2. Literature Review

The use of multitarget-directed ligands (MTDLs) in the treatment of complex, multifactorial disorders such as AD is becoming more recognized by medicinal chemists worldwide. By fusing two pharmacophores with distinct biological activity on separate targets, a hybrid structure or molecule known as MTDLs is created. This hybrid technique has been utilized to build a wide range of novel drugs.

The creation of MTDLs is acknowledged as one of the most promising drug discovery strategies to treat AD-like conditions with a complicated etiology.[1,2] Even with great affinity and selectivity for their targets, drugs that only operate on one target may not have a satisfactory impact on the enigmatic etiology of the disease. When compared to a molecule that is just targeted, an MTDL with balanced but modest affinities for the targets can nonetheless have a greater positive impact. MTDLs are better at changing the intricate balance of the cellular network because they have simultaneous impacts on several therapeutic targets.[3,4]

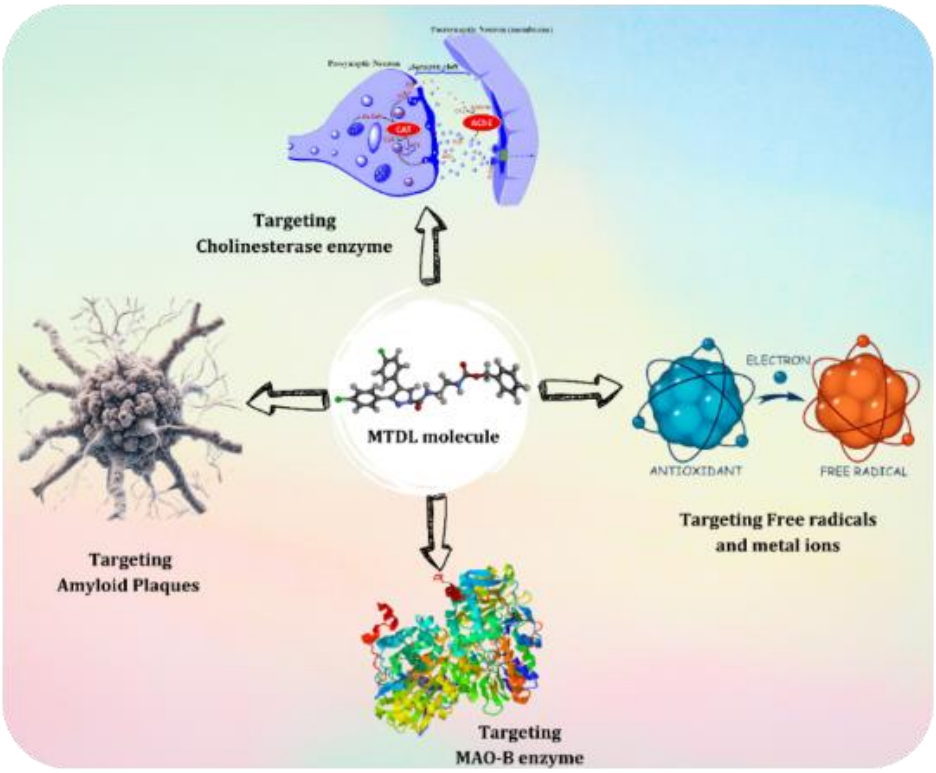


Fig 2.1: MTDL molecule possessing effects on multiple targets of AD.

The majority of the MTDLs that have been reported for the treatment of AD are cholinesterase inhibitors that have affinity for both dual binding sites catalytic active site (CAS) and peripheral anionic site (PAS), in addition to some other potential or property like

metal chelating, antioxidant, Aβ aggregation inhibition, β-site APP cleaving enzyme (BACE-1), and monoamine oxidase-B (MAO-B) (**Fig 2.1)**. The majority of these MTDLs were created by altering the structure of the available AChE inhibitors.[2]

Several newly created MTDLs with various scaffolds including pyrazole, benzyl piperidine, carbamate, benzofuran, vicinal diaryl and urea have been discussed in the following section.

2.1. Pyrazole scaffold-based anti-AD agents

2.1.1. Pyrazole as cholinesterase inhibitor

Gopinath Gutti *et al.*[5] using *de novo* drug design approach, new 3,5-diarylpyrazoles and spiropyrazoline derivatives were reported as acetyl cholinesterase inhibitors. Compounds (**6)** and (**7)** among them produced favourable outcomes. Compound (**6)** showed IC_{50} values of 1.937 ± 0.066 µM against AChE and 1.166 ± 0.088 µM against BuChE, respectively. Similarly, compound **(7)** showed IC_{50} values were 0.464 ± 0.166 µM and 0.754 ± 0.121 µM, respectively. Propidium iodide was greatly displaced from PAS-AChE by both of the compounds. At 50 µM, it exhibited 90% cell viability, and at 20 µM, there was a successful reduction in metal-induced $A\beta_{1-42}$ aggregation. According to *in vivo* investigations, compounds (**6)** and (**7**) were safer for brain tissues, dramatically reduced the generation of Aβ, and did not modify locomotor activity.

Compound	AChE IC_{50}	BuChE IC_{50}
6	1.937 ± 0.066 µM	1.166 ± 0.088 µM
7	0.464 ± 0.166 µM	0.754 ± 0.121 µM

Multi-target directed edaravone-*N*-benzylpyridinium hybrid compound was designed and synthesized by Zondagh *et al.*[6]. Of the synthesized derivatives, compounds **8** showed the most promise as MTDL candidates for AD, exhibiting remarkable antioxidant and selective

AChE inhibitory activities (IC_{50} values of X=F 1.9 µM and X=Cl 1.2 µM, respectively). Compounds were also predicted to have a promising BBB permeability through in silico prediction.

Compound 8	AChE IC_{50}
8a-F	1.9 µM
8b-Cl	1.2 µM

Omania *et al.*,[7] reported a series of pyrazolopyridine scaffold as MTDL to treat AD. Compound (**9**) showed remarkable activity as *h*AChE inhibitors with IC_{50} values of 0.16 µM and *h*BuChE IC_{50} values 0.17 µM which is eight and two-fold more active than the reference compound rivastigmine, respectively. Compound (**9**) GSK3β inhibition with IC_{50} value of 26 µM compared to L807mts. Compound (**9**) also showed 66.0 % tau protein aggregation inhibitors; and $A\beta_{1-42}$ self-aggregation inhibitors with 79.0% respectively. Furthermore, compound (**9**) proved to have the ability to chelate bio-metals such as Fe^{2+}, Cu^{2+}, and Zn^{2+} preventing their oxidative damage in the brain of AD patients.

Compound	*h*AChE IC_{50}	*h*BuChE IC_{50}	GSK3β IC_{50}	Tau protein aggregation	$A\beta_{1-42}$ self-aggregation
9	0.16 µM	0.17 µM	26 µM	66.0 %	79.0%

Siti Munirah Mohd Faudzi *et al.*[8] created and assessed novel series of diarylheptanoids' pyrazole, phenylpyrazole, and pyrazoline analogues both computationally and physiologically for the purpose of treating AD by inhibiting cholinesterase. Due to its pyrazole scaffold, compound (**10)** (IC_{50} 0.11 ± 3.7 µM) proved to be suited for the selective and efficient inhibition of BChE. Furthermore, compound (**10)** exhibited action against AChE, with an IC_{50} value of 0.3 ± 0.0 µM.

