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Innovations in acetylcholinesterase inhibitors: emerging strategies for Alzheimer's disease therapy (2019–2024)

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Alzheimer's disease (AD) is a prevalent irreversible neurological degenerative disorder and one of the considerable health hazards in the aged population worldwide. It is estimated that AD population will reach 74.4 million by 2030, and the prevalence is anticipated to reach 131.5 million by 2050, representing an enormous burden on health organizations. AD is a familiar type of dementia characterized by gradual memory loss and cognitive function impairment. AD is a complex multifactorial disease, and the cholinergic hypothesis is the primary theory explaining its pathogenesis. It was observed that the brains of AD patients showed a sharp decrease in cholinergic neurons and a remarkable deficiency in acetylcholine levels. Thus, acetylcholinesterase inhibitors (AChEI) are considered a promising strategy for AD treatment. The FDA approves four AChEI for AD treatment: tacrine, donepezil, rivastigmine, and galantamine. This review article focuses on the latest advances in the development of new AChEI inhibitors from 2019 to 2024, encompassing single-targeted, multi-targeted, and naturally derived inhibitors as potential treatments for AD. It highlights new molecules with promising inhibitory activity against acetylcholinesterase, underscoring their therapeutic potential in AD management.

Keywords:
acetylcholine inhibitors, Alzheimer's, disease, cholinergic hypothesis

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Introduction

Alzheimer's disease (AD) is a prevalent neurological degenerative disorder, representing around 60–80% of total dementia cases worldwide [1]. In 2024, about 6.9 million Americans aged 65 and older are inflicted with AD [2]. It is anticipated that AD population will reach 74.4 million by 2030 and 131.5 million by 2050, which will act as a considerable burden on health organizations [3]. AD is the seventh leading cause of death in the United States and the fifth leading cause of death among Americans aged 65 and older in 2021 [2].

AD is distinguished by gradual memory loss, impairment of cognitive functions, and deterioration of cholinergic functions [4]. The primary pathological hallmarks of AD are extracellular aggregation of β-amyloid protein, neurofibrillary tangles, and gradual neuronal loss. Many hypotheses have evolved to illustrate the pathogenesis of this multifactorial disease, including the cholinergic hypothesis, amyloid hypothesis, and tau hypothesis.

However, the cholinergic hypothesis is the first and extensively studied hypothesis interpreting its pathogenesis [5]. Clinical studies revealed that the brains of AD patients showed a sharp decrease of cholinergic neurons, a remarkable deficiency in acetylcholine levels, and a considerable reduction in the activity of acetylcholine transferase [6]. It was reported that the rate of cognitive deterioration and cholinergic degeneration is raised in age-related neurological degenerative diseases, including AD [4]. Besides, the abnormal cholinergic alteration resulted in many pathological conditions like anomalous tau protein phosphorylation and apoptosis of nerve cells [4]. Acetylcholine is a substantial neurotransmitter in the brain, is synthesized by cholinergic neurons, and reacts with both nicotinic (ionotropic) and muscarinic (metabotropic) receptors [7]. Due to its wide distribution, it is responsible for many physiological roles in different body compartments, including brain function (like attention and memory) [8]. Acetylcholine is involved in the cholinergic signal transduction concerning memory and learning capability. Structurally, acetylcholine is an ester generated in nerve cells from choline and acetyl CoA via choline acetyltransferase [9]. However, its hydrolysis into choline and acetic acid is catalyzed by cholinesterase enzymes [10].

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Cholinesterases are a family of enzymes that hydrolyze the choline esters, substantial for the appropriate functioning of the human nervous system [10]. Cholinesterases comprise acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is an intrinsic enzyme in vertebrates and humans, primarily found in hematopoietic cells and nerve endings [11]. AChE is responsible for regulating nerve signal transmission by catalyzing the hydrolysis of acetylcholine at neuromuscular junctions. BuChE (nonspecific cholinesterase) is structurally similar to AChE and primarily present in plasma. However, AChE hydrolyzes acetylcholine more rapidly than BuChE [10].

The 3D structure of AChE (from *Torpedo californica* – TcAChE) (Fig. 1) showed that the active site is located in the center, appears as 20°A deep long aromatic gorge and consists of distinct subsites [12] (Fig. 2): the esteratic subsite (also known as a catalytic triad) contains Ser203, Glu334, and His447 residues (where choline ester hydrolysis to acetate and choline is taken place), anionic subsite (uncharged, lipophilic, contains aromatic amino acids residues like Trp86 and binds to the quaternary nitrogen of acetylcholine, other ligands, and substrates), oxyanion hole (binds to negatively charged oxygen ions and enhances the effectiveness of AChE catalysis process), acyl pocket (provides substrate selectivity), peripheral anionic subsite (is located 15°A from catalytic triad) and omega loop (disulfide linkage and covers the active site). It was also reported that cholinesterase enzymes are involved in many other processes, such as the aggregation of beta-amyloid [13,14]. Therefore, AChE is considered an auspicious therapeutic target for AD.

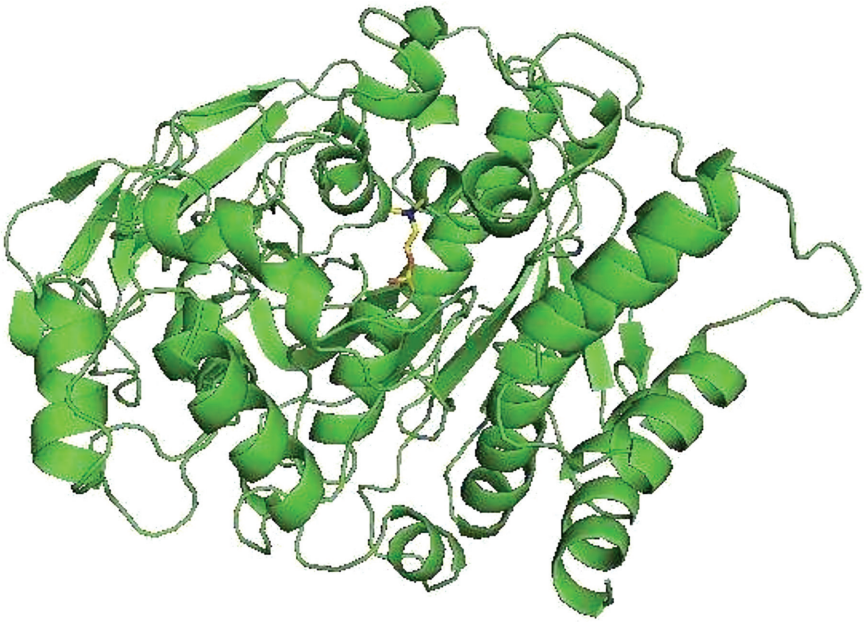
Many efforts have been devoted to the evolution of potential acetylcholinesterase inhibitors (AChEIs). This review article has concentrated on the latest advances in the evolution of novel promising AChEIs for AD remedy through the last 5 years. Particularly, it shows the molecular structures of these novel inhibitors, including single-targeted, multi-targeted, and naturally derived inhibitors, their inhibitory activity values, and the ligand-target interactions using molecular docking studies.

Single-target acetylcholinesterase inhibitors

FDA approves four AChEIs for AD therapy including tacrine, donepezil, galantamine, and rivastigmine [15]. These inhibitors are used as reference compounds for the discovery and development of novel potential compounds for AD therapy. Tacrine, donepezil, and rivastigmine demonstrated inhibitory activity against AChE with in vitro IC_{50} values of 77, 6.7, and 4.3 nM, respectively [16].

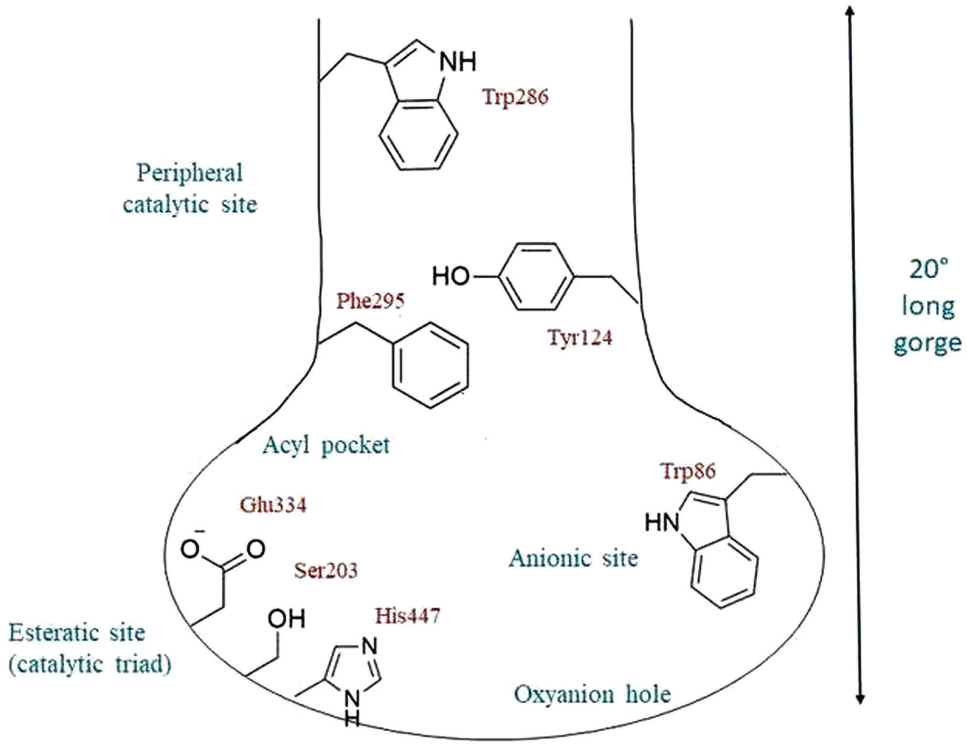
Tacrine (1,2,3,4-tetrahydroacridin-9-amine, Fig. 3) is a tetrahydroacridine derivative, approved in 1993 and withdrawn from the market due to its hepatotoxicity in 2013 [17]. It inhibits both AChE and BuChE and

Figure 1



Crystal structure of acetylcholinesterase enzyme (TcAChE) (PDB: 2ace) showed as a green cartoon, demonstrating acetylcholine in the active site (yellow sticks). The figure is prepared using Pymol molecular graphics software.

