Chapter 2: Literature Review

2.1. Current therapies for AD

Current pharmacotherapies for Alzheimer's disease (AD) encompass acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonists. Presently, AD has no cure, and FDA-approved drugs for managing AD often come with significant side effects. AChE has emerged as a promising target for AD treatment. Butyrylcholinesterase (BChE), a closely related enzyme, also regulates cholinergic neurotransmission by breaking down ACh. Recent research indicates that targeting brain-specific BChE reduces Aβ levels and enhances cognitive function in animal models, and holds potential for neuroprotective and disease-modifying therapy in AD, as both AChE and BChE levels undergo significant changes during the disease's progression.

2.1.1. Cholinesterase inhibitors (ChEIs)

The current treatments available for AD can be divided into two main categories: acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonists. Among the AChE inhibitors, donepezil and galantamine are reversible and selective, while rivastigmine, a carbamate derivative, acts as a pseudo-irreversible inhibitor of both AChE and BChE, with some preference towards BChE. Donepezil, an FDA-approved drug since 1996, has been deemed safe and well-tolerated, interacting with the active and peripheral anionic sites (PAS) of AChE through aromatic interactions. Galantamine, an alkaloid from the Amaryllidaceae family, competitively inhibits AChE and allosterically interacts with nicotinic acetylcholine receptors (nAChR), enhancing ACh release. Rivastigmine, a newer generation carbamate derivative, shows a greater affinity for brain AChE compared to peripheral AChE, but its inhibition potency is relatively weak. Tacrine, the first

licensed cholinesterase inhibitor, was withdrawn due to inefficiency in modifying AD progression and adverse effects like hepatotoxicity.

These drugs offer symptomatic relief during the early stages of AD onset, typically for 1-2 years, but they do not address the underlying pathological factors driving neurodegeneration. They are unable to compensate for neuronal loss in later disease stages, limiting their effectiveness primarily to the early phases of AD.

2.1.2. Noncompetitive N-methyl-D-aspartate (NMDA) antagonists

Memantine, originally synthesized by Eli Lilly and Company in 1968, is an ionotropic non-competitive antagonist of NMDA receptors and is sanctioned for treating moderate to severe Alzheimer's disease (AD). It stands as the sole NMDA antagonist prescribed clinically to alleviate symptoms and improve the quality of life for AD patients. Nonetheless, memantine's clinical efficacy is modest, and it may induce side effects such as dizziness, restlessness, constipation, ocular issues, confusion, rash, and urinary incontinence. Second-generation derivatives of memantine are currently under development, exhibiting improved neuroprotective properties.

Aducanumab, marketed as Aduhelm, is a monoclonal antibody targeting amyloid beta (Aβ) aggregates in AD. Approved by the US-FDA in June 2021, it aims to dismantle aggregated Aβ fibrils into smaller, non-toxic filaments. It is indicated for individuals with mid-stage cognitive impairment or mild dementia due to AD. Suvorexant, sold as Belsomra, is an antagonist of orexin receptors used for treating insomnia, authorized by the US-FDA in August 2014. However, it is not recommended for individuals with narcolepsy, liver impairment, or during pregnancy.

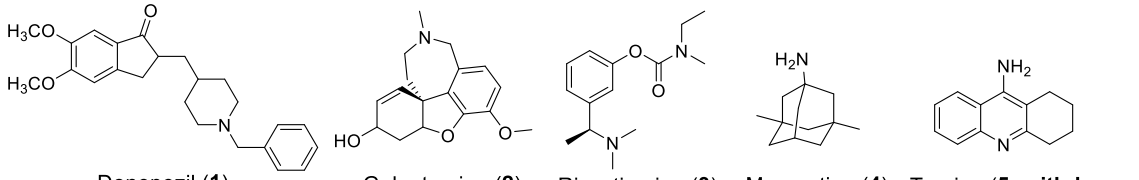

Figure 2.1. Chemical structures of the drugs for the treatment of AD.

