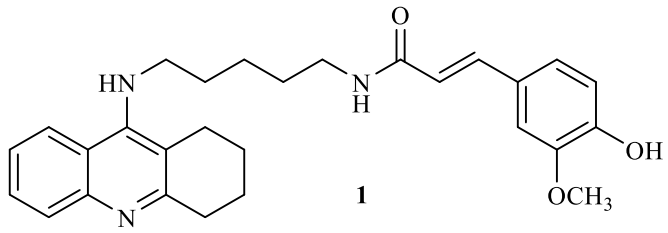
## 2.1 Ferulic acid as a multitargeted pharmacophore

Ferulic acid (4-Hydroxy-3-methoxy cinnamic acid) is an abundant antioxidant reported to be beneficial in the prevention and/or treatment of various disorder linked to oxidative stress such as AD, Diabetes, Cancer cardiovascular and atherosclerosis [Higuchi M. 2014].

Pi et al. have reported a hybrid compound by linking ferulic acid to tacrine as multifunctional agents **1.** The results of the *in-vitro* study suggested that compound having anti-cholinesterase and AChE induced Aβ aggregation inhibition potential. Additionally, the compound showed neuroprotection ability against $A\beta_{1-40}$ induced toxicity on the PC-12 neuroblastoma cell line. The *in-vivo* results revealed that the compound significantly enhanced cognitive function evaluated through the Morris water maze test. Further, the compound also showed Antioxidant property in the *ex-vivo* experiment indicated by an increased level of CAT and SOD while AChE and MDA levels decreased in the brain [Pi et al. 2012].

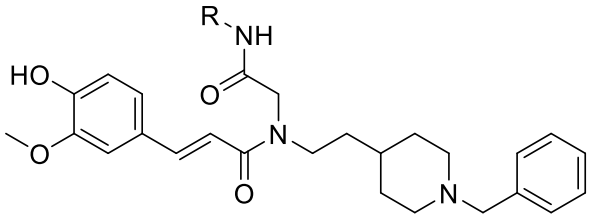


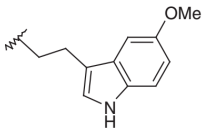
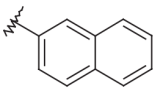

**1**

| | 100 μM | 50μM |
|---|---|---|
| AChE induced Aβ inhibition | 50.27% | 20.23% |

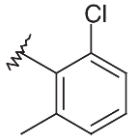
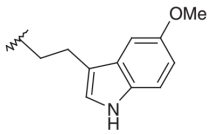
Benchekroun et al. have reported a series of ferulic acid and donepezil hybrid, having ChEs inhibitory potential with antioxidant property. Among the synthesized analogs, compounds **3–6** exhibited inhibition against *ee*AChE in nanomolar concentration slightly lower activity than donepezil, while compounds **2–6** showed considerably higher inhibition of against *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].



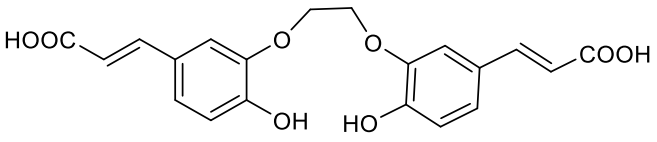
R =   Bn

| | **2** | **3** | **4** |
|---|---|---|---|
| *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 =  

| | **5** | **6** |
|---|---|---|
| *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 |

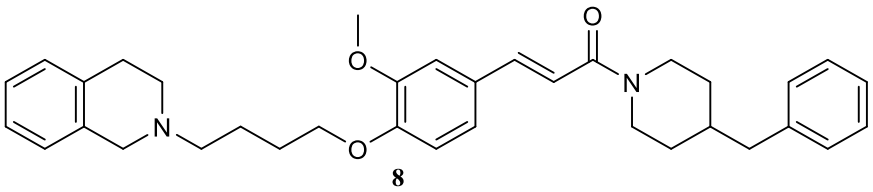
Jung et al. have reported a dimeric derivative of ferulic acid **7** showed amelioration of cognitive dysfunction attenuated the $A\beta_{1-42}$-induced memory impairment in mice and APP/PS1 mutant transgenic mice model evaluated in passive avoidance, novel arm entry, and Y maze test**.** The compound also found anti-inflammatory properties in the *ex-vivo* experiment [Jung et al. 2016].