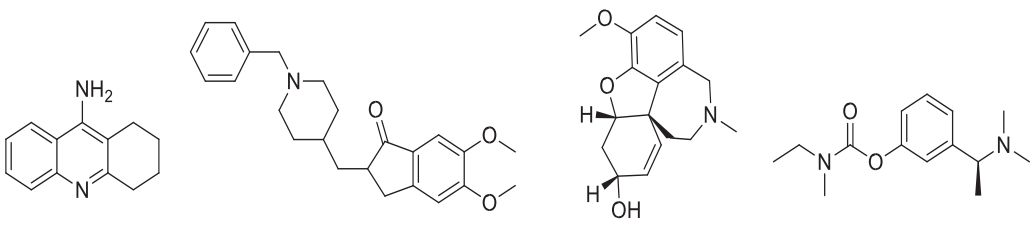
Figure 2



Diagrammatic representation of the active site of AChE. AChE, acetylcholinesterase.

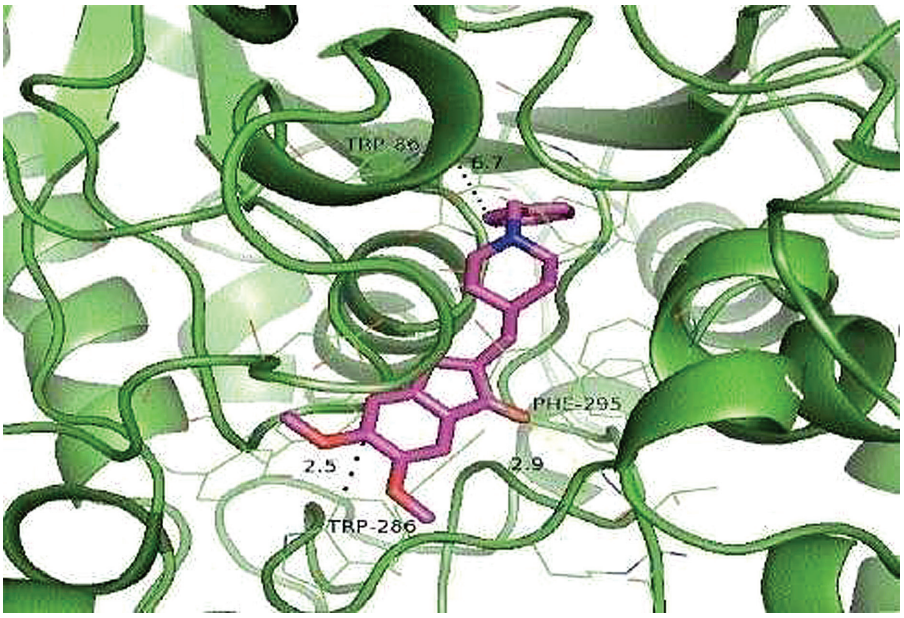
interacts with Phe330 and Trp84 amino acid residues in the anionic subsite of AChE. Donepezil (2-((1-benzylpiperidin-4-yl)methyl)-5,6dimethoxy2,3dihydro-1H-inden-1-one, Fig. 3), is a piperidine derivative, approved in 1996 and used as a reversible inhibitor of AChE for patients with mild to moderate AD [18]. Docking studies revealed that donepezil inhibits both the peripheral and active site of *T. californica* AChE due to its distinct chemical structure (Fig. 4) [19]. Galantamine(4aS,6R,8aS)-3-methoxy-11-methyl-5,6, 9,10,11,12 hexahydro-4aH-benzo [2,3] benzo-furo[4,3-cd]azepin-6-ol, Fig. 3), is a phenanthrene alkaloid extracted from botanical source (bulbs of the Amaryllidaceae) and approved in 2003 as a competitive and reversible inhibitor of AChE for treatment of AD [20]. It was reported that galantamine also binds to nicotinic cholinergic receptors and is effective for cognitive impairment associated with AD treatment [21]. Rivastigmine (3-(1-(dimethylamino)ethyl)phenyl ethyl(methyl) carbamate, Fig. 3) is a carbamate derivative (noncompetitive pseudo-irreversible inhibitor of cholinesterase) and was approved for AD in 2000. It is indicated for the medication of mild to moderate Alzheimer's dementia. It is utilized as a dual inhibitor of AChE and BuChE and has a structural similarity with physostigmine [22]. Rivastigmine interacts with AChE through its carbamate group and occupies its estratic subsite. Consequently, AchE cleaves rivastigmine, producing different phenolic compounds that are promptly excreted from the body.

Figure 3



Chemical structure of Food & Drug Administration (FDA)-approved acetylcholinesterase inhibitors.

Figure 4

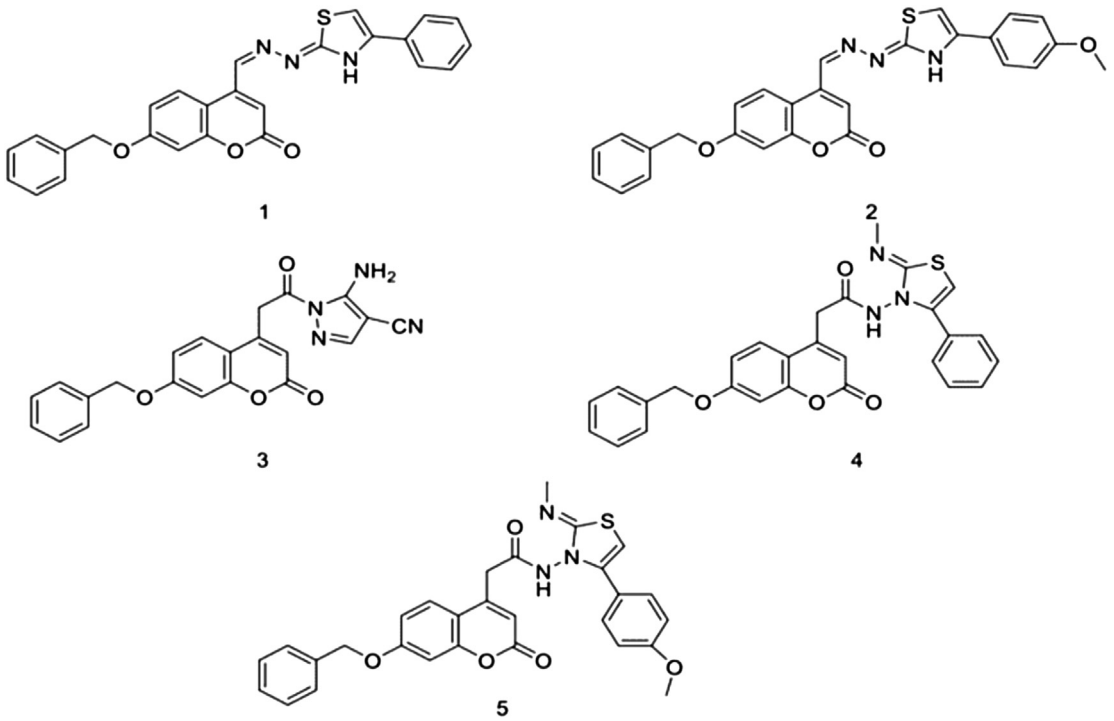


Docked pose of donepezil into AChE active site (PDB: 4EY7), donepezil showed as sticks (magenta) and AChE active site represented in green cartoons. The figure was prepared using Pymol molecular graphics software. AChE, acetylcholinesterase.

Coumarins derivatives

Coumarins (benzopyran-2-one) are privileged structures widely used in the pharmaceutical industry, exhibiting various biological activities, including antioxidants, anticancer, and AChEIs [23]. They have various sites for substitution, making them charming compounds for the design and development of novel biologically active compounds [23]. Amin and colleagues synthesized a series of novel 7-benzyloxycoumarins that showed significant inhibitory activity against AChE. In vitro study demonstrated that compounds 1, 2, 3, 4, and 5 (Fig. 5) exhibited auspicious inhibitory activity against AChE with in vitro IC_{50} values of 0.451, 0.625, 0.466,

Figure 5



Coumarin derivatives (compounds 1–5) as acetylcholinesterase inhibitors.

0.500, and 0.590 μM, respectively (Table 1), even superior to donepezil (reference drug). An in vivo study was performed on compounds 2, 3, and 4 and emphasized remarkable memory enhancement in mice (scopolamine animal model). Moreover, docking studies were carried out and showed that the synthesized coumarins are located in both the peripheral anionic site (PAS) and catalytic anionic site (CAS) of the AChE active site, exhibiting comparable binding mode to donepezil (co-crystallized ligand). The benzyloxy group formed π–π stacking interactions with Trp86 residues, while the carbonyl group of the coumarin scaffold interacted with Tyr337, Asp74, and Tyr341 via a water-mediated hydrogen bond. Additionally, two hydrogen bonds were observed between the benzyloxy oxygen and Ser203 amino acid residue and between the coumarin carbonyl group and Tyr341 [23].

