2. LITERATURE REVIEW

2.1. FDA approved drugs for the treatment of AD

Currently, four FDA-approved medications are available in the market for the treatment of AD. Three of the four medications available are AChE inhibitors (donepezil, galantamine, and rivastigmine), and the fourth is an *N*-methyl-D-aspartate (NMDA) receptor antagonist (memantine). A combination of donepezil with memantine is also approved for the treatment of moderate to severe AD. These medications give only symptomatic relief to the patients, as they are neither disease-modifying agents nor they cure the disease.

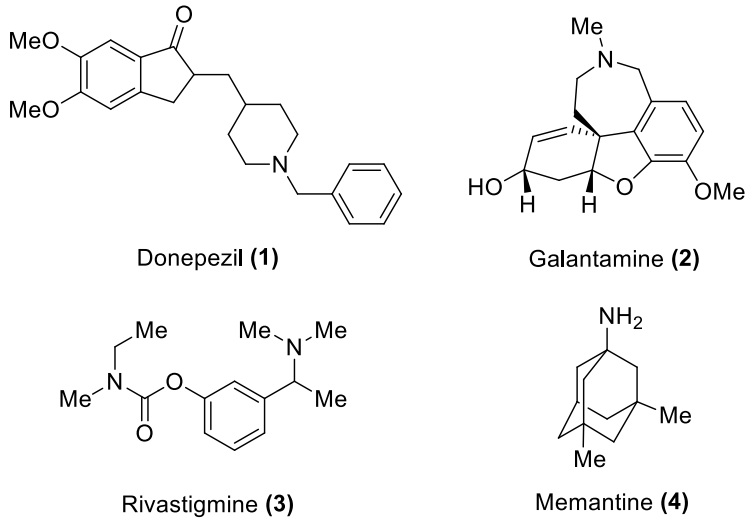


Figure 2.1. FDA-approved medications **(1-4)** for the treatment of AD.

Tacrine was first FDA-approved in 1993 for treatment of AD, but later on, it was withdrawn because of its hepatotoxicity.[52] Donepezil (**1**) was approved in 1996 for AD treatment and it is a centrally acting reversible and selective AChE inhibitor. It was developed by Eisai and partner Pfizer, and is sold with the trade name Aricept.[53] Galantamine (**2**) is an alkaloid used for the treatment of mild to moderate AD. It is a competitive and reversible nicotinic ACh receptor (nAChR) antagonist approved by the FDA in 2001 for the treatment of AD.[54] Rivastigmine (**3**) was developed by Marta Weinstock-Rosin of the Pharmacology Department, The Hebrew University of Jerusalem. It is a semi-synthetic derivative of physostigmine and inhibits both ChEs.[55] Memantine (**4**) is a non-competitive glutaminergic NMDA receptor blocker. It

was first synthesized by Eli Lilly in 1968. Memantine is sold under various trade names like Axura, Akatinol, and Namenda for the treatment of AD.[56]

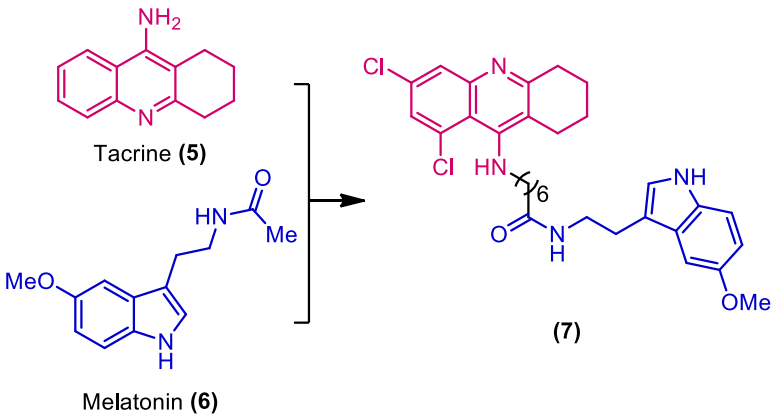
2.2. Reported MTDLs for treatment of AD

To combat AD-like diseases having a complex etiology, development of multitarget-directed ligands (MTDLs) is recognized as one of the most assuring drug discovery approaches.[57-59] Drugs acting on a single target even though they have high affinity and selectivity for their targets might not influence the mysterious etiology of the disease satisfactorily. An MTDL having moderate but balanced affinities for the targets can still exert more beneficial effects compared to a single-targeted molecule. Concurrent effects on several therapeutic targets make MTDLs superior in altering the complex equilibrium of the cellular network.[60,61] A mild and balanced activity on multiple therapeutic targets might secure higher safety and reduce the risk of therapeutic resistance.[62] In the following section, various recently developed MTDLs with different scaffolds have been summarized.

2.2.1. Indole based anti-AD agents

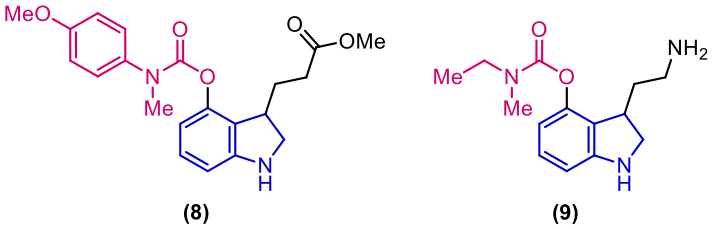
Indole ring is present in many natural compounds having important biological activities.[63] Melatonin is an indole ring containing pineal neurohormone whose levels decrease in AD patients.[64] It possesses strong free radical scavenging properties[65] and offers protective effects against Aβ-induced apoptosis[66] and glutamate-induced excitotoxicity.[67] Many indole based MTDLs were reported in the literature for the treatment of AD as described below.

Rodríguez-Franco M. I. *et. al.* focused on the designing of compounds that were having AChE inhibitory and antioxidant activity in a single-molecule.[68] The authors designed novel tacrine-melatonin hybrids by linking tacrine (**5**) and melatonin (**6**) with suitable linkers. These novel tacrine-melatonin hybrids showed potent AChE inhibitory activity with high oxygen radical scavenging capacity. Additionally, these hybrids were predicted to be BBB permeable by PAMPA assay.



