Chapter 2
(Review of Literature)

2.1. *N*-Benzylpiperidines: Development as multitargeted ligands in AD

Donepezil (**1**) is the most potent AChE inhibitor and highly prescribed drug for the treatment of AD. Donepezil bears the *N*-benzylpiperidine nucleus as a core group. The structural framework of donepezil has been reported to suggest that a modification in this *N*-benzylpiperidine group drastically reduces the AChE inhibitory activity [Kryger et al. 1999]. Also, *N*-benzylpiperidine ring of donepezil was observed to be extended into the CAS, which is catalytic pocket of AChE [Caliandro et al. 2018, Costanzo et al. 2016]. Apart from these observations, *N*-benzylpiperidine ring possesses basic nitrogen moiety, which gets protonated at physiological pH, and leads to its increased affinity towards AChE and aspartate dyad of BACE-1 along with significant BBB permeability due to acid-base equilibrium [Peauger et al. 2017]. All these findings have prompted us to select *N*-benzylpiperidine nucleus as a core group.

Kryger et al. have explained the importance and SAR about modification in the *N*-benzylpiperidine ring of donepezil. The structure of donepezil has three major groups: the benzyl moiety, the piperidine nucleus, and the dimethoxyindanone moiety (Figure 2.1).

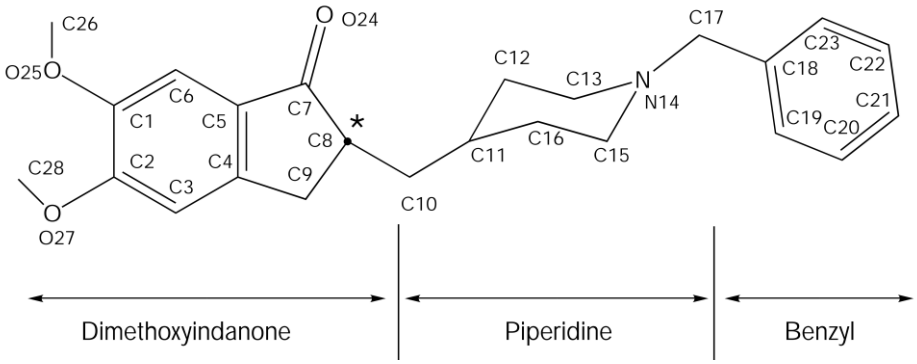


Figure 2.1. Schematic structural representation of (*R,S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]methylpiperidine (Donepezil, **1**, AChE IC_{50} = 5.7 nM). *C8 is the chiral carbon [Kryger et al. 1999].

The *N*-benzylpiperidine ring represents the core group of donepezil structure, and SAR findings revealed that any substitution at the benzyl group or any

modification/replacement of the piperidine ring leads to drastic decline in AChE inhibition. Cyclohexane (**2,** Figure 2.2) in place of a benzyl ring cannot form a π–π stacking interaction with Trp84, resulting in reduced affinity. Piperazine (**3**, Figure 2.2) in place of piperidine lowers the affinity of the resulting analog by ~19-fold, whereas piperidine with nitrogen at the opposite position (in place of E2020 C10, **4**, Figure 2.2) lowers the affinity by ~90-fold. Piperazine, although containing nitrogen at a position suitable for binding with Phe330, possesses a different charge distribution, whereas piperidine with nitrogen at the opposite position does not allow a quaternary-π interaction with Phe330 to occur. The presence of a single rotatable bond between benzyl and piperidine is also optimal for significant interactions of benzyl moiety with the important CAS residues [Kryger et al. 1999].

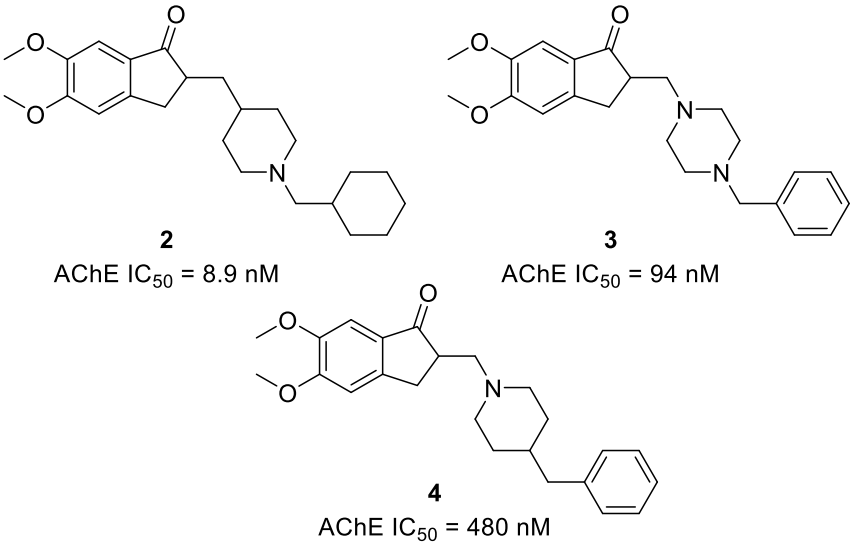
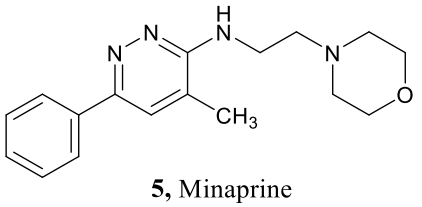