Aminobenzohydrazide derivatives

Various derivatives of aminobenzohydrazide were developed and estimated for their inhibitory activity against AChE and BuChE by Almaz *et al.* [24]. Compound 6 (4-amino-3bromo5fluorobenzo hydrazide) showed potent inhibitory activity against AChE and BuChE with an in vitro IC_{50} values of 0.59 and 0.15 μM (Table 1), respectively (Fig. 6). Docking studies were performed on compound 6 to verify its binding mechanism with AChE. The hydrazide group was involved in a charged interaction with Glu198 residue, a hydrogen bond with Gly116, and a cation–cation interaction with Trp82. Additionally, the benzene ring formed π–T-shape interactions with Tyr333 and Trp82 amino acids, while Phe293, Phe334, and His443 were involved in alkyl interactions with the bromide group [24].

N-benzylpyrrolidine derivatives

El Khatabi *et al.* [25], designed and developed a series of N-benzylpyrrolidine derivatives as potent AChEIs using three-dimensional quantitative structure–activity relationship techniques (CoMFA and CoMSIA models) to illustrate the structural characteristics essential for AChE inhibitory activity. Compound 7 (Fig. 7) showed the superior predicted activity among the synthesized compounds. All compounds were subjected to molecular modeling studies and MD simulation to investigate their binding affinity and dynamic behavior. Compound 7 occupied the AChE active site and established two hydrogen bonds with Tyr121 and Asp72 via its NH linker while the hydrophobic $OCF(C_2H_5)_2$ substituent formed a π-sigma effect with Phe 290 residue [25].

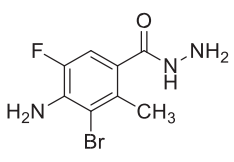
Pyrrole derivatives

Pyrrole derivatives display diverse therapeutic efficacy besides being a part of different biological molecules like vitamin B12 and alkaloids [26]. Thus, 39 compounds of polysubstituted pyrrole derivatives were designed, synthesized, and evaluated as potent AChEIs by Pourtaher and colleagues. Among these synthesized compounds, compound 8 (Fig. 8) exhibits superior activity against AChE (in vitro $IC_{50} = 2.95 \pm 1.31$ μM) (Table 1) [26]. The computational molecular modeling study demonstrated that compound 8 fitted perfectly into the AChE active site where the acetamide group stayed in the anionic subsite, forming three hydrogen bond interactions with Tyr124 and Trp86 residues, whereas the para hydroxyl group was directed toward the PAS subsite, forming two hydrogen bonds with Phe295 and Tyr341 [26].

Lophine derivatives

A novel series of lophine (2,4,5-triphenyl-1H-imidazole) hybrids (connected to natural-based D-

Figure 6



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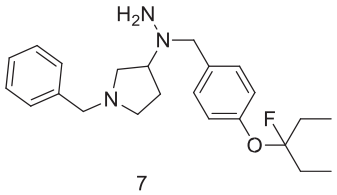
Aminobenzohydrazide derivative (compound 6) as an acetylcholinesterase inhibitor.

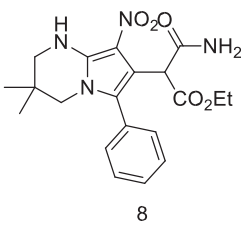
Figure 7



7

N-benzylpyrrolidine derivative (compound 7) as an acetylcholinesterase inhibitor.

Figure 8



8

Pyrrole derivative (compound 8) as an acetylcholinesterase inhibitor.

Table 1 IC_{50} values of the proposed acetylcholinesterase inhibitors on acetylcholinesterase and other receptors

Compound numbers	Inhibitor	IC_{50} values				References
		AChE	BuChE	MAO-A	MAO-B	
1	7-benzyloxy-4-{[(4-phenylthiazol-2(3H)-ylidene)hydrazono]methyl}-2H-chromen-2-one	0.451 μM	–	–	–	[1]
2	7-benzyloxy-4-({[4-(4-methoxyphenyl)thiazol-2(3H)-ylidene]hydrazono} methyl)-2H-chromen-2-one	0.625 μM	–	–	–	[1]
3	5-amino-1-[2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-1H-pyrazole-4-carbonitrile	0.466 μM	–	–	–	[1]
4	2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)-N-(2-methylimino-4-phenylthiazol-3(2H)-yl)acetamide	0.500 μM	–	–	–	[1]
5	2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)-N-[4-(4-methoxyphenyl)-2-methyliminothiazol-3(2H)-yl]acetamide	0.590 μM	–	–	–	[1]
6	4-amino-3-bromo-5-fluorobenzohydrazide	0.59 μM	0.15 μM	–	–	[3]
8	2-(2-(4-chlorophenyl)-1-(4-hydroxyphenyl)-5-(methylthio)-4-nitro-1H-pyrrol-3-yl)-2-cyanoacetamide	2.95±1.31 μM	–	–	–	[2]
9	N^1 –(1-O-Methyl-5-deoxy-2,3-O-isopropylidene-β-D-ribofuranoside)-5-(N^7-(2,4,5-triphenyl-1H-imidazol-1-yl)heptan-1-amine)	2.75 μM	–	–	–	[4]
10	N-(2,4,5-Trichlorophenyl)-2-(4-ethyl-5-(3-chlorophenyl)-4H-1,2,4-triazo-3-ylthio)acetamide	5.41±0.24 μM	7.52±0.18 μM	–	–	[5]
11	N-(4-Iodophenyl)-2-(4-phenyl-5-(3-chlorophenyl)-4H-1,2,4-triazol-3-ylthio)acetamide	13.57±0.31 μM	–	–	–	[5]
14	Ethyl 5-(4-methacryloylpiperazine-1-carbonyl)-2,5-dimethyl-4,5-dihydrofuran-3-carboxylate	5.79 μM	–	–	–	[6]
15	Diethyl 5,5′-(piperazine-1,4-dicarbonyl)bis(2,5-dimethyl-4,5-dihydrofuran-3-carboxylate)	3.89 μM	–	–	–	[6]
16	Ethyl 2,5-dimethyl-5-(4-(2,6,6-trimethyl-4-oxo-2,3,4,5,6,7-hexahydrobenzofuran-2-carbonyl) piperazine-1-carbonyl)-4,5-dihydrofuran-3-carboxylate	5.07 μM	–	–	–	[6]
17	2-(4-(Furan-2-carbonyl)piperazine-1-carbonyl)-2,6,6-trimethyl-2,3,6,7-tetrahydrobenzofuran-4 (5H)-one	4.30 μM	–	–	–	[6]
18	Ethyl 5-(4-(furan-2-carbonyl)piperazine-1-carbonyl)-2,5-dimethyl-4,5-dihydrofuran-3-carboxylate	2.24 μM	–	–	–	[6]
19	{7-[3-(Benzyl-methyl-amino)-propoxy]-6-methoxy-3-nitro-quinolin-4-yl}-(2-chloro-phenyl)-amine	0.86 μM	2.6 μM	–	–	[7]
20	(E)-4-methyl-7-(4-(4-(3-(thiophen-2-yl)-acryloyl)-phenoxy)-butoxy)–2H-chromen-2-one	0.42±0.019 μM	–	–	–	[8]
21	6-(2-((5-((4-Methoxyphenyl)amino)-1,3,4-thiadiazol-2-yl)sulfanyl)acetyl)-2H-benzo[b][1,4] thiazin3(4H)-one	0.027 μM	–	–	–	[9]
22	6-(2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)sulfanyl)acetyl)-2H benzo[b][1,4] thiazin3(4H)-one	0.025 μM	–	–	–	[9]
23	3-((3-Acetylphenyl)amino)-1-(benzofuran-2-yl)prop-2-en-1-one	0.058 μM	–	–	–	[10]
24	1-(Benzofuran-2-yl)-3-((2-hydroxyphenyl)amino)prop-2-en-1-one	0.086 μM	–	–	–	[10]
27	Villocarine A	14.45 μM	13.94 μM	–	–	[11]
28	Palmatine	0.74 μmol/l	–	–	–	[12]
29	Berberine	0.52 μmol/l	–	–	–	[12]
30	Jatrorrhizine	0.51 μmol/l	–	–	–	[12]
31	Penicinoline E	68.5 μM	–	–	–	[13]
32	Penicinoline	87.3 μM	–	–	–	[13]
34	5-methoxy-2-methyl-3-tricosyl-1,4-benzoquinone	37.7±1.5 to 370.0±2.9 μM		–		[14]
35	1-O-methylemodin					
36	(3β,5α,6α,22E)-3-hydroxy-5,6-epoxy-7-one-8(14),22-dien-ergosta					
41	Murranganone	79.1 μM	74.3 μM	–	–	[15]
40	Paniculatin	31.6 μM	–	–	–	[15]

(*Continued*)

Table 1 (*Continued*)

Compound numbers	Inhibitor	IC_{50} values				References
		AChE	BuChE	MAO-A	MAO-B	
43	Kolaflavanone	–	3.6 mM	–	–	[16]
44	Ginkgetin	3.2 mM	–	–	–	[16]
45	Ethyl asterrate	20.1 μM	–	–	–	[17]
46	Methyl asterrate	23.3 μM	–	–	–	[17]
47	Asterric acid	66.7 μM	–	–	–	[17]
57	N-methyl-N-(4-(((6-(methyl(prop-2-yn-1-yl)amino)hexyl)oxy)methyl)benzyl)-1,2,3,4-tetrahydroacridin-9-amine	1.57 μM	0.43 μM	2.30 μM	4.75 μM	[18]
58	N-(2-Hydroxyphenyl)quinoline-8-sulfonamide	0.58±0.05 μM	1.72±0.68 μM	1.25±0.45 μM	1.09±0.65 μM	[19]
Reference compounds	Tacrine	77 nM	–	–	–	[20]
	Donepezil	6.7 nM or 0.049 μM	–	–	–	[20]
	Rivastigmine	4.3 nM	–	–	–	[20]
	Galantamine	1.142±0.027 μM	–	–	–	[8]

AChE, acetylcholinesterase; BuChE, butyrylcholinesterase.

xylose, D-ribose, and D-galactose by a methylene chain) were synthesized by Lopes's B and collaborators in an attempt to develop potent cholinesterase inhibitors [27]. The study showed that all synthesized compounds showed considerable inhibitory activity against BuChE while only compound 9 (lophine connected to the D-ribose derivative through a heptylene chain, Fig. 9) manifested an inhibitory activity against AChE with an in vitro IC_{50} value of 2.75 μM (Table 1) [27].