**7**

Sang et al. have designed sixteen novel ferulic acid based hybrids with different alkyl amine as multi-targeted ligands. The most active compound of the series (*E*)-1-(4-

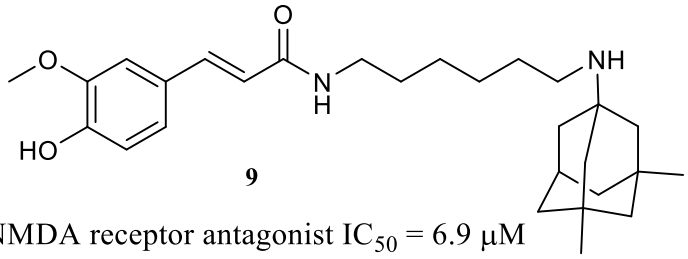
benzylpiperidin-1-yl)-3-(4-(4-(3,4-dihydroisoquinolin-2(1H)-yl)butoxy)-3-methoxyphenyl)prop-2-en-1-one **8** showed selective BChE inhibition ($IC_{50}$ value of 0.021 mM for equine serum BChE, 8.63 mM for rat BChE and 0.07 mM for human serum BChE), and impressive AChE inhibition ($IC_{50}$ 2.13 mM for electric eel AChE, 1.8 mM for rat AChE and 3.82 mM) for human erythrocytes AChE. The compound has Aβ anti-aggregation (50.8 ± 0.82%) and disaggregation (38.7 ± 0.65%) properties along with moderate antioxidant (0.55 eq of Trolox), neuroprotective ability and significant BBB permeability. Further, the *in-vivo* evaluation suggested that the compound did not show acute toxicity and reverse the scopolamine induce cognitive dysfunction in mice [Sang et al. 2017].



**8**

equine serum BChE $IC_{50}$ = 0.021 μM ee AChE $IC_{50}$ =2.13 μM
ratBChE $IC_{50}$ = 8.63 μM ratAChE $IC_{50}$ = 1.8 μM
human serum BChE $IC_{50}$ = 0.07μM h erythrocytes AChE $IC_{50}$ =3.82 μM

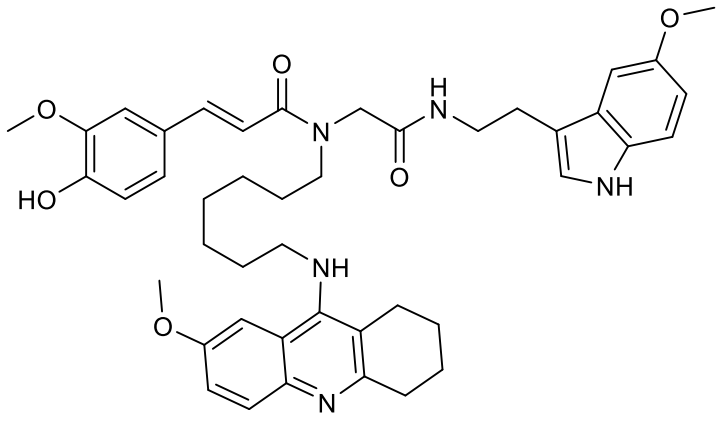
Rosini et al. have developed a series of ferulic acid hybrids conjugate with memantine. These two pharmacophores are attached to a variable chain of carbon(1-6). Most potent derivative **9** (-N-(6-(((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)amino)hexyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide) antagonised the over activation of NMDA receptor ($IC_{50}$= 6.9 μM). Results of the *in-vitro* study suggested that the compound. I have devoid of toxicity on SHSY-5Y and H4SW at a dose of 20 mM, significant antioxidant properties at the concentration of 10 mM and showed neuroprotection against $H_2O_2$ insult. Compound limits the Aβ production by stimulating APP processing in favor of α-secretase pathway [Rosini et al. 2019].



**9**

NMDA receptor antagonist $IC_{50}$ = 6.9 μM

Benchekroun et al. have investigated a series of ferulic acid derivative conjugated with two pharmacophores, melatonin, and modified tacrines. The hybrid **10** possessed significant cholinesterase inhibition $IC_{50}$ (hAChE) = 1290 ± 70 nM; $IC_{50}$ (hBChE) = 234 ± 8 nM, CNS permeability and strong antioxidant property. Besides, the compound showed non-hepatotoxic at (100, 300, and 1000 μM), and neuroprotective ability at (1 μM) against lethal insults induced by $H_2O_2$ (300 μM), $A\beta_{1-40}$ and $A\beta_{1-42}$ (30 μM) dose [Benchekroun et al. 2016].

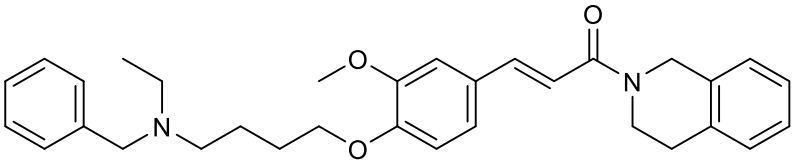


**10**

*h*AChE $IC_{50}$ = 1290 nM,
*h*BChE $IC_{50}$ = 234 nM.

