Literature Review

Chapter 2

2. Literature Review

2.1. Current therapies for AD

The current pharmacotherapies for AD include AChE inhibitors and NMDA receptor antagonists. Although there is no cure for AD, the FDA-approved medications used for its management are often associated with significant side effects. AChE is considered a promising target for AD treatment, and BChE, a closely related enzyme, also plays a role in regulating cholinergic neurotransmission by hydrolyzing ACh (88). Recent research suggests that targeting BChE in the brain not only reduces Aβ levels in transgenic mice but also enhances cognitive function in these animals (89). As AD progresses, the levels of both AChE and BChE change significantly, leading to their exploration as potential targets for neuroprotective and disease-modifying therapies.

2.1.1. Cholinesterase inhibitors (ChEIs)

The available treatments for AD are classified into two categories: AChE inhibitors and NMDA receptor (N-methyl-D-aspartate) antagonists (90). Currently, there are no cures for AD, and the FDA-approved drugs for managing the disease are associated with significant side effects. The AChE inhibitors include reversible and selective drugs like DPZ and galantamine, along with the pseudo-irreversible AChE and BChE inhibitor RIV, a carbamate derivative with slight selectivity for BChE (**Figure 2.1**). DPZ, an FDA-approved drug introduced in 1996, has been deemed safe and well-tolerated (91). It interacts with the active and peripheral anionic sites (PAS) of AChE through aromatic interactions. The N-benzyl piperidine and indanone groups of DPZ interact with Trp84 in the anionic subsite and the indole ring of Trp279 in the PAS of the enzyme. This pharmacological profile has spurred interest in developing other N-benzylpiperidine derivatives as potential anti-AD agents. Galantamine, an alkaloid from the Amaryllidaceae family, acts as a competitive and

reversible inhibitor of AChE, while also interacting allosterically with nicotinic ACh receptors (nAChR) to enhance ACh release. Although galantamine has a lower potency (IC_{50} = 800 nM), its unique dual mechanism and lower toxicity contribute to developing novel analogs. Rivastigmine (RIV) is a newer carbamate derivative that is a pseudo-irreversible inhibitor of cholinesterases. It has a ten-fold greater affinity towards brain AChE than peripheral AChE, although it is a weak inhibitor overall. Tacrine, the first licensed cholinesterase inhibitor approved in 1993, was later withdrawn from the market due to poor efficacy in reversing disease progression and adverse effects such as hepatotoxicity (92). These drugs provide symptomatic relief during the early stages (1-2 years) of AD, but they do not address the underlying pathophysiological causes of neurodegeneration. Furthermore, they cannot compensate for neuronal loss in the later stages of the disease, limiting their efficacy primarily to the early stages of AD.

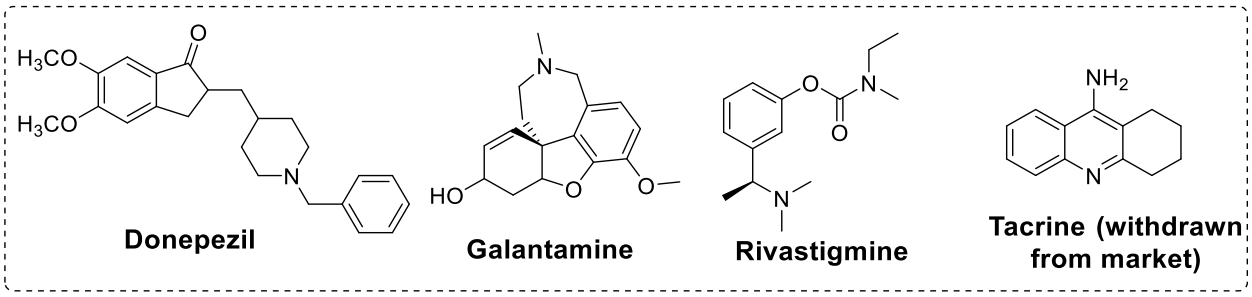

Figure 2.1: Structure of marketed drugs reported as cholinesterase inhibitors.

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

Memantine (1-amino-3,5-dimethyladamantane), a derivative of amino-alkyl cyclohexane, was first synthesized by Eli Lilly and Company (Indianapolis, IN) and patented in 1968. It is a non-competitive antagonist of the NMDA receptor and is approved for treating moderate to severe AD (**Figure 2.2.)** (93). Memantine is the only NMDA antagonist clinically prescribed to offer symptomatic relief and improve the quality of life for AD patients. However, its clinical effectiveness is limited, and it is associated with side effects such as dizziness, occasional restlessness/agitation, constipation, ocular issues, confusion,

rash, and urinary incontinence (94). Currently, second-generation derivatives of memantine are under development, with improved neuroprotective properties compared to the original compound (95).

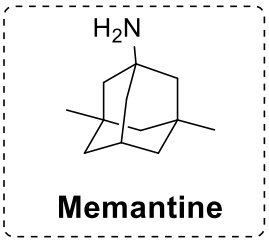

Figure 2.2: Structures of the marketed drug reported as NMDA receptor antagonists.

2.2. Antioxidant therapy for AD

Antioxidant therapy is emerging as a potential approach for AD treatment. Compounds like vitamin E, vitamin C, β-carotene, and lipoic acid (**Figure 2.3**) have been explored for their ability to reduce oxidative damage by neutralizing free radicals. However, the results of antioxidant therapies have been inconsistent across different studies (96). Enzymatic antioxidants, such as glutathione peroxidase and superoxide dismutase, are critical in counteracting oxidative stress (97). Mitochondrial-targeted antioxidants, including α-lipoic acid, coenzyme Q10, and Szeto-Schiller peptides, promise to protect neuronal cells and improve cognitive function (98). Additionally, dietary supplements like omega-3 fatty acids, caffeine, and curcumin have been studied for their potential benefits in AD, with some animal models indicating reduced amyloid-beta (Aβ) accumulation and oxidative stress (99). Traditional herbal antioxidants, such as alkaloids from Coptidis rhizoma, Silibinin from milk thistle, and Ginkgo biloba, have shown potential anti-AD effects, but their efficacy in humans remains unclear (100). Antioxidants like melatonin, selegiline, and estrogen have also been investigated for their potential to slow AD progression due to their antioxidative properties (101). While antioxidant therapies offer promise for managing AD, further validation through rigorous clinical trials is needed to confirm their safety and effectiveness. Combining various

antioxidants and targeting specific cellular pathways, such as mitochondrial function, may result in more effective interventions for treating and managing AD.

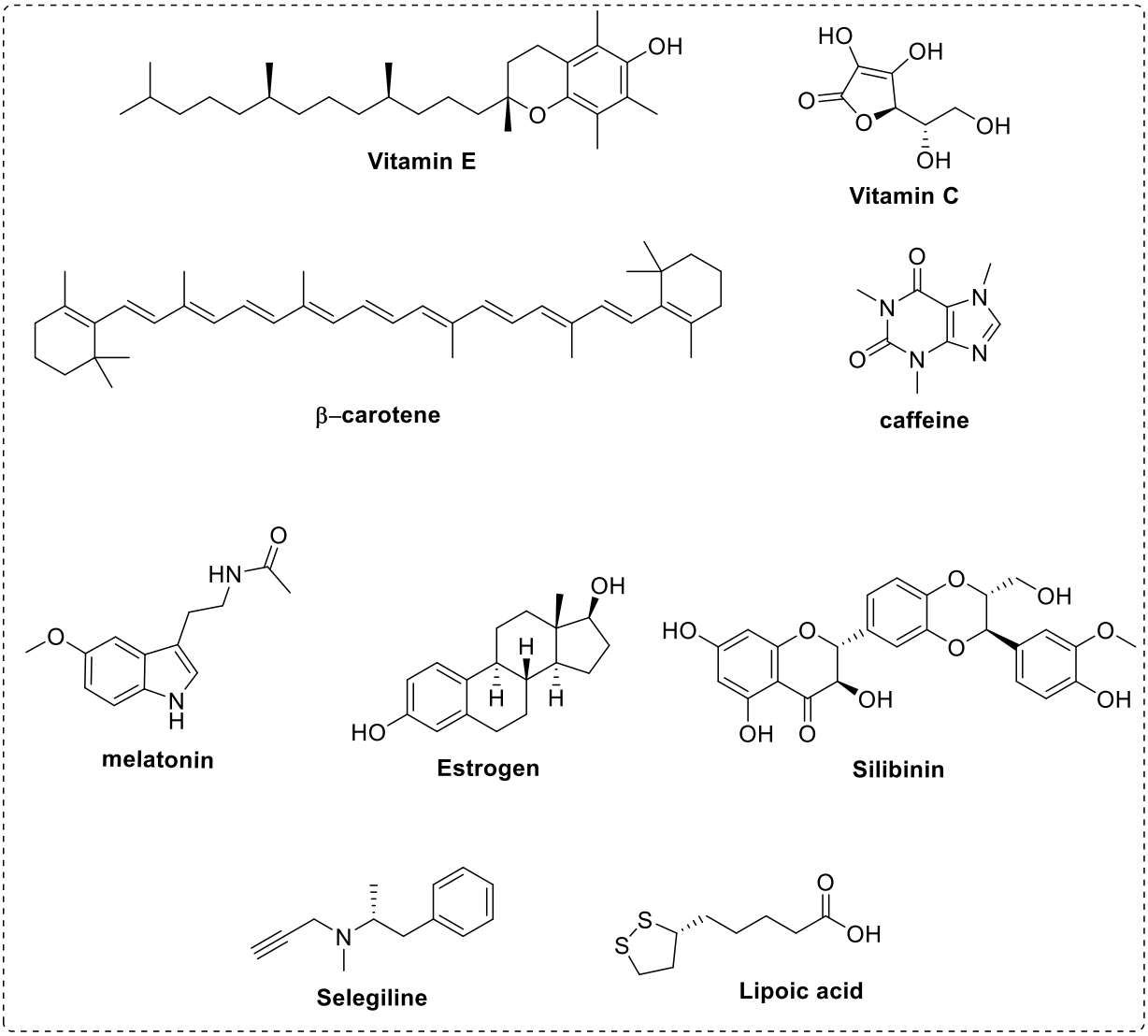

Figure 2.3: Structure of compounds reported in antioxidant therapy of AD.