Compd	*h*AChE IC_{50} (nM)	*h*BuChE IC_{50} (nM)	ORAC
5	350 ± 10	40 ± 2	< 0.01
6	--	--	2.3 ± 0.1
7	0.008 ± 0.0004	7.8 ± 0.4	2.5 ± 0.1

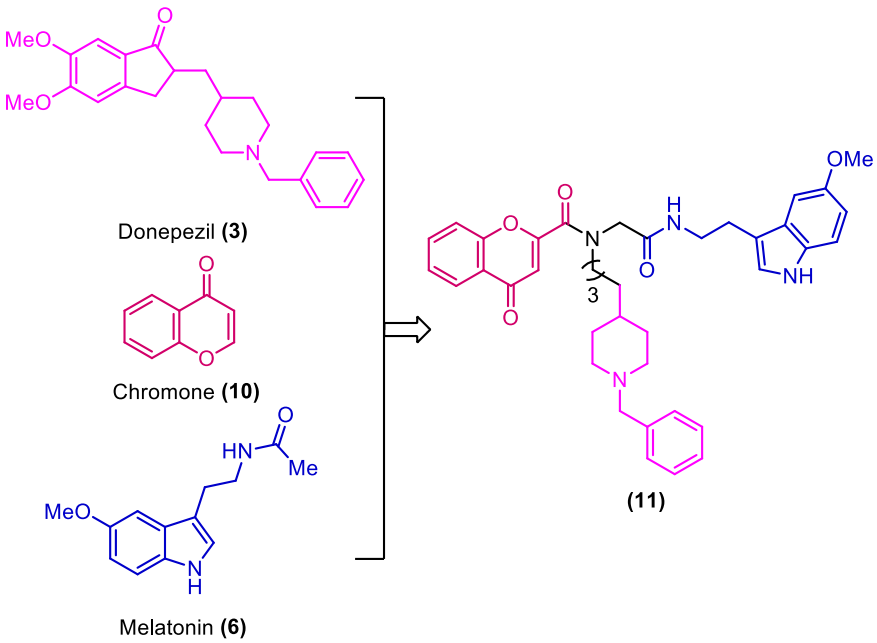
Amongst them, compound (**7**) showed excellent human AChE inhibitory activity (IC_{50} value of 8 pM) and antioxidant activity (ORAC value of 2.5 trolox equivalents).[68]



Compd	*h*AChE IC_{50} (µM)	*h*BuChE IC_{50} (µM)	% radical scavenged (DPPH)
8	1.20 ± 0.01	4.60 ± 0.04	30.4 ± 0.4 at 10 µM conc.
9	0.40 ± 0.04	0.20 ± 0.01	36.3 ± 3.5 at at 10 µM conc.

Yanovsky I. *et. al.* reported substituted indoline derivatives as ChEIs and antioxidant agents.[69] These derivatives exhibited significant radical scavenging activity for different radicals and provided neuroprotection against H_2O_2-induced toxicity. In most of the synthesized compounds, incorporation of *N*-methyl-*N*-ethyl and *N*-methyl-*N*-methoxyphenyl carbamate moieties at the phenyl ring of the indoline enhanced the ChE inhibitory activity. Compounds (**8** and **9**) showed notable AChE inhibitory activity (IC_{50} values of

1.20 μM and 0.40 μM, respectively) and BuChE inhibitory activity (IC_{50} values of 4.60 μM and 0.20 μM, respectively).



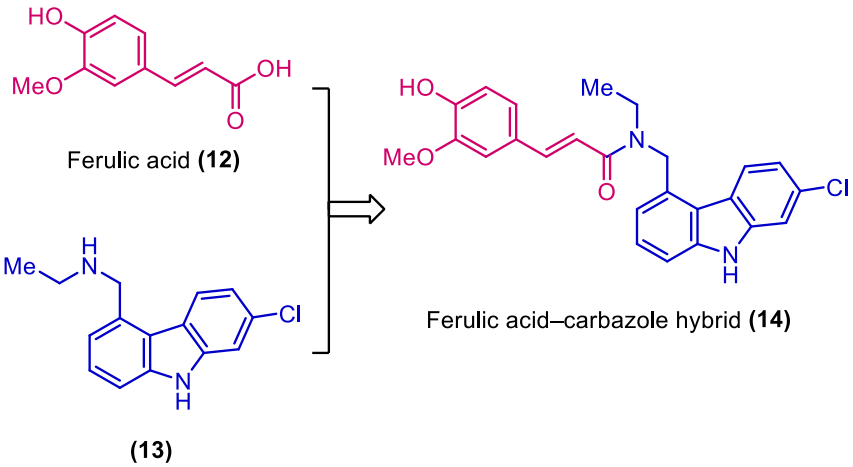
Compd	AChE IC_{50} (μM)	BuChE IC_{50} (nM)	MAO-B IC_{50} (μM)	ORAC
11	1.73 ± 0.34	11.90 ± 0.05	21.29 ± 3.85	3.04 ± 0.36

Pachon A. I. *et. al.* reported novel donepezil-chromone-melatonin hybrids as potential multifunctional anti-AD agents.[70] They merged the important pharmacophores from donepezil (AChE inhibition), chromone (MAO-B inhibition) and melatonin (antioxidant) into a single scaffold to obtain multifunctional activity from a single compound. One representative compound (**11**) exhibited notable BuChE inhibitory activity (IC_{50} value of 11.90 nM), *h*AChE inhibitory activity (IC_{50} value of 1.73 μM), moderate MAO-B inhibitory activity (IC_{50} value of 21.29 μM) and excellent antioxidant activity (ORAC; 3.04 trolox equivalent).

2.2.2. Carbazole-based anti-AD agents

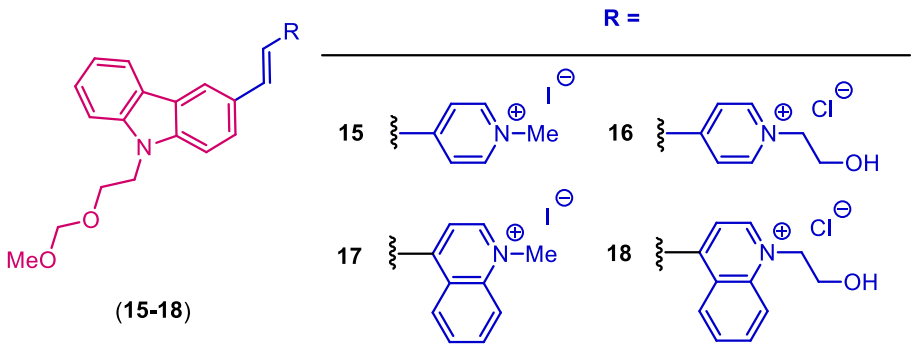
Carbazole is an important nitrogen containing heterocycle widely present in many phytochemicals with an array of biological activities associated with AD. The notable biological activities like antioxidant activity and cholinesterase inhibitory activity associated with the carbazole structure drew the attention of researchers to use carbazole as a lead scaffold for

designing of novel anti-AD agents. Many research groups reported carbazole-based multifunctional anti-AD agents as described below:



Compd	AChE IC_{50} (µM)	BuChE IC_{50} (µM)	FRSA value at 10 µM
12	--	--	63.1 ± 5.5
13	2.1 ± 0.6	1.9 ± 0.2	60.1 ± 3.0 **(12 + 13)**
14	1.9 ± 0.8	3.1 ± 0.4	66.0 ± 5.1

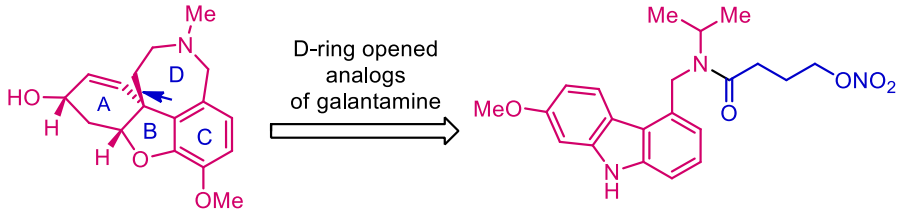
Fang L. *et. al.* designed and synthesized a series of ferulic acid-carbazole hybrids to obtain synergic action of ferulic acid moiety as antioxidant and carbazole moiety as ChE inhibitor.[71] Most of the synthesized hybrids showed moderate to good cholinesterase inhibitory activities in Ellman's assay. Compounds (**13** and **14**) displayed good AChE inhibitory activity (IC_{50} values of 2.1 µM and 1.9 µM, respectively) but their BuChE inhibitory activity (IC_{50} values of 1.9 µM and 3.1 µM, respectively) was better than that of galantamine (AChE, IC_{50} value of 8.5 µM; BuChE, IC_{50} value of 28.1 µM). The hybrid **14** exhibited good antioxidant activity [free radical scavenging activity (FRSA) value of 66.0 % at 10 µM concentration] which was similar to that of ferulic acid (FRSA value of 63.1 % at 10 µM concentration). While the anti-oxidant potential of the compound (**13**) in presence of ferulic acid (FRSA value of 60.1 % at 10 µM concentration) was comparatively lower than that of the hybrid (**13**) and ferulic acid alone.



(**15-18**)

Compd	15	16	17	18
$F_{peptide}/F_{dye}$	1.5	3.3	3.2	6.3
$F_{peptide}/F_{dye}$	4.2	10.5	23.5	81.5
K_d (μM)	194	459	49	92

Yang. *et. al.* developed carbazole-based cyanine fluorophores **15-18** as Aβ aggregation inhibitors.[72] This novel series of fluorophores exhibited sharp fluorescence increment when bonded with Aβ peptides and fibrils. Among them, compound (**18**) had shown fluorescence enhancement ratio of 6.3 and 81.5 for Aβ peptides and fibrils, respectively having a dissociation constant (K_d) value of 92 μM for Aβ fibrils. Additionally, compound (**18**) was not toxic to the neuronal cells and conferred a protective effect against neurotoxicity induced by Aβ oligomers and fibrils. Its BBB permeability exhibited its potential as anti-AD agent.

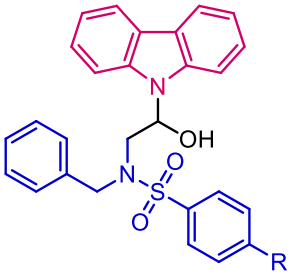


Gallantamine **(2)** **(19)**

Compd	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
2	10.53 ± 3.11	39.03 ± 12.11
19	2.21 ± 0.51	2.50 ± 0.90

Fang. L. *et. al.* reported novel dibenzofuran/carbazole-NO donor hybrids contemplated as the D-ring opened analogs of galantamine.[73] *In vitro* enzyme inhibition study pointed out that the compound (**19**) having a nitrate moiety in the structure conferred good inhibitory activity for AChE (IC_{50} value of 2.21 μM) and BuChE (IC_{50} value of 2.50 μM). Compound (**19**)

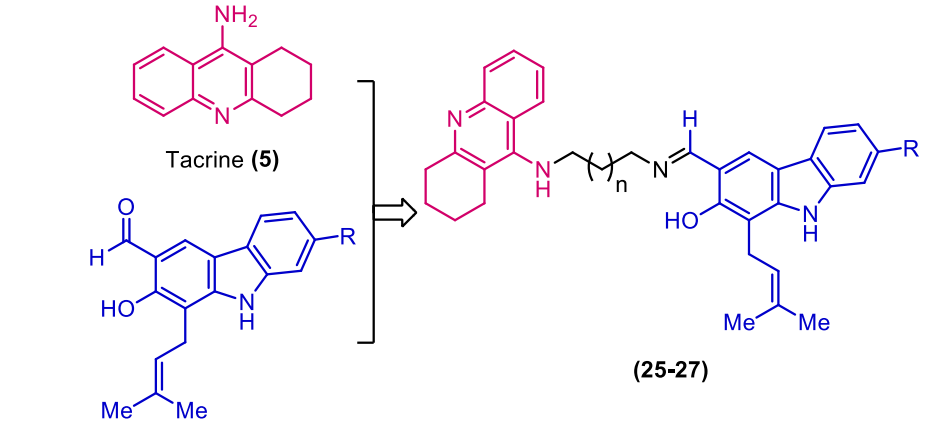
also released a relatively low concentration of NO *in vitro* and was non-toxic to neuronal cells, providing a neuroprotective effect against the Aβ-induced toxicity. Additionally, it displayed notable spatial memory-improvement in cognition impaired adult rats.



(20-22)

Compd	R	BACE-1 IC_{50} (μM)	$logBB_{pred}$
20	-H	1.9	-0.61
21	-Cl	1.7	-0.65
22	-OMe	1.6	-0.67

Bertini S. *et. al.* reported a novel series of sulfonamide derivatives of carbazole as BACE-1 inhibitors.[74] Introduction of various substituted benzyl groups on the nitrogen of the arylsulfonamide group resulted into compounds with good BACE-1 inhibitory activity. Compounds (**20-22**) exhibited the highest BACE-1 inhibitory activity with IC_{50} values in the range of 1.6 μM to 1.9 μM among the compounds of the series. Additionally, the predicted logBB values pointed out satisfactory BBB permeation of these compounds.