Compound	AChE IC_{50}	BuChE IC_{50}
10	0.3 ± 0.0 µM	0.11 ± 3.7 µM

Tarana Umar *et al.*[9] examined pyrazolo[3,4-*b*]pyridine derivatives having potential to treat AD. Aβ aggregation inhibitors 2-(piperazin-1-yl)-*N*-(1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide and potent and selective AChE inhibitors were designed and developed by them. The substance potential to prevent Cu (II)-mediated and self-mediated Aβ aggregation, as well as their inhibitory action on AChE and BuChE were evaluated. To show that 4-substituted piperazine-pyrazolo-pyridin-3-yl-acetamide analogues have the potential to be multi-target drug ligands with antioxidant properties, the study evaluated the analogues' efficacy against important targets related to Alzheimer's disease, such as AChE inhibition and Aβ aggregation/disaggregation, *in vitro*. With IC_{50} values of 4.8 nM and 4.9 nM, respectively, compound (**11)** and (**12)** were determined to have the highest AChE inhibition activity and the best selectivity for AChE inhibition. Compound (**13)** exhibited a remarkable 79.47% activity against the suppression of Aβ aggregation. Further research on compound (**13)** is warranted due to its potential as a lead molecule for MTDL treatment. Compound (**13)** showed IC_{50} value for AChE was 0.045 ± 0.010 µM.

Compound	AChE IC_{50}	Aβ aggregation inhibition
11	4.8 nM	-
12	4.9 nM	-
13	45 nM	79.47%

Using the Cu(OTf)2 catalyst, Parham Taslimi *et al.*[10] synthesized compounds that were subsequently recognized as AChE and BuChE enzymes inhibitors, with Ki values ranging from 60.17 to 91.27 for AChE and BuChE inhibition. These compounds were synthesized as 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives. Examining the inhibition data for the cholinesterase enzymes, compound (1**4)** was the best inhibitor, with IC_{50} values of 68.25 ± 13.84, 60.17 ± 9.47 nM and Ki values of 94.37 and 98.25 for AChE and BuChE respectively.

Compound	AChE IC_{50}	BuChE IC_{50}
14	68.25 ± 13.84 nM Ki 94.37	60.17 ± 9.47 nM Ki 98.25

2.1.2. Pyrazole as amyloid inhibitor

Nguyen Quoc Thai *et al.*[11] synthesized chemical CID 9998128 compound (**15**), a tiny molecule. The substance showed a promising multitarget activity for AD through *in vitro* and *in silico* studies. The compound was found binding strongly to both $A\beta_{42}$ fibrils and β-secretase, with the van der Waals interaction outweighing the electrostatic interaction in binding affinity. The results demonstrated that all of the complexes under study were unstable due in large part to the compound's indazole moiety. With IC_{50} and DC_{50} values of 42.6 µM and 22.7 µM, respectively, compound **(15)** has shown *in vitro* trials to block Aβ42's amyloid fibrillization and to be capable of eliminating $A\beta_{42}$ fibrils. With an EC_{50} value of 15 µM, the chemical demonstrated a dose-dependent reduction in the activity of the β-site amyloid precursor protein cleaving enzyme (BACE-1).

Compound	$A\beta_{42}$ IC_{50}	$A\beta_{42}$ DC_{50}	BACE-1 EC_{50}
15	42.6 µM	22.7 µM	15 µM

Pedro Gonzalez-Naranjo *et al.*[12] reported a new class of multitargeting 5-substituted indazole compounds that function as BACE1 and cholinesterase inhibitors. In the fight against AD, the therapeutic potential of multitarget medications was quite encouraging. AChE/BuChE and BACE1 enzyme *in vitro* inhibitory assays were included in the pharmacological assessment. Furthermore, Oxygen Radical Absorbance Capacity (ORAC) tests was used to assess the antioxidant properties. Furthermore, studies were conducted to examine the anti-inflammatory characteristics of Raw 264.7 cells and the neuroprotective effects on human neuroblastoma SH-SY5Y cells. The results showed that some of these 5-substituted indazole derivatives, compounds **(16)** and **(17)**, concurrently inhibit BACE1 and AChE/BuChE. The compounds with the highest potencies against BuChE were compounds **(16)** and **(17)**, with 0.17 ± 0.05 µM and 0.57 ± 0.2 µM, respectively. However, with IC_{50} values of 2.1 ± 0.2 µM and 1.9 ± 0.1 µM, respectively, these compounds were found to be most effective against BACE1. Additionally, a few indazole compounds demonstrated

antioxidant and neuroprotective qualities against Aβ-induced cell death in human neuroblastoma SH-SY5Y cells.

Compound	BuChE IC_{50}	BACE-1 IC_{50}
16	0.17 ± 0.05 µM	2.1 ± 0.2 µM
17	0.57 ± 0.2 µM	1.9 ± 0.1 µM

Khambete *et al.*[13] reported novel compounds containing the *N*-acetylpyrazoline moiety to inhibit Aβ aggregation. Five compounds containing pyrazoline were created and designed, and compound (**18)** demonstrated a significant inhibition of Aβ42 aggregation at a concentration of 20µM, with a 76% inhibition, respectively. Further, two more series of compounds with or without an ethylene linker, were synthesized and assessed. Compound (**19)** and (**20)** had the highest percentage of Aβ aggregation, with 84.6 and 84.1 % inhibition, respectively.

Compound	Aβ aggregation inhibition (%)
18	76%
19	84.6%
20	84.1%

2.1.3. Pyrazole as MAO inhibitor

Badr Jismy *et al.*[14] repported new MAO inhibitors from derivatives of pyrimido[1,2-*b*]indazoles. A number of compounds including derivatives of pyrimidoindazole were produced and subsequently subjected to biological and *in silico* evaluation. Compound (**21)** showed the highest potency against the MAO-B target among the reported series of compounds, with an IC_{50} value of 0.130 ± 0.008 µM. It also showed minimal toxicity and neuroprotective properties in a cell-based Parkinson's disease model.

Compound	MAO-B IC_{50}
21	0.130 ± 0.008 µM
22	0.063 ± 0.0042 µM

Nair *et al*[15] investigated the inhibitory action of MAO-A and B for compounds of halogenated pyrazolines. With an inhibitory concentration of IC_{50} 0.063 ± 0.0042 µM, 3-(4-ethoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole, compound (**22)** had the best efficacy against MAO-B among the reported series of compounds. According to the Selectivity Index (SI), compound (**22)** was found to be the most selective in relation to MAO-B. Additionally, it is reported as a competitive and reversible inhibitor of MAO-B.

Compound	*h*MAO-A IC_{50}	*h*MAO-B IC_{50}
23	3.63 ± 0.50 µM	0.38 ± 0.06 µM
24	3.03 ± 0.60 µM	0.44 ± 0.028 µM

Paolo Guglielmi *et al.*[16] synthesized a modest library of new *N*-acetyl/*N*-thiocarbamoylpyrazoline derivatives in order to create novel MAO inhibitors. Compounds (**23)** and (**24**) from the synthesized compounds exhibited a strong and noteworthy inhibition of *h*MAO-A and *h*MAO-B, with IC_{50} values of 3.63 ± 0.50 µM, 3.03 ± 0.60 µM, 0.38 ± 0.06 µM, and 0.44 ± 0.028 µM respectively.