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

Iron chelation therapy has gained attention as a potential treatment for AD, with evidence suggesting that iron accumulation may contribute to the disease's pathogenesis. Iron chelators are drugs that bind excess iron in the body and aid in its removal. This approach has been successfully used for years in conditions such as sickle cell disease and beta-thalassemia, where iron overload can cause complications (102). Deferoxamine, one of the oldest and most established iron chelators, has been clinically used for over three decades and

effectively reduces iron levels. However, it requires subcutaneous or intravenous administration, which can be inconvenient for patients. Its potential side effects, including visual and ototoxicity, may limit its long-term use (103). Deferasirox, an oral iron chelator, offers a more convenient alternative to deferoxamine (**Figure 2.4**). It is considered more patient-friendly due to its oral administration and lower risk of side effects. However, deferasirox is typically more expensive than other iron chelators, potentially limiting its accessibility for some patients (104). Deferiprone (**Figure 2.4**) is another oral iron chelator that is easier to administer than deferoxamine but requires weekly blood monitoring to detect potential side effects, which can be a drawback (105). Despite the promise of iron chelation therapy in treating iron overload disorders, its effectiveness and safety in AD need careful evaluation. Further research is necessary to better understand the role of iron chelation in AD and identify which patients are most likely to benefit. Ongoing clinical trials aim to determine the optimal dosing, duration, and combinations of iron chelators for AD treatment. Additionally, research is focused on developing new iron chelators with enhanced safety and efficacy profiles.

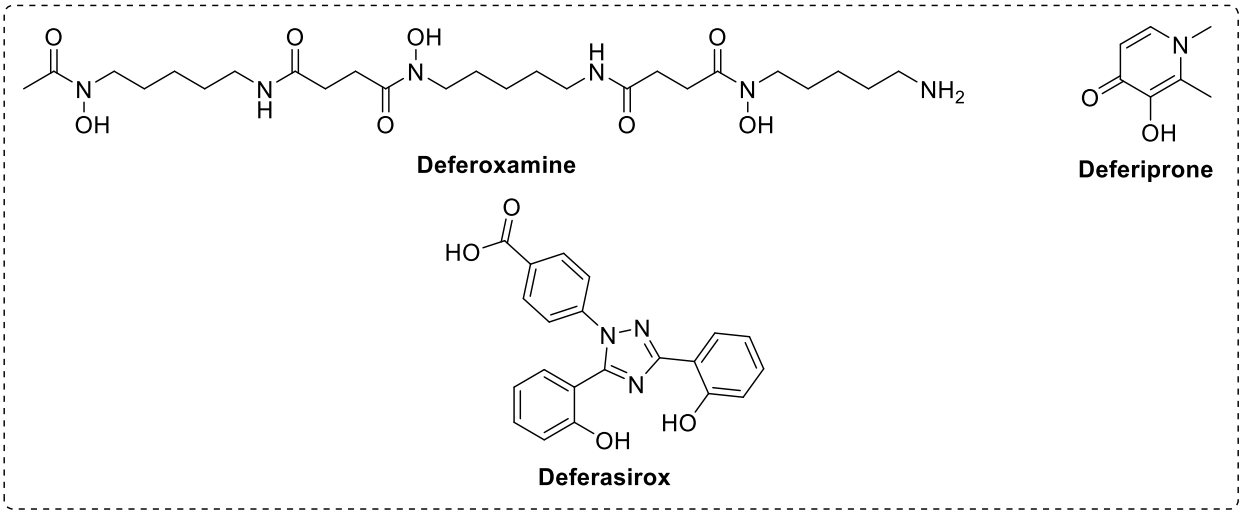

Figure 2.4: Structure of metal chelators under investigation.

2.4. Monoclonal antibodies in AD treatment

Aducanumab, a recently FDA-approved monoclonal antibody, effectively targets amyloid-beta (Aβ) proteins and reduces plaque accumulation in patients with AD. While it effectively lowers existing amyloid plaques, it does not prevent the initial aggregation of these proteins (106). Similarly, Lecanemab is another promising monoclonal antibody that has demonstrated potential in reducing amyloid levels and slowing cognitive decline in early-stage AD. However, its use is linked to various adverse effects, and the requirement for frequent dosing makes the treatment financially challenging due to its high cost (107).

2.5. Literature review related to urea and carbamate-based hybrids for the management of AD

This literature review examines the potential of urea and carbamate derivatives as promising multifunctional therapeutic agents for AD. AD is a progressive neurodegenerative disorder that affects millions of people worldwide, with its prevalence projected to increase significantly by 2050. The disease's hallmark features include cholinergic neuronal loss, amyloid plaque accumulation, tau protein hyperphosphorylation, oxidative stress, and neuroinflammation. Current FDA-approved drugs, such as AChE inhibitors (DPZ, RIV) and NMDA receptor antagonists (memantine), provide symptomatic relief without addressing underlying disease mechanisms.

RIV, an FDA-approved drug, is a potent, selective, uncompetitive, reversible BChE inhibitor and a moderate AChE inhibitor. It is a carbamate derivative structurally similar to physostigmine. Its mechanism of action involves reversible binding to cholinesterases, thereby inactivating enzymes like AChE and BChE. This inhibition prevents the hydrolysis of ACh, leading to an increased concentration of ACh at cholinergic synapses. Notably, RIV exhibits relatively higher specificity for brain AChE and BChE than those found in peripheral tissues.

2.5.1. Binding mechanism of RIV towards the cholinesterase enzyme

Carbamates are a class of synthetic compounds derived from carbamic acid, characterized by a carbonyl group directly bonded to nitrogen (N) and oxygen (O). The carbamate scaffold has demonstrated diverse pharmacological activities, including anti-AD properties (108). It serves as a crucial pharmacophore for the anti-cholinesterase activity of RIV. Carbamate derivatives act as pseudo-irreversible inhibitors of AChE (109). by interacting with the enzyme's catalytic active site (CAS). At the catalytic site, the carbamate moiety undergoes cleavage by amino acid residues Ser203, His447, and Glu334, resulting in

the formation of phenol and a carbamylated Ser203 residue, which blocks the cholinesterase site (**Figure 2.5**)(71).

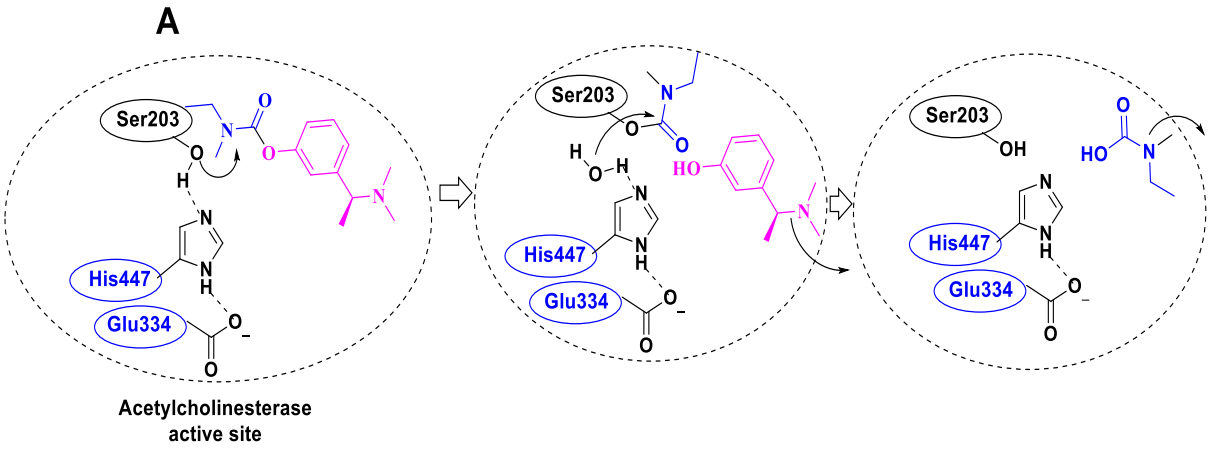

Figure 2.5: Interaction of RIV with the active site of ChE.

2.6. Literature available based on urea-based scaffolds for the management of AD

2.6.1. Fluorenyl-based urea derivatives

In 2024, Zahra Rezaei et al. designed and synthesized fluorenyl-based urea derivatives as selective inhibitors of BChE and β-amyloid aggregation (110) (**Figure 2.6**). These compounds were designed to target both cholinergic deficits and Aβ pathology in AD. This study reports synthesizing and evaluating a novel series of 2,4-disubstituted quinazoline derivatives for their inhibitory activity against AChE and BChE enzymes and their antioxidant properties. Biological assays identified compounds **1**, **2**, and **3** as potent BChE inhibitors, with IC_{50} values of 0.52, 6.74, and 3.65 µM, respectively, demonstrating high selectivity for BChE over AChE. Kinetic analysis revealed a mixed-type inhibition mechanism, suggesting these compounds interact with both the catalytic active site and the peripheral anionic site of BChE and AChE. Molecular docking and dynamic simulation studies confirmed favorable binding interactions with BChE active sites. Additionally, antioxidant screening showed that these compounds possess strong radical scavenging activity. These findings indicate that compounds **1**, **2,** and **3** are promising lead candidates for

developing potent, selective BChE inhibitors with antioxidant properties for AD management.

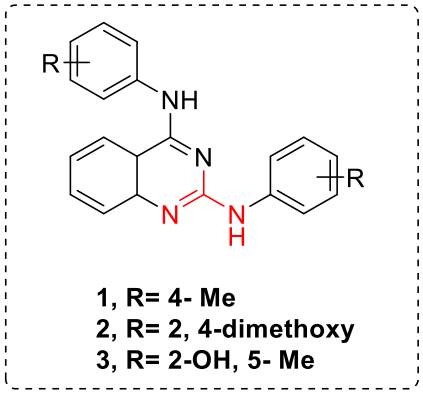

Figure 2.6: Structure of lead fluorenyl-based urea derivatives reported by Zahra Rezaei et al.

2.6.2. Pyrazinyl ureas derivatives

Elkamhawy, Park, et al. (2018) reported the synthesis and biological evaluation of pyrazinyl ureas as an inhibitor of β-amyloid (Aβ)-induced mitochondrial permeability transition pore (mPTP) opening (111). Compound **4** demonstrated superior neuroprotective properties to the standard Cyclosporin A (CsA) in preventing Aβ-induced mPTP opening. Among these, 1-(3-(benzyloxy)pyrazin-2-yl)-3-(3,4-dichlorophenyl)urea showed exceptional efficacy in maintaining mitochondrial function and cell viability, as confirmed by ATP and MTT assays. Additionally, a hERG channel assay indicated a safe cardiotoxicity profile for compound **4 (Figure 2.7)**. Molecular docking studies using the CDocker algorithm provided a plausible explanation for the differences in the compounds' efficiency in reducing green-to-red fluorescence, a marker for mPTP closure. This study highlights these compounds as some of the most promising pyrazinyl urea-based mPTP blockers identified to date.