Sang et al. have reported a series of novel multitargeted ferulic acid derivatives. The most potent compound **11** elicited selective BChE inhibition ($IC_{50}$ = 8.9 nM), as well as MAO-A ($IC_{50}$ = 6.3) and MAO-B inhibitor (8.6 μM) respectively. Further, the *in-vitro* assessment showed Aβ aggregation inhibition potential (53.9%) and disaggregation potential (43.8%), free radical scavenging property (ORAC = 0.52 equiv), and neuroprotection ability against toxicity mediated through $A\beta_{1-42}$ with significant BBB

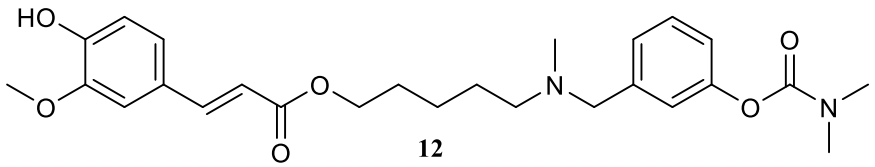
permeability. In the behavioral experiment results of passive avoidance, the test indicated that this compound reversed the scopolamine-induced cognitive dysfunction [Sang et al. 2018].



**11**

BChE $IC_{50}$ = 8.9 nM
MAO-A $IC_{50}$ = 6.3 μM, MAO-B $IC_{50}$ = 8.6 μM
% Aβ inhibition: = 53.9% and disaggregation = 43.8%

In a recent study, Lan et al. have designed novel ferulic acid derivatives for the treatment of AD. The most active analogs of this series **12,** (-5-((3-((dimethylcarbamoyl)oxy)benzyl)(methyl)amino)pentyl-3-(4-hydroxy-3-methoxyphenyl)acrylate, exhibited ChE inhibition ($IC_{50}$, 19.7 nM for hAChE and 0.66 μM for hBChE), and self-induced Aβ aggregation inhibition potential (49.2% at 20 μM). Further, compound showed good antioxidant (1.26 trolox equiv), and remarkable neuroprotection ability shown on PC 12 neuroblastoma cell line against $H_2O_2$ and Aβ-induced toxicity. The compound showed good interaction with CAS and PAS site of AChE. These results corroborated with *in-silico* experiments. [Jin-Shuai Lan 2020].



**12**

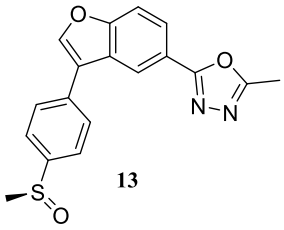
hAChE $IC_{50}$ = 19.7 nM, hBChE $IC_{50}$ = 0.66 mM
% Ab inhibition: Self- = 49.2%
Antioxidant ORAC = 1.26 Trolox equivalant

### 2.2. 1,3,4-Oxadiazoles as a multitargeted pharmacophore

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

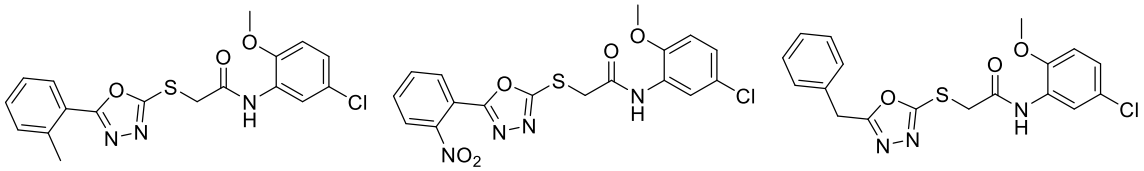
target enzymes. Moreover, several studies suggested 1,3,4-oxadiazole be a suitable pharmacophore acting against multiple targets involved in AD.

Saitoh et al. have reported a potential inhibitor **13,** of GSK-3β and CDK-5. Compound **13** also inhibited tau phosphorylation, APP metabolism, and ameliorated cognitive dysfunction in 3xTg-AD mice [Onishi et al. 2011, Saitoh et al. 2009].



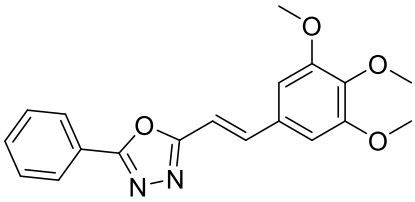
**13**

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, **14** and **15** exhibited AChE inhibition activity with $IC_{50}$ values of 34.61 and 40.21 µM, respectively, while compound **16** showed maximum BChE inhibition ($IC_{50}$ = 33.31 µM). Compound **15** exhibited considerable inhibition of LOX (69.67%) [Rehman et al. 2013].



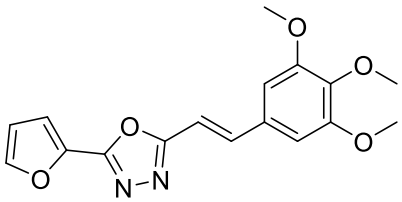
**14** **15** **16**

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 **17, 18, 19,** and **20** 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 compounds showed good interaction at PAS and CAS residues of the AChE enzyme [Kamal et al. 2014].