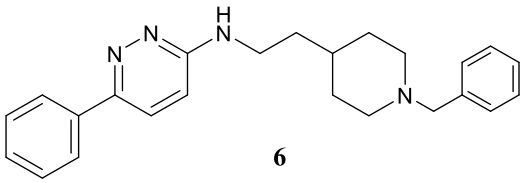
Figure 2.2. Structures of donepezil analogs (**2–4**) with modification of benzyl and piperidine moieties [Kryger et al. 1999].

The superior pharmacological profile of donepezil has prompted several researchers to develop its congeners, and extensive research efforts have been made to explore and develop the *N*-benzylpiperidine analogs with the modification particularly made at the terminal indanone nucleus.

Contreras et al. have studied the minaprine (**5**) derivatives and found that the most active compound of the series has *N*-benzylpiperidine ring as a pharmacophoric feature for the AChE inhibition. The designed compounds bear a *N*-benzylpiperidine ring at one end, and other end was substituted with a pyridazine ring with an aminoalkyl chain. Among all the derivatives, investigated, 3-[2-(1-benzylpiperidin-4-yl)ethylamino]-6-phenylpyridazine (**6,** Figure 2.3), which shows an IC_{50} of 0.12 μM on purified AChE (electric eel), was found to be one of the most potent inhibitor, representing a 5000-fold increase in potency compared to minaprine. These findings proved the importance of *N*-benzylpiperidine moiety and confirmed that any modification or replacement of *N*-benzylpiperidine with other moieties would lead to a drastic decrease the AChE inhibitory potency [Contreras et al. 1999].



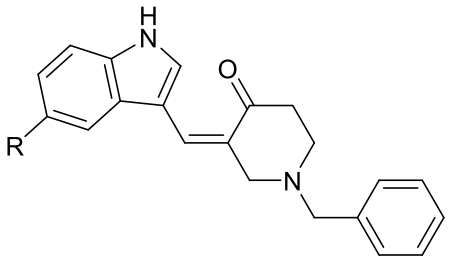
5, Minaprine
*ee*AChE IC_{50} = 600 μM
*rat*AChE IC_{50} = 85 μM



6
*ee*AChE IC_{50} = 0.12 μM
*h*AChE IC_{50} = 0.14 μM
*h*BChE IC_{50} = 0.70 μM

Figure 2.3. Structures of minaprine and its most potent analog [Contreras et al. 1999].

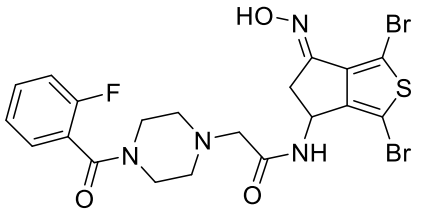
Andreani and coworkers modified the donepezil by replacing the indanone ring with indole nucleus and introducing the double bond connecting the basic benzylpiperidine nucleus and indole ring (**7–9**, Figure 2.4). The results suggested very lower inhibitory potential of compounds against AChE compared to standard donepezil and tacrine. The reason for reduced potency was cited as loss of interaction of compounds with Trp279 residue. Another reason mentioned was the rigidity of the molecule that might have hindered the penetration of compounds into AChE gorge [Andreani et al. 2001].



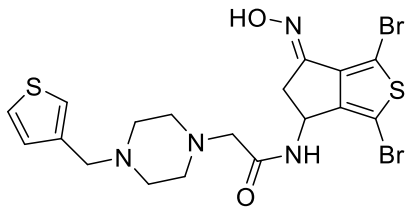
R, **7** = H; IC_{50} = 8 μM
R, **8** = OCH_3; IC_{50} = 6 μM
R, **9** = $OCH_2C_6H_5$; IC_{50} = 25 μM

Figure 2.4. Structures of *N*-benzylpiperidine analogs tethered with variably substituted indole moieties [Andreani et al. 2001].

Omran et al. have designed a series of donepezil hybrids with modification in central amide functionality along with terminal indanone portion. The most potent compounds **(10** and **11**, Figure 2.5**)** of the series showed AChE inhibition with an IC_{50} = 0.06 μM and 0.14 μM, respectively, having cyclopentathiophene nucleus. Among the tested compounds, piperazines derivatives were found to be optimal for AChE inhibitory potency instead of *N*-benzylpiperidine ring [Omran et al. 2005].