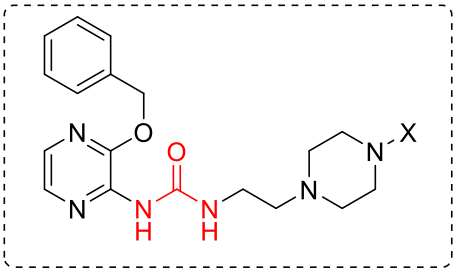

Figure 2.7: Structure of lead pyrazinyl ureas derivatives reported by Park, et al.

2.6.3. Propargylamine-modified pyrimidinylthiourea derivatives as multifunctional molecules for AD

Xu, Wang, et al. (2018) designed and synthesized a series of novel propargylamine-modified pyrimidinylthiourea derivatives as multifunctional molecules for AD treatment (112). Among these derivatives, compound **5** exhibited strong selective inhibitory activity against AChE (IC_{50} value of 0.324 μM) and MAO-B (IC_{50} value of 1.427 μM). Molecular docking studies revealed that the pyrimidinylthiourea moiety of this compound binds to the catalytic active site of AChE, while the propargylamine moiety interacts directly with the flavin adenine dinucleotide (FAD) of monoamine oxidase-B (MAO-B). Additionally, the compound demonstrated mild antioxidant properties, effective metal-chelating ability, and significant inhibitory activity against $A\beta_{1-42}$ aggregation. It also showed moderate neuroprotective effects, low cytotoxicity, and favorable blood-brain barrier (BBB) permeability. These findings suggest that this compound has the potential to be a multifunctional molecule for treating AD (**Figure 2.8**).

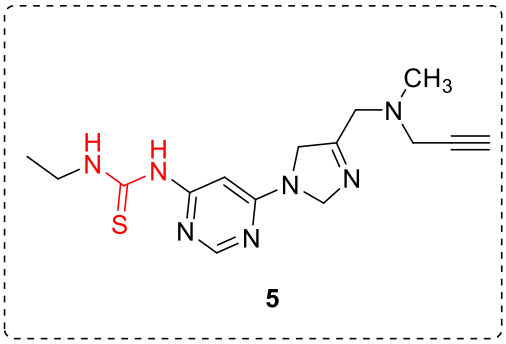

Figure 2.8: Structure of lead propargylamine-modified pyrimidinylthiourea derivatives reported by Xu, Wang, et al.

2.6.4. 7-Methoxytacrine (7-MEOTA)-amantadine urea derivatives

Spilovska et al. (2015) developed a series of cholinesterase inhibitors designed as dual-binding site heterodimers for the treatment of AD (113). The study focused on 7-

methoxytacrine (7-MEOTA)-amantadine urea derivatives, which were synthesized and evaluated *in-vitro* for their inhibitory activity against *h*AChE and *h*BChE. These compounds were compared with a series of 7-MEOTA-amantadine thioureas. The heterodimers featured varying linker lengths connecting the 7-MEOTA and amantadine moieties. Compared to 7-MEOTA, the newly synthesized compounds demonstrated improved inhibitory activity against both ChE. However, the urea derivatives did not exhibit the expected enhancement in inhibitory potency and showed IC_{50} values comparable to those of the thiourea derivatives (**Figure 2.9**).

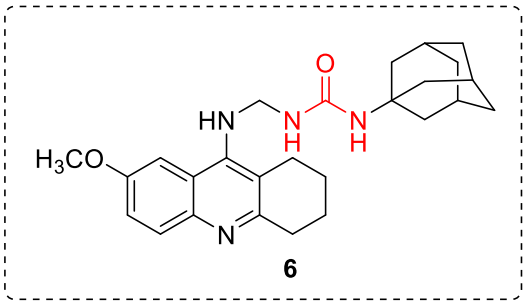

Figure 2.9: Structure of lead 7-methoxytacrine (7-MEOTA)-amantadine urea derivatives reported by Spilovska et al.

2.6.5. Benzofuranylthiazole-arylurea derivatives

Kurt, Gazioglu, et al. (2015) synthesized and evaluated a series of new benzofuranylthiazole derivatives containing an aryl-urea moiety as dual inhibitors of AChE and BChE (114). The synthesized compounds were also tested for their cupric-reducing antioxidant capacity (CUPRAC) and ABTS cation radical scavenging abilities. The results demonstrated that all the compounds exhibited inhibitory activity against both AChE and BChE with 1-(4-(5-bromobenzofuran-2-yl)thiazol-2-yl)-3-(2-fluorophenyl)urea showing the strongest inhibition of AChE (IC_{50} = 3.85 μM) and 1-(4-iodophenyl)-3-(4-(5-nitrobenzofuran-2-yl)thiazol-2-yl)urea showing the strongest inhibition of BChE (IC_{50} = 2.03 μM). A few compounds with IC_{50} values of 0.2, 0.5, and 1.13 μM exhibited better ABTS cation radical scavenging ability than the standard quercetin (IC_{50} = 1.18 μM). Molecular docking studies

suggest that the compound **7** bearing thiazole ring interacts well with AChE and BChE enzymes (**Figure 2.10**).

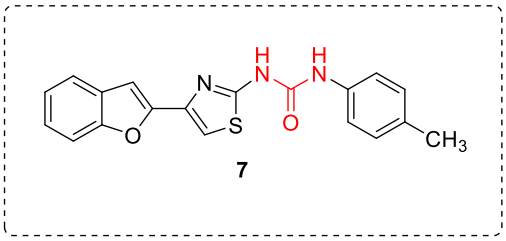

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Figure 2.10: Structure of lead benzofuranylthiazole derivatives reported by Kurt, Gazioglu, et al.

2.6.6. Methylsulfonyl -1,3-diaryl-urea derivatives

Zarghi et al. (2008) designed and synthesized a series of 1,3-diaryl-urea derivatives containing a methylsulfonyl pharmacophore at the para-position of the N-1 phenyl ring, along with a substituted phenyl ring at the N-3 position, to evaluate their potential as selective cyclooxygenase-2 (COX-2) inhibitors (115). *In-vitro* studies of COX-1/COX-2 isozyme inhibition revealed that 1-(4-methylsulfonylphenyl)-3-(4-methoxyphenyl) urea **(8)** exhibited potent inhibitory activity, with an IC_{50} of 9.2 μM for COX-1 and 0.17 μM for COX-2, achieving a COX-2 selectivity index comparable to that of the reference drug celecoxib (COX-2 IC_{50} = 0.17 μM, COX-2 SI = 405). The structure-activity relationship data demonstrated that the urea moiety is an effective scaffold for designing novel acyclic 1,3-diarylurea derivatives with selective COX-2 inhibitory properties. Furthermore, recent studies indicate that the progression of AD may be reduced in some users of NSAIDs. Chronic administration of selective COX-2 inhibitors may, therefore, slow AD progression while minimizing gastrointestinal side effects (**Figure 2.11**).

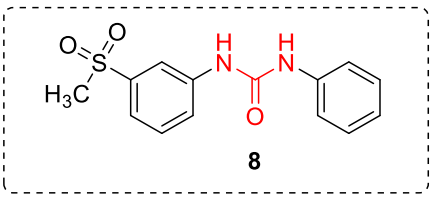

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Figure 2.11: Structure of lead 1,3-diaryl-urea derivatives reported by Zarghi et al.

2.7. Literature reported based on the carbamate fragment (RIV scaffold)

This literature review highlights the potential role of the carbamate fragment, a core scaffold of RIV, as an inhibitor of ChEIs in AD. According to different studies, cholinesterase inhibitors are one of the important therapies for the treatment of AD. It was also noted that the carbamate moiety, chemically and functionally similar to ACh, binds to the active site of both cholinesterases like organophosphorus inhibitors (116). Thus, carbamates bind to AChE reversibly, which effectively protects the neurons by carbomylation of AChE at neuronal synapses and enhances neurotransmission (117). Recent, reports have shown that novel carbamate is an active pharmacophore of cannabidiol (CBD) and RIV for treating AD by targeting ChEs such as AChE and BChE (118, 119). Therefore, the focus is on significant advances in designing and developing various molecules with carbamate scaffold or its derivative that could be a potential agent for treating AD.

2.7.1. Tryptamine-carbamate hybrids

Toublet F.X. and colleagues recently designed and synthesized a novel series of tryptamine-carbamate hybrids, selecting the tryptamine core for its antioxidant properties and the carbamate scaffold for ChE inhibition (120)(**Figure 2.12**). Seven compounds were systematically developed and evaluated for their ChE inhibitory activity and 5-HT_6 receptor binding. Among these, compound **9** displayed the highest ChE (BChE) inhibition with an IC_{50} value of 0.97 μM and strong 5-HT_6 receptor antagonistic activity (Ki = 11.4 nM). Additionally, the Lineweaver-Burk plot indicated that compound **9** acts as a mixed-type inhibitor of BChE. These findings suggest that compound **9** holds potential as a lead compound for further development as an anti-AD agent.

