Chapter 2

(Review of Literature)

2.1 1,3,4-Oxadiazoles as multitarget directed ligands in AD

The 1,3,4-Oxadiazole scaffold has emerged as a suitable pharmacophore to design MTDL against AD owing to its planner ring orientation and H-bond acceptor ability. The structural features in the 1,3,4-Oxadiazole scaffold make it an appropriate candidate to interact active site pocket of the multiple enzymes involved in the disease development and progression.

Saitoh and Onishi et al. have investigated compound **1** (1,3,4-Oxadiazole scaffold) with GSK (IC_{50} = 37 & 53 nM against GSK-3α and GSK-3β respectively) and CDK-5 inhibitory activity (Figure 2. 1). Compound **1** was also investigated for multitargeting potentials *in vivo* which demonstrated tau phosphorylation reduced APP metabolism. Compound **1** also showed amelioration of cognitive dysfunction in transgenic AD mice model [Onishi et al. 2011, Saitoh et al. 2009].

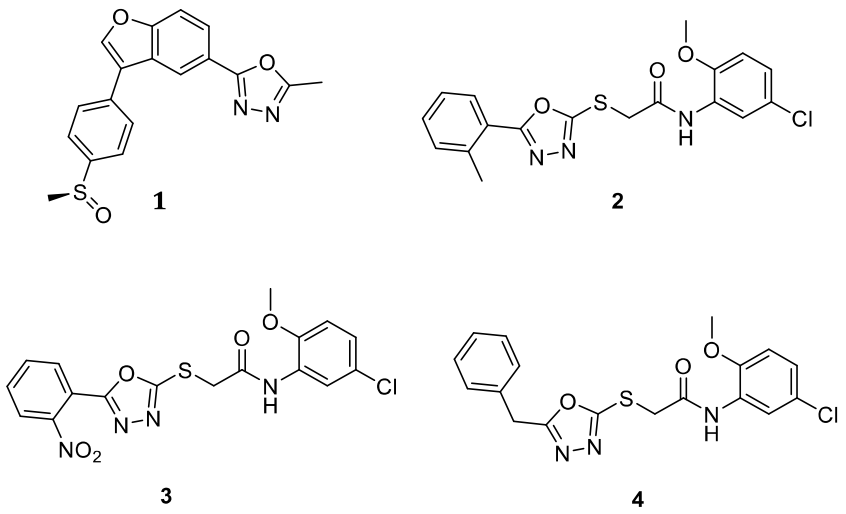


Figure 2. 1 1,3,4-Oxadiazole compound as GSK-3β, CDK-5 inhibitor (1), ChE, and LOX inhibitors (**2-5**).

Rehman et al. reported 1,3,4-oxadiazole-2-yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide derivatives as LOX and ChE inhibitors. Compounds **2** (IC_{50} = 34.61 μM) and **3** (IC_{50} = 40.21 μM) from their synthesized products demonstrated good AChE

inhibitory activity. In comparison, compound **4** showed the highest BChE inhibitory (IC_{50} = 33.31 μM) activity (Figure 2. 1). Compound **3** from their series also exhibited considerable % LOX inhibitory activity (69.67%) [Rehman et al. 2013].

Kamal et al. have demonstrated AChE inhibitory activity of the (*E*)-2-aryl-5-(3,4,5-trimethoxystyryl)-1,3,4-oxadiazoles and (*E*)-2-aryl-5-(2-benzo[d][1,3]diox-ol-5-yl)vinyl)-1,3,4-oxadiazoles derivatives, Compounds **5** (IC_{50} = 24.89μM), **6** (IC_{50} = 13.72μM), **7** (IC_{50} = 37.65 μM), and **8** (IC_{50} = 19.63 μM) amongst their synthesized derivatives showed moderate AChE inhibitory activity (Figure 2. 2). Further molecular docking interaction of the compounds containing 1,3,4-oxadiazoles scaffold also demonstrated its binding within the AChE active site [Kamal et al. 2014].

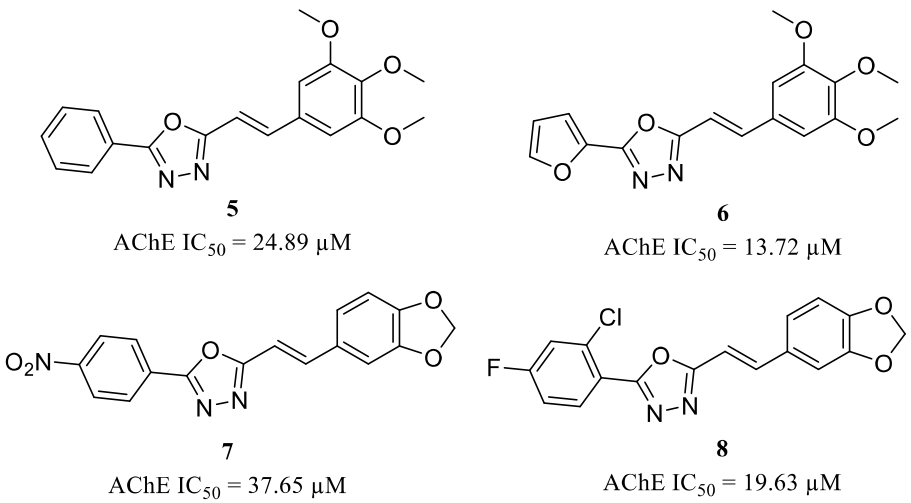


Figure 2. 2. 1,3,4-Oxadizole scaffold containing analogous (**5-8**)

Mei et al. have reported a series of 1,3,4-Oxadiazoles scaffolds containing molecular hybrids. They tested their synthesized compounds for neuroprotective activity against Aβ-induced toxicity. Compound **9** from their series exhibited significantly higher % cell viability than the other compounds **10, 11,** and **12**, which have also demonstrated good % cell viability (Figure 2. 3) [Mei et al. 2017].

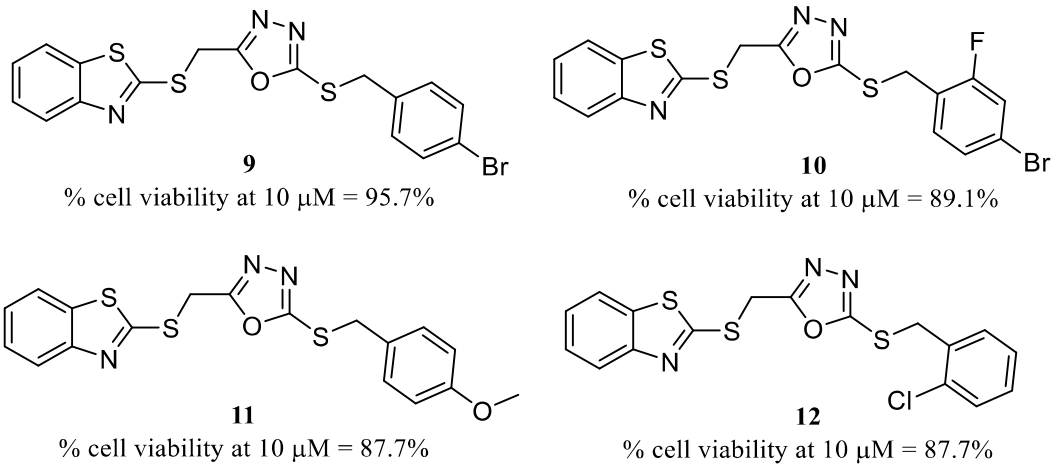


Figure 2. 3. Novel benzothiazole tethered 1,3,4-oxadiazole hybrids (**9-12**).

Rehman et al. showed the AChE inhibitory potential of 3-piperidinyl-1,3,4-oxadiazoles derivatives. Compound **13** (IC_{50} = 3.64 μM) amongst their synthesized derivatives exhibited moderate AChE inhibitory potential and minimal % hemolysis (9.8 %) as compared to standard Triton-X 100 (99.27%) (Figure 2. 4) [Rehman et al. 2018].

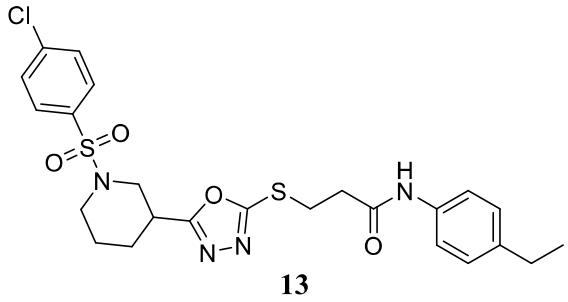


Figure 2. 4. 3-Piperidinyl-1,3,4-oxadiazole molecular hybrid (**13**) with AChE inhibitory potential.

Tripathi et al. reported substituting 1,3,4-oxadiazoles tethered 2-aminopyrimidine novel molecular hybrids as MTDL. They investigated the anti-AChE and anti-Aβ aggregation potential of the compounds. Compounds **14** (pIC_{50} = 6.52, Ki = 0.17 μM) among their series showed significant anti-hAChE and anti-Aβ aggregation activity (Figure 2. 5). Compound **14** also demonstrated effective AChE-PAS binding, good BBB permeability

in PAMPA-BBB assay, antioxidant potential and an appreciable amelioration of scopolamine-induced cognitive impairment in mice models [Tripathi et al. 2019b].