10
AChE IC_{50} = 0.06 μM

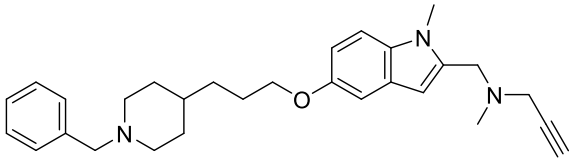


11
AChE IC_{50} = 0.14 μM

Figure 2.5. The cyclopentathiophene analogs of donepezil [Omran et al. 2005].

Bolea et al. have 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 **12** (Figure 2.6) showed balanced dual cholinergic inhibition with IC_{50} values in submicromolar range (*ee*AChE = 0.35 μM; *eq*BChE = 0.46 μM). Additionally, compound **12** elicited significant MAO inhibition (MAO-A, IC_{50} = 5.2 nM; 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 **12** (self-induced = 47.8%; *h*AChE-induced = 32.4%) by thioflavin T assay [Bolea et al. 2011].

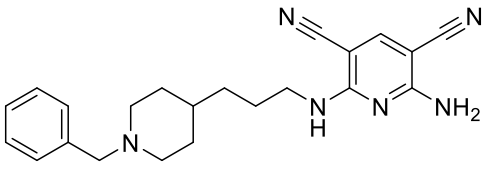


12

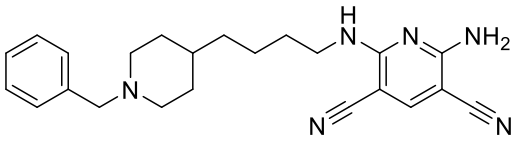
*ee*AChE IC_{50} = 0.35 μM, *eq*BChE IC_{50} = 0.46 μM
MAO-A IC_{50} = 5.2 nM, MAO-B IC_{50} = 43 nM
% Aβ inhibition: Self- = 47.8% and AChE-induced = 32.4%

Figure 2.6. The *N*-benzylpiperidine and indolylpropargylamine tethered multitargeted hybrid [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 (**13**, IC_{50} = 0.0094 μM; **14**, IC_{50} = 0.070 μM) elicited nanomolar inhibitory potency against hAChE. Compounds **13** and **14** (Figure 2.7) 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 PAMPA-BBB assay [Samadi et al. 2012].



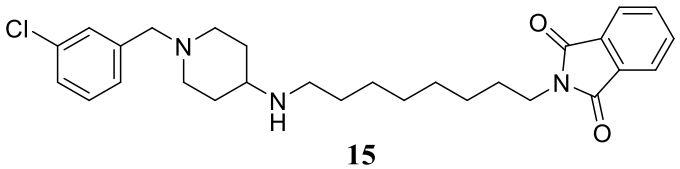
13
hAChE IC_{50} = 0.0094 μM
hBACE-1 IC_{50} = 6.6 μM



14
hAChE IC_{50} = 0.070 μM
hBACE-1 IC_{50} = 1.7 μM

Figure 2.7. Molecular hybrids of *N*-benzylpiperidine and 2-aminopyridine-3,5-dicarbonitrile [Samadi et al. 2012].

Malawska and coworkers have designed twenty-eight novel donepezil-based hybrids containing *N*-benzylpiperidine nucleus combined with a phthalimide or indole moieties as multitargeted ligands. The most active compound of the series (2-(8-(1-(3-chlorobenzyl)piperidin-4-ylamino)octyl)-isoindoline-1,3-dione) (**15**, Figure 2.8) showed selective BChE inhibition (IC_{50} = 0.72 μM) along with Aβ anti-aggregation activity (72.5% inhibition at 10 μM concentration). The compound also has significant BBB permeability [Więckowska et al. 2015].



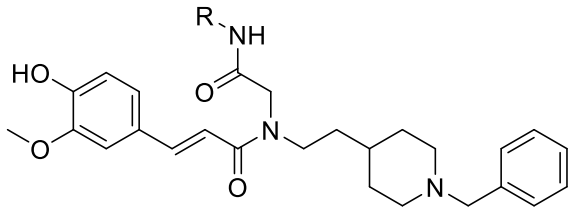
15

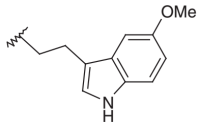
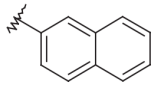
*eq*BChE IC_{50} = 0.72 μM

% Aβ inhibition = 72.5% at 10 μM

Figure 2.8. The *N*-benzylpiperidine and indole molecular hybrid with multitargeted activities against AD [Więckowska et al. 2015].

Benchekroun et al. have designed ferulic acid-based molecular hybrids of *N*-benzylpiperidine with dual ChE inhibition and antioxidant potency. Among the synthesized analogs, **17–20** exhibited nanomolar inhibition of *ee*AChE slightly lower than donepezil, while compounds **16–20** (Figure 2.9) 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].