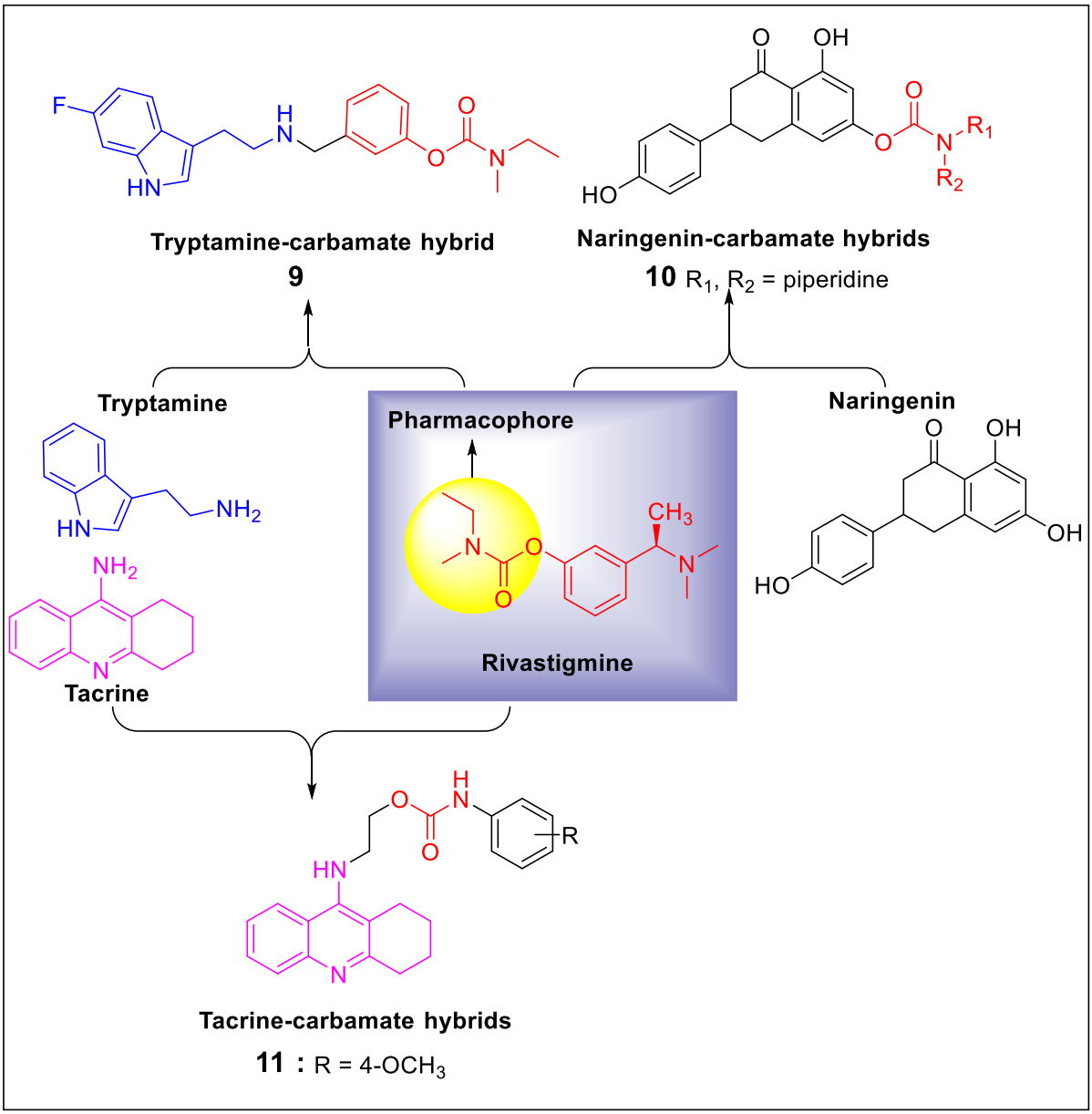

Figure 2.12: Design of tryptamine, naringenin and tacrine carbamate hybrids.

2.7.2. Naringenin carbamate hybrids

Wu J. and colleagues recently designed, synthesized, and biologically evaluated a novel series of naringenin-carbamate hybrids, selecting naringenin for its antioxidant properties and the carbamate scaffold for ChE inhibition (121)(**Figure 2.13**). Enzyme assays demonstrated that the synthesized derivatives exhibited moderate to good inhibitory activity against AChE and BChE, with IC_{50} values ranging from 12.91 to 62.52 µM for AChE and 0.094 to 13.72 µM for BChE. Among these, the most promising compound, hybrid **10**, showed the highest inhibitory activity against BChE with an IC_{50} of 0.094 ± 0.0054 µM.

Enzyme kinetic and molecular modeling studies confirmed that compound **10** binds to both the CAS and PAS of BChE. Additionally, all derivatives exhibited strong free radical scavenging activity compared to vitamin C. Compound **10** also demonstrated metal chelation and Aβ modulation properties. These biological findings suggest compound **10** is a potential anti-AD agent for managing the condition.

2.7.3. Tacrine-carbamate hybrids

Ozge-Ozten and colleagues developed a novel series of tacrine-carbamate hybrids, where tacrine (a ChE inhibitor) was combined with carbamate to enhance the ChE inhibitory activity of the resulting compounds (122)(**Figure 2.13**). Among these hybrids, compound **11** exhibited potent ChE inhibitory activity, with IC_{50} values of 22.15 nM for AChE and 16.96 nM for BChE. Molecular docking studies further revealed that compound **11** interacted with the CAS and PAS of ChE, forming interactions with amino acid residues His478, Gly153, and Glu233. The systematic structure-activity relationship (SAR) analysis showed that factors such as electronegativity, the position of halogens, and electron-withdrawing groups significantly influence ChE inhibitory activity.

2.7.4. Cannabidiol−carbamate hybrids

Jiang and colleagues recently introduced a novel series of cannabidiol-carbamate hybrids as potential therapeutic agents for AD (123). The strategy involved designing innovative ChE inhibitors using a fragment reassembly approach, coupling the cannabidiol core with RIV (a ChE inhibitor) to impart ChE inhibitory properties to the synthesized molecules (**Figure 2.14**). A total of 17 compounds were systematically developed and evaluated for ChE inhibition. While most derivatives showed limited activity against AChE, they demonstrated strong inhibitory activity against BChE. Among these, compound **12** emerged as a highly selective BChE inhibitor, exhibiting an IC_{50} of 5.30 nM and pseudo-irreversible inhibition with a dissociation constant (Kd) of 13 nM. Additionally, compound **12**

displayed antioxidant properties, neuroprotective effects, and effective BBB permeability. SAR analysis revealed that introducing a carbamate moiety enhanced ChE inhibition, while chain fatty amines, tert-benzylamines, and 4-halogen substitutions significantly boosted BChE inhibitory activity. Molecular docking studies indicated compound **12** formed p–π interactions with Met437 and Trp430 in BChE. In vivo experiments demonstrated that compound **12** effectively mitigated scopolamine-induced cognitive impairment in the Morris water maze (MWM) test and improved cognitive deficits in an $A\beta_{1\text{-}42}$-induced model, highlighting its anti-AD potential.

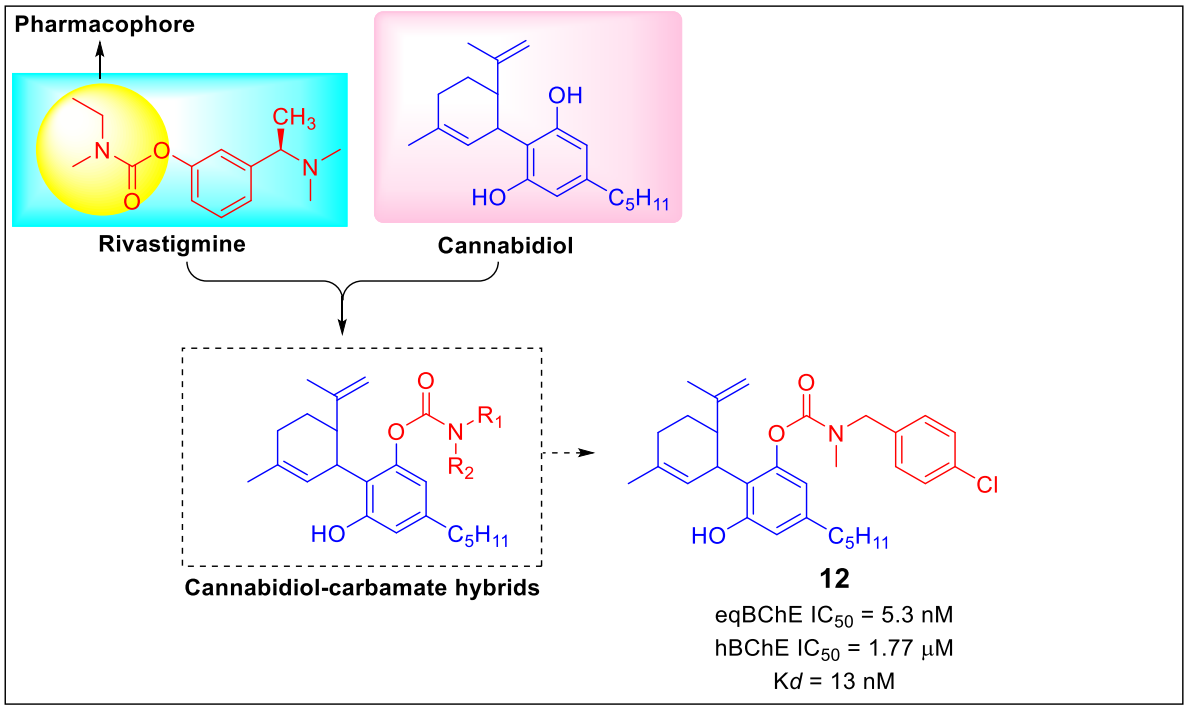

Figure 2.13: Design and SAR study of cannabidiol-carbamate hybrids.

2.7.5. Arylcarbamate-N-acyl hydrazone hybrids

In 2021, Diego A.S.Y. and colleagues synthesized a novel series of arylcarbamate-N-acylhydrazones by incorporating a hydrazone group into the carbamate fragment (120). They systematically developed 12 compounds and evaluated for inhibitory activity against AChE and BChE. The enzyme assay results showed that compounds **13-16, 18,** and **20** demonstrated the highest selectivity towards BChE with weak AChE inhibition (**Figure 2.15**). Compound **15** (IC_{50} = 0.07 μM) exhibited the most potent BChE inhibitory activity, 50

times more effective than DPZ (IC_{50} = 3.54 μM). The SAR study revealed that the aromatic ring's carbamate group significantly influences the analog's ChE inhibitory activity. Additionally, enzyme kinetics analysis showed compound **15** acts as a competitive inhibitor. Molecular docking studies supported the in vitro findings, showing that compound **15** interacts with Tyr128 and Trp82 through π-π interactions, and His438, Gly121, and Glu197 via CH···O interactions. Based on these results, compound **15** has the potential to be a promising lead for further development in AD treatment.