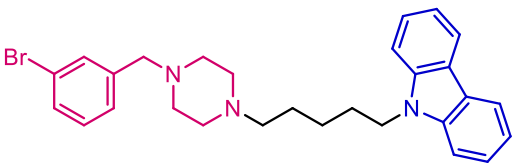
Tacrine **(5)**

Heptaphylline (R = -H) **(23)**
7-Methoxyheptaphylline (R = -OMe) **(24)**

(25-27)

Compd	n	R	AChE IC_{50} (μM)	BuChE IC_{50} (μM)	ABTS assay IC_{50} (μM)
25	3	$-OCH_3$	0.48 ± 0.14	52.14 ± 6.08	8.34 ± 1.68
26	3	-H	0.95 ± 0.27	19.37 ± 0.54	11.24 ± 0.48
27	1	$-OCH_3$	1.03 ± 0.23	64.32 ± 11.9	9.77 ± 0.03

Songsiang U. *et. al.* reported heptaphylline **23** and 7-methoxyheptaphylline **24**, extracted from the root of *Clausena Harmandiana*, as antioxidant agents.[75] Based on these findings, Thiratmatrakul S. *et. al.* designed novel carbazole-tacrine hybrids as potential MTDL agents by linking tacrine (**5**) with heptaphylline (**23**) and 7-methoxyheptaphylline (**24**).[76] Among the designed compounds, compound (**25**) exhibited notable AChE inhibitory (IC_{50} value of 0.48 µM) and mild BuChE inhibitory (IC_{50} value of 52.12 µM) activities. Molecular modeling studies revealed that the compound (**25**) could interact with both the sites of AChE. Compound (**25**) was also endowed with potent 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging activity (IC_{50} value of 8.34 µM). Furthermore, compound (**25**) reduced Aβ-induced and oxidative stress-induced neurotoxicity and improved the scopolamine-induced memory impairment in mice.



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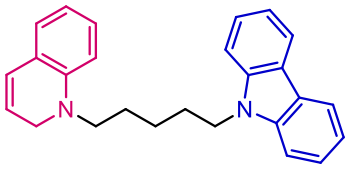
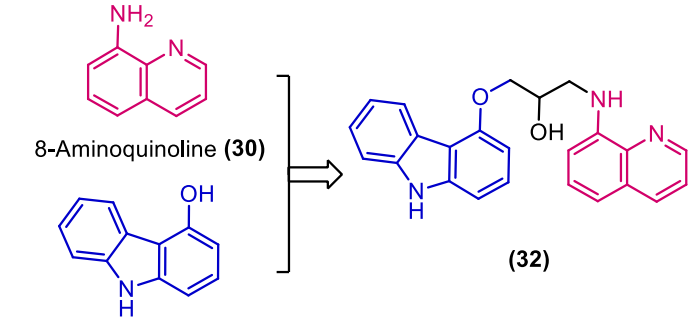


(29)

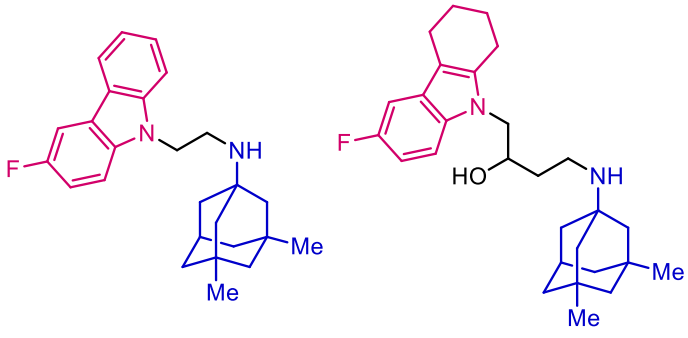
Compd	AChE IC_{50} (µM)	BuChE IC_{50} (µM)	$Aβ_{1-42}$ aggregation inhibition	
			Self-induced	AChE-induced
28	1.4 ± 0.19	5.1 ± 1.02	19.5 ± 1.3	19.2 ± 3.4
29	0.11 ± 0.18	0.02 ± 0.11	27.4 ± 1.8	24.9 ± 6.4

Choubdar N. *et. al.* reported a series of compounds having a carbazole scaffold attached to benzylpiperazine, benzylpiperidine, quinoline, isoquinoline or pyridine moieties through an aliphatic linker as anti-AD agents.[77] All the synthesized compounds displayed good ChEs inhibitory activities having IC_{50} values in micromolar to nanomolar range. Among them, the quinolinium salt **29** exhibited significant AChE and BuChE inhibitory activities (IC_{50} values of 0.11 µM and 0.02 µM, respectively). Replacement of the benzylpiperazine moiety (compound **28**) with quinoline (compound **29**) significantly improved the ChE inhibitory activity. This enhancement in the activity could be due to the formation of a stable cationic center on the quinoline moiety and favorable π-stacking interactions between the quinoline moiety and the PAS of the enzyme. Compound (**29**) also showed moderate

inhibition of self-induced and AChE-induced $A\beta_{1-42}$ aggregation (27.4 and 24.9 % of inhibition at 100 µM concentration, respectively). Compound (**29**) also offered protection against H_2O_2-induced toxicity in PC12 cells.



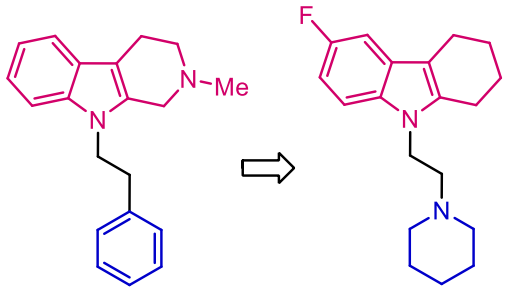
Zhang X. *et. al.* reported a multifunctional carbazole–aminoquinoline hybrid by linking 8-aminoquinoline (**30**) (metal chelator) with 4-hydroxycarbazole (**31**) (neuroprotectant).[78] Compound (**32**) exhibited selective copper chelation, copper-induced $A\beta_{1-42}$ aggregation inhibition, and moderate self-induced $A\beta_{1-42}$ aggregation inhibition. In addition, compound (**32**) exhibited significant protection of at HT22 cells at 10 µM concentration against glutamate-induced toxicity.



Activity	33	34
AChE IC_{50} (µM)	> 20	> 20
BuChE IC_{50} (µM)	0.74 ± 0.02	5.43 ± 0.39
ABTS radical scavenging activity (%)	15.3 ± 1.3 at 100 µmol/L	48.4 ± 3.5 at 100 µmol/L

Makhaeva G. *et. al.* reported a novel series of aminoadamantane-carbazole hybrids linked by different linkers as anti-AD agents.[79] The presence of a linker was found to be important for the BuChE inhibitory and radical-scavenging activity. Hybrids with flexible linkers, such as ethylene (compound

33) and 2-hydroxypropylene (compound **34**) showed selective BuChE inhibition (IC_{50} values of 0.74 µM and 5.43 µM, respectively). These hybrids possessed moderate free radical-scavenging activity at a concentration of 100 µmol/L.