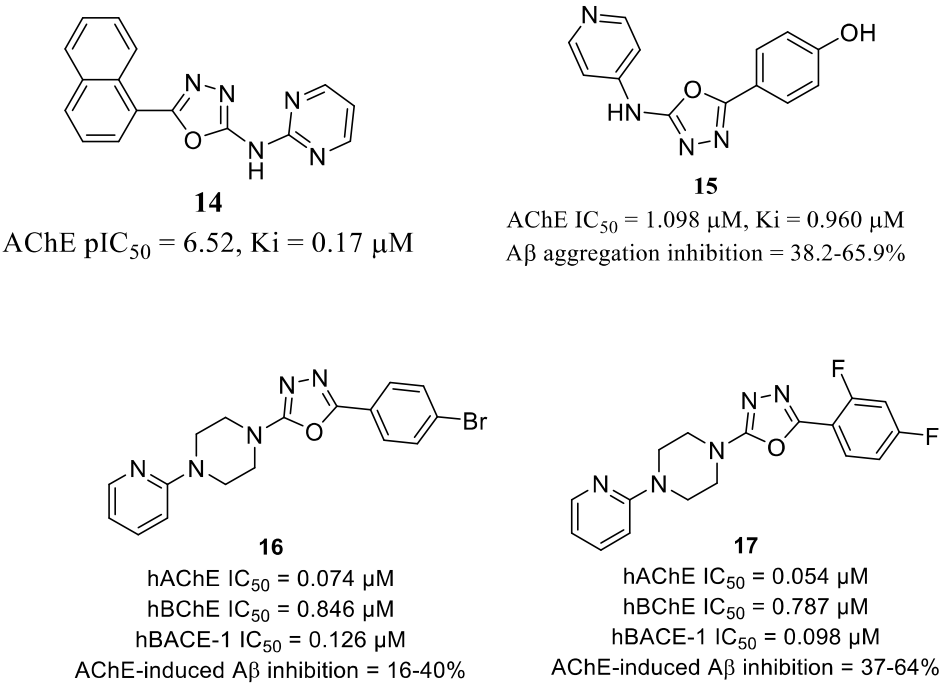


Figure 2. 5. A multifunctional molecular hybrids of 1,3,4-oxadiazoles (**14-17**) to treat AD.

Mishra et al. reported the design and synthesis of 4-aminopyridine-linked substituted 1,3,4-oxadiazoles derivatives as multifunctional agents. Compound **15** (Figure 2. 5) amongst their series appeared to be the most potent AChE (IC_{50} = 1.098 μM) inhibitor and exhibited mixed type-noncompetitive enzyme inhibition. Furthermore, compound **15** showed significant anti-Aβ aggregation potential in self- and AChE-induced (38.2–65.9% respectively) thioflavin T experiments. The *in vivo* investigations of compound **15** also demonstrated a substantial reversal of memory functions in the scopolamine-induced mice model. At the same time, the *ex vivo* studies of the brain homogenate showed the antioxidant potential of the compound [Mishra et al. 2019].

Tripathi A. et al. showed the design, synthesis, and biological evaluation of 2-pyridyl piperazine linked 5-phenyl-1,3,4-oxadiazoles molecular hybrids as MTDL for AD

therapy. Among their synthesized derivatives, compound **16** (hAChE, IC_{50} = 0.074 μM; hBChE, IC_{50} = 0.846 μM and BACE-1, IC_{50} = 0.126 μM) and **17** (hAChE, IC_{50} = 0.054 μM; hBChE, IC_{50} = 0. 0.787 μM and BACE-1, IC_{50} = 0. 0.098 μM) demonstrated balanced multifunctional inhibitory activity (Figure 2. 5). Both these compounds exhibited self- and AChE-induced Aβ aggregation inhibition potential in Thioflavin T assay with comparable BBB permeability as compared to standard donepezil in PAMPA-BBB assay. Both these compounds also demonstrated neuroprotective activity against Aβ induced oxidative stress and AChE-PAS binding in PI displacement assay. The *in vivo* study suggested that these compounds had restored memory functions in cognitively impaired rat models while demonstrating the antioxidant potential of the compounds in ex vivo studies [Tripathi et al. 2019a].

Sharma et al. demonstrated the synthesis and biological evaluation of N-benzyl piperidine linked 1,3,4-oxadiazoles molecular hybrids as MTDL for AD therapeutics. The most promising compounds from their series **18** (hAChE, IC_{50} = 0.055 μM; hBChE, IC_{50} = 0.186 μM and BACE-1, IC_{50} = 0.146 μM) and **19** (hAChE, IC_{50} = 0.086 μM; hBChE, IC_{50} = 0.143 μM and BACE-1, IC_{50} = 0.114 μM) showed multifunctional inhibitory profiles of the compounds (Figure 2. 6). In addition to this, both the compounds also demonstrated good BBB permeability via PAMPA-BBB assay, significant AChE-PAS binding in PI displacement assay, self- and AChE-induced anti-Aβ aggregation potential via thioflavin T assay and neuroprotective properties against SH-SY5Y cell lines. Both these compounds also demonstrated amelioration of cognitive deficits in scopolamine and Aβ-induced animal models of AD. The ex vivo studies of both these compounds also suggested antioxidant potential and capability of reducing molecular expression of Aβ and BACE-1 enzymes [Sharma et al. 2019b].

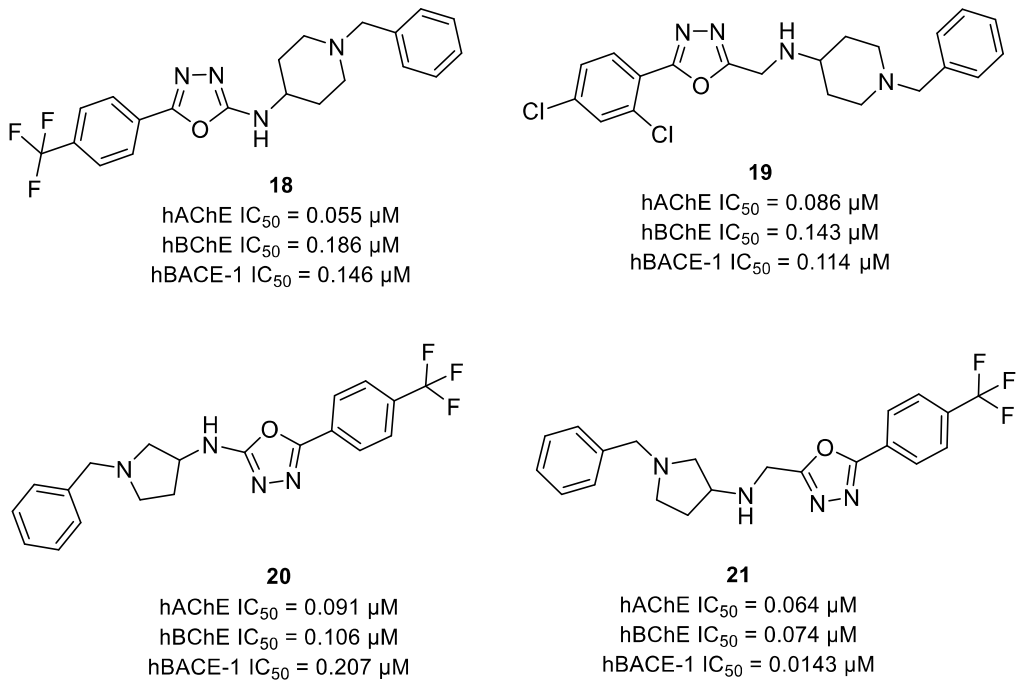
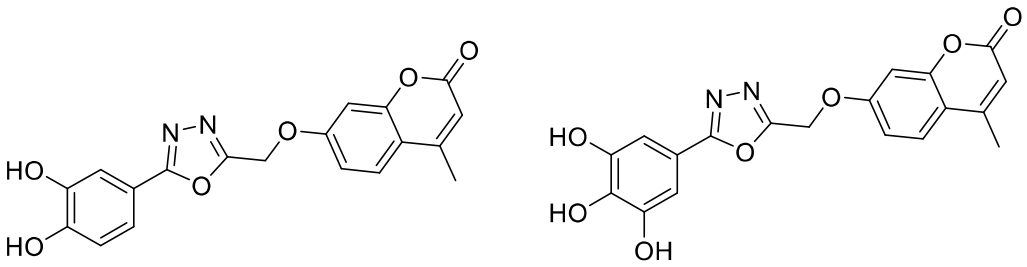


Figure 2. 6. A N-benzylpyrrolidine linked 1,3,4-oxadiazoles (18-21) MTDL to treat AD.

Choubey et al. reported N-benzyl pyrrolidine and 1,3,4-oxadiazole fused derivatives as multifunctional molecular hybrids. The most potent compounds **20** (hAChE, IC_{50} = 0.091 µM; hBChE, IC_{50} = 0.106 µM and BACE-1, IC_{50} = 0.207 µM) and **21** (hAChE, IC_{50} = 0.064µM; hBChE, IC_{50} = 0.074 µM and BACE-1, IC_{50} = 0.143 µM) from their series demonstrated a considerable multifunctional inhibitory potency against tested enzymes (Figure 2. 6). Bothe these compounds also suggested Aβ aggregation inhibition potential in self- and AChE-induced thioflavin T assay, excellent BBB permeability, good AChE-PAS binding and neuroprotective properties of the compounds against SH-SY5Y cell lines. The in vivo studies of both compounds further demonstrated improvements in memory functions in scopolamine and Aβ-induced cognitive deficit animal models. At the same time, the ex vivo studies showed the antioxidant potential of both compounds [Choubey et al. 2021].

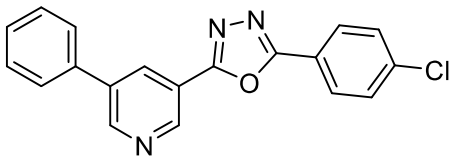


22
AChE IC_{50} = 29.56 µM
BuChE IC_{50} = 45.87 µM
COX % Inhibition = 65.22 %

23
AChE IC_{50} = 28.68 µM
BuChE IC_{50} = 47.39 µM
COX % Inhibition = 71.34%

Figure 2. 7. A multifunctional molecular hybrids of 1,3,4-oxadiazoles (**22-23**) for AD therapy.