Umut Salgin-Goksen *et al.*[17] reported 1-[2-(5-substituted-2-benzoxazolinone-3-yl)acetyl]-3,5-disubstitutedphenyl-2-pyrazoline and N′-(1,3-disubstitutedphenylallylidene)-containing compounds skeleton for the inhibition of MAO enzyme (2-(5-substituted-2-benzoxazolinone-3-yl)). Compounds (**25)** and **(26)** exhibited the best selectivity and inhibition against MAO-A, according to an *in vitro* assessment, with IC_{50} values of **(25)** 0.009 µM and **(26)** 0.001 µM, respectively.

Compound	*h*MAO-A IC_{50}
25	0.009 µM
26	0.001 µM

2.2. Benzyl piperidine scaffold based anti-AD agents

It is well known that already marketed drug Donepezil **(1)** which is one of the most commonly prescribed molecule in treatment of AD contains two pharmacophores, a 4,5-dimethoxyindanone ring which is attached to a benzyl piperidine ring with the help of a methyl spacer. Benzyl piperidine is responsible for binding at the CAS site of the enzyme thereby inhibiting the esterase enzyme. Therefore, a thorough literature was conducted on benzyl piperidine and its activities on various targets of AD which is described as follows.

Rochais *et al.*[18] announced the use of a few donecopride analogs as MTDLs to treat AD. A range of nanomolar IC_{50} values was observed for all the compounds in the series, indicating potential ChE inhibitory action. In comparison to the reference donepezil (IC_{50} value of 6 nM), compound (**27)** showed the most effective and selective AChE inhibition in the series, with an IC_{50} value of 8.5 nM.

Novel *N*-benzylpiperidine-indole hybrids with a carboxamide linker were reported by Wang *et al.*[19] Compound (**28)**, which has a spacer of two carbons, demonstrated adequate inhibition of AChE and BuChE. It also showed good antioxidant activity (3.28 Trolox equivalent by ORAC test), with an average suppression of Aβ aggregation of 56.3% at 20 mM. Additionally, compound (**28)** chelated metal ions were able to pass across the blood-brain barrier and reduced the oxidative stress-induced mortality of PC12 cells. A mixed-type inhibitor that binds to both the CAS and PAS of AChE concurrently is represented by the molecular docking studies of compound (**28)**. Based on the propargylamine's indole derivative and the donepezil's benzylpiperidine part, Bautrisa-Aguilera *et al.*[20] reported a variety of multi-target compounds. *N*-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl) methyl)prop-2-yn-1-amine (**29)** was found to be the most effective inhibitor among reported series.

Compound	AChE IC_{50}	BuChE IC_{50}	MAO-A IC_{50}	MAO-B IC_{50}
28	273 nM	73 nM	-	-
29	190 nM	830 nM	5.5 nM	56 nM

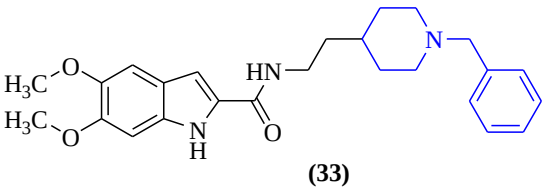
Tripathi *et al.*[21] linked *N*-benzylpiperidine and 5-phenyl-1,3,4-oxadiazole hybrids with -NH and -$NHCH_2$ linkers to generate multi-target hybrids. This allowed them to produce unique donepezil hybrids. 1,3,4-Oxadiazole increased the binding affinity toward the PAS, it was found that this led to the *N*-benzylpiperidine moiety being extended deeper into the CAS of AChE and BuChE, producing effective dual inhibitors of ChE. *h*AChE, *h*BuChE, and β-secretase-1 (*h*BACE-1) were all significantly inhibited by compounds (**30)** and (**31)** out of those studied.

Compound	*h*AChE IC_{50}	*h*BACE-1	BACE-1
30	0.086 µM	0.143 µM	0.114 µM
31	0.055 µM	0.186 µM	0.146 µM

`Donepezil hybrids compound (**32)** was reported by Wu *et al.* [22,23]. In theoretical pharmacokinetic study, compound **(32)** showed appropriate druglikeness features and moderate inhibition against MAOs and ChEs (rMAO-A, IC_{50} 6.2 µM; rMAO-B, IC_{50} 10.2 µM; *ee*AChE, IC_{50} 1.8 µM; *eq*BuChE, IC_{50} 1.6 µM). High affinity for chelating copper and zinc ions as well as modest in vitro antioxidant capabilities were demonstrated by hybrids (**32)**.

Compound	*ee*AChE IC_{50}	*ee*BuChE	*hr*MAO-A	*hr*MAO-B
32	1.8 µM	1.6 µM	6.2 µM	10.2 µM

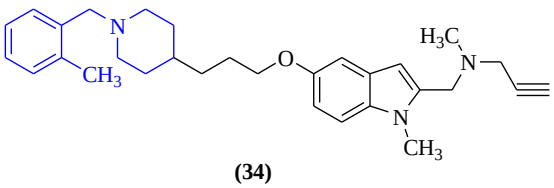
Razia *et al.*,[24] reported indole-piperidine derivatives as MTDLs for AD. The 5,6-dimethoxy-indole *N*-(2-(1-benzylpiperidine) carboxamide compound **(33)** was found to inhibit *h*AChE and *h*BACE-1 with IC_{50} values of 0.32 and 0.39 µM, respectively having a mixed-type inhibition with K_i values of 0.26 µM and 0.46 µM, respectively. The PAMPA-BBB assay, demonstrated CNS permeability of compound (**33)**, thus making the compound suitable for further investigation.



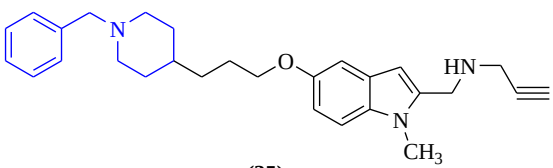
(33)

Compound	*ee*AChE IC_{50}	*eq*BuChE	*h*AChE	*h*BACE-1
33	0.52 µM	19.16 µM	0.32 µM	0.39 µM

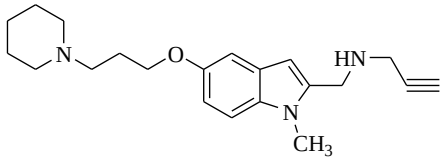
Samadi *et al.*[25,26] along with Bautista *et al.*[20,27] reported compound (**34)** as a strong, effective, and irreversible MAOI in the nanomolar range (*h*MAO-A, IC_{50} 6.3 nM; *h*MAO-B, IC_{50} 183.6 nM). It was also BBB permeable and showed inhibitory action of ChEs which was moderate and nearly equipotent (*h*AChE, IC_{50} 2.8 µM; *h*BuChE, IC_{50} 4.9 µM). Every kind of linker had benefits in terms of selectivity and inhibitory efficacy when it comes to inhibiting MAOs. Compound (**35)** was the most powerful (IC_{50} 5.5 nM, SI = 27 versus *h*MAO-B) and moderately potent (IC_{50} 190 nM) inhibitor of *ee*AChE and *eq*BuChE, respectively, and *h*MAO-A selective inhibitor (IC_{50} 830 nM) in this series. Pyridine or naphthyridine substitution for the indole core dramatically reduced the inhibition of MAOs but increased that of ChEs. Furthermore, compound (**36)** demonstrated modest potency over MAOs but efficient and selective *ee*AChE (IC_{50} 16 nM) inhibitory actions. The pharmacophore of histamine receptor subtype 3 (H3R) antagonists was then fitted to the ASS234 and consisted of an alkyl spacer, a tertiary basic amine, and an indolylpropargylamine moiety. With good *in vitro* pharmacological characteristics (*h*MAO-A, IC_{50} 145 nM; *h*MAO-B, IC_{50} 78 nM; *h*AChE, IC_{50} 0.53 µM; *h*BuChE, IC_{50} 1.69 µM; hH3R: Ki 10.80 nM), (**36)** is a BBB permeable antioxidant and neuroprotectant. Comparing contilisant (standard antioxidant drug for AD) to ASS234, the latter showed lower hydrophilicity and better *in vitro* radical scavenging capabilities (ORAC 3.74 TE). Furthermore, contilisant outperformed donepezil and ASS234 in the *in vivo* $A\beta_{1\text{-}42}$ oligomers caused cognitive impairment test on the Y-maze and radial arm-maze.