(35) **(36)**

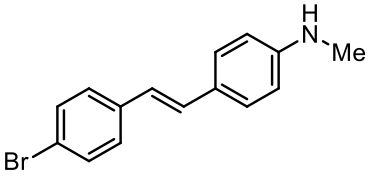
Activity	35	36
AChE IC_{50} (µM)	> 100	> 100
BuChE IC_{50} (µM)	7.9	0.11 ± 0.01
BACE-1 % inhibition	-	18.8 ± 0.1 at 50 µM conc.

Ghobadian R. *et. al.* reported a novel series of 1,2,3,4-tetrahydro-9*H*-carbazole derivatives as anti-AD agents.[80] *In vitro* enzyme inhibition study displayed that these compounds possessed selective BuChE inhibitory activity. Compound (**36**) displayed excellent BuChE inhibitory activity (IC_{50} value of 0.11 µM) with selectively index of 892.86 for BuChE. The parent scaffold 2,3,4,9-tetrahydro-1*H*-carbazole did not show significant BuChE inhibitory activity (IC_{50} value of 89.13 µM). So, the presence of heterocyclic amine fragment seems to govern the BuChE inhibitory activity. The presence of piperidinyl moiety (compound **36**) resulted into more potent BuChE inhibitory activity than pyrolidinyl and morpholinyl moieties. This was following the *p*Ka values for the nitrogen (*p*Ka: 8.006, 9.945 and 10.042 for morpholine, pyrrolidine, and piperidine, respectively). Compound (**36**) was also endowed with moderate BACE-1 inhibitory potential. It showed significant protection of PC12 cells at 10 µM concentration against H_2O_2-induced toxicity.

2.2.3. Stilbene-based anti-AD agents

Resveratrol and pterostilbene are naturally occurring stilbene derivatives possessing multiple anti-AD properties i.e. Aβ aggregation inhibitory, neuroprotective and antioxidant activities. In the last two decades, several stilbene derivatives have been developed as Aβ imaging probes and Aβ aggregation inhibitors. In combination with other bioactive moieties, these stilbene hybrids showed cholinesterase inhibitory, metal chelating, Aβ aggregation inhibitory, and free radical scavenging activities.

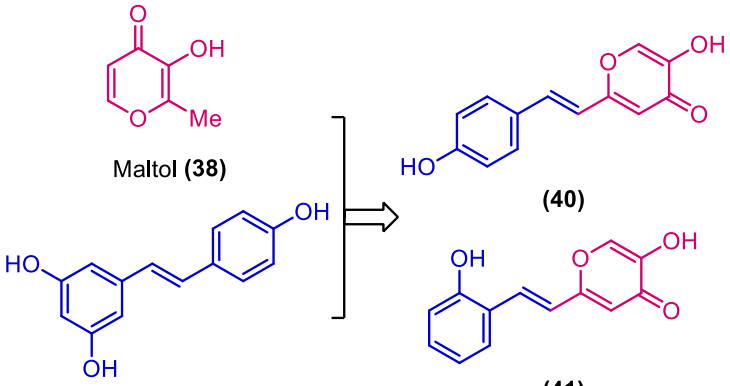
Hong M. C. *et. al.* reported stilbene derivatives as amyloid plaques' imaging agents.[81] All the reported compounds were evaluated *in vitro* for the detection of $A\beta_{40}$ fibrils. Among them, four stilbenes conferred higher than six-fold enhancement in fluorescence intensity ($F_{peptide}/F_{dye}$) and they were further studied for specific bindings and amyloid plaque imaging.



(37)

Activity	37
$F_{peptide}/F_{dye}$	25.8
K_d (μM)	1.13 ± 0.37

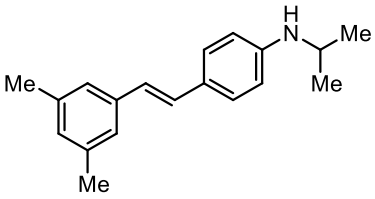
Among the synthesized compounds, compound (**37**) exhibited high fluorescence responsiveness ($F_{peptide}/F_{dye}$ value of 25.8) and strong binding affinity (K_d value of 1.13 μM), which are two critical requirements for any ideal imaging agent.



Compd	ABTS assay IC_{50}	Inhibition of self-induced Aβ aggregation	
		% Inhibition	IC_{50} (μM)
40	1.94 ± 0.71	68.46 ± 2.02 at 20 μM conc.	7.20 ± 0.72
41	1.18 ± 0.043	63.56 ± 2.40 at 20 μM conc.	8.29 ± 0.91

Cheng G. *et. al.* reported a novel series of hybrid compounds by amalgamation of a metal chelating moiety maltol (**38**) with stilbene part of natural product resveratrol (**39**).[82] Among the synthesized compounds, compounds (**40** and **41**) exhibited significant free radical scavenging effect (ABTS assay; IC_{50} values of 1.94 μM and 1.18 μM, respectively). These compounds were also endowed with significant metal chelating potential and

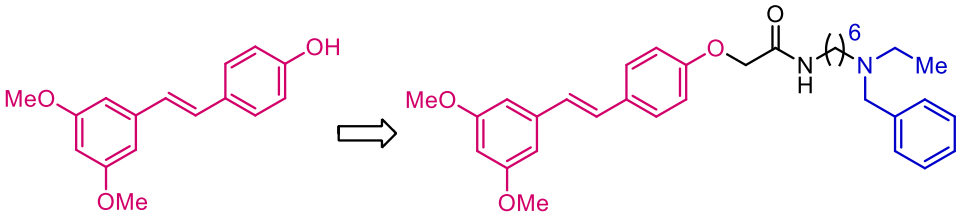
notable self-induced and metal ion-induced $A\beta_{1-42}$ aggregation inhibitory activities.



(42)

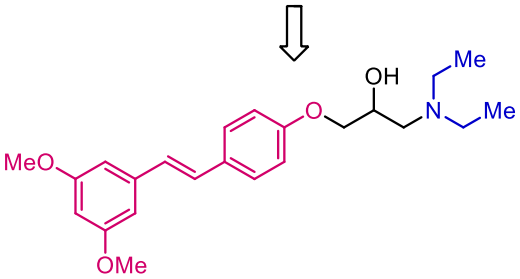
Activity	42
% $A\beta_{1-42}$ aggregation inhibition	71.65 ± 3.32 at 20 µM conc.
ORAC	4.12 ± 0.17

Lu C. *et. al.* synthesized various MTDL stilbene derivatives as anti-AD agents.[83] Some of the synthesized compounds exhibited notable antioxidant activity as well as good $A\beta_{1-42}$ aggregation inhibitory activity. Amongst, compound (**42**) exhibited good $A\beta_{1-42}$ aggregation inhibition (71.65 at 20 µM concentration) and potent anti-oxidant activity (ORAC value of 4.2). Additionally, in cytotoxicity study, compound (**42**) showed lesser cytotoxicity than resveratrol at 60 µM concentration.



