2 Literature Review

2.1 Current treatments of Alzheimer's disease (AD)

Current pharmacological treatments for AD include acetylcholinesterase (ACh) inhibitors and NMDA receptor antagonists. Although these drugs provide symptomatic relief, they do not cure the disease and may cause significant side effects. ACh inhibitors prolong the action of acetylcholine, a key neurotransmitter for memory and learning. Butyrylcholinesterase (BChE), similar to ACh, also regulates cholinergic neurotransmission by hydrolyzing acetylcholine [89]. Recent research indicates that targeting BChE in the brain can reduce amyloid-beta (Aβ) levels and improve cognitive function in animal models, suggesting potential therapeutic benefits [90]. As AD progresses, levels of both AChE and BChE change, prompting further research into inhibiting these enzymes for neuroprotection and disease modification. By precisely modulating AChE and BChE activity, researchers aim to enhance cholinergic signaling and potentially slow AD progression. This dual-targeting strategy provides a more comprehensive approach to addressing AD pathology and its impact on brain function.

2.1.1 Cholinesterase inhibitors

Current treatments for AD primarily target the cholinergic hypothesis, utilizing cholinesterase inhibitors like donepezil, rivastigmine, and galantamine. These medications offer symptomatic relief but do not address the underlying causes of the disease. [91, 92]. Among the AChE inhibitors, donepezil (**DPZ**), known as Aricept, and galantamine are reversible and selective inhibitors, while rivastigmine (**Figure** 2.1) is a pseudo-irreversible inhibitor of both AChE and BChE. Donepezil, an FDA-approved drug for AD since 1996, is known for its safety and tolerance [93]. Through aromatic interactions, it interacts with the catalytic active site (CAS) and peripheral anionic sites (PAS) of AChE. These interactions involve the N-benzylpiperidine

and indanone groups of **DPZ**, which engage with Trp84 and Trp279 of AChE, respectively. This unique profile highlights the potential for developing new N-benzylpiperidine derivatives as anti-AD agents[94]. Galantamine, an alkaloid sourced from the Amaryllidaceae family, acts as a competitive and reversible inhibitor of AChE. Additionally, it allosterically modulates nicotinic acetylcholine receptors (nAChR), enhancing the release of acetylcholine. Although its potency is moderate (IC_{50} = 800 nM), its dual mechanism of action and lower toxicity have inspired the creation of novel analogs. Rivastigmine, a newer carbamate derivative, is a covalent inhibitor of AChE and showed a higher affinity for brain AChE than its peripheral counterpart. Although it is a weaker inhibitor overall, it offers a tenfold greater affinity for brain AChE, which enhances its therapeutic potential. Tacrine, the first licensed AChE inhibitor approved for AD in 1993, was removed from the market due to limited efficacy and severe hepatotoxicity [95]. These medications provide symptomatic relief in the early stages of AD, typically for the initial 1-2 years after symptom onset, but they do not target the root causes of neurodegeneration. Therefore, their pharmacological effects and efficacy are limited to the initial stages of the disease, offering minimal assistance in addressing neuronal loss as the disease progresses.

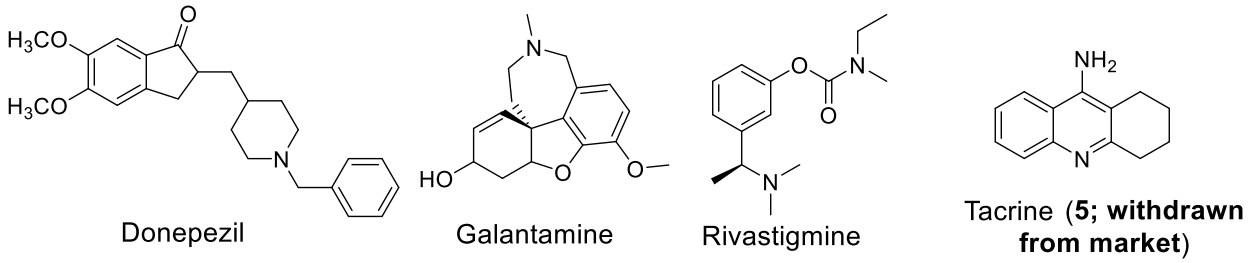
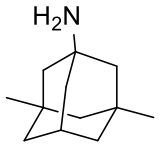


Figure 2.1: Structure of marketed drugs reported as cholinesterase inhibitors.

2.1.2 NMDA receptor antagonist

Memantine, or 1-amino-3,5-dimethyladamantane, is an amino-alkyl cyclohexane derivative patented by Eli Lilly and Company in 1968. It is a non-competitive NMDA receptor antagonist, helping to regulate synaptic plasticity and memory function. By inhibiting excessive activation of the NMDA receptor, memantine may protect against excitotoxicity [96]. Memantine (**Figure 2.2**) is approved for treating moderate to severe AD and is the only NMDA receptor antagonist used clinically to provide symptomatic relief and improve the quality of life for patients. This therapy offers an alternative to acetylcholinesterase inhibitors typically used in earlier AD stages [97]. However, memantine's clinical efficacy is limited, and it is associated with side effects such as dizziness, agitation, constipation, ocular issues, confusion, rash, and urinary incontinence [98]. These adverse effects can impact patient compliance and may restrict long-term use. To improve outcomes, researchers are developing second-generation memantine derivatives that aim for better neuroprotective properties and enhanced efficacy while minimizing side effects. These new compounds are intended to offer more effective treatment options for AD patients.



Memantine

Figure 2.2: Structures of the marketed drug reported as NMDA receptor antagonists.

2.2 Antioxidant therapy for the treatment of AD

Antioxidant therapy is an emerging approach for the treatment of AD. The compounds such as vitamin E, vitamin C, β-carotene, and lipoic acid (**Figure** 2.3) were investigated for their

capacity to mitigate oxidative damage by neutralizing free radicals. Nevertheless, the outcomes of antioxidant therapies across various studies have been inconsistent [99]. Enzymatic antioxidants, including glutathione peroxidase and superoxide dismutase, are crucial in neutralizing oxidative stress [100]. In contrast, mitochondrial-targeted antioxidants such as α-lipoic acid, coenzyme Q10, and Szeto Schiller peptides exhibit promise in safeguarding neuronal cells and enhancing cognitive function [101]. Additionally, dietary supplements like omega-3 fatty acids, caffeine, and curcumin have been examined for their potential positive effects on AD, with some animal models showing decreased amyloid-beta (Aβ) accumulation and oxidative stress [102]. Traditional herbal antioxidants, including alkaloids from Coptidis Rhizoma and Silibinin derived from milk thistle and Ginkgo biloba, have demonstrated potential anti-AD activity; however, their efficacy in human subjects remains elusive [103]. Antioxidants such as melatonin, selegiline, and estrogen have also been studied for their possible role in decelerating AD progression due to their antioxidative properties [104]. Although antioxidant therapies hold promise for managing AD, further validation through rigorous clinical trials is essential to establish their safety and effectiveness. Combining different antioxidants and strategies targeting specific cellular pathways, such as mitochondrial function, may lead to more effective interventions for treating and managing AD.