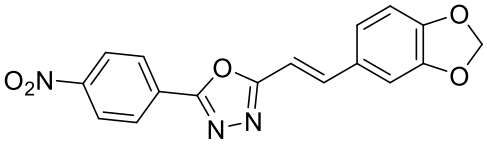
**17**

AChE $IC_{50}$ = 24.89 µM



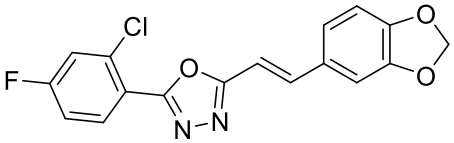
**18**

AChE $IC_{50}$ = 13.72 µM



**19**

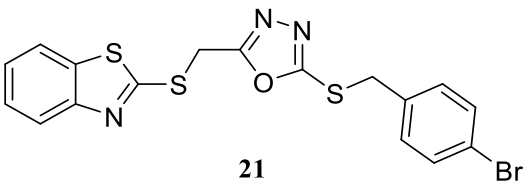
AChE $IC_{50}$ = 37.65 µM



**20**

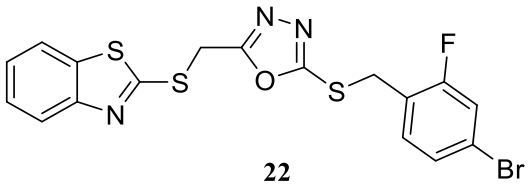
AChE $IC_{50}$ = 19.63 µM

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 **21** exhibited significantly higher neuroprotective activity, whereas compounds **22–24** showed slightly moderate activity [Mei et al. 2017].



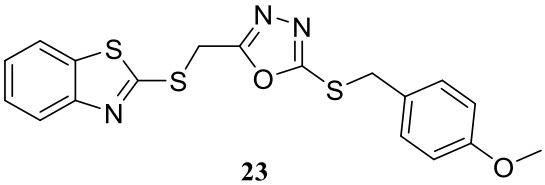
**21**

% cell viability at 10 µM = 95.7%



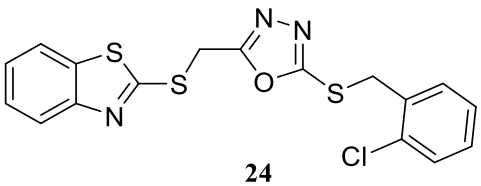
**22**

% cell viability at 10 µM = 89.1%



**23**

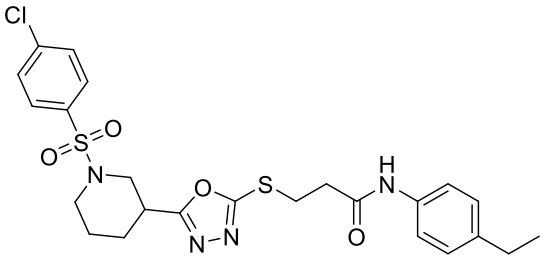
% cell viability at 10 µM = 87.7%



**24**

% cell viability at 10 µM = 87.7%

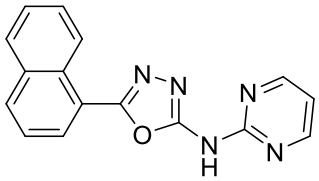
Rehman et al. have synthesized a series of 3-piperidinyl-1,3,4-oxadiazoles and evaluated their AChE inhibitory potential. The results showed compound **25** exhibited moderate AChE inhibitory activity with $IC_{50}$ value of 3.64 µM [Rehman et al. 2018].



**25**

AChE $IC_{50}$ = 3.64 μM

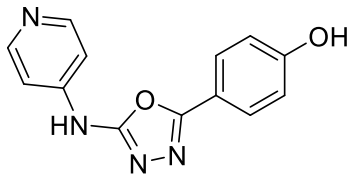
Tripathi et al. have designed and synthesized novel hybrids bearing a 2-aminopyrimidine moiety linked to substituted 1,3,4-oxadiazoles, and investigated AChE and Aβ aggregation inhibitory potential. Among the synthesized fifteen compounds, **26** exhibited significant hAChE ($pIC_{50}$ = 6.52, Ki = 0.17 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. 2019].



**26**

AChE $pIC_{50}$ = 6.52, Ki = 0.17 mM

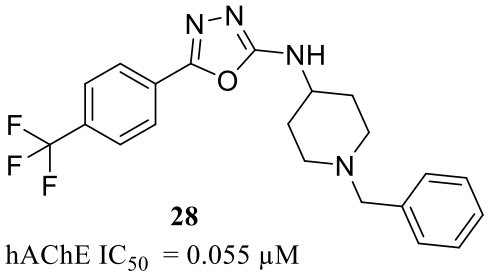
Mishra et al. have designed and synthesized fifteen molecular hybrids of 4-aminopyridine and substituted 1,3,4-oxadiazoles as potential AChE and Aβ aggregation inhibitors. The compound **27** was found to be the most potent inhibitor of AChE ($IC_{50}$ = 1.098 μM) with a non-competitive mechanism. Also, **27** 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 revealed considerable antioxidant activity of compound **27** [Mishra et al. 2019].