Pterostilbene (**43**)

(**44**)



(45)

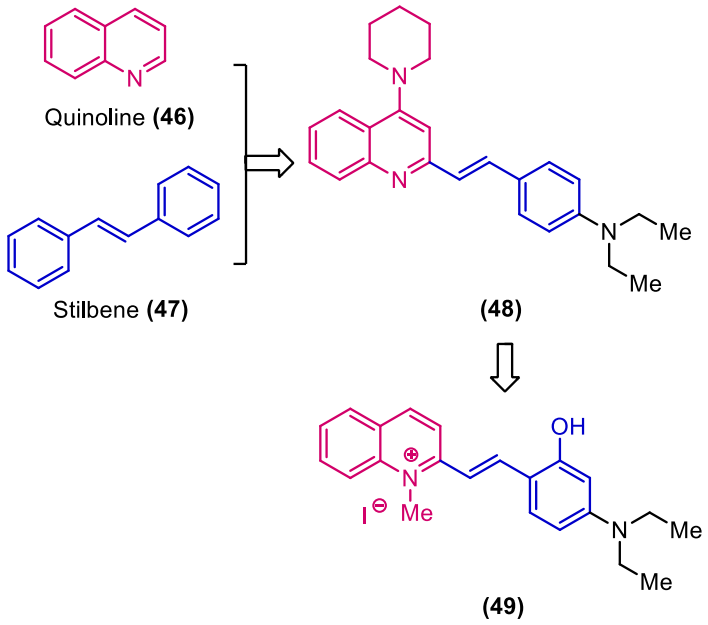
Compd	AChE IC_{50} (µM)	BuChE IC_{50} (µM)	ORAC	% Inhibition of Aβ aggregation	
				Self-induced	AChE-induced
44	0.06 ± 0.03	28.04 ± 1.71	0.51	32.4 ± 1.0 at 25 µM conc.	19.3 ± 0.3 at 25 µM conc.
45	24.04 ± 1.48	-	1.20	40.23 ± 1.2 at 25 µM conc.	-

Pterostilbene **43**, is a phenolic compound available naturally from blueberries with notable biological activities i.e., Aβ aggregation inhibition, antioxidant, and neuroprotective activities. These activities make pterostilbene

a starting scaffold for designing of multifunctional agents for AD. Pterostilbene, itself is a weak ChE inhibitor which limits its usage as an anti-AD agent, whereas a tertiary amine fragment is an indispensable pharmacophore for ChE inhibition, which allows cation-π interactions between the compound and CAS of the enzymes.

A research group of Li Y. *et. al*. reported a novel series of pterostilbene-O-acetamido-alkylbenzylamines as ChEIs for the treatment of AD.[84] The Ellman's assay results revealed that most of these compounds could efficiently inhibit both cholinesterases. In the series, compound (**44**) showed excellent AChE and moderate BuChE inhibition (IC_{50} value of 0.06 μM and 28.04 μM, respectively). Compared to the previously reported pterostilbene derivatives, incorporation of amide in side-chain considerably enhanced the inhibitory activity for both the ChEs. The inhibitory activity was correlated with the length of the linker, as expanding the length of the linker resulted in a better activity. Compounds possessing 4- or 6-methylene linker between benzylamine and amide showed better inhibition of AChE than those with 2- or 3-methylene linkers. Compound (**44**) also showed moderate self-induced and AChE-induced Aβ aggregation inhibitory activity (32.4 and 19.3 % inhibition at 25 μM concentration, respectively). A compound possessing 4- or 6-methylene linkers between amide and benzylamine showed improved inhibition of AChE than the one possessing 2- or 3-methylene linker. Additionally, compound (**44**) was endowed with moderate antioxidant activity with an ORAC value of 0.51.

Zheng Y. *et. al.* reported a series of pterostilbene β-amino alcohol derivatives as multifunctional anti-AD agents.[85] In vitro enzyme inhibition assay displayed that most of the compounds displayed moderate AChE inhibitory activity. Among them, compound (**45**) exhibited a moderate AChE inhibitory activity (IC_{50} value of 24.04 μM). In addition, compound (**45**) was endowed with good antioxidant activity and showed substantial protective effect to PC-12 cell against toxicity induced by H_2O_2. Furthermore, it also exhibited self-induced $A\beta_{1-42}$ aggregation inhibitory activity and showed good *in vitro* BBB permeability.

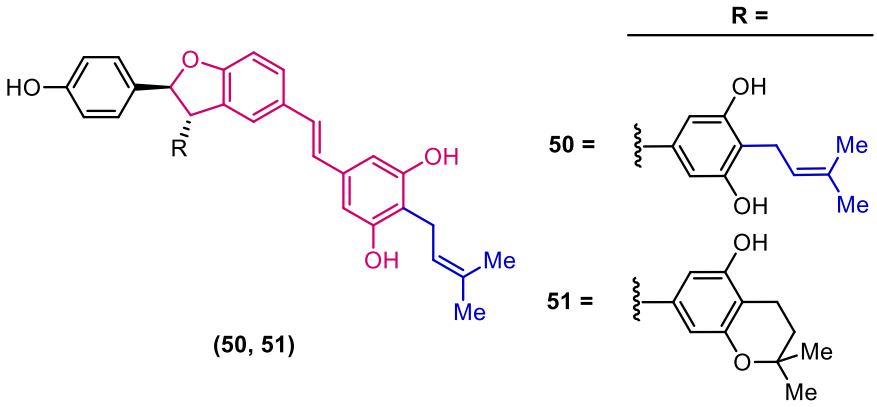


Compd	AChE IC_{50} (μM)	BuChE IC_{50} (μM)	ORAC	Inhibition self-induced Aβ aggregation	
				% Inhibition	IC_{50} (μM)
48	64.0 ± 0.1	0.2 ± 0.1	3.9 ± 0.1	83.9 ± 1.9 at 20 μM conc.	9.7 ± 1.2
49	1.5 ± 0.1	1.1 ± 0.1	1.6 ± 0.1	91.3 ± 0.1 at 20 μM conc.	-

Several stilbene derivatives have been reported in literature showing strong affinity to Aβ and inhibiting Aβ aggregation. Quinoline moiety has been reported as a pharmacophore in many anti-AD agents i.e., tacrine, clioquinol, methylene blue, berberine derivatives. On the basis of these literature reports, Wang X. Q. *et. al.* reported novel 2-arylethenylquinoline derivatives by fusion of quinoline ring with the stilbene moiety for the treatment of AD.[86] Among the reported compounds, compound (**48**) showed significant BuChE inhibitory activity (IC_{50} value of 0.2 μM) in comparison to AChE inhibitory activity (IC_{50} value of 64 μM). It was also endowed with good antioxidant (ORAC value of 3.9) and biometal chelating potential. Compound (**48**) inhibited self-induced $A\beta_{1-42}$ aggregation effectively (IC_{50} value of 9.7 μM) and exhibited a potential to disassemble the $A\beta_{1-42}$ fibril.