R =			Bn
	16	**17**	**18**
*ee*AChE IC_{50} =	No inhibition	37.2 nM	51.05 nM
*eq*BChE IC_{50} =	10.39 nM	182.0 nM	105.28 nM
Antioxidant ORAC =	8.71 μM Trolox/μM	5.63 μM Trolox/μM	4.80 μM Trolox/μM

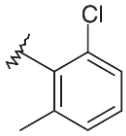
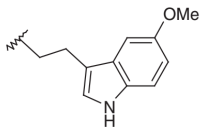
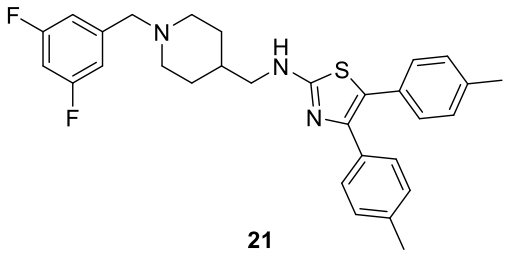
R =		
	19	**20**
*ee*AChE IC_{50} =	29.3 nM	75.0 nM
*eq*BChE IC_{50} =	52.3 nM	20.1 nM
Antioxidant ORAC =	5.32 μM Trolox/μM	8.48 μM Trolox/μM

Figure 2.9. Ferulic acid-based *N*-benzylpiperidine hybrids [Benchekroun et al. 2015].

Shidore et al. have designed a series of hybrid structures connecting *N*-benzylpiperidine nucleus with diarylthiazole moiety as potential multitarget-directed ligands for the treatment of AD. The most potent lead molecule of the series *N*-[(1-(3,5-difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(p-tolyl)thiazol-2-ylamine (**21**, Figure 2.10) elicited significant AChE (IC_{50} = 0.30 μM) and BChE (IC_{50} = 1.84 μM) along with AChE-induced Aβ aggregation inhibition, antioxidant, and anti-apoptotic activities. The compound was designed by tethering *N*-benzylpiperidine with diarylthiazole moiety through an amide linkage. The *N*-benzylpiperidine ring was extended deep into the CAS, while diaryl moiety interacted with PAS residues. The *in vivo* behavioral studies showed amelioration of cognitive dysfunction in Y-maze and

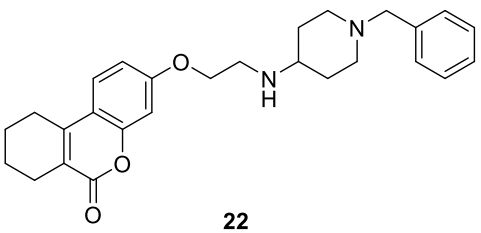
Morris water maze test by compound **21**, and the *ex vivo* studies established its antioxidant activity [Shidore et al. 2016].



21
*h*AChE IC_{50} = 0.30 μM
*eq*BChE IC_{50} = 1.84 μM
% Aβ inhibition = 27.65%(10 μM concentration)

Figure 2.10. A molecular hybrid of *N*-benzylpiperidine and diarylthiazole as potential multitargeted ligand against AD [Shidore et al. 2016].

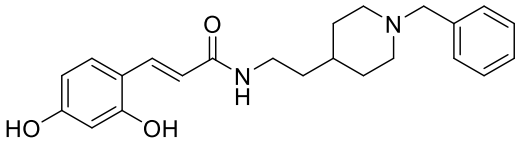
Xie et al. have combined *N*-benzylpiperidine moiety with coumarin to investigate the inhibitory potential against ChEs and MAO-B. Among the tested fourteen compounds, compound **22** was found to be the most potent dual ChE inhibitor with IC_{50} values 0.87 μM and 0.93 μM for *ee*AChE and *eq*BChE, respectively. Compound **22** also elicited balanced inhibition of *h*MAO-B with IC_{50} value of 2.62 μM. Enzyme kinetic studies revealed that compound **22** was a mixed type of AChE and a competitive-type of MAO-B inhibitor. Compound **22** also showed appreciable BBB permeability and found nontoxic against SH-SY5Y cell lines. All these results signified compound **22** (Figure 2.11) as promising multitargeted lead candidate for the treatment of AD [Xie et al. 2016].



22
*ee*AChE IC_{50} = 0.87 μM
*eq*BChE IC_{50} = 0.93 μM
*h*MAO-B IC_{50} = 2.62 μM

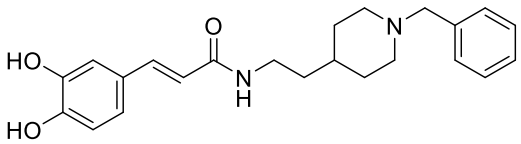
Figure 2.11. A molecular hybrid of *N*-benzylpiperidine moiety of donepezil and coumarin [Xie et al. 2016].