**27**

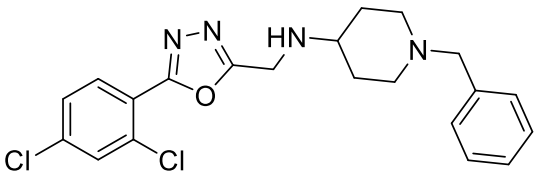
AChE $IC_{50}$ = 1.098 mM, Ki = 0.960 mM
Ab aggregation inhibition = 38.2-65.9%

In a recent experiment, Sharma et al. have designed, synthesized and biologically evaluated molecular hybrids of *N*-benzylpiperidine and 1,3,4-oxadiazole as multifunctional agents to treat AD. Among the synthesized thirty two compounds, **28** and **29** exhibited balanced inhibition of hAChE ($IC_{50}$, **28** = 0.055 µM; **29** = 0.086 µM), hBChE ($IC_{50}$, **28** = 0.186 µM; **29** = 0.143µM), BACE-1 ($IC_{50}$, **28** = 0.146 µM; **29** = 0.114 µM), and AChE-induced Aβ aggregation (NFI, **28** = 17–56%; **29** = 16–38%). 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 [Sharma et al. 2019].



**28**

hAChE $IC_{50}$ = 0.055 µM
hBChE $IC_{50}$ = 0.186 µM
hBACE-1 $IC_{50}$ = 0.146 µM
AChE-induced Aβ inhibition = 17-56%



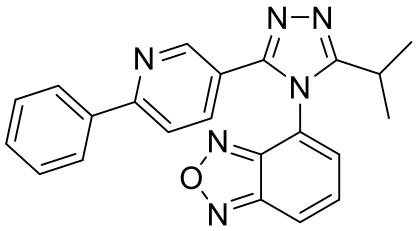
**29**

hAChE $IC_{50}$ = 0.086 µM
hBChE $IC_{50}$ = 0.143 µM
hBACE-1 $IC_{50}$ = 0.114 µM
AChE-induced Aβ inhibition = 16-38%

### 2.3 Pyridyl ring as a multitargeted pharmacophore

Harada et al. have reported a Compound **30,** (4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazole) novel glycine transporter-1 inhibitor, potently

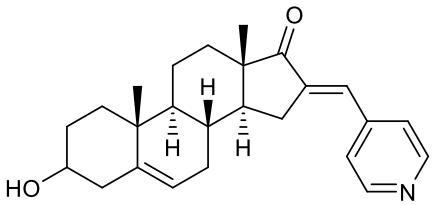
inhibited rat GlyT1 ($IC_{50}$=92 nM) with appreciable CNS permeability. Also, compound **30** (0.1–3 mg/kg, p.o.) enhanced scopolamine-induced working memory deficit evaluated on mice in the Y-maze experiment and Morris water maze experiment performed to assessed improvement in spatial learning deficit in the old aged rat at (0.1 mg/kg, p.o.) [Harada et al. 2012].



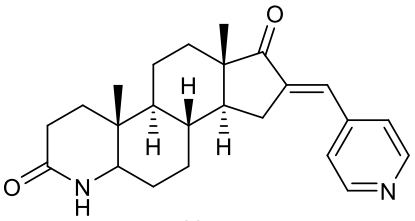
**30**

GlyT1 $IC_{50}$ = 92nM

Singh et al. have reported a series of 16-Arylideno Steroids conjugate with pyridine derivatives for the treatment of AD. Compounds **31** and **32** showed prominent leads among all the derivative and exhibited reversed the LPS induced learning and memory deficit which was determined by Morris water maze and elevated maze test. Compounds **31** and **32** at 5mg/kg showed significant anti-inflammatory activity in compound with celecoxib (20 mg/kg) and quite better than dexamethasone (5 mg/kg). The *ex-vivo* experiment results suggested considerable suppression of acetylcholinesterase, nitrative stress and LPS-induced oxidative stress level in brain homogenates [Singh and Bansal 2017].