George et al. reported coumarin linked 1,3,4-oxadiazole MTDL as an anti-AD agent. Compounds **22** (AChE, IC_{50} = 29.56 µM; BChE, IC_{50} = 45.87 µM, and COX % inhibition = 65.22 %) and **23** (AChE, IC_{50} = 28.68 µM; BChE, IC_{50} = 47.39 µM, and COX % inhibition = 71.34 %) among their reported derivatives demonstrated multifunctional inhibitory activity against the AChE, BuChE and COX enzymes (Figure 2. 7). Additionally, most of the compounds from their synthesized derivatives exhibited good antioxidant along with anti-inflammatory activity [George et al. 2022].



24
AChE IC_{50} = 50.87 nM
BuChE IC_{50} = 4.77 nM

Figure 2. 8 A dual-targeting molecular hybrids of 1,3,4-oxadiazoles (**24**) to treat AD.

Elghazawy et al. showed the discovery of 1,3,4-oxadiazole derivatives as dual-targeting agents effective in AD. Compound **24** (AChE, IC_{50} = 50.87 nM; and BuChE, IC_{50} = 4.77 nM) from their reported compounds was found to be dual AChE and BuChE

inhibitor in nanomolar potencies (Figure 2. 8). Further, in vivo and ex vivo study of the compound **24** in rat animal model also demonstrated anti-AChE and BuChE inhibitory activity, and antioxidant potential of the compound in brain tissue homogenates along with reduced DNA damage and neurotoxicity levels. The in vivo experiment also demonstrated lower Aβ levels in rate brain homogenates after treatment with compound **24** suggesting the ability of the compound to improve memory deficits in experimental animal models [Elghazawy et al. 2022].

Pflégr et al. demonstrated the synthesis and biological evaluation of 5-aryl-1,3,4-oxadiazole-2-amines as dual AChE and BChE inhibitors. Compounds **25-29** from their work exhibited moderate dual ChE inhibition (Figure 2. 9). Most of the compounds from their work demonstrated moderate AChE and poor BChE inhibitory activity. While compound **29** exhibited good AChE and BChE inhibitory activity [Pflégr et al. 2022].

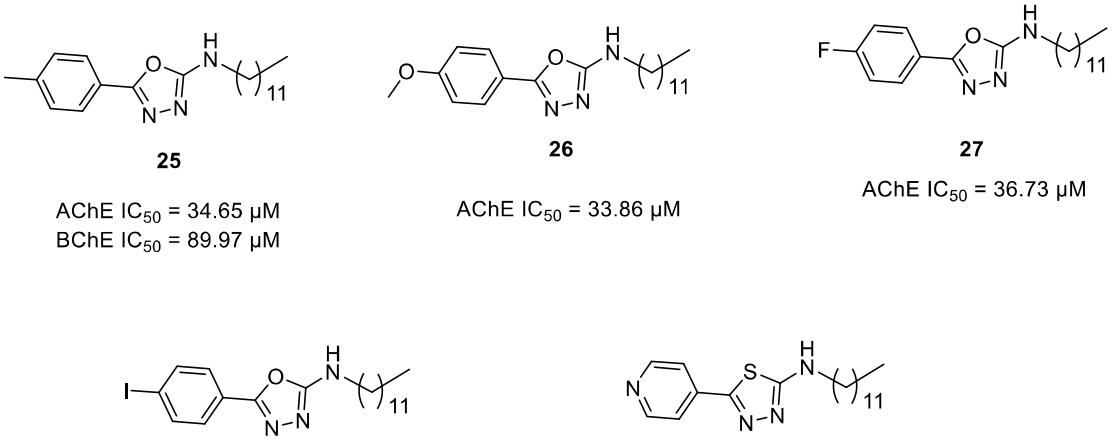


Figure 2. 9 1,3,4-oxadiazoles/thiazole molecular hybrids (25-28) as dual ChE inhibitors against AD.

Naseem et al. demonstrated the therapeutic potential of 1,3,4-oxadiazole compounds for the treatment of AD. Compounds **30** (AChE, IC_{50} = 0.83 μM; MAO-A IC_{50} 1.44

μM; MAO-B IC_{50} = 1.04 μM and BChE % inhibition = 15.23 %), **31** (AChE, IC_{50} = 1.96 μM; MAO-A IC_{50} = 1.47 μM; MAO-B IC_{50} = 1.91 μM and BChE % inhibition = 10.54 %), **32** (AChE, IC_{50} = 1.11 μM; MAO-A IC_{50} = 1.21 μM; MAO-B IC_{50} = 2.02 μM and BChE % inhibition = 15.23 %) and **33** (AChE, IC_{50} = 2.67 μM; MAO-A IC_{50} = 0.11 μM; MAO-B IC_{50} = 3.08 μM and BChE % inhibition = 15.78 %) exhibited AChE, BChE, and MAO inhibitory activity in in vitro experiments (Figure 2. 10). The MAO inhibitory activity of compounds **33** was found to be superior over other compounds of the series [Naseem et al. 2023].

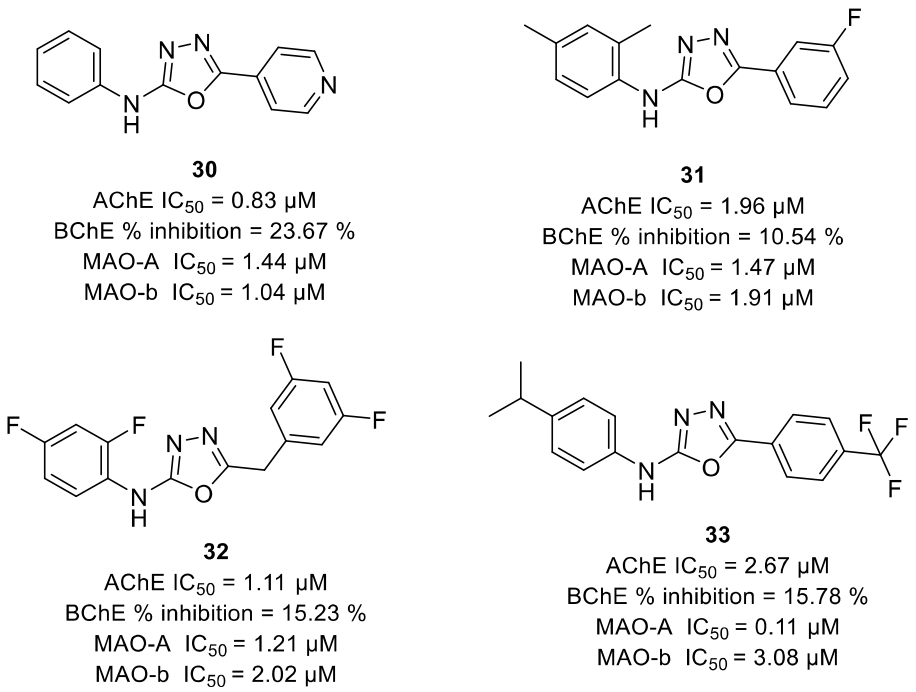
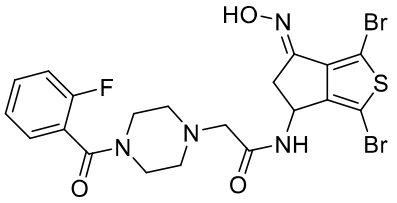


Figure 2. 10. Potential MTDL of 1,3,4-oxadiazoles (**25-28**) for the treatment of AD.

2.2 Piperazines and Benzylpiperidine as MTDL

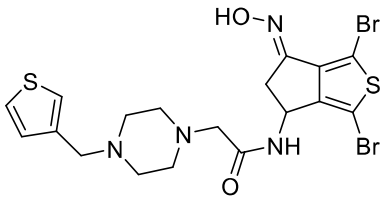
Omran at al. reported the synthesis and biological evaluation of indanones and thiaindanones compounds related to donepezil. The most potent compounds **34** and **35** from their series having cyclopentathiophene nucleus showed AChE inhibition (**34**, IC_{50} = 0.06 μM and **35**, IC_{50} = 0.14 μM). Among the tested compounds, piperazine

derivatives were found to be optimal for AChE inhibitory potency (Figure 2. 11) instead of *N*-benzylpiperidine ring [Omran et al. 2005].



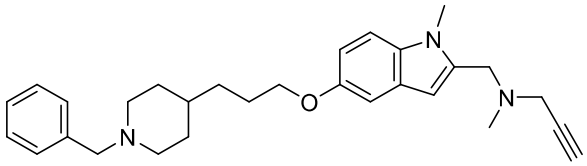
34

AChE IC_{50} = 0.06 μM



35

AChE IC_{50} = 0.14 μM



36

*ee*AChE IC_{50} = 0.35 μM, *eq*BChE IC_{50} = 0.46 μM

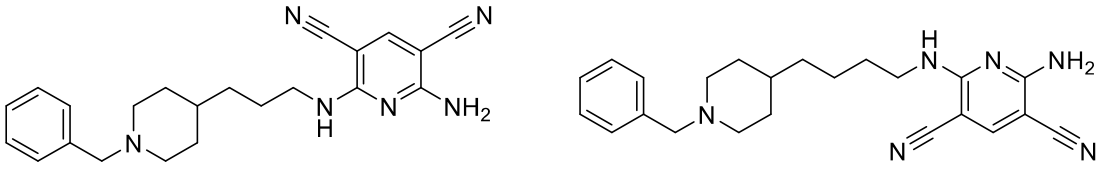
MAO-A IC_{50} = 5.2 nM, MAO-B IC_{50} = 43 nM

% Aβ inhibition: Self- = 47.8% and AChE-induced = 32.4%

Figure 2. 11. The piperazine tethered cyclopentathiophene (**34-35**) and benzylpiperidine substituted analogs (**36**) as multifunctional agents against AD.