Triazole derivatives

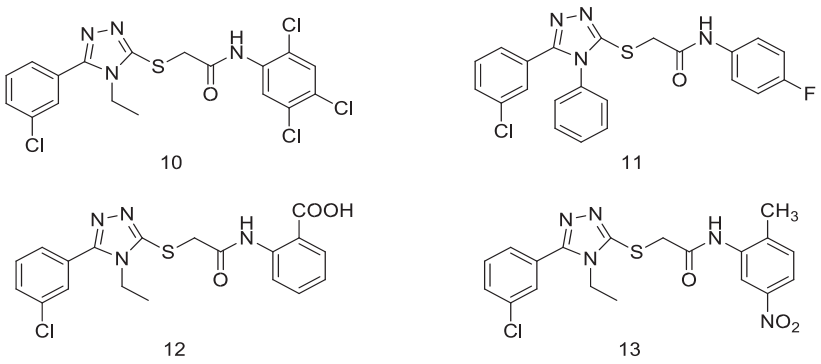
Triazoles are heterocyclic nitrogenous compounds that represent an interesting and attractive area for drug discovery and development. Triazoles have a unique structure due to the presence of three nitrogen atoms (electron-rich ring), which enables them and their derivatives to form a network of various interactions with the targeted enzymes or receptors inside the biological systems. Besides, the triazole scaffold exhibited good ADME properties, low toxicity, and high bioavailability. As long as two series of 1,2,4 triazole derivatives were designed, developed, and investigated for their biological activity against AChE and BuChE by Riaz *et al.* [28]. Figure 10 showed the most potent synthesized compounds (compounds 10, 11, 12, and 13); however, compounds 10 and 11 exhibited the superior AChE inhibitory activity with an in vitro IC_{50}=5.41±0.24 and 13.57±0.31 μM (Table 1), respectively [28]. Additionally, compound 10 exhibited significant BuChE inhibitory activity with an in vitro IC50 value of 7.52±0.18 μM. Molecular modeling studies were performed to explore the binding site interactions, and compound 10 was fitted into the AChE active site and stabilized by different nonbonded interactions. For instance, one of the triazole nitrogens interacted with both Arg296 and Phe295 through two hydrogen bond interactions, while NH of amide moiety and sulfur atom were involved in hydrogen bonds with Tyr124 and Phe295, respectively. Besides, π–alkyl interactions were formed between the triazole ring and Val294, while a T-shaped π–π stacking interaction was

Figure 9

9

Lophine derivative (compound 9) as an acetylcholinesterase inhibitor.

Figure 10



Triazole derivatives (compounds 10–13) as acetylcholinesterase inhibitors.

observed between the trichloro substituent and Tyr337 [28].

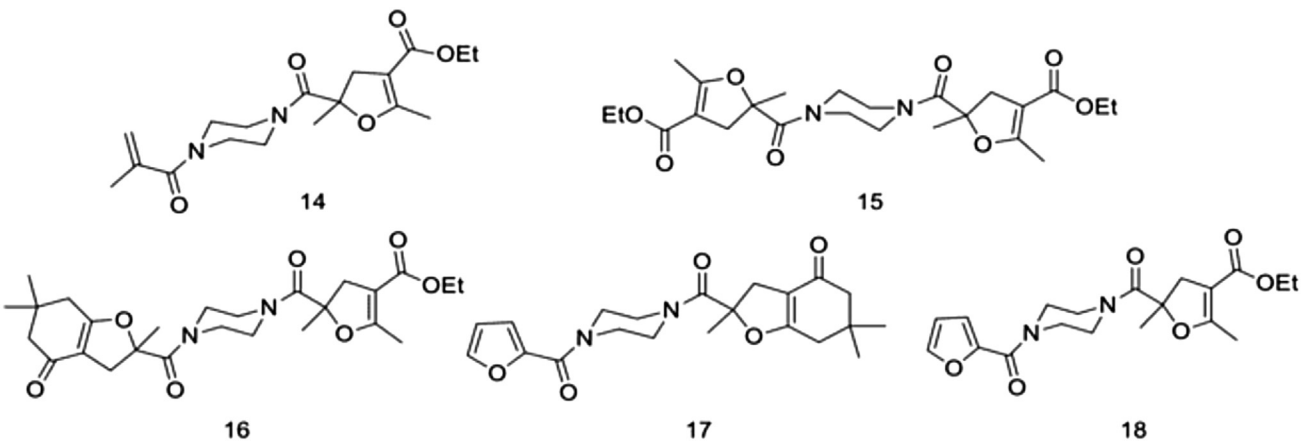
Dihydrofuran derivatives

Dihydrofuran moiety and its derivatives have drawn great attention in the drug discovery area due to their distinct therapeutic activities [29]. Thus, Sari and Yilmaz synthesized novel piperazine-substituted dihydrofuran derivatives by radical cyclization (mediated by $Mn(OAc)_3$) of 1,3-dicarbonyl compounds such as dimedone with novel unsaturated piperazine and homopiperazine [29]. All synthesized compounds were estimated for their AChE inhibitory activity using in vitro Ellman technique. Compound 14 (in vitro IC_{50}=5.79 μM), compound 15 (in vitro IC_{50}=3.89 μM), compound 16 (in vitro IC_{50}=5.07 μM), compound 17 (in vitro IC_{50}=4.30 μM), and compound 18 (in vitro IC_{50}=2.24 μM, Fig. 11) exhibited the superior AChE inhibitory activity (Table 1). Additionally, molecular modeling studies were carried out for the most active compounds to investigate the ligand–enzyme interactions using donepezil as a standard drug. For instance, ligand–protein interactions of compound 18 showed that the two methyl groups of dihydrofuran interacted with several amino acids in the active site like Tyr124, Tyr341, and Phe338 through πalkyl interactions, while the furan ring formed a π–π interaction with Trp86. Furthermore, π–alkyl interactions were observed between piperazine moiety and Phe338 and Tyr337 in a way similar to the piperidine moiety of the standard drug (donepezil) [29].

Aminoquinoline derivatives

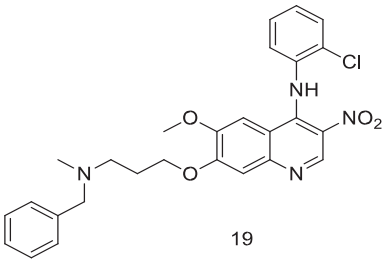
Quinolines and their derivatives showed diverse pharmacological activities, including anticancer, antimalarial, antibacterial, and anti-Alzheimer. Therefore, a new series of aminoquinoline compounds was developed and assessed as dual inhibitors for both AChE and BuChE [30]. Compound 19 (Fig. 12) was endowed with remarkable inhibitory activity against AChE (in vitro IC_{50}=0.86 μM) and BuChE (in vitro IC_{50}

Figure 11



Dihydrofuran derivatives (compounds 14–18) as acetylcholinesterase inhibitors.

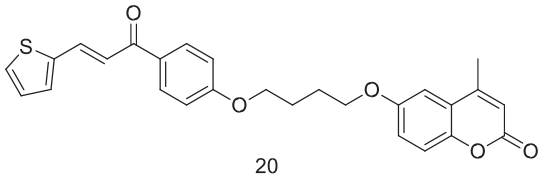
Figure 12



19

Aminoquinoline derivative (compound 19) as an acetylcholinesterase inhibitor.

Figure 13



20

Thiophene chalcone-based coumarin derivative (compound 20) as an acetylcholinesterase inhibitor.

values=2.6 μM). Docking studies elucidate that compound 19 fitted into the AChE active site and formed a π–π-stacking interaction through N-methylbenzylamine with amino acid Trp84 within the CAS subsite, a π–alkyl interaction through its 4-N-phenyl ring with amino acid residue Ile287 and a π–cation and a π–anion interactions between the nitro group and amino acid residue Trp279. Additionally, a carbon-hydrogen bond and three π–π-stacking interactions were observed between the quinoline scaffold and Tyr70, Trp279, and Tyr121, respectively. More and above, compound 19 was docked into the active site of BuChE, showing distinct interaction with all five regions of the BuChE active site [30].

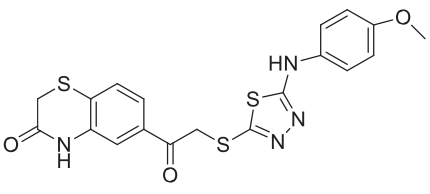
Thiophene chalcone-based coumarin derivatives

Chalcone ((E)-1,3-diphenyl-2-propene-1-one) is a major intermediate in the development of various pharmacologically active compounds, including antiviral, anticancer, antibacterial, anti-inflammatory, antidepressant, and monoamine oxidase inhibitor [31]. Hasan and colleagues designed and synthesized eight distinct chalcone-based coumarin analogs. All the synthesized compounds were subjected to in vitro assessment as AChE inhibitors and insilico molecular modeling investigation [31]. Among these compounds, compound 20 (Fig. 13) showed the superior inhibitory activity with an in vitro IC_{50}=0.42±0.019 μM as opposed to galantamine (control drug), which exhibited an in vitro IC_{50} value of 1.142±0.027 μM (Table 1). Ligand–protein interactions showed that compound 20 fitted into both CAS and PAS subsites of the AChE active site and stabilized by different ligand–protein interactions like hydrogen bonds, π–π stacking interactions, and π–alkyl interactions. The coumarin ring was directed toward the CAS of the AChE enzyme, where its carbonyl oxygen formed two hydrogen bonds with Gly121 and Gly122 residues, and its phenyl moiety established π–π interactions with Tyr337 and Tyr341 residues. Chalcone moiety is orientated toward the PAS subsite of the AChE enzyme, establishing two hydrogen bonds with Tyr72 and Trp286 residues through its carbonyl oxygen and two π–π interactions with Trp286 residue through its phenyl ring. Additionally, thiophene moiety formed π–π interactions with His287 residue [31].