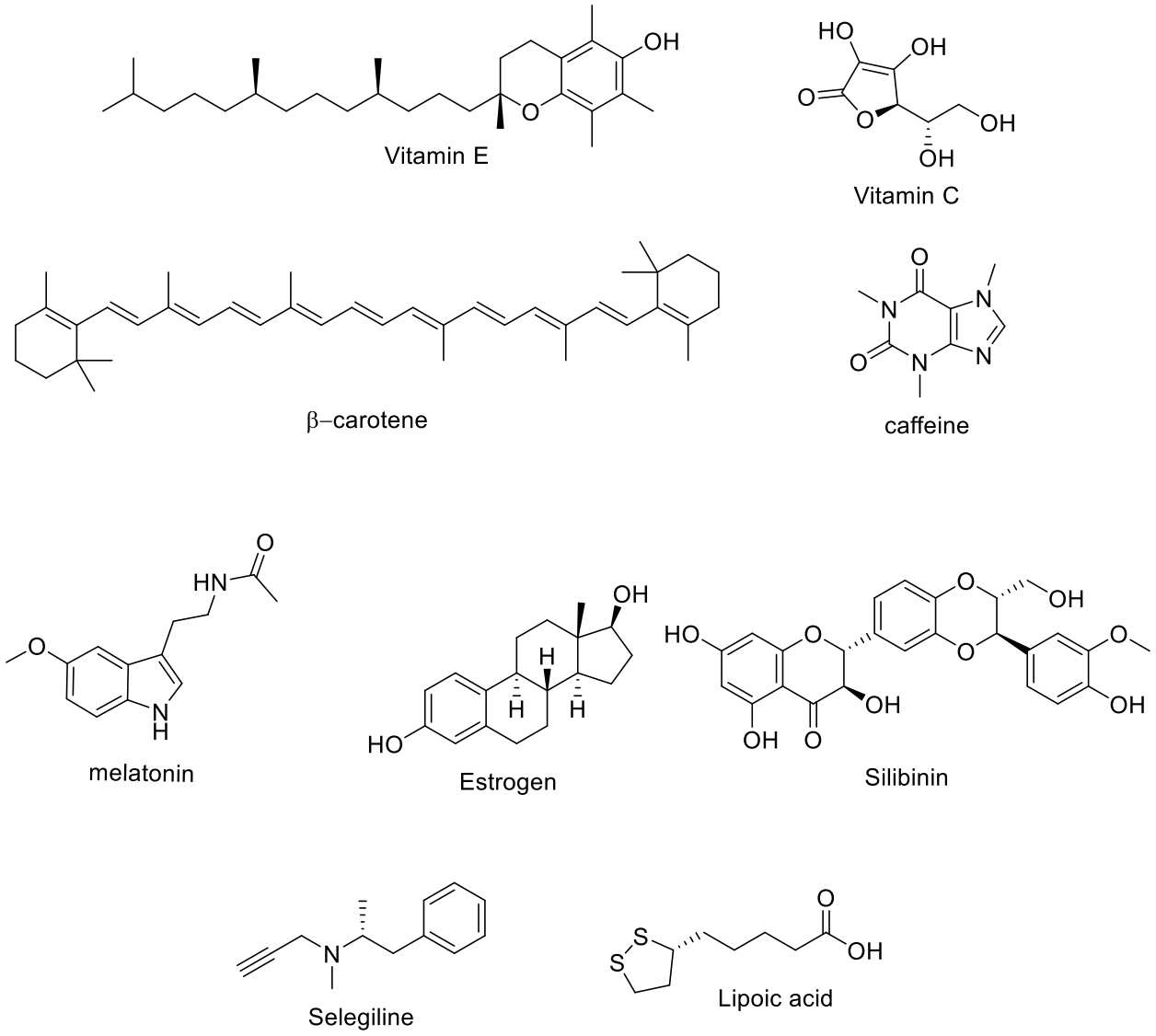


Figure 2.3: Structure of compounds reported in antioxidant therapy of AD.

2.3 Metal (iron) chelators in a clinical trial for the treatment of AD

Iron chelation therapy has emerged as a potential approach to treating AD, as researchers have found evidence suggesting that iron accumulation may play a role in the disease's pathogenesis. Iron chelators are drugs that bind to excess iron in the body and facilitate its removal. This treatment has been successfully used for years in conditions such as sickle cell disease and major beta-thalassemia, where iron overload can lead to complications [105]. Deferoxamine is

one of the oldest and most well-established iron chelators, with over three decades of clinical use. It is known to reduce iron levels in patients effectively. However, it requires subcutaneous or intravenous administration, which can be burdensome for patients. Its potential side effects include visual and ototoxicity, which may limit its long-term use [106]. Deferasirox is an oral iron chelator, making it more convenient for patients than deferoxamine (**Figure 2.4**). It is generally considered patient-friendly due to its oral administration and lower risk of side effects. However, deferasirox can be more costly than other iron chelators, which may impact its accessibility for some patients [107]. Deferiprone (**Figure 2.4**) is another oral iron chelator that is easier to administer than deferoxamine. It requires weekly blood monitoring to track potential side effects, which can be its limitation [108]. Nevertheless, its oral administration offers a more convenient option for patients. While iron chelation therapy has shown promise in treating iron overload syndromes, its efficacy and safety in AD require careful evaluation. Continued research is needed to fully understand the role of iron chelation therapy in AD and determine which patients may benefit the most. Clinical trials may help to establish the optimal dosing, duration, and combinations of iron chelators for treating AD. Research into new iron chelators with improved safety and efficacy profiles is ongoing.

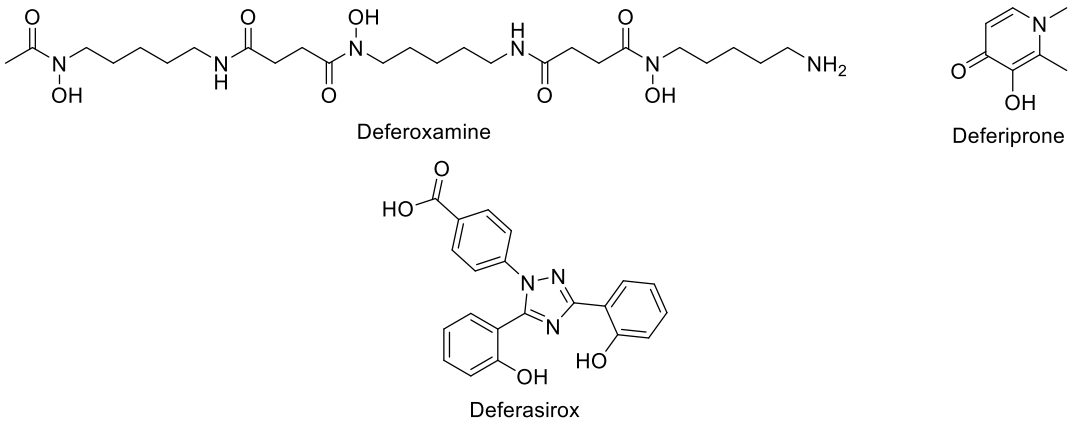


Figure 2.4: Structure of metal chelators under investigation.

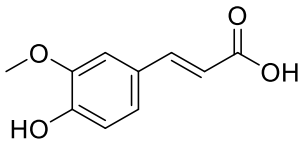
2.4 Monoclonal antibodies in AD treatment

Aducanumab, a monoclonal antibody recently approved by the FDA, effectively targets amyloid-beta (Aβ) proteins and reduces plaque formation in AD patients. Despite its efficacy in lowering existing amyloid plaques, it does not prevent the initial aggregation of these proteins [109]. Similarly, lecanemab is another promising monoclonal antibody that has shown potential in lowering amyloid markers and mitigating cognitive decline in early-stage AD. However, its use is associated with several adverse events, and the need for frequent dosing can make the treatment financially burdensome due to its high cost [110].

2.5 Role of ferulic acid (FA) in AD treatment

Ferulic acid (**Figure 2.5**) is a naturally occurring compound in various plants, including cereals, fruits, vegetables, and medicinal plants. It is part of the hydroxycinnamic acids, a subfamily of the polyphenol class. **FA** is abundant in the bran and germ portions of grains such as wheat, maize, rice, barley, oats, rye, and millet and is also present in fruits like blueberries and grapes and vegetables such as red beet, radish, spinach, and tomatoes. **FA** is found in beverages like coffee, tea, and wine and medicinal plants such as Lavandula angustifolia, Teucrium spp., and Angelica sinensis. **FA** was first isolated from Ferula assa-foetida, a medicinal plant in Ayurveda and traditional medicine. Chemically, **FA** ([E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid) possesses a phenolic core with a para-substitution and an ortho-methoxy group, allowing it to occur in both cis and trans isomers. **FA** is synthesized in plant cells through the shikimate pathway, which leads to the production of primary and secondary metabolites. **FA**, recognized for its antioxidant and anti-inflammatory effects, has been investigated as a potential neuroprotective agent in AD. In vitro studies have demonstrated that **FA** can protect neurons from amyloid beta-induced toxicity and mitigate

inflammation. In vivo studies using animal models have revealed that **FA** enhances memory and cognitive function, reduces Aβ deposition, and lowers neuroinflammation and oxidative stress.



Ferulic acid (1)

Figure 2.5: Structure of ferulic acid.