Estrada et al. have 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 (**23**, Figure 2.12) and caffeic acid-based hybrids (**24**, Figure 2.12) displayed balanced biological profiles, with IC_{50} in the low-micromolar and submicromolar range for hChEs and hMAOs, and an antioxidant potency comparable to vitamin E. Additionally, the caffeic acid-based hybrid **24** stimulated the differentiation of adult subgranular zone-derived neural stem cells into a neuronal phenotype, showing a great neurogenic effect [Estrada et al. 2016].



23

IC_{50}, *h*AChE= 0.99 μM, *h*BChE = 0.26 μM
IC_{50}, *h*MAO-A = 5.5 μM, *h*MAO-B = 8.3 μM



24

IC_{50}, *h*AChE= 1.75 μM, *h*BChE = 0.69 μM
IC_{50}, *h*MAO-A = 3.5 μM, *h*MAO-B = 6.0 μM

Figure 2.12. Umbelic and caffeic acid-based molecular hybrids of *N*-benzylpiperidine [Estrada et al. 2016].

Costanzo et al. have developed donepezil analogs with dual inhibitory potential against AChE and BACE-1. The most promising candidates of the designed analogues, (E)-2-((1-benzylpiperidin-4-yl)methylene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (**25**, Figure 2.13) and (E)-2-((1-benzylpiperidin-4-yl)methylene)-5-methoxy-2,3-dihydro-1H-inden-1-one (**26**, Figure 2.13) exhibited significant inhibition of AChE (IC_{50} = 0.058 μM, 0.043 μM) and BACE-1 (IC_{50} = 0.697 μM, 0.333 μM), respectively [Costanzo et al. 2016].

The 2-methoxy group was removed in compound **26**. This rigidification of the donepezil resulted in a likely entropy-enthalpy compensation with solvation effects contributing primarily to AChE binding affinity. Molecular docking studies also revealed the better binding affinity of these compounds to the BACE-1 active pocket.

Overall the study has suggested that these rigid molecules could be the new structural designed template for dual inhibition strategy against AChE and BACE-1 [Caliandro et al. 2018].

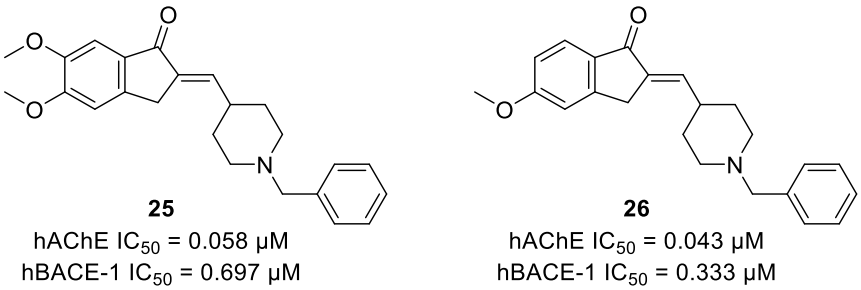


Figure 2.13. Donepezil-like multitargeted compounds as AChE and BACE-1 inhibitors [Caliandro et al. 2018, Costanzo et al. 2016].

Piemontese et al. have investigated novel donepezil-based molecular hybrids. The hybrids were designed by conjugating benzylpiperidine/benzylpiperazine moiety with derivatives of bioactive heterocycles (benzimidazole or benzofuran). Among the total seven synthesized hybrids, **27**, **28** and **29** (Figure 2.14) exhibited micromolar inhibition of hAChE at IC50 values of 4.2, 6.9, and 4.0 μM, respectively. Additionally, these hybrids also elicited moderate anti-Aβ aggregatory and neuroprotective activity. Moderate antioxidant activity was also observed by these molecular hybrids [Piemontese et al. 2018].

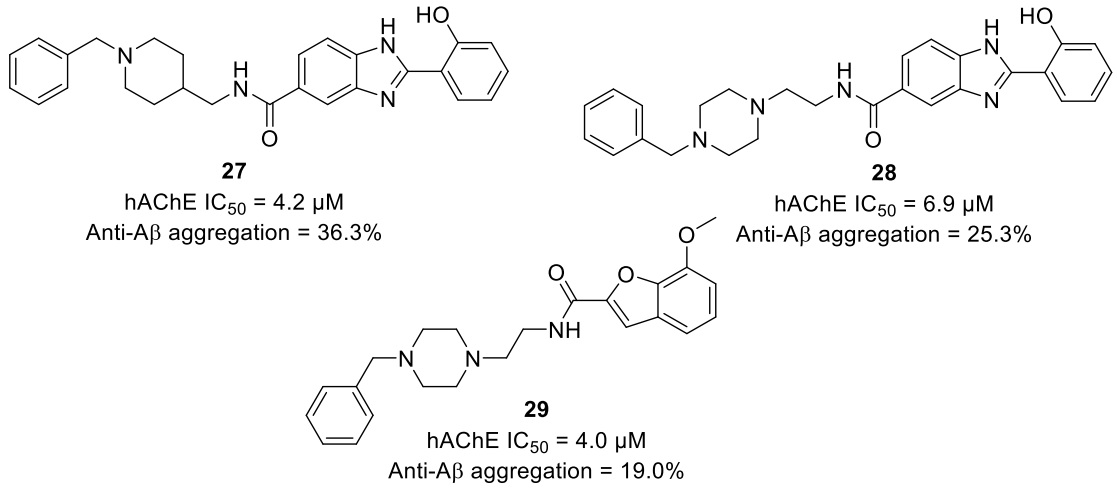
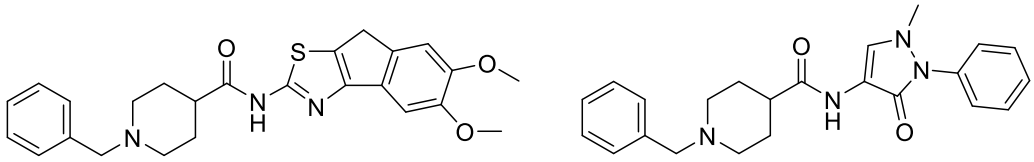


