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RECENT ADVANCES OF CHOLINESTERASE INHIBITORS PLAYING A CRITICAL ROLE IN THE TREATMENT OF ALZHEIMER'S DISEASE (2020-2022)

ALZHEİMER HASTALIĞININ TEDAVİSİNDE KRİTİK BİR ROL OYNAYAN KOLİNESTERAZ İNHİBİTÖRLERİNDEKİ SON GELİŞMELER (2020-2022)

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ABSTRACT

Alzheimer's disease (AD) is a common neurodegenerative disorder which has a catastrophic effect on the brain. It significantly affects people's daily life, especially the population over the age of 65, with problems such as neuron death, memory loss, cognitive disorders, and cholinergic dysfunction. However, despite the drug development studies which have been conducted recently quite intensively, an effective drug molecule that can completely cure people or stop the stage of the disease has not been found yet, unfortunately. One of the reasons for this is the complexity of understanding of how the pathology of the disease arises. The most accepted theory for AD is the cholinergic hypothesis. Acetylcholine (ACh), an important neurotransmitter, is metabolized by two cholinesterase (ChE) enzymes named acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). With AD, ChE activity increases and thus the degradation of acetylcholine is triggered. In addition, it is understood that the peripheral anionic region (PAS) of the AChE also caused the formation of Aβ-peptide fibrils. Therefore, based on this hypothesis, the most effective approach in treatment is the use of cholinesterase inhibitors (ChEI), which try to restore ACh levels by increasing them at cholinergic synapses. Furthermore, it focused on the design of multifunctional molecules. In conclusion, considering the complex nature of the disease and its effects, it is clear that more studies are needed in this area. In this study, important and remarkable studies in recent years have been included and it aimed to contribute to future drug research and development studies.
Keywords: Alzheimer's disease (AD), cholinesterase inhibitors (ChEIs), acetylcholinesterase inhibitors (AChEIs), butyrylcholinesterase inhibitors (BuChEIs), multifonctional drugs, multi-target directed ligands

ÖZ

Alzheimer hastalığı (AD) oldukça sık rastlanılan ve majör yıkıcı bir etkiye sahip olan nörodejeneratif bir hastalıktır. Özellikle 65 yaş üstü nüfusu büyük oranda tutan, hasta kişinin nöron ölümü, hafıza kaybı, bilişsel ve kolinerjik işlev bozuklukları gibi sıkıntılarla günlük yaşamını önemli derecede etkileyen bir durumdur. Fakat son dönemde yapılan ilaç geliştirme çalışmalarının oldukça yoğun yürütülmesine rağmen, hala kişileri tamamen iyileştirebilecek veya hastalığın evresini tamamen durdurabilecek etkili bir ilaç molekülü maalesef bulunamamıştır. Bunun sebeplerinden biri de hastalığın patolojisinin nasıl ortaya çıktığının anlaşılamaması ve karmaşıklığı ile ilgilidir. Alzheimer hastalığı için kabul gören en önemli teori kolinerjik hipotezdir. Önemli bir nörotransmitter olan asetilkolin (ACh), asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BuChE) isimli iki kolinesteraz enzimi (ChE) tarafından metabolize edilir. AD ile birlikte ise ChE aktivitesi artmış ve dolayısıyla asetilkolinin yıkımı tetiklenmiştir. Ayrıca AChE'in periferal anyonik bölgesi (PAS)'nin Aβ-peptid fibrillerinin oluşmasına da neden olduğu anlaşılmıştır. Bu sebeple bu hipoteze dayanarak tedavideki en etkili yaklaşım, kolinerjik sinapslarda ACh seviyesini arttırarak eski haline döndürmeye çalışan kolinesteraz inhibitörlerinin (ChEI) kullanılmasıdır. Üstelik multifonksiyonel moleküllerin tasarımları üzerine de odaklanılmıştır. Sonuç olarak hastalığın karmaşık yapısı ve etkileri göz önüne alındığında, bu alanda daha fazla çalışma yapılması gerekmektedir. Bu çalışmada son yıllardaki önemli ve dikkat çeken çalışmalar yer almış olup, bundan sonraki ilaç araştırma ve geliştirme çalışmalarına katkı sağlaması amaçlanmıştır.
Anahtar kelimeler: Alzheimer hastalığı (AD), kolinesteraz inhibitörleri (ChEIs), asetilkolinesteraz inhibitörleri (AChEIs), butirilkolinesteraz inhibitörleri (BuChEIs), multifonksiyonel ilaçlar, çoklu hedefe yönlendirilmiş ligandlar

INTRODUCTION

Dementia, a major neurodegenerative disorder, is a disease that significantly affects a person's memory, behavior, mental abilities, and social life (1). In 1906, Alzheimer's disease (AD) was first defined as the condition of a serious disease of the cerebral cortex by the German psychiatrist Alois Alzheimer, after the examination of the brain autopsy of a patient who had some personality differences and lost his memory before he died (2, 3). It is the most common type of dementia worldwide,

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which is chronic-permanent and very common especially in people over the age of 65, although the exact cause is unknown, the pathology can first be detected by;

- the accumulation of intracellular tau proteins (by hyperphosphorylation) and extracellular beta-amyloid (Aβ) aggregates,
- the occurrence of neuronal degeneration with neurofibrillary tangles (NFTs) after upregulation of β-sesterase,
- oxidative stress,
- neuroinflammation,
- the low levels of acetylcholine (ACh) neurotransmitter in the hippocampal and cortical region of the brain (1, 4-8).

For this reason, neuronal death and damage to the hippocampal part of the brain occur with the changes as a result of AD. Besides, symptoms which are characterized by insidious onset and slow progress, such as agitation, disinhibition, apathy, difficulty in making decisions, difficulty in finding words, severe speech disorder and forgetfulness, psychosis, anxiety and sleep disturbance may occur and it is highly progressive (2, 4, 7). Physiological differences between the normal brain of a healthy person and the brain of a person with AD are shown in Figure 1. Advanced age, gender, genetic factors (70% probability), head injuries, vascular disorders, obesity, diabetes, infections, lifestyle and environmental factors (heavy metals, air pollution and others) can be counted as threatening situations that may cause the disease (3). Although it is considered to affect the elderly population, a familial AD caused by a gene mutation caused death in a 28-year-old (9). As stated in the World Alzheimer's report of 2021, there are more than 55 million Alzheimer's patients worldwide today, and this number is estimated to reach 78 million by 2030 (1). Dementia in the world population data in 2016; It was reported as the 5th largest cause of death among 2.4 million deaths and the 2nd largest one in people over 70 years of age (5). The World Health Organization has stated that AD may be more common than cancer, cardiovascular diseases and AIDS within the next century (10).

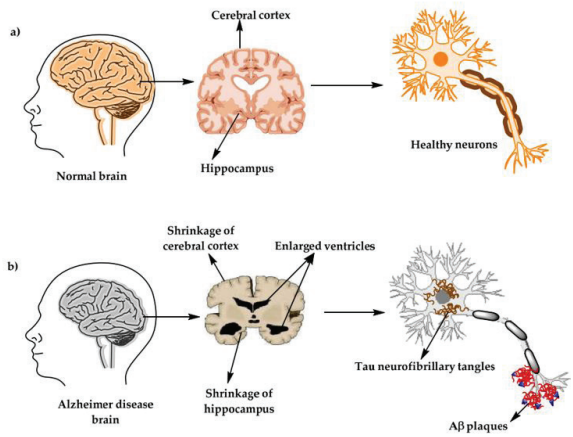


Figure 1: Physiological differences between the normal brain of a healthy person and the brain of a person with Alzheimer's disease (3).