(34)



(35)



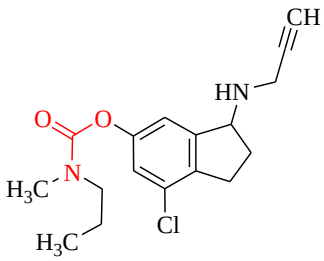
(36)

Compound	AChE IC_{50}	BuChE	MAO-A	MAO-B
34	2.8 µM	4.9 µM	6.3 µM	183.6 nM
35	190 nM	830 nM	5.5 nM	150 nM
36	0.53 µM	1.69 µM	0.14 µM	10.80 nM

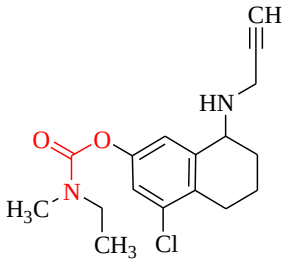
2.3. Carbamate moiety-based anti-AD agents

A medication from the AChE inhibitor family, Rivastigmine **(2)** was authorized for clinical use in the treatment of AD in 2000. It is a pseudo-irreversible ChEI that inhibits BuChE in addition to having less selectivity for AChE than donepezil. Numerous cholinesterase inhibitors based on rivastigmine that contain the carbamate group of rivastigmine as their active pharmacophore have been documented in the literature mentioned as follows:

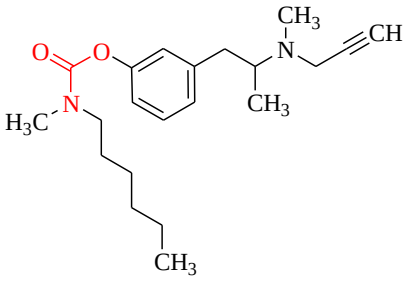
Sterling *et al.* [28] created and synthesized many new *N*-propargylphenethylamines and N-propargylaminoindans that have a carbamoyl group replaced. Actually, rivastigmine-rasagiline/selegiline hybrids **(37–39)** with strong MAO and ChE inhibitory action were used to generate these compounds. It's interesting to note that all four of these hybrids (IC_{50} values 43.9, 52.4, and 3.06 nM, respectively) showed strong AChE inhibition.



(37)



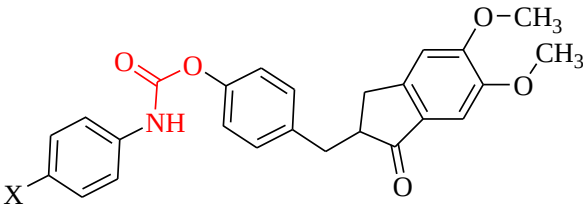
(38)



(39)

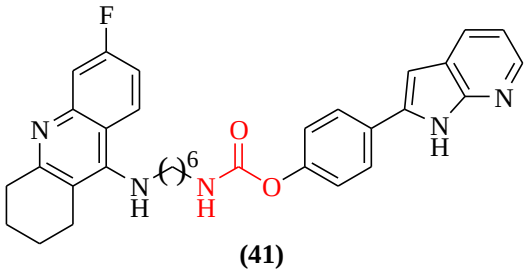
Compound	AChE IC_{50}
37	43.9 nM
38	52.4 nM
39	3.06 nM

Mohammad Shahrivar Gargari *et al*,[29] used the pharmacophore-based design technique to synthesize a novel series of indanone–carbamate hybrid as AChE and BuChE inhibitors. The compound **(40)** produced potent AChE inhibitory activity which had an IC_{50} value of 3.04 µM. Furthermore, compound **(40)** was also found to be potent inhibitor of $A\beta_{1-40}$ aggregation.



(40)

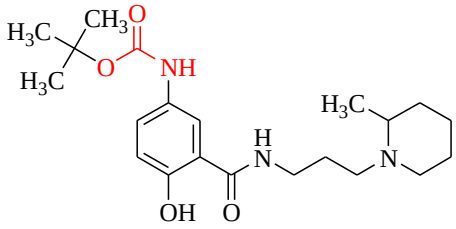
Liu *et al.,*[30] developed a series of tacrine-sibiriline based carbamate derivatives and evaluated as potential AD agents. Compound (**41**) exhibited potent inhibitory activities against *h*AChE IC_{50} 30.35 ± 2.07 nM and *h*BuChE IC_{50} 48.03 ± 6.41 nM with minimal neurotoxicity. Further investigations demonstrated compound (**41**) to exhibit a remarkable capacity to safeguard PC12 cells against H_2O_2-induced apoptosis and effectively suppressed the production of reactive oxygen species (ROS). It also decreased the levels cytokines in an inflammation model of BV2 cells induced by lipopolysaccharide (LPS). In the scopolamine-induced AD mouse model, compound (**41**) (20 mg/kg) significantly reduced ChEs activity in the brain of the mice.



(41)

Compound	*h*AChE IC_{50}	*h*BuChE IC_{50}
41	30.35 ± 2.07 nM	48.03 ± 6.41 nM

Lopez *et al,*[31] reported that compound **(42)** can inhibit AChE and β-secretase, inhibiting the aggregation of Aβ and production of fibrils (fAβ) from $Aβ_{1-42}$. Compound **(42)** demonstrated an IC_{50} value of 15.4 nM for β-secretase-1 inhibition and a Ki value of 0.17 µM for acetylcholinesterase inhibition. Compound **(42)** showed capacity to suppress amyloidogenesis in an *in vivo* model following scopolamine treatment. The findings indicated that, in astrocytes, compound **(42)** had a somewhat protective effect against $Aβ_{1-42}$ because it reduced the levels of free radicals and TNF-α in cell cultures.