2.5.1 Ferulic acid in AD: *In-vitro* studies

In-vitro studies have explored the therapeutic potential of **FA** for AD by using various cell models, showcasing its neuroprotective capabilities. These studies have assessed the impact of **FA** on neuronal cells, microglial cells, and other cell lines relevant to AD. Kikugawa et al. found that pretreatment with **FA** in primary cerebral cortical neurons protected them from amyloid beta ($A\beta_{25–35}$)-induced cytotoxicity. **FA** also inhibited the aggregation of $A\beta_{25–35}$, $A\beta_{1–40}$, and $A\beta_{1–42}$, destabilizing pre-aggregated amyloid beta, indicating its potential to reduce amyloid beta toxicity [111]. In microglial cells activated by lipopolysaccharide (LPS), **FA** exhibited anti-inflammatory effects by suppressing the release of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1, and nitric oxide (NO). The treatment also decreased the activities of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), underscoring its anti-inflammatory properties [112]. Studies in Neuro-2a cells exposed to hydrogen peroxide (H_2O_2) showed that **FA** treatment downregulated the expression of genes associated with inflammation and apoptosis, including iNOS, eNOS, COX-2, IL-1β, caspase-9, and BCL-2 [113]. **FA** also upregulated the brain-derived neurotrophic factor (BDNF) gene, suggesting its ability to support neuronal survival and plasticity. In the pheochromocytoma PC12 cell line, a

commonly used model for neuroprotective research, **FA** inhibited the production of pro-inflammatory cytokines TNF-α and IL-1β induced by LPS and suppressed phosphodiesterase 4 (PDE4) activity. It also downregulated cAMP-response element binding protein (CREB) and phosphorylated CREB (pCREB) induced by LPS [114]. In hypoxia-stressed PC12 cells, FA improved cell viability through its antioxidant effects, such as free radical scavenging and increased superoxide dismutase (SOD) activity. Moreover, **FA** decreased intracellular free calcium levels, lipid peroxidation, and prostaglandin E2 (PGE2) release while reducing the activation of p-p38 MAPK, caspase-3, and COX-2, demonstrating its anti-apoptotic and anti-inflammatory properties [115]. These *in-vitro* studies suggest that **FA** exerts multiple neuroprotective effects, including antioxidative, anti-inflammatory, antiapoptotic, and anti-amyloid activities. These findings support its potential as a therapeutic agent for AD, but further research is needed to elucidate its mechanisms of action and to apply these findings clinically.

2.5.2 Ferulic acid in AD: *In-vivo* studies

In vivo studies have examined the therapeutic potential of **FA** in various animal models of AD, such as transgenic mice and intraventricular injections of amyloid beta (Aβ). These investigations have demonstrated that **FA** may enhance cognitive function, decrease amyloid beta accumulation, and mitigate neuroinflammation and oxidative stress. Yan et al. pretreated mice with **FA** (14–19 mg/kg/day) for four weeks, observing that it alleviated the detrimental effects of intracerebroventricular Aβ injection in the hippocampus. The study's outcome included attenuation of neuroinflammation and gliosis and improvements in memory deficits by **FA** [116]. In another study, **FA** treatment (0.006% w/v in drinking water) for four weeks in mice resulted in reduced microglia activation and interferon-gamma (IFN-γ) release in the

hippocampus after intracerebroventricular injection of $A\beta_{1-42}$ [117]. **FA** also reduced the hippocampus's endothelial nitric oxide synthase (eNOS), 3-nitrotyrosine, and IL-1α-mediated immunoreactivity [114]. Jin et al. reported that administration (50–250 mg/kg/day) in rats for four weeks significantly decreased IL-1β levels and increased phosphorylation of ERK and Akt, thereby reducing inflammation and promoting pro-survival signaling [118]. **FA** also inhibited the apoptotic cascade, particularly the activation of caspases 9, caspase3, and caspase7, following intracerebroventricular injection of $A\beta_{1-40}$ [119]. Other studies have highlighted the neuroprotective effects of **FA** in rat models, including improvements in memory and cognitive performance, and reductions in oxidative stress and neuroinflammation [120]. **FA** also demonstrated the ability to decrease amyloid beta plaques, protect against neuronal death, and promote neuronal survival [121]. These *in-vivo* studies suggest that **FA** may provide therapeutic benefits for AD by targeting various pathological processes, such as neuroinflammation, oxidative stress, and amyloid beta deposition. Nevertheless, further research is necessary to validate these findings and to optimize dosing and treatment regimens for clinical application.

2.5.3 FA in clinical trials

Clinical trials assessing the therapeutic potential of **FA** in AD have been limited, primarily investigating its combination with other agents, such as the dietary supplement Feru-guard® 100M, which contains *Angelica archangelica* and **FA** in a 1:5 ratio [122]. Kimura et al. conducted a 4-week open-label trial involving patients with frontotemporal lobar degeneration and dementia with Lewy bodies. Participants who took daily Feru-guard® (3.0 g/day) experienced significant improvements in behavioral and psychological symptoms, such as delusions, hallucinations, aggression, and anxiety [123]. Matsuyama et al. conducted an open-

label study from 2014 to 2018, recruiting patients with mild cognitive impairment (MCI). Participants received Feru-guard® 100M (1.5 g/day) for 48 weeks. Although changes in amyloid beta deposition, brain volume, and cognitive function were monitored, no significant differences were observed between the intervention and control groups [124]. Kudoh et al. performed a randomized, double-blind, placebo-controlled trial involving MCI patients aged 65 to 85. The active group received Feru-guard® (200 mg **FA** and 40 mg *Angelica archangelica* extract daily), while the placebo group received a placebo. The active group exhibited significant cognitive improvements at 24 and 48 weeks, suggesting potential therapeutic benefits [122]. While these trials indicate the cognitive benefits of Feru-guard® in older adults with mild cognitive impairment (MCI), further research involving larger sample sizes and more extended intervention periods is essential to establish the efficacy and safety of FA in treating AD.

2.6 Ferulic acid hybrids with their neuroprotective effects

In 2008, Lei Fang et al. synthesized tacrine–ferulic acid hybrids to enhance cholinesterase Inhibitory (ChEI) activity compared to tacrine. Compound **2** displayed the highest inhibition against both AChE and BChE. Enzyme kinetic studies indicated reversible and noncompetitive inhibition for AChE and reversible competitive inhibition for BChE by the hybrids. Compounds **2** and **3** (**Figure 2.6**) showed promising inhibitory activity against eeAChE with IC_{50} values of 4.4 ± 1.7 nM and 9.6 ± 2.1 nM, respectively, surpassing tacrine (IC_{50} value 45.1 ± 6.9 nM). However, for eqBChE inhibition, compounds **2** and **3** exhibited lower IC_{50} values of 6.7 ± 1.6 nM and 12.7 ± 2.6 nM, respectively, compared to tacrine (IC_{50} value 5.1 ± 1.0 nM) [125]. Moreover, these compounds demonstrated potent antioxidant properties, suggesting their potential for AD treatment through a multi-target approach.

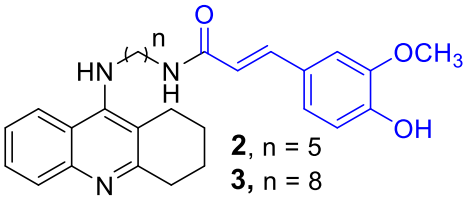


Figure 2.6: Structure of lead FA derivative reported by Lei Fang et al [125] .

In 2011, Jiang et al. synthesized a new series of hybrid molecules combining FA and berberine analogs to develop multi-targeted drug ligands (MTDLs) for AD. These compounds were evaluated for their activity against cholinesterases (ChEs), Aβ aggregation, and antioxidant activity. Among them, compound **4** (**Figure 2.7**) showed notable inhibition of both AChE and BChE with IC_{50} values of 3.21 ± 0.15 µM and 2.40 ± 0.04 µM, respectively. Additionally, compound **4** exhibited significant antioxidant activity (3.43 ± 0.11 TE) and demonstrated potential to inhibit Aβ aggregation [126].

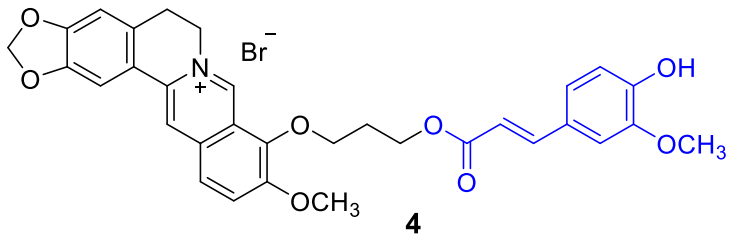


Figure 2.7: Structure of lead FA derivative reported by Jiang et al [126].