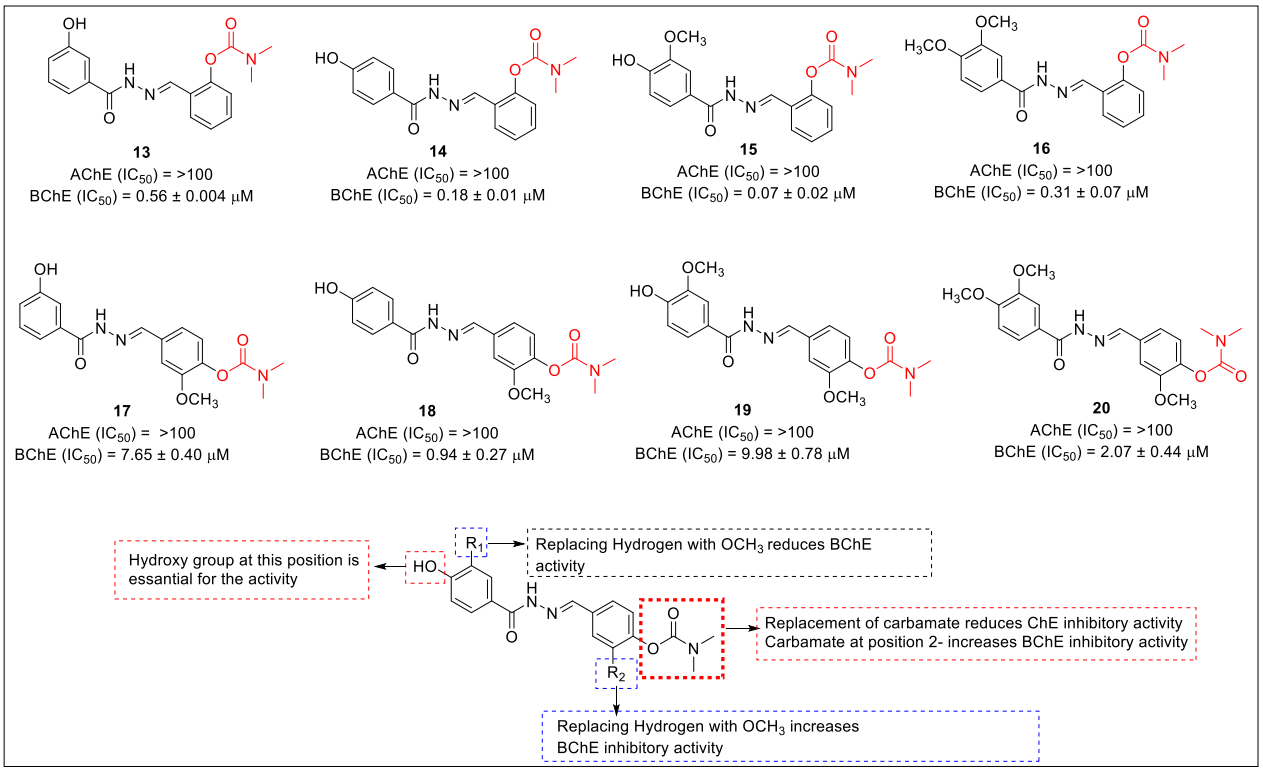

Figure 2.14: Structures and SAR of arylcarbamate-N-acyl hydrazone hybrids.

2.7.6. Ferulic acid-O-carbamoyl hybrids

In 2020, Sang et al. designed and synthesized a series of novel ferulic acid-O-carbamoyl derivatives as potential multifunctional candidates for AD therapy (124). These compounds were developed using a hybrid approach, combining ferulic acid (a known antioxidant) with RIV to endow the molecules with multifunctional properties (**Figure 2.15**). Most of the synthesized hybrids exhibited significant inhibitory activity against *h*AChE and *h*BChE, with IC_{50} values ranging from 6.8 ± 0.37 to 32.1 ± 2.2 μM and 0.97 ± 0.02 to 36.7 ±

3.6 μM, respectively. Among the 21 synthesized compounds, derivative **21** emerged as the most potent dual inhibitor of AChE and MAO-B, with IC_{50} values of 0.97 μM for AChE and 5.3 μM for MAO-B. SAR analysis indicated that introducing N-di-substituted (carbamoyl-NR1R2) groups enhanced ChE inhibitory activity. Compound **21** inhibited Aβ aggregation by 58.2% and disaggregated preformed Aβ fibrils by 43.3% in self-mediated Aβ aggregation assays. Molecular docking studies on *h*BChE (PDB code: 4TPK) revealed interactions with residues Trp82, Gln119, Pro285, Ser287, Val288, Ala328, and Phe329. Furthermore, *in-vivo* studies demonstrated that compound **21** significantly improved learning and memory in a scopolamine-induced amnesia model. Overall, the findings suggest that compound **21** holds promise as a multifunctional lead candidate for AD treatment.

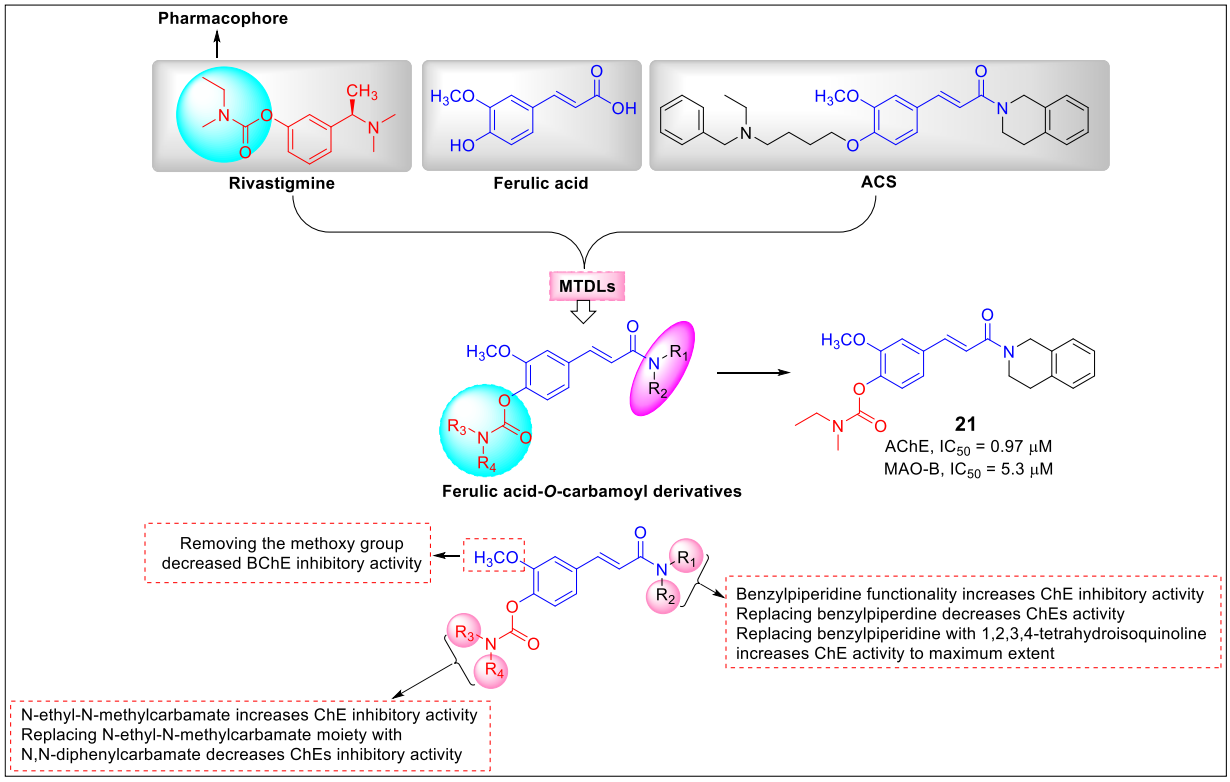

Figure 2.15: Design and SAR study of ferulic acid-O-carbamoyl derivatives.

2.7.7. N-alkylpiperidine carbamate hybrids

In 2020, Kosak and colleagues developed a novel series of N-alkylpiperidine derivatives as potential anti-AD agents (108). They systematically designed, synthesized, and evaluated 36 compounds for their inhibitory activities against AChE, BChE and monoamine

oxidases (MAO-A and MAO-B). Among these, four compounds exhibited notable inhibitory activity: compound **22** demonstrated IC_{50} values of 7.31 μM for AChE, 0.56 μM for BChE, and 26.1 μM for MAO-B; compound **25** showed IC_{50} values of 2.25 μM for AChE and 0.81 μM for BChE; compound **23** was a selective BChE inhibitor with an IC_{50} of 0.06 μM; and compound **24** selectively inhibited MAO-B with an IC_{50} of 0.18 μM. The SARs of the 1,3-disubstituted N-alkylpiperidine-carbamates are illustrated in **Figure 2.16**. Enzyme kinetic studies revealed that compound **23** inhibits non-covalently, compound **24** acts as an irreversible MAO-B inhibitor, and compound **22** functions as a reversible inhibitor. Compounds **22, 23, 24,** and **25** exhibited good BBB permeability and cytoprotective effects in SH-SY5Y cells. Notably, compounds **22** and **23** protected neuronal cells from $A\beta_{1-42}$ induced cytotoxicity, highlighting their potential as therapeutic candidates for AD.

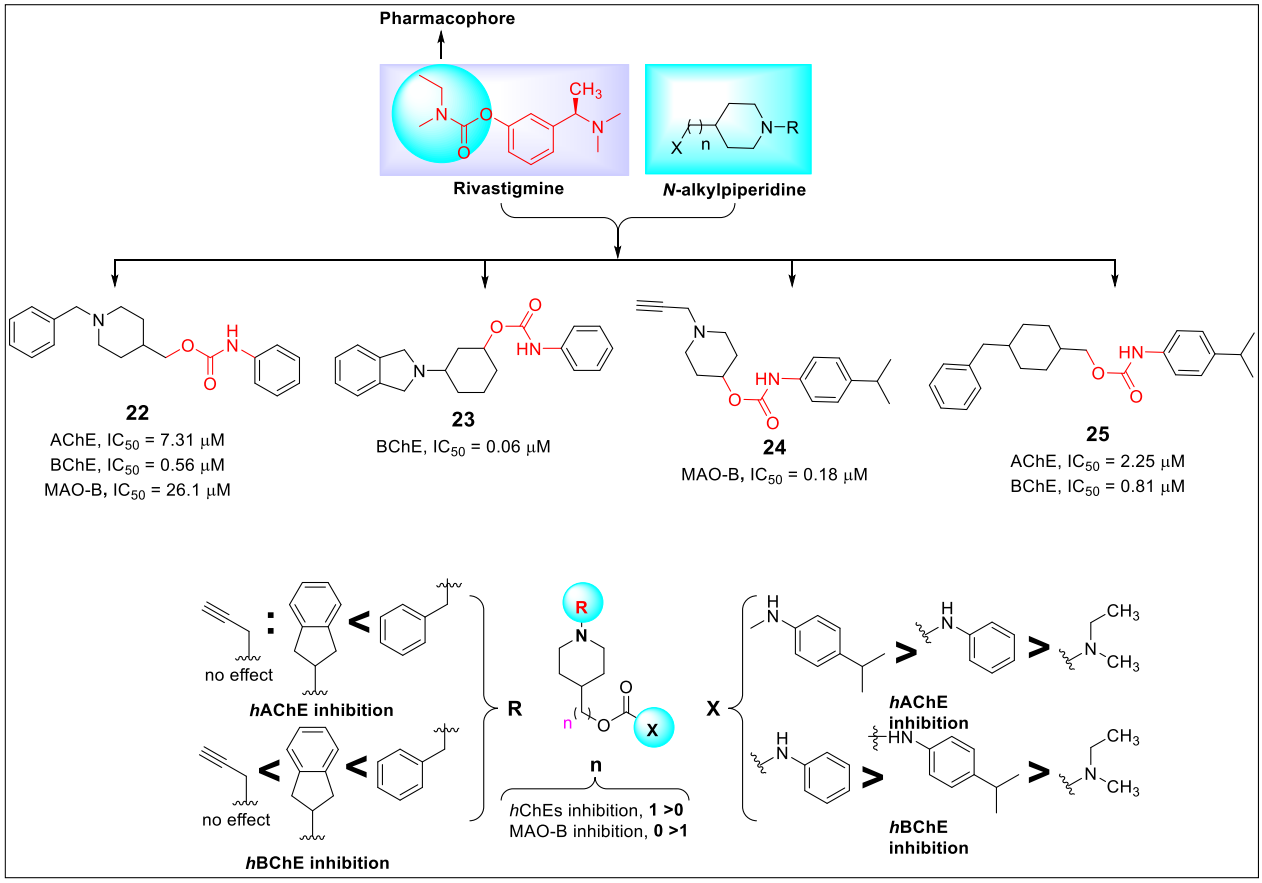

Figure 2.16: Design and SAR study of N-alkylpiperidine carbamate hybrids.