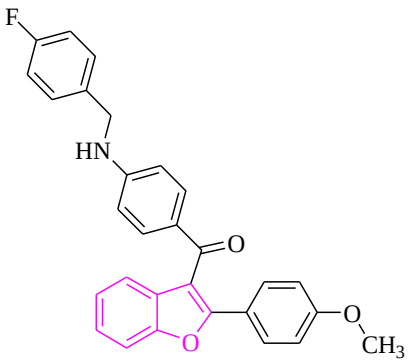
(42)

Compound	BACE-1	AChE Ki
42	15.4 nM	0.17 µM

2.4. Benzofuran moiety-based anti-AD agents

One common alkaloid used to treat AD is galantamine **(3).** The Food and Drug Administration (FDA) authorized it in 2001 to treat Alzheimer's disease. AChE is competitively and reversibly inhibited by galantamine.[32] Galantamine has an antioxidant action in addition to its anti-AChE activity, which allows it to be used as a multi-target strategy against the development and progression of AD.[33] Reports on various galantamine-based cholinesterase inhibitors including the benzofuran group, is described below:

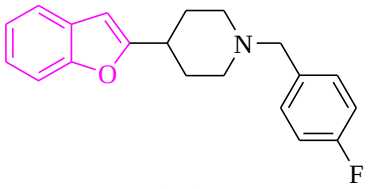
Montanari S. *et al*,[34] synthesized 2-arylbenzofuran derivatives and evaluated these derivative towards cholinesterases and cannabinoid receptors. Compound (**43)** showed best inhibitory activity against cholinesterases and cannabinoid receptors. Compound (**43)** showed butyrylcholinesterase IC_{50} value 13.4 ± 0.5 µM. Compound (**43)** also evaluated in human SH-SY5Y neuroblastoma cells using the MTT assay showed the neuroprotective activity.



(43)

Compound	BuChE IC_{50}
43	13.4 ± 0.5 µM

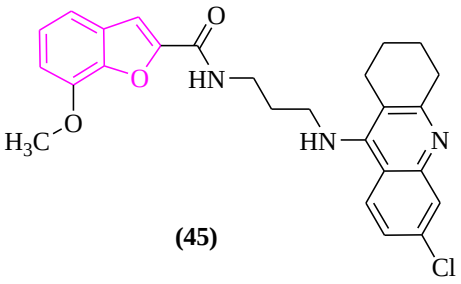
Chowdhury *et al,*[35] designed, synthesized and evaluated a series of benzofuran piperidine derivatives as multifunctional Aβ antiaggregant to treat AD. Among them compound **(44)** showed $A\beta_{25\text{-}35}$ aggregation inhibition 57.71% and *h*AChE IC_{50} value 21 mM. In addition, compound **(44)** stops the production of intracellular ROS and the cell death that $A\beta_{25\text{-}35}$ peptides cause in SH-SY5Y cells. Compound **(44)** showed 80.8% neuroprotective and 10.81 ± 1.08% antioxidant activity. Based on all data, compound **(44)** was concluded as potent multifunctional Aβ antiaggregant, useful candidate for the treatment of AD.



(44)

Compound	*h*AChE IC_{50}	$A\beta_{25\text{-}35}$ inhibition
44	13.4 ± 0.5 µM	57.71%

Fancellu, G. *et al,* [36] developed a novel range of hybrids by connecting the well-known AChE inhibitor tacrine to derivatives of benzofurans. Compound **(45)** demonstrated AChE IC_{50} value 0.13 µM and Aβ aggregation inhibition 57.8%. The benzofuran framework aims to endow the conjugate molecules with ability for inhibition of AChE and amyloid-beta peptide aggregation, in addition to providing metal (Fe, Cu) chelating ability and concomitant anti-oxidant activity among the new tacrine-benzofuran derivatives.

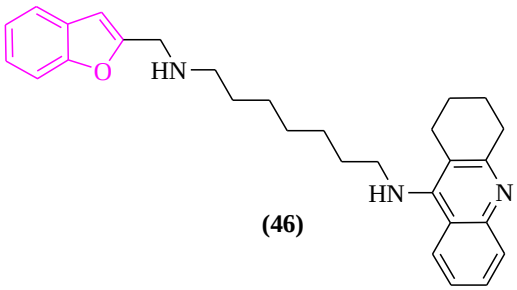


(45)

Compound	AChE IC_{50}	Aβ inhibition
45	0.13 µM	57.8%

Zha, X. *et al,*[37] assessed novel tacrine-benzofuran hybrids to be used in AD therapy. The majority of the hybrids between tacrine and benzofuran had strong inhibitory effects on AChE and β-secretase-1. Compound **(46)** among the 26 hybrids had sub nanomolar selective inhibitory activity against *h*AChE, with an IC_{50} value of 0.86 nM, and against human β-

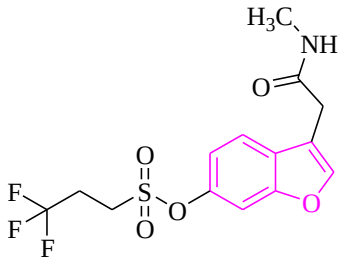
secretase-1 (hBACE-1), with an IC_{50} value of 1.35 µM. Compound **(46)** functioned as a sluggish, tight-binding, mixed-type inhibitor, according to kinetic tests. Research conducted *in vivo* verified that compound **(46)** did not demonstrate noteworthy hepatotoxicity.



(46)

Compound	AChE IC_{50}	*h*BACE-1
46	0.86 nM	1.35 µM

Pisani *et al.*,[38] reported novel family of compounds with a 6′-substituted *(E)*-2-(benzofuran-3(2*H*)-ylidene)-*N*-alkylacetamide skeleton as monoamine oxidase inhibitors. Outstanding affinities to MAO-A/B were demonstrated by 6′-sulfonyloxy derivatives. The related 6′-benzyloxy compounds (**47)** demonstrated significant MAO inhibition and reversed selectivity profile. By utilizing docking simulations and targeted structural alterations, important molecular factors contributing to the high affinity for both MAO isoforms were found.



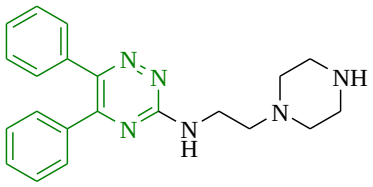
(47)

Compound	MAO-A	MAO-B
47	9.1 ± 0.9 nM	17 ± 2.4 nM

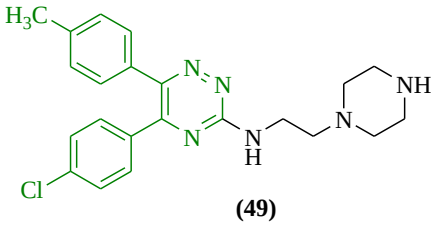
2.5. Vicinal diaryl scaffold-based anti-AD agents

Accordingly, Sinha *et al.*[39] reported a unique series of 2,4-triazine, 5,6-diaryl-1 derivatives and assessed each compound's anticholinesterase action. Using the Ellman approach, *in vitro* tests were conducted to assess the impact of ChE inhibition. Compounds (**48-49)** were proved to be potent inhibitors of AChE and BuChE. When compared to

BuChE, these compounds showed superior efficacy and selectivity towards AChE. Compound (**48)** (IC_{50} AChE 4.23 µM and IC_{50} BuChE 13.3 µM), compound **49** (IC_{50} AChE 5.79 µM and IC_{50} BuChE 163.4 µM). Moreover, compounds (**49)** and (**50)** in the active cite of AChE and hBuChE were shown to have a very good binding activity by molecular docking experiments.