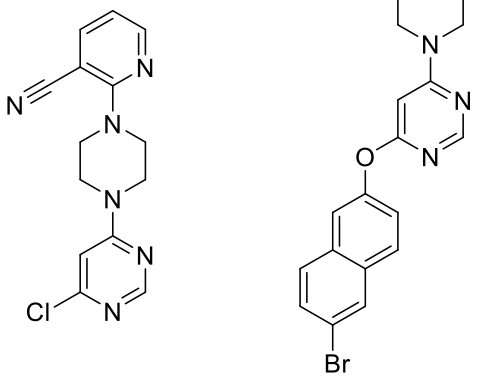
**31**



**32**

| | | |
|---|---|---|
| μmol of AchE/min. mg of protein = | 0.0063 | 0.0059 |
| mol of MDA/mg of protein = | 6.3 | 6.1 |
| nitrite concn(μg/mL) = | 595.8 | 578.5 |
| μmol of GSH/mg of protein = | 0.033 | 0.035 |
| SOD units/mg of protein = | 5.9 | 6.2 |
| catalase activity/min = | 4.9 | 5.1 |

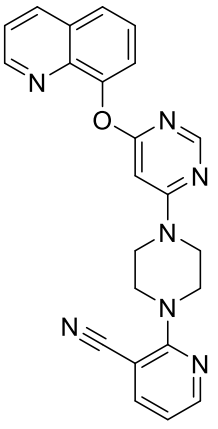
Kumar et al. have reported the synthesis and screening of pyrimidine analogs in combination with a triazolopyrimidine scaffold where compounds **33, 34** and **35** derived from synthon. The research described that 2-(4-(6-(quinolin-8-yloxy)pyrimidin-4-yl)piperazin-1-yl)nicotinonitrile **35** displayed the maximum inhibitory activity comparable to standard drugs tacrine and donepezil. In the report, *in-silico* and enzyme kinetic study revealed that the compound has a bivalent-binding mode of mechanism [Kumar et al. 2018].



**33**
*ee*AChE $IC_{50}$ = 0.67 μM

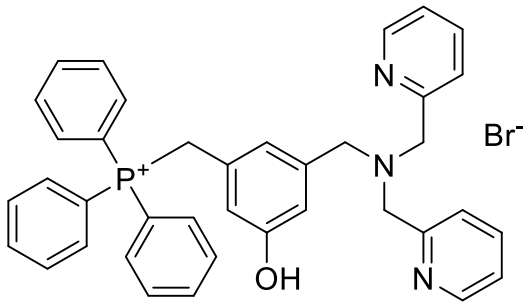


**34**
0.161 μM



**35**
0.036 μM

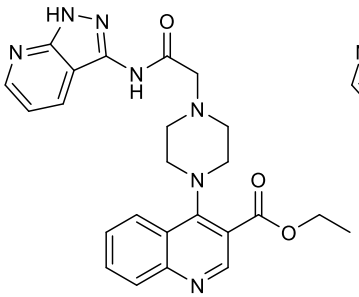
Zhu et al. have reported pyridine amine derivative,3-bis(pyridin-2ylmethyl)aminomethyl-5-hydroxybenzyltriphenylphosphonium bromide **36** as a potential inhibitor of AChE, self and metal-induced Aβ aggregation with little neurotoxicity. In addition, i*n-vivo* experiment on transgenic *C. elegans* showed down regulation of mRNA levels with reduced AChE activity and ROS level. Gene screening analysis suggested that **36** upregulated mRNA of hsp-16.2, hsp-60, and hsf-1gene, against the accumulation of abnormal protein. Moreover, the results of the Morris water maze test (APP/PS1 mice model) signified that the compound could enhance learning and memory [Zhu et al. 2019].




**36**

Self-induced Aβ aggregation inhibition $IC_{50}$ = 12.53 μM
Metal-induced Aβ aggregation inhibition $IC_{50}$ = 27.42 μM

Umar et al. have developed 1H-pyrazolo[3,4-b]pyridine derivatives as anti-AD. The most potential compounds **37** and **38,** demonstrated the selective anti AChE, with little BChE inhibition property. Besides, Compound **39** showed the inhibition against Aβ aggregation (self and metal-induced) up to 81.65% along with antioxidant, and metal chelation property [Umar et al. 2019].



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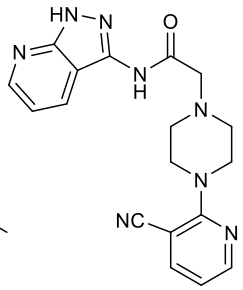
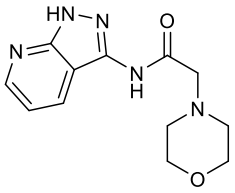


38

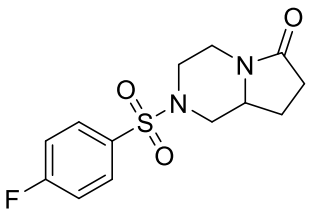


39

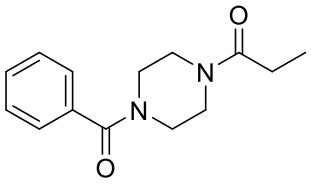
| | | | |
|---|---|---|---|
| *ee*AChE $IC_{50}$ = | >100 µM | 0.0048 µM | 0.0049 µM |
| *eq*BChE $IC_{50}$ = | >100 µM | >100 µM | >100 µM |
| Antioxidant = | 0.274µM Trolox/µM | 0.650µM Trolox/µM | 0.362µM Trolox/µM |
| % Aβ inhibition= | 81.64 % | 50.60 % | 50.84% |

## 2.4 Piperazines as a multitargeted pharmacophore

Manetti et al. have reported the synthesis of 4-substituted-1-acyl piperazine compounds by chemical modification of 4-substituted 1,4-diazabicyclo[4.3.0] nonan-9-ones, where compounds **40** and **41** had uplifted the concentration of ACh in rat cerebral cortex. The compound **41** (0.001 mg/kg; sc) was elicited better nootropic activity without motor incoordination. The studies reflected that piperazine derivatives to be a new class of nootropic agents [Manetti et al. 2000].