2.7.8. Chalcone-O-carbamate hybrids

Sang et al. recently reported the development of a series of 12 novel multifunctional molecules designed for AD management (125). The design strategy involved coupling chalcone with carbamate to incorporate ChE inhibition, antioxidant properties, and metal chelation capabilities into the synthesized compounds (**Figure 2.17**). The *in-vitro* biological evaluation included ChE and MAO inhibition studies, antioxidant activity, $A\beta_{1\text{-}42}$ modulation, and metal-chelation properties. Among these, compounds **26,** and **27** demonstrated the most potent BChE inhibitory activities, with IC_{50} values of 3.1 µM and 1.2 µM, respectively. **26** and **27** also exhibited MAO-B inhibitory activities with IC_{50} values of 1.3 µM and 3.7 µM, respectively. Both compounds effectively modulated $A\beta_{1\text{-}42}$ aggregation, achieving 63.9% and 53.1% inhibition rates, respectively. **26** selectively chelated copper (Cu^{2+}) and aluminum (A^{l3+}) showed strong neuroprotective effects against H_2O_2-induced cell death in PC12 cells. Finally, *in-vivo* studies revealed that compound **26** significantly improved learning and memory in a scopolamine-induced cognitive impairment model.

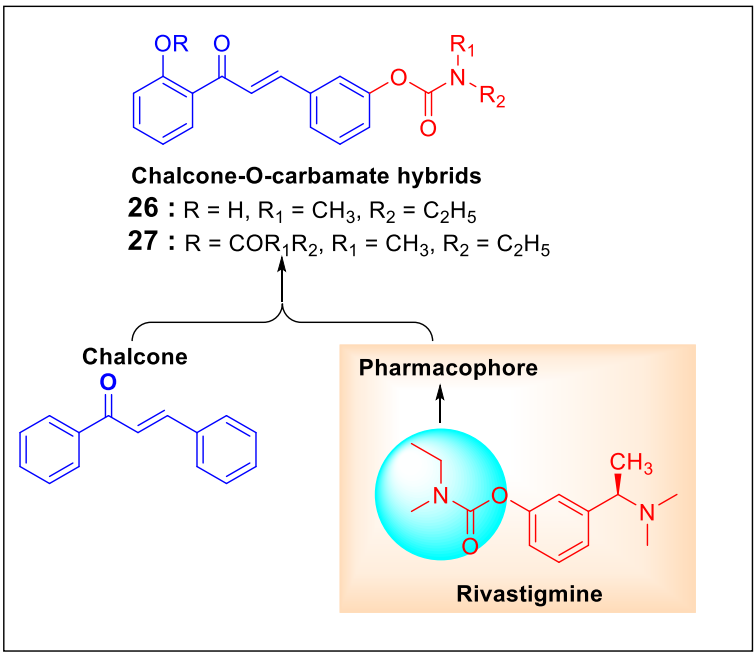

Figure 2.17: Design of chalcone, quinoline, pyridine, and diosgenin-carbamate hybrids.

2.7.9. Smilagenin carbamate hybrids

In 2019, Zhang et al. developed a series of smilagenin-carbamate hybrids as potential anti-AD agents (126). They synthesized 14 hybrid compounds, with analogue **28** exhibiting the most significant neuroprotective effect, showing 40.5 ± 2.3% cell viability at 10 µM (**Figure 2.18**). Additionally, compound **28** demonstrated strong cytoprotective properties, protecting against H_2O_2-induced cell death, oxygen-glucose deprivation-induced cytotoxicity in SH-SY5Y neuroblastoma cells, and lipopolysaccharide-induced nitric oxide (NO) release in RAW264.7 cells.

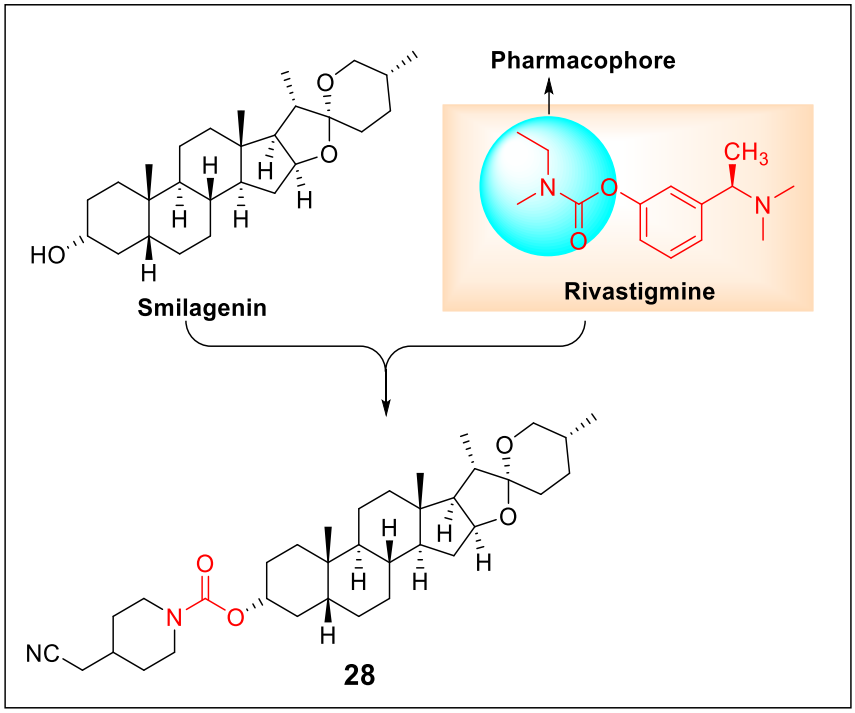

Figure 2.18: Design of smilagenin, and arctigenin-carbamate hybrids.

2.7.10. Pyranoisoflavone-carbamate hybrids

In 2019, Chuanhai Wu and colleagues developed a new series of naturally inspired pyranoisoflavone derivatives as potential BChE inhibitors (127). They synthesized 26 analogs and evaluated them against AChE and BChE. The results showed that all the analogs exhibited better BChE inhibitory activity, with IC_{50} values ranging from 13.10% inhibition at 10 µM to 0.093 ± 0.001 µM. Among them, compounds **29,** and **30** displayed the highest activity against BChE, with IC_{50} values of 0.22 ± 0.01 µM for compound **29** and 0.093 ±

0.001 µM for compound **30**. Enzyme kinetics studies revealed that both compounds exhibited mixed-type of inhibition. Molecular docking studies showed that compound **29**, containing the carbamate moiety, interacted with the catalytic site, forming hydrogen bonds with Ser198 (2.1 Å) and His438 (2.9 Å). A similar docking pattern was observed for compound **30,** which formed a hydrogen bond between the carbonyl group and Gly117 (2.9 Å), while its heptyl group interacted through π-alkyl interactions with Tyr332 and Trp430. Cytotoxicity studies indicated that compounds **29,** and **30** were safe at concentrations up to 1 µM, but at 5 µM, both compounds caused a decrease in cell viability (64.2% and 66.2%, respectively) (**Figure 2.19**).

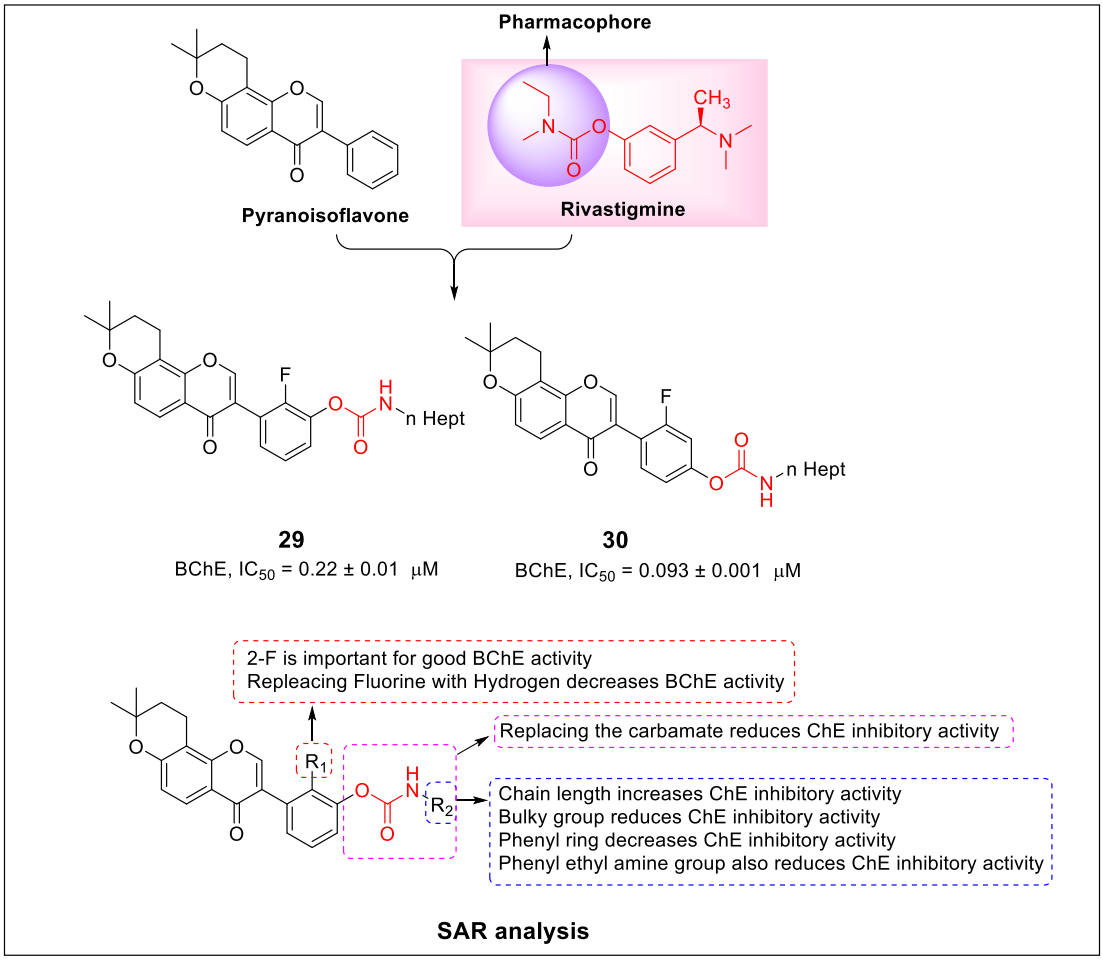

Figure 2.19: Design and SAR study of pyraoisoflavone and 4-aminochalcone-RIV hybrids**.**

2.7.11. Salicylanilide and 4-chlorophenol-based N-monosubstituted carbamate hybrids

In 2018, Krátký and colleagues synthesized 20 novel salicylanilide N-monosubstituted carbamates and evaluated their *in-vitro* inhibitory activity against *ee*AChE and *eq*BChE (128) (**Figure 2.20**). All the synthesized compounds exhibited mild to moderate ChE inhibitory activity, with IC_{50} values ranging from 5 to 235 μM. Among the hybrids, compounds **31, 32,** and **33** demonstrated stronger ChE inhibition compared to RIV against both enzymes, with IC_{50} values of 43.88 ± 0.69, 45.51 ± 0.69, and 51.50 ± 0.54 μM for AChE, respectively. The SAR study revealed that N-phenyl-ethyl carbamates had the highest inhibitory activity against BChE compared to the other analogs.