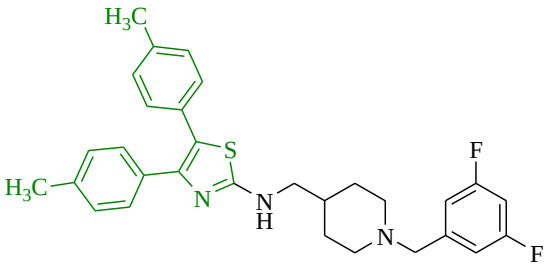
(48)



(49)

Compound	AChE IC_{50}	BuChE IC_{50}
48	4.23 µM	13.3 µM
49	5.79 µM	163.4 µM

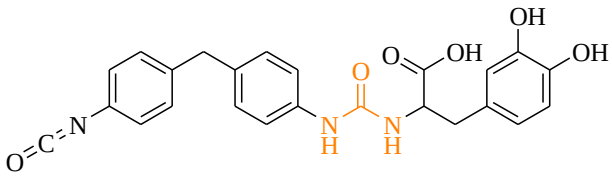
In order to create a new class of multitarget-directed ligands that may be used to treat Alzheimer's disease (AD), Shidore *et al.*[40] the pharmacophoric characteristics of diarylthiazole and the cholinesterase inhibitor donepezil were combined. Among the compounds, compound (**50)** had the greatest activity IC_{50} value of 0.30 ± 0.01 µM for AChE and 1.84 ± 0.03 µM for BuChE, indicating considerable *in vitro* anticholinesterase (anti-ChE) activity. In the experiments on enzyme kinetics, compound (**50)** exhibited mixed inhibition of AChE. A few substances had discernible antioxidant and antiapoptotic qualities *in vitro*, along with moderate to strong suppression of AChE-induced $A\beta_{1-42}$ aggregation. Significant *in vivo* anti-ChE and antioxidant effects were demonstrated by compound (**50)**. Moreover, compound (**50)** showed *in vivo* neuroprotection by reducing aberrant levels of $A\beta_{1-42}$, p-Tau, cleaved caspase-3, and cleaved PARP proteins, hence reducing $A\beta_{1-42's}$ toxicity.



(50)

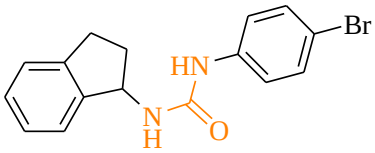
Compound	AChE IC_{50}	BuChE IC_{50}
50	0.30± 0.01 µM	1.84 ± 0.03 µM

2.6. Urea derivative-based anti-AD agents



(51)

A novel class of urea/thiourea derivatives were effectively reported via the reaction of selective isocyanates/isothiocyanates with L-3-hydroxytyrosine. By using the 1,1-diphenyl1-2-picrylhydrazyl (DPPH) radical test and the ferric reducing antioxidant power assay, all synthesized compounds were evaluated for their antioxidant activity. Additionally, their molecular docking interaction patterns were examined against the enzymatic proteins 1N8Q and 3NRZ. These studies have also revealed that the compounds may be bound to the amino acid residues ASP490 and ASP361 in chain A of the 1N8Q protein and GLN1194 in chain L of the 3NRZ protein, where they may be responsible for potential antioxidant activity. Promising antioxidant activity showed by urea derivative compound (**51)**. [41]



(52)

Lachhi *et al.* [42] reported a number of 1-(2,3-dihydro-1*H*-indan-1-yl)-3-arylurea/thiourea derivatives via using 2,3-dihydro-1*H*-inden-1-amine with different aryl isocyanates/isothiocyanates. Following an *in-vitro* screening process to assess their antioxidant activity against NO and DPPH free radicals, compound (**52)** was identified as a potent antioxidant. The recorded *in silico* binding affinities showed to have a strong association with the *in vitro* antioxidant findings when the acquired *in vitro* results were compared with the results of the molecular docking, ADMET, QSAR, and bioactivity studies conducted for them. The strong hydrogen bonding connections between produced ligands and the ARG 160 residue of the protein tyrosine kinase (2HCK) enzyme were shown by molecular docking research, and this contact effectively inhibits the enzyme.

2.7. References

(1) Agis-Torres, A.; Sollhuber, M.; Fernandez, M.; Sanchez-Montero, J. Multi-Target-Directed Ligands and Other Therapeutic Strategies in the Search of a Real Solution for Alzheimer's Disease. *Curr. Neuropharmacol.* **2014**, *12* (1), 2–36.

(2) Oset-Gasque, M. J.; Marco-Contelles, J. Alzheimer's Disease, the "One-Molecule, One-Target" Paradigm, and the Multitarget Directed Ligand Approach. *ACS Chem. Neurosci.* **2018**, *9* (3), 401–403.

(3) Csermely, P.; Agoston, V.; Pongor, S. The Efficiency of Multi-Target Drugs: The Network Approach Might Help Drug Design. *Trends Pharmacol. Sci.* **2005**, *26* (4), 178–182.

(4) Richard Morphy, J. The Challenges of Multi-Target Lead Optimization. In *Designing Multi-Target Drugs*; The Royal Society of Chemistry, **2012**; pp 141–154.

(5) Gutti, G.; Kumar, D.; Paliwal, P.; Ganeshpurkar, A.; Lahre, K.; Kumar, A.; Krishnamurthy, S.; Singh, S. K. Development of Pyrazole and Spiropyrazoline Analogs as Multifunctional Agents for Treatment of Alzheimer's Disease. *Bioorg. Chem.* **2019**, *90*, 103080.

(6) Zondagh, L. S.; Malan, S. F.; Joubert, J. Design, Synthesis and Biological Evaluation of Edaravone Derivatives Bearing the N-Benzyl Pyridinium Moiety as Multifunctional Anti-Alzheimer's Agents. *J. Enzyme Inhib. Med. Chem.* **2020**, *35* (1), 1596–1605.

(7) Waly, O. M.; El-Sayed, S. M.; Ghaly, M. A.; El-Subbagh, H. I. Multi-Targeted Anti-Alzheimer's Agents: Synthesis, Biological Evaluation, and Molecular Modeling Study of Some Pyrazolopyridine Hybrids. *Eur. J. Med. Chem.* **2023**, *262*, 115880.

(8) Mohd Faudzi, S. M.; Leong, S. W.; Auwal, F. A.; Abas, F.; Wai, L. K.; Ahmad, S.; Tham, C. L.; Shaari, K.; Lajis, N. H.; Yamin, B. M. In Silico Studies, Nitric Oxide, and Cholinesterases Inhibition Activities of Pyrazole and Pyrazoline Analogs of Diarylpentanoids. *Arch. Pharm. (Weinheim).* **2021**, *354* (1), 1–13.

(9) Umar, T.; Shalini, S.; Raza, M. K.; Gusain, S.; Kumar, J.; Seth, P.; Tiwari, M.; Hoda, N. A Multifunctional Therapeutic Approach: Synthesis, Biological Evaluation, Crystal Structure and Molecular Docking of Diversified 1H-Pyrazolo[3,4-b]Pyridine Derivatives against Alzheimer's Disease. *Eur. J. Med. Chem.* **2019**, *175*, 2–19.

(10) Taslimi, P.; Turhan, K.; Türkan, F.; Sedef Karaman, H.; Turgut, Z.; Gulcin, İ. Cholinesterases, α-Glycosidase, and Carbonic Anhydrase Inhibition Properties of 1H-Pyrazolo[1,2-b]Phthalazine-5,10-Dione Derivatives: Synthetic Analogues for the Treatment of Alzheimer's Disease and Diabetes Mellitus. *Bioorg. Chem.* **2020**, *97* (February), 1–7.