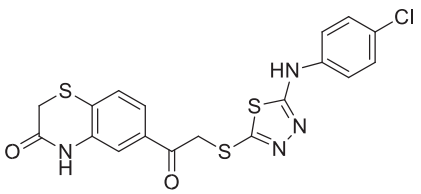
Thiadiazole derivatives

New thiadiazole hybrids analogs with benzothiazines were developed and estimated for their inhibitory activity against AChE [32]. In vitro Ellman technique revealed that compounds 21 and 22 (Fig. 14) showed considerable AChE inhibitory activity with in vitro IC_{50} values of 0.027 and 0.025 μM (Table 1), respectively. Furthermore, the results of the molecular docking studies illustrated that compounds 21 and 22 fitted into the AChE active site with a comparable binding pattern to donepezil. Particularly, the benzothiazine ring of

Figure 14



21



22

Thiadiazole derivative as acetylcholinesterase inhibitors (compounds 21–22).

compound 21 interacted with Trp286 residues through a π–π interaction, while the carbonyl moiety and the amino group of the benzothiazine ring were involved in two hydrogen bonding interactions with Ser293 amino acid residue. Additionally, a π–π interaction was established between thiadiazole moiety and Tyr337 [31].

Benzofuran derivatives

New benzofuran derivatives were developed and estimated as AChEIs using in vitro assay by Abd El-Karim *et al.* [33]. Compounds 23 and 24 (Fig. 15) demonstrated remarkable AChE inhibitory activity with in vitro IC_{50} values of 0.058 and 0.086 μM (Table 1), respectively, while donepezil (control drug) displayed in vitro IC_{50} value of 0.049 μM. To investigate the compounds' binding affinity to AChE, the most active compounds (23 and 24) were docked into the AChE active site using donepezil as a control drug. Results of docking studies showed that compounds 22 and 23 fitted into the active sites in similar binding patterns to those of the control drug (donepezil). The 3D and 2D ligand interaction of the selected AChEIs are shown in Fig. 16.

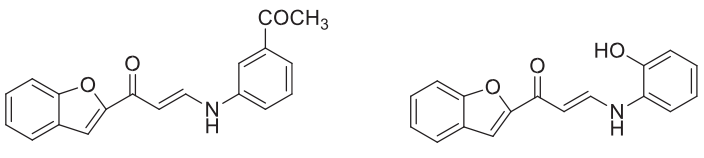
Natural derived inhibitors

Alkaloids

Alkaloids are a diverse group of chemicals that typically have the characteristic of containing nitrogen atom(s) in a cyclic ring. This category of metabolites is likely the greatest in terms of ChE inhibitory action at lower concentrations [34]. An analysis of the leaves of Malaysian *Uncaria attenuata* Korth (from the Rubiaceae family) revealed the presence of a natural corynanthe-type oxindole alkaloid called isovillocarine D (compound 25, Fig. 17), along with two previously identified indole alkaloids, geissoschizine methyl ether (compound 26, Fig. 16), and villocarine A (compound 27, Fig. 17). The isolated compounds (compounds 25–27) exhibited cholinesterase inhibition of moderate to weak intensity, with in vitro IC_{50} values below 50 μM. Compound [27] exhibited the highest inhibitory action against BuChE and AChE, with in vitro IC_{50} values of 13.94 and 14.45 μM (Table 1), respectively, then compounds 26 and 25. Compound 27 exhibits dual inhibition with a selectivity value of ~1, while compounds 25 and 22 specifically inhibit BuChE. Besides, the molecular modeling studies demonstrated that compound 27 engaged with the active site of BuChE and AChE primarily through the establishment of hydrogen and hydrophobic bonds with the essential amino acid residues, specifically with the ethylidene side chain and indole moiety [35]. Song and colleagues showed that the in vitro IC_{50} values for palmatine (compound 28, Fig. 17), berberine (compound 29, Fig. 17), and jatrorrhizine (primary components of two traditional Chinese medicines, *Mahonia bealei* and *Mahonia fortunei*) (compound 30, Fig. 16) were 0.74, 0.52, and 0.51 μmol/l (Table 1), respectively. In this regard, it is important to mention that the effectiveness of the aforementioned compounds is superior to that of galantamine, a well-established inhibitor of AChE, with a concentration of 0.81 μmol/l. Also, the findings indicate that the process of hydrogen addition on the B ring might lead to a substantial reduction in the effectiveness of compounds. But simply, the arrangement of atoms on the B ring is extremely important for inhibiting AChE [36].

Endophytes are abundant providers of chemically and bioactive unique substances that possess significant potential for medical and agricultural applications. In addition, they can generate bioactive compounds that are identical or comparable to those found in the host plants. Therefore, the pursuit of a natural, environmentally, and economical source of potent AChEIs from endophytes has garnered significant interest among researchers [37]. In this regard, Chen and colleagues conducted a study showing that pyrrolyl 4-quinolone alkaloids, namely penicinoline E (compound 31, Fig. 17) and penicinoline (compound 32, Fig. 16), obtained from the mangrove-derived fungus *Penicillium steckii* SCSIO 41025 (Trichocomaceae), had mild inhibitory effects on