Bolea et al. has investigated multitargeted hybrids of *N*-benzylpiperidine and indolylpropargylamine against AChE, BChE, and MAO-A/B. These molecular hybrids were designed in a way to interact simultaneously with CAS and PAS of AChE and occupy the active binding pocket of MAO. Among synthesized hybrids, compound **36** (Figure 2. 11) showed balanced dual cholinergic inhibition with IC_{50} values in the submicromolar range (*ee*AChE = 0.35 μM; *eq*BChE = 0.46 μM). Additionally, compound **36** elicited significant MAO inhibition (MAO-A, IC_{50} = 5.2 nM and MAO-B, IC_{50} = 43 nM), which might be beneficial in restoring serotoninergic neurotransmission and antidepressant activity. Moreover, the results also indicated remarkable anti-Aβ aggregatory activity of compound **36** (self-induced = 47.8%; *h*AChE-induced = 32.4%) by thioflavin T assay [Bolea et al. 2011].

Samadi et al. have designed molecular hybrids of *N*-benzylpiperidine and 2-aminopyridine-3,5-dicarbonitrile. Among the eight synthesized compounds, six of them showed micromolar to submicromolar inhibition of AChE, while two of the compounds (**37**, IC_{50} = 0.0094 μM; **38**, IC_{50} = 0.070 μM) elicited nanomolar inhibitory potency against hAChE. Compounds **37** and **38** (Figure 2. 12) exhibited a lower hBChE inhibitory profile compared to other compounds of the series with AChE selectivity of 703 and 24, respectively. All these tested compounds also showed appreciable brain permeability in the PAMPA-BBB assay [Samadi et al. 2012].



37
hAChE IC_{50} = 0.0094 μM
hBACE-1 IC_{50} = 6.6 μM

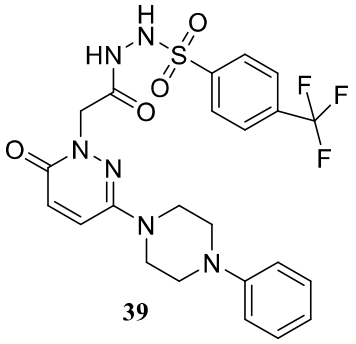
38
hAChE IC_{50} = 0.070 μM
hBACE-1 IC_{50} = 1.7 μM

Figure 2. 12. Molecular hybrids of *N*-benzylpiperidine and 2-aminopyridine-3,5 dicarbonitrile as multifunctional hybrids (**37-38**).

Önkol et al. have reported *N*'-[(4-aryl)sulfonyl]-2-[4-(aryl) piperazine]-3(2H)-pyridazinone-2-ylacetohydrazide derivatives as potential ChEIs. Compounds **39, 40,** and **42** exhibited AChE inhibitory activities, whereas compound **41** showed less inhibitory activity (Figure 2. 13). The results reflected that compounds bearing -CF_3 on the 4th position of phenylsulfonyl ring had increased AChE inhibitory activity [Önkol et al. 2013].

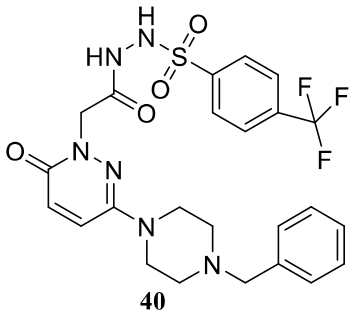
Mohammadi-Farani et al. (2013) reported the synthesis of phthalimide derivatives linked with N-benzylpiperidine to mimic the donepezil structure. Among their series, compound **43** (Figure 2. 14) was found to be the most potent AChE inhibitor (IC_{50} = 0.91 μM). The representative compound from their work **43** shows the presence of an

ortho-chloro group on the benzyl ring and was assumed that the potency of the compound was due to the presence of electron-withdrawing groups on phthalimide derivatives (Figure 2. 14). The molecular modeling study also suggested the similar binding of the compound **43** to that of donepezil in the active site of AChE [Mohammadi-Farani et al. 2013]



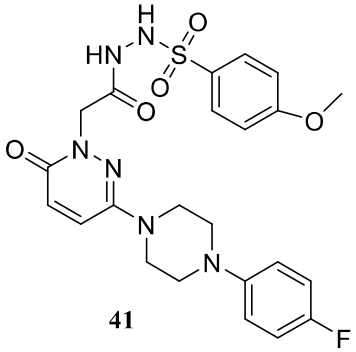
39

*ee*AChE IC_{50} = 72.46 (0.05mM)



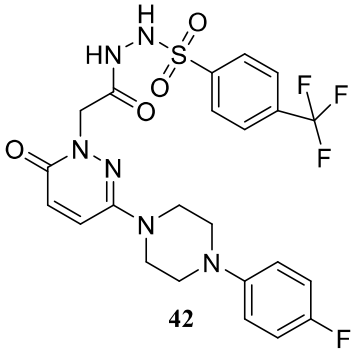
40

*ee*AChE IC_{50} = 80.30 (0.05mM)



41

*ee*AChE IC_{50} = 17.87 (0.05mM)

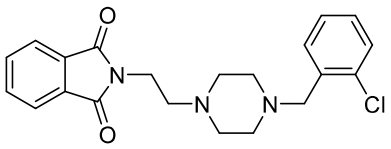


42

*ee*AChE IC_{50} = 84.07 (0.05mM)

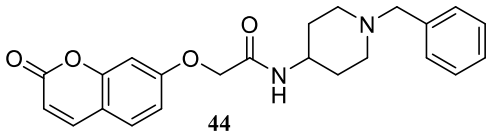
Figure 2. 13. Piperazine linked molecular hybrids (**39-42**) as MTDLs for AD treatment.

Alipour et al. have reported the design and synthesis of a group of 7-hydroxycoumarin analogs tethered with piperazine or piperidine through an amide linker and evaluated for ChE inhibitory potential. Among the reported compounds (**44-47**), **44** exhibited the maximum inhibition and selectivity for AChE (Figure 2. 14). The compound **44** also elicited antioxidant and neuroprotective activities. The docking study revealed the significant interactions of **44** with CAS of AChE [Alipour et al. 2014].



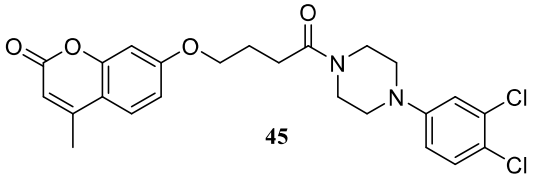
43

AChE IC_{50} = 0.91 μM



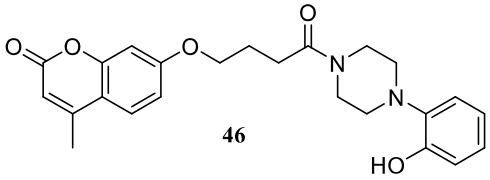
44

*ee*AChE IC_{50} = 1.6 μM
*eq*BChE IC_{50} = 42 μM



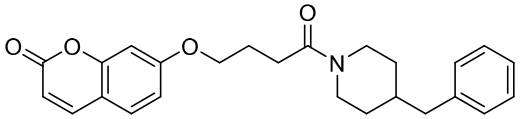
45

*ee*AChE IC_{50} = 6.9 μM
*eq*BChE IC_{50} = 387 μM



46

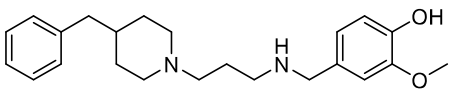
*ee*AChE IC_{50} = 4.38 μM
*eq*BChE IC_{50} = 250 μM



47

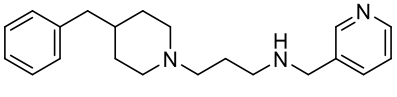
*ee*AChE IC_{50} = 6.2 μM
*eq*BChE IC_{50} = 184 μM

Figure 2. 14. Phthalimide (**43**) and Coumarin (**44-47**) link piperazine and *N*-benzylpiperidine derivatives as multifunctional molecular hybrids.



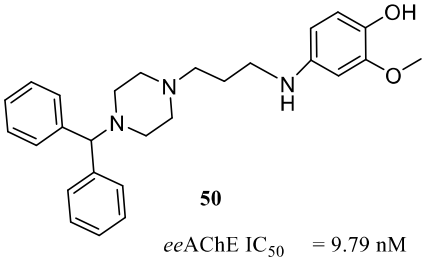
48

*ee*AChE IC_{50} = 11.53 nM
*eq*BChE IC_{50} = 3.87 nM
% Ab inhibition = 71.35 %



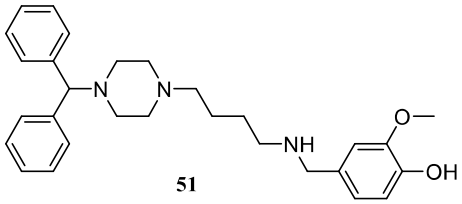
49

*ee*AChE IC_{50} = 2.13 nM
*eq*BChE IC_{50} = 81.70 nM
% Ab inhibition = 88.81%



50

*ee*AChE IC_{50} = 9.79 nM
*eq*BChE IC_{50} = 18.95 nM
% Ab inhibition = 54.16%



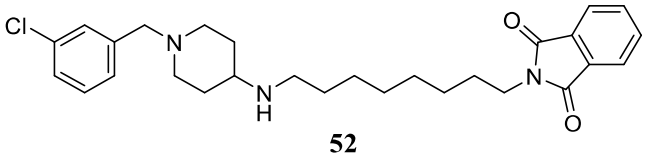
51

*ee*AChE IC_{50} = 11.44 nM
*eq*BChE IC_{50} = 4.17 nM
% Ab inhibition = 42.18%

Figure 2. 15 Molecular hybrids of *N*-benzylpiperidine (**48-49**) and piperazine derivatives (**50-51**) as multifunctional agents for AD therapy.