(11) Thai, N. Q.; Bednarikova, Z.; Gancar, M.; Linh, H. Q.; Hu, C.-K.; Li, M. S.; Gazova, Z. Compound CID 9998128 Is a Potential Multitarget Drug for Alzheimer's Disease.

ACS Chem. Neurosci. **2018**, *9* (11), 2588–2598.

(12) González-Naranjo, P.; Pérez, C.; González-Sánchez, M.; Gironda-Martínez, A.; Ulzurrun, E.; Bartolomé, F.; Rubio-Fernández, M.; Martin-Requero, A.; Campillo, N. E.; Páez, J. A. Multitarget Drugs as Potential Therapeutic Agents for Alzheimer's Disease. A New Family of 5-Substituted Indazole Derivatives as Cholinergic and BACE1 Inhibitors. *J. Enzyme Inhib. Med. Chem.* **2022**, *37* (1), 2348–2356.

(13) Khambete, M. P.; Khare, L. P.; Kapadia, A. B.; Degani, M. S. Exploring the Potential of Pyrazoline Containing Molecules as Aβ Aggregation Inhibitors in Alzheimer's Disease. *Drug Metab. Pers. Ther.* **2020**, *35* (3), 20190031.

(14) Jismy, B.; El Qami, A.; Pišlar, A.; Kos, J.; Gobec, S.; Knez, D.; Abarbri, M. Pyrimido [1, 2-b] Indazole Derivatives: Selective Inhibitors of Human Monoamine Oxidase B with Neuroprotective Activity. *Eur. J. Med. Chem.* **2021**, *209*, 112911.

(15) Nair, A. S.; Oh, J. M.; Koyiparambath, V. P.; Kumar, S.; Sudevan, S. T.; Soremekun, O.; Soliman, M. E.; Khames, A.; Abdelgawad, M. A.; Pappachen, L. K.; Mathew, B.; Kim, H. Development of Halogenated Pyrazolines as Selective Monoamine Oxidase-B Inhibitors: Deciphering via Molecular Dynamics Approach. *Molecules* **2021**, *26* (11), 1–17.

(16) Guglielmi, P.; Carradori, S.; Poli, G.; Secci, D.; Cirilli, R.; Rotondi, G.; Chimenti, P.; Petzer, A.; Petzer, J. P. Design, Synthesis, Docking Studies and Monoamine Oxidase Inhibition of a Small Library of 1-Acetyl- and 1-Thiocarbamoyl-3,5-Diphenyl-4,5-Dihydro-(1h)-Pyrazoles. *Molecules* **2019**, *24* (3), 1–21.

(17) Salgin-Goksen, U.; Telli, G.; Erikci, A.; Dedecengiz, E.; Tel, B. C.; Kaynak, F. B.; Yelekci, K.; Ucar, G.; Gokhan-Kelekci, N. New 2-Pyrazoline and Hydrazone Derivatives as Potent and Selective Monoamine Oxidase A Inhibitors. *J. Med. Chem.* **2021**, *64* (4), 1989–2009.

(18) Rochais, C.; Lecoutey, C.; Gaven, F.; Giannoni, P.; Hamidouche, K.; Hedou, D.; Dubost, E.; Genest, D.; Yahiaoui, S.; Freret, T. Novel Multitarget-Directed Ligands (MTDLs) with Acetylcholinesterase (AChE) Inhibitory and Serotonergic Subtype 4 Receptor (5-HT4R) Agonist Activities as Potential Agents against Alzheimer's Disease: The Design of Donecopride. *J. Med. Chem.* **2015**, *58* (7), 3172–3187.

(19) Wang, J.; Wang, Z.-M.; Li, X.-M.; Li, F.; Wu, J.-J.; Kong, L.-Y.; Wang, X.-B. Synthesis and Evaluation of Multi-Target-Directed Ligands for the Treatment of Alzheimer's Disease Based on the Fusion of Donepezil and Melatonin. *Bioorg. Med. Chem.* **2016**, *24* (18), 4324–4338.

(20) Bautista-Aguilera, O. M.; Esteban, G.; Bolea, I.; Nikolic, K.; Agbaba, D.; Moraleda, I.; Iriepa, I.; Samadi, A.; Soriano, E.; Unzeta, M. Design, Synthesis, Pharmacological Evaluation, QSAR Analysis, Molecular Modeling and ADMET of Novel Donepezil–Indolyl Hybrids as Multipotent Cholinesterase/Monoamine Oxidase Inhibitors for the

Potential Treatment of Alzheimer's Disease. *Eur. J. Med. Chem.* **2014**, *75*, 82–95.

(21) Sharma, P.; Tripathi, A.; Tripathi, P. N.; Singh, S. Sen; Singh, S. P.; Shrivastava, S. K. Novel Molecular Hybrids of N-Benzylpiperidine and 1, 3, 4-Oxadiazole as Multitargeted Therapeutics to Treat Alzheimer's Disease. *ACS Chem. Neurosci.* **2019**, *10* (10), 4361–4384.

(22) Wu, M.-Y.; Esteban, G.; Brogi, S.; Shionoya, M.; Wang, L.; Campiani, G.; Unzeta, M.; Inokuchi, T.; Butini, S.; Marco-Contelles, J. Donepezil-like Multifunctional Agents: Design, Synthesis, Molecular Modeling and Biological Evaluation. *Eur. J. Med. Chem.* **2016**, *121*, 864–879.

(23) Wang, L.; Esteban, G.; Ojima, M.; Bautista-Aguilera, O. M.; Inokuchi, T.; Moraleda, I.; Iriepa, I.; Samadi, A.; Youdim, M. B. H.; Romero, A. Donepezil+ Propargylamine+ 8-Hydroxyquinoline Hybrids as New Multifunctional Metal-Chelators, ChE and MAO Inhibitors for the Potential Treatment of Alzheimer's Disease. *Eur. J. Med. Chem.* **2014**, *80*, 543–561.

(24) Banoo, R.; Nuthakki, V. K.; Wadje, B. N.; Sharma, A.; Bharate, S. B. Design, Synthesis, and Pharmacological Evaluation of Indole-Piperidine Amides as Blood−Brain Barrier Permeable Dual Cholinesterase and β-Secretase Inhibitors. *Eur. J. Med. Chem.* **2024**, *266*, 116131.

(25) Samadi, A.; Chioua, M.; Bolea, I.; De Los Ríos, C.; Iriepa, I.; Moraleda, I.; Bastida, A.; Esteban, G.; Unzeta, M.; Gálvez, E. Synthesis, Biological Assessment and Molecular Modeling of New Multipotent MAO and Cholinesterase Inhibitors as Potential Drugs for the Treatment of Alzheimer's Disease. *Eur. J. Med. Chem.* **2011**, *46* (9), 4665–4668.

(26) Samadi, A.; De Los Ríos, C.; Bolea, I.; Chioua, M.; Iriepa, I.; Moraleda, I.; Bartolini, M.; Andrisano, V.; Gálvez, E.; Valderas, C. Multipotent MAO and Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease: Synthesis, Pharmacological Analysis and Molecular Modeling of Heterocyclic Substituted Alkyl and Cycloalkyl Propargyl Amine. *Eur. J. Med. Chem.* **2012**, *52*, 251–262.