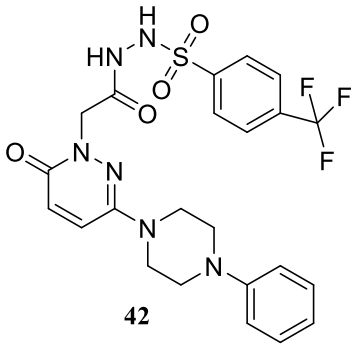


40



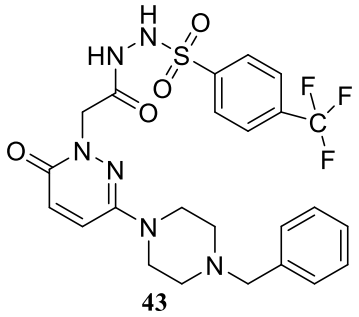
41

Önkol et al. have reported *N*'-[(4-aryl)sulfonyl]-2-[4-(aryl) piperazine]-3(2H)-pyridazinone-2-ylacetohydrazide derivatives as potential ChEIs. The compounds **42, 43** and **45** exhibited AChE inhibitory activities, whereas compounds **44** showed less inhibitory activity. The results reflected that compounds bearing -$CF_3$ on the 4$^{th}$ position of phenylsulfonyl ring had increased AChE inhibitory activity [Önkol et al. 2013].



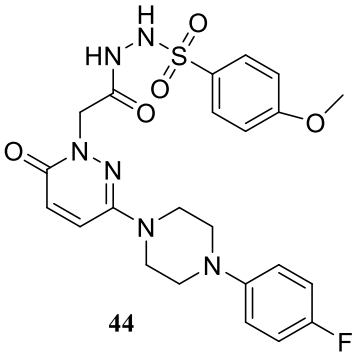
**42**

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



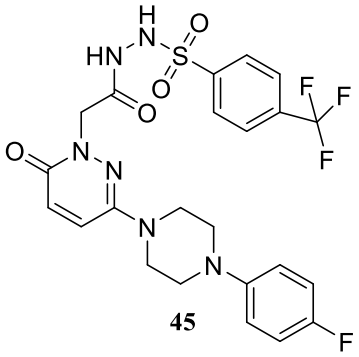
**43**

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



**44**

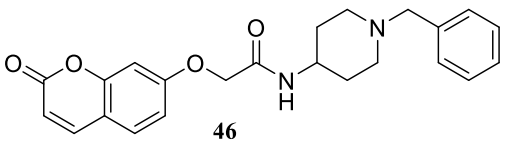
*ee*AChE $IC_{50}$ = 17.87 (0.05mM)



**45**

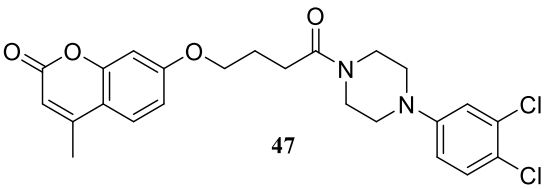
*ee*AChE $IC_{50}$ = 84.07 (0.05mM)

Alipour et al. have reported the design and synthesis of a group of 7-hydroxycoumarin analogs tethered with piperazine or piperidine through amide linker and evaluated for ChE inhibitory potential. Among the reported compounds (**46-49**), **46** exhibited the maximum inhibition and selectivity for AChE. The compound **46** also elicited antioxidant and neuroprotective activities. The docking study revealed the significant interactions of **46** with CAS of AChE [Alipour et al. 2014].