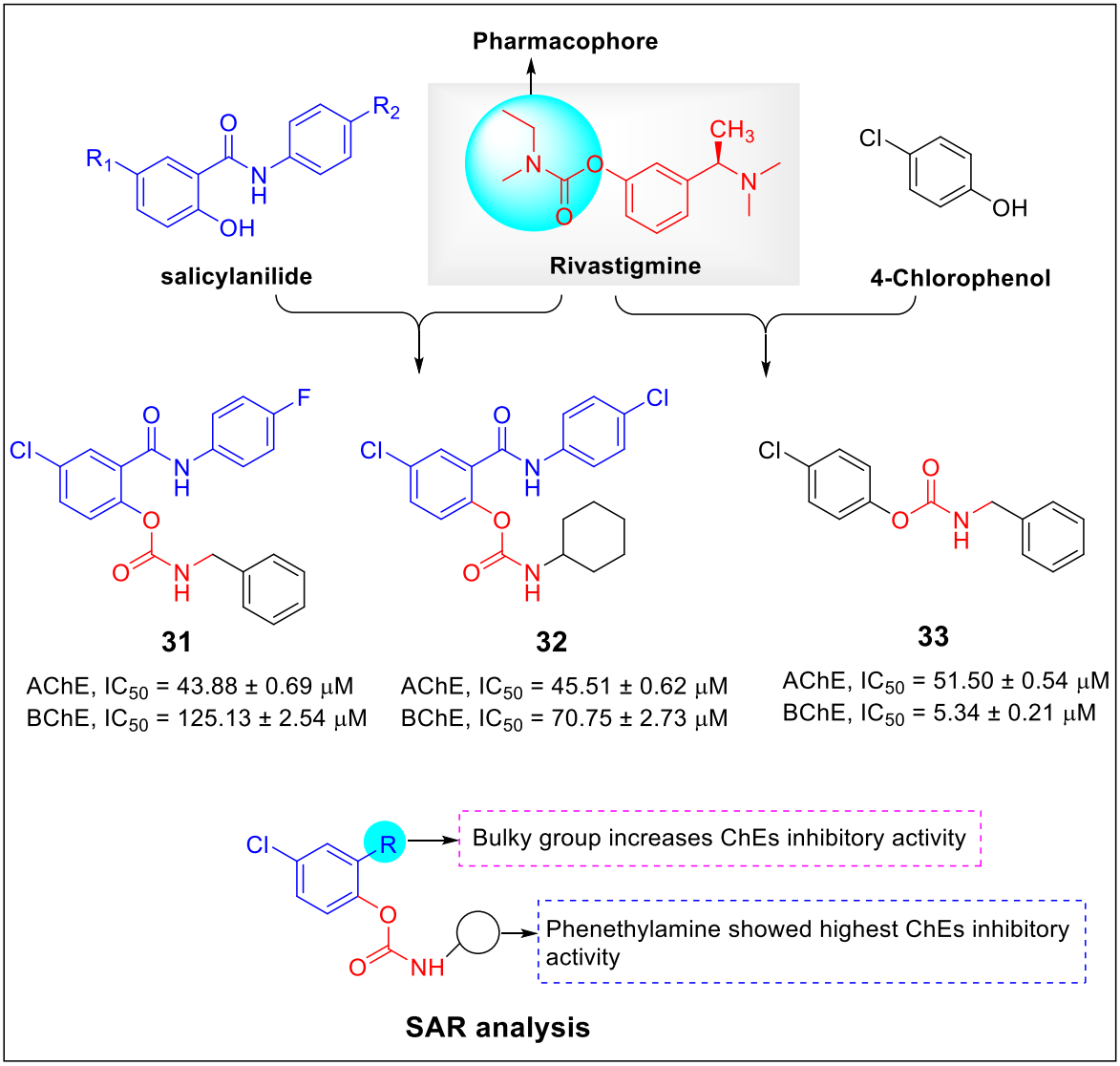

Figure 2.20: Design and SAR study of salicylanilide and 4-chlorophenol-based carbamate hybrids.

2.7.12. 2-methoxy-phenyl dimethyl-carbamate hybrids

In 2014, Li Y. and colleagues investigated a series of RIV-curcumin hybrids for their ability to inhibit AChE and BChE (129). Most synthesized analogs demonstrated good to excellent ChE inhibitory activity. Among them, compound **35** exhibited strong activity with an IC_{50} value of 0.097 µM against AChE, which is 20 times more potent than RIV. The SAR study indicated that para-substituted analogs containing piperidine and morpholine favored AChE inhibition. Compounds **34, 35,** and **36** displayed Aβ modulation in TEM assays, free radical scavenging activity in the ABTS assay, and potent Cu^{2+} ion chelation in vitro. Overall, the findings suggest that compound **35** could be a promising multifunctional drug lead (MTDL) for treating AD (**Figure 2.21**).

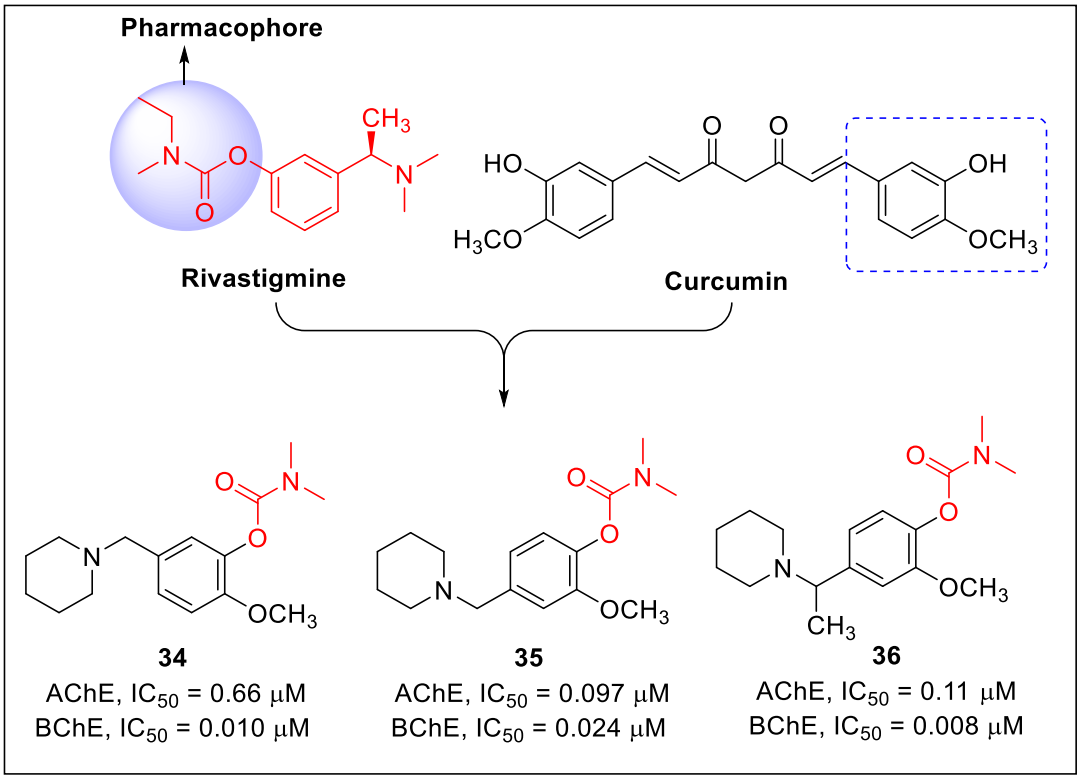

Figure 2.21: Design of 2-methoxy-phenyl dimethyl-carbamate hybrids.

2.7.13. Miscellaneous hybrids

In 2017, Nesi G. *et al.* developed a new series of naturally inspired multifunctional molecules by coupling carbamate with gallic acid, lipoic acid, and chromone-carboxylic acid

to address the multifactorial nature of AD (130). They systematically developed nine molecules and evaluated them against AChE, BChE, radical scavenging activity assay (DPPH), and self-induced Aβ aggregation assay. Among all, lipoic acid derivatives showed the most potent BChE inhibitory activity with IC_{50} values of 0.35 μM for **37**, 0.378 μM for **38**, and 0.34 μM for **39**, respectively. Furthermore, the SAR study revealed that long-chain lipoic acid is well tolerated for BChE compared to gallic acid and chromone-carboxylic acid. Cytotoxicity study revealed that **37**-**39** were safe candidates up to 100 μM in HT22 cells (**Figure 2.22**).

Bajda M. *et al.*, in the year 2018, designed, synthesized, and biologically evaluated another series of carbamate derivatives with N-phenylpiperazine, N-benzylpiperazine, and 4-benzylpiperidine moieties (131). A pharmacological study revealed that most developed analogs exhibited significant activity against BChE. Among all compounds, **46** (3-(4-phenyl-piperazin-1-ylmethyl)-phenyl phenylcarbamate) exhibited IC_{50} = 2.00 μM against BChE (**Figure 2.23**). Lipophilicity and ADMET profiles were accessed using computer programs for all synthesized analogs. Furthermore, a molecular docking study was performed to check binding interaction with BChE.