It has been stated in the literature that insertion of positive charge in the structure increased the Aβ aggregation inhibitory activity. Based on the activities of the previously reported 2-arylethenylquinoline derivatives, the authors planned to introduce *N*-methyl group in the basic scaffold to develop

novel 2-arylethenyl-*N*-methylquinolinium derivatives, and assessed them for their anti-AD activities. Incorporation of *N*-methyl moiety in the quinoline framework notably enhanced anti-AD potential of the compounds. SAR study confirmed significance of positively charged nitrogen in the quinoline ring, particularly for Aβ aggregation inhibition and ChEs inhibition. Compound (**49**) showed good AChE and BuChE inhibitory activity (IC_{50} values of 1.5 μM and 1.1 μM, respectively) and noteworthy Aβ aggregation inhibitory activity (91.3 % at 20 μM concentration) in the series. Compound (**49**) also protected the neuronal cells against glutamate-induced cytotoxicity by inhibiting ROS generation and enhancing the GSH level in HT22 cells.[87]

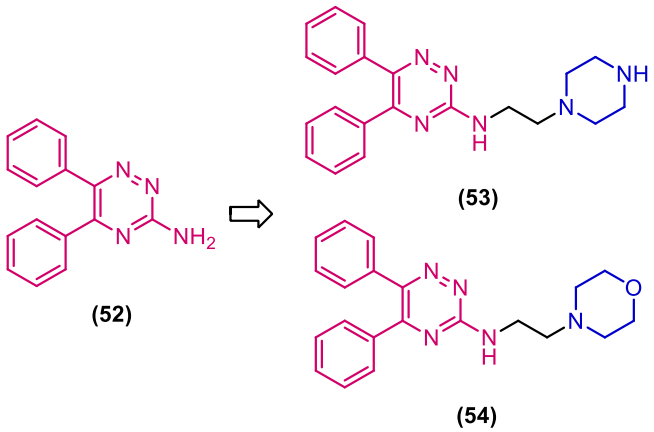


Compd	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	DPPH IC_{50} (μM)	Trolox equivalent	
				ABTS	FRAP
50	2.60 ± 0.04	0.92 ± 0.11	46.95 ± 0.16	1.43 ± 0.14	1.74 ± 0.11
51	8.12 ± 0.05	3.93 ± 0.13	35.33 ± 0.11	1.70 ± 0.21	1.97 ± 0.04

Tang Y. W. *et. al.* reported isoprenylated resveratrol derivatives as anti-AD agents.[88] All the reported compounds showed moderate MAO-B inhibitory activity. Compounds (**50** and **51**) showed good MAO-B (IC_{50} values of 0.92 μM and 3.93 μM, respectively) inhibitory activities. Compounds (**50** and **51**) exhibited better antioxidant activity in DPPH assay (IC_{50} values of 46.95 μM and 35.33 μM, respectively), ABTS assay (1.43 and 1.70 trolox equivalent, respectively) and FRAP assay (1.74 and 1.97 trolox equivalent, respectively). In cell line studies, both the compounds showed very less cytotoxicity and provided protection against neurotoxicity induced by ROS to PC12 cells. The PAMPA-BBB permeation assay results supported the ability of the compounds to penetrate the BBB.

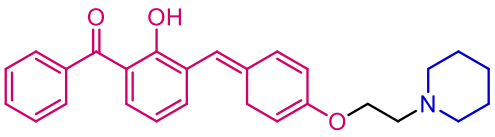
2.2.4. Tertiary amine fragment containing anti-AD agents

The tertiary amine fragment is an indispensable pharmacophore for cholinesterase inhibitory activity, which could enhance the interactions between the compound and the active sites of ChEs. The nitrogen of the tertiary amine gets protonated at physiological *p*H and forms strong cation-π interaction with the aromatic amino acid residues of the active site of enzymes. Several authors reported novel anti-AD agents in which biologically significant scaffolds were attached with various secondary amines through suitable linkers. Some of the reports are discussed below:



Compd	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
52	102.5	250.2
53	4.23	13.3
54	5.79	163.4

Sinha. A. *et. al.* reported substituted vicinal diaryltriazine derivatives as novel ChEIs as anti-AD agents.[89] The basic diaryltriazine scaffold was lacking in significant level of ChE inhibitory activity. The authors reported that when tertiary heterocyclic amines were tethered to the diaryltriazine framework, the resulting compounds exhibited a significant improvement in ChE inhibitory activity. Representative compound (**53**), showed IC_{50} values of 4.23 μM and 13.3 μM for AChE and BuChE respectively, and compound (**54**) showed IC_{50} values of 5.79 μM and 163.4 μM for AChE and BuChE respectively. Furthermore, these compounds exhibited neuroprotection in scopolamine-induced amnesic mice and $A\beta_{1-42}$-induced Alzheimer's rat models.



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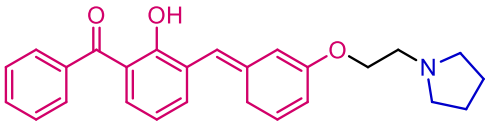
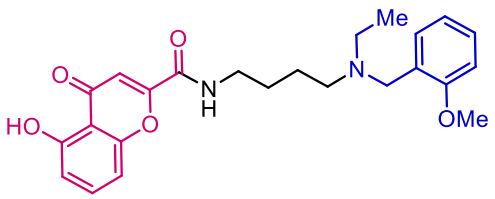


(56)

Compd	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
55	1.6 ± 0.2	2.7 ± 0.6
56	3.5 ± 0.3	0.6 ± 0.1

Leong S. *et. al.* designed and synthesized diarylpentenedione analogs possessing heterocyclic amines as potent dual ChEIs.[90] The Ellman's assay revealed that amongst all the forty-one reported compounds, compounds (**55** and **56**) possessed the highest inhibitory activity against both AChE and BuChE. SAR study of the compounds suggested that the basic scaffold diarylpentenedione was selective towards AChE inhibition, while the presence of heterocyclic chain was critical for both AChE and BuChE inhibitory activities. The Lineweaver-Burk plots and docking results confirmed that both the compounds could simultaneously bind to both PAS and CAS of the enzymes.

