).

Familial AD (FAD) occurs due to the autosomal dominant genetic mutations in amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) genes, usually progresses rapidly and is manifested between 30-65 years of age (15, 16). Whereas, sporadic AD (SAD), typically unfolds at the later stage of life typically after the age of 65 and is generally influenced by multiple factors related to genetic, environmental, and various other comorbidities (17). Genome-wide association studies (GWAS) and genome-wide meta-analyses have pin pointed various genetic risk factors related to SAD, indicating pathways in immune response, lipid metabolism, amyloid beta (Aβ) plaque formation, NFTs formation, and endocytosis, still various factors are yet to be discovered. Various non-genetic factors like sedentary lifestyle, psychosocial factors, environment surroundings may heighten the risk of developing AD. Thus, making it a challenging task to pinpoint the direct root cause of AD pathology (18, 19).

Moreover, different AD subtypes (typical and atypical) often display different clinical symptoms. Also, various pathological features like Aβ plaques, neuro fibrillary tangles (NFTs), synaptic and neuronal loss, and neuroinflammation and their occurrence greatly affect the progression of AD (20). Neuronal cell death is particularly rampant in the regions associated

with memory function, like hippocampus, leading to the initial memory loss and confusion and is often related to the preliminary signs of AD. The brain volume starts to shrink with the progression as neurons continue to degenerate, affecting the regions responsible for language, reasoning, and social behavior leading to more pronounced cognitive hindrance and exhibit personality changes, confusion, difficulty with daily task. In the severe advanced stages, most of the cerebral cortex tissues shrink, leading to cognitive and functional impairments to the point where they fail to communicate, recognize loved ones, and or carry out daily activities (21, 22).

Therefore, developing an exhaustive theoretical framework that can link the underlying genetic factors, molecular mechanics, and clinical phenotypes of AD is an ever-ending challenge. Current shortcomings in the field of AD research hinder researchers from understanding its complete pathophysiology. Furthermore, the high failure rate associated with the clinical trials conducted, makes it a challenging task at hand to effectively validate newer hypotheses, primarily due to the fact that multiple theories are attributed to such diseases (14).

1.3.1. Cholinergic Hypothesis

The cholinergic hypothesis is one of the earliest and most significant theory related to the pathogenesis of the AD. Acetylcholine (ACh) is the key cholinergic neurotransmitter associated with learning, memory and other cognitive functions. The central cholinergic nervous system primarily manages the ACh levels by governing the biosynthesis and regulating its release into the synapses (23). Basic forebrain cholinergic neurons (BFCNs) are linked with the cognitive functions like learning and memory. The nerve growth factor (NGF) is responsible for the survival of BFCNs, and are primarily located in the cerebral cortex and hippocampus region of the brain, the region is enriched with the nicotinic acetylcholine receptors (NAChR). There is a significant degeneration of the BFCN in the AD brain and the degree of dementia is directly

related to the synaptic loss between the basal forebrain and the target tissues of the hippocampus and cortex, primarily responsible for the loss of memory (24). The brains of AD patients exhibit severe neurodegeneration and decline in cholinergic neurons which ultimately leads to the severe deficiency of ACh. This is primarily due to the significant downregulation of the choline acetyltransferase (ChAT) activity in the AD affected patients (25). **Figure 1.3** represents the schematic diagram for the cholinergic hypothesis. Acetylcholinesterase (AChE) is another enzyme responsible for breaking down acetylcholine into choline and acetate for choline reuptake. Thus, with this understanding of the cholinergic pathway and how its dysregulation occurs in AD brain it led to the discovery and development of several AChE inhibitors, which prevents the breakdown of already available acetylcholine in the synapse, temporarily improving the cholinergic neurotransmission. Thus, providing with symptomatic treatment for improving cognition and functioning of neurons but does not modify or cure the root cause of AD (26).

AChE can also directly bind to PS-1 enzyme, linked to the process of Aβ production by enhancing its expression, leading to the increased levels of Aβ, which gradually contributes to the progression of cognitive dysfunction. Also, aberrant changes in the central cholinergic system can induce abnormal phosphorylation of the Tau protein, nerve cell inflammation, cell apoptosis, neurotransmitter and neurohormone system imbalance and other pathological anomalies, but the understanding of the mechanism of action is still a mystery to the researchers (27).

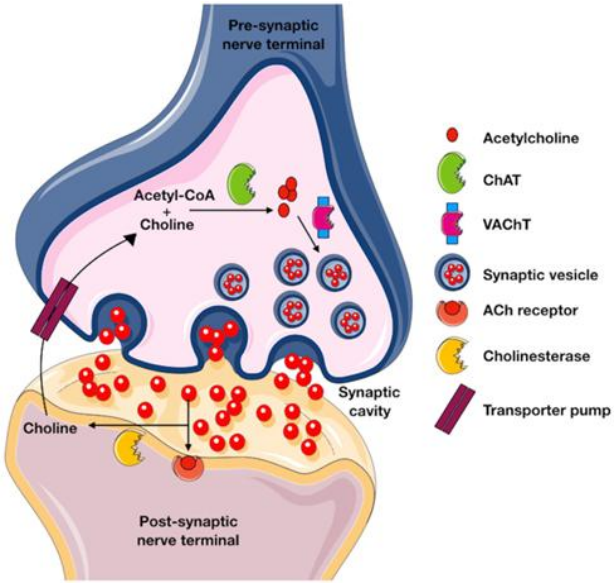


Figure 1.3. The Cholinergic hypothesis in AD. It suggests that a deficiency in the neurotransmitter acetylcholine plays a central role in the disease's cognitive symptoms. In AD, the brain's cholinergic neurons degenerate, reducing acetylcholine levels, impairing memory and cognition. Reprinted with permission from Bagwe et al. Copyright, License Number 5938630748306, dated Dec 30, 2024, Current Molecular Biology Reports (28).

1.3.2. Amyloid Cascade Hypothesis

Aβ peptide consists of about 37-49 amino acids that are generated from the metabolic breakdown of the APP, an integral transmembrane glycoprotein primarily found in the central nervous system (CNS) playing a crucial role in a wide aspects of bio-activities like development of nerve cell, signalling and other aspects necessary to maintain homeostasis within the neurons (29). APP is generally cleaved by β-secretases and γ-secretases to produce amino acid residue peptides, Aβ, that plays the central role in the amyloid cascade hypothesis and the amyloidogenic fibrillary forms are the key components in the formation of amyloid plaques found in the AD brain (30).

Metabolic breakdown of APP follows two different biochemical pathways namely amyloidogenic and non-amyloidogenic pathways. The first step of it involves the enzymes α-secretase (nonamyloidogenic pathway) or β-secretase (amyloidogenic pathway), which cleaves

the APP producing membrane-tethered α- or β-C terminal fragments (CTFs). Further in the second stage these formed α- and β-CTFs undergo an intramembrane cleavage by γ-secretase, generating P3 (3 kDa) and Aβ (4 kDa) peptides respectively. Thus, the amyloidogenic breakdown of APP occurs due to the subsequent cleavages by β- and γ-secretase at the N and C termini of Aβ, respectively. The 99-amino-acid C-terminal fragment of APP (C99) which is produced by β-secretase cleavage of APP can be internalized and processed by γ-secretase at multiple sites to generate fragments of 43, 45, 46, 48, 49 and 51 amino acids that are further cleaved to produce the final forms of Aβ, namely the Aβ40 (40-amino-acid) and the Aβ42 (42-amino-acid), in endocytic compartments. C99 cleavage by γ-secretase results in the formation of APP intracellular domain (AICD), which regulates gene expression by translocating to the nucleus for the induction of an apoptotic genes. The cleavage of APP/C99 by caspases produces a neurotoxic peptide (C31). The abundance of β-site APP cleaving enzyme in neurons can accelerate the amyloidogenic pathway of APP degradation in the brain resulting in neuronal impairment. Both α- and β-secretase have an opposite effect in the generation of Aα and Aβ due to their competitive cleavages of APP. Protein kinase C stimulation can upregulate the activity of the α-secretase and decrease the generation of Aβ (**Figure 1.4**) (31).

Thus, a homeostasis between the two biochemical pathways must be maintained for the anabolism of Aβ. The key differences seem to be that the Aβ peptides, and most of its oligomers are neurotoxic, whereas Aα displays neuroprotective role. Therefore, a physiological imbalance of this mechanism due to the ageing factor may be the starting point for the overproduction of Aβ peptides. Aβ accumulation can further interfere with the axonal transport of acetylcholine in the cholinergic neurons, hinder ACh release and may ultimately lead to the cholinergic neuronal cell death (29, 32).