Saxena J. *et al.* in the year 2015 developed a new series of ethynyl phenyl carbonates and carbamates as dual-action AChEIs and anti-inflammatory agents (132). All the developed molecules showed mild to moderate AChE inhibitory activity with an IC_{50} range of 28–86 μM. Compound **47** was the most potent candidate, with IC_{50} = 28.4 μM for AChE (**Figure 2.23**). Furthermore, these molecules can potentially treat neuroinflammation and dually target AD by restoring cholinergic balance and suppressing inflammation.

Kratký M. *et al.*, in the year 2021, developed a novel series of MTDL by combining carbamate, propargylamine, and salicylic scaffolds to impart ChE and MAOs inhibitory and antioxidant properties to developed analogs (133). They systematically evaluated all the

developed compounds in vitro against AChE, BChE, and MAO. All the compounds act as dual inhibitors. Most of the analogs were found to be potent inhibitors of AChE, among the developed molecules **48, and 49** exhibited most promising activity for BChE (25.10 and 26.09 μM) (**Figure 2.23**). Furthermore, *in-silico* prediction of physicochemical parameters acknowledges that the candidate would be active after oral administration and able to reach brain tissue.

In the year 2016, Edgar Sawatzky *et al*. reported a new series of pseudo-irreversible BChE inhibitors by coupling of tetrahydroquinazoline scaffold with a carbamate fragment (27). They systematically designed 26 derivatives and biologically evaluated them for *eq*BChE and *h*AChE inhibition assay (**Figure 2.23**). All the developed analogs exhibited mild to excellent ChE inhibitory activity in enzyme assay experiment with IC_{50} value from 2.1 - 196.1 μM. Among all compounds, **50** was the most potent inhibitor of *eq*BChE (K_C = 14.3 nM) and *h*BChE (K_C = 19.7 nM). The SAR study revealed that pyridyl residues decreased ChE inhibition activity and the thiophene residues as less polar bioisosteres increased ChE inhibition more than 5-fold to IC_{50} against *eq*BChE of 22 nM for **51** and 14 nM for **50**. Furthermore, the molecular simulation study proved how the tetrahydroquinazoline scaffold containing carbamate moieties interacted with the active site of AChE. Moreover, the neuroprotection study revealed high radical scavenging properties of the phenolic heterocycle carrier with carbamate fragment. In summary, compound **50** is the most potent lead for developing other pseudo-irreversible enzyme inhibitors.

In 2021, Scheiner M. et al., the same research group developed a new series of MTDLs as a pseudo-irreversible BChE inhibitor for managing AD (134, 135). The obtained hybrids were investigated in *in-vitro* for their *h*BChE and *h*AChE inhibition, enzyme kinetics, and antioxidant physicochemical properties (DPPH, ORAC, metal chelating). All the developed analogs were good to excellent inhibitors of *h*BChE with IC_{50} values from 5.03 ±

0.2 - 8.19 ± 0.2 nM. All the developed analogs (**52-60**) exhibit excellent activity with IC_{50} values of 6.32 ± 0.03 nM for **52**, 6.90 ± 0.01 nM for **53**, 6.69 ± 0.02 nM for **54**, 6.94 ± 0.02 nM for **55**, 6.78 ± 0.02 nM for **56**, 6.66 ± 0.03 nM for **57**, 6.35 ± 0.04 nM for **58**, 6.10 ± 0.05 nM for **59**, and 5.03 ± 0.02 nM for **60**, respectively. Furthermore, the antioxidant assay revealed that ferulic acid and trolox fragment-containing compounds showed excellent antioxidant potential. In addition, *in-vitro* assays were applied to investigate antioxidant effects using murine hippocampal HT22 cells and immunomodulatory effects on the murine microglial N9 cell line. The MTDLs retained their antioxidative properties compared to the parent antioxidant-moieties *in-vitro* and the inhibition of *h*BChE was maintained in the submicromolar range. Representative compounds were tested in a pharmacological AD mouse model and demonstrated high efficacy at doses as low as 0.1 mg/kg. Considering all the results, the author concluded that MTDLs might be a potential candidate for the management of AD.

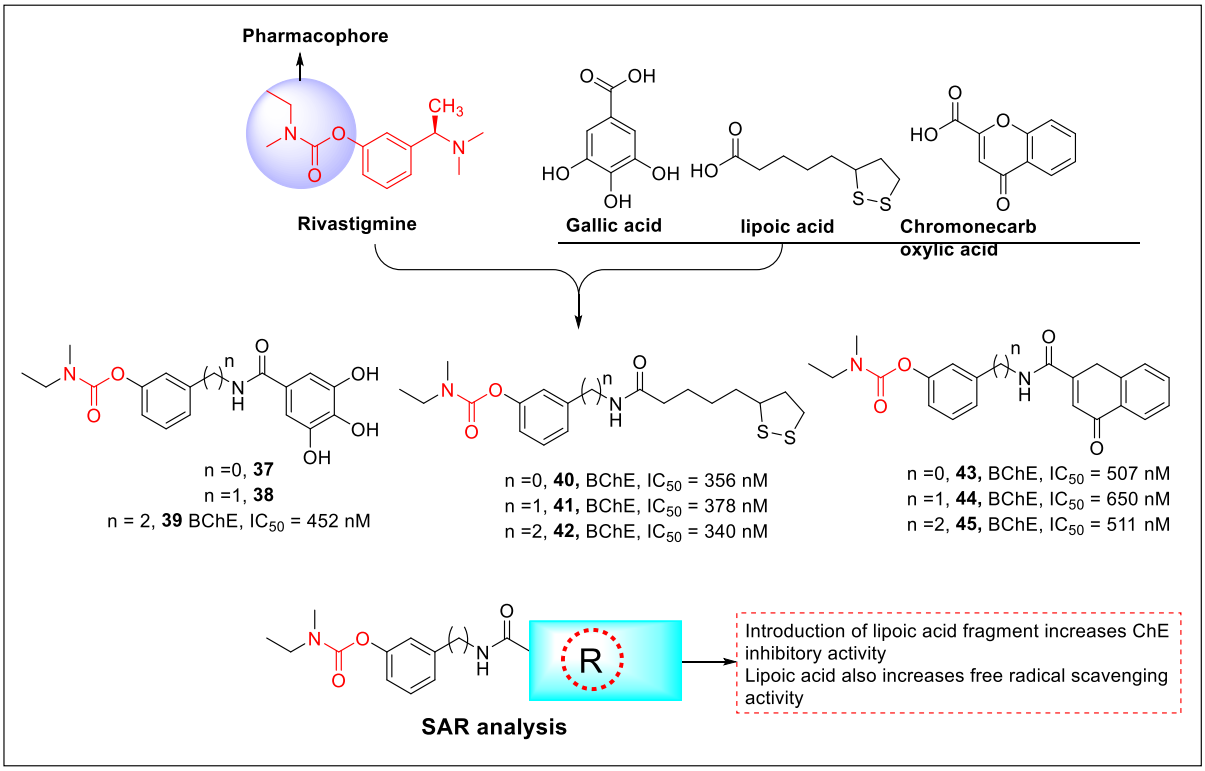

Figure 2.22: Miscellaneous hybrids analogues (67-75).

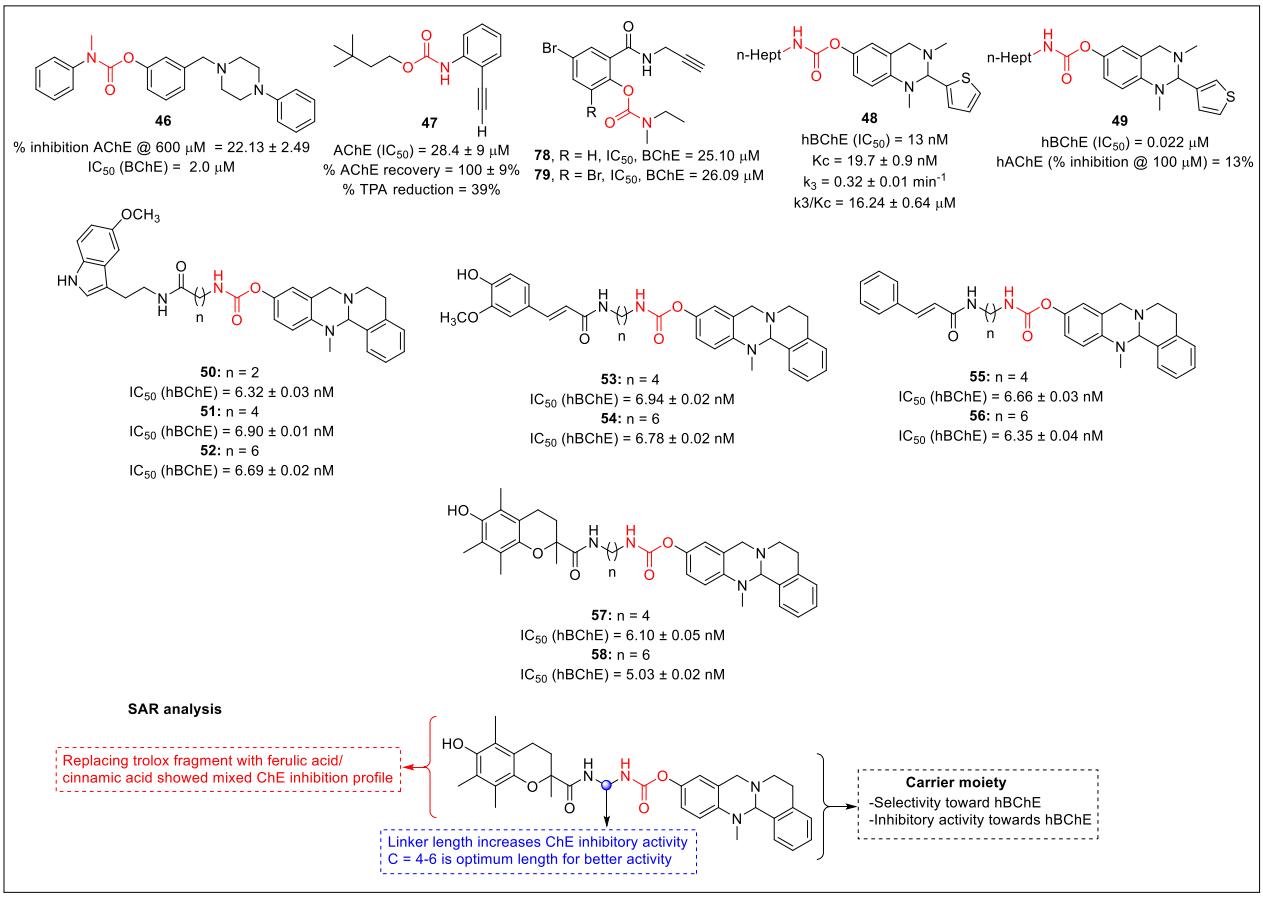

Figure 2.23: Miscellaneous hybrids analogues (50-58).