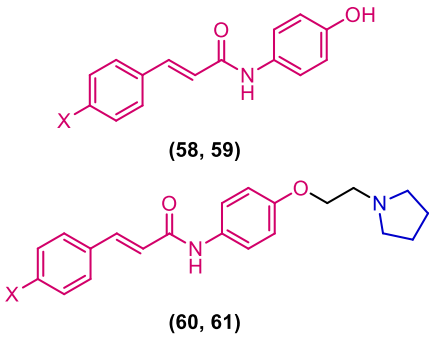
Liu Q. *et. al.* reported a series of novel chromone-2-carboxamide derivatives for the treatment of AD.[91] All the reported compounds exhibited good AChE inhibitory activity and displayed high selectivity for AChE over BuChE.



(57)

Compd	*Rat*AChE IC_{50} (μM)	*Rat*BuChE IC_{50} (μM)	ORAC	% Inhibition of Aβ aggregation	
				Self-induced	Cu^{2+}-induced
57	0.07 ± 0.01	51.50 ± 1.87	0.83	59.2 ± 1.6 at 25 μM conc.	48.3 ± 1.7 at 25 μM conc.

Among the reported chromane-2-carboxaminde derivatives, compound (**57**) showed significant AChE inhibitory activity (IC_{50} value of 0.07 µM). It also showed excellent self-induced and Cu^{2+}-induced Aβ aggregation inhibition (59.2 and 48.3 % inhibition, respectively). Additionally, it was also endowed with good metal chelation potential and moderate antioxidant activity.

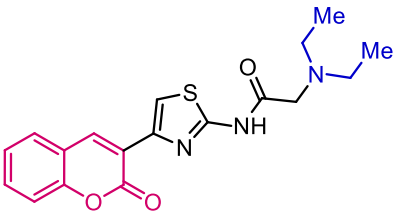


(58, 59)

(60, 61)

Compd	X	AChE IC_{50} (µM)	BuChE IC_{50} (µM)
58	-F	> 500	> 500
59	-Cl	> 500	> 500
60	-F	1.11 ± 0.08	51.7 ± 3.26
61	-Cl	1.40 ± 0.11	45.6 ± 3.10

Gao X. H. *et. al.* reported a novel series of halo-substituted cinnamic acid derivatives having tertiary amine side chain as ChEIs for the treatment of AD.[92] Compounds containing tertiary amine side chain displayed moderate to good AChE inhibition. Compounds with alicyclic amines like pyrrolidine and piperidine in the side chain exhibited better AChE inhibitory activity than the compounds with aliphatic amines like diethylamine in the side chain. The halogen group present in the structure had a notable effect on selectivity as well as on inhibitory activity on AChE. Compounds having para-substituted chlorine or fluorine showed better AChE inhibition. Among all the synthesized compounds, compound (**60**) exhibited the best selective AChE inhibitory activity (IC_{50} value of 1.11 µM, SI value of 46.58). It is interesting to note that the parent compounds (**58** and **59**) without the tertiary amine moiety in their structures exhibited poor inhibitory activity against AChE.

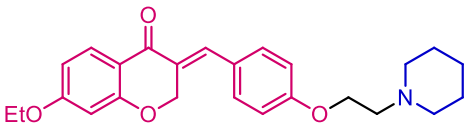


(62)

Compd	AChE IC_{50} (nM)	BuChE IC_{50} (µM)
62	43 ± 02	178.50 ± 0.03

Sonmez F. *et. al.* reported a novel series of coumarin-thiazole derivatives as anti-AD agents.[93] Amongst them, compound (**62**) showed excellent selective AChE inhibitory activity (IC_{50} value of 43 nM, SI value of

4151.16). Kinetic analysis of AChE inhibition suggested that the compound (**62**) was a mixed-type AChE inhibitor. Additionally, it did not show any toxicity in the cell-line toxicity assay.



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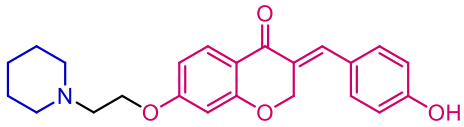
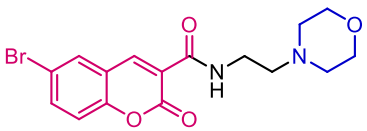


(64)

Compd	AChE IC_{50} (µM)	BuChE IC_{50} (µM)
63	0.122	--
64	1.18 ± 0.09	5.6 ± 0.4

Shamsimeymandi R. *et. al.* reported novel chroman-4-one derivatives having cyclic amine side chains in their structures as ChEIs.[94] Among the synthesized compounds, compound (**64**) having piperidinyl moiety in structure conferred good AChE (IC_{50} value of 1.18 µM) and BuChE (IC_{50} value of 5.6 µM) inhibitory activities. SAR study confirmed that the substitution of electron-donating groups on the aryl ring attached to the benzylidene moiety showed more ChE inhibition in comparison to the compounds having electron-withdrawing groups. Molecular modeling studies of the compound (**64**) showed notable interactions of the compound within the active sites of ChEs that supported the results obtained during the *in vitro* study.



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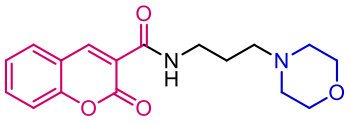

(66)

Compd	AChE IC_{50} (µM)	BuChE IC_{50} (µM)
65	6.21 ± 0.03	12.09 ± 0.02
66	8.26 ± 0.03	7.65 ± 0.01

Tehrani M. *et. al.* reported a series of coumarin-3-carboxamide derivatives as ChEIs.[95] Most of the hybrids possessed good AChE inhibitory and BuChE inhibitory activities. Amongst them, compound (**65**) (AChE: IC_{50} value of 6.21 µM, BuChE: IC_{50} value of 12.09 µM) and compound (**66**) (AChE: IC_{50} value of 8.26 µM, BuChE: IC_{50} value of 7.65 µM) exhibited the most potent ChE inhibitory activities in the reported series.

In conclusion, a better understanding of the etiology of the AD, and biological activities associated with the above-discussed compounds could guide us in designing of some novel MTDLs as anti-AD agents. This thesis work contains designing, synthesis and biological evaluation of some novel MTDLs as anti-AD agents as detailed in **Chapter-3** Aims and Objectives, **Chapter-4** Results and Discussion and **Chapter-5** Experimental sections.