In 2012, Yao Chen *et al*. reported a series of nitric oxide (NO)-donating tacrine–ferulic acid hybrid compounds were synthesized and investigated for their potential as potent cholinesterases (ChEs) inhibitors. Among these compounds, **5** demonstrated notable inhibitory activity against AChE IC_{50} = 10.9 nM and BChE IC_{50} = 17.7 nM. Compound **5** (**Figure 2.8**) exhibited activity correlated with NO production in vascular relaxation assays, comparable to

the reference isosorbide dinitrate (ISDN). Compound **5** effectively reverses scopolamine-induced cognitive impairment in animal models [127].

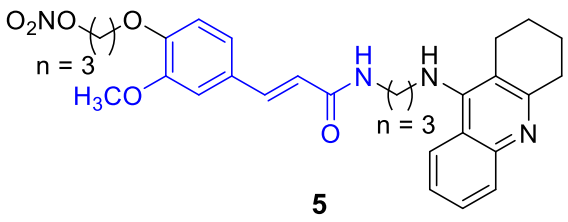


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Figure 2.8: Structure of lead FA derivative reported by Yao Chen et al [127].

In 2015, Benchekroun et al. designed and developed multifunctional hybrid derivatives that combined the pharmacophores of donepezil (**DPZ**) and FA, aiming to exhibit cholinesterase inhibition and antioxidant properties simultaneously. These N-benzylpiperidine (NBP) derivatives were also intended to offer potential therapeutic benefits by targeting both pathways. Among these compounds, derivative six emerged as an up-and-coming candidate. It featured N-(1-chloro-1-methyl) benzyl amide substitutions and demonstrated robust inhibition of eeAChE, with an IC_{50} value of 24.8 ± 4.1 nM, as assessed using Ellman's protocol, with **DPZ** (IC_{50} = 20.8 ± 2.1 nM) serving as a comparative standard. Moreover, compound **6** (**Figure 2.9**), characterized by a methoxy alkyl indole structure with an ethylene linker, displayed remarkable selectivity against eqBChE, with an IC_{50} value of 10.39 ± 0.48 nM, representing a substantial improvement over **DPZ** (IC_{50} = 2057 ± 290 nM). Additionally, compound **6** exhibited potent antioxidant activity, as evidenced by its high oxygen radical absorbance capacity (ORAC) value of 8.71 ± 0.20, determined using Trolox as a reference standard and fluorescein as a quantitative marker. These findings underscored the remarkable dual functionality of compound **6**, which demonstrated significant inhibition of eqBChE and potent antioxidant effects, surpassing the efficacy of reference drugs such as **FA** or melatonin

[128]. The researchers concluded that the initial exploration of structure-activity relationships (SAR) provided valuable insights for designing novel compounds with enhanced pharmacological properties, suggesting promising avenues for future research in this domain.

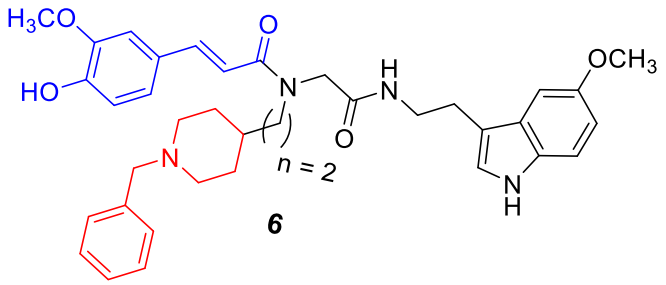
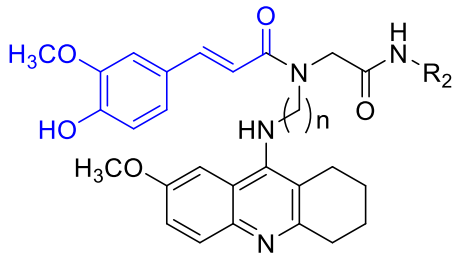


Figure 2.9: Structure of lead FA derivative reported by Benchekroun et al [128].

Tacrine, the first FDA-approved drug for AD treatment, was withdrawn due to hepatotoxicity. To address this concern, Mohamed Benchekroun and colleagues developed ferulic acid-tacrine hybrids with enhanced efficacy and reduced side effects. In 2015, they synthesized 14 compounds, and each compound exhibited less toxicity compared to tacrine. Compound **7** (**Figure 2.10**), identified as a 9-amino-7-methoxy-1,2,3,4-tetrahydroacridine (7-MEOTA) derivative, exhibited enhanced cell viability (59.4 ± 4.7%) in cell cultures, indicating reduced cytotoxicity. Moreover, compound **7** showed no discernible toxicity in neuroblastoma SH-SY5Y cells up to a concentration of 10 µM. Regarding cholinesterase inhibitory activity, compound **7** displayed notable potency with IC_{50} values of 22.2 ± 1.6 nM against AChE and 70.5 ± 0.2 nM against BChE. Its antioxidant potential, assessed via the ORAC-FL assay, revealed a moderate ORAC value of 4.29 ± 0.19 Trolox equiv., indicating some degree of free radical scavenging capability. Compound **7** exhibited significant inhibition (65.6 ± 0.9%) of self-induced $A\beta_{1-42}$ aggregation, suggesting potential therapeutic relevance in addressing AD pathology. Compound **7** emerged as a promising candidate, demonstrating favorable attributes

such as acceptable blood-brain barrier permeability and efficacy in mitigating Aβ aggregation [129]. Its selectivity and potency against BChE further emphasize its potential to enhance cognitive function in individuals with moderate AD.



7: n = 8, R^2=2'-napthyl

Figure 2.10: Structure of lead FA derivative reported by Mohammad Benchekroun et al [129].

In 2015, Digiacomo et al. synthesized a novel series of tacrine-derived compounds, incorporating caffeic acid (CA), **FA**, and lipoic acid (LA), and evaluated them for their potential therapeutic effects in AD. Among all compounds, **8** (**Figure 2.11**) exhibited significant antioxidant capacity, surpassing CA alone. Additionally, it effectively inhibited the self-induced Aβ aggregation, a key pathological feature of AD. Moreover, compound 8 demonstrated sub-micromolar inhibition of AChE (IC_{50} = 0.15 µM) and BChE (IC_{50} = 0.36 µM) enzymes. Furthermore, compound **8** displayed inhibition of beta-secretase 1 (BACE1), an enzyme involved in the production of amyloid-beta peptides. Additionally, at 30 µM, compound **8** exhibited DPPH radical scavenging activity up to 90.36 ± 1.4 %, indicating its antioxidant potential. Moreover, it demonstrated copper chelating properties, suggesting a potential role in metal ion dyshomeostasis implicated in AD pathology. Most notably, compound **8** showed potent neuroprotective effects against glutamate-induced cell death with low toxicity in HT22 cells, underscoring its potential therapeutic relevance in preventing neuronal loss, a hallmark of AD progression [130].

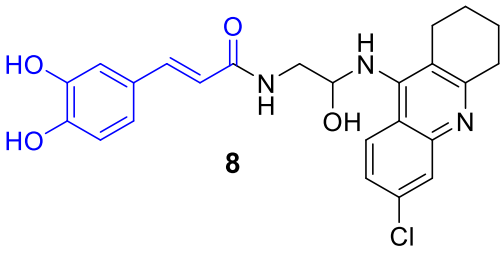


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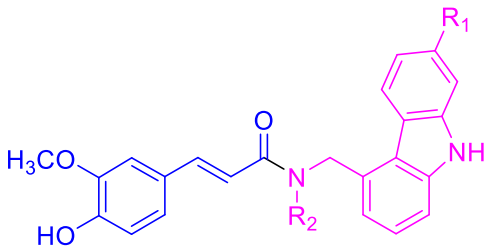
Figure 2.11: Structure of lead FA derivative reported by Digiacomo et al [130].