Figure 2.14. Donepezil-based hybrids of *N*-benzylpiperdine/benzylpiperazine moiety with benzimidazole or benzofuran [Piemontese et al. 2018].

In a recent study, Greunen et al. have designed novel *N*-benzylpiperidine carboxamide derivatives as potential ChE inhibitors for the treatment of AD. The two most active analogs of the series (compounds **30** and **31**, Figure 2.15) 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].



30
hAChE IC_{50} = 0.41 µM

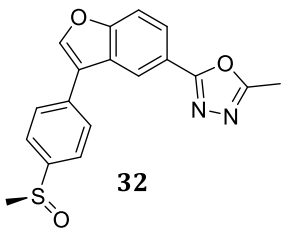
31
hAChE IC_{50} = 5.94 µM

Figure 2.15. Novel *N*-benzylpiperidine carboxamide analogs [van Greunen et al. 2019].

2.2. 1,3,4-Oxadiazoles: Development as multitargeted ligands in AD

The planer ring conformation and H-bond acceptor ability of 1,3,4-oxadiazole makes it a suitable candidate for achieving the requisite orientation within the active pocket of target enzymes. Moreover, several studies suggested 1,3,4-oxadiazole be a suitable pharmacophore acting against multiple targets involved in AD.

Saitoh et al. have reported a potential inhibitor (**32,** Figure 2.16) of GSK-3β and CDK-5. Compound **32** also inhibited tau phosphorylation, APP metabolism, and ameliorated cognitive dysfunction in 3xTg-AD mice [Onishi et al. 2011, Saitoh et al. 2009].

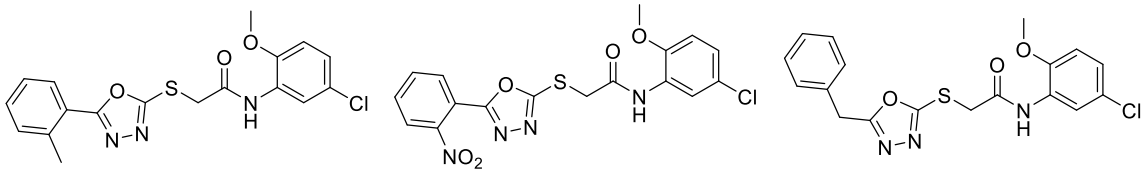


32

Figure 2.16. 1,3,4-Oxadiazole as GSK-3β and CDK-5 inhibitor [Saitoh et al. 2009].

Rehman et al. have investigated a series of 1,3,4-oxadiazole-2-yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanylacetamide derivatives with ChE and LOX inhibitory potential.

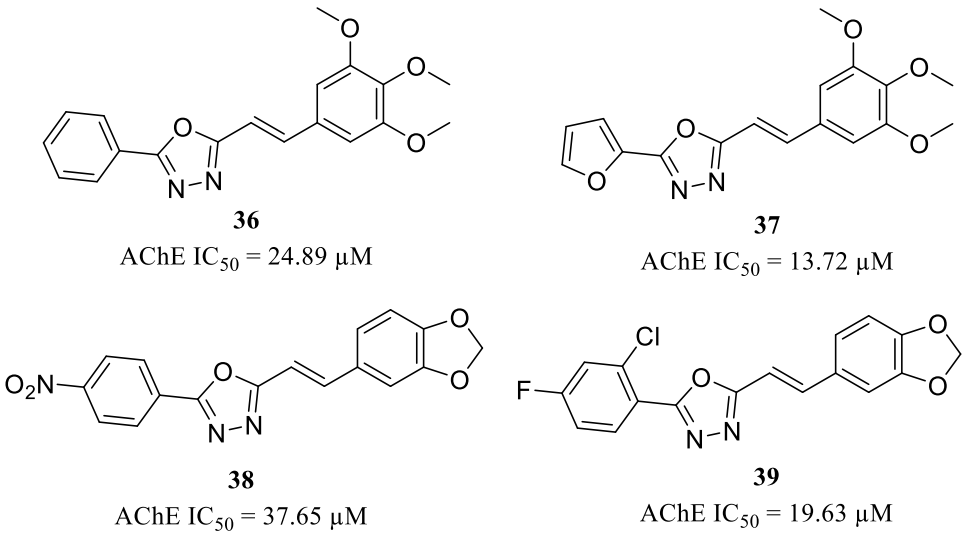
Among the synthesized compounds, **33** and **34** (Figure 2.17) exhibited AChE inhibition activity with IC_{50} values of 34.61 and 40.21 µM, respectively, while compound **35** showed maximum BChE inhibition (IC_{50} = 33.31 µM). Compound **34** exhibited considerable inhibition of LOX (69.67%) [Rehman et al. 2013].