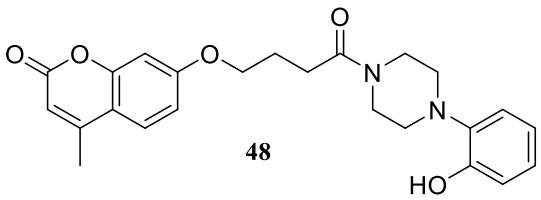
**46**

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



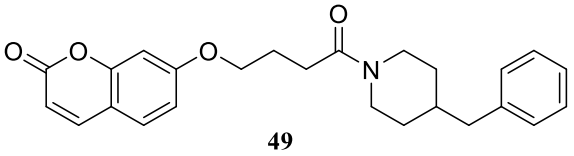
**47**

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



48

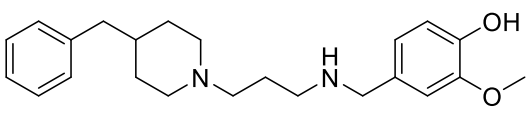
*ee*AChE $IC_{50}$ = 4.38 μM
*eq*BChE $IC_{50}$ = 250 μM



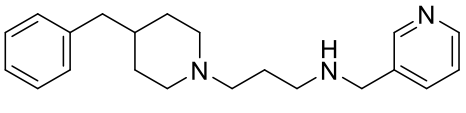
49

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

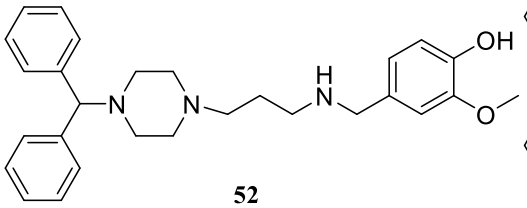
Meena et al. have reported the synthesis of a novel series of piperidine and piperazine derivatives and developed as potential ChEIs with anti-Aβ aggregation and radical scavenging activities. The compounds **50-53** displayed significant inhibition of AChE, self-induced Aβ aggregation, with potent oxygen radical absorbance capacity compared to standard Trolox [Meena et al. 2015].



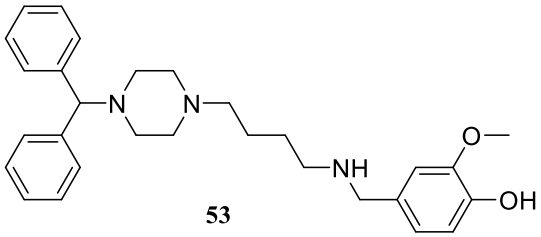
50



51



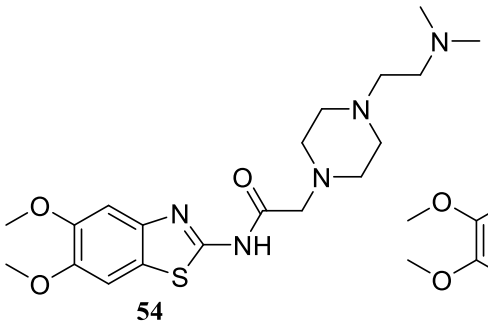
52



53

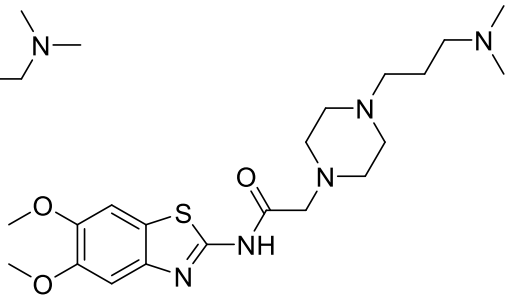
| | 50 | 51 | 52 | 53 |
|---|---|---|---|---|
| *ee*AChE $IC_{50}$ | = 11.53 nM | 2.13 nM | 9.79 nM | 11.44 nM |
| *eq*BChE $IC_{50}$ | =3.87 nM | 81.70 nM | 18.95 nM | 4.17 nM |
| Antioxidant | = 4.45μM Trolox/μM, | 1.18μM Trolox/μM, | 2.68μM Trolox/μM, | 2.78μM Trolox/μM |
| % Ab inhibition | = 71.35 % | 88.81% | 54.16% | 42.18% |

Benzothiazole–piperazine derivatives were reported by Özkay et al. as potential AChEIs. The piperidine moiety was bioisosterically replaced with piperazine, where compounds **54** and **55** showed potential inhibition on AChE. The *in-silico* study also reported a favorable interaction of compounds **54** and **55** with AChE [Özkay et al. 2016].



**54**

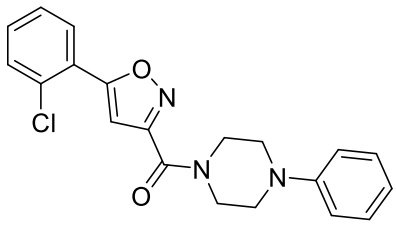
*ee*AChE $IC_{50}$ = 0.0426mM



**55**

*ee*AChE $IC_{50}$ = 0.0576mM

In a recent investigation, the design and synthesis of arylisoxazole-phenylpiperazines were reported by Saeedi et al. and investigated them as potential ChEIs. The compound **56** showed the highest inhibition of AChE and BACE-1 with neuroprotective activity. *In-silico* study suggested active site interactions with CAS and PAS residues of AChE by compound **56** [Saeedi et al. 2019].



**56**

AChE $IC_{50}$ = 21.85 mM

BChE $IC_{50}$ = 76.76 mM