Meena et al. have reported the synthesis of a novel series of piperidine and piperazine derivatives developed as potential ChEIs with anti-Aβ aggregation and radical scavenging activities (Figure 2. 15). The compounds **48-51** displayed significant inhibition of AChE, self-induced Aβ aggregation, with potent oxygen radical absorbance capacity compared to standard Trolox [Meena et al. 2015].

Więckowska et al. have reported design and synthesis of twenty-eight novel donepezil-based molecular hybrids of N-benzylpiperidine attached with phthalimide or indole scaffold as multitarget directed ligands (Figure 2. 16). Compound **52** (2-(8-(1-(3-chlorobenzyl)piperidin-4-ylamino)octyl)-isoindoline-1,3-dione) showed selective BChE inhibitory activity (IC_{50} = 0.72 μM) along with Aβ aggregation inhibition (72.5% at 10 μM concentration). Additionally compound has also exhibited BBB permeability and significant cognitive improvements in scopolamine-treated animal models [Więckowska et al. 2015].



52

*eq*BChE IC_{50} = 0.72 μM

% Aβ inhibition = 72.5% at 10 μM

Figure 2. 16. The *N*-benzyl piperidine and indole molecular hybrid (**52**) with multitargeted activities against AD.

Benchekroun et al. has designed ferulic acid-based molecular hybrids of *N*-benzylpiperidine with dual ChE inhibition and antioxidant potency. Among the synthesized analogs, **53–57** exhibited nanomolar inhibition of *ee*AChE slightly lower than donepezil, while compounds **53–57** (Figure 2. 17) showed considerably higher inhibition of *eq*BChE than donepezil in the nanomolar range. Additionally, all

molecular hybrids signified remarkably higher antioxidant activity compared to standard ferulic acid and melatonin [Benchekroun et al. 2015].

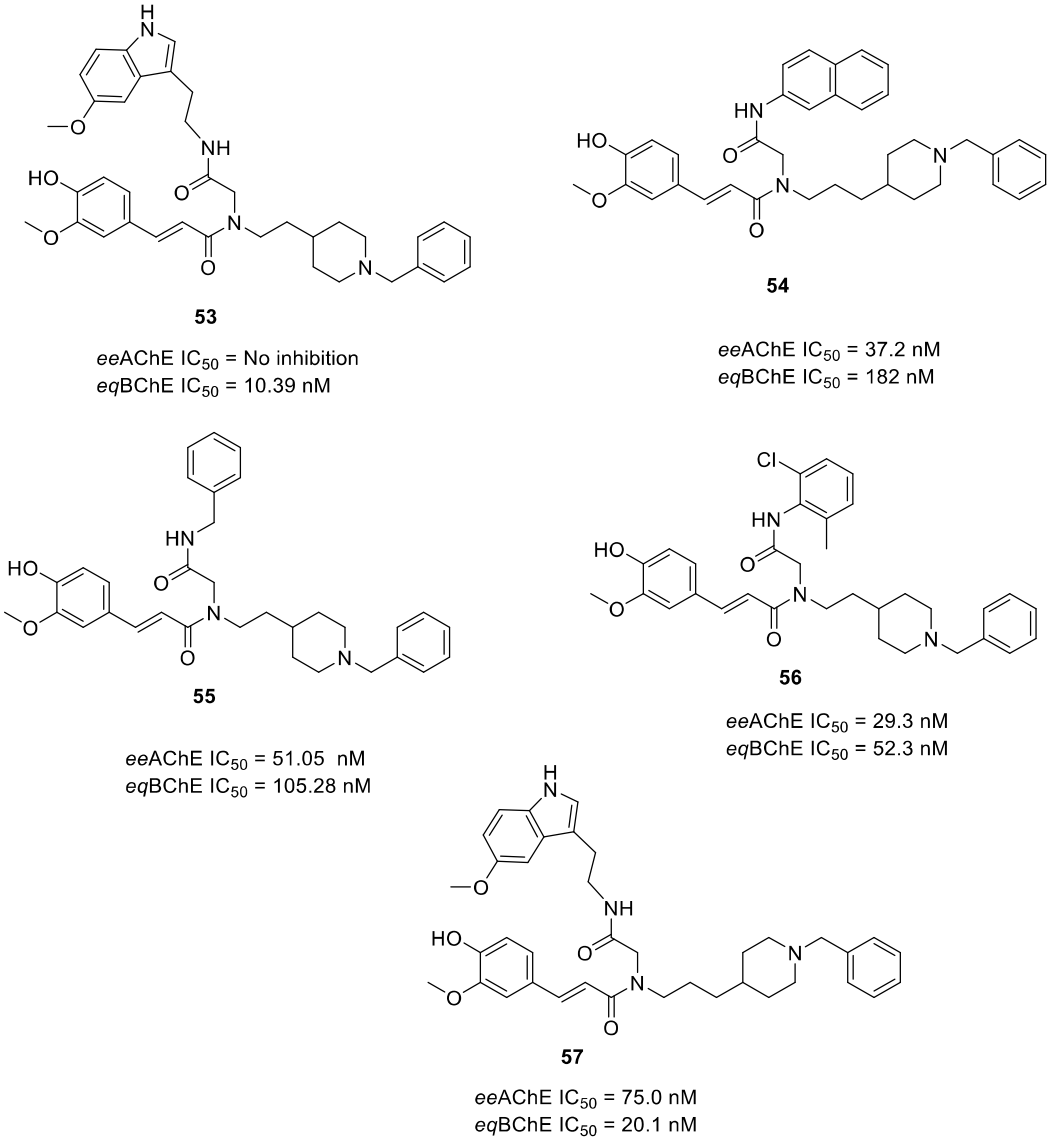
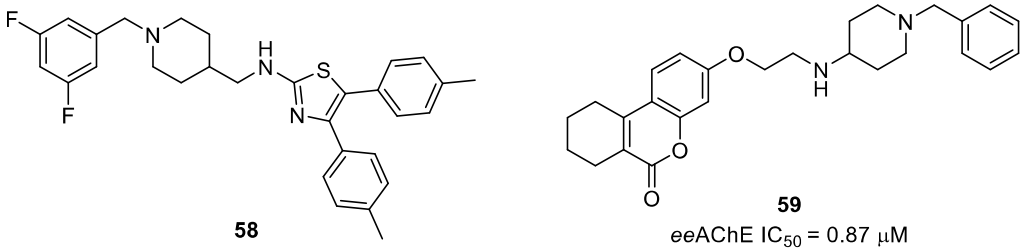


Figure 2. 17. Ferulic acid-based *N*-benzyl piperidine hybrids (**53-57**) as MTDLs.

Shidore et al. have reported a series of *N*-benzylpiperidine-linked diarylthiazole molecular hybrids as potential multifunctional ligands for AD treatment. The most potent molecule among their series **58** (*N*-[(1-(3,5-difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(p-tolyl)thiazol-2-ylamine) elicited significant AChE (IC_{50} = 0.30 μM) and BChE (IC_{50} = 1.84 μM) inhibitory activity along with Aβ aggregation

inhibition, anti-apoptotic and antioxidant activities (Figure 2. 18). The molecular docking studies also suggested that *N*-benzylpiperidine ring was interacting with the CAS residues, while diaryl moiety was interacting with AChE-PAS residues. The *in vivo* Y-maze and Morris water maze test also supported the MTDL profile of the compounds and exhibited reversal of memory deficits in experimental animals. The ex vivo studies also demonstrated additional antioxidant properties of the compound [Shidore et al. 2016].



58
*h*AChE IC_{50} = 0.30 μM
*eq*BChE IC_{50} = 1.84 μM
% Aβ inhibition = 27.65%(10 μM concentration)

59
*ee*AChE IC_{50} = 0.87 μM
*eq*BChE IC_{50} = 0.93 μM
*h*MAO-B IC_{50} = 2.62 μM

Figure 2. 18. *N*-benzylpiperidine linked diarylthiazole (**58**) and coumarin compound (**59**) as potential multitargeted ligands against AD.

Xie et al. have combined *N*-benzylpiperidine moiety with coumarin to investigate the inhibitory potential against ChEs and MAO-B. Among the tested fourteen compounds, compound **59** was found to be the most potent dual ChE inhibitor with IC_{50} values of 0.87 μM and 0.93 μM for *ee*AChE and *eq*BChE, respectively. Compound **59** also elicited balanced inhibition of *h*MAO-B with an IC_{50} value of 2.62 μM. Enzyme kinetic studies revealed that compound **59** was a mixed type of AChE and a competitive-type of MAO-B inhibitor (Figure 2. 18). Compound **59** also showed appreciable BBB permeability and was found nontoxic against SH-SY5Y cell lines. All these results signified compound **59** as a promising multitargeted lead candidate for the treatment of AD [Xie et al. 2016].

Estrada et al. has designed cinnamic acid-based hybrids of *N*-benzylpiperidines as multitargeted ligands against ChEs (hAChE and hBChE) and MAO (A/B) along with significant antioxidant potential. The umbelic acid **60** (AChE, IC_{50} = 0.99 μM and BChE, IC_{50} = 0.26 μM), and caffeic acid-based molecular hybrids **61** (AChE, IC_{50} = 1.75 μM and BChE, IC_{50} = 0.69 μM), demonstrated AChE and BChE inhibitory activity in lower micromolar to the submicromolar range, additionally, antioxidant potency of both compounds were comparable to vitamin E (Figure 2. 19). Further. Compounds also demonstrated MAO inhibitory activity in the micromolar range supporting the multifunctional potential of the compounds. The caffeic acid-based hybrid **61** also showed neurogenic effects in adult subgranular zone-derived neural stem cells [Estrada et al. 2016].