2.2. Antioxidant therapy for AD

Oxidative stress (OS) has been strongly implicated as a significant contributing factor responsible for neurodegeneration in AD. One of the early events in the neurodegeneration pathway related to AD is increased oxidative stress [84, 85]. The key role played by OS in AD pathogenesis is well established in the literature [86]. Oxidative stress is defined as dyshomeostasis between ROS/RNS and the cells' antioxidant ability to neutralize them. It is caused either by producing reactive oxygen species (ROS) or due to failure in the elimination of ROS [87]. OS arises due to an imbalance that occurs at a molecular/cellular level when free radical production exceeds antioxidant scavenging capacity [85, 88]. The electrons leaked from the mitochondrial membrane react with oxygen to form superoxide anions ($O_2^{\cdot -}$). These superoxide radicals further react and generate other ROS forms like hydrogen peroxide (H_2O_2) and hydroxyl ion (OH^-). Whereas ROS like superoxide ($O_2^{\cdot -}$) and H_2O_2interacts with nitric oxide (NO) to generate peroxynitrite anion (RNS). Overproduction of reactive species (ROS/RNS) leads to compromised antioxidant function and induces toxicity *via* lipid peroxidation, oxidation of proteins, DNA, and RNA damage [89]. Overproduction of ROS/RNS is highly reactive towards nucleic acid, proteins, lipid, and other molecules in the brain, which governs the principle factors in the progressive neuronal damage in AD's pathophysiology [90]. In AD, overproduction of ROS in neurons is likely to be associated with significantly increased oxidative damage and mitochondrial dysfunction [91, 92].

Natural products are the major sources of therapeutic agents for diseases, including neurodegenerative disorders [93].There are a variety of natural constituents, including caffeic acid (CAA; **7**), curcumin (**8**), cinnamic acid (CIA; **9**), kaempferol (**10**), butein (**11**) sharing the ferulic acid (**FA, 6**) structural motif that can provide neuroprotection under neurodegenerative conditions as shown in figure 2.2. Polyphenols constitute one of the most important groups of natural metabolites of plants.

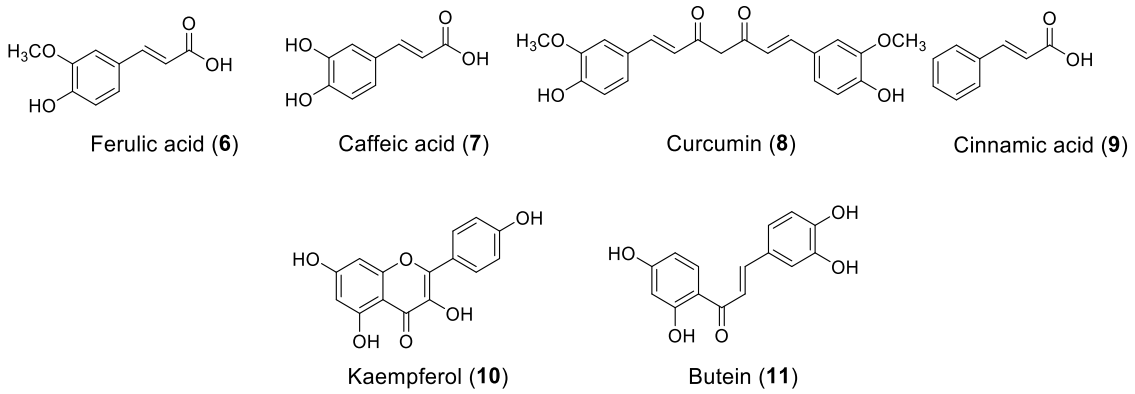

Figure 2.2. Chemical structures of the natural products are known for the anti-AD effect.