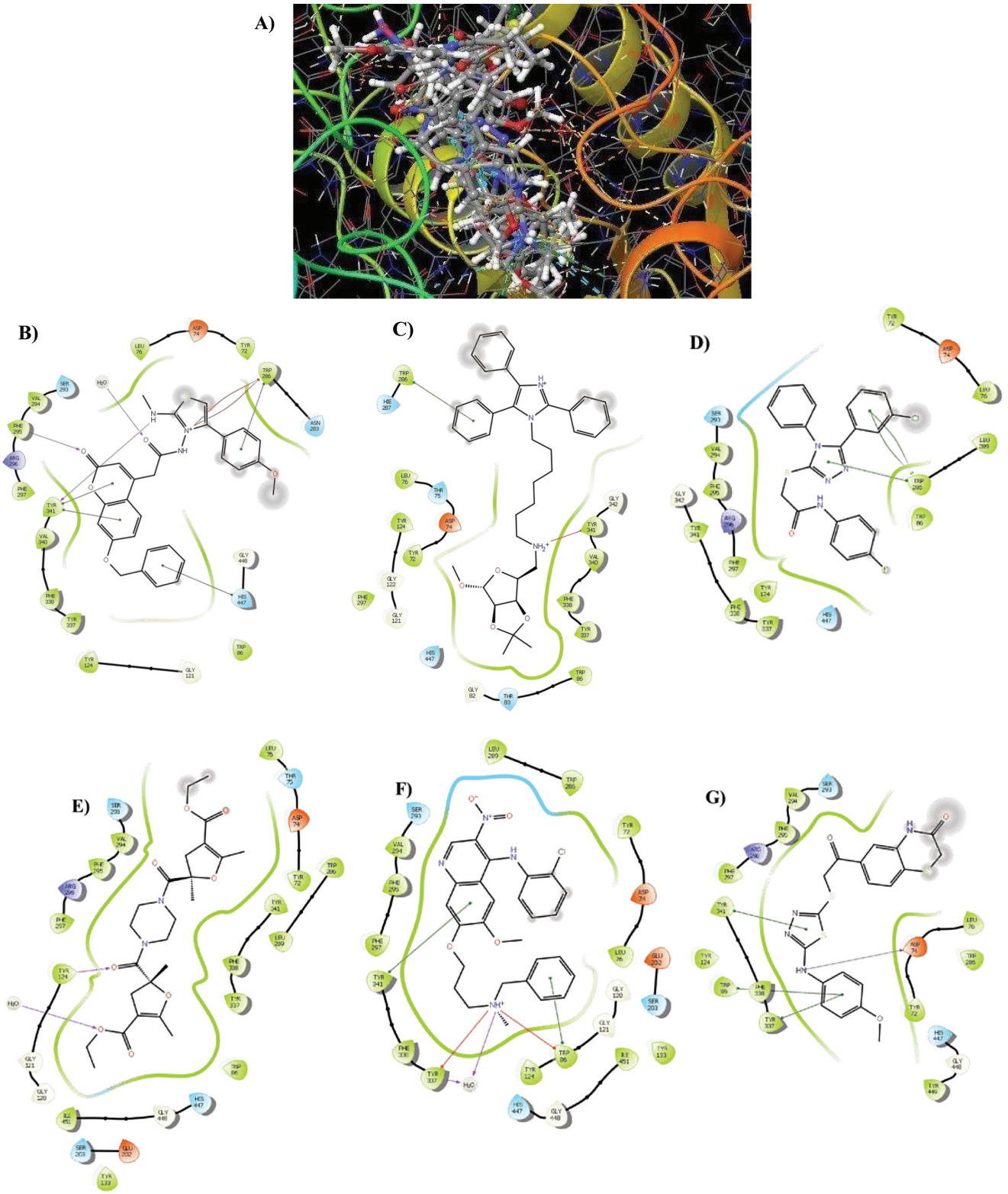
Figure 15



23 24

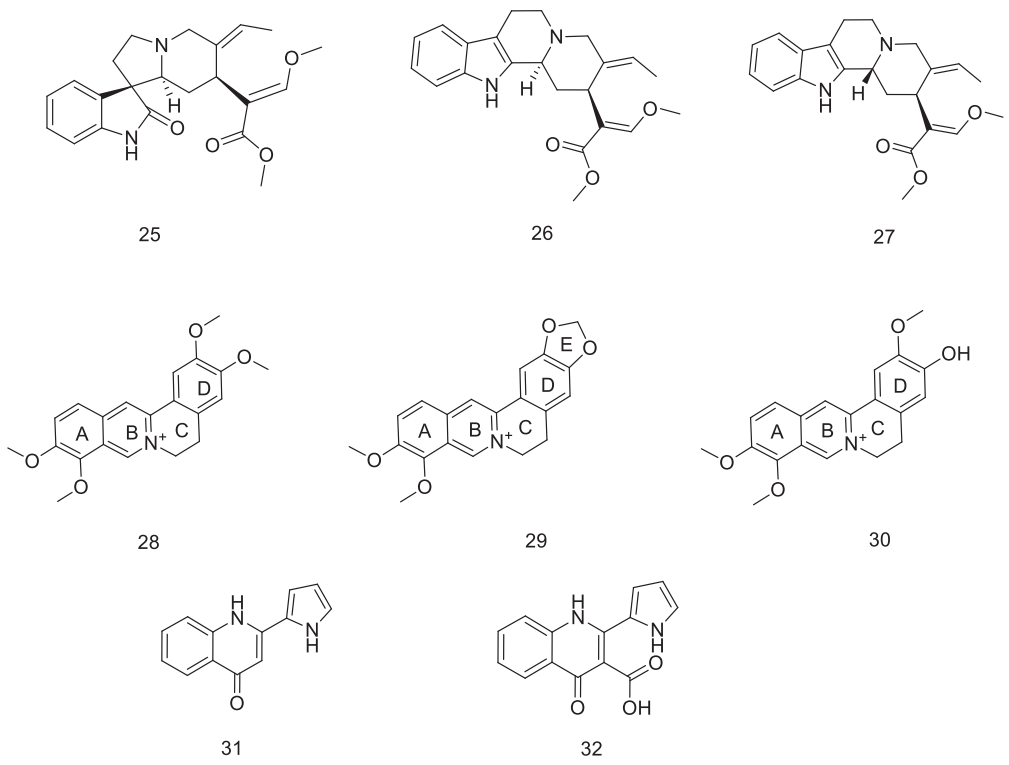
Benzofuran derivatives as acetylcholinesterase inhibitors (compounds 23–24).

Figure 16



(a) 3D superimposition of certain AChEIs in the active site of crystal structure of AChE (PDB: 4EY7); 2D ligand interaction of compound 5 (b); compound 9 (c); compound 11 (d); compound 15 (e); compound 19 (f); compound 21 (g); within substrate binding site of AChE. AChE, acetylcholinesterase.

Figure 17



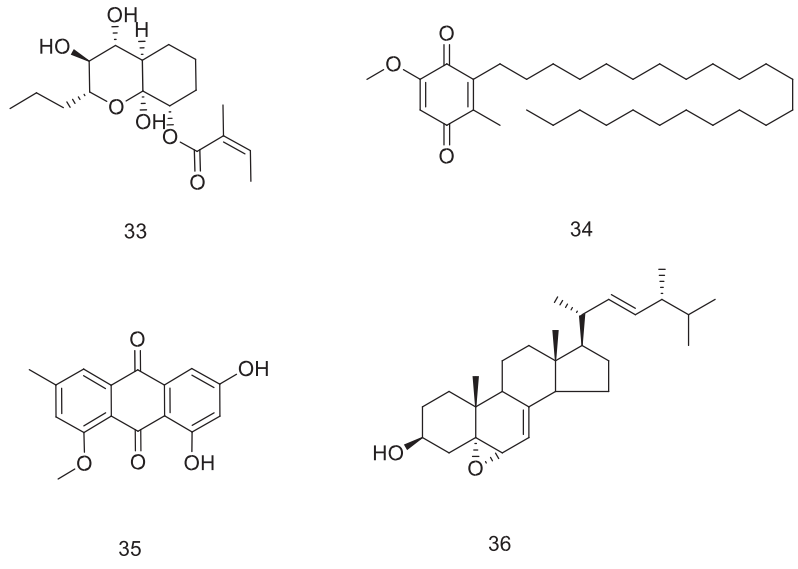
Alkaloids as acetylcholinesterase inhibitors (compounds 25–32).

AChE. The in vitro IC_{50} values for penicinoline E and penicinoline were 68.5 and 87.3 μM (Table 1), respectively [38].

Polyketides

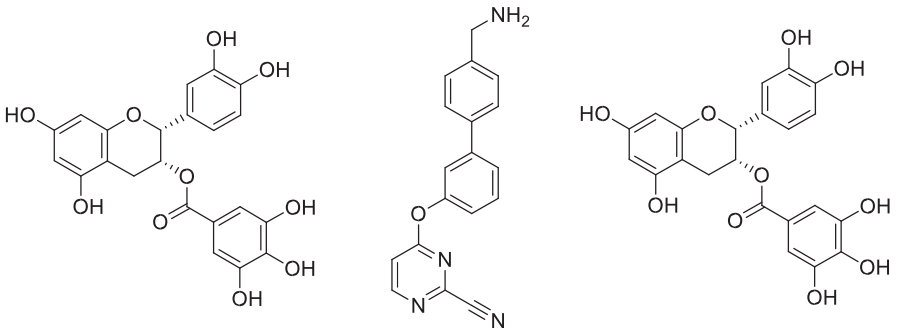
Biasetto and colleagues reported that Koninginin T (compound 33, Fig. 18), derived from the endophytic fungus *Phomopsis stipata* found in *Styrax camporum* Pohl Family Styracaceae, was discovered to effectively inhibit the activity of AChE at a concentration of 10.0 μg. Galantamine was utilized as a positive control at a concentration of 1.0 μg [39]. Another study by Li and colleagues showed that polyketides, 5-methoxy-2-methyl-3-tricosyl-1,4-benzoquinone (compound 34, Fig. 17) and 1-O-methylemodin (compound 35, Fig. 18) along with

Figure 18



Polyketides as acetylcholinesterase inhibitors (compounds 33–36).

Figure 19



37 38 39

Polyphenols as acetylcholinesterase inhibitors (compounds 37–39).

steroid (3β,5α,6α,22E)-3-hydroxy-5,6-epoxy-7-one-8 (14),22-dien-ergosta (compound 36, Fig. 18), which were derived from the endophytic fungus *Chaetomium sp*. YMF432, found in *Huperzia serrata*, had considerable inhibitory activities against AChE with in vitro IC_{50} values ranging from 37.7±1.5 to 370.0 ±2.9 μM [40].

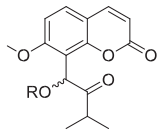
Polyphenols

One of the earliest discoveries that natural compounds from apple, scientifically known as *Malus domestica* (Rosaceae), showed interactions with BuChE and AChE in their active pockets compared to the chosen control drug rivastigmine and had the potential to be used as therapies for neurological illnesses, such as AD. (-)-Epicatechin gallate (compound 37, Fig. 19) and 4-((4'-(Aminomethyl)-[1,1'-biphenyl]-3-yl) oxy) pyrimidine 2carbonitrile (compound 38, Fig. 19) interacted and showed binding affinity with AChE (−12.2 and −11.6 kcal/mol) which better in comparison to the reference medication rivastigmine (−7.8 kcal/mol). Moreover, the interaction between Folic acid (compound 39, Fig. 19) and BuChE had a binding affinity of −10.0 kcal/mol, which was superior to that of the rivastigmine (−6.8 kcal/mol) [41].

Coumarins

Coumarins are compounds that result from the fusion of a benzene ring with an α-pyrone ring [42]. It was noted that compounds isolated from *Murraya paniculata* (Orange Jasmine) leave paniculatin (compound 40, Fig. 20), and murranganone (compound 41, Fig. 20) functioned as mixed-type inhibitors of BuChE and AChE enzymes, and the presence of the ketone group at the C-2' position could play a crucial role in determining the ability to inhibit cholinesterase. Besides, the isopropyl group in

Figure 20



40 R=$COCH_2CH(CH_3)_2$
41 R= H

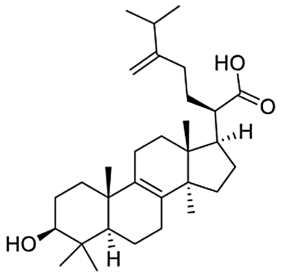
Coumarins as acetylcholinesterase inhibitors (compounds 40–41).

compounds (compound 40) and (compound 41) facilitated π–alkyl, alkyl–alkyl, and hydrophobic interactions with aromatic amino acids, including tryptophan, tyrosine, and phenylalanine. This highlights the significance of this group in binding to the active sites of BuChE and AChE proteins. Murranganone (compound 41) had the highest level of activity against BuChE, with an in vitro IC_{50} value of 74.3 μM, while paniculatin (compound 40) had the superior activity against AChE with an in vitro IC_{50} value of 31.6 μM, followed by murranganone (compound 41) with an in vitro IC_{50} value of 79.1 μM (Table 1) [43].

Terpenes

Terpenes are produced through biosynthesis using isoprene units, which have a chemical formula of C_5H_8, and terpenoids are the substances that are obtained after the chemical modification of terpenes [44]. Pratap and colleagues clarified in their study that triterpenoid component (MS-1) (compound 42, Fig. 21) that was isolated from the medicinal plant *Curculigo orchioides*, is a particularly potent inhibitor of DmAChE (binding energy is 7.91 kcal/mol). Moreover, this binding energy surpasses that of donepezil, tacrine, rivastigmine, and galantamine,

Figure 21



42

Terpenes as acetylcholinesterase inhibitors (compound 42).

which are FDA-approved medications classified as cholinesterase inhibitors [45].