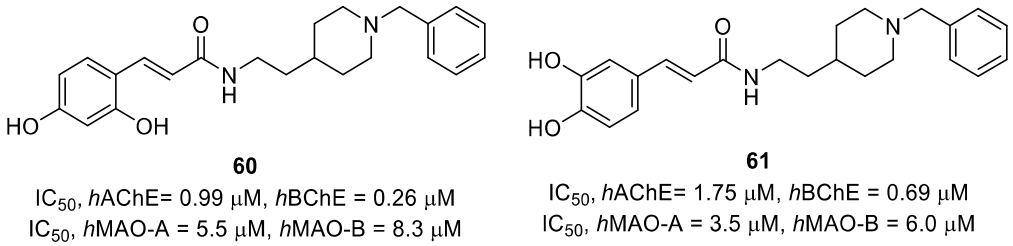


Figure 2. 19. Umbelic (**60**) and caffeic acid-based molecular hybrids (**61**) of *N*-benzylpiperidine.

Costanzo et al. showed the development and evaluation of some donepezil-based molecular hybrids as dual AChE and BACE-1 inhibitors. The most promising candidates **62** (AChE, IC_{50} = 0.058 μM, BACE-1, IC_{50} = 0.333 μM)**,** and **63** (AChE, IC_{50} = 0.697 μM, BACE-1, IC_{50} = 0.043 μM) among their designed hybrids exhibited significant AChE and BACE-1 inhibition (Figure 2. 20) [Costanzo et al. 2016].

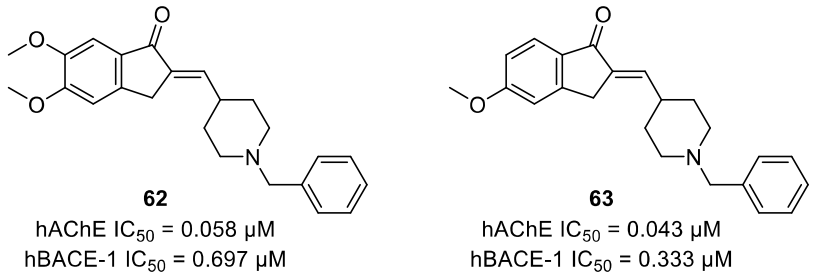


Figure 2. 20. Molecular hybrids of *N*-benzylpiperidine (**62-63**) as MTDLs.

Benzothiazole–piperazine derivatives were reported by Özkay et al. as potential AChEIs. The piperidine moiety was bioisosterically replaced with piperazine, where compounds **64** and **65** showed potential inhibition on AChE (Figure 2. 21). The *in silico* study also reported a favorable interaction of compounds **64** and **65** with AChE [Özkay et al. 2016].

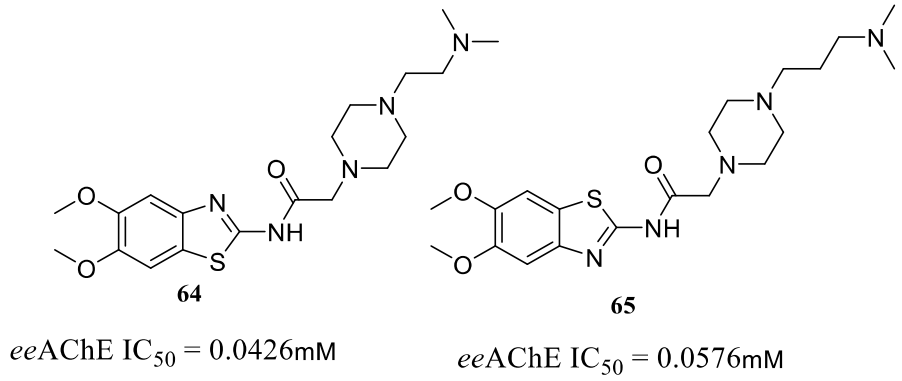


Figure 2. 21. Benzthiazole linked piperazine compounds (**64-65**) as MTDLs.

Mishra et al. explained the design development of some novel donepezil derivatives as MTDL for the treatment of AD. Compounds **66** (AChE IC_{50} = 0.045 µM and BChE IC_{50} = 3.0 µM), **67** (AChE IC_{50} = 0.034 µM and BChE IC_{50} = 1.96 µM), and **68** (AChE IC_{50} = 0.025 µM and BChE IC_{50} = 2.43 µM) amongst their synthesized compounds appeared with multifunctional inhibitory potential (Figure 2. 22). The AChE inhibitory activity of the compound **68** was also found to be better than standard drug donepezil (AChE IC_{50} = 0.039 µM). The compounds **66-68** were also found to possess better Aβ

aggregation inhibition as compared to curcumin. Additionally, compounds **66-68** also exhibited non-neurotoxic and neuroprotective properties of the compounds [Mishra et al. 2017a].

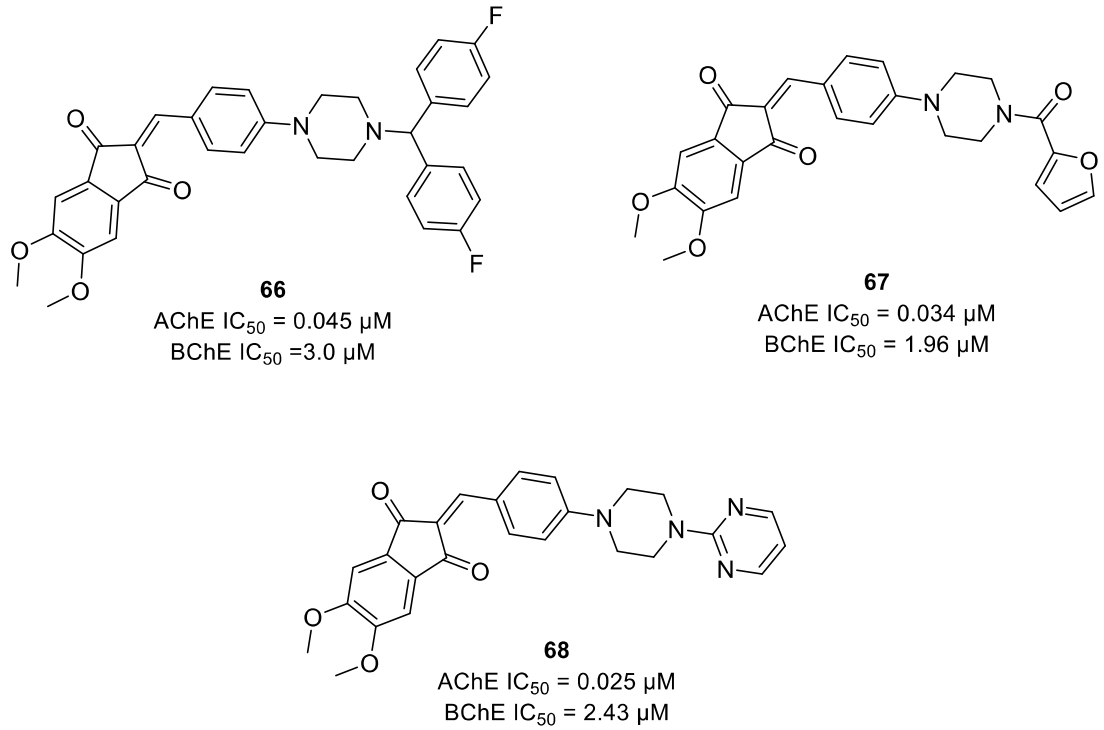


Figure 2. 22. Piperazine derivatives (**66-68**) as multifunctional agents for AD treatment.

Ozadali-Sari et al. reported synthesis biological evaluation and molecular modeling studies of the piperazine-linked benzimidazole derivatives. Amongst their synthesized compounds **69** (AChE IC_{50} = 34.85 µM and BChE IC_{50} = 5.18 µM), and **70** (AChE IC_{50} = 97.2 µM and BChE IC_{50} = 5.22 µM) exhibited considerable ChE inhibitory activity (Figure 2. 23). Both the compounds also demonstrated self-induced Aβ-aggregation inhibition comparable to donepezil. Additionally, compounds **69** and **70** also exhibited neuroprotective and non-neurotoxic properties of the compounds [Ozadali-Sari et al. 2017].

Monjas et al. have reported donepezil-based L- and D-glutamic acid derivatives for AD and cerebral ischemia therapy. Compound **71** (N-Cbz-L-Glu(OEt)-[NH-2-(1-benzylpiperidin-4-yl)ethyl]) from their work exhibited hAChE (IC_{50} = 4.99 µM), and hBChE (IC_{50} = 0.40 µM) inhibitory activity. Compound **71** also demonstrated L-type VDCC inhibitory activity (63%) with an IC_{50} = 90.1 µM (Figure 2. 23). Additionally compound **71** also showed neuroprotective and good BBB permeability in vitro. [Monjas et al. 2017]

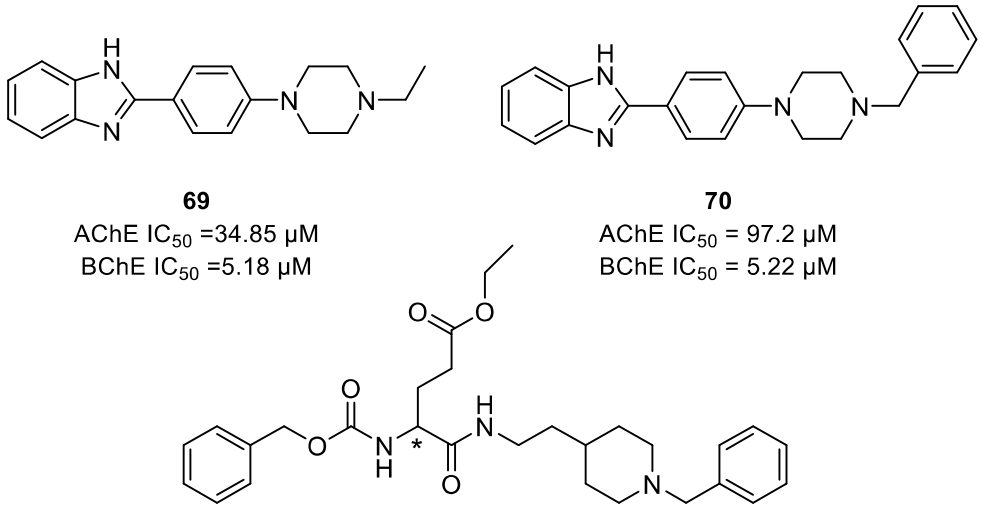
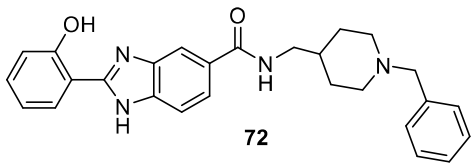


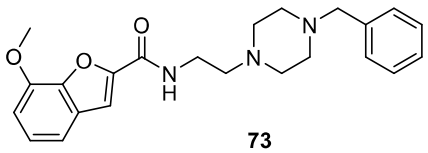
Figure 2. 23. Piperazine linked benzimidazole compounds (69-70) and L- and D-glutamic acid linked benzylpiperidine compound (**71**) as MTDL for AD therapy.