(27) Bautista-Aguilera, O. M.; Samadi, A.; Chioua, M.; Nikolic, K.; Filipic, S.; Agbaba, D.; Soriano, E.; De Andrés, L.; Rodríguez-Franco, M. I.; Alcaro, S. N-Methyl-N-((1-Methyl-5-(3-(1-(2-Methylbenzyl) Piperidin-4-Yl) Propoxy)-1 H-Indol-2-Yl) Methyl) Prop-2-Yn-1-Amine, a New Cholinesterase and Monoamine Oxidase Dual Inhibitor. *J. Med. Chem.* **2014**, *57* (24), 10455–10463.

(28) Sterling, J.; Herzig, Y.; Goren, T.; Finkelstein, N.; Lerner, D.; Goldenberg, W.; Miskolczi, I.; Molnar, S.; Rantal, F.; Tamas, T. Novel Dual Inhibitors of AChE and MAO Derived from Hydroxy Aminoindan and Phenethylamine as Potential Treatment for Alzheimer's Disease. *J. Med. Chem.* **2002**, *45* (24), 5260–5279.

(29) Shahrivar-Gargari, M.; Hamzeh-Mivehroud, M.; Hemmati, S.; Mojarrad, J. S.; Tüylü

Küçükkılınç, T.; Ayazgök, B.; Dastmalchi, S. Hybridization-based Design of Novel Anticholinesterase Indanone–Carbamates for Alzheimer's Disease: Synthesis, Biological Evaluation, and Docking Studies. *Arch. Pharm. (Weinheim).* **2021**, *354* (7), 2000453.

(30) Liu, Y.; Ma, C.; Li, Y.; Li, M.; Cui, T.; Zhao, X.; Li, Z.; Jia, H.; Wang, H.; Xiu, X. Design, Synthesis and Biological Evaluation of Carbamate Derivatives Incorporating Multifunctional Carrier Scaffolds as Pseudo-Irreversible Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease. *Eur. J. Med. Chem.* **2024**, *265*, 116071.

(31) Camarillo-López, R. H.; Hernández Rodríguez, M.; Torres-Ramos, M. A.; Arciniega-Martínez, I. M.; García-Marín, I. D.; Correa Basurto, J.; Méndez Méndez, J. V.; Rosales-Hernández, M. C. Tert-Butyl-(4-Hydroxy-3-((3-(2-Methylpiperidin-Yl) Propyl) Carbamoyl) Phenyl) Carbamate Has Moderated Protective Activity in Astrocytes Stimulated with Amyloid Beta 1-42 and in a Scopolamine Model. *Molecules* **2020**, *25* (21), 5009.

(32) Santos, G. S.; Sinoti, S. B. P.; de Almeida, F. T. C.; Silveira, D.; Simeoni, L. A.; Gomes-Copeland, K. K. P. Use of Galantamine in the Treatment of Alzheimer's Disease and Strategies to Optimize Its Biosynthesis Using the in Vitro Culture Technique. *Plant Cell, Tissue Organ Cult.* **2020**, *143*, 13–29.

(33) Santos, T. C. dos; Gomes, T. M.; Pinto, B. A. S.; Camara, A. L.; Paes, A. M. D. A. Naturally Occurring Acetylcholinesterase Inhibitors and Their Potential Use for Alzheimer's Disease Therapy. *Front. Pharmacol.* **2018**, *9*, 1192.

(34) Montanari, S.; Mahmoud, A. M.; Pruccoli, L.; Rabbito, A.; Naldi, M.; Petralla, S.; Moraleda, I.; Bartolini, M.; Monti, B.; Iriepa, I. Discovery of Novel Benzofuran-Based Compounds with Neuroprotective and Immunomodulatory Properties for Alzheimer's Disease Treatment. *Eur. J. Med. Chem.* **2019**, *178*, 243–258.

(35) Chowdhury, S. R.; Gu, J.; Hu, Y.; Wang, J.; Lei, S.; Tavallaie, M. S.; Lam, C.; Lu, D.; Jiang, F.; Fu, L. Synthesis, Biological Evaluation and Molecular Modeling of Benzofuran Piperidine Derivatives as Aβ Antiaggregant. *Eur. J. Med. Chem.* **2021**, *222*, 113541.

(36) Fancellu, G.; Chand, K.; Tomás, D.; Orlandini, E.; Piemontese, L.; Silva, D. F.; Cardoso, S. M.; Chaves, S.; Santos, M. A. Novel Tacrine–Benzofuran Hybrids as Potential Multi-Target Drug Candidates for the Treatment of Alzheimer's Disease. *J. Enzyme Inhib. Med. Chem.* **2020**, *35* (1), 211–226.

(37) Zha, X.; Lamba, D.; Zhang, L.; Lou, Y.; Xu, C.; Kang, D.; Chen, L.; Xu, Y.; Zhang, L.; De Simone, A. Novel Tacrine–Benzofuran Hybrids as Potent Multitarget-Directed Ligands for the Treatment of Alzheimer's Disease: Design, Synthesis, Biological Evaluation, and X-Ray Crystallography. *J. Med. Chem.* **2016**, *59* (1), 114–131.

(38) Pisani, L.; Barletta, M.; Soto-Otero, R.; Nicolotti, O.; Mendez-Alvarez, E.; Catto, M.;

Introcaso, A.; Stefanachi, A.; Cellamare, S.; Altomare, C.; Carotti, A. Discovery, Biological Evaluation, and Structure-Activity and -Selectivity Relationships of 6′-Substituted (E)-2-(Benzofuran-3(2H)-Ylidene)-N- Methylacetamides, a Novel Class of Potent and Selective Monoamine Oxidase Inhibitors. *J. Med. Chem.* **2013**, *56* (6), 2651–2664.

(39) Sinha, A.; Tamboli, R. S.; Seth, B.; Kanhed, A. M.; Tiwari, S. K.; Agarwal, S.; Nair, S.; Giridhar, R.; Chaturvedi, R. K.; Yadav, M. R. Neuroprotective Role of Novel Triazine Derivatives by Activating Wnt/β Catenin Signaling Pathway in Rodent Models of Alzheimer's Disease. *Mol. Neurobiol.* **2015**, *52*, 638–652.

(40) Shidore, M.; Machhi, J.; Shingala, K.; Murumkar, P.; Sharma, M. K.; Agrawal, N.; Tripathi, A.; Parikh, Z.; Pillai, P.; Yadav, M. R. Benzylpiperidine-Linked Diarylthiazoles as Potential Anti-Alzheimer's Agents: Synthesis and Biological Evaluation. *J. Med. Chem.* **2016**, *59* (12), 5823–5846.

(41) Kollu, U.; Avula, V. K. R.; Vallela, S.; Pasupuleti, V. R.; Zyryanov, G. V.; Neelam, Y. S.; Chamarthi, N. R. Synthesis, Antioxidant Activity and Bioinformatics Studies of L-3-Hydroxytyrosine Templated N-Alkyl/Aryl Substituted Urea/Thioureas. *Bioorg. Chem.* **2021**, *111*, 104837.

(42) Lachhi Reddy, V.; Avula, V. K. R.; Zyryanov, G. V; Vallela, S.; Anireddy, J. S.; Pasupuleti, V. R.; Chamarthi, N. R. Hunig's Base Catalyzed Synthesis of New 1-(2,3-Dihydro-1H-Inden-1-Yl)-3-Aryl Urea/Thiourea Derivatives as Potent Antioxidants and 2HCK Enzyme Growth Inhibitors. *Bioorg. Chem.* **2020**, *95*, 103558.

****