2.3. Piperine Background

In a vast world of spices, black pepper stands out as one of the most used spices with its characteristic pungent flavor which can be attributed to the molecule piperine, formally known as (2E,4E)-5- (1,3-benzodioxol-5-yl)-1-(1-piperidinyl)-2,4-pentadien-1-one. The amount of piperine in black pepper kernels falls between 1-5% and can be extracted using maceration and polar organic solvents followed by purification techniques such as column chromatography and recrystallization. Piperine belongs to the class of molecules known as alkaloids and is derived from the black pepper plant known as Piper nigrum, in the Piperaceae family. Alkaloids are naturally occurring compounds that are typically weakly basic in nature and contain at least one nitrogen atom. In fact, the previously mentioned FDA-approved cholinesterase inhibitor, galantamine, is an alkaloid. A variety of other alkaloids have been known to elicit medicinal benefits for the treatment of diseases

including malaria, cancer, diabetes, and cardiac dysfunction. Piperine is no different and has been extensively studied for its beneficial roles in chronic diseases, preservatives, and perfumes. For instance, piperine has been demonstrated to enhance the bioavailability of other therapeutic compounds, such as curcumin, through its ability to alter drug metabolism by inhibiting certain major cytochrome P450 (CYP) isoforms such as CYP3A4 and CYP3A5. 120, 121 On its own, piperine possesses a vast portfolio of biological activities including anti-inflammatory, anti-cancer, anti-larvicidal, anti-viral, antidepressant, pesticide, and anti-AD activity. Structurally, piperine contains a piperidine ring attached via an amide bond to a conjugated pi-bond system composed of a trans alkene and a methylenedioxyphenyl (MDP) bicyclic ring system. There are several isomers of piperine known as isopiperine, (Z, E), chavicine (Z,Z), and isochavicine (E, Z). However, the bioactive form of piperine is the (E, E) isomer and its geometric isoforms do not exhibit any pungency. In terms of stability, piperine may undergo isomerization upon exposure to light over extended periods and this process is directly dependent on light intensity and time exposed. Major metabolites of piperine are processed by CYP1A2 and include the hydrolyzed products; piperic acid and piperidine, as well as others such as piperonal, vanillic acid, piperonyl alcohol and piperonylic acid. Nevertheless, piperine has been reported to undergo minimal phase I hepatic metabolism. This is likely because of its CYP3A enzyme inhibition abilities and because CYP1A2 is not as abundant within the body as some of the other CYP isoforms. For example, when administered orally in rats, Ren et al. demonstrated that piperine crosses the BBB into the brain effectively at the 33 clinically relevant dose of 35 mg/kg. Although piperine has proven itself as an effective bioenhancer, improving the bioavailability of at least 16 drugs by amounts ranging from 0.54-fold to 20-fold, its own bioavailability has only been reported to be 25.4% and 23.2% in rats and mice respectively. Even so, the pre-clinical

reported levels of brain penetration and distribution in combination with its known neuroprotective effects against AD and other CNS diseases makes it a promising molecular template for further development and optimization.

2.4. PA as a potential therapeutic agent for AD

2.4.1. Synthesis of piperine analogues as AChE and BChE inhibitors for the treatment of Alzheimer's disease

Rungnapha Saeeng investigates the conversion of piperine, an alkaloid from the black pepper plant, into piperine amide derivatives through condensation with various piperazine derivatives and secondary amines. These derivatives were evaluated for their potential in Alzheimer's disease therapy, focusing on antioxidant properties and inhibition of AChE and BChE activities. Most synthesized compounds demonstrated promising antioxidant behavior and potent inhibition of AChE and BChE activities. Notably, para-pyridyl piperazine 3c showed the highest AChE inhibition (IC50 = 51.7 μM), while piperine amide derivatives 3v, 3x, and 3z exhibited superior BChE inhibitory activities compared to galantamine, with IC_{50} values ranging from 4.0 to 15.4 μM. The most active compounds displayed competitive and noncompetitive inhibitions against AChE and BChE activities, respectively. Docking studies revealed favorable binding energies towards the target enzymes. These findings suggest the potential for developing novel anti-Alzheimer's drugs utilizing natural piperine derivatives.

2.4.2. PA derivatives as potential therapeutic agent for AD

BhanuKiran *et. al.*, 2023, has reported synthesis of few naturally inspired multitarget-directed legends from piperine for the management of Alzheimer's disease. Scheme 1, Figure 2.3 (Bhanukiran *et. al.*, 2023) of his studies reported 10 compounds (PC01-10) as

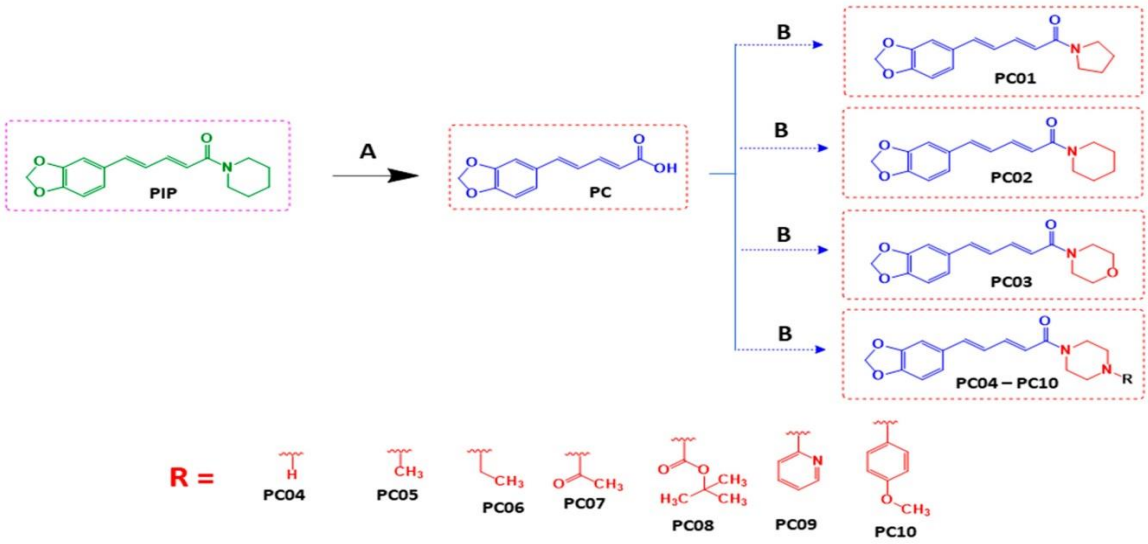

Figure 2.3: Piperine Derivatives (PC01–PC10), Bhanukiran *et. al.*, ACS Chem. Neurosci. 2023, 14, 2743−2760

Whereas scheme 2 (Bhanukiran *et. al.*, 2023) Figure 2.4 of his experiment lead to synthesize 26 compounds (PD01-26) as

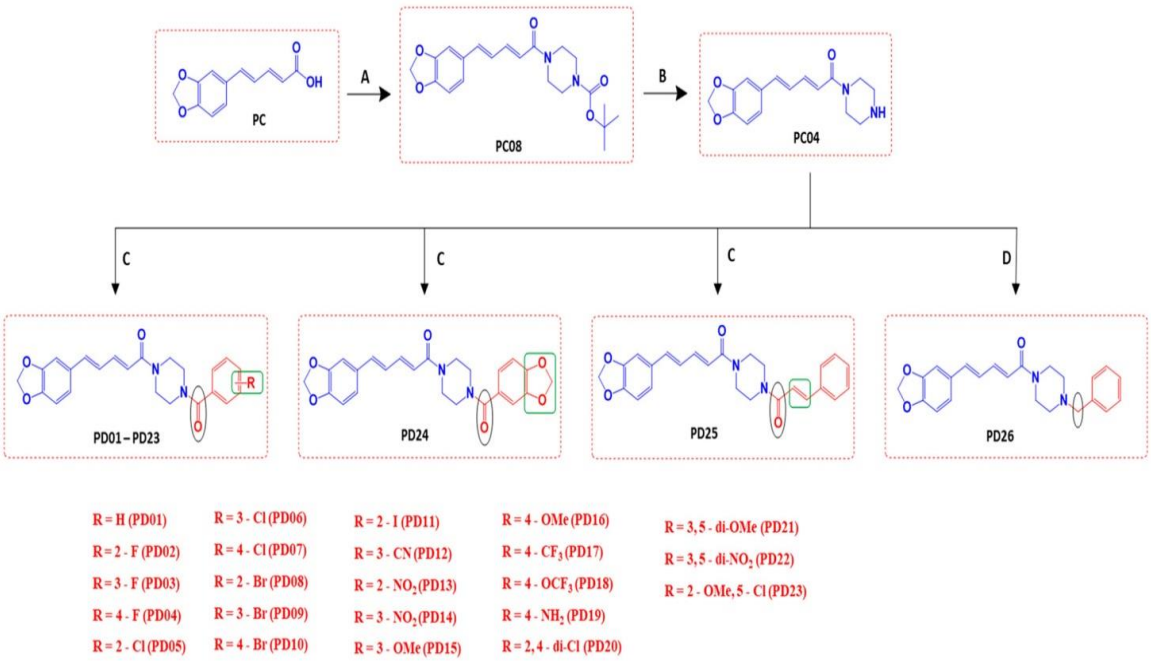

Figure 2.4: Piperine Derivatives (PD01–PD26), Bhanukiran *et. al.*, ACS Chem. Neurosci. 2023, 14, 2743−2760

The results from *in vitro*, *in silico* and *in vivo* studies in his experiment suggested (2E,4E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(4-(4-chlorobenzoyl)- piperazin-1-yl)penta-2,4-dien-1-one (PD07) having most potent activity comparable to the standard with less toxicity.

2.4.3. Vasicine 3-OH pyrrolidine derivatives as therapeutic agent for AD

In another experiments Bhanukiran *et. al.* further synthesized multifunctional 3-OH pyrrolidine analogs (VA01-VA25) through a semisynthetic approach using pyrroloquinazoline alkaloid vasicine as a precursor compound. The study also reports *4-bromo-N-(2-((3-hydroxypyrrolidin*-1-yl*)methyl)phenyl)benzamide* (VA10) as a potential molecule among all the synthesized derivatives, identified through *in-silico*, *in-vitro*, and *in-vivo* studies. They followed the scheme of synthesis as

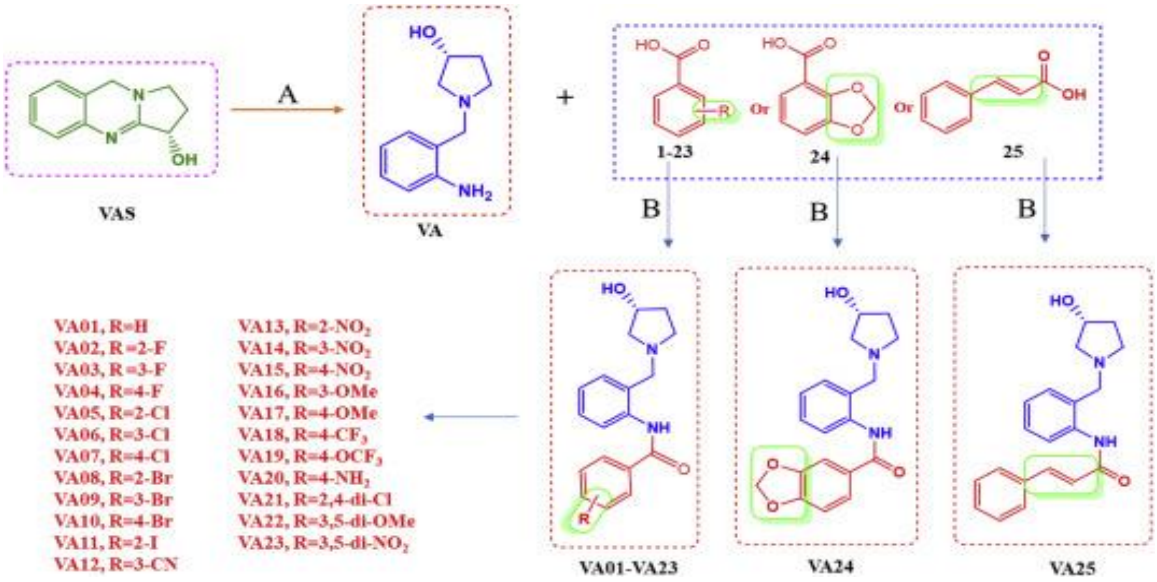

Figure 2.5: 3-OH pyrrolidine analogs (VA01-VA25), Bhanukiran *et.al.* European Journal of Medicinal Chemistry 249 (2023) 115145