33 **34** **35**

Figure 2.17. 1,3,4-Oxadiazoles as ChE and LOX inhibitors [Rehman et al. 2013].

Kamal et al. have synthesized a library of thirty two compounds of (*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, and investigated their AChE inhibitory activity. Among them, compounds **36, 37, 38,** and **39** (Figure 2.18) elicited moderate AChE inhibition with IC_{50} values of 24.89, 13.72, 37.65, and 19.63 µM, respectively. The molecular docking study revealed active site interactions of these compounds with AChE [Kamal et al. 2014].



36
AChE IC_{50} = 24.89 µM

37
AChE IC_{50} = 13.72 µM

38
AChE IC_{50} = 37.65 µM

39
AChE IC_{50} = 19.63 µM

Figure 2.18. 1,3,4-Oxadizole analogs with AChE inhibitory potential from the library of (*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 [Kamal et al. 2014].

Mei et al. have reported the design, synthesis of molecular hybrids of benzothiazole tethered 1,3,4-oxadiazoles as Aβ targeted compounds in AD. The compound **40** exhibited significantly higher neuroprotective activity, whereas compounds **41–43** (Figure 2.19) showed slightly moderate activity [Mei et al. 2017].

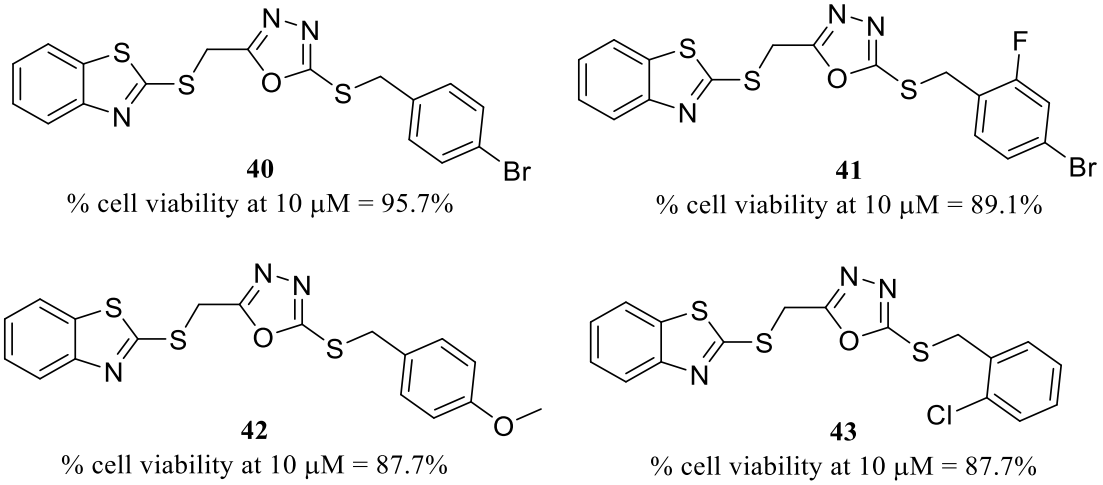


Figure 2.19. Novel benzothiazole tethered 1,3,4-oxadiazole hybrids [Mei et al. 2017].

Rehman et al. have synthesized a series of 3-piperidinyl-1,3,4-oxadiazoles and evaluated their AChE inhibitory potential. The results showed compound **44** (Figure 2.20) exhibited moderate AChE inhibitory activity with IC_{50} value of 3.64 μM [Rehman et al. 2018].