Current Alzheimer's drug therapy

Acetylcholine is the key for both the central nervous system (CNS) and autonomous nervous system, the biomarker in AD, which is of such importance, and responsible for memory and learning tasks in the brain by providing communication between two nerve cells. Acetylcholinesterase enzyme plays an important role in the regulation of acetylcholine and the first theory is the cholinergic hypothesis based on it (4, 11). As it is known, cholinergic transmission is quite significant in cerebral cortical development and activity, cerebral blood flow, sleep-wake cycle, memory, learning and cognition. Furthermore, the biological response to acetylcholine-transmitted warning signals in the cholinergic system is given by a post-synaptic receptor (muscarinic or nicotinic receptor) (12). In order to increase the density of ACh, which decreases with the increase of acetylcholinesterase enzyme as a result of Alzheimer's disease in brain cholinergic synapses and also in neuromuscular junctions, to make up for the cholinergic loss, and to increase cholinergic transmission, it is attempted to block acetylcholinesterase and butyrylcholinesterase enzymes that break down this neurotransmitter into choline and acetate (2, 4, 13, 14). Butyrylcholinesterase enzyme, which is the isoenzyme of acetylcholinesterase, a member of the cholinesterase enzyme family (also known as serum cholinesterase and contains 65% homologous amino acid residues with AChE) is a minor element in the brain of a healthy person, while it is increased due to plaque and tangles in the brain of the patient with AD. And therefore impaired cholinergic transmission can also be treated by inhibition of BuChE (15, 16). But in advanced AD, on the contrary, the AChE level is severely reduced due to severely damaged neurons (15). Since the BuChE enzyme, which is also called as pseudocholinesterase, is synthesized in the liver and then distributed in the plasma, it is less specific to acetylcholine, and AChE activity plays a more dominant role in acetylcholine hydrolysis at neuromuscular junctions and cholinergic synapses (5, 15, 17). It was reported that BuChE-rich neurons in the human cerebral cortex were only half as large as neurons with dense AChE (15). Additionally, when the structure of AChE was examined, it was understood that the peripheral anionic site (PAS) of the enzyme also causes the formation of Aβ-peptide fibrils. Thus, it was also studied on the designs of molecules that would interact with the AChE enzyme binding site like the PAS and prevent the formation of Aβ-peptide fibrils. What makes AChEI the most effective and most successful treatment method is not only the fact that these drug molecules increase cholinergic transmission, but also the fact that it creates a dual solution by preventing Aβ synthesis, accumulation and formation of aggregates (18).

In summary, AChE enzyme is preferred as a target in drug development due to its structure and relationship with acetylcholine, but BuChE enzyme also gains importance as a target to be inhibited because it will increase to 105-165% of normal condition in advanced AD (15).

Researchers have discovered a catalytic anionic site (CAS) at the bottom of a narrow passage, in which the hydrophobic amino-

acid side chains are located. CAS consists of a few subsites: the anionic site, where the interaction with ACh takes place, the esteratic site (ES), where three residues of the catalytic triad are included, the oxyanion hole, and the acyl pocket, which provides substrate selectivity (12). The catalytic triad (serine, histidine and an acidic residue which is also a glutamate) is included in AChE enzym's catalytic site and has a crucial importance (12, 15). A choline-binding site have hydrophobic tryptophan residues while it is expected to have anionic groups. Another significant subunit known as the peripheral anionic site (PAS) has also been found approximately 15A˚from the CAS (12). Structural features of the AChE enzyme are shown in Figure 2.

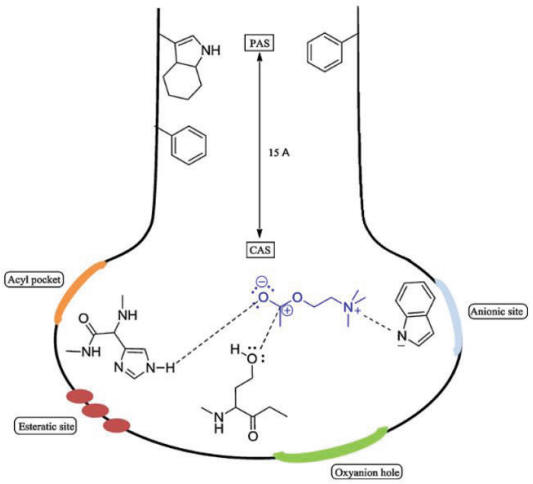


Figure 2: Structural features of the AChE enzyme (as known as serine hydrolase) (12).

Although the communication of the substrate with the active site seems to take a long time based upon the path it will take, it is considered that this reaction takes place very quickly thanks to the electric field of AChE. Despite electrical field reduction attempts, the rate of the enzyme has not changed. In the literature, it was noted that AChE hydrolyzes about 25000 AChs per second (19). The serine amino acid containing the hydroxyl group (acts as a nucleophile) and the histidine amino acid containing the imidazole ring (acts as a base) in the structure of the AChE hydrolyze ACh in its ester structure and allow the release of choline and the formation of the intermediate Acetly-AChE, and subsequently let the intermediate product hydrolyze to release acetate. The AChE reaction is shown in Figure 3.

The acetylcholine neurotransmitter is synthesized from choline and acetyl-coenzyme A in the cytoplasm of neurons by the enzyme choline acetyltransferase (ChAT) and is transported via the vesicular acetylcholine transporter (VAChT). According to Cholinergic Hypothesis; In the 1970s, it was thought that the source of the problem in cholinergic transmission was the enzyme choline acetyltransferase (ChAT), which enabled the synthesis of acetylcholine. However, later studies pointed out the AChE enzyme. Discovery of interactions between AChE and Aβ peptide supported this idea (3). The cholinergic synapse is shown in Figure 3.

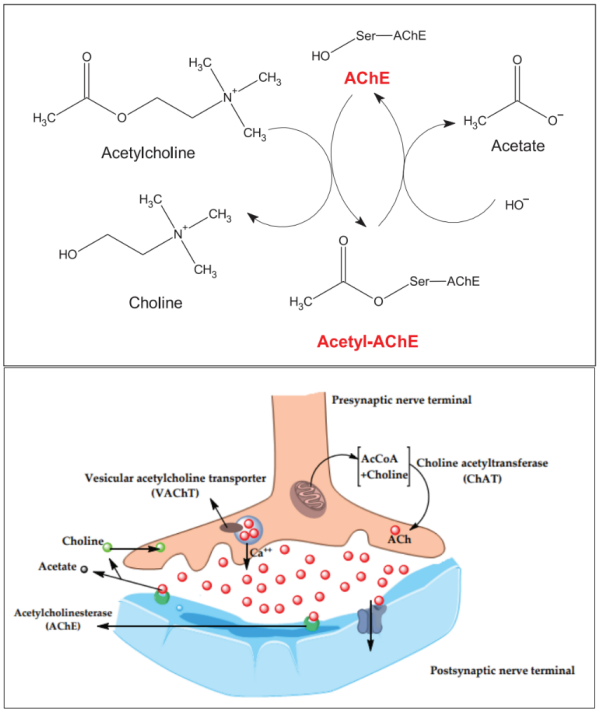


Figure 3: The acetylcholinesterase (AChE) reaction (19). And the cholinergic synapse (3).

Unfortunately, the progression of Alzheimer's disease cannot be stopped or completely cured with current medications, but they are helpful in reducing symptoms and improving the person's quality of life (8). In addition, cholinergic-acting cholinesterase enzyme inhibitor (ChEI) molecules, which are also approved by the Food and Drugs Administration (FDA), constitute an important group of drugs in the treatment in the early stages of Alzheimer's disease (2, 4, 16). These are:

- Tacrine: It has the tetrahydroacridine structure and it was the first FDA approved ChEI (in 1993). It inhibits both AChE and BuChE.
- Donepezil: It is an indanonebenzylpiperidine derivative (FDA approval in 1996) and it selectively inhibits AChE.
- Galantamine: It is an alkaloid derivative (FDA approval in 2003) and it selectively inhibits AChE.
- Rivastigmine: It is a carbamate derivative (FDA approval in 2006) and it makes non-specific inhibition (2, 9, 14).

Apart from ChEIs, memantine, a non-competitive antagonist of glutamate N-methyl-D-aspartate (NMDA) receptors, which was FDA-approved (in 2003) is also used in the treatment of AD (5, 8, 9). Nevertheless, as well as their benefits, these molecules may also have serious peripheral cholinergic side effects such as gastrointestinal discomfort, bradycardia, diarrhea, loss of appetite, nausea, vomiting, sweating, muscle cramps, fatigue, increased frequency of bowel movements, insomnia, headache, dizziness, loss of consciousness, excitement, extreme fear, hallucination, urinary incontinence and bronchoconstriction.

Thus, new drug molecules are being developed in order to improve the selectivity and side-effect profiles (2, 4, 6, 8, 13). The chemical structures of traditional ChEIs are shown in Figure 4.

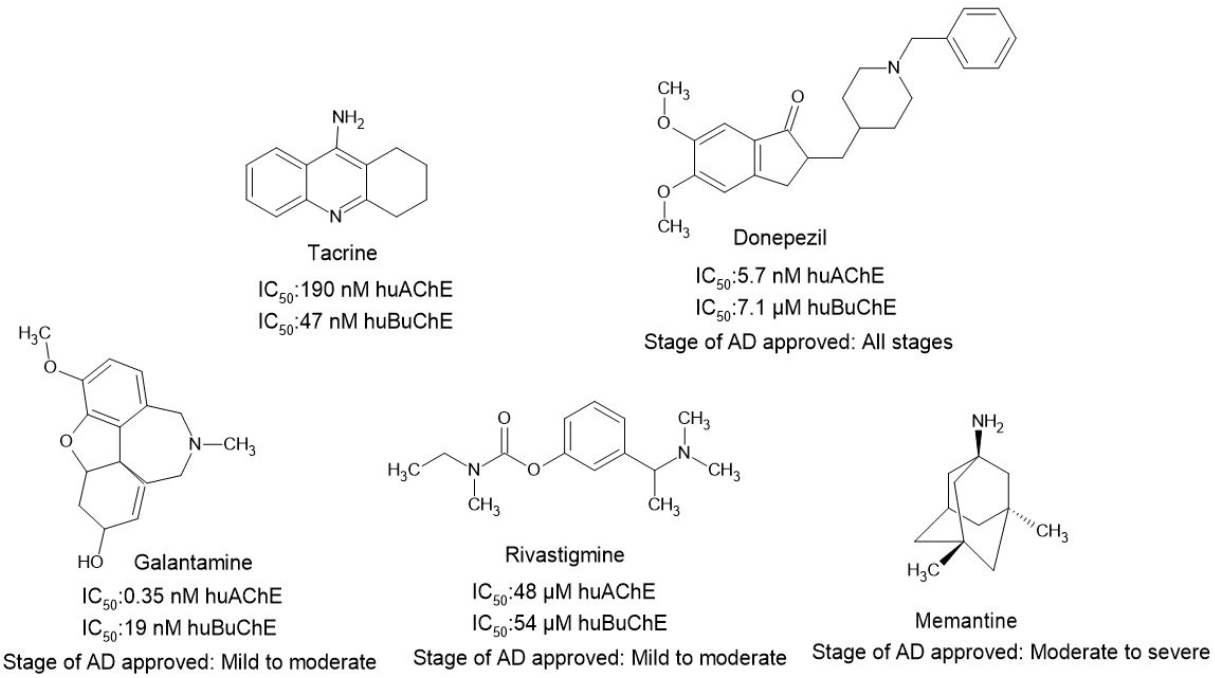


Figure 4: Chemical structures of cholinesterase enzyme inhibitor (ChEI) drug molecules and their inhibitory values against human acetylcholinesterase enzyme (huAChE) and human butyrylcholinesterase enzyme (huBuChE), and non-competitive antagonist of glutamate N-methyl-D-aspartate (NMDA) receptors, memantine (5, 14).

While donepezil, rivastigmine and galantamine are currently used clinically for Alzheimer's disease, tacrine was withdrawn from the market due to its severe hepatotoxic effect and drug-drug interactions (2, 14). In a study it was discovered that memantine and donepezil molecules can also be used as a dual combination in the treatment (8). In another study, it was noted that in a situation where donepezil or galantamine molecules cannot improve, cognition and behavior with the rivastigmine molecule was better, and if the progression of the disease continued, the combination therapy of adding memantine molecule to the treatment (rivastigmine + memantine) was also beneficial (20).

In drug research, AChE is an enzyme system targeted not only for AD, but also for the treatment of neurological diseases such as Lewy body dementia, Parkinson's disease dementia, schizophrenia, and diseases such as myasthenia gravis, glaucoma, and anticholinergic poisoning (21, 22).

The first of the methods focused on in drug development studies is the synthesis of analogs and derivatives of molecules synthesized from nature, the second is the synthesis of analogs and derivatives of molecules synthesized from existing drug molecules, the third is the design of molecules that will interact with the valuable parts of the AChE enzyme binding site like the PAS, and finally the use of methods such as structure-based pharmacophore modeling, molecular docking, etc. (22).

Therapeutic approaches that focus on solving and understanding AD and trying to stop or change the progress of the disease are; a) stopping oxidative damage to neurons; b) stopping or reducing the increase of amyloid protein; c) fixing the loss in the cholinergic system; d) increasing or restoring the amount of acetylcholine (3, 23).

It is understood from the studies conducted in recent years that both AChE and BuChE, which are important drug targets in the central nervous system, were of great importance. Although the focus is on AChE at the beginning of AD, while this enzyme decreases, the BuChE enzyme level increases and takes over the task of AChE. Therefore, ACh level is closely related to both enzyme systems. For this reason, the design of Dual-Target Inhibitors has gained importance and studied by researchers. The molecule **1** in the Figure 5 inhibited both AChE (eeAChE IC_{50} = 0.39 µM) and BChE (eqBChE IC_{50} = 0.28 µM) enzymes (by binding to the CAS and PAS regions of enzymes), and the molecule was reported to have lower cytotoxicity than tacrine. Therefore, it is thought to be promising in AD as a safe and multipotent molecule (24).

Considering the effective molecules in the treatment, some compounds formed as a result of hybridization (these are shown in Figure 5) have been discovered to be highly potent AChE and BuChE inhibitor molecules (14).

In a study where researchers designed and synthesized a series of acridine derivatives containing 1,3,4-thiadiazole moiety, it was observed that all new molecules inhibited both ChE enzymes but had high selectivity against AChE. It has been noted that the molecules **6** and **7** in Figure 6 have better inhibitory

power and much lower hepatotoxicity than tacrine. It has been reported that the molecules provide inhibition by binding to site 2, a new allosteric site of AChE, instead of site 1 (CAS/PAS) (25).

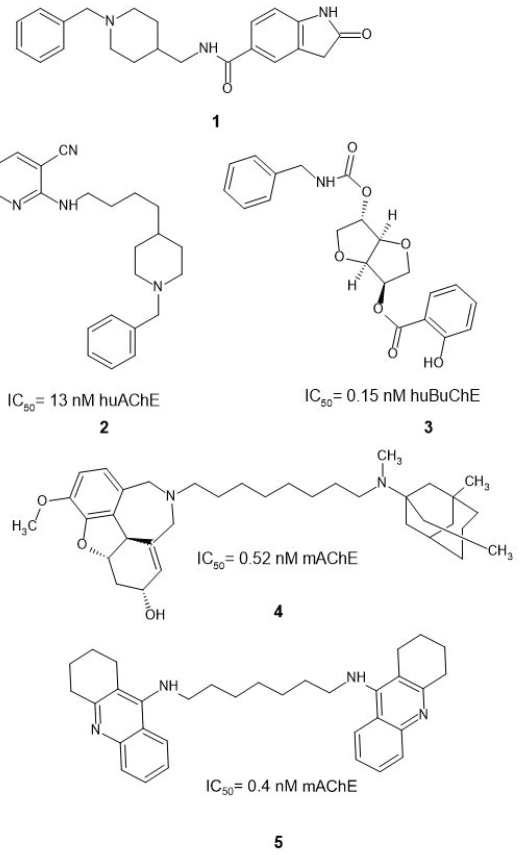


Figure 5: Examples of molecules and potencies discovered to inhibit potent AChE and BuChE (human AChE (huAChE), human BuChE (huBuChE), murine AChE (mAChE) (14, 24).

In order to prevent hepatotoxicity caused by the free amine group in tacrine, new tacrine analogues were looked for. It has been stated that these analogs are safer molecules with low toxicity compared to tacrine, and that molecules bind to the enzyme through hydrogen bond interactions and provide inhibition. The IC_{50} values of the compound **8** and **9** with piperazine containing acetamide and butyrylamide chains have shown in the Figure 6 were 0.52±0.03 and 0.73±0.04 µM, 0.71±0.04 and 1.01±0.03 µM, respectively, and showed an inhibition effect against AChE and BuChE respectively (10).

One of the AD treatment goals by researchers is to prevent Aβ aggregation. Normally, the Aβ peptide structure consists of 39-42 amino acids but it was discovered that oligomers consisting of the Aβ1–42 peptide are much more toxic, so the formation of Aβ1–42 must be stopped. For this purpose, in a study designed to evaluate novel multifunctional inhibitors, it was noted that the most potent molecule (molecule **12** is shown in Figure 7) had 100 times more selectivity against BuChE, while it showed IC_{50}: 180 nM value against AChE. In addition, it was understood that all four synthesized molecules showed preventive and neuroprotective activities against Aβ accumulation. It was an important step for drug candidate molecules that could be used in AD (26).

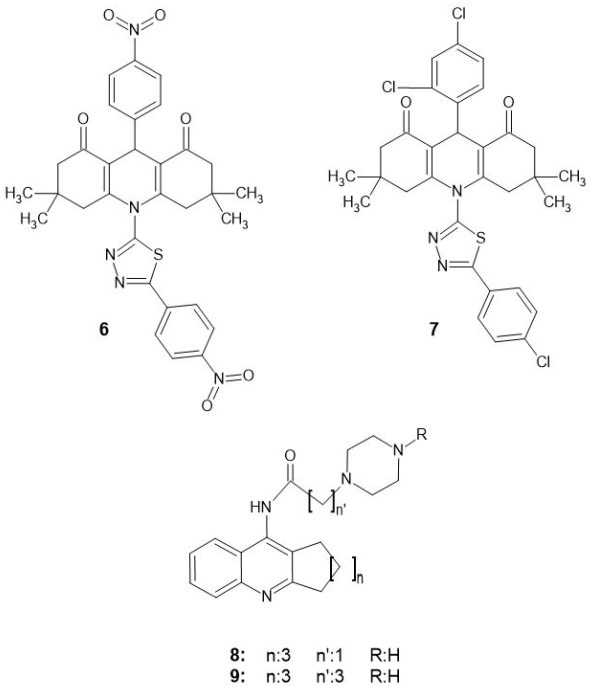


Figure 6: The chemical structures of acridine derivatives containing 1,3,4-thiadiazole moiety (molecule **6** and **7**), analogues of the tacrine (molecule **8** and **9**) (10, 25).

We are in an era when multi-target molecules are in demand. Therefore, based on tramiprosate, which has selective anti-Aβ oligomers aggregating activity, a series of novel compounds based on tramiprosate was designed and synthesized. In the designed compounds, the addition of a pyridinium/isoquinolinium ring to the tramiprosate moiety increased the efficiency of the molecules to the binding sites in ChE enzymes, but sulfonic acid moiety prevented this binding. It has been reported that new derivatives synthesized by removing this fragment inhibit over 10% Aβ aggregation at 1 µM concentration, while they can inhibit 70% Aβ on ChEs. The most effective molecule with dual inhibition (with >85% inhibition, at 100 µM), low cytotoxicity and anti-Aβ aggregation (18% inhibition) properties was shown in the Figure 7 as compound **15**. In addition, according to an experiment performed on AD mice, this molecule crossed the blood brain barrier, suggesting that it may be useful for drug molecules *in vivo* (27).

In another study, amine, oxime, ether, epoxy and acyl derivatives of the benzobicyclo[3.2.1]octene were synthesized and most of them were found to have a higher selectivity capacity against BuChE. Among the synthesized compounds, compound **16** in Figure 7 showed the highest AChEI effect with an IC_{50}=8.3 µM. Compound **17** in Figure 7, on the other hand,

has 5 times stronger inhibition properties against BuChE than Huperzine A (28).

Studies showed that zinc accumulates in high concentration in the brains of people with AD. While the Zn^{2+} ion is necessary for neural functions in a healthy individual under normal conditions, excessive accumulation of this ion causes the aggregation of neurotoxic Aβ, oxidative stress and the secretion of high levels of proinflammatory cytokines. For this reason, studies for metal chelation therapy are being tried. In a study synthesizing 8-substituted derivatives of the sampangine alkaloid, which had strong antibacterial and antifungal effects, multifunctional agents that could better cross the blood-brain barrier and selectively chelate Zn^{2+} ion, as well as high AChE enzyme inhibitory activity, were detected. The most successful derivative in the study is given in the Figure 7 as compound **19** with IC_{50}: 0.27±0.01μM against AChE (29).

AChEI and anti-Aβ aggregation properties of phosphoshazine and phosphazide derivatives were also discovered and synthesized. It was noted that a coumarin phosphazide derivative compound (it is shown in Figure 7 as compound **20**) could bind to MMP-2 (Matrix metalloproteinase-2) and Zn^{2+}-induced Aβ42 aggregation, PAS and CAS at the same time, which caused AChE inhibition and had low toxicity data. Matrix metalloproteinases, which should be at lower levels in the human brain under normal conditions, unfortunately increase in AD and cause neurotoxicity together with Aβ. According to this known fact, adding extra "MMP-2 inhibitory" activity to the discovered molecule is a great improvement in terms of multifunctional drug developments and should be studied (30).

In recent years, drug candidates obtained from natural sources related to AChE inhibition were investigated. Some examples are; the galantamine molecule that has been isolated from *Galanthus nivalis* and Huperzine A alkaloid obtained from a medicinal plant named *Huperzia serrata* (22). The chemical structures of Huperzine A and neuroprotective dimeric AChE-Is derived from the tacrine and/or huperzine A fragment are shown in Figure 8 (31). In addition, while the polysaccharides (are shown in Figure 8) contained in *Pleurotus ostreatus* (aka Oyster Mushroom) are known for their antitumor and antioxidant properties, they are proved to improve learning and memory impairment by reducing cognitive deficits, AChE activity and oxidative stress in AD (5).

Apart from plants, bioactive inhibitors were also found in marine sponges. Moreover, it was demonstrated that these compounds have a better side-effect profile than synthetically produced molecules and provide neuroprotective activity with a very good bioavailability. Examples of these are gracilin derived from *Spongionella gracilis*, a marine sponge that has therapeutic potential in AD. (The chemical structures of the Gracilin A derivative molecules are shown in Figure 8) For this reason, marine life has become very interesting for researchers due to its important effects on the pathogenesis of AD in various *in vivo* and *in vitro* studies (9).

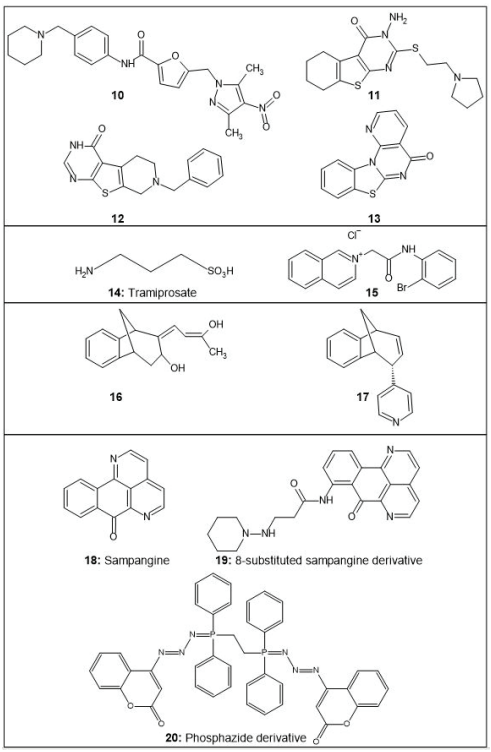


Figure 7: The chemical structures of novel multifunctional inhibitor molecules **10**-**13** (26). The chemical structures of tramiprosate and the novel compound based on tramiprosate (compound **15**) (27). The chemical structures of compound **16** and **17** (28). The chemical structures of sampangine, 8-substituted sampangine derivative and phosphazide derivative (29, 30).

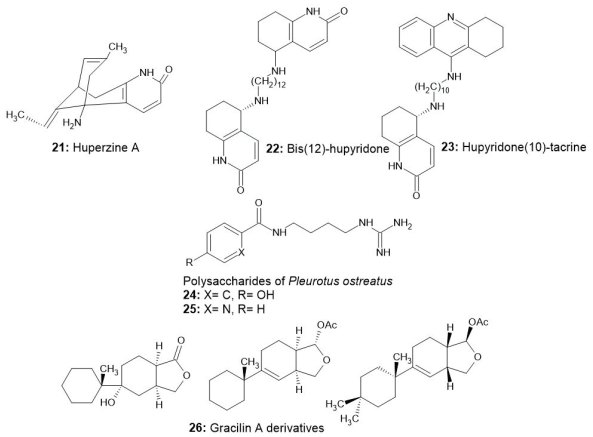


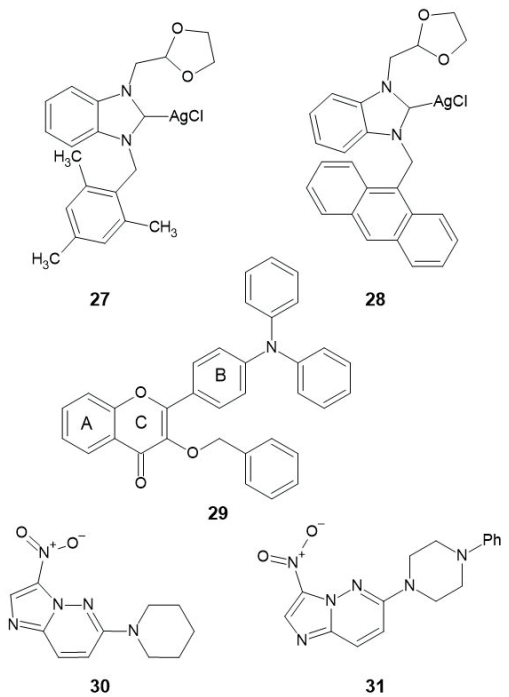
Figure 8: The chemical structures of Huperzine A, AChEIs derived from the tacrine and/or huperzine A fragment, polysaccharides of *Pleurotus ostreatus* and Gracilin A derivative molecules (5, 9, 31).

In the last decade, studies on the antimicrobial activity of silver salts have emerged. Since antimicrobial, antiproliferative, anticancer, anti-HIV, antioxidants, and anti-inflammatory properties of N-Heterocyclic Carbenes (NHC) are known, new NHC salts containing benzimidazole-moeities and their silver (I) complexes were prepared. Compounds **27** and **28** in Figure 9 were

measured with IC_{50} values of 8.56±1.17 μM and 5.05±0.30 μM against AChE and BChE, respectively, and were identified as potent inhibitors. They also showed moderate antibacterial and antifungal activities (32).

A number of 3-benzyloxyflavone derivatives have been synthesized and observed to have potent dual inhibitory effects against AChE and BChE. Overall, these compounds turned out to be more active against AChE than BChE. Due to the presence of a bulky and hydrophobic diphenylamino group substituent at the 4-position of the B ring, the molecule **29** in Figure 9 interacted with active pockets of both enzymes and became the most potent ChE enzyme inhibitor (33).

Polar nitro and amino groups substitutes were added to imidazo[1,2-b]pyridazine compounds, which is a pharmacophore group for analgesic and anti-inflammatory properties, and their biological activities were measured. The substituted 3-nitro-6-amino-imidazo [1,2-b] pyridazine compounds (**30** and **31**) in the Figure 9 showed strong AChE inhibition. In addition, it has been reported that these compounds are multifunctional compounds that cause antiproliferative, anti-migratory and anti-inflammatory activities at high doses (34).



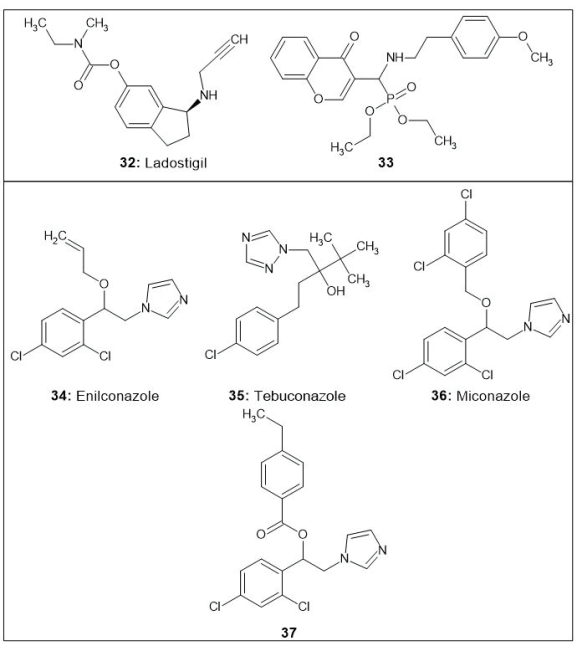
27 28 29 30 31

Figure 9: The chemical structures of N-Heterocyclic Carbenes salts containing benzimidazole-moeities and their silver (I) complexes (molecule **27** and **28**), the 3-benzyloxyflavone derivative (molecule **29**) and the substituted 3-nitro-6-amino-imidazo[1,2-b]pyridazine compounds (molecule **30** and **31**) are shown in Figure 9 (32-34).

Ladostigil, a synthetic molecule, is a pluripotential neuroprotective drug that inhibits cholinesterase enzymes and brain selective monoamine oxidase -A and -B. Therefore, it is thought to be beneficial especially for dementia with depression and it has come up to phase IIb studies (35). While some ChEIs such as rivastigmine have a short half-life and serious dose-related side effects are encountered in long-term use, the side-effect profile of Ladostigil has been found to be better. However, the potential of this compound to cross the blood-brain barrier is weak (21). The chemical structure of the Ladostigil is shown in Figure 10.

It has been reported that the N-substituted α-aminophosphonate-bearing chromone moiety molecule in Figure 10 (compound **33**) shows AChE enzyme inhibition with an IC_{50}=0.103±0.24 μM that is twice as potent as tacrine, 35 times more than galantamine, and 50 times more potent than rivastigmine. In addition, the compound has the ability to bind to the CAS and PAS regions of both ChE enzymes and has DNA damage protection efficacy. In the study, it has been also observed that the synthesized aliphatic amine analogs provide better inhibition against AChE, while aromatic amine analogs provide better inhibition against BuChE (36).

It has been discovered that enilconazole, one of the antifungal drugs, provides 43% inhibition of AChE at 0.3 μg/ml, tebuconazole provides dose-dependent inhibition of AChE and BChE and miconazole provides dual ChE inhibition, improving memory disorders in the brain of mice with AD. Based on the fact that the azole ring has ChE enzyme inhibitory activity as well as antifungal, antiviral, antibacterial, antitubercular, anticonvulsant, anti-inflammatory effects, compound **37** in Figure 10 that provides AChE inhibition with IC_{50}: 8.77μM has been reported. In particular, it has been reported that these molecules provide inhibition by allowing the imidazole ring to bind with important residues of ChE enzymes (17). The chemical structures of enilconazole, tebuconazole, miconazole and compound **37** are shown in Figure 10.



32: Ladostigil **33** **34**: Enilconazole **35**: Tebuconazole **36**: Miconazole **37**

Figure 10: The chemical structure of Ladostigil and the N-substituted α-aminophosphonate-bearing chromone moiety molecule (compound **33**) (35- 36). The chemical structures of enilconazole, tebuconazole, miconazole and compound **37** (17).

The carbazole-coumarin hybrid compound shown in Figure 11 as compound **38** shows high inhibition and selectivity towards AChE and BuChE enzyme. According to the study, the length of the binding site affects the degree of AChE enzyme inhibition, and the moiety of coumarin affects the degree of BuChE enzyme inhibition. It has been discovered that the synthesized molecule provides this inhibition by interacting with amino acids in both the CAS and PAS regions of AChE. In conclusion, selective and dual binding site inhibitors of AChE are promising for future drug designs of AD, by increasing cholinergic stimulus and additionally preventing Aβ aggregation (37).

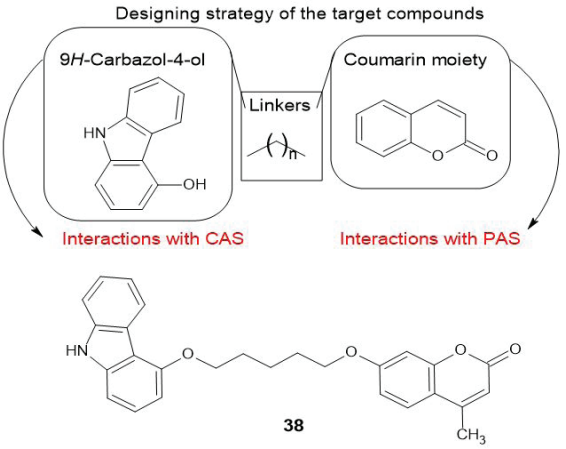


Figure 11: Designing strategy of the target compounds and the chemical structure of compound **38** showing high selectivity and high inhibitory activity towards both AChE and BuChE enzymes (37).

The compounds in Figure 12 are included in the literature as new original patented AChE inhibitor compounds (5).

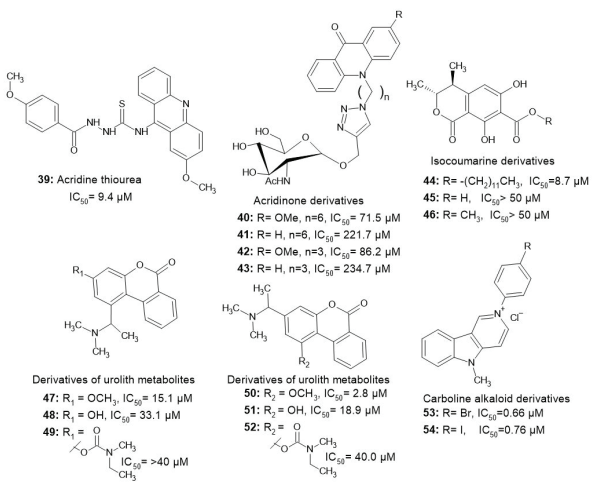


Figure 12: The chemical structures of new original patented AChE inhibitor compounds and their respective IC_{50} values for AChE) (5).

In a study based on the ChE enzyme inhibitor activities of rings containing nitrogen and sulfur atoms and the information that polyphenols inhibit oxidative stress, multi-targeted 1,3,4-thiadiazole-1,3-benzenediol conjugates **(55-59)** in Figure 13 were synthesized and it was discovered that they have many valuable biological activities. Molecules **55, 56, 57 and 58** were shown to have stronger and more selective inhibitory activity against the AChE enzyme (with an IC_{50} = 29–76 nM) than against the BuChE enzyme. This sensitivity to both enzymes was supported by docking studies and thus they were determined to be dual active inhibitors. In this case, the molecules containing amine groups (**55-58**), targeted the CAS region of AChE like the tacrine molecule. The thiadiazole ring and the phenyl groups in their structures showed activity by interacting with the Trp84 part and –Phe330 part of the enzyme, respectively. In addition, the interaction of OH functional groups by making hydrogen bonds was also important. Considering this data, the side-effect profiles of these molecules (**55-59**) are better, as they form reversible enzyme-inhibitor complexes with non-covalent interactions, and it was also observed that they did not show cytotoxicity at this concentration. Molecule **58** was also found to be better as BuChEI than the others. Besides ChE inhibition activity of synthesized molecules, it was also noted that their anti-amyloidogenic effects (except for molecule **58**) were better than curcumin, and molecules **55, 56 and 59** inhibited oxidation as well as quercetin. Another advantage of these molecules is that they have metal ion (such as Cu^{2+}, Fe^{3+} and Zn^{2+} ions) chelating activities and their blood-brain barrier permeability is reasonable (except for molecule **56**). Thus, it can be said that molecules **(55-59)** in this study are effective in many ways to target AD. And it has been a very important and valuable development for researchers (38).

In a study, a series of carbamate derivatives of N-salicyloyl tryptamine were synthesized. It was discovered that molecule **60** in Figure 13 showed properties as a mixed type reversible dual inhibitor of AChE and BChE, also increased anti-inflammatory cytokines IL-4 and inhibited $A\beta_{1-42}$ aggregation (39). In another study synthesizing a novel series of (4-(1,2,4-oxadiazol-5-yl) phenyl)-2-aminoacetamide derivatives, multifunctional agents that inhibit BuChE, have anti-Aβ aggregation properties and inhibit neuroinflammation was discovered. The most active molecule (BuChEI IC_{50}:1.28±0.18 µM) was noted as the compound **61** in Figure 13 (40).

In another study, acetylated triterpenoic acids and 1,3- or 1,4-diazabicyclo[3.2.2]nonanes were combined. It has been discovered that the synthesized compounds have low AChEI activity, but they are very strong active compounds to the BuChE enzyme (42). The chemical structures of triterpenoic acids are shown in Figure 14.

In another interesting study, tacrine-linked triazole glycoconjugates were synthesized and it was expected to reduce the effect of tacrine on known hepatotoxicity. It was reported that the desired effect (non-toxicity) is provided in the evaluated molecules and as a result of molecular modeling, molecule **62** in Figure 13 as the most active molecule as AChEI (41).

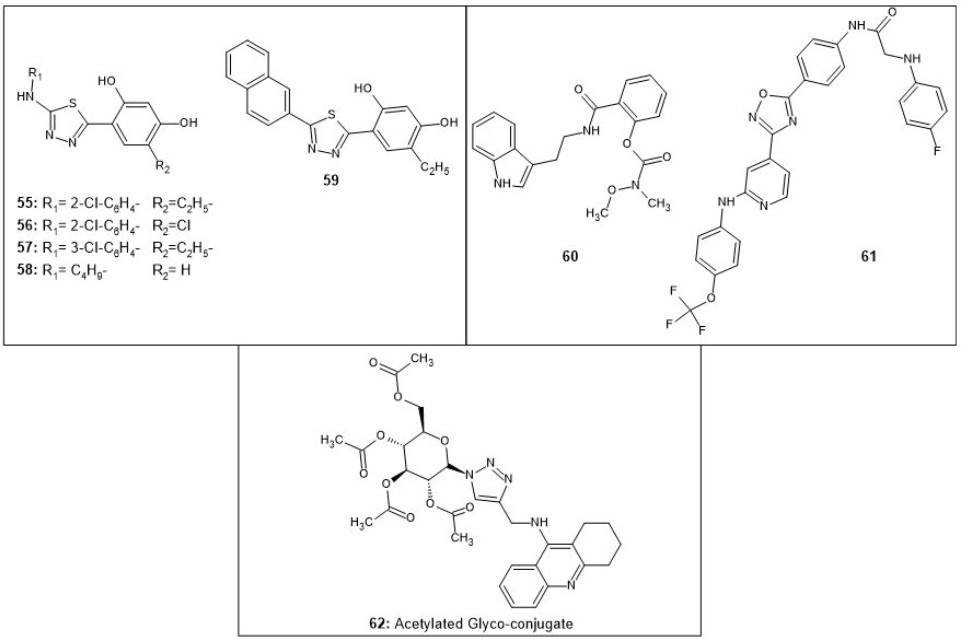


Figure 13: The chemical structure of 1,3,4-thiadiazole-1,3-benzenediol conjugates **55-59** (38). The chemical structures of carbamate derivative of N-salicyloyl tryptamine (compound **60**) and (4-(1,2,4-oxadiazol-5-yl)phenyl)-2-aminoacetamide derivative (compound **61**) (39- 40). The chemical structure of the tacrine linked triazole glycoconjugate (compound **62**) (41).

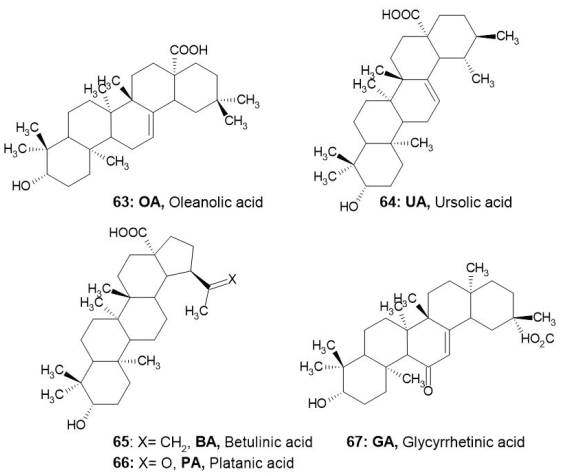


Figure 14: The chemical structures of triterpenoic acids (42).

Recent current studies have also aimed to treat multiple diseases. In a study considered in this context, both AChE and carbonic anhydrase (hCA) enzymes were studied to target people with both AD and Parkinson's disease. For this purpose, a group of new bis-ureido-substituted sulfaguanidine and sulfsoxazole derivatives were obtained. As a result of *in silico* and *in vitro* studies, it was found that compounds **68** and **69** in Figure 15 also have ABTS radical scavenging activity (at the rate of 70% and 78%, respectively) as well as enzyme inhibitor activity. Thanks to the elimination of free radicals that cause cellular damage and inhibitory effects of the molecules on hCAs and AChE enzymes, these molecules can be beneficial to both targeted diseases and shed light on the future (43).

In another study targeting dual enzyme inhibition, sulfonamide-pyrrole-3-one conjugates were synthesized. Inhibitory activities of the compounds against hCA I, hCA II and AChE enzymes were investigated. It has been of interest that many derivatives are more potent inhibitors than existing drugs. Compound **70** in Figure 15 is the best AChEI molecule in the study. With these discoveries, researchers are seeking solutions to many metabolic diseases (44).

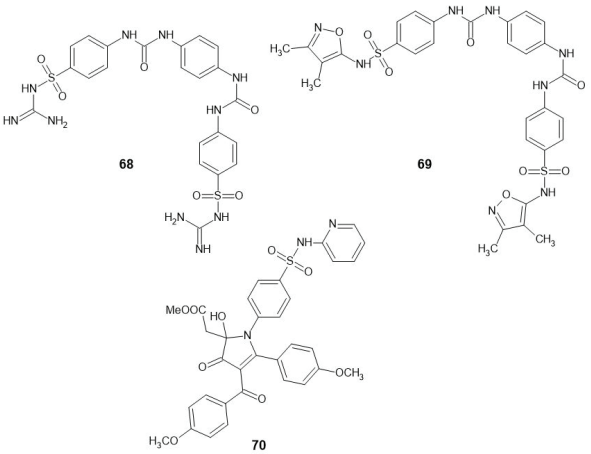


Figure 15: The chemical structures of bis-ureido-substituted sulfaguanidine and sulfsoxazole derivatives (compound **68** and **69**, respectively), sulfonamide-pyrrole-3-one conjugate (compound **70**) (43, 44).

Hybrids were designed in a different study after it was discovered that intervention in many aspects of AD, which has a comp-

lex multifactorial etiology, is important. The hybrid molecules consisted of TPPU, which is a soluble epoxide hydrolase (sEH) inhibitor, and 6-chlorotacrine (and huprine Y), which inhibits AChE enzyme. When the leader molecule given in Figure 16 as compound **73** was administered to a mouse with AD, it was observed that it had high brain permeability and water solubility and decreased toxicity in the nervous system (45).

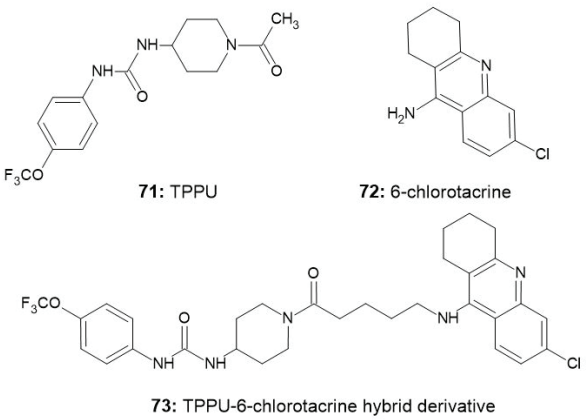


Figure 16: The chemical structures of TPPU, 6-chlorotacrine and TPPU-6-chlorotacrine hybrid derivative (45).

A group of researchers working on a series of pyrazole and pyrazolone derivatives proved that the compound **74** in Figure 17 provided a strong inhibition by connecting with the catalytic and important residues of the AChE enzyme (His 447 and Ser 203) through *in silico* studies. This data was also supported by *in vitro* studies, and it was discovered that compound **74** (IC_{50} value of 0.38 ± 0.019 mg/mL ($p < 0.05$) had close and good results with rivastigmine (IC_{50} value of 0.36 ± 0.018 mg/mL ($p < 0.05$) (46).

In a study in which another heterocyclic ring was involved and polysubstituted pyrroles were synthesized, it was found that compound **75** in Figure 17 was not very effective in BuChE inhibition, but it had affinity with H-bond and hydrophobic interactions especially against the AChE enzyme and provided significant inhibition (IC_{50} value of 2.95± 1.31 µM) appeared (47).

In another study, which was aimed to proceed based on Donepezil, an FDA-approved molecule, a change was made on the heterocyclic ring. A new compound was formed by adding the pyridine ring instead of the phenyl ring of the known donepezil molecule. It was revealed that this compound **76** in Figure 17 binds to PAS and CAS pockets of AChE and shows mixed inhibition as strong as the reference molecule (48).

In another important and valuable study, a group of 2-(2- oxoethyl)pyrimidine-5-carboxamide derivatives was synthesized based on the Donecopride molecule. According to studies in the literature, Donecopride is a serotonin subtype 4 receptor agonist, and a molecule that has been discovered to have low inhibition against BuChE and selective and strong enzyme inhibition against AChE. When the biological activities of the synthesized molecules were examined, it was understood that compound **78** in Figure 18 had lower BuChE enzyme inhibition, but had a stronger inhibition property against the AChE enzyme than Huperzine-A. As a result of this information, an *in silico* study was performed and it was revealed that compound **78** interacts with both the CAS and PAS regions of AChE and causes a complex inhibition. Researchers thought that the aryl ketone moiety of Donecopride was important in this activity. Moreover, the fact that the compound **78** has drug-like properties by providing Lipinski's rule of five is an important discovery for future research (49).

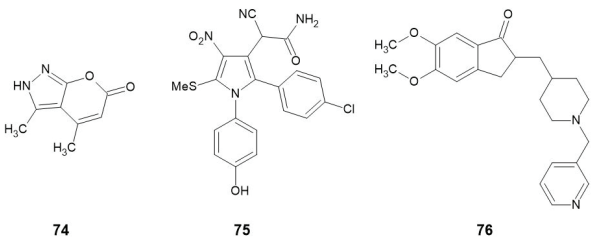


Figure 17: The chemical structure of 3,4-dimethylpyrano[2,3-c] pyrazol-6(2H)-one (compound **74**), polysubstituted pyrrole derivative (compound **75**) and compound **76** based on donepezil (46-48).

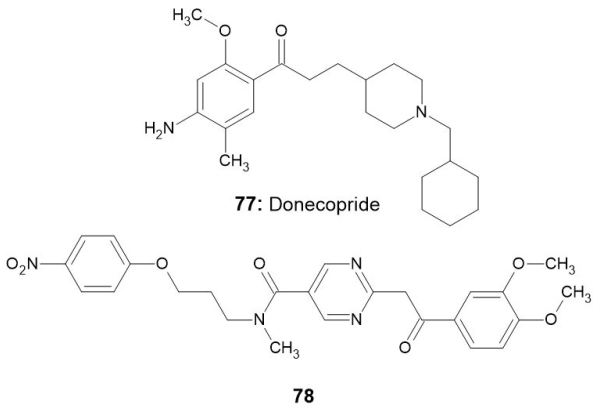
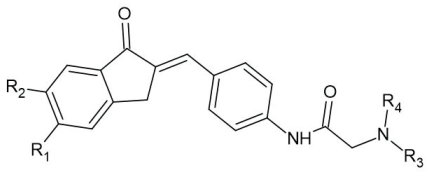


Figure 18: The chemical structures of donecopride and the novel compound based on donecopride (49).

In another recent study that adopted the "one drug for multiple targets" approach, a group of molecules containing an indan-1-one fragment was synthesized based on the donepezil molecule and these molecules were tested with *in vitro* and *in silico* studies. As a result of the study, it was found that some of the synthesized molecules inhibited both ChE enzymes and monoamine oxidase (MAO) B, had anti-amyloidogenic effects, and also prevented oxidative stress with antioxidation activity. The molecules **79-81** in Figure 19 inhibited the MAO-B enzyme as well as the AchE enzyme and for this reason, these molecules were found valuable in terms of AD. On the other hand, it was suggested that molecules **82-84** in Figure 19, which are the other molecules synthesized in the study, could be the leading drug molecules in the treatment of Parkinson's disease with their MAO B inhibition activities (50).



79: R_1= OCH_3 R_2= H R_3= CH_3 R_4= C_3H_7
80: R_1= H R_2= OCH_3 R_3= CH_3 R_4= C_3H_7
81: R_1= OCH_3 R_2= OCH_3 R_3= CH_3 R_4= C_3H_7
82: R_1= OCH_3 R_2= H R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$
83: R_1= H R_2= OCH_3 R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$
84: R_1= OCH_3 R_2= OCH_3 R_3= CH_3 R_4= $CH_2CH_2CH_2N(CH_3)_2$

Figure 19: The chemical structures of new donepezil-based indan-1-one derivatives (50).

CONCLUSION

In the light of the information obtained, while 24 million of the current world population has Alzheimer's disease and it is estimated that these Figures will increase 4 times in 2050, the drug groups approved to solve this problem that threatens public health are extremely limited (3). Researchers are trying to obtain molecules with potent and wide therapeutic potential through the development of novel multifunctional ChEIs. In addition, it is desired that these molecules have optimal pharmacodynamics and low side-effect profile. For this reason, in new drug development studies, researchers try to change the chemical structures of existing drugs and develop them by combination/hybridization. As a result of our study, it has been detected that as a multifactorial disease with complex pathology, AD has been demonstrated by important and remarkable literature studies in recent years that it can be targeted and resolved with multifunctional drugs and with the multi-target-directed ligand strategy. Using this strategy, the researchers have aimed to achieve multiple targets with one multifunctional molecule. The present review was written to draw attention to this issue.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.R.Y., N.U.G.; Data Acquisition- E.R.Y.; Data Analysis/Interpretation- E.R.Y.; Drafting Manuscript- E.R.Y.; Critical Revision of Manuscript- E.R.Y., N.U.G.; Final Approval and Accountability- E.R.Y., N.U.G.

Conflict of Interest: The authors have no conflict of interest to declare.

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