In 2016, Fang et al. developed a series of carbazole-coupled FA hybrids via amide linkage as novel multifunctional anti-AD agents, combining antioxidant properties from the FA moiety and ChE inhibitory activity from the carbazole moiety. The SAR analysis suggested that electron-withdrawing groups on the carbazole moiety and small alkyl substituents on the side chain favored ChE inhibition. Compound **9** (**Figure 2.12**) showed significant inhibitory activity against eeAChE (IC_{50} = 2.1 ± 0.6 µM), *h*AChE (IC_{50} = 5.1 ± 0.8 µM), eqBChE (IC_{50} = 1.9 ± 0.2 µM), and *h*BChE (IC_{50} = 7.9 ± 0.5 µM). Additionally, it exhibited potent antioxidant activity in the DPPH-FRSA assay (41.8 ± 4.2% inhibition at 1µM concentration). Moreover, compound **9** showed pronounced neuroprotective properties against H_2O_2-induced toxicity, indicating its potential as a multifunctional therapeutic candidate for AD [131].



9: R_1 -Cl, R_2 -CH_2CH_3

Figure 2.12: Structure of lead FA derivative reported by Fang et al [131].

In 2016, Estrada et al. developed compound **10** by coupling N-benzyl-piperidine (NBP) with various substituted cinnamic acid (CA) derivatives. Compound 10 (**Figure 2.13**), which contains FA fragments, exhibited potent inhibitory activity against *h*AChE (IC_{50} = 0.39 ± 0.05 µM) and unexpectedly preferential inhibition of hBChE (IC_{50} = 76 nM). Compound **40** demonstrated superior hBChE inhibition (IC_{50} = 0.076 ± 0.01 µM) compared to donepezil (**DPZ**) (IC_{50} = 2.50 ± 0.07 µM), suggesting its potential as a lead compound for addressing various factors associated with AD pathology [132].

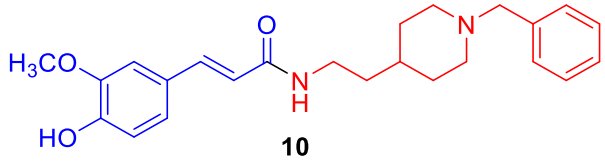


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Figure 2.13: Structure of lead FA derivative reported by Estrada et al [132].

In 2016, Benchekroun et al. synthesized and assessed the efficacy of ferulic acid−tacrine−melatonin hybrids (FATMHs) and lipoic acid−tacrine−melatonin hybrids (LATMHs) as potential therapeutic agents for AD. Among the synthesized compounds, compound **11** (**Figure 2.14**) exhibited pronounced activity as a cholinesterase inhibitor, with IC_{50} values against *h*AChE = 1290 ± 70 nM and hBChE = 234 ± 8 nM, respectively. Additionally, compound **11** demonstrated robust antioxidant properties, evidenced by its Trolox equivalent (TE) value 9.11, and displayed the potential to penetrate the BBB. Furthermore, compound **11** exhibited neuroprotective effects against H_2O_2 (300 µM) induced oxidative stress. Moreover, at a concentration of 3 µM, compound **11** effectively stimulated the Nrf2 transcriptional pathway in AREc32 cells. Compound **11** displayed lower potency as a cholinesterase inhibitor compared to Tacrine. However, unlike tacrine, it demonstrated a favorable non-hepatotoxic profile across varying doses (100, 300, and 1000 µM).

Consequently, compound **11** emerges as a promising candidate warranting further development for AD therapy [133].

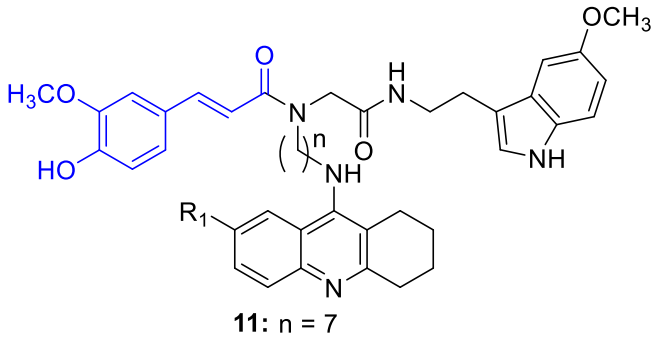


Figure 2.14: Structure of lead FA derivative reported by Bencheroun et al [133].

In 2016, Fu Ye et al. conducted a rational design and synthesis of a novel series of ferulic acid-tacrine piperazine hybrids by incorporating an alkyl chain linker. The SAR investigation revealed that a tacrine-containing ether linkage is crucial for activity. Compound **12** (**Figure 2.15**), featuring a C5-linker between **FA** and the piperidine moiety, demonstrated the highest inhibition, with IC_{50} values of 61.7 nM for AChE and 106.9 nM for BChE. Furthermore, **12** was tested for its modulation of Aβ1–42 peptide properties and protective effects against Aβ-induced Neuro-2A cell injury [134].

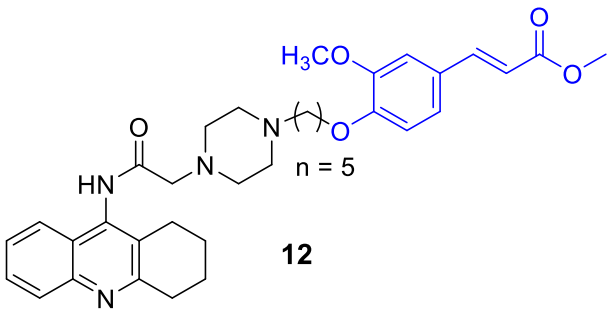
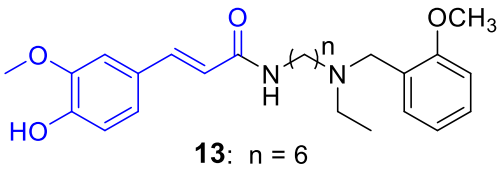


Figure 2.15: Structure of lead FA derivative reported by Fu Ye et al [134].

2016 Pan et al. synthesized novel ferulic acid-memoquin hybrids as multifunctional anti-AD agents. Compound **13** (**Figure 2.16**) exhibited potent AChE inhibition (IC_{50} = 3.2 μM), a significant reduction in amyloid species aggregation (49.3% at 50 μM), and robust anti-ROS activity (ORAC-FL value: 0.9-1.3). It demonstrated dose-dependent neuroprotective effects in SH-SY5Y neuronal cells and against H_2O_2-induced PC12 cell injury. Compound **13** also efficiently crossed the blood-brain barrier, indicating promise as a multifunctional anti-AD agent [135].



13: n = 6

Figure 2.16: Structure of lead FA derivative reported by Pan et al [135].

In 2016, Kikugawa et al. synthesized water-soluble derivatives of FA, denoted as compounds **14** and **15** (**Figure 2.17**). To enhance the solubility of these derivatives, they utilized FA esterase from *Aspergillus niger* during the synthesis process. Compound **14** manifested markedly superior water miscibility compared to FA, whereas compound **15** demonstrated enhanced water solubility relative to its glycerol derivative **14**. Both derivatives exhibited neuroprotective attributes against Aβ-induced neurotoxicity in cortical neurons, attributable to their capacity to impede Aβ aggregation and disrupt preformed Aβ fibrils. Notably, their antioxidant potency approximated that of FA, implying a plausible contribution to neuroprotection. *In-vivo* experimentation via contextual fear conditioning tests elucidated that orally administered compounds **14** and **15** effectively shielded hippocampal neurons from Aβ-induced cytotoxicity without eliciting adverse effects at concentrations up to 2 mM. These findings underscore the prospective therapeutic utility of water-soluble derivatives **14** and **15**

in AD management, with conceivable applications across medical, nutritional, and cosmetic sectors [111].

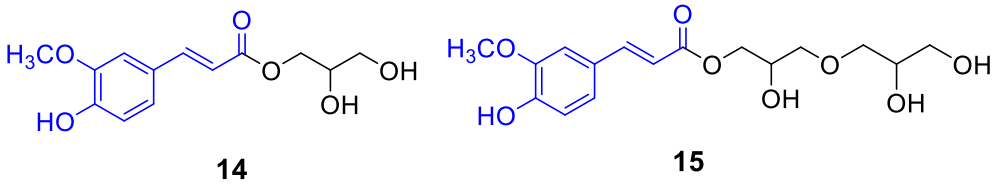


Figure 2.17: Structure of lead FA derivatives reported by Kikugawa et al [111].