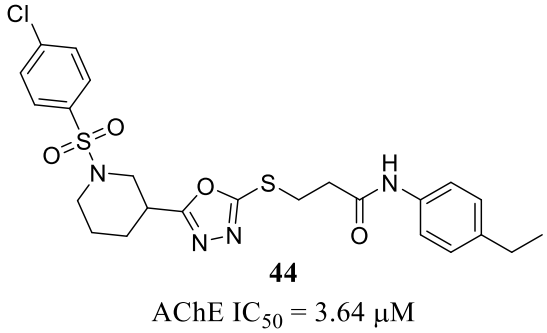
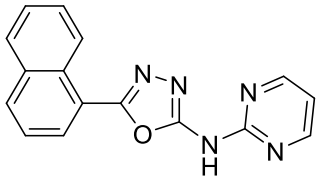


Figure 2.20. 3-Piperidinyl-1,3,4-oxadiazole hybrid with AChE inhibitory activity [Rehman et al. 2018].

Tripathi et al. have designed and synthesized novel hybrids bearing a 2-aminopyrimidine moiety linked to substituted 1,3,4-oxadiazoles, and investigated AChE and Aβ aggregation inhibitory potential. Among the synthesized fifteen compounds, **45**

(Figure 2.21) exhibited significant hAChE (pIC_{50} = 6.52, Ki = 0.17 μM) and Aβ aggregation inhibition. Additionally, this novel hybrid exhibited significant PAS-AChE binding, appreciable BBB permeability, amelioration of cognitive impairment in mice, and remarkable antioxidant activity [Tripathi et al. 2019b].

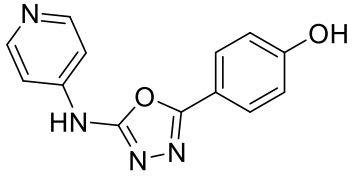


45

AChE pIC_{50} = 6.52, Ki = 0.17 μM

Figure 2.21. A multifunctional hybrid with 2-aminopyrimidine linked 1,3,4-oxadiazole to treat AD [Tripathi et al. 2019b].

Mishra et al. have designed and synthesized fifteen molecular hybrids of 4-aminopyridine and substituted 1,3,4-oxadiazoles as potential AChE and Aβ aggregation inhibitors. Amongst them, compound **46** (Figure 2.22) was found to be the most potent inhibitor of AChE (IC_{50} = 1.098 μM) with a noncompetitive mechanism. Also, compound **46** exhibited significant inhibition of AChE-induced Aβ aggregation (38.2–65.9%) in thioflavin T assay. The *in vivo* study in mice signified amelioration of scopolamine-induced cognitive dysfunction and *ex vivo* study of brain homogenates established considerable antioxidant activity of compound **46** [Mishra et al. 2019].



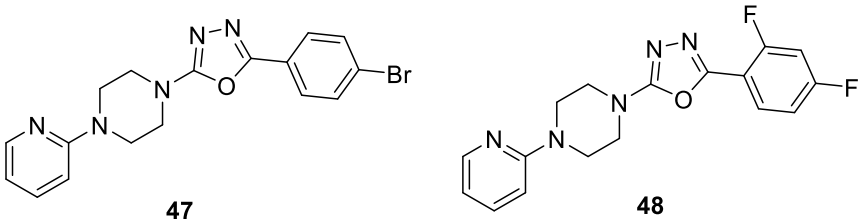
46

AChE IC_{50} = 1.098 μM, Ki = 0.960 μM

Aβ aggregation inhibition = 38.2-65.9%

Figure 2.22. A molecular hybrid of 4-aminopyridine and 1,3,4-oxadiazole [Mishra et al. 2019].

In a recent experiment, Tripathi et al. have designed, synthesized and biologically evaluated molecular hybrids of 2-pyridylpiperazine and 5-phenyl-1,3,4-oxadiazoles as multifunctional agents to treat AD. Among the synthesized thirty compounds, **47** and **48** (Figure 2.23) exhibited balanced inhibition of hAChE (IC_{50}, **47** = 0.074 µM; **48** = 0.054 µM), hBChE (IC_{50}, **47** = 0.846 µM; **48** = 0.787 µM), BACE-1 (IC_{50}, **47** = 0.126 µM; **48** = 0.098 µM), and AChE-induced Aβ aggregation (NFI, **47** = 16–40%; **48** = 37–64%). Additionally, these molecular hybrids exhibited appreciable BBB permeation, PAS-AChE binding, and neuroprotective effect against Aβ induced oxidative stress toward SH-SY5Y cell lines. The *in vivo* investigation in rats showed improvement in learning and memory, and the *ex vivo* study of hippocampal brain homogenates proved the antioxidant potential of these novel hybrids [Tripathi et al. 2019a].



47
hAChE IC_{50} = 0.074 µM
hBChE IC_{50} = 0.846 µM
hBACE-1 IC_{50} = 0.126 µM
AChE-induced Aβ inhibition = 16-40%

48
hAChE IC_{50} = 0.054 µM
hBChE IC_{50} = 0.787 µM
hBACE-1 IC_{50} = 0.098 µM
AChE-induced Aβ inhibition = 37-64%

Figure 2.23. Molecular hybrids of 4-aminopyridine and 1,3,4-oxadiazole [Tripathi et al. 2019a].