Flavonoids

Flavonoids are a group of polyphenols that have a diphenylpropane (C_6-C_3-C_6) structure and are categorized into subclasses according to the linkage between the two aromatic rings, the level of oxidation, and the functional groups present on the third ring [34]. Sadeghi and colleagues reported that the results of enzyme inhibition show that Kolaflavanone (compound 43, Fig. 22) from *Garcinia kola* (Clusiaceae family) and Ginkgetin (compound 44, Fig. 22) from *Selaginella willdenowii* (Selaginellaceae family) had significant inhibitory effects on BuChE and AChE, respectively, which aligns with the findings of molecular docking. Kolaflavanone (compound 43) demonstrated a potent inhibitory action on BuChE, with an in vitro IC_{50} value of 3.6 mM, and the Kolaflavanone–BuChE complex had a docking score of −7.48 kcal/mol, which is expected to surpass that of the donepezil–BuChE complex. Also, Ginkgetin (compound 44) demonstrated a potent inhibitory action on AChE with in vitro IC_{50} of 3.2 mM, and The Ginkgetin–AChE complex displayed a higher level of docking score of −8.72 kcal/mol in comparison to donepezil [46].

Asterric acid derivatives

Xiao and colleagues conducted a study showing that three natural chemicals, namely ethyl asterrate compound (45, Fig. 23), methyl asterrate (compound 46, Fig. 23), and asterric acid (compound 47, Fig. 23), which exhibit a diphenyl ether structure, were extracted from the endophytic fungal strain *Talaromyces aurantiacus* FL15 of *H. serrata* leaves and displayed strong inhibitory effects on AChE, with in vitro IC_{50} values of 20.1, 23.3, and 66.7 μM, respectively. The results indicated that the three asterric acid derivatives demonstrated significant selectivity and moderate inhibition of AChE activity. Their inhibition of AChE was attributed to the esterification R groups located on the 8-carbon parent nucleus [47]. The 3D and 2D ligand interaction of the selected natural product are shown in Fig. 24.

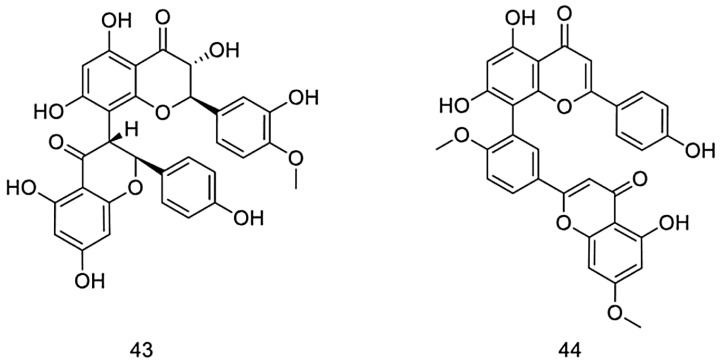
Multi-target acetylcholinesterase inhibitors

AD is a multifactorial disease, and multiple enzymes and pathways are involved in its pathophysiology. This triggers the evolution of multi-target ligands, which involve joining more than one pharmacophore in a single compound (can act concomitantly on multiple targets for AD) [48,49]. In the last decades, multi-targeted drugs have emerged as a prospective strategy for developing effective treatment for AD [49].

Multi-target inhibitors for cholinesterases and β-secretase (BACE-1)

According to the amyloid hypothesis, the accumulation of beta-amyloid (Aß) is the major cause of the pathogenesis of AD [50]. Aβ is the peptide that is formed by proteolytic cleavages of larger β amyloid precursor protein, βAPP. In the amyloidogenic

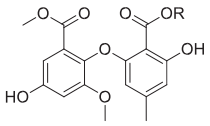
Figure 22



43 44

Flavonoids as acetylcholinesterase inhibitors (compounds 43–44).

Figure 23

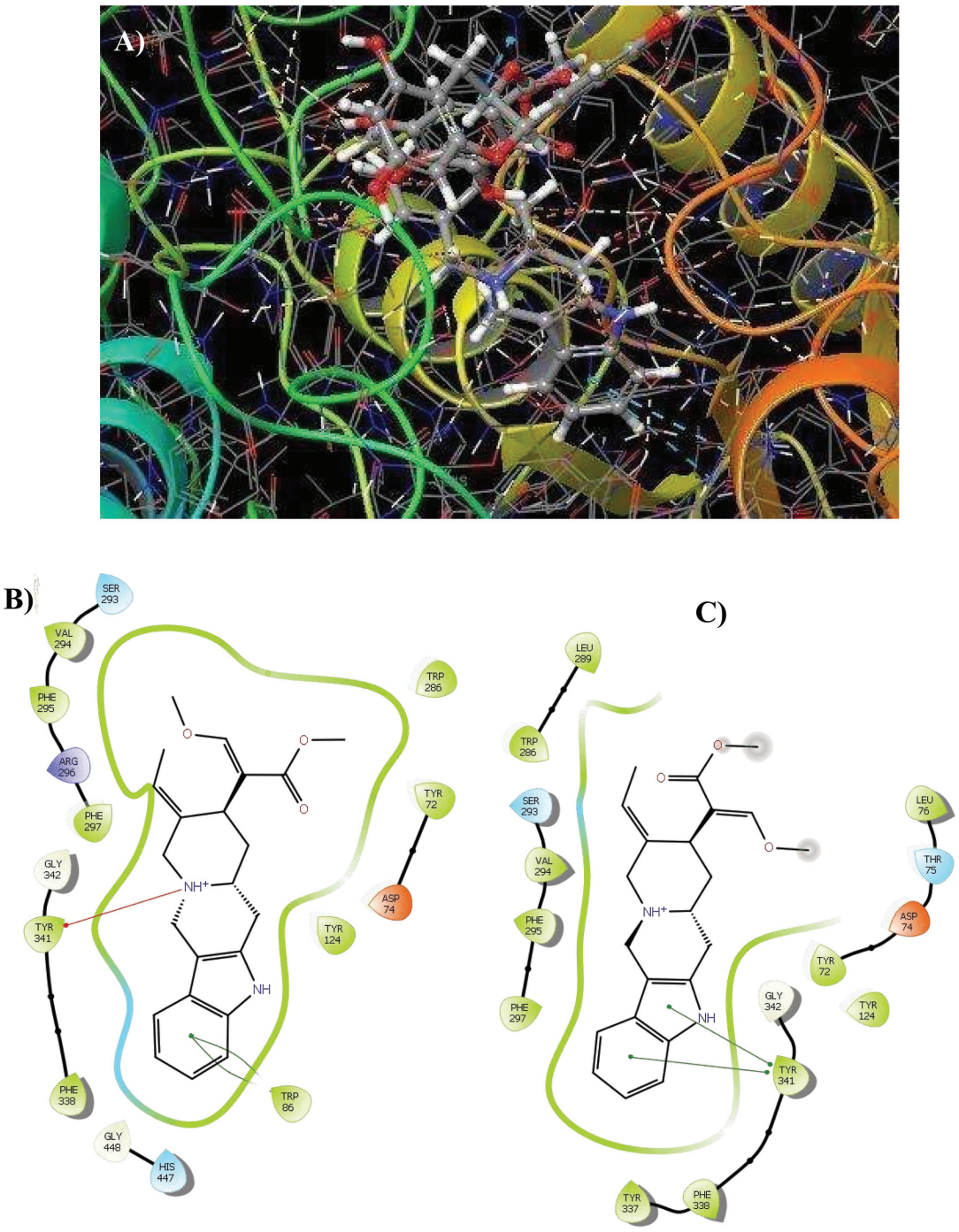


45 R=CH2CH3
46 R=CH3
47 R=H

Asterric acid derivatives as acetylcholinesterase inhibitors (compounds 45–47).

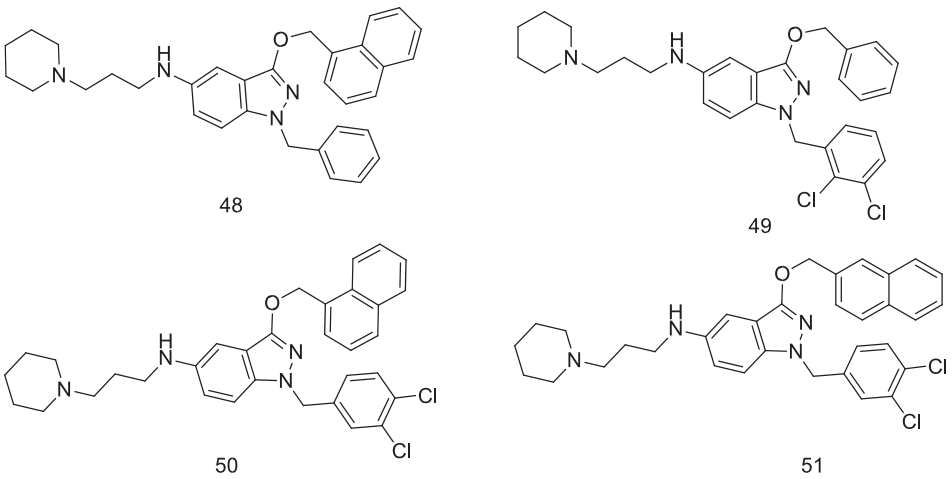
pathway, APP is firstly cleaved by β-secretase enzyme (BACE-1), producing an N-terminal Aβ fragment and a C-terminal fragment, C99. The C-terminal fragment is subjected to further cleavage by γsecretase generating βamyloid peptides and AICDs [51]. Additionally, it was reported that βsecretase inhibitors not only inhibit Aβ aggregation and deposition but also they can decrease the potential toxicity of APP-cleaving products like AICDs and enhance synaptic and cognitive functions [52]. On the other side, in vivo

Figure 24



(a) 3D superimposition of certain selected AChEI in the active site of crystal structure of AChE (PDB: 4EY7); 2D ligand interaction of compound 27 (b); compound 39 (c) within substrate binding site of AChE. AChE, acetylcholinesterase.