Piemontese et al have reported a structure-based design of synthesis and biological evaluation of some donepezil-like molecular hybrids. Their work demonstrated compounds **72** and **73** containing benzylpiperazine moiety had a better AChE inhibitory activity as compared to the piperidine analogs (Figure 2. 24). They also found that the compound containing benzylpiperazine ring had Aβ aggregation, antioxidant, and reduced Aβ-induced cell toxicity. [Piemontese et al. 2018]



72

AChE IC_{50} μM= 4.2 ± 0.8
Aβ1–42 aggregation inhibit. (%) =36.3%

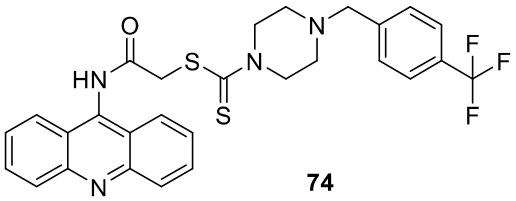


73

AChE IC_{50} μM= 4.0 ± 0.4
Aβ1–42 aggregation inhibit. (%) =19 %

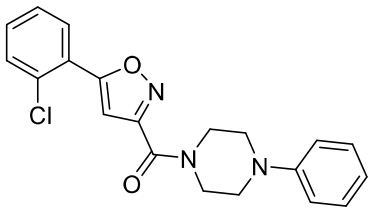
Figure 2. 24. Donepezil-like molecular hybrids of *N*-benzylpiperidine linked benzimidazole (**72**) and benzofuran (**73**) compounds and 2-aminopyridine-3,5-dicarbonitrile.

Hussein W et.al, in their work, demonstrated the synthesis and biological evaluation of some tacrine-based molecules linked with piperazines via the (thiocarbamoylthio) acetamide group which had been expected to be responsible for enhanced ChE activity. The compound **74** containing 4-trifluoromethyl benzylpiperazine scaffold demonstrated good AChE and BChE inhibitory activity with good BBB permeability as compared to standard Tacrine and Donepezil (Figure 2. 25) [Hussein et al. 2018].



74

hAChE IC_{50} μM= 41.12 ± 1.69
hBChE IC_{50} μM= 0.014 ± 0.005



75

AChE IC_{50} = 21.85 mM
BChE IC_{50} = 76.76 mM

Figure 2. 25. Tacrine (**74**) and isoxazole (**75**) linked benzylpiperazine derivatives and as multifunctional agents for AD treatment.

Saeedi et al. explored the design and synthesis and biological evaluation of aryl isoxazole-phenylpiperazines as potential ChEIs. Compound **75** showed the highest inhibition of AChE and BACE-1 with neuroprotective activity (Figure 2. 25). *In silico* study suggested active site interactions with CAS and PAS residues of AChE by compound **75** [Saeedi et al. 2019].

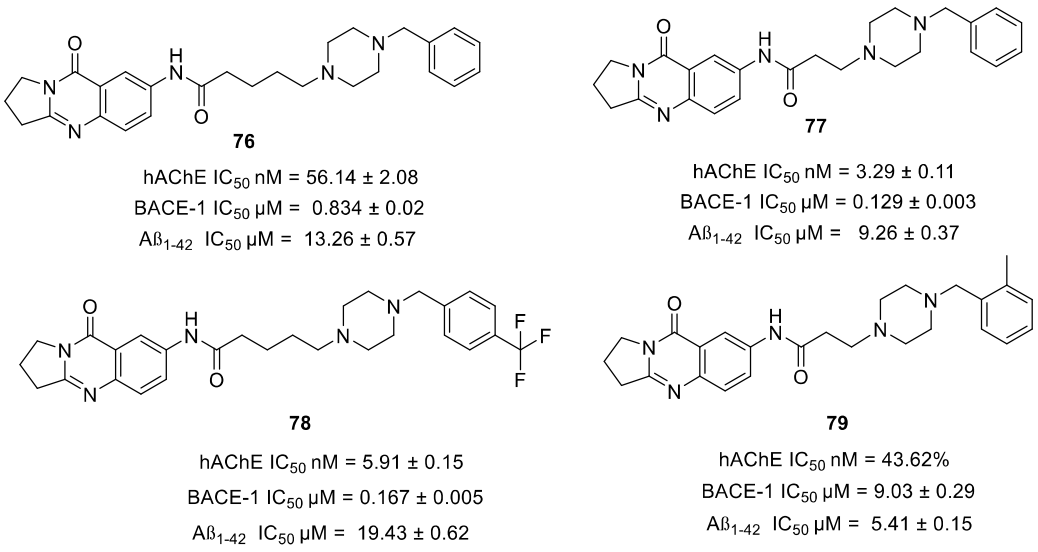


Figure 2. 26. Benzylpiperazine linked deoxyvasicinone derivatives as multifunctional agents (**76-79**) for AD treatment.

Du H. et al. suggested the design and synthesis of different deoxyvasicinone-benzylpiperazine fused derivatives as MTDL. The compounds **76-79** among their series exhibited hAChE, BACE-1, and Aβ aggregation inhibition potential. They have also demonstrated that compounds **76** and **78** possess low cytotoxicity along with neuroprotective effects against SH-SY5Y cell lines (Figure 2. 26) [Du et al. 2019].

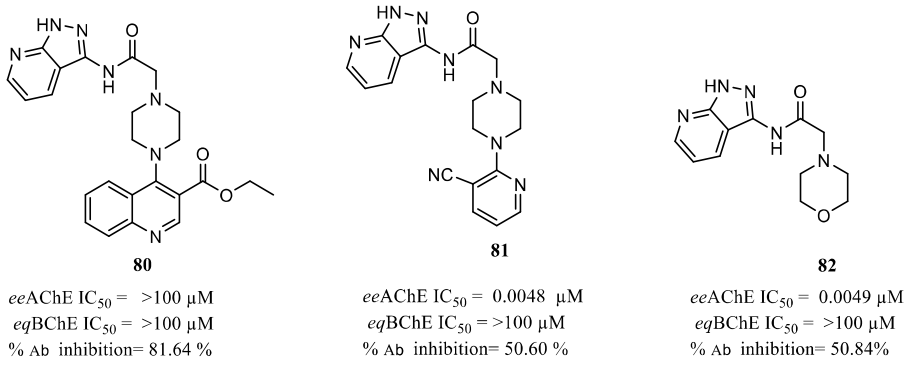
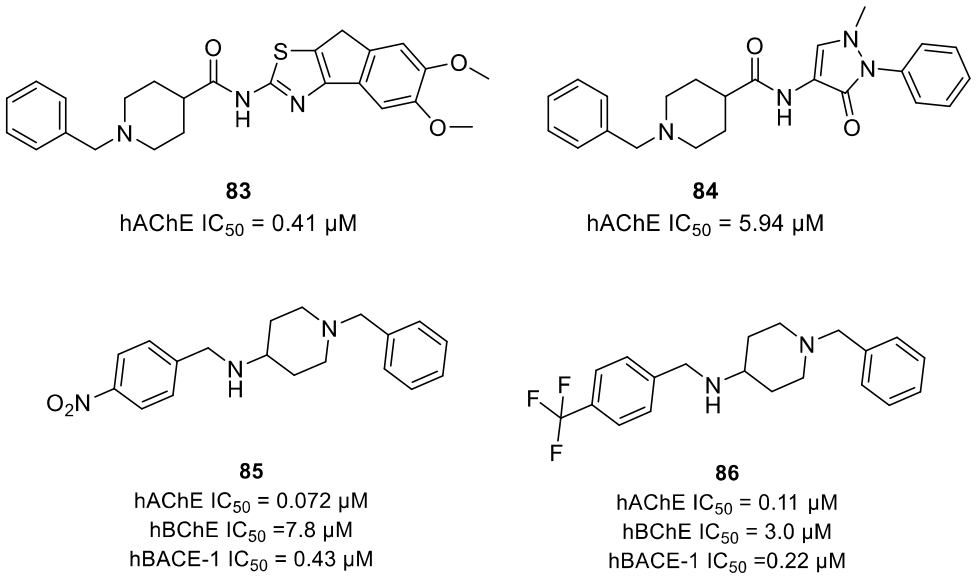


Figure 2. 27. Molecular hybrids of 1H-pyrazolo[3,4-b]pyridine as MTDL for AD therapy (**80-82**).

Umar et al. have developed 1H-pyrazolo[3,4-b]pyridine derivatives as anti-AD. The most potential compounds **80** and **81,** demonstrated the selective anti-AChE, with little

BChE inhibition property. Besides, Compound **82** showed Aβ aggregation (self and metal-induced) inhibition potential up to 81.65% along with disaggregation of 79.47%, antioxidant, and metal chelation properties (Figure 2. 27) [Umar et al. 2019].