In 2016, Jung et al. synthesized compound **16**, a novel dimeric derivative of ferulic FA, aiming to enhance pharmacological efficacy against pathogenic Aβ oligomers in AD. Evaluation in murine models demonstrated a significant reversal of $A\beta_{1-42}$-induced memory impairment with 30 mg/kg/day intra-gastric administration for five days, while a lower dose showed no significant effect. In the transgenic AD model, 3 and 30 mg/kg/day of compound **16** (**Figure 2.18**) administered via drinking water for three months notably improved novel object recognition memory. Quantitative analysis indicated reduced $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the frontal cortex without affecting IL-1β levels. Survival of the treatment group for three months at 30 mg/kg confirmed safety. Compound **16** showed potential as a safe and effective therapeutic agent for AD [136].

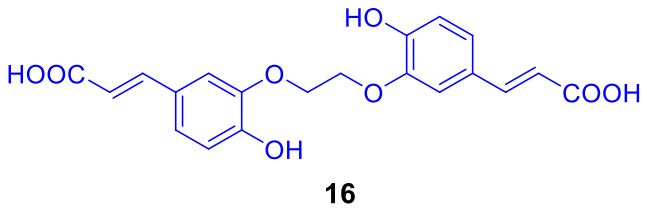


Figure 2.18: Structure of lead FA derivative reported by Jung et al [136].

In 2016, Wei Xu and colleagues developed a new series of derivatives of Donepezil-ferulic acid hybrids (DFAHs) using the MTDLs approach to address various stages of AD, ranging from mild to severe. These derivatives underwent assessments for their ability to inhibit ChE, act as antioxidants, penetrate the blood-brain barrier (BBB), chelate metals, and provide neuroprotection. In vitro SAR studies found that compounds containing N-benzylpiperidine (NBP) linked with FA at the terminal ends of long alkyl chain spacers exhibited superior inhibition of ChEs. Compounds containing both OH and OCH_3 groups, exemplified by compounds **17** and **18** (**Figure 2.19**), demonstrated moderate inhibition of enzymes compared to their analogs with only one of these groups. The positioning of the OH or OCH_3 group also influenced ChE inhibition, with compound **18** harboring methoxy (OCH_3) and hydroxy (OH) groups, showing notable inhibition against both AChE and BChE. Compounds **17** and **18** exhibited potent ABTS radical scavenging activity, indicating their antioxidant properties. Based on their promising antioxidant activity and potential for enzymatic inhibition, compound **18** was selected for further evaluation. In-silico studies suggested compound **18** could interact with the peripheral anionic site (PAS) and catalytic active site (CAS) of AChE, demonstrating dual binding site inhibitor activity. Di-methoxy and OH groups on the phenylpropanoid and core amide moieties were associated with compound **18** potential for metal chelation. Furthermore, results from the BBB-parallel artificial membrane permeability assay (PAMPA) indicated that compound **18** could effectively cross the BBB. These findings suggest that compound **18** holds promise as a multifunctional lead compound with potential neuroprotective effects against AD [137].

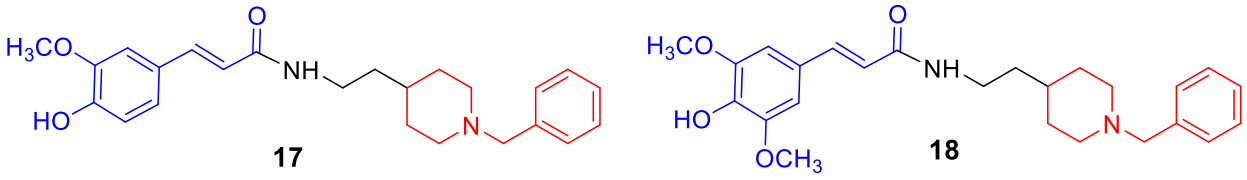


Figure 2.19: Structure of lead FA derivatives reported by Wei Xu et al [137].

In 2017, Liu et al. explored the role of the phenolic hydroxyl group in the design of anti-AD drug candidates. They synthesized novel ferulic acid-benzamide derivatives with tertiary amine side chains, drawing inspiration from chalcone's potential as AChE inhibitors. The majority of these compounds demonstrated potent AChE inhibitory activity, surpassing the standard drug Rivastigmine (AChE IC_{50} = 10.54 ± 0.86 μmol/L and BChE IC_{50} = 0.26 ± 0.08 μmol/L). Compounds **19** and **20** (**Figure 2.20**) exhibited the highest AChE inhibition potency, with IC_{50} values of 0.71 ± 0.08 μmol/L and 1.11 ± 0.17 μmol/L, respectively. Compound **20** displayed the most potent BChE inhibition activity, with an IC_{50} of 2.23 ± 0.18 μmol/L. The SAR analysis revealed no significant impact on AChE inhibition activity with varying alkyl chain lengths in the phenolic hydroxyl group of **FA** but a notable change in BChE inhibition property. Enzyme kinetic and molecular docking studies indicated mixed-type inhibition activity of **20** against AChE [138]. These findings suggest that compound **20** has potential as an anti-AD agent.

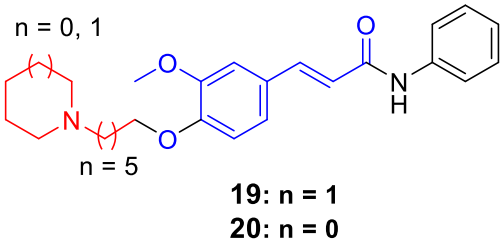
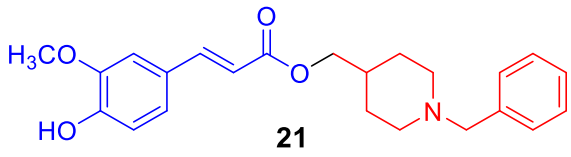


Figure 2.20: Structure of lead FA derivative reported by Liu et al [138].

In 2017, Dias et al. synthesized and evaluated a series of novel feruloyl-donepezil ester derivatives, amalgamating N-benzylpiperidine (NBP) from donepezil's pharmacophore with **FA** subunits, aiming at potential therapeutic interventions for AD. Through a comprehensive array of in vitro assays encompassing antioxidant activity, metal chelation, anti-inflammatory effects against reactive oxygen species (ROS)-induced neuronal toxicity, and amyloid beta (Aβ)-induced toxicity, as well as enzyme kinetics, molecular docking, and cholinesterase inhibition, the researchers conducted extensive investigations. Among the tested derivatives, compound **21** (**Figure 2.21**) demonstrated the highest inhibitory activity against eeAChE, with respective IC_{50} values of 0.46 μM. Compound **21** manifested a non-competitive inhibitory mechanism and potent antioxidant activity, primarily attributed to activating the Keap1/Nrf2/ARE pathway in neuronal SH-SY5Y cells. Moreover, it effectively mitigated ROS formation in a concentration-dependent manner. Compound **21** demonstrated selective chelation of cupric and ferrous ions and exhibited neuroprotective effects against $A\beta_{1-42}$ oligomers-induced neuronal cell death and inflammation in various animal models. These findings collectively indicate the potential of compound **21** as a lead multi-target-directed ligand (MTDL) in treating AD [139].

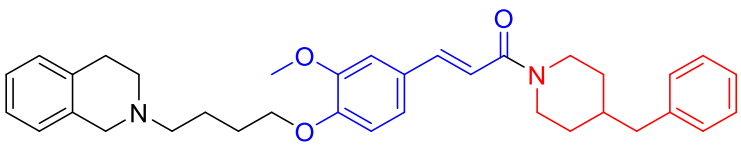


21

Figure 2.21: Structure of lead FA derivative reported by Dias et al [139].