Figure 25



Multi-target inhibitors for cholinesterases and β-secretase (compounds 48–51).

and in vitro studies showed that selective inhibition of BuChE reduces the production of Aβ peptide and APP protein [52]. Thus, González-Naranjo and colleagues were inspired to synthesize novel compounds exhibiting multitarget properties, including BACE-1, BuChE, and AChE. Novel 5-substituted indazole derivatives were synthesized and estimated against AChE, BuChE, and BACE-1. In vitro studies revealed that the piperidinopropylaminoindazole derivatives (compound 48, 49, 50, and 51, Fig. 25) act as simultaneous inhibitors for AChE, BuChE, and BACE-1 enzymes [52]. Additionally, these compounds showed antioxidant characteristics and provoked anti-inflammatory and neuroprotective effects [52].

Multi-target inhibitors for cholinesterases and monoamine oxidases

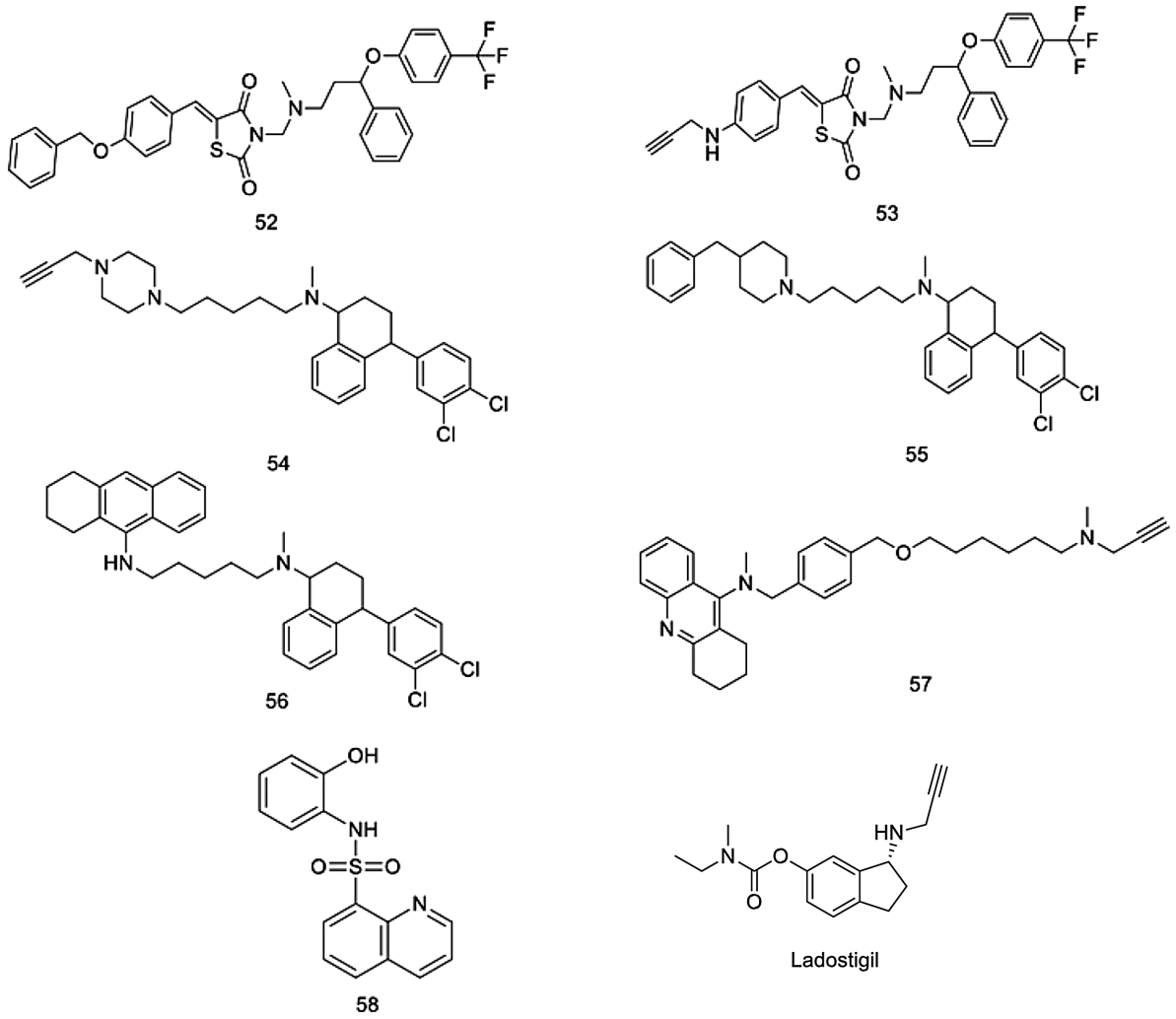
Monoamine oxidase (MAO) is the enzyme that degrades the amine neurotransmitters, including serotonin, norepinephrine, and dopamine, generating reactive oxygen species and hydrogen peroxide (by Fenton reaction) that may cause the death of neuronal cells [53]. It includes two isoforms, MAO-A and MOA-B. It was proposed that MAO is involved in the pathophysiology of different neurodegenerative diseases [54]. MAO inhibitors have protruded as a promising therapeutic approach for impeding the progress of neurological degenerative disease [54]. MAO-B inhibitors are extensively utilized for the treatment of AD [55]. Thus, dual inhibitors of cholinesterases and monoamine oxidases have emerged as an auspicious approach for the treatment of AD.

Ladostigil (Fig. 26) is a propargylamine multifunctional ligand targeting both cholinesterases and monoamine oxidases [56]. Recent studies in pharmacology and chemistry resulted in the evolution of such a multi-target compound, making it a promising compound for clinical trials. It is an aminoindan analog of the selective irreversible MAO-B inhibitor (rasagiline) and is considered a rivastigmine-rasagiline hybrid. It shows 100 times inhibition for BuChE over AChE in rates while demonstrating 71 and 66% inhibitions for MAO-B and MAO-A, respectively, in rats for 2 weeks [56].

Novel hybrids of fluoxetine and sertraline were synthesized and estimated as concomitant inhibitors for both cholinesterases and monoamine oxidases by Nadeem *et al.* [57]. In vitro assay demonstrated that compounds 52, 53, 54, 55, and 56 (Fig. 26) exhibited concomitant inhibitory activity against AChE, BuChE, MAO-A, and MAO-B with in vitro IC_{50} values in the submicromolar to nanomolar values. Molecular modeling studies showed that the active compounds occupied both CAS and PAS subsites of the AChE active site. Additionally, all synthesized compounds occupy the large active site cavity of the MAO-B enzyme [58].

In 2024, Huang *et al.* [59] designed and synthesized a novel series of tacrine-selegiline hybrids as multi-target inhibitors against cholinesterases and monoamine oxidases. All synthesized hybrids were evaluated for their inhibitory activity against cholinesterases and monoamine oxidases using in vitro techniques. Compound 57 (Fig. 26) showed potent and balanced inhibitory activity against AChE, BuChE, MAO-A, and MAO-B with in vitro IC_{50} values of 1.57, 0.43, 2.30, and 4.75 μM (Table 1), respectively. Molecular modeling studies revealed that compound

Figure 26



Multi-target inhibitors for cholinesterases and monoamine oxidases (compounds 52–58 and ladostigil).

57 could occupy both CAS and PAS of the AChE active site and demonstrated binding affinity to BuChE and MAO-B. Furthermore, in vivo evaluation revealed that compound 57 could enhance the cognitive functions of tested mice (scopolamine-induced memory impairment animal model), showing no acute toxicity [59].

Novel quinoline–sulfonamide derivatives were designed, synthesized, and estimated for their dual inhibitory activity against cholinesterase and monoamine oxidases by Jalil *et al.* [60]. In vitro assay revealed that compound 58 showed concomitant inhibitory activity against AChE, BuChE, MAO-A, and MAO-B with in vitro IC_{50} values 0.58±0.05, 1.72±0.68, 1.25±0.45, and 1.09 ±0.65 μM (Table 1), respectively. Compound 58 (Fig. 26) was docked into the AChE active site to investigate the binding affinity, oxygen of sulfonamide moiety formed a hydrogen bond with Gly116, sulfur atom formed a π–sulfur interaction with Phe329 residue, and methoxy hydrogen interacted with Tyr332 by a carbon-hydrogen bond. Moreover, all synthesized quinolines interacted with amino acid residues of MAO-B active site via van der Waals forces, hydrophobic interactions besides hydrogen bonds [60].

Conclusion

The present review highlights the latest advancements in the development of novel single-targeted, multi-targeted, and naturally derived AChEIs. Various chemical scaffolds, including coumarins, chalcones, piperazines, aminoquinolines, lophines, dihydrofurans, and triazoles, have demonstrated significant inhibitory activity against AChE, and their interactions with the enzyme's active site have been elucidated. This research underscores the potential of these diverse scaffolds as lead

compounds for the development of effective AChEIs. Additionally, the study emphasizes the promising role of endophytic fungi as a source of unique natural products with cholinesterase-inhibiting properties. Given the complexity of AD pathophysiology, with multiple pathways and enzymes involved, multi-targeted ligands have emerged as a promising therapeutic strategy. Consequently, current research is expanding beyond AChE inhibition to include other critical targets, such as BACE-1 and MAO enzymes. This review not only provides a comprehensive overview of recent progress but also underscores the critical need for continued innovation in the search for effective treatments for AD.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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