83
hAChE IC_{50} = 0.41 µM

84
hAChE IC_{50} = 5.94 µM

85
hAChE IC_{50} = 0.072 µM
hBChE IC_{50} =7.8 µM
hBACE-1 IC_{50} = 0.43 µM

86
hAChE IC_{50} = 0.11 µM
hBChE IC_{50} = 3.0 µM
hBACE-1 IC_{50} =0.22 µM

Figure 2. 28. Novel *N*-benzylpiperidine carboxamide derivatives (**83-84**) and benzylamine linked multifunctional hybrids (**85-86**)

In a recent study, Greunen et al. designed novel *N*-benzylpiperidine carboxamide derivatives as potential ChE inhibitors for the treatment of AD. The two most active analogs of the series (compounds **83** and **84**, Figure 2. 28) afforded *in vitro* AChE inhibition with IC_{50} values of 0.41 and 5.94 µM, respectively. In silico molecular docking and dynamics study showed similar binding patterns of these hybrids with donepezil against AChE [van Greunen et al. 2019].

Sharma et al. in their work reported N-benzylpiperidine analogs as MTDL for the treatment of AD. Compound **85** (hAChE IC_{50} = 0.072 µM; hBChE IC_{50} = 7.8 µM; and hBACE-1 IC_{50} = 0.43 µM) and **86** (hAChE IC_{50} = 0.11 µM; hBChE IC_{50} = 3.0 µM; and hBACE-1 IC_{50} = 0.22 µM) among their designed compounds exhibited balanced multifunctional potential in micromolar to submicromolar range (Figure 2. 28).

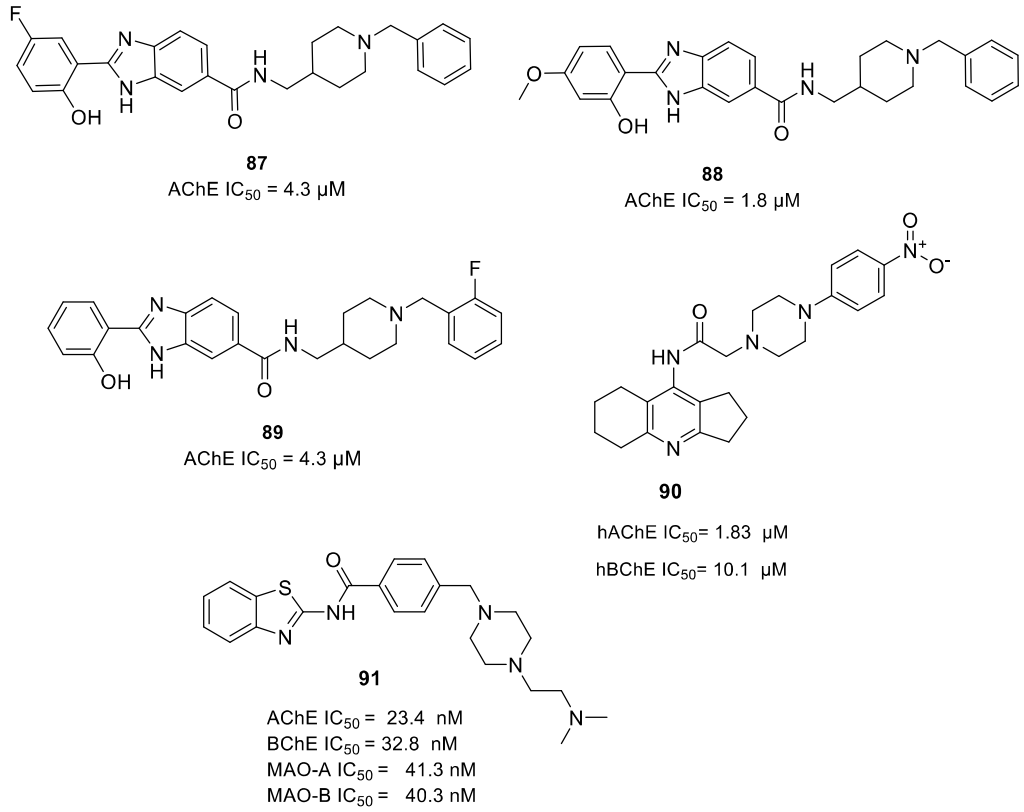


Figure 2. 29. Molecular hybrids of *N*-benzylpiperidine tethered benzimidazole (**87-89**) and amridine (**90**) and benzthiazole (**91**) linked piperazine analogs as MTDL for AD treatment.

Additionally, both compounds have also demonstrated anti-Aβ aggregation in self-and AChE-induced thioflavin T assay with excellent BBB permeability in PAPMA-BBB assay. Both compounds also demonstrated AChE-PAS binding in PI-displacement assay and the neuroprotective potential of the compound against SH-SY5Y cell lines. The in vivo results of compound **86** demonstrated reversal of cognitive dysfunction in scopolamine and Aβ-induced animal models. While ex vivo studies on brain homogenate demonstrated the antioxidant potential of the compounds [Sharma et al. 2019a].

Chaves et al. reported the synthesis and in vitro biological studies of hydroxy benzimidazole-donepezil (mimetic) hybrids as multitarget-directed ligands for AD treatment. Compounds **87** (IC_{50} = 1.8 µM), **88** (IC_{50} = 1.8 µM), and **89** (IC_{50} = 1.8 µM)

from their synthesized compounds demonstrated good AChE inhibitory activity in the micromolar range. Furthermore, compounds 79, 80, and 81 also exhibited anti-Aβ aggregation potential in self and Cu (II) induced thioflavin T experiments, Additionally, compounds **87-89** have shown increased cell viability and neuroprotective activity against SH-SY5Y cell lines (Figure 2. 29) [Chaves et al. 2020].

Makhaeva et al. reported amridine-piperazine hybrids as multifunctional agents for AD therapy. Their work demonstrates compound **90** (hAChE IC_{50}= 1.83 µM and hBChE IC_{50}= 10.1 µM) as the most potent ChE inhibitory compound (Figure 2. 29). Along with ChE inhibition, compound **90** also showed AChE-PAS binding in PI-displacement assay, antioxidant activity, and radical scavenging activity additionally demonstrated multifunctional property of the compound [Makhaeva et al. 2021].

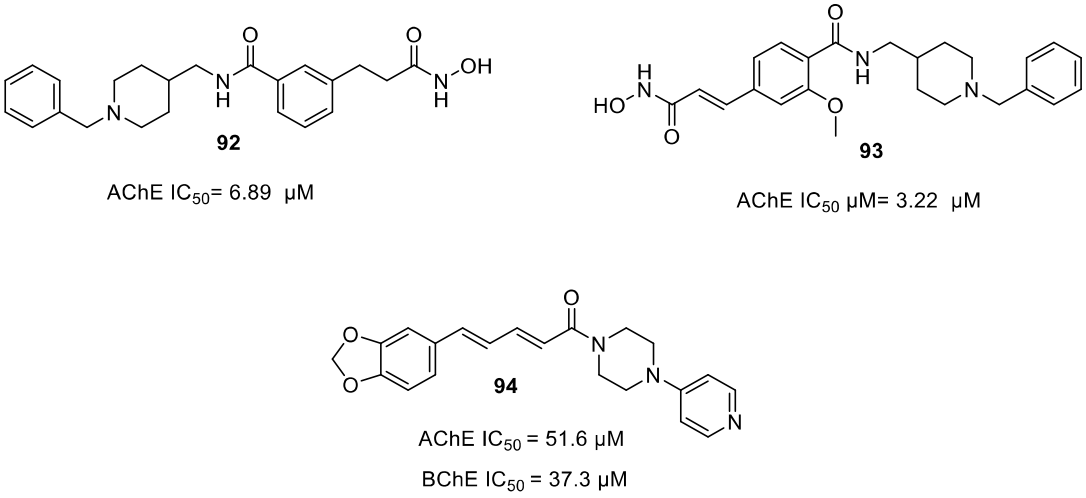


Figure 2. 30. Molecular hybrids of *N*-benzylpiperidine (**92-93**) and piperin linked piperazine compounds as dual ChE inhibitors.

Karaca et al. have reported novel benzothiazole liked piperazine derivatives as MTDL for AD treatment. The most active compound from their series **91** exhibited good ChE (AChE IC_{50} = 23.4 nM and BChE IC_{50} = 32.8 nM) and MAO (MAO-A IC_{50} = 41.3 nM and MAO-B IC_{50} = 40.3 nM) dual inhibitory activity (Figure 2. 29). Additionally

compound **91** also exhibited anti-Aβ aggregation and non-neurotoxic profile of the compound suggesting overall multifunctional inhibitory property of the compound against AD [Karaca et al. 2022].

Qin et al. have demonstrated N-benzylpiperidine derivatives as multifunctional inhibitors. Compound **92** (HDAC IC_{50} = 0.17 μM, AChE IC_{50} = 6.89 μM), and **93** (HDAC IC_{50} = 0.17 μM, AChE IC_{50} = 6.89 μM), among their tested derivatives, exhibited multifunctional inhibitory activity including free radical scavenging, metal chelation and anti-Aβ activity (Figure 2. 30). Both compounds also demonstrated neuroprotective and AChE selectivity profile suggesting MTDL ability of the compounds [Qin et al. 2023].

Jaipea et al. recently reported piperine analogs as dual ChE inhibitors. Compound **94** was the most potent ChE inhibitor. The pharmacokinetic study of the compound **94** against AChE also revealed competitive inhibition while it showed noncompetitive inhibition against BChE enzymes (Figure 2. 30). The inhibition constant (Ki) value of the compound also demonstrated its binding with ChE enzymes [Jaipea et al. 2023].