In 2017, Sang et al. designed and synthesized novel FA-O-alkylamine derivatives using an MTDLs approach to develop anti-AD agents. Among these derivatives, compound **22** (**Figure 2.22**) demonstrated notable inhibition activity against eeAChE with an IC_{50} of 2.13 ± 0.01 μM,

as well as compelling inhibitory effects on BChE, mainly equine BChE (eqBChE) with an IC_{50} of 0.021 ± 0.01 µM and a selectivity index (SI) value of 101. Moreover, compound 22 exhibited significant anti-ROS activity with an ORAC-FL value of 0.55 ± 0.01 eq. of Trolox. It also displayed dose-dependent inhibition of self-induced amyloid ($A\beta_{1-42}$) species aggregation, achieving a maximum inhibition of 68.7 ± 0.76% at 50 mM. Additionally, compound **22** demonstrated selective inhibitory activity against human BChE (huBChE) compared to human AChE (huAChE), with an IC_{50} of 0.07 ± 0.01 µM for huBChE and 3.82 ± 0.05 µM for huAChE. Molecular modeling studies suggested compound **22** interacts with multiple binding sites on BChE. Furthermore, compound **22** exhibited neuroprotective effects in rescuing PC12 cells from H_2O_2-induced oxidative cell injury and demonstrated safety in acute toxicity studies in mice at doses up to 1000 mg/kg. In a step-down passive avoidance test in mice with scopolamine-induced memory deficits, compound **22** effectively reversed the memory impairment. These findings underscore compound **22** as a potential multi-target agent for AD treatment [140].

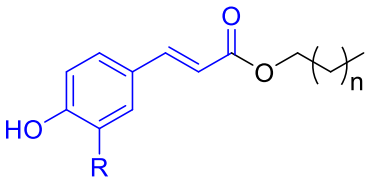


22

Figure 2.22: Structure of lead FA derivative reported by Sang et al [140].

In 2018, Micheles et al. identified compounds naturally occurring in R. rosea extract, notably β-sitosterol-β-D-glucoside (BSSG), which were isolated and subjected to chemical reactions to synthesize compound **23** to investigate its potential impact on memory enhancement. The experiment conducted on Drosophila larvae revealed that memory scores increased in a dose-

dependent manner upon treatment with compound **23** (**Figure 2.23**). It also demonstrated effectiveness in rodent models with enhanced excitability in mouse hippocampal CA1 neurons and improved hippocampus-dependent memory in treated mice. Notably, compound **23**, as an ester derivative, exhibited greater lipophilicity than its precursor, FA, facilitating its efficient permeation across the blood-brain barrier. The combined findings from initial experimental studies involving *R. rosea* plant supplementation (via extract or dried root parts) and the synthesis of derivatives support the idea of compound **23** possessing memory-enhancing properties. Consequently, it suggests potential clinical utility in addressing memory-related disorders, such as AD [141].

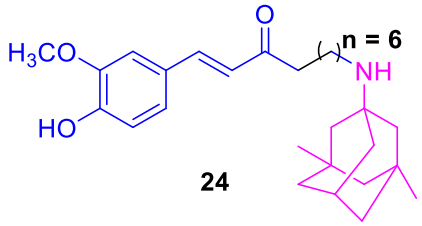


23 if R = OMe, n = 18

Figure 2.23: Structure of lead FA derivative reported by Michels et al [141] .

2019 Rosini et al. designed compounds targeting NMDAR activity, which is crucial for synaptic plasticity. Memantine, an FDA-approved anti-AD medication, inspired the synthesis of hybrid derivatives with FA to counteract Aβ-mediated neuronal death and oxidative stress. Compound **24** (**Figure 2.24**), featuring a hexamethylene spacer, exhibited potent NMDAR inhibition (IC_{50} = 6.9 μM) and voltage-dependent behavior akin to memantine. It demonstrated significant free radical scavenging and activated Nrf2/HO-1 pathways, reducing oxidative stress. Additionally, it boosted soluble amyloid precursor protein α (sAPPα) levels, suggesting non-amyloidogenic pathway activation. Compound **24** showed a multifunctional profile,

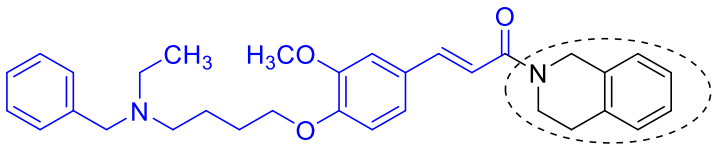
including NMDAR antagonism, antioxidant activity, and APP stimulation, making it a promising lead for AD treatment [142].



24

Figure 2.24: Structure of lead FA derivative reported by Rosini et al [142].

In 2019, Sang et al. introduced a series of FA derivatives conjugated with substituted 1,2,3,4-tetrahydroisoquinoline, following an MTDL approach to tackle AD. Compound **25** (**Figure 2.25**) emerged as a notable candidate, demonstrating significant inhibitory activity against BChE (IC_{50} = 8.9 nM). It also showed an inhibitory effect against monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B) with IC_{50} = 6.3 µM and 8.6 µM, respectively. Additionally, compound **25** exhibited promising effects on amyloid-β (Aβ) aggregation, with 59.3% inhibition and 43.8% disaggregation. Furthermore, it demonstrated significant antioxidative activity, as indicated by an oxygen radical absorbance capacity (ORAC) value of 0.52 equivalents. Notably, compound **25** displayed low acute toxicity and favorable brain permeability. A zebrafish model of AD demonstrated efficacy in mitigating $AlCl_3$-induced dyskinesia and provided potent neuroprotection against $A\beta_{1-40}$-induced vascular injury. These findings underscore the potential of compound **25** as a lead candidate for developing a multifaceted anti-AD therapeutic agent [143].



25

Figure 2.25: Structure of lead FA derivative reported by Sang et al [143].

In 2019, Mo J. et al. synthesized hybrid molecules combining FA and quinolone and assessed them for their cholinesterase inhibition activity. Compounds **26** and **27** (**Figure 2.26**) emerged as the most potent inhibitors against AChE and BChE. Compound **26** exhibited higher inhibition activity against AChE (IC_{50} = 0.62 ± 0.17 μM) compared to **27** (IC_{50} = 3.28 ± 1.32 μM), while its activity against BChE (IC_{50} = 1.18 ± 0.14 μM) was slightly lower compared to **27** (IC_{50} = 0.93 ± 0.40 μM). Enzyme kinetic studies revealed competitive inhibition by both compounds against AChE and BChE. Molecular docking studies suggested that both compounds simultaneously occupy the CAS and PAS of AChE and BChE, with the U-shaped conformation of **27** predicted to better bind with BChE's active site, indicating selectivity towards BChE. Compound **27** exhibited superior radical quenching ability in the DPPH assay compared to **28**. Compound **27** showed no cytotoxicity in HepG2 cell lines and displayed moderate neuroprotective activity against H_2O_2-induced oxidative stress in PC12 cells. These findings designate **27** as a lead molecule and highlight the potential of quinolone-based scaffolds as multi-target drug candidates for developing new anti-Alzheimer agents [144].

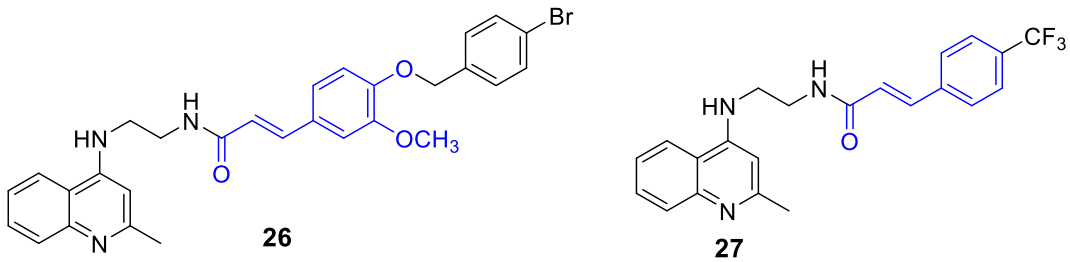
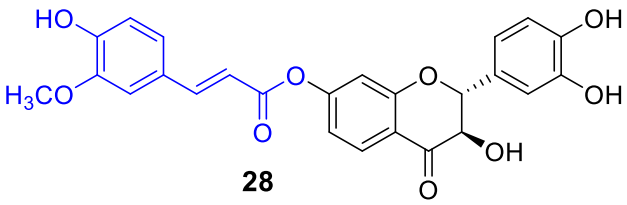


Figure 2.26: Structure of lead FA derivatives reported by Mo J et al [144].

