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REVIEW ARTICLE

BACE-1 Enzyme: A Promising Target for Alzheimer's Disease

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ABSTRACT

Alzheimer's Disease (AD) is a progressive dementia-type neurological brain disease that causes the destruction of cells in the brain. According to WHO 2022 data, there are more than 55 million AD patients in the world. This number is expected to increase to 139 million in 2050. Since the BACE-1 enzyme, a member of the type I transmembrane aspartic proteinase enzyme family, causes proteolytic cleavage of APP and leads to the production of Aβ peptides in the brain parenchyma, compounds that can inhibit this enzyme are thought to be useful in the treatment of AD. Compounds with an inhibitory effect on the BACE-1 enzyme are generally categorized in two groups: peptidomimetic and nonpeptidomimetic. Peptidomimetic BACE-1 enzyme inhibitors include statin- and norstatin-based compounds, as well as derivatives bearing hydroxyethylene, hydroxyethylamine and carbinamine structures. Nonpeptidomimetic BACE-1 inhibitors carry acyl guanidine, 2-aminopyridine, aminoimidazole, amino/iminohydantoin, aminothiazoline, aminooxazoline, aminoquinoline, piperazine fragments. Apart from these synthetic compound groups, some natural products that inhibit BACE-1 enzyme have also been discovered. LY2811376, LY2886721, RG7129, BI 1181181, JNJ-54861911, LY3314814, MK-8931, E2609, CNP-520, LY3202626, PF-06751979, CTS21166, and HPP854 are BACE-1 inhibitors entering clinical trials. Despite intensive efforts and a significant number of compounds entering clinical trials, none of the BACE-1 inhibitors have been approved and made available for treatment.

Contents

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1. Introduction

The Alzheimer's disease (AD) is a progressive, high prevalence, dementia-type neurological disease that causes the destruction of brain cells [1]. Depending on the stage of the disease, it is characterized by disinterest, depression, disturbance of communication and orientation, poor judgement, difficulty swallowing and walking, and failure to perform daily activities such as behavioral changes [2].

AD is a major health problem in the world, both in developed and developing countries, due to its ever-increasing prevalence and difficulties in treatment, especially among people over 60. While incurring severe costs such as patient care and medication costs, illness-related accidents are also a major problem [1].

According to data released by the World Health Organization (WHO) in 2022, there are more than 55 million known Alzheimer's patients worldwide. This number is expected to rise to 78 million by 2030 and 139 million by 2050 [3]. AD also has a considerable negative impact on the affected lives of families and their budgets for social and health services. According to 2018 data, the global cost of healthcare is $1 trillion and is expected to double by 2030 [4].

Although there is no medication available to stop, reduce or treat AD, rivastigmine, an FDA-approved N-methyl-D-aspartate (NMDA) receptor antagonist, and acetylcholinesterase (AChE) inhibitors, galantamine and donepezil are used to alleviate the symptoms of disease [5]. The existing single-target small molecules have not been sufficiently effective in the treatment and progression of AD. Therefore, studies are also under way on multi-targeted directed ligands (MTDLs) to slow the progression of the disease [6]. One of the treatment approaches for AD is the development of β-secretase (BACE) enzyme inhibitors, which act on the amyloid precursor protein (APP), which produces the neurotoxic amyloid β (Aβ) protein. Insoluble Aβ protein aggregates cause plaque accumulation and neurodegeneration. Therefore, the development of BACE-1 enzyme inhibitors is an important approach in the treatment of AD [7].

1.1. The Amyloid Hypothesis

APP breaks down by certain enzymes to form the Aβ protein, the metabolic product. The resulting Aβ protein causes the formation and accumulation of amyloid plates. These amyloid plates lead to neuropathological changes. The amyloid hypothesis is based on this formation. APP is a pioneering protein that can be metabolized by α-, β- and γ-secretase. These enzymes metabolize APP by breaking it down at different points. α-secretase cuts the APP from its center point and forms an extracellular protein as a product of metabolism. β- secretase and γ-secretase cut off the APP from the amino end and the carboxyl end, respectively, and produce Aβ as a product of metabolism [8].

While it is not a protein that causes the formation of amyloid plaque, such as Aβ, which is caused by the breakdown of α-secretase and APP, this breakdown also prevents the production of Aβ. For this reason, studies on α-secretase modulators are aimed at preventing the formation of Aβ [1].

It breaks down by β-secretase, or BACE enzyme, causing the formation of APP, Aβ, and amyloid plates. At this point, β-secretase acts as a speed limiting enzyme in the formation of Aβ. BACE-1 enzyme inhibitors also inhibit the formation of Aβ by inhibiting β-secretase. This is why these enzyme inhibitors are often involved in the development of new drug molecules [9]. The γ-secretase enzyme inhibitors are another group of inhibitors that have been studied because they inhibit the formation of Aβ, such as the BACE-1 enzymes. These enzyme inhibitors are of great importance in the development of drugs for the treatment of AD [10].

Advanced glycation end-product receptor (RAGE) is a transmembrane receptor that increases the activity of β- and γ-secretases. RAGE also activates oxidative stress. It is believed to contribute to the formation of Aβ by increasing the activity of β- and γ-secretases, as well as having an active role in the transport of amyloid to the brain. For this reason, RAGE antagonists are an important component in the study of AD treatment [11].

1.2. Cholinergic Hypothesis

According to the cholinergic hypothesis, loss of cholinergic function in the central nervous system (CNS) causes cognitive decline characterised by AD [12].

1.3. Tau Hypothesis

Tau is a protein involved in the organization and stabilization of microtubules and plays an active role in neuronal transport. Hyperphosphorylated tau protein becomes a helical filament and causes the formation of neurofibril tangles [13]. These neurofibril tangles disrupt the organization and stabilization of microtubules and cause disruption of axonal transport. This situation also causes cell death as it disrupts intercellular communication [14].

1.4. Excitotoxicity Hypothesis

Glutamatergic neurons form the main excitatory system in the brain, playing an important role in memory, learning and synaptic stretch. Glutamate is the main neurotransmitter that generates CNS excitatory responses. Overactivation of glutamate receptors causes excitotoxicity, which can result in neuronal dysfunction and death [15].

1.5. Neuroinflammation Hypothesis

There are high levels of pro-inflammatory mediators in the brain and CSF (cerebrospinal fluid) of AD patients. With

excessive secretion of pro-inflammatory cytokines and chemokines, brain resident macrophages are activated. As a result of this activation, tumor necrosis factor-alpha (TNFα), interleukin-8 (IL-8), transforming growth factor-β (TGF-β), macrophage inflammatory protein-1α (MIP-1α) and insoluble forms of Aβ plaques increase [16]. Thus, neuroinflammation occurs. Neuroinflammation refers to the inflammatory response that may be caused by internal pathological movements in the CNS or external factors [17].

2. The BACE-1 Enzyme

The BACE-1 enzyme belongs to the type I transmembrane family of aspartic proteinases and causes the production of Aβ peptides in the brain parenchyma, causing the proteolytic division of APP [18]. BACE-1; BACE-2, is a complex protein with structural similarities to many enzymes found in different parts of the human body, such as pepsin, renin, catepsin D, and catepsin E. [19]. Therefore, ensuring selectivity in BACE-1 inhibition without affecting other proteases is crucial for the development of effective BACE-1 inhibitors and the elimination of non-targeted side effects [19, 20]. The BACE-1 enzyme consists of a total of five basic fields, including the 501 amino acid and the signal peptide domain, the pro-peptide domain, the catalytic domain, and the transmembrane domain and the cytosolic domain. The signal peptide domain of BACE-1 carries it to the endoplasmic reticulum, where the pro-peptide domain is divided by the furin protease. The transmembrane domain then causes the translocation of the protein into the Golgi complex, which leads to posttranslational processing and the full maturity of the BACE-1 enzyme. The transmembrane domain facilitates the binding of BACE-1 to the plasma membrane. The catalytic domain contains the active aspartate residues of the BACE-1 enzyme. [21].

The BACE-1 enzyme also has a N-terminal, a C- terminal, and an interface area that connects these terminal regions. [7]. The substrate bonding area is located between the N and C terminal lobes with different bonding pockets, and so the movement in both lobes affects the catalyst properties of BACE-1 [22].

BACE-1 has a fairly large active zone consisting of a number of sub-zones called cell. The sub pockets S1, S2, S3, S4, S1', S2', S3', S4 form the ligand bonding area. Hydrophobic pockets S1 and S3 are adjacent to each other and contain the remains of Leu30, Phe108, Ile110, Ile1, Trp115. The S2 and S4 pockets contain residues of hydrophilic amino acids, including Lys9, Ser10, Thr72, Gln73, Thr231, Thr232, Arg235, Arg307 and Lys321. The S3 and S4 subregions include remains such as Pro70, Thr72, Glu125, Arg128, Agr195 and Trp197. The S2 subregion consists of residues such as Ser35, Val69, Tyr71, Ile126 and Tyr198 in hydrophobic and amphiphilic structures. The subregion S1 includes the hydrophobic Ile226 and Val332 residues as well as the main catalytic residue Asp32 and Asp228 [23].

The BACE-1's bonding pocket contains three important parts, including the catalytic aspartic acid residues, which are very important for the proteolytic activity of BACE-1, the wing, which is the most flexible part of the bonding region and controls access to the substrate, and the 10-second cycle, located near the S3 pocket (Figure 1) [7].The catalytic binaries Asp32 and Asp228 are found in the ligand binding regions and are very important for the proteolytic activity of the enzyme. The binding of a ligand to these two amino acids increases the binding affinity and thus strength [24]. For full activation, the enzyme gains mono protonation in active form (Ghosh and Osswald 2014). The four oxygen atoms of Asp32 and Asp228 in the BACE-1 active region are not equal due to their different chemical environments, and there is a possible set of different protonated states. Analyses show that only mono and diprotonated derivatives are suitable for the design of the potent inhibitor [25].

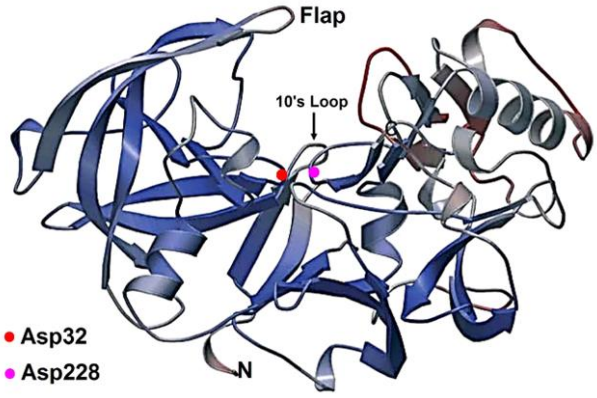

Figure 1 Three important components of the BACE-1 enzyme: catalytic aspartic acid residues, wing and 10-second cycle point [26]

The active region of BACE-1 is protected by a β-connection point, which forms most of the binding region between Val67 and Glu77 in the N-terminal lobes. It is commonly known as the "wing" and is the most flexible part of the active zone that controls the access of the substrate to the active area through conformational changes. When the active region is in inactive form, the wing tends to be in open conformation. However, the presence of a substrate or inhibitor keeps the wing in closed conformation (Figure 2) [24].

Tyr71 is an important remnant on the wing, which connects to the binding region and becomes harmonious to the nature and shape of the ligand. This residue contributes to the change in the position of the wing in relation to the catalytic aspartic acid residue, as well as to the entry and exit of the substrate or ligand into the active area. With its flexibility, the Tyr71 plays an important role in the open or closed conformation of the BACE-1 [24]. Tyr71's hydroxyl group has the ability to form a hydrogen bond with the amino group of the Trp76 side chain. In this connection, the Tyr71 separates the underpockets S1 and S2, allowing the BACE-1 to transition to closed conformation. When the wing is removed from the residues of catalytic aspartic acid, the hydrogen bond between the Tyr71 and the Trp76 remains disappears, in which case it cannot replace the underpockets of Tyr71 S1 and S2 and the pockets cannot be separated. This enables the transition of BACE-1 to open conformation. This suggests that in the presence of the hydrogen bond between the Tyr71 and the Trp76 remains, BACE-1 is in closed conformation, and in the absence of this hydrogen link, Bace-1 is open conformation [27].

Dynamic simulation studies on BACE-1 have shown that both outdoor and indoor conformations of BACE1 are freely accessible at room temperature. This proves the flexibility of

the Tyr71 remains. This unique structure and composition of BACE-1 plays an important role in the design of various inhibitors [25].

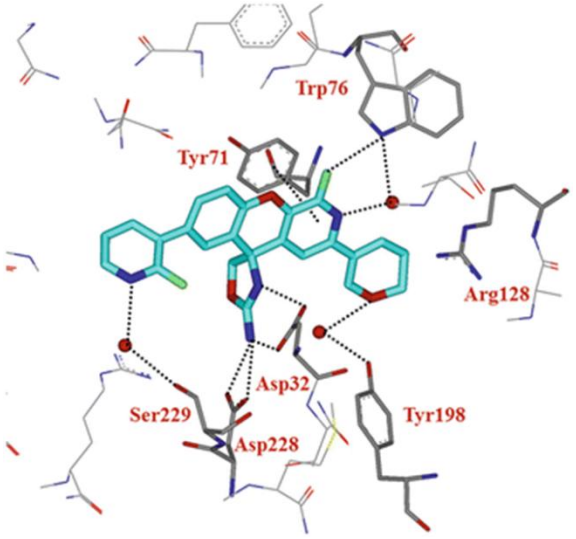

Figure 2 The active region of BACE-1 is protected by a β-connection point, which forms most of the binding region between Val67 and Glu77 in the N-terminal lobes. It is commonly known as the "wing" and is the most flexible part of the active zone that controls the access of the substrate to the active area through conformational changes. When the active region is in inactive form, the wing tends to be in open conformation. However, the presence of a substrate or inhibitor keeps the wing in closed conformation [24]

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3. BACE-1 Enzyme Inhibitors

The BACE-1 enzyme, which supports the production of Aβ peptides due to the division of APP, is believed to be involved in the development of AD by these properties. This is why studies are being conducted on BACE-1 enzyme inhibitors in the treatment of AD. BACE-1 inhibitors, both peptidomimetic and nonpeptidomimetic, were developed in these studies. In addition, natural compounds that inhibit the BACE-1 enzyme have been studied.

3.1. Peptidomimetic BACE-1 Enzyme Inhibitors

The structure-oriented designs of the BACE-1 inhibitors have been successful in developing highly potent and specific inhibitors in the peptidomimetic structure [28]. Peptidomimetic inhibitors are designed to replicate the enzyme's natural substrate. This increases the subregional identity, hydrogen bonding, and hydrophobic interactions [29].

The inhibitory effect of these molecules is caused by the transition isoster. These compounds have been developed by following the shortening of the substrate-based peptide, and also by replacing the easily divisible amide bond with an indivisible transition state isotope such as hydroxyethylamine, statins, hydroxyethylene, isophthalamide, norstatine, and homostatin [30].

In the free BACE-1, a catalytic residue of aspartic acid binds hydrogen molecules with water to keep them in place. When encountered with a substrate molecule, one of the aspartic acid nucleophils attacks the water molecules, while the other activates the carbonyl of the peptide bond. This forms a tetrahedral intermediate, and a new N and C terminal, consisting of two substrate parts, breaks the bond of carbonyline regeneration (Figure 3) [31].

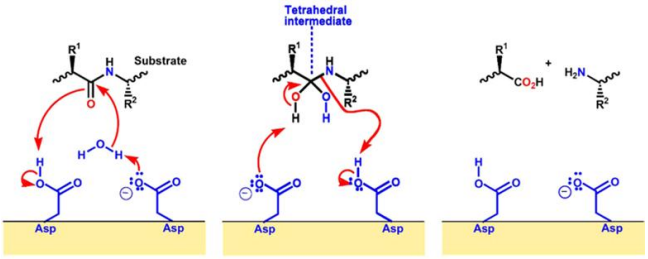


Figure 3 Mechanism of action of catalytic aspartic acid residues [31]

This approach has been applied to design first generation potent BACE-1 inhibitors, and further studies have focussed on lowering molecular weight and maintaining binding interactions while reducing peptidic properties [32].

3.1.1. BACE-1 enzyme inhibitors based on statins and norstatins

Statin and norstatin (hydroxymethylcarbonyl) are classic transition isotopes for aspartyl protease inhibitors. Statin is used because of the leucine side chain at S1 position, which mimics the Swedish mutant APP, which shows high affinity for BACE-1. Statin-based inhibitors were developed in the early 2000s and showed an IC_{50} of less than 10 μM. Molecular placement studies have shown that the isolatine and its field fit well to the subregions S3 and S2 respectively. Similarly, the isobutyl group is found in the S1 pocket. The compound, a phenyl statin-based inhibitor, is designed to place a large, hydrophobic substitution at position 1, P1 (Figure 4) [31]. The inclusion of N-acetyl leucine in the PABA and N-terminal ends of C-terminals has been observed to contribute to enzyme inhibition. A modeling study of a benzyl-substituent inhibitor in the active region of BACE-1 showed that the phenylstatine transition isoster forms hydrogen bonds with catalytic aspartic acid residues [33].

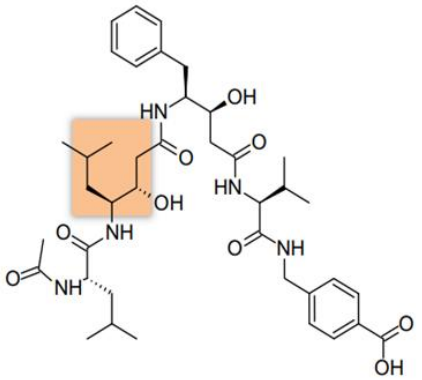


Figure 4. Compound 1. IC_{50} = 21 μM [30]

Inhibitors based on phenyl norstatine have been developed, as in compound 2 (Figure 5), to better pharmacokinetic properties by reducing molecular weight. One of these compounds, the compound 3, has shown an inhibitor

potential with an IC_{50} value of 0.20 µM. However, at a pH of 3.5 to 5.5, these compounds are unstable due to the migration of the oxalyl group from the side chain to the N-terminal. Compound 1 has a strong inhibitory effect, while the compound contains enough acidic and polar parts to reduce the passage through the 3 cell membrane. Therefore, further optimization has been done to increase the tissue cell permeability of this inhibitor and the permeability from blood-brain barrier (BBB). Among the carboxylic acid isosteres, 1H-tetrazole in compound 4 showed the most promising results and also showed an improved performance in terms of chemical stability, retaining their potency compared to the original analogues (Figure 6) [34].

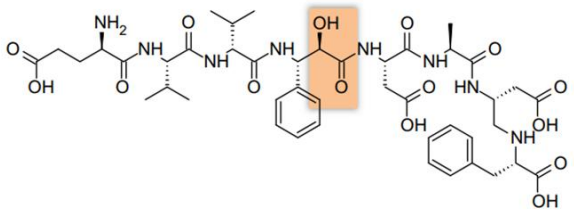


Figure 5. Compound 2. IC50 = 50 µM [33].

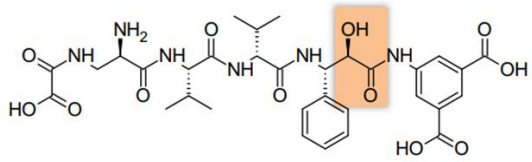


Figure 6. Compound 3. IC_{50} = 0.20 µM [33]

Modeling studies have demonstrated that the replacement of phenyl norstatine with phenyl thionorstatine has a similar hydrogen bond interaction with the residues of aspartic acid, and provides inhibition of up to 99% at 2 µM concentrations. The addition of a dichlorobenzene structure, which serves as a bridge between the wing region and the active region, increases lipophility and optimizes. Compound 5 is a compound optimized in this way (Figure 7) [32].

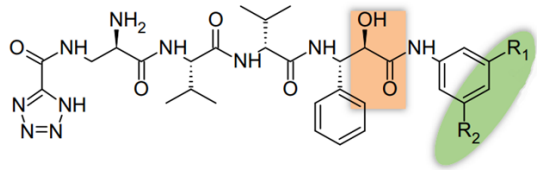


Figure 7. Compound 4 R_1 and R_2 = Tetrazole, IC_{50} = 1.2 µM. Compound 5 R_1 and R_2 = Cl, IC_{50} = 14 µM [34]

3.1.2. Hydroxyethylene isosteric-based BACE-1 enzyme inhibitors

Hydroxyethylene is one of the direct dipeptide isotopes replaced by the entire carbon structure and hydroxyl group placed in the catalytic binary of the divisible amide bond. [30]. These inhibitors are based on the Leu-Ala structure in the divisible amide region, which mimics the peptide substrate. OM99-2, compound 6, is the first substrate inhibitor developed for the BACE-1 enzyme. This inhibitor is designed by using a hydroxyethylene nucleus to imitate the substrate. The crystalline structure of OM99-2 indicates that the catalytic aspartate is located in the center of the pocket and that the central hydroxyl group interacts with the active region. With the further development of substrate-based inhibitors, OM99-3, compound 7 (Figure 8), with an IC_{50} value of 0.3 µM, was designed to replace compounds 6 (Figure 8) with IC_{50} values of 1.6 µM. This activity of substrate-based inhibitors has formed an important drug design model for further inhibitor development. But despite their powerful inhibitory activity, the peptide structure and large molecular weight of these molecules limit their therapeutic effects [35].

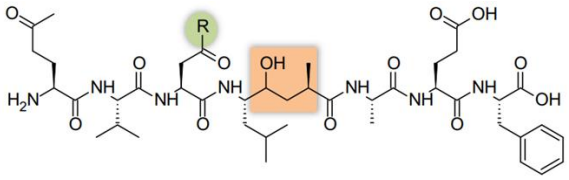


Figure 8. Compound 6 (OM99-2). IC_{50} = 1.6 µM. R = -NH_2. Compound 7 (OM99-3). IC_{50} = 0.3 µM. R = -OH [35]

Based on the X-ray structure of compound 6 with BACE-1, a selective inhibitor has been developed. These studies led to the design of compound 7 with an IC_{50} value of 0.3 µM. The crystalline structure of compound 8 (Figure 9) in the active region BACE-1 shows that one of the nitrogen-hydrogen bonds in the pyrazole structure forms a hydrogen bond with Thr232, the other methyl group of pirazole with the subregion S3, the sulphone with Arg235 and the active flat water. This interaction demonstrates the inhibitor's highly selective properties [7, 36]. Based on these interactions, a strong peptidomimetic inhibitor of 0.12 µM IC_{50} was formed by designing a hydroxyethylene with a change in the pyrazole ring at P3 position [37].

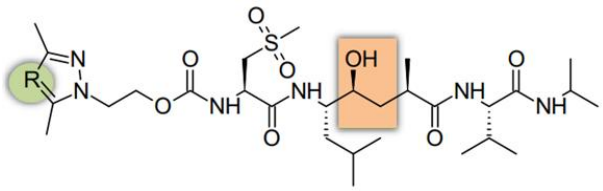


Figure 9. Compound 8. IC_{50} = 0.3 µM. R = C. Compound 9. IC_{50} = 0.12 µM. R = O [37]

Further optimization studies have been carried out with the incorporation of the leu-ala isoster, isophthalamide components and hydrophobic group (methyl benzylamide) into the structure, and a compound 10 (Figure 10) has been obtained with a high potential IC_{50} value of 39 µM for BACE-1. [38]. Further studies have synthesized a series of hydroxyethylene-based compounds to explore potential interactions with the active regions of the enzyme. Of the 30 compounds in this series, the strongest was compound 11 (Figure 11). This compound has a more voluminous substitution on the hydroxycytylene nucleus than other compounds, and is therefore believed to cover the enzyme's large binding region. Peptide structure and high molecular weight are restrictive factors that reduce their potential as a therapeutic agent in these compounds, as in other compound forms [39].

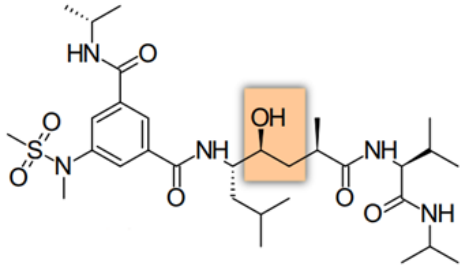


Figure 10. Compound 10. IC_{50} = 39 µM [38]

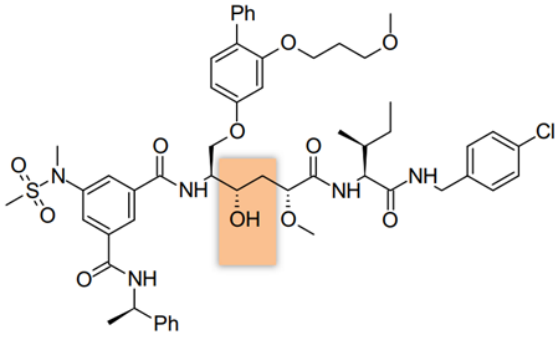


Figure 11. Compound 11. IC_{50} = 39 µM [39]

3.1.3. Hydroxyethylamine-based BACE-1 inhibitors

Extensive research has been carried out on BACE-1 inhibitors based on hydroxyethylamine transition isotope [30]. The hydroxyethylamine isoster has less amino bonds than the homo and norstatine isoster. This isoster reduces the peptide character and also has a low molecular weight that increases the inhibitor's cellular absorption, metabolic stability, and brain penetration capacity [40].

The isoster typically binds to the active region by a double hydrogen bond from the isosteric hydroxyl group to the catalytic aspartate pair in the parallel plane. The addition of basic amines to the hydroxyethylene series to enhance interaction with the negative-loaded catalytic region improved binding effectiveness, potency, pharmacokinetic and pharmacodynamic properties [7, 19].

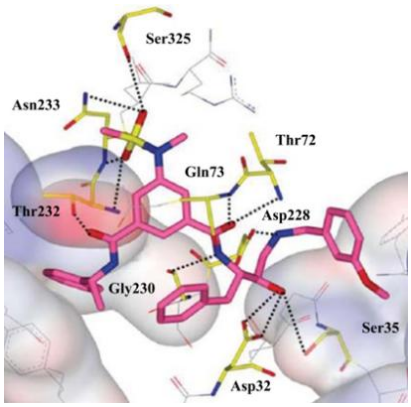


Figure 12. X-ray crystalline structure of the inhibitor-enzyme complex for compound 18 [41]

The first inhibitor with hydroxyethylamine isoster is compound 12 (Figure 13) with a 15 µM IC_{50} value. The X-rays of the inhibitor-enzyme complex have been found to be crystallized, occupying the subregion S3 of α-methyl benzyl amide, interacting with the amine groups Thr232 and Asn233 of sulfonamide oxygen, the hydroxyl group Asp32 and the α-amino group of hydroxyethylamine, and the hydrogen bond with Asp228, as well as catalytic aspartic acids. These studies suggest that hydroxyethylamine isostere is a potential candidate for BACE-1 inhibitors [30, 34].

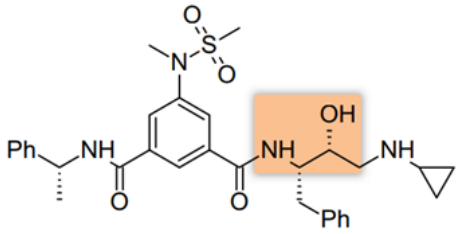


Figure 13. Compound 12. IC_{50} = 15 µM [34]

As a result of advanced optimization studies, the compound 13 (Figure 14) showed strong inhibitory activity at 20 µM IC_{50} for BACE-1, but was unable to pass through the central nervous system (CNS) due to high P-gp flow rates. Incorporation of basic amine-containing carbocyclics gives compounds 14, 15 and 16 (Figure 15), which show IC50 values of 5 nM, 3 nM and 8 nM for BACE-1, respectively [32].

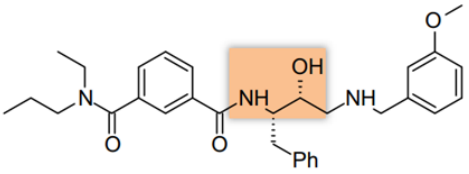


Figure 14. Compound **13**. IC_{50} = 20 µM [34]

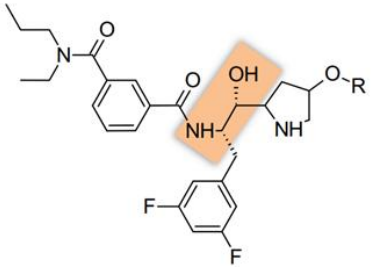


Figure 15. Compound 14. IC_{50} = 5 µM. R = -Benzyl. Compound 15. IC_{50} = 3 µM. R = - Phenyl. Compound 16. IC_{50} = 8 µM. R = (-Et)2 [30]

The compound obtained by adding indole to this nucleus has a value of 17 (Figure 16), 20 µM IC_{50}. However, this compound shows weak brain penetration and therefore lacks centralized activity *in vivo*. Compound 19 (Figure 17) was obtained with the combination of isophthalamide and 3-methoxybenzylamine of the compound 18 (Figure 16) with hydroxyethylamine isoster, a potent BACE-1 inhibitor with an IC_{50} value of 10 µM for BACE-1 [34, 41].

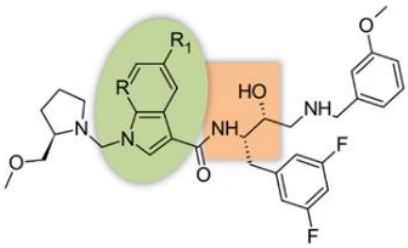


Figure 16. Compound 17. IC_{50} = 18 µM. R = -C, R1 = -CN. Compound 18. IC_{50} = 20 µM. R = -N, R1 = $-CH_3$ [33]

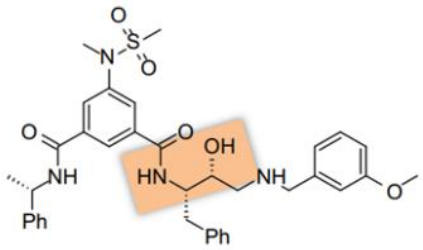
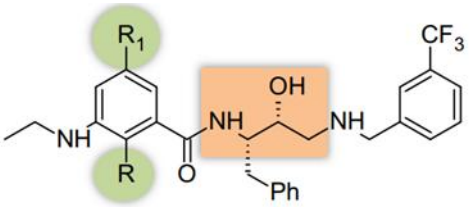


Figure 17. Compound 19. IC_{50} = 10 µM [41]

With the design of a series of derivatives carrying pyrolidinone and triflоromethyl benzyl structures in the S2 and S2 subregions, the compound 20 (Figure 18) was achieved, a powerful inhibitor with a 40 µM IC_{50} value with good enzyme activity. The pyrolidinone ring replaces the sulfonamide group in the S2 bag, thereby increasing enzyme binding efficiency [35].



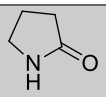
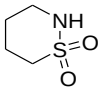
Compound	R	R1	IC50
20	H		40 nM
21	F		4 nM

Figure 18. Molecular structure of compound 20 and 21 [35]

The replacement of the lactam group with a cyclic sulfonamide in the non-primary part resulted in the production of the compound 21 (Figure 18), which inhibits the enzyme very strongly. The addition of benzoamide flora in its composition increases the cellular activity of the compound, showing a 4 µM IC_{50} value [32].The key feature in the development of hydroxyethyl amin isoster-based inhibitors is that the state-isoster based inhibitor prefers the S-configuration at the hydroxyl center, while the derivative of hydroxyethyl amine prefers R-configure at the Hydroxy-center so that the enzyme can interact with the catalytic aspartic acid at the active site [25].

3.1.4. Carbinamine (aminoethylene) based BACE-1 inhibitor

Analogs of carbinamine, a primary amine, have been synthesized to increase BBB penetration [30]. The compound 22 (Figure 19) is developed from the hydroxyethylamine isoster, which is derived from the conversion of hydroxyethylamine to the primary amine. The amine interacts with the catalytic center, replacing the hydroxyl group in hydroxyethylene structure, and has a good activity against enzymes at 26 µM IC_{50} [33]. The inhibitor is bound to the enzyme *via* the primary amine contained on the proton, which interacts with the deprotoned aspartic acid residue of the catalytic centre. With advanced optimization using 3, 5-diflorophenyl belt, a pioneering compound 23 (Figure 20) has been developed with an activity equivalent of 90 µM IC_{50} [41].

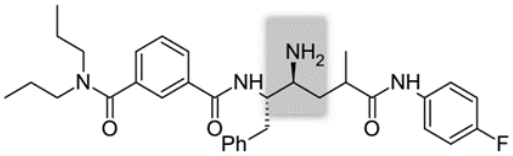


Figure 19. Compound 22. IC_{50} = 26 µM [33]

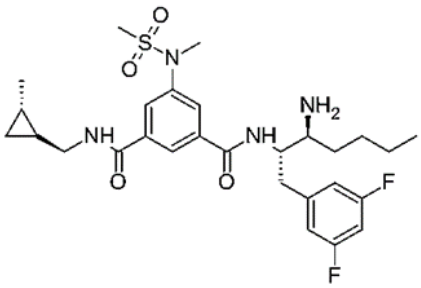


Figure 20. Compound 23. IC_{50} = 90 µM [41]

The next inhibitor of the series is compound 24 (Figure 21) carrying the 1, 3, 4-oxadiazole ring, which has been shown to be very potent for BACE-1 with a 12 µM IC_{50} value [41]. Although these compounds are very potent, they have a high P-gp flow ratio and only 1% oral bioavailability. When given in combination with ritonavir to inhibit metabolism, oral bioavailability increased to 83% [32].

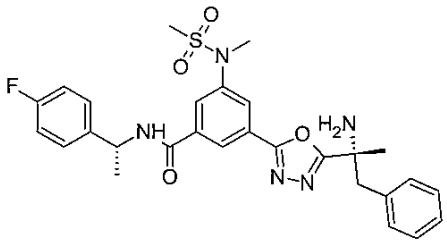


Figure 21. Compound 24. IC_{50} = 12 µM [41]

3.2. Nonpeptidomimetic BACE-1 Enzyme Inhibitors

Non-peptide inhibitors have been developed using modelling methods such as High Throughput Screening (HTS), substrate-based design, fragment-based approaches, and computational scanning [20]. The main objective of developing a non-small-molecular peptide inhibitor is to develop an inhibitor with small molecular size and weight, with improved CBD permeability, reduced peptide character and P-gp flow ratio, and better metabolic stability [32].

The common feature of these inhibitors of different classes is that they can carry acyl guanidine, amino/iminohydantoin, aminothiazole, aminoxazoline, and aminochinoline nuclei. [7, 20].

3.2.1. BACE-1 inhibitors based on acyl guanidine

Compared to non-peptidomimetic inhibitors, acyl guanidine has a high BACE-1 strength and reduced P-gp flow ratio and better pharmacokinetic properties [38, 42].

Using the HTS approach, a compound 25 with an acyl guanidine structure showing a value of 370 nM IC_{50} has been synthesized. The crystalline structure of the enzyme-

inhibitor complex showed that the acyl guanidine moiety and the catalysing active region of the enzyme formed four hydrogen bonds. With the optimization of compound 25, compounds 26 (Figure 22) with 110 nM inhibitor activity have been obtained, but this compound has less selectivity for BACE-1. The crystalline structure of the complex interacted with the hydrogen bond between the acyl guanidine moiety and the aspartic acid residue in the active region, while the substituted amine of acyl guanidine extended to the S1 pocket and to the para-n-propyloxyphenyl, forming a hydrogen link with the residues in the S1-S3 subregion.

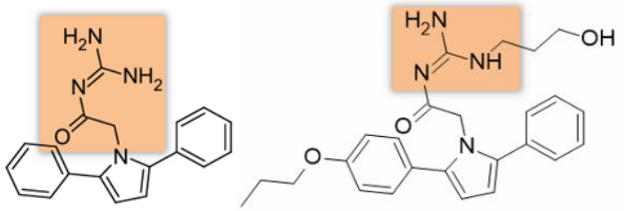
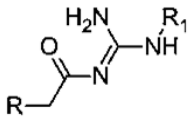


Figure 22. Compund 25. IC_{50} = 370 nM. Compound 26. IC_{50} = 110 nM [38, 43]

The compound 27 (Figure 23) was discovered by virtual scanning of compounds carrying indole guanidine structure and the composite 28 (Figure 23) was obtained by optimization of this compound, showing strong inhibitory activity against the enzyme. When the crystalline structure of the enzyme and compound 27 was studied, it was found that acyl guanidine was bound to catalytic aspartate and formed a direct hydrogen bond with the side chain residues, as well as a cation π-interaction of the indole moiety with Arg235. All these interactions suggest that the inhibitor binds to the enzyme in a semi-closed wing conformation. The compound 28 has similar interactions, and the substituting of indol with nitrile enables it to form a hydrogen bond with Ser328 which plays a stronger inhibitory role [38].



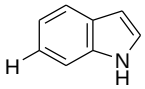
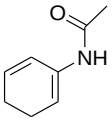
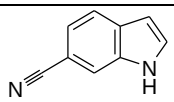
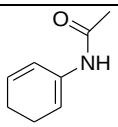
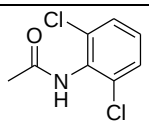
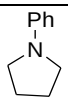
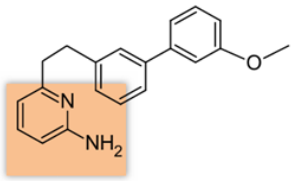
	R	R1	IC50
27			101 nM
28			44 nM
29			250 nM

Figure 23. Molecular structure of compounds 27, 28 and 29 and IC_{50} values [38]

The optimization of guanidine-containing compounds with the addition of a 4-acyl pyrrolidine group resulted in the synthesis of a stronger compound 29 (Figure 23) and the achievement of unique U-shaped conformations directed by the pyrolidine to the S2 subpocket [32].

3.2.2. 2-Aminopyridine-based BACE-1 inhibitors

The acyl guanidine group was replaced by 2-aminopyridine to enhance drug-like properties [41]. The compound has been found to have an effect of 2500 nM IC_{50} on the BACE-1 enzyme 30 (Figure 24). The 3-methoxy-biaryl moiety turns the conformation of the wing region into an open conformation due to the rotation of Tyr71 over the aminopyridine. The curvature in the biaryl region fills the non-primary hydrophobic pocket, while the methoxy substitution replaces the water molecules in the S3 pocket and forms a hydrogen bond with the nitrogen nitrogen of Gly13 [20].



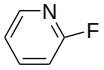
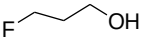
	R	R_1	R_2	IC_{50}
33		H	CF_3	3800 nM
34	H	- OCF_3		34 nM

Figure 24. Compound 30. IC_{50} = 2500 nM [20]

The pyrol ring Tyr71 found in inhibitors such as compound 31 (Figure 25) helps stabilize the complex by creating a π interaction with it [32]

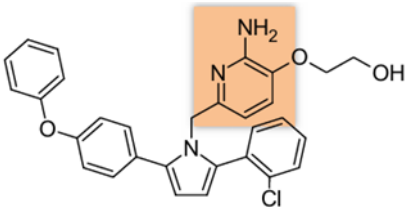


Figure 25. Compound 31 [20]

3.2.3. Aminoimidazole-based BACE-1 inhibitors

A series compound has been developed using the HTS approach, showing strong BACE-1 activity with the structure of aminoimidazole [44].

An enzyme modeling study of aminoimidazole showed that the amino group forms a hydrogen bond with the catalytic active region. Nonsubstantial nitrogen of the imidazole ring forms electrostatic and hydrogen bond interactions with the side chains of Asp32 and Asp228, respectively. In addition, the flora of the benzyl and polymethoxy groups stabilizes the complex by forming hydrogen bonds with the side chain residue in the active region. The compound 32 (Figure 26) thus developed has an IC_{50} value of 740 nM [45].

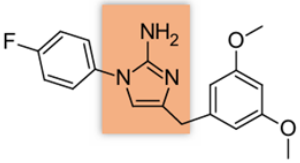


Figure 26. Compound 32. IC_{50} = 740 nM [45]

A new aminoimidazole series has been developed to increase cell permeability and reduce flow properties. In this series there are compounds 33 and 34 (Figure 27). These compounds have been found to bind with BACE-1 in an open position, which helps to increase metabolic stability and reduce the polar surface area for the inhibitor. [45, 46].

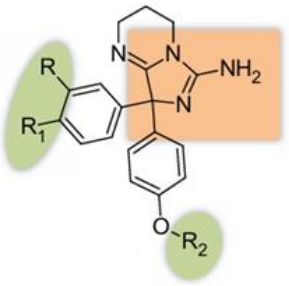


Figure 27. Molecular structure of compounds 33 and 34 and IC_{50} values [46]

3.2.4. Amino/aminohydantoin-based BACE-1 inhibitors

New methods such as substrate-based design, HTS, and fragment-based design have been used in the development of BACE-1 inhibitors. In studies of molecular docking of various polypeptides with BACE-1, aspartic acid residues Asp32 and Asp228 acted as hydrogen base donors (HBDs). Therefore, combinations with hydrogen bonds and amino/imino groups have shown potential interactions with the enzyme [47].

A series of cyclic acetyl guanidine BACE-1 inhibitors have been developed based on the high ligand effectiveness and selectivity of the diphenyl imino hydantoin group, as well as their appropriate pharmacokinetic properties [48]. The compound 35 (Figure 28), an analogue of the structure of immunohydantoin chloropyridine, has shown good selectivity for BACE-1 and strong inhibitory activity (IC_{50} value 21 nM). The BACE-1 subpockets have been optimized to improve ligand binding efficiency. The inclusion of the propenyl group in the chloro substituent resulted in a stronger interaction in the S3 subpockets due to the verticality of propenyl group. This resulted in a five-fold increase in the potent of the compound 36 (Figure 28), with an IC_{50} value of 5.4 nM. Thus, the compound has shown greater selectivity for BACE-1 [49].

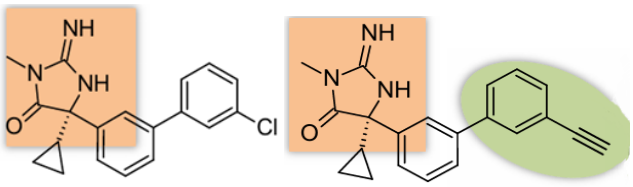


Figure 28. Compound 35. IC_{50} = 21 nM [48] Compound 36. IC_{50} = 5.4 nM [49]

The compound 37 (Figure 29) resulting from the inclusion of the spiropiperidine fragment in the structure has an IC_{50} value 280 nM. The X-ray crystalline structure of the enzyme-inhibitor complex revealed that the inhibitor fits upper than the active pocket and prevents the wing from closing completely onto the inhibitor. In addition, the S2 and S3 lower pockets are left empty. Compound 37 (Figure 29) is also less selective for BACE-1 than other aspartyl proteases [50].

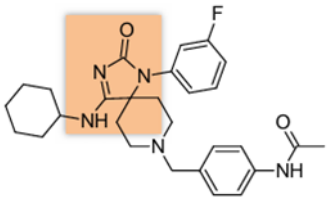


Figure 29. Compound 37. IC_{50} = 280 nM [50]

3.2.5. BACE-1 inhibitors based on aminothiazoline and aminooxazoline

The compound 38 (Figure 30) with the aminothiazole structure has an IC_{50} value of 380 nM. This inhibitor binds to the enzyme with the hydrogen bonds between the nitrogen in the aminothiazole group and the catalyst active region and the methoxyphenyl group that mimics Trp76, thus providing complex stability [51, 52].

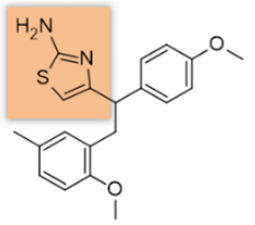


Figure 30. Compound 38. IC50 = 380 nM [51, 52]

Aminoxazoline derivatives carrying xanthene ring have been developed as BACE-1 inhibitors. These groups of compounds have high enzyme potential with reduced P-gp flow ratio and increased metabolic stability [53]. Compound 39 (Figure 31) containing 3-aza-4-floroxanthenes has been found to be a very powerful BACE-1 inhibitor that significantly reduces Aβ levels in rats and non-human species [32].

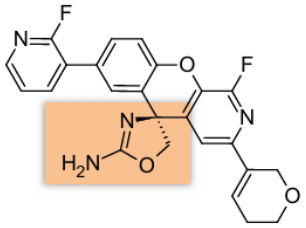


Figure 31. Compound 39. IC_{50} = 0.3 nM [54]

The crystalline structure of the enzyme-inhibitor complex suggests that the 3-azaxanthene nucleus has a strong hydrogen-binding interaction with the catalytic active region. In addition, there is a hydrogen bond between dihydropyrane oxygen and Tyr198, which further stabilizes the complex and strengthens the binding interactions [54]. With further optimization studies, the amino-azine compound 40 (Figure 32) has been obtained. This compound has a strong effect against BACE-1 and greater bioavailability with a value of 12 nM IC_{50} [55, 56].

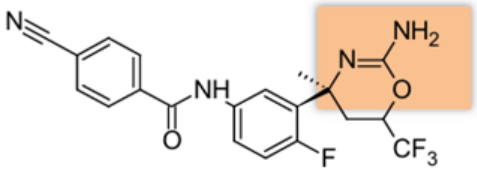
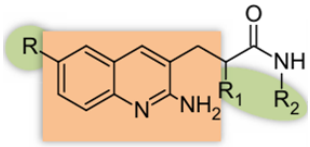


Figure 32. Compound 40. IC_{50} = 12 nM [55, 56]

3.2.6. Aminoquinoline -based BACE-1 inhibitors

In this class, a library of powerful BACE-1 inhibitors has been designed using the 2-aminoquinoline nucleus. For this purpose, SAR studies were used, including X-ray crystallography and fragment scanning, including molecular modeling [57].



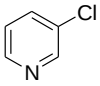
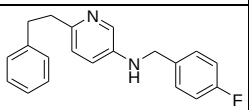
	R	R1	R2	IC50
41		H		74 nM
42		- CH3	- C2H5C(CH3)	11 nM
43		- CH3	- C2H5C(CH3)	0.8 nM

Figure 33. Molecular structure and IC50 values of compounds 41, 42 and 43 [58]

The inclusion of an alkyl amide in the 3-position of the aminoquinoline provided access to the S2 pocket. It has been found that monosubstantial amides are more active than disubstituted derivatives. The compound 41 (Figure 33) containing an N-cyclohexyl substitution has been shown to have strong inhibitory properties, and the compounds have shown expanded interaction in the S2 subregion of the enzyme. Further optimization to improve the pharmacokinetic properties resulted in achieving compounds 42 (Figure 33) with 3-chloro-2-pyridinil substantive at 6 positions. Although it is a powerful inhibitor, the compound has poor metabolic stability and a high P-gp efflux ratio [58].

The aminoquinoline derivative of compound 43 (Figure 33) is a 350-fold-increased selectivity derivative by optimization studies. [32].

3.2.7. Piperazine-based BACE-1 inhibitors

A potent BACE-1 inhibitor has been developed by incorporating a piperazine ring into the peptidomimetic BACE-1 inhibitor. This modification resulted in hydrogen binding interactions with many residues in the enzyme pocket and an IC_{50} of 0.249 nM [59]. The addition of the phenyl ring also created hydrophobic interactions leading to increased brain permeability of the drug [60].

Compounds 44 and 45 (Figure 34) with values of 0.128 nM and 0.023 nM IC_{50} respectively were obtained using molecular placement and structural modification [41]. The X-ray crystalline structure of the enzyme inhibitor complex shows that one of the nitrogens in the piperazine is linked to Asp32 and the substituted nitrogen in the piperazine to Asp228 through a hydrogen bond, while the aryl group with the subregion S2 provides greater binding effectiveness and stability to the complex. The compound 46 and 47 (Figure 35), with an IC_{50} value of 79 nM, showed an interaction similar to that seen in previous inhibitors [30].

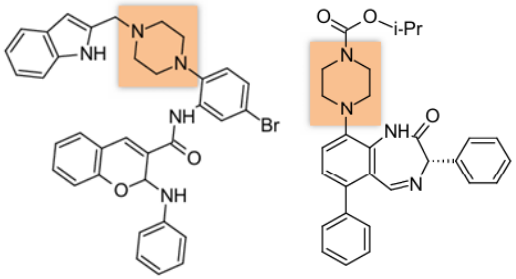


Figure 34. Compound 44. IC_{50} = 0.08 nM. Bileşik 45. IC_{50} = 0.289 nM [41]

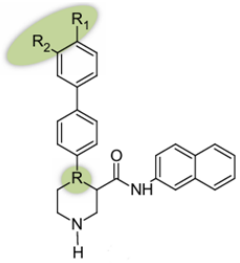


Figure 35. Compound 46. IC_{50} = 0.03 nM. R=-C, R_1=-H. R_2=-H. Bileşik 47. IC_{50} = 0.023 nM. R=-N, R_1=-F, R_2=-Cl [30]

The inclusion of a piperidine ring into the BACE-1 inhibitor has resulted in good inhibiting properties. Data on the X-ray crystalline structure of the complex piperidine inhibitors with BACE-1 have shown that the protonated piperidine nitrogen is located between two catalytic residues of aspartic acid. The substituents at 3-position reach the S1-S3 subregion and the 4-aryl ring is connected to a new bonding pocket underneath the enzyme wing. The compound 48 (Figure 36) designed using this approach is a derivative with an IC_{50} value of 0.035 nM [61].

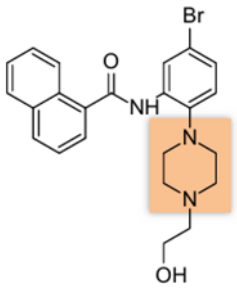


Figure 36. Compound 48. IC_{50} = 79 nM [61]

In a study using the MTDL approach, synthesis and biological activity studies of chalcone derivatives bearing N-aryl piperazine fragment were carried out. The properties of the synthesized compounds to inhibit the AChE, butylcholinesterase (BuChE), BACE-1 enzymes and aggregation of Aβ have been studied. Compounds with phenylpiperazine structure have shown moderate inhibition of BACE-1. Compound 49 (Figure 37), a bromo derivative bearing a nonsubstituted benzylpiperazine structure, was found to be a potent inhibitor of BACE-1 [62].

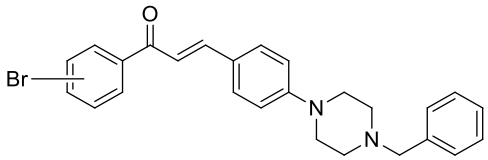


Figure 37. Compound 49. IC_{50} = 46.60 nM [62]

In a study assessing the biological activities of compounds carrying the 1,2,4-triazole ring and benzylpiperazine moiety, the compound 51 (Figure 38) have been determined to have anti-Aβ-aggregation properties and are non-neurotoxic. Compound 51 improved eye functions in the Alzheimer's phenotypic drosophila model and corrected behavioral defects in the rat model induced by Aβ. Molecular dynamic simulation studies have shown that the compound 51 forms a stable bond with the AChE and BACE-1 enzymes [63].

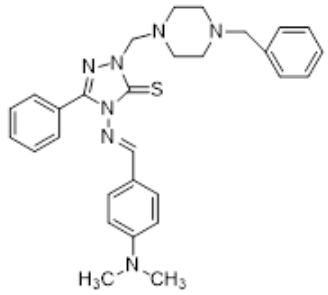


Figure 38. Compound 51 IC_{50} = 0.709 µM [63]

3.3. Other BACE-1 Inhibitors

3.3.1. Some Other Compounds Showing BACE-1 Inhibitory Activity

A study assessing the inhibitory properties of indol-piperidine amide derivatives of AChE and BACE-1 found that the N-(2-(1-benzylpiperidin-4-il)ethyl)-5,6-dimethoxy-1H-indole-2-carboxamide (compound 52 (Figure 39)) inhibits AChE and BACE-1 with IC_{50} values of 0.32 and 0.39 respectively [6].

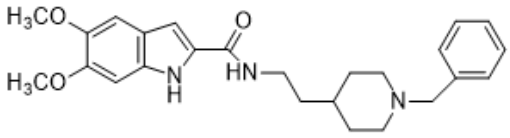


Figure 39. Compound 52 IC_{50} = 0.39 µM [6]

The inhibitory properties of these derivatives against the BACE-1 enzyme have been studied by synthesizing certain derivatives containing 2-aminobenzimidazole with an ether moiety. In this study, effective inhibitors were synthesized with EC_{50} values ranging from 0.05 to 2.71 µM. Compound 53 (Figure 40) has been found to be the most potent BACE-1 inhibitor. This derivative has also shown high selectivity over BACE2 and catepsin D [64].

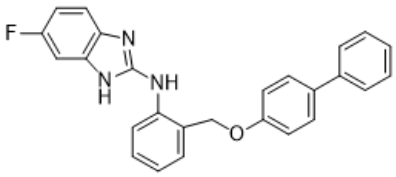


Figure 40. Compound 53. IC_{50} = 0.67 µM [64]

A study based on the MTDL approach found that 54 and 55 compounds, which are GABA transporter (GAT) inhibitors, inhibit various AD-related activities. Compound 54 (Figure 41) has been found to be effective in preventing BACE-1 and Aβ40 aggregation. This compound has also been found to have inhibitory activities of mGAT1, mGat4 and BuChE. The compound 55 (Figure 41) has been discovered to have high inhibitory activity of BACE-1, Aβ aggregation, mGAT1, mGat4 and BuChE. Studies in mice found that the compound 54 and 55 had anti-amnesial properties in the model of amnesia induced by scopolamine [65].

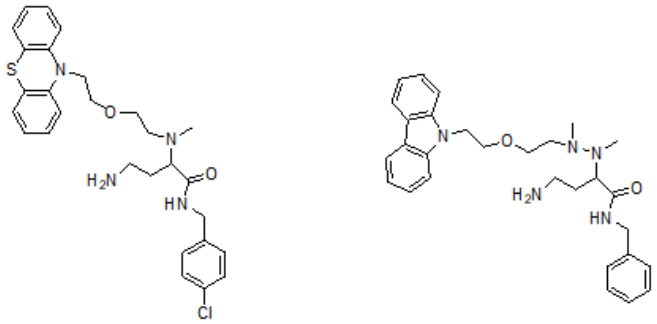


Figure 41. Compound 54 IC_{50} = 1.57 µM. Compound 55 IC_{50} = 9.42 [65]

In a different study, four small-molecular BACE-1 inhibitors were selected and their molecular dynamics simulations and binding energies analyzed in a virtual environment. As a result of the study, four molecules have been found to bind to BACE-1 in a generally stable way. According to the free-binding energy analysis, 56 (Figure 42) of these molecules have the highest binding affinity with BACE-1. Hydrogen bond analysis showed that it forms a limited number of bonds between BACE-1 and small molecules [66].

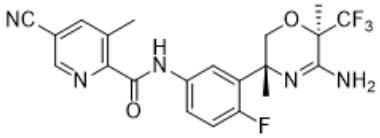


Figure 42. Compound 56. IC_{50} = 5 µM [66]

3.3.2. Natural Compounds Investigated for BACE-1 Inhibitor Activity

Along with the development of synthetic compounds for the treatment of AD, it is estimated that natural compound containing polyphenolic groups such as flavonoids, terpenoids, ginsenosides, and alkaloids could also be used. [7, 67, 68].

The polymethoxy flavons found in black ginger (*Kampferia parviflora*) are known for their antioxidant, anti-cancer, and fatigue relieving properties. The most potent BACE-1 inhibitor was found to be 5,7,4'-trimethoxyflavone (TMF) (Figure 43) with an IC_{50} of 0.569 nM. The other components in the extract have been found to have significant BACE-1 inhibitor activity [69].

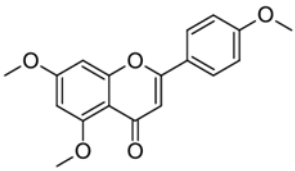


Figure 43. 5,7,4'-Trimetoxyflavone (TMF) IC_{50} = 0.569 nM [69]

Leea indica leaves have been used as an antispasmodic, anti-cancer and antidiarrheal medicine [70]. 40 molecules have been isolated from various parts of this plant [71]. A virtual scan of the isolated compounds identified ursolic acid and lupeol, two molecules of triterpenes with a high degree of binding affinity to BACE-1. In SAR studies, the BACE-1 enzyme has been observed to bind ursolic acid with residues of Asn233 and Thr232, while lupeol (Figure 44) binds to Gly11 by hydrogen. Although both of these compounds have hydrophobic interactions with Tyr71 residues in the wing region, they have not been found to interact with the catalytic aspartic acid residue (Asp32 and Asp228). Lupeol has been found to be more efficient in eliminating BBB than ursolic acid [72].

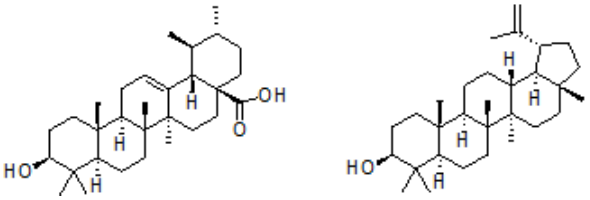


Figure 44. The chemical formulas of ursolic acid and lupeol

Compound 57 (Figure 45) isolated from *Morusihou* bark is a noncompetitive inhibitor of BACE-1 with an IC_{50} value of 0.034 nM. Molecular modeling studies show that the resorcinol moiety in the compound has a hydrophobic interaction with the hydrogen bond Asp228 and the prenyl group [73].

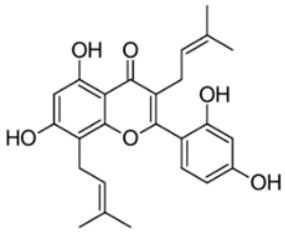


Figure 45. Compound 57. IC_{50} = 0.034 nM [73]

Curcumin obtained from turmeric (Compound 58 (Figure 46)) has been found to reduce the accumulation of hippocampal β-amyloid, which is important in the treatment of AD [74–76].

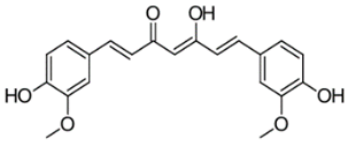


Figure 46. Compound 58. IC_{50} = 0.025 nM [74, 76]

Terpenoids are a type of natural product that exhibits BACE-1 inhibitor activity. Diterpenes and triterpenes have been studied extensively. The source of one of these compounds, *Spongionella gracilis*, contains four diterpenes with strong activity. The compound 59 (Figure 47), obtained from *Spongionella gracilis* reduced β-amyloid levels by 64% with a value of 0.01nM IC_{50} [77, 78].

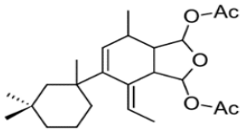


Figure 47. Compound 59. IC_{50} = 0.01 nM [77, 78]

The carbazole alkaloid compound (Compound 60 (Figure 48)) obtained from *Murraya koenigii* leaves with an IC_{50} value of 0.017 nM was found to be an effective BACE-1 inhibitor and beneficial for cognitive function [32].

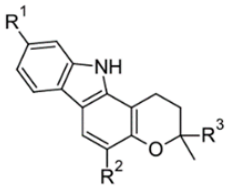


Figure 48. Compound 60. IC_{50} = 0.017 nM [78]

The antioxidant, BACE-1 and AChE enzyme inhibitory activities of the compounds found in the root, bulb and above-ground parts of *Clivia miniata* plant belonging to Amaryllidaceae family were investigated. Research has found that the root extraction of the plant is more effective in terms of BACE-1 inhibitor activity than other parts [79].

Emblica officinalis (Amla) is used in Indian medicine to treat diabetes, hyperlipidemia, and neurological diseases. A study has been conducted to study the potential of the active ingredients of *E. officinalis* in the treatment of AD. Results of molecular dynamic simulation analysis show that quercetin and rutine (Figure 49) have the potential to inhibit the BACE-1 protein and may be effective in the treatment of AH [80].

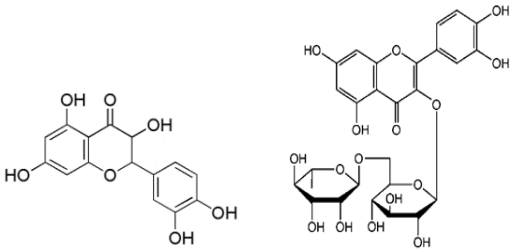


Figure 49. The chemical structures of quercetin and rutine

Selaginella doederleinii (Selaginellaceae) is a plant widely used in Chinese herbal medicine as an anti-inflammatory, anti-cancer and cardioprotective agent. Various triflavonoids have been successfully isolated from *S. doederleinii*, including triflavonoids of selajin with a trimeric structure. An *in vitro* study using the Floresan Resonance Energy Transfer (FRET) technique showed that compound 61 (Figure 50) is the most potent BACE-1 inhibitor with 0.75 μM IC_{50} [81].

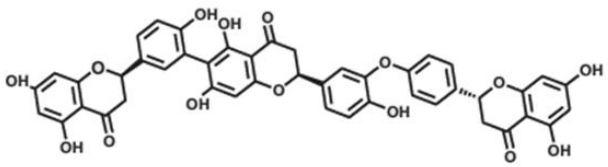


Figure 50. Compound 61. IC_{50} = 0.75 μM [82]

In a study on the BBB permeability of flavonoid, anthraquinone and cinnamic acid derivatives, ponsiretin, danthrone, chrysophanol and N-p-coumaroyl tyramine (Figure 51) were identified as some of the most effective BACE-1 inhibitors. The study also found that these compounds are seen as potential options for the treatment of AD and Parkinson's disease. These compounds are thought to be a model for the development of new BACE-1 inhibitors [83].

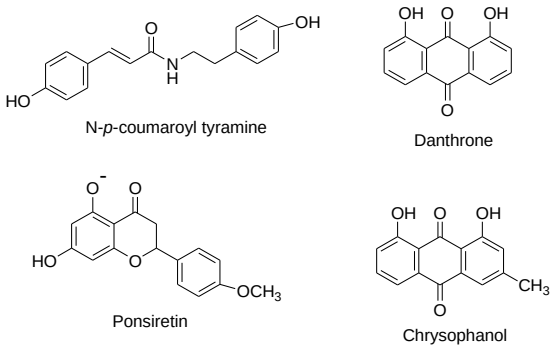


Figure 51. The molecular structure of N-*p*-coumaroyl thyramine, danthrone, ponsiretin, and chrysophanol

The methyl chalcone derivative of hesperidin (Compound 62 (Figure 52)), a plant product, was synthesised and its effect on Aβ was investigated. In these studies, it was observed that compound 62 increased cell viability, reduced oxidative stress, prevented macromolecular damage, calmed mitochondrial dysfunction and exhibited anticholinesterase activity. Studies have also shown that compound 62 can be a powerful inhibitor of BACE-1 and inhibit the formation of toxic Aβ peptides [84].

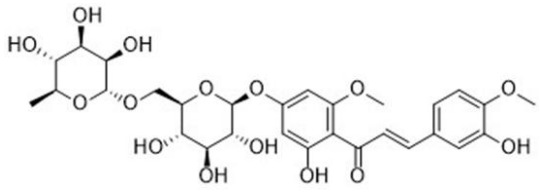


Figure 52. The chemical structure of compound 62

3.3.3. BACE-1 Inhibitors Entering Clinical Trials

3.3.3.1. LY2811376

LY2811376 (Figure 53) is the first small molecular BACE-1 inhibitor developed by Eli Lilly and entered clinical trials. In 2009, a translational phase I clinical study was conducted to investigate the safety, pharmacodynamic and pharmacokinetic profiles of oral LY2811376. The results showed a significant decrease in Aβ levels in plasma and cerebrospinal fluid [85]. However, further research has been stopped due to additional toxicological data reporting damage to the pigment epithelium in eyes of the rats. Despite the negative results of LY2811376, this molecule provided the first clinical evidence that BACE-1 is a reasonable target for AD. LY2811376 is currently used as a selective BACE-1 inhibitor only in experimental studies [86].

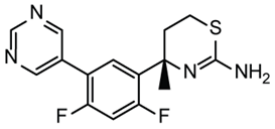


Figure 53. Molecular structure of LY2811376

3.3.3.2. LY2886721

LY2886721 (Figure 54) is a second-generation BACE-1 inhibitor developed by Eli Lilly. It is the first BACE-1 inhibitor to reach phase II clinical trials. Results from Phase I clinical trials show that LY2886721 is generally safe and well tolerated in different dosage regimes [87]. However, the study was discontinued due to the observation of abnormal levels on liver enzymes. Therefore, more human studies have been stopped for patients with AD and mild cognitive impairment [88].

Animal studies are being conducted to investigate the potential effects of LY2886721. A study in this direction found that the drug improved glucose homeostasis, increased hepatic gluconeogenesis and insulin sensitivity, and had negative effects on APP development. BACE-1 inhibitors have been suggested to be potentially used in the treatment of pathologies associated with type 2 diabetes mellitus [89].

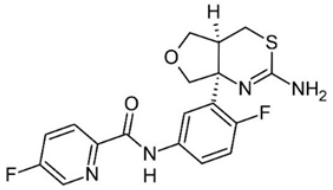


Figure 54. Molecular structure of LY2886721

3.3.3.3. RG7129 (RO5508887)

RG7129 (Figure 55) is a BACE-1 inhibitor produced by Roche and administered by mouth. Roche launched three phase I clinical trials in late 2011 and 2012 to evaluate the safety, pharmacokinetics and pharmacodynamics of RG7129 in healthy participants. However, clinical trials were stopped in late 2013 due to liver toxicity [90].

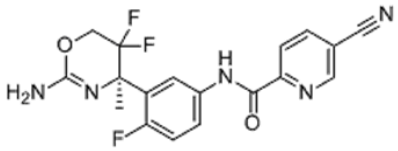


Figure 55. Molecular structure of RG7129

3.3.3.4. BI 1181181

BI 1181181 (Figure 56) is a small molecular BACE-1 inhibitor, discovered by Vitae Pharmaceuticals and developed by Boehringer Ingelheim. In pre-clinical studies, the drug has been shown to significantly reduce Aβ levels in rats and goats [91, 92].

Three Phase I clinical trials were launched in 2014 to investigate safety, tolerability, pharmacokinetics and pharmacodynamics of BI 1181181 in healthy participants. Two of the three phase I studies have been completed. The results show that a single dose of BI 1181181 is well

tolerated and there is a significant and sustained decrease in the Aβ level in the cerebrospinal fluid. The pharmacokinetics were dose-proportionate, not influenced by food and were consistent with dosing once a day [93, 94].

The third phase I study aimed primarily at investigating the safety and tolerability of repeated and increased oral doses of BI 1181181 (given once a day for 10 days). However, the study was discontinued after skin reactions were among some participants. In 2015, another phase I study aimed at investigating the effects of different BI 1181181 doses on the kinetics of midazolam, warfarin, omeprazole and digoxin in a single dose was withdrawn. Currently, BI 1181181 is not mentioned in any other experimental or clinical study [95].

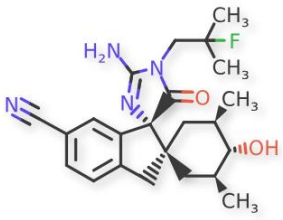


Figure 56. Molecular structure of BI 1181181

3.3.3.5. JNJ-54861911 (Atabecestat)

JNJ-54861911 (Figure 57) is a BACE-1 inhibitor developed by Janssen. This molecule has a long history of clinical trials with promising results, and phase II/III studies have been conducted. Janssen conducted a series of phase I studies that began in 2013. These studies aimed to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic effects of single and multiple dose regimes on healthy subjects. Serious adverse effects such as the compound's QT/QTc range, interactions with other medicines and foods have also been studied. Two Phase I studies aimed at investigating the safety, tolerability, pharmacokinetic and pharmacodynamic effects of the drug in prodromal AD patients and the Aβ levels in the cerebrospinal fluid in asymptomatic individuals. Results of Phase I studies show that JNJ-54861911 is generally well tolerated, is effective on CNS, and provides a high and stable decrease in Aβ levels. [96–98].

However, in 2014 and 2015, Janssen launched two phase II studies to assess the long-term safety and tolerability of various JNJ-54861911 doses in early-term AD patients. In addition, a Phase II/III study was conducted in 2015 to evaluate the effectiveness of JNJ-54861911 in slowing cognitive decline in Aβ-positive asymptomatic subjects with high risk of AD. However, both studies in 2015 were terminated and stopped in 2018 due to reports of liver toxicity in subjects. Preliminary and complete trial results were later published and revealed that treatment with JNJ-54861911 did not yield any benefit compared to a placebo, caused an increase in liver enzymes, and had a tendency to decline in cognition. However, after 6 months of treatment, evidence of reversibility has been found [99–101].

In 2018, a study has been conducted to investigate inflammatory immune response through T-cell in subjects who have previously administered JNJ-54861911 or their metabolites, which is thought to be a means of mediating liver toxicity. However, a liver biopsy from one of the volunteers with elevated liver enzymes was discontinued because there were signs of inflammation along with an increase in T and B cell infiltrations and hepatocyte death [102]. Subsequent findings showed that T-cell clones sensitive to the metabolite JNJ-54861911 were detected in patients suffering from liver toxicity, indicating an immune-based mechanism for the observed liver enzyme elevations [103]. By 2022, the use of JNJ-54861911 for AD in clinical or experimental trials has been discontinued [95].

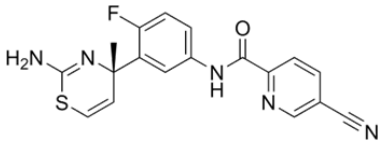


Figure 57. Molecular structure of JNJ-54861911 (Atabecestat)

3.3.3.6. LY3314814 (AZD3293, Lanabecestat)

LY3314814 (Figure 58) is a BACE-1 and BACE-2 inhibitor developed in collaboration with AstraZeneca and Eli Lilly. Pre-clinical data from various animal models have shown promising results supporting the progress of LY3314814 into clinical trials [104, 105].

From 2012 to 2017, a number of phase I studies of LY3314814 have been conducted to evaluate the safety, tolerability, drug interactions, pharmacokinetic and pharmacodynamic profiles of various dosages and dosage regimes. Results from Phase I trials show that LY3314814 is generally safe and well tolerated, with a significant decrease in plasma and cerebrospinal fluid Aβ levels in different drug formulations [106–108].

A phase II/III trial (NCT02245737) called the 'AMARANTH' trial to investigate the safety and efficacy of LY3314814 was conducted in 2014 for a period of 104 weeks in early-stage AD treatment. In 2016, the 'AMARANTH' study was followed by two additional phase III studies (NCT02972658 and NCT02783573), with a total of more than 4,300 participants in the three studies. However, an independent assessment in 2018 revealed that these trials were less likely to succeed after they were completed, leading to the termination of the trials [106–108].

The results of Phase III studies were later published and confirmed the findings of the independent assessment. The treatment of LY3314814 has been associated with cognitive impairment and decreased brain volume, although it does not slow down cognition or functional decline and does not alter the progression of the disease. [109–111]. LY3314814's trials for AD are currently stopped [95].

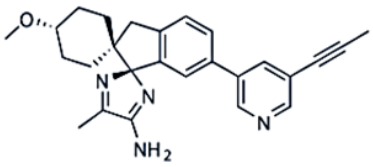


Figure 58. Molecular structure of LY3314814

3.3.3.7. MK-8931 (MK-8931-009, Verubecestat)

MK-8931 (Figure 59) is a small molecular BACE-1 and BACE-2 inhibitor developed by Merck. Three phase I clinical trials (NCT01496170, NCT01537757 and NCT-02910739) have been conducted to assess safety, tolerability, pharmacokinetics and pharmacodynamics. Results from Phase I studies show that MK-8931 has potential in the treatment of AD. It is generally well tolerated and has been

able to reduce the average cerebrospinal fluid concentrations of Aβ proteins [112, 113].

In 2012, Merck launched the EPOCH study (NCT01739348) as a Phase II study, which was later extended to Phase III. The study involved more than 2,000 participants to evaluate the effectiveness and safety of MK-8931 for 78 weeks in people with mild to moderate AD. This was followed by the APECS study (NCT01953601), another Phase III study that investigated the safety and efficacy of MK-8931 in prodromal AD. However, both EPOCH and APECS trials have been stopped. The published results showed that MK-8931 had no benefit in reducing cognitive or functional decline in patients with mild to moderate AD. On the contrary, various adverse effects related to treatment, including cognitive impairment, loss of brain volume, falls and injuries, suicidal thoughts, weight loss, sleep disorders, rash and hair color changes, have been observed. However, MK-8931 has no adverse effect on retinal thickness, and there has been some improvement in verbal fluidity functions [114–118]. Work on MK-8931 stopped in 2022 [95].

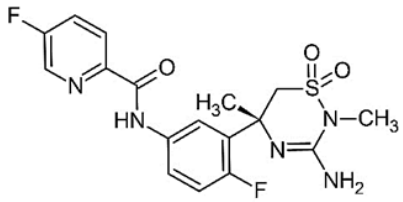


Figure 59. Molecular structure of MK-8931

3.3.3.8. E2609 (Elenbecestat)

E2609 (Figure 60) is a small molecular, selective BACE-1 inhibitor. It was developed by Biogen and Eisai Co. Ltd. Pre-clinical data have shown that E2609 significantly reduces Aβ levels and does not cause hypopigmentation, a side effect observed with other BACE-1 inhibitors such as MK 8931 [119].

Ten phase I clinical trials have been conducted from 2011 to 2017 to assess the safety, tolerability, pharmacokinetics, pharmacovigilance, drug and food interactions of the various doses and regimes of E2609. Results of some phase I studies showing that E2609 is generally well tolerated have been published. Furthermore, no limitation or dose adjustment was required when administered in conjunction with CYP3A inhibitors [120–122].

A Phase II study to evaluate the safety and efficacy of E2609 in participants with mild-to-moderate cognitive impairment with AD, prodromal AD or AD-related dementia was launched in December 2014. The initial results of the study showed that E2609 was generally well tolerated and no unexpected safety concerns emerged. Also, no liver toxicity has been observed. The trial also showed a significant decrease in the amount of Aβ. [123]. Two multi-centric phase III studies, known as 'MissionAD1' and 'MisionAD2', were launched in 2016 to evaluate the effectiveness and safety of E2609 in early-stage AD patients and were conducted in parallel with the phase II study. However, all trials in 2019 were discontinued due to negative risk-to-risk ratio, lack of evidence of potential effectiveness, and worse adverse event profile of E2609 compared to placebo [121, 123, 124].

Phase III results were presented at the 2021 International Conference of the Alzheimer's Association. The results showed no evidence of the effectiveness of the treatment, even in very light subjects, in the early termination of the 'MissionAD' program [124]. In 2022, the work of E2609 was stopped [95].

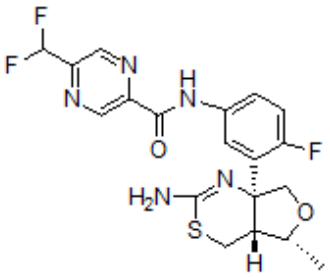


Figure 60. Molecular structure of E2609

3.3.3.9. CNP-520 (Umibecestat)

CNP-520 (Figure 61) is a BACE-1 inhibitor in capsule form developed by Novartis and Amgen. Initial data showed that CNP520 reduced Aβ levels and accumulation in the brain and cerebrospinal fluid in rats, dogs, and APP-transgenic mice. It has also shown sufficient safety without signs of depigmentation, retina, liver or cardiovascular toxicity. In 2015, a multi-centric phase II study (NCT02576639) was conducted on subjects aged 60 and over. The study revealed that CNP520 is generally safe and well tolerated, resulting in a sharp dose-dependent decrease in Aβ levels in the cerebrospinal fluid. [125]. Two registered phase II/III studies, called 'Generation 1' and 'General 2', were terminated in 2019 due to safety concerns such as cognitive impairment, brain atrophy and weight loss related to CNP-520. However, follow-up after discontinuation of drug therapy has shown that these adverse events are reversible. At the moment, work on CNP-520 has been stopped [95].

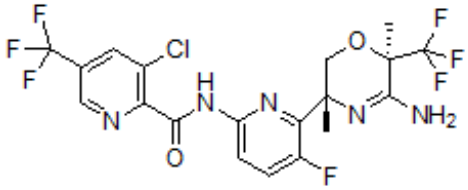


Figure 61. The molecular structure of CNP-520

3.3.3.10. LY3202626

LY3202626 (Figure 62) is a BACE-1 inhibitor developed by Eli Lilly. Pre-clinical studies have shown that LY3202626 can reduce concentration-dependent Aβ expression in primary neural cultures in mice with PDAPP. Also, after oral treatment, PDAPP was found to reduce hippocampal and cortical Aβ levels in mice and hunting dogs [126, 127].

Three phase I trials (NCT02323334, NCT02555449 and NCT-03023826) were conducted on healthy subjects between 2014 and 2017. Phase I trials showed that LY3202626 is generally well tolerated in all tested doses. Maximum plasma concentration was reached 3 hours after administration. LY3202626 passed BBB and produced a dose-dependent decrease in both Aβ40 and Aβ42 in plasma and cerebrospinal fluid. It has been found to be metabolized

primarily through O-demethylation and amide hydrolysis. [128, 129].

In 2016, the clinical study 'NAVIGATE-AD' phase II (NCT02791191) evaluated the safety and effects of LY3202626 on tau in patients with mild AD. The published results showed that LY3202626 was generally well tolerated, but had no significant effect compared to placebo, and the study was closed [130].

In the same year, LY3202626 was removed from another phase II study (NCT03367403) called 'TRAILBLAZER-ALZ', which was evaluated in early-term symptomatic AD patients. In 2022, LY3202626 was discontinued.

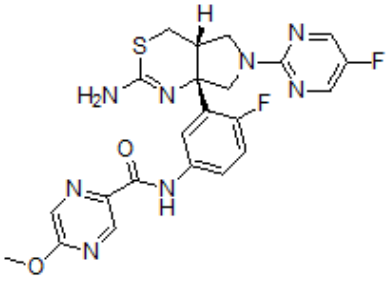


Figure 62. Molecular structure of LY3202626

3.3.3.11. PF-06751979

PF-06751979 (Figure 63), developed by Pfizer, is a small molecular BACE-1 inhibitor with high selectivity for BACE-1, compared to BACE-2 [131]. The safety, tolerability, pharmacokinetic and pharmacodynamic properties of PF-06751979 were evaluated in three phase I studies (2015 NCT02509117, 2016 NCT02793232 and 2017 NCT03126721) [132].

The published results of Phase I studies indicate that PF-06751979 is generally well tolerated in all tested doses and that the adverse events are mild to moderate. The pharmacokinetic parameters remained consistent in once-daily dosing regimes and no significant food effect was observed. Pharmacodynamic analysis showed a concentration-related decrease in the cerebrospinal fluid and Aβ in plasma. The greatest decreases were observed with a dose of 275 mg once daily. These results support further clinical development. However, in January 2018, Pfizer announced that it had completed developments in the field of neurology, including PF-06751979. As a result, the compound has not progressed to further phase II or III trials and studies have been stopped by 2022 [132].

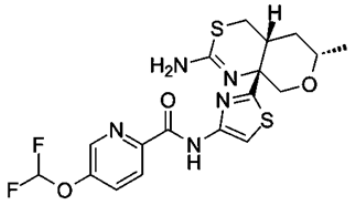


Figure 63. Molecular structure of PF-06751979

3.3.3.12. CTS21166

CTS21166 (Figure 64) is a BACE-1 inhibitor developed by CoMentis in conjunction with Astellas Pharma. CTS21166 significantly reduced Aβ levels and accumulation in APP-transgenic mice [133].

A single registered phase I study (NCT00621010) was conducted in 2008 to assess the safety and tolerability of CTS21166 in healthy male volunteers. However, no further records of human studies or the results of the experiment have been found.

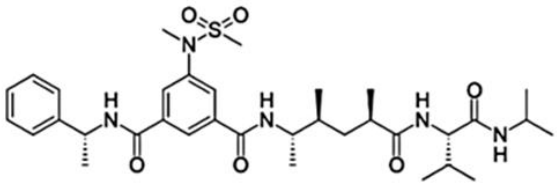


Figure 64. The molecular structure of CTS21166

3.3.3.13. HPP854

HPP854 is a BACE-1 inhibitor developed by High Point Pharmaceuticals. There are no pre-clinical studies of HPP854. Only one registered phase I study (NCT01482013) was carried out in 2011 to assess safety, tolerability, pharmacokinetic and pharmacodynamic relations, as well as cerebrospinal fluid and plasma concentrations. However, the results of the study have not been published or announced, and no records of any further human study have been found.

4. Nanotechnology: Unveiling the Potential in Targeting BACE-1 Enzyme for Alzheimer's Disease

As the battle against Alzheimer's disease rages on, scientists are harnessing the power of nanotechnology to explore novel therapeutic avenues. Among the key targets in this endeavor is BACE-1, a pivotal player in the formation of amyloid-beta plaques characteristic of AD pathology [134].

Nanotechnology, with its capacity to manipulate materials at the nanoscale, offers a unique toolkit for precise intervention at the molecular level. In the context of AD, nanotechnology presents a promising platform for targeted drug delivery, enhanced imaging techniques, and innovative therapeutic strategies [135].

In the quest to develop effective BACE-1 inhibitors, nanotechnology provides avenues for overcoming traditional pharmacological challenges. Nanoformulations offer improved bioavailability, enhanced blood-brain barrier permeability, and reduced off-target effects, thereby augmenting the therapeutic potential of BACE-1 inhibition [136, 137].

Nanoparticle-based drug delivery systems hold immense promise in delivering BACE-1 inhibitors to the brain with precision. Functionalized nanoparticles can traverse the blood-brain barrier, enabling targeted drug delivery to specific regions affected by AD pathology. Furthermore, nanocarriers can encapsulate BACE-1 inhibitors, shielding them from enzymatic degradation and prolonging their circulation time in the body [138].

Lipid-based nanocarriers, such as liposomes and lipid nanoparticles, offer a versatile platform for BACE-1 inhibitor delivery. By incorporating BACE-1 inhibitors into lipid bilayers, these nanocarriers can exploit endogenous cellular uptake mechanisms to facilitate