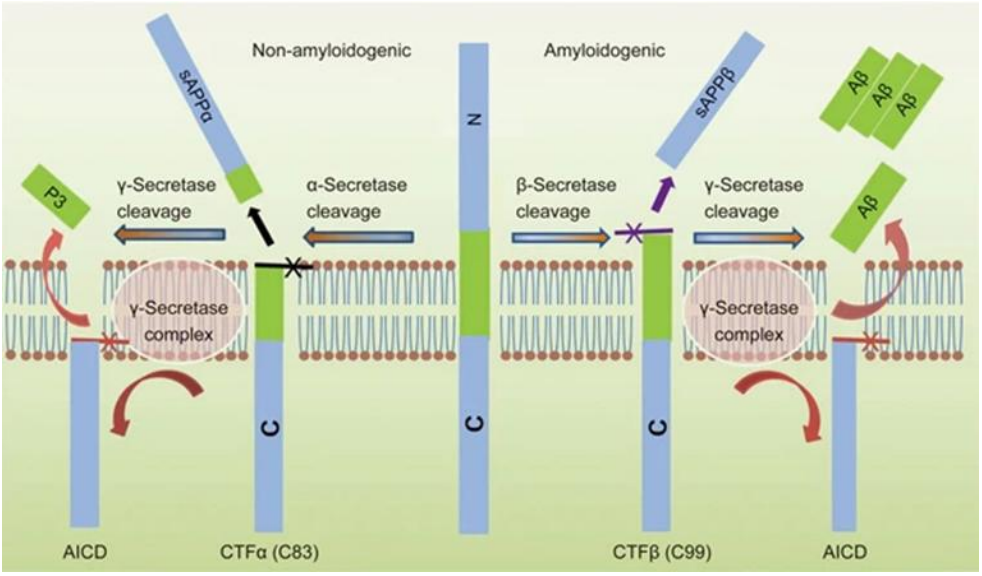


Figure 1.4. The amyloid beta cascade. The processing of amyloid precursor protein via two main pathways: non-amyloidogenic and amyloidogenic. In the non-amyloidogenic pathway, APP is sequentially cleaved by membrane-bound α-secretases, which cut within the Aβ region, resulting in the generation of two fragments: the membrane-anchored α-C-terminal fragment (CTFα or C83) and the soluble N-terminal fragment (sAPPα). Subsequently, CTFα is processed by γ-secretases, producing extracellular P3 and the AICD. Conversely, the amyloidogenic pathway involves the stepwise cleavage of APP by β-secretases followed by γ-secretases. β-Secretase activity generates the membrane-associated β-C-terminal fragment (CTFβ or C99) and the soluble N-terminal fragment (sAPPβ). The subsequent action of γ-secretases on CTFβ leads to the formation of extracellular Aβ peptides and AICD. Reprinted with permission from Bagwe et al. Copyright, License Number 5930700717288, dated Dec 16, 2024 Acta Pharmacologica Sinica (29).

1.3.3. Tau Hypothesis

Tau is one of the microtubule-associated proteins that falls under the classification of intrinsically disordered proteins. It is responsible for maintaining the stability of the tubulin assembly, is a part of the cell's cytoskeleton and is vital for maintaining the cell shape and cell

division. Tau, belonging to the family of intrinsically disordered proteins, it does not retain a fixed three-dimensional geometrical conformation when not bound to the microtubules (33). The human Tau gene is localized in chromosome 17. mRNA alternative splicing, with or without exons 2, 3, and 10 results in six different Tau isoforms that are expressed in the adult human brain, where exon 10 contains the microtubule-binding region (34). Inclusion of the exon 10 produces 4-repeat (4R) Tau isoforms, whereas deletion of exon 10 produces 3-repeat (3R) Tau isoforms. 3R and 4R Tau isoforms are expressed in a healthy adult brain and are primarily located in the axonal part of the neurons under normal physiological conditions stabilizing the microtubule assembly. Whereas, during the diseased state, the 3R and 4R Tau are hyperphosphorylated by the kinase enzyme and begins to accumulate (35). Ultra structurally, unique twisted fibrils with ~80 nm periodicity appearing as paired helical filaments (PHFs) or related straight filaments (SFs) are observed which collectively form the neurofibrillary tangles NFTs in neuronal cell bodies, while they are attributed to as threads if they are formed in dendrites or axons (36). These formed tangles can disrupt the normal functioning of the neuronal signal transport and impair cellular functioning, ultimately progressing towards neurodegeneration and cognitive decline (**Figure 1.5**). The underlying mechanism by which NFTs cause neuronal cell death isn't completely understood, but the presence of NFTs directly correlates with the severity of the loss of cognition in AD patients (37).

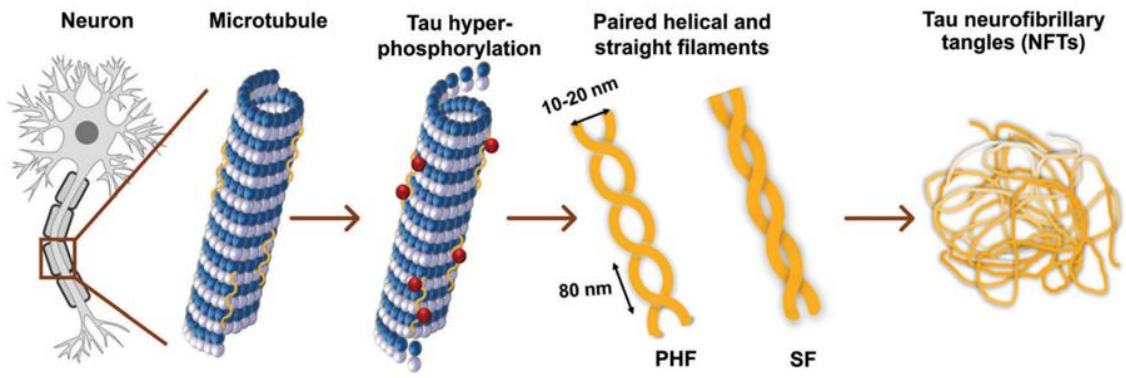


Figure 1.5. Tau hypothesis in AD. Normally, Tau helps stabilize microtubules, which are essential for neuronal structure and function. However, in AD, Tau proteins become hyperphosphorylated due to the action of kinases and phosphatases, causing them to detach from microtubules and aggregate into NFTs. These tangles disrupt neuronal transport and impair cellular function, ultimately leading to neurodegeneration and cognitive decline. Reprinted with permission from Verwilst et al., Chem. Soc. Rev. (38).

1.4. Current Diagnostics for AD

AD diagnosis is primarily divided into a two-step process where the first preliminary step consists of diagnosing the patient for dementia and differentiating it from other psychological factors like delirium, depression, concomitant physical illness, the effects of a severely impoverished environment, and the normal memory loss that are associated with aging. Once, the preliminary diagnosis confirms the patient is having dementia as a clinical syndrome, the second step consists of identifying the root cause of the dementia. AD being the most common cause followed by other neurodegenerative disorders like vascular dementia, Lewy-body dementia, and frontal lobe dementia. The first noticeable signs and symptoms in patients suffering from AD are manifested as decline in cognition, disorientation in time and place, confusion and impaired memory (39).

Since, such neurodegenerative disorders are multifactorial in nature and very limited understanding of its pathophysiology and root cause of appearance is not yet properly understood, making it a challenging task for finding an early-stage and more effective ways to diagnose AD. Tremendous amount of research and scientific advances have been made in the last few decades with expanded use of positron emission tomography (PET) and magnetic resonance imaging (MRI), as well as in the identification of biomarkers in cerebrospinal fluid (CSF) and more recently serum. Despite the efforts, only limited number of advanced

diagnostics are available to the public at elevated prices due to the complexity of the disease and diagnostic procedures (40). Thus, precise diagnosis of such neurodegenerative disorder is paramount for the proper therapeutic management and prognosis of AD.

Neuroimaging techniques have gained tremendous attention given to the fact that it is a non-invasive technique used to unravel the hidden specifications about the pathophysiological changes of the brain. Multimodal imaging methods have emerged to (differentially) diagnose AD and have significant advancements in the field of neuroimaging in the past two decades. Aβ-PET, and more recently Tau PET which can visualize and quantify molecular pathology at an early stage are two most popular biomarkers used for the imaging of AD brain. Also, the early synaptic dysfunction and neuronal injury, can be measured with FDG-PET as the functional imaging biomarkers (41, 42).

Amyloid pathology assessed through PET imaging is a well-established prognostic biomarker in individuals with mild cognitive impairment (MCI) with a sensitivity of 82% (95% confidence interval (CI)) and a specificity of 56% (95% CI) for distinguishing patients with stable MCI from those who progress to dementia. Among cognitively normal individuals, the amyloid positivity has been directly linked to an elevated risk of cognitive decline and eventual progression to dementia (43). However, to classify individuals as amyloid-positive or amyloid-negative is yet a topic to debate. Emerging evidence indicates that amyloid plaques tend to accumulate in specific brain regions, leading to the upstream of amyloid pathology. Although amyloid positivity has been well-established and linked to the subsequent cognitive decline in cognitively normal individuals, the rate of progression appears to depend on the presence and extent of neurodegeneration. Furthermore, amyloid positivity well correlates with the higher rates of brain atrophy in affected individuals. While amyloid pathology is a critical factor in determining the likelihood of person affected with AD, it alone is insufficient for concluding

the presence of AD as various other factors are needed to be considered like predicting the timing and progression of cognitive impairment, as these are strongly influenced by the rate of neurodegeneration (44).

The deposition of NFTs, composed of hyperphosphorylated and aggregated Tau proteins, has a better biological correlation with the neuronal loss and is highly evident with the timing and severity of clinical symptoms of AD as compared to Aβ aggregation (45). Recent advancements in Tau PET imaging have enabled the researchers to visualize the Tau pathology, though some limitations still persist which needs to be overcome like the development of Tau tracers has been a challenging task due to Tau's intracellular localization, its low abundance, and the presence of multiple isoforms of Tau (46). Early Tau tracers exhibited off-target binding in regions such as the basal ganglia and choroid plexus. However, newer Tau tracers have been developed to have improved specificity, minimizing off-target binding in intra-parenchymal regions. Given the strong evidence that NFTs are better correlated to the symptoms associated with onset of AD as compared to Aβ, Tau PET imaging could play a pivotal role for the diagnosis of patients who are at elevated risk of inevitable clinical decline (47).

In AD related dementia, characteristic patterns of glucose hypometabolism is detected using fluorodeoxyglucose (FDG)-PET and can also predict the progression to dementia in individuals with MCI (48). However, FDG-PET imaging alone cannot confirm and rule out AD dementia in patients with MCI but in case of a cognitively normal person, baseline hypometabolism in lateral temporo-parietal and posterior cingulate-precuneus regions has been shown to predict the onset of MCI or dementia with great success rate of about 70–80%. Although FDG-PET offers potential as a biomarker for clinical trials, the observed changes in cognitively normal person are often subtle and unnoticeable and in case of abnormal brain or person with MCI base threshold have not yet well established (49).

1.5. Need of New and Effective Diagnostic Biomarker for AD

AD is a slowly progressive neurodegenerative disease with no available effective treatment. It is possible to distinguish an early-onset AD that affects a limited number of subjects of young age, and a sporadic or late-onset form of the disease that affects the vast majority of subjects who are diagnosed with AD. As life expectancy has increased considerably over the past century, the number of people diagnosed with AD has grown exponentially. So, AD and AD-related pathologies represent a huge social and economic burden. Additional investigations are much needed to help identify the very early signs of AD, better understand the course of the disease, and, most importantly, clarify which biomarkers reliably predict AD development and which may be linked either to other conditions or to normal brain aging (50, 51).

Many Aβ and Tau PET tracers are already in use for the clinical diagnosis of AD, but still have room for major improvements with respect to lack of imaging biomarkers with high affinity and selectivity (52). In the current scenario, various imaging procedures undoubtedly focus on the detection of the abnormal features of the AD, [^{18}F]fluoro-2-deoxy-D-glucose (FDG) PET has been relied upon for many years to diagnose AD by tracking the levels of cerebral hypo-glucose metabolism in brain (48). Furthermore, three Aβ tracers, [^{18}F]flutemetamol (Vizamyl), [^{18}F]florbetaben (AV-1, Neuraceq), and [^{18}F]florbetapir (Amyvid) have been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for clinical use (53). Also, for tracking the deposition of Tau tangle several first generation Tau tracers like [^{18}F]FDDNP, [^{18}F]AV1451 (also called [^{18}F]flortaucipir), and [^{11}C]PBB3, and the 2-arylquinolines derivative tracers include [^{18}F]THK523, [^{18}F]THK5105, [^{18}F]THK5117 [^{18}F]THK5317, and [^{18}F]THK5351 have been developed among which [^{18}F]flortaucipir, is the first PET Tau tracer that was recently approved to detect Tau inclusions in AD brain by the FDA. With the scientific advancements, second generation Tau tracers with improved binding

properties have been developed giving rise to [^{18}F]MK6240, [^{18}F]PM-PBB3, [^{18}F]RO948, [^{18}F]PI-2620, [^{18}F]JNJ311, and [^{18}F]Genentech Tau Probe 1 (GTP1) (54).

To date, there have been several approved Aβ tracers, and emerging Tau PET tracers with improved specificity and binding properties for the detection of NFTs in AD. Amyloid and Tau PET helps to uncover the interplay between Aβ, Tau and neurodegeneration in longitudinal studies of the disease progression. Aβ PET has been established as diagnostic tool for assisting clinical diagnosis, while the diagnostic value for Tau imaging has yet to be further demonstrated. Finding an optimal imaging biomarker remains a demanding task, as there are several prerequisites for PET tracers targeting at central nervous system, including structural requirements such as the size to pass the blood brain barrier (BBB), pharmacokinetic properties, sensitivity and stability of the chemical for imaging. Off-target binding of Tau radiotracers, e.g., to Monoamine oxidase B (MAO-B), is a concern for first-generation Tau PET tracers. Other off-target binding sites like [^{18}F]MK6240 tends to bind to neuromelanin and melanin-containing cells (55-57).

PET imaging of Aβ and Tau has enabled the early and definitive diagnosis of AD and monitoring of disease progression. Although various Aβ PET tracers are in preclinical research and a few for clinical use, only one Tau PET imaging biomarker is currently in the clinic. With the above scientific progression of the neuroimaging of the various biomarkers of AD pathophysiology, **Figure 1.6.** represents the appearance of the hallmark biomarkers with respect to the progression of the AD, where it can be seen that the neurodegeneration starts 20-30 years before the appearance of any visual symptoms of AD (58) and most of the hallmarks seems to be significant in the later stages of the progression where the patient has already progressed to the mild cognitive impairment. Often leading to delay in the therapeutic

interventions which otherwise might have halt the progression earlier improving the quality of life (59).

Therefore, a more definitive suitable biomarker for the early-stage detection of AD is of utmost importance. This calls for an urgent scientific advancement in the field of AD brain imaging as to identify more definitive biomarkers and develop improved and more sensitive PET imaging tracers for the early-stage diagnosis of AD.

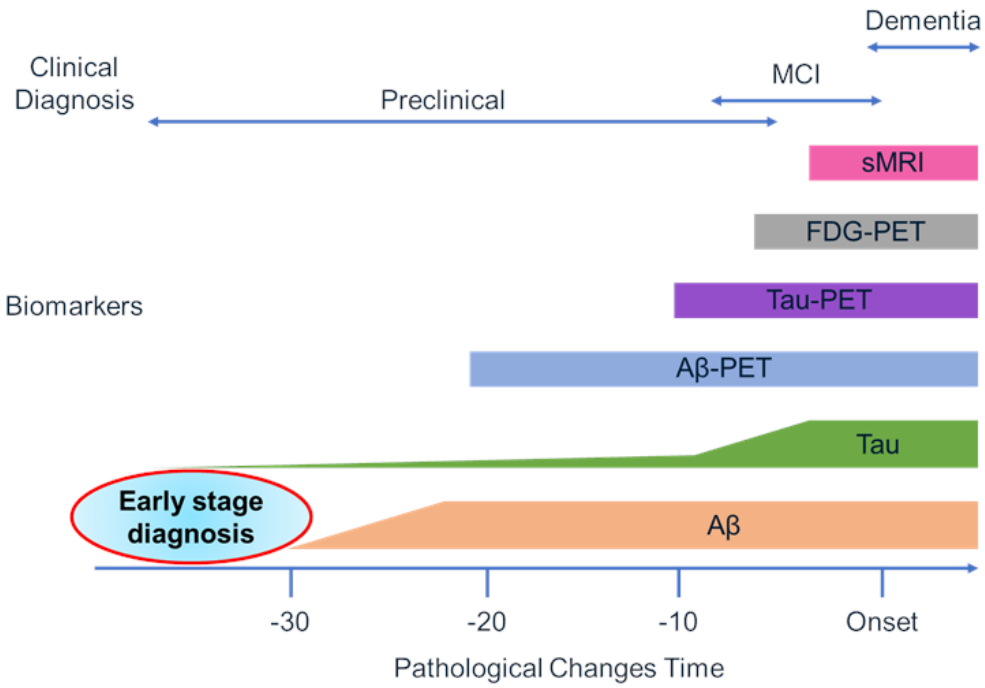


Figure 1.6. The AD continuum with corresponding pathological changes, biomarkers and clinical diagnosis.

1.6. Choline Acetyltransferase as a Potential Biomarker

ACh is the first ever reported neurotransmitter and is used by all the cholinergic neurons, playing a key role in the signal transduction within the peripheral and central nervous systems (60). The cholinergic neurons are found in almost all the regions of the brain and given the vast distribution pattern, any dysfunction in its regulation can significantly impact on the normal functioning of the CNS. The cholinergic system is generally associated with the crucial

physiological processes, like attention, memory, learning and sensory information. It has been well documented that the cholinergic system plays a significant role in the memory and learning process. Thus, backing the fact that these neurons are degenerated in AD, contributing to the overall memory loss in the AD patients (61, 62).

Cholinergic neurotransmission revolves around the homeostasis of the Ach synthesis, storage, transportation and degradation. ChAT is the key cholinergic biosynthesizing enzyme found in the cytoplasm of the cholinergic neurons where is utilizes choline and acetyl-coenzyme A (acetyl-CoA) to produce ACh, followed by its transport with the help of vesicular acetylcholine transporter (VAChT) from the cytosol into synaptic vesicles. Finally, when the cholinergic neurons are depolarized, ACh is exocytosed from synaptic vesicles and released into the synaptic cleft, to activate both the muscarinic and nicotinic receptors (63). Also, the excess ACh present at the synaptic cleft is rapidly broken down by another enzyme AChE liberating choline and acetate. For each molecule of AChE, 5000 molecules of ACh are hydrolyzed, making it one of the most kinetically efficient enzymes. The released choline from the ACh hydrolysis is simultaneously reuptaken into the presynaptic cholinergic neuron by an active transport system from the synaptic cleft for the synthesis of new molecules of ACh (64).

Any downregulation in the cholinergic pathway can interfere with all the aspects of cognition and memory, primarily in the cortex and hippocampus region. Cortex is responsible for the attention and decision-making skills needed in daily tasks. AD brain has been extensively studied and reports indicates signs of severe neurodegeneration and reduction of cholinergic neurons and a severe deficiency of ACh levels; denoting the downregulation of ChAT activity (65). Thus, restoration of the cholinergic neuronal health after injury is currently a clinical therapeutic approach towards the treatment of AD and is a valid biomarker for the development of potential PET tracers for monitoring the health of cholinergic neuron.

1.7. Structure of Choline Acetyltransferase

ChAT is a 69 kDa enzyme, a relatively big and complex monomeric protein that is encoded from six different mRNAs, termed H-, M-, N1-, N2-, R-, and S-ChAT. ChAT belongs to the family of the CoA-dependent acyltransferase superfamily. In such group of protein, the active site is located deep within the interface between two domains and is only reachable to the substrates through the tunnels at the entry points on either side of the enzyme surface (66). The synthesis of acetylcholine by ChAT occurs in series of reaction where the acetyl group present on the acetyl-CoA is transferred to the choline, where acetyl-CoA acts as a leading substrate, where an essential amino acid HIS324 in hChAT is known to be the catalytic site of the reaction taking place (67, 68).

The hChAT structure have been resolved by the molecular replacement using the rat enzyme coordinates as reference structure. hChAT consists of two domains, each comprising of a six-stranded β-sheet surrounded by α-helices, with the same pseudo-2-fold symmetry noted originally for the related carnitine acetyltransferase. The catalytic amino acid HIS324 lies in between the interface of these two domains, at the center of the tunnel that runs across the enzyme (**Figure 1.7**). The binding domain consists of amino acid residues 1 to 89 and 392 to 615, making most of the interactions with the two endogenous substrates, acetyl-CoA and choline, during the reaction. The catalytic domain makes up of amino acid residues from 90 to 391 including the HIS324 and other residues that are important for catalysis. The choline entrance site of the tunnel is more than large opening required to accommodate the choline, accompanying a vestibule with significant negative electrostatic potential, adjacent to the opening of choline binding site. This may be due to the fact that choline is a cation and the negatively charged vestibule may play a vital role is attracting the choline molecule into the close vicinity of the choline entrance site in order for the reaction to occur for biosynthesis of

acetylcholine (63). Likewise, the CoA binding site of the tunnel is primarily governed by the pyrophosphate and 3′-phosphoadenosine moieties lie on the surface of a shallow and opening groove on the binding domain. Amino acid LYS403 and LYS407 that possess positively charged side chain can interact directly with the negatively charged 3′- phosphate of CoA. Also, it is evident that the CoA binding site has a neutral or negative electrostatic potential, the surface surrounding the tunnel entrance for CoA has a relatively strong positive electrostatic potential. CoA, when binds to the tunnel of hChAT brings about conformational changes, rotating the catalytic domain by approximately 1.5° about an axis that runs through the active site of the enzyme. Such conformational changes are not observed in the ChAT-choline complex. Thus, CoA binding is causing a small but significant domain movement of the hChAT which might be necessary for the forward reaction of the biosynthesis of acetylcholine (63).

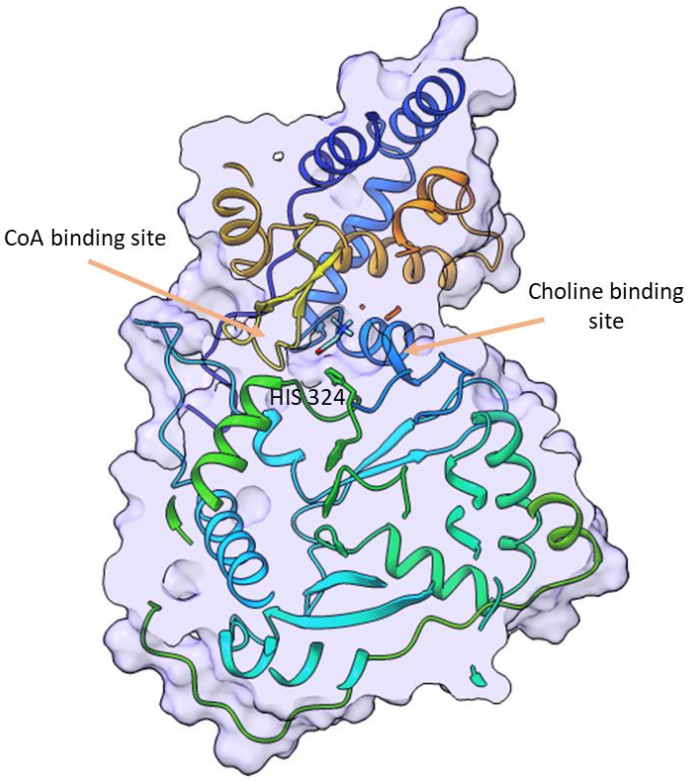


Figure 1.7. Structures of ligand bound human choline acetyltransferase (PDB ID = 2FY3). Showing the catalytic amino acid residue HIS324 along with the CoA and the choline binding site in the binding tunnel of ChAT.

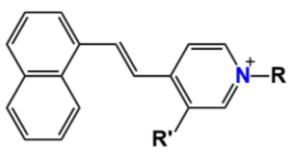
1.8. Previously Reported Choline Acetyltransferase Inhibitors

ChAT was first described by David Nachmansohn and A. L. Machado in 1943. Despite the huge potential to be a valid drug target and several extensive research there have been very limited number of ChAT inhibitors reported in the literature due to which validating the ChAT as a drug target have not been successful yet. Despite the shortcomings, some potent groups of inhibitors of ChAT have been reported in the literature.

Naphthylvinylpyridine (NVP) is a naphthalene derivative possessing potent anticholinergic activity where its mechanism of action suggests the inhibition of ChAT enzyme. Quaternary derivatives of 4-(l-naphthylviny1)-pyridine (NVP) are considered to be the most potent inhibitors of the enzyme ChAT. The first series of NVP analogues were reported by Cavallito et al. in 1967 (69).

DeBernardis et al. in 1987, reported their investigation on the side arm of (Naphthylviny1) pyridinium inhibitors and its effect on ChAT inhibition, about 30 NVP derivatives were reported and all of them displayed potent ChAT inhibition, with varied specificity measured by the IC_{50} AChE/IC_{50} ChAT ratio. Among the series, the most potent NVP inhibitors of ChAT were alkyl and amide derivatives. Alkyl derivatives were found to possess the best potency among the synthesized series, compounds **1-4**, containing a lipophilic side arm. Likewise, the simple amides like compounds **5** and **6** displayed equivalent potency close to the alkyl derivatives also having the best selectivity to ChAT among the reported series (**Table 1.1)** (70).

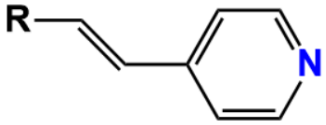
Table 1.1. List of NVP derivatives with selective ChAT inhibition.



Compound	R'	R	X	IC_{50} ChAT X 10^{-7} M	IC_{50} AChE X 10^{-7} M	IC_{50} AChE / IC_{50} ChAT
1	H	CH_3	I	3.9	300	77
2	H	CH_2CH_3	Br	8.6	151	18
3	H	$CH_2CH_2CH_3$	Br	1.9	175	92
4	H	$CH_2CH_2CH_2CH_3$	Br	3.7	39	11
5	H	CH_2CONH_2	I	2.7	800	296
6	CH_3	CH_2CONH_2	I	3.4	450	132

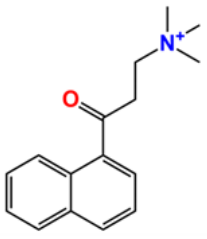
Baker et al. in 1970, reported 4-stilbazole derivatives as irreversible inhibitors of ChAT. Total 43 analogues were synthesized and tested against ChAT from rabbit brain among which the most potent compound from the series was compound **7** (3',4'-dichloro-4-stilbazole) and was 230 times more potent than the parent compound 4-stilbazole and 910 times more effective than the substrate choline. Some other promising compounds from the series, compound **8-10** having 80- and 130- times more potent than the substrate choline were also identified (**Table 1.2**) (71).

Table 1.2. List of stilbazole derivatives with selective ChAT inhibition.

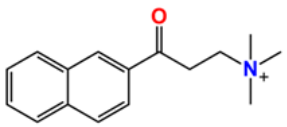


Compound	R	IC_{50} ChAT (μM)	IC_{50} AChE (μM)
7	3,4-$Cl_2C_6H_3$	1.1	> 120
8	3-ClC_6H_4	7.8	1000
9	3-$CH_3C_6H_4$	12	>240
10	3-$CH_3OC_6H_4$	13	1000

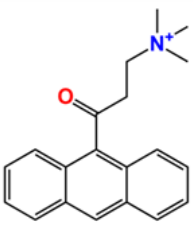
Rama Sastry et al. in 1988, reported potent 2-(α-naphthoyl) ethyltrimethylammonium (α-NETA) derivatives as potent ChAT inhibitors, among the series four quaternary ammonium compounds, α-NETA, 2-([β-naphthoyl)ethyltrimethylammonium (β-NETA), 2-(9'-anthroyl)ethyltrimethylammonium (9'-AETA) and 2-benzoylethyltrimethylammonium (BETA) were found to be potent inhibitors of ChAT with IC_{50} value of α-NETA, 9; β-NETA, 76; 9'-AETA, 32; BETA, 3.1 μM. Thus, α-NETA and related derivatives exhibit good potency of inhibition against ChAT (72).



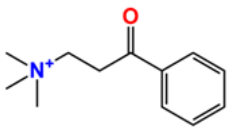
11 α-NETA



12 β-NETA

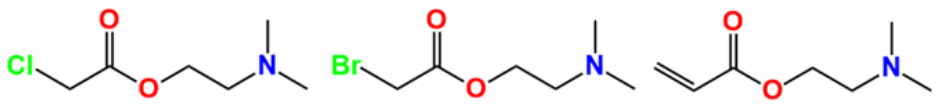


13 9'-NETA



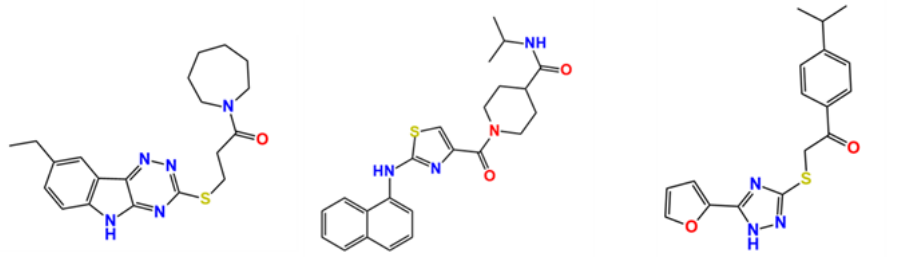
14 BETA

Rowell et al. in 1975, reported some tertiary alkylaminoethyl esters having potent ChAT inhibitory activity. Where the SAR study indicated three critical criteria, a terminal cationic head on the amine terminal, ability to stabilize the partial negative charge on the acyl end and the α-carbon on the acyl end must have a leaving group attached. The three potent derivatives **15** (N,N-dimethylaminoethyl chloroacetate (Cl-DMA)), **16** (N,N-dimethylaminoethyl bromoacetate (Br-DMA)) and **17** (N,N-dimeihylaminoethyl acrylate (acryl-DMA)) had cell free ChAT extracted from brain acetone powder IC_{50} (M x 10^{-4}) value of 1.29, 5.80 and 5.02 respectively. Displaying reversible and uncompetitive nature of inhibition respect to the substrate. Cl-DMA and acryl-DMA were also tested against AChE and butyrylcholinesterase (BuChE) and were found to be 20- 150-fold and 3- 5-fold less potent respectively compared to ChAT inhibition (73).



15 Cl-DMA **16** Br-DMA **17** acryl-DMA

Kumar et al, in 2017 identified and reported some novel inhibitors of ChAT using in-silico structure based virtual screening of Asinex Gold/Platinum small molecule library. Which were tested for in vitro potency among which compound B1 (ASN 07441713) was found to be the most potent and displayed IC_{50} value of 7.02 µM and K_i value of 9.44 µM against ChAT. Two other compounds also emerged as hits, B4 (BAS 11101702) with IC_{50} of 16.50 µM and K_i value of 11.93 µM against ChAT and compound E1 (BAS 03014741) with IC_{50} of 15.18 µM and K_i value of 25.37 µM against ChAT. The compounds were also tested against AChE and BuChE and did not inhibit any of the two enzymes indicating selectivity towards ChAT enzyme (74).



18 B1
ChAT IC_{50} = 7.02 µM

19 B4
ChAT IC_{50} = 16.50 µM

20 E1
ChAT IC_{50} = 15.18 µM

1.9. Computational Modeling in Drug Design

The drug discovery and development process involve a series of rigorous and complex process which is a time consuming, risky and costly affair to begin with and may take up to 10-15 years with approximated investment of US$800 million (75). With the above scenario, computational modeling, termed as computer aided drug design (CADD) comes into play and is extensively applied to the field of drug discovery revolutionizing the process and has a pivotal role in the early-stage drug discovery process to accelerate the early-stages of drug development. Thus, accelerating the process of identification of novel hit-to-lead molecules and novel drug candidate while significantly reducing the associated cost and time as compared to the traditional methodologies (76). CADD can be defined as a battery of computer-assisted techniques used for the design and discovery of novel compounds possessing desired properties to achieve a desirable action. It includes a variety of processes which involves target protein identification, virtual screening of small molecule database, optimizing lead molecules and evaluation of new compounds for their potential ADMET properties (77). Which can efficiently and precisely reduce the number of hit compounds that are to be evaluated in wet lab experiment, ultimately leading to the smarter utilization of available resources and focus on novel scaffolds having the best potential to be developed into a drug, alleviating the time and cost involved with the traditional wet lab experimental procedures (78).

Based on the principles of the in-silico drug screening, CADD can be broadly classified into structure-based drug design (SBDD) and ligand-based drug design (LBDD) (79). SBDD methods can be categorized to be having three major techniques namely molecular docking, molecular dynamics (MD) simulation and de novo drug design (80). Molecular docking protocol aids in deducing how the small molecules bind with the target protein of interest and docking a library of compound researchers can identify the best compounds with highest binding affinity, rapidly screening a large number of compounds saving the wet lab resources and narrowing down the search from a large library (81, 82). Complimenting the molecular docking studies the results can be further evaluated using the MD simulations unraveling the dynamic behavior of the complex formation between the compounds under investigation with the target protein with respect to time, aiding researchers understand the stability and feasibility of the drug target interactions (80, 83). LBDD method consists of quantitative–structure–activity relationship (QSAR) model, similarity searching, and pharmacophore modeling. QSAR correlates the chemical structures of compounds with their biological activities, allowing researchers to predict the activity of new molecules based on known compounds. Pharmacophore modeling, on the other hand, identifies the essential chemical features necessary for a molecule to interact with a specific target (84).

CADD has emerged to be an indispensable tool in modern drug discovery. Its primary objective is to virtually evaluate extensive compound libraries, identifying initial hit compounds, advancing them into lead candidates, or optimizing existing leads. This optimization process aims to enhance their pharmacological and physicochemical properties, including absorption, distribution, metabolism, excretion, toxicity (ADMET), and pharmacokinetic (PK) characteristics, thereby transforming bioactive molecules into viable drug candidates (85). Despite some drawbacks associated with CADD due to approximations of results, it has become a cornerstone of drug development due to its capacity to accelerate the early-stages of drug

discovery pipelines by leveraging insights into receptor-ligand interactions, structural optimization, and energy minimization.

However, challenges such as improvement of scoring functions, accounting for molecular flexibility and solvent effects, addressing poorly characterized targets, and enhancing computational performance still persists. Overcoming these limitations requires sustained advancements in chemical and structural biology, bioinformatics, and computational methodologies. By addressing these shortcomings, the efficiency and accuracy of virtual drug discovery tools can be significantly improved, unlocking the full potential of computational approaches in pharmaceutical research (76).

1.9.1. Molecular Docking

Molecular docking is an extensively validated in-silico technique widely applied in the field of drug discovery process. Docking has proved to be a crucial tool at an early stages of drug discovery process to elucidate the mode of binding interactions through which ligands interact with the biologically relevant target protein (86). It involves predicting the binding interactions between the small molecules and a target protein at atomic level. Enabling the researchers to study the underlying molecular mechanics behind the binding interaction leading to the desired change in physiological action. It often requires a high-resolution 3D representation of the target protein as a starting structure to perform docking obtained through techniques like X-ray crystallography, Nuclear Magnetic Resonance Spectroscopy, or Cryo-Electron Microscopy (87).

The primary objective of molecular docking is to predict the binding affinity of ligands to the receptor binding site of interest and to identify the best possible geometrical conformation favored during the binding interaction. The generated binding poses are referred to as the conformations of the ligand molecule within the active cavity obtained from the computationally modelled hypothetical conformation in the molecular docking protocol. The

search algorithm and the scoring function are two important of the docking protocol. It utilizes various fast and accurate search algorithms to predict and generate various favorable conformations for the ligand in the active binding site of the protein target and further a scoring function is used to evaluate the physicochemical properties and the thermodynamic affinity of each generated binding pose and rank then accordingly from most favorable to least favorable pose (88). Ultimately, while molecular docking provides invaluable insights, it is most effective when combined with experimental data and other computational methodologies in the drug discovery journey.

1.9.2. Molecular Dynamics

Molecular dynamics simulations predict the movement of bio-molecular system like protein ligand complexes on atomic level with respect to time under physiological conditions. Capturing the vast range of crucial biologically relevant motions like conformational changes occurring during protein folding and ligand binding process to the target protein, revealing the minute changes at atomic levels associated with the biological significance (83). The basic ideology of MD simulations can be described as the starting three-dimensional geometrical conformation of all the components in the biomolecular system are known at atomic level, thus, one can calculate the force exerted on each atom affecting its spatial position by all the other atoms as a function of time with the help of Newton's laws of motion (89). Thus, unraveling the mechanics of how these biomolecules will undergo minute conformational changes at atomic level, in response to biologically relevant response to factors such as incorporation of a ligand. Furthermore, researchers are estimating the binding affinity of a drug molecule to its target by the help of extensive MD simulation studies, giving insights into how well a potential drug might work. Making MD simulations an advanced and powerful tool in the CADD arsenal (90).

1.9.3. Artificial Intelligence in Drug Discovery

AI has been revolutionizing various sectors from the past few decades and its applicability to the pharmaceutical sector is no different and is accelerating the drug discovery process and making precision medicine possible at the present times as and revolutionizing the pharmaceutical healthcare sector. AI can be termed as a technology driven system comprising of advanced tools and networks that can mimic the human intelligence. Traditional drug discovery is a laborious and resource exhaustive and time taking process, which usually takes more than a decade to bring a new drug to market. However, application of AI to process the ever-increasing pool of chemical database in a fraction of time, identify patterns, and make predictions with precision is significantly accelerating this process, reducing costs, and improving the probability of success (91).

AI can be particularly effective in the early stages of drug discovery, such as target identification narrowing down to the key target protein of interest and screening of large dataset of compounds. Machine learning algorithms can effectively analyze large dataset of complex biological data, such as genomics, proteomics, and clinical datasets, to efficiently pinpoint potential drug targets with high precision. AI can also effectively accelerate the compound screening process from large datasets at the initial phase of drug discovery for hit identification, reducing the need for time-consuming laboratory experiments (**Figure 1.8**) (92).

Furthermore, deep learning models can efficiently predict the pharmacokinetics and toxicity of drug candidates early in the process. This predictive power helps in selecting the most promising compounds and reducing the risk of late-stage failures due to adverse side effects (93, 94). In addition, AI can assist in the design of novel compounds by leveraging generative models, which learn from existing molecular structures to create new molecules with desired properties (95). In clinical development, AI algorithms are being used to design and optimize

more efficient clinical trial protocols and monitor clinical data in real-time for any adverse side effects by identifying suitable patient populations and response to the clinical trial. These applications not only expedite the trial process but also improve the likelihood of trial success (96). In conclusion, AI is revolutionizing drug discovery and development process by enhancing the speed, accuracy, and cost-effectiveness of developing new therapies. From identifying drug targets to optimizing clinical trials, AI-driven technologies are reshaping the drug discovery landscape, offering unprecedented opportunities for the development of safer and more effective drugs (97, 98).

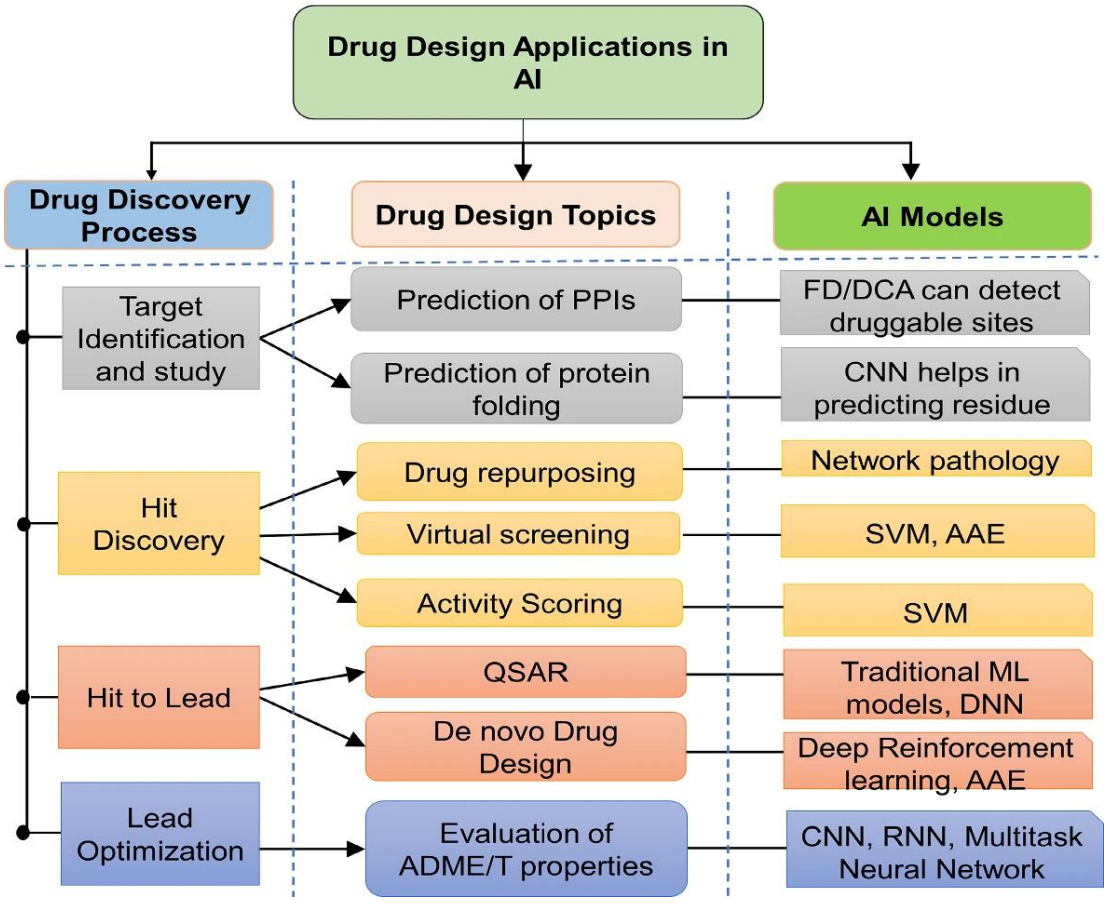


Figure 1.8. Role of Artificial Intelligence (AI) in drug discovery. Reprinted with permission from Reference Dara et al. Copyright, License Number 5930730699733, dated Dec 16, 2024, Artificial Intelligence Review (99).

1.10. Piperidine: a versatile heterocyclic compound

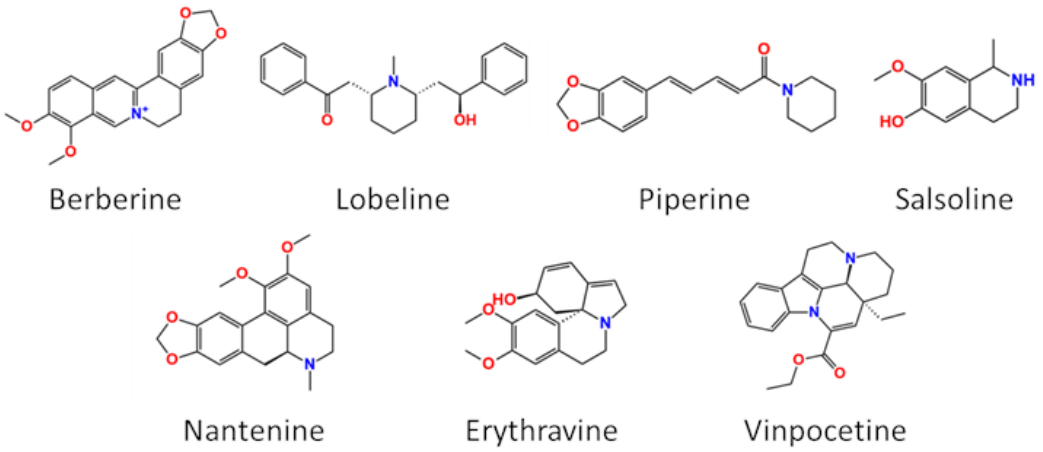
Piperidine also termed as hexahydro pyridine ($C_5H_{11}N$), a six-membered heterocyclic ring containing one nitrogen atom in its core and five carbon atoms that are sp3-hybridized, having a molecular weight of 85.15, liquid with a characteristic odor, miscible with water and soluble in organic solvents, a heterocyclic amine widely found in building blocks of various organic compounds and is a prominent scaffold found in wide range of medicinal compounds. Was first isolated from the alkaloid piperine, extracted from black pepper piper nigrum. Piperidine itself is also present in trace amount in black pepper (0.0086% of dry weight) and also in tobacco, but can be very abundantly found in *Psylocaulon absimile* (4.5% of dry weight), an African toxic plant.

Piperidine and pyridine scaffold are two of the most used heterocyclic fragments making up the FDA approved drugs. Piperidine is considered as one of the crucial scaffolds that is widely explored in the production of drug molecules, piperidine and related byproducts display various important features that can be beneficial in the development of wide range of medications and have successfully found its application in the development of anticancer, analgesic, antimicrobial, anti-inflammatory, and antipsychotic agents. Piperidines can be found in various biologically active compounds like anopterine, pergoline, scopolamine and morphine. Making it one of the most extensively studied compounds for synthetic feasibility as development of newer drugs containing such six-membered heterocyclic ring is becoming more and more successful. Making it an interesting scaffold for the development of newer drug molecules in the field of medicinal chemistry.

1.10.1. Role of Piperidines in Neurodegenerative disease

Most of them play important roles in the human body and have great biologic qualities. Piperidine derivatives play a crucial role in the development of anti-Alzheimer's drugs since

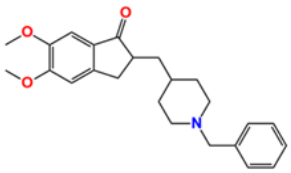
many natural compounds containing piperidine have been found to be active against neurodegenerative diseases. Berberine, an isoquinoline alkaloid have found to be valuable in several neuropsychiatric and neurodegenerative diseases. Likewise, Lobeline an alkaloidal compound containing piperidine ring, obtained from *lobelia inflata*, have been claimed to demonstrate neuroprotective effects by protecting the dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which in turn lowers the nigral dopaminergic levels. Piperine is another naturally occurring alkaloid obtained from long pepper (*Piper longum*) and black pepper (*Piper nigrum*) have been reported to have inhibitory effect on the enzymes acetylcholinesterase (AChE) and β-secretase, displaying cognitive enhancement.



Another isoquinoline alkaloidal member, Salsoline derived from the *Chenopodiaceae* family, is thought to have AChE inhibitory activity, displaying improved cognition in AD. Nantenine derived from the fruit of *Nandina domestica* is an aporphine alkaloid that has been reported to alter the calcium influx causing hyperexcitability, affecting the activity of neurons. Erythravine, an erythrine byproduct obtained from *Erythrina mulungu* flowers, and Vinpocetine, an alkaloid isolated from *Vinca minor* have been claimed to treat cognitive impairment caused by AD.

Various synthetic piperidine based compounds have been developed and studied as potential drug candidates for AD. Gunhild et al. studied the effect of Donepezil on the development of

apathy and other neuropsychiatric disorders in patients with Alzheimer's disease. It was proved that Donepezil could cause a great reduction of apathy that reached over 6 months and thus had a considerable favorable effect(100, 101).



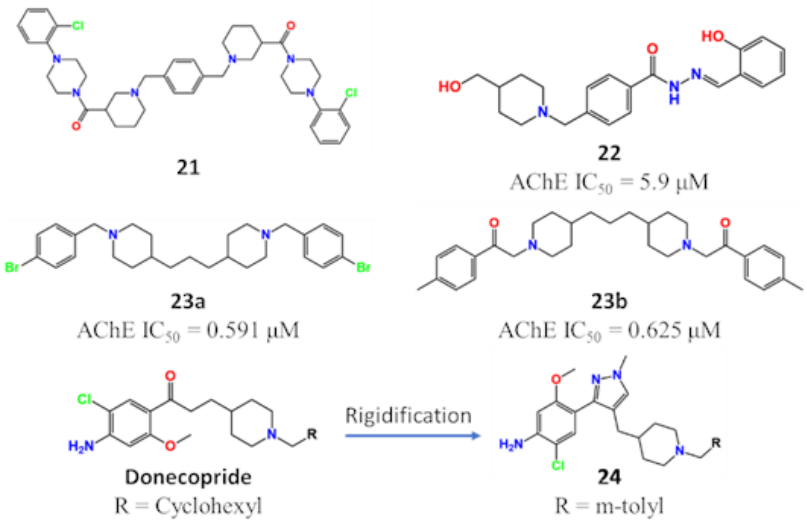
Donepezil

Khairia et al. designed, synthesized and reported a series of new piperidine based compounds having potential inhibitory activity on acetylcholinesterase. These derivatives exhibited potential to be AchE inhibitors as well as had good radical scavenging properties. Compound **21** exhibited the best free radical scavenging properties among the series with % inhibition value of 99.51%(102).

Vaz et al. reported a series of 4-hydroxymethyl piperidine-N-benzyl-acyl-hydrazone derivatives, as multi-target directed ligands (MTDLs). Targeting AChE enzyme, cellular antioxidant and neuroprotective activities. Compound **22** was found to be the best selective inhibitor of AChE with IC_{50} = 5.9 μM, and had non-competitive type of binding. Along with antioxidant activity with IC_{50} value of 7.45 μM against the neuronal t-BOOH-induced oxidative stress and neuroprotective ability against neurotoxicity elicited by both t-BOOH and OAβ1-42 and was also able to interfere in the Aβ1-42 aggregates formation process (103, 104).

Ahmed et al. reported a group of 1,3-di-4- piperidylpropane derivatives. In vitro AChE inhibition study revealed compounds **23a** and **23b** to be potent inhibitors of AChE with IC_{50} values of 0.591 μM and 0.625 μM, respectively. Both the compounds indicated dual binding mechanism interacting with the peripheral anionic site (PAS) and catalytic anionic site (CAS) of AChE, as deduced from the docking studies (105).

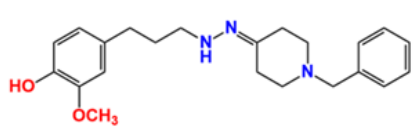
Lecoutey et al. reported some conformationally constrained donecopride analogues and replacing the cyclohexyl group of the donecopride scaffold with m-tolyl group, which resulted in a novel phenyl pyrazole compound **24** displaying dual mode of inhibition for the 5-HT6R and AChE. Docking study revealed that the presence of substituted benzyl group on the piperidine ring improved its ability to form more hydrophobic contacts at the active sites of AChE and 5-HT6R, deduced from in-silico docking studies (106).



Ortiz et al. reported a group of ten cinnamoyl-N-acyl hydrazone-donepezil derivatives, assessed for their possibility as multifunctional ligands. The IC_{50} values for the best active compounds **25a** and **25b** against AChE were 13.04 and 9.1 µM respectively, indicating good potential as inhibitors. Also, the binding mode was similar to that of donepezil during the docking study (107).

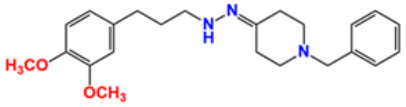
Sadeghian et al. reported some new carbazole-benzyl piperidine hybrids as potential inhibitors of AChE and BuChE enzymes. Among the series compound **26a** (IC_{50} = 16.5 µM for AChE and IC_{50} = 0.59 µM for BuChE) and **26b** (IC_{50} = 26.5 µM for AChE and IC_{50} = 0.18 µM for BuChE) exhibited the best inhibitory potential against AChE and BuChE. The compounds were also screened for anti-secretase (BACE1) activities, where **26b** displayed moderated BACE1

inhibition of 24.5% at 50 µM. Both the compounds **26a and 26b** also displayed some metal chelation capabilities and interacted with the Zn^{2+} ion (108).



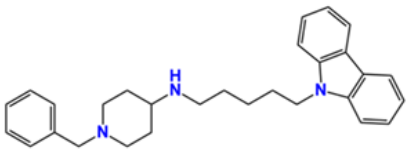
25a

AChE IC_{50} = 13.04 µM



25b

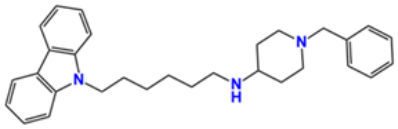
AChE IC_{50} = 9.1 µM



26a

AChE IC_{50} = 16.5 Mm

BuChE IC_{50} = 0.59 µM



26b

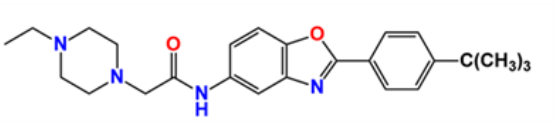
AChE IC_{50} = 26.5 µM

BuChE IC_{50} = 0.18 µM

Celik et al. reported some novel benzoxazole derivatives having p-tert-butyl at position 2 and acetamide bridging 4-substituted piperazine/piperidine at position 5. The compounds were screened against AChE, BuChE, and Tyrosinase, to tackle AD and related symptoms, of which compound **27** was found to have the best BuChE inhibition of 54 ± 0.75% at 50 mM (109).

Lalut et al. reported the pharmacomodulation study on denecopride, which is a preclinical drug candidate and is a known AChE inhibitor along with having potential 5-HT4R agonist response. They performed scaffold rigidification and the obtained derivatives were tested in vitro. Compound **28a** (3-[2-[1-(cyclohexylmethyl)-4-piperidyl]ethyl]-4-methoxy-1,2-benzoxazole) was discovered and had the best activity of IC_{50} value of 63.5 nM for AChE and K_i value of 59 nM against the 5-HT4R. Also compound **28b** displayed promising potency of IC_{50} value of 97.3 nM towards AChE and K_i value 37 nM for the 5-HT4R (110).

Van et al. reported some N-benzylpiperidine carboxamide analogues, of which compound **29** 1-benzyl-N-(5,6-dimethoxy-8Hindeno[1,2-d]thiazol-2-yl)piperidine-4-carboxamide was found to have the best AChE inhibitory activity with IC_{50} = 0.41 ± 1.25 µM (111).



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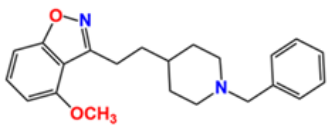
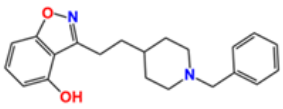


28a

AChE IC_{50} = 63.5 µM

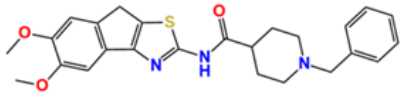
5-HT4R Ki = 59 µM



28b

AChE IC_{50} = 97.3 µM

5-HT4R Ki = 37 µM



29

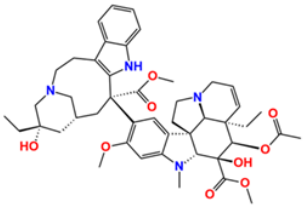
AChE IC_{50} = 0.41 µM

1.10.2. Biological importance of Piperidine based molecules in other disease

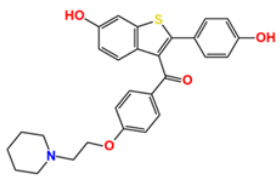
Besides the neuroprotective application potential of piperidines, it has found to be beneficial in numerous other diseases with enormous biological significance. Nitrogen containing heterocyclic rings are prevalent in various drug molecules and play a crucial role in its bioactivity. Such compounds can find its application in a wide range of pharmacological diseases like anticancer, analgesic, antimicrobial, anti-inflammatory, anti-tubercular, anti-Alzheimer's effects and others.

Potential anti-cancer agents, vinblastine, an alkaloidal compound naturally occurring in *Vinca Rosea* exhibits antineoplastic activity by inhibiting the mitosis at metaphase causing cell death (112). Raloxifene is another anti-cancer agent classified as second-generation of selective estrogen receptor modulators (SERM), which is used for the prevention of risk of invasive breast carcinoma in postmenopausal women (113).

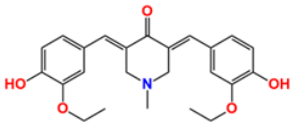
Khairia et al. reported a piperidine analogue compound **30** displaying chemo-preventive effect by successfully decreasing the population of cancer cells (114). Suvankar et al. reported twenty-five piperidine analogues as potent free radical scavengers exhibiting a promising anticancer property. From the series, compound **31** was found to be having interaction to the ctDNA via intercalation, and was the most potent antitumor agent (115).



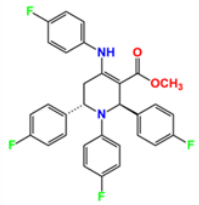
Vinblastine



Raloxifene

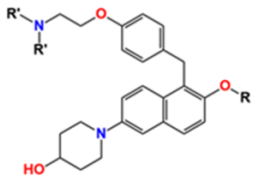


30



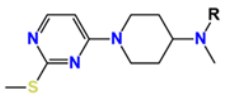
31

Amitabh et al. reported some piperidine derivatives along with its related aryl methyl ethers displaying potent cytotoxic activity on breast cancer cell line MCF-7. Compounds **32a** and **32b** emerged to be the best performing hits and were further tested on the murine L1210, human HeLa, and CEM cell lines and were found to have better activity than tamoxifen and raloxifene (116).



Compound	R	R'-R'
32a	Me	$-(CH_2)_2-CH(CH_3)-(CH_2)_2-$
32b	Me	$-(CH_2)_6-$

Yanqun et al. identified some piperidine based analogues through in silico virtual screening as novel inhibitors of HSP70. Compounds **33a-e** displayed significant inhibition of breast cancer cells (117).



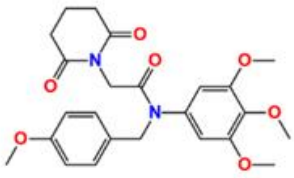
Compound	R
33a	P-cyanobenzyl
33b	2-chloro-6-fluorobenzyl
33c	2,4-dichlorobenzyl
33d	2,6-dichlorobenzyl
33e	2-chlorobenzyl

Dong et al. reported some novel piperidine derivatives that were evaluated for their anticancer properties and compound **34** was found to be the best performing hit, possessing

antiproliferative activity and its mechanism of action was deduced to be causing interference with the tubulin polymerization (118). Jin et al. reported some N-(piperidine-4-yl) benzamide based derivatives with promising anticancer activity. Compound **35** was found to have the best potency with an IC_{50} value 15-times more than Sorafenib (119).

Xin et al. synthesized a series of innovative 5-phenyl-N-piperidine ethanone-4,5-dihydropyrazole derivatives and were evaluated for anticancer activity. Compound **36** was found to have the best potency against SGC-7901and MGC-803 cell lines (120).

Benaka et al. reported some novel diphenyl (piperidine-4-yl) methanol analogues that were assessed for their antiproliferative activity on HT-29, HeLa,16 MCF-7 and HepG2 cell lines. Where compound **37** was concluded to displayed the best cytotoxic activity (121).



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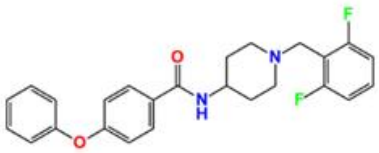

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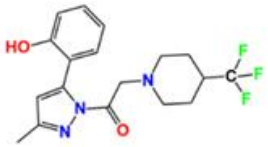

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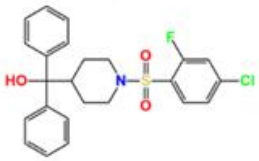
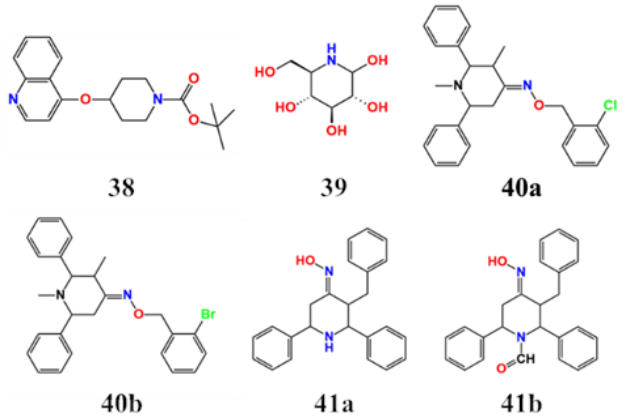


37

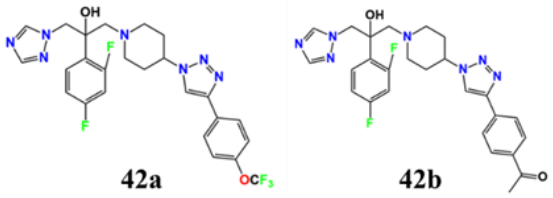
To explore the antiviral potential of piperidine derivatives, Guoxin et al. reported compound **38** as potent antiviral agent effectively inhibiting diversity of influenza virus strains by interfering with the replication process of influenza virus at its early to middle stages (122).

Some piperidine scaffold containing compounds reported as potential antimicrobial agents as follows. AD et al. reported few novel piperidine bearing compounds isolated from the Streptomyces ficellus strain and were evaluated for its antibacterial proficiency. Compound **39** (Nojirimycin) was finally identified to be promising antimicrobial agent against S. lutea, S. aureus and E. coli., and is a prototype compound for the development of newer class of antibiotics (123).

Chennan et al. synthesized and evaluated a series of piperidine-4-one oxime derivatives for their antimicrobial activity. Compound **40a** was found to be most active antifungal agent against Aspergillus favus and compound **40b** was effective against Candida-51 and was found to be better preforming as compared to the reference drug amphotericin B regarding minimum inhibitory concentration (MIC) (124). Jayaraman et al. synthesized and evaluated a series of piperidone derivatives for their antifungal activity against various strains of bacteria. Compound **41a** and **41b** was found to be best effective against B. subtilis, E. coli, K. pneumoniae and S. faecalis (125).



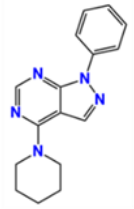
Piperidine scaffold have also been reported to possess antifungal properties by Zhigan et al. designed a series of novel antifungal piperidinyl triazole derivatives and evaluated them for antifungal activity against wide variety of fungi, and compound **42a** and **42b** were found to be most active (126).



Yanjiang et al. reported compound **43** as a potent BMP up regulator that regulates BMP2 and PTGS2 levels, which may aid in treating pulmonary arterial hypertension. SK et al. synthesized and reported some piperidinyl urea derivatives and compound **44** evolved to be the best compound having good epoxide hydrolase inhibition, thereby, can aid to control the elevated

blood pressure effectively. Signifying the importance of piperidine scaffold having antihypertensive properties.

Takeo et al. reported their investigation of some novel piperidine derivative having good antipsychotic activity, compound **45** was found to possess good affinity towards several dopamine receptors, exhibiting atypical antipsychotic effect in animal models. Ling et al. reported a class of new benzoxazole piperidine derivatives, from which compound **46** emerged to have the best affinity for dopamine D2, serotonin 5-HT1A and serotonin 5HT2A receptors, making it a promising scaffold for the development of potent antipsychotic agent.



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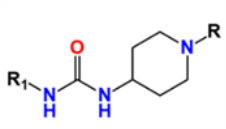
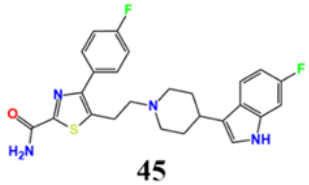


44

R = Adamantyl

$R_1 = COCH_3$



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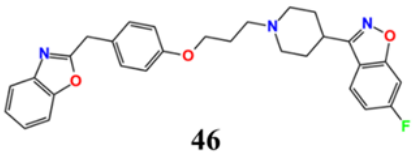

46

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