In 2020, S. Gunesch et al. synthesized compound **28** through the incorporation of a 7-O-ester hybrid of the flavonoid taxifolin with **FA**, employing regio-selective synthesis techniques. Compound **28** (**Figure 2.27**) demonstrated notable neuroprotective properties against various forms of cell death, including oxytocin and ferroptosis, in murine hippocampal HT22 cells, surpassing the efficacy of taxifolin. At a concentration of 10 μM, it significantly enhanced cell survival and increased total glutathione (TSH) levels. Additionally, compound **28** mitigates lipopolysaccharide (LPS)-induced neuroinflammation in BV-2 mouse microglial cells, exhibiting superior efficacy compared to FA. When combined with taxifolin, compound **28** displayed a marked over-additive effect relative to mixtures containing **FA** with taxifolin. In a mouse model, compound **28** succeeded in reversing memory deficits induced by oligomerized $A\beta_{25-35}$ peptide. The improved cognitive performance and efficient BBB permeability make compound **28** a promising candidate for treating neurodegenerative disorders [145].

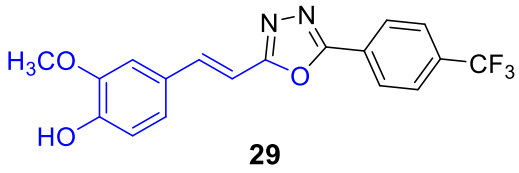


28

Figure 2.27: Structure of lead FA derivative reported by Gunesch et al [145] .

In 2020, Tripathi [146] et al. synthesized compounds based on **FA** and 1,3,4-oxadiazole to target multiple factors associated with AD. Compound **29** (**Figure 2.28**) emerged as a potent inhibitor of AChE (IC_{50} = 0.068 μM) and BChE (IC_{50} = 0.255 μM), as well as BACE-1 (IC_{50} = 0.255 μM). It exhibited dual binding sites at AChE and demonstrated BBB-crossing ability. Compound **29** inhibited self-induced and AChE-mediated Aβ aggregation, showed neuroprotective effects on neuroblastoma cells, and reversed cognitive dysfunction in mice's

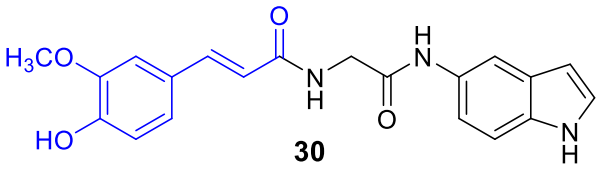
scopolamine-induced Y maze experiment. *Ex-vivo* studies indicated antioxidant and brain activity, and in an ICV rat model, compound **29** enhanced learning and memory behavior with excellent oral absorption. These findings highlight compound **29** as a promising candidate for further development as an anti-AD agent [146].



29

Figure 2.28: Structure of lead FA derivative reported by Tripathi et al [146].

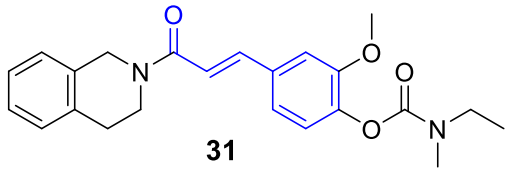
In 2020, Singh et al. conducted a systematic SAR investigation to address the shortcomings associated with **FA**, such as weak AChE and BChE interaction, poor blood-brain barrier permeability, and low logP value. Through rational design, they developed novel diamide compounds, including compound **30** (**Figure 2.29**), which exhibited potent inhibition of *h*AChE (IC_{50}= 5.74 ± 0.13 µM) and eqBChE (IC_{50} = 14.05 ± 0.10 µM) compared to FA. Molecular docking and dynamics studies indicated the ability of compound 30 to bind to the active sites of AChE and BChE stably. *In-vivo* studies demonstrated that oral administration of compound **30** reversed scopolamine-induced memory deficit in mice and reduced AChE and BChE levels in the brain. Moreover, *ex-vivo* studies showed that **30** reduced oxidative stress markers and modulated $A\beta_{1\text{-}42}$ aggregation. These findings highlight compound **30** as a promising lead molecule for developing multifunctional anti-Alzheimer agents [147].



30

Figure 2.29: Structure of lead FA derivative reported by Singh et al [147].

In March 2020, Sang et al. introduced 21 novel compounds with an O-carbamoyl ceramide core, utilizing the MDTL strategy to assess their neuroprotective activity in an AD model. Most compounds showed significant inhibition against *ee*AChE and *eq*BChE. Compound **31**(**Figure** 2.30) displayed notable *h*BChE inhibition (IC_{50} = 0.92 μM), significant MAO-B inhibition (IC_{50} = 5.3 μM), and substantial inhibition of Aβ aggregation (58.2%). Compound **31** demonstrated favorable recovery in $AlCl_3$-induced dyskinesia and potent neuroprotection against $A\beta_{1-40}$-induced vascular injury in a zebrafish AD model. PET-CT imaging with radio-labeled derivative **31** indicated good brain permeability and rapid uptake. Compound **31** exhibited neuroprotection without cytotoxicity up to 50 μM in Aβ-induced SH-SY5Y cells. It also showed reduced pathogenic tau levels, increased APP clearance rate, and improved cognitive impairment in a scopolamine-induced model. These results underscore **31** as a potential multifunctional therapeutic against various stages of AD pathology. [148].

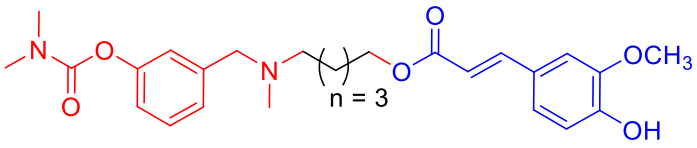


31

Figure 2.30: Structure of lead FA derivative reported by Sang et al [148].

In 2020, Lan et al. developed a series of compounds derived from FA, incorporating benzyl amino and carbomyl groups of rivastigmine. Compound **32** (**Figure** 2.31) demonstrated the most prominent cholinesterase inhibition activity, with an IC_{50} value of 19.7nM for *h*AChE and 0.66 μM for hBChE. In vitro studies revealed that compound **32** inhibited $A\beta_{1-42}$ self-induced aggregation by 49.2% at 20 μM, suggesting the beneficial role of hydroxyl and methoxy groups in inhibiting Aβ aggregation. Compound **32** exhibited the highest antioxidant activity (1.26 Trolox equivalents), comparable to FA (1.12 Trolox equivalents), and showed a

neuroprotective effect against H_2O_2-induced cytotoxicity. Enzyme kinetics studies reveal that **32** binds on both CAS and PAS binding sites of AChE and could effectively penetrate BBB, suggesting its potential as a therapeutic agent for AD [149].



32

Figure 2.31: Structure of lead FA derivative reported by Lan et al [149].

In 2021, Singh et al. introduced a novel series of multifunctional cholinesterase inhibitors based on FA for AD management. In this report, they further explored the SAR of these molecules to enhance cholinesterase inhibition and antioxidant properties. Three series of novel compounds were synthesized, focusing on replacing the phenylpiperazine ring with benzylpiperazine, increasing the linker length between FA and the substituted phenyl ring, and replacing the indole moiety with tryptamine. Compound **33** (**Figure 2.32**) emerged as a lead molecule with potent inhibitory activity against both AChE ($IC_{50} = 0.96 \pm 0.14$ μM) and BChE ($IC_{50} = 1.23 \pm 0.23$ μM), outperforming the previously identified lead molecule EJMC-7a. Molecular docking and dynamics studies revealed strong interactions between **33** and critical residues in AChE and BChE active sites. Additionally, **33** exhibited three times more potent antioxidant activity than EJMC-7a and was able to chelate iron. It attenuated H_2O_2-induced toxicity in SH-SY5Y cells and demonstrated efficient BBB permeability, tested by PAMPA assay. Furthermore, in an *in-vivo* study, compound **33** effectively reversed the effect of scopolamine in animal models. These findings suggest that compound **33**, a naturally inspired

multifunctional molecule, holds potential as a novel therapeutic agent for AD management [150].

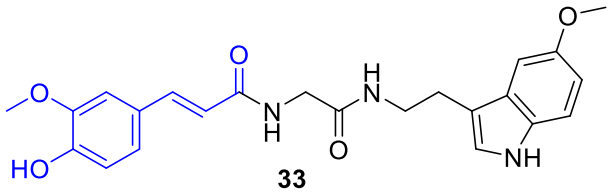


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Figure 2.32: Structure of lead FA derivative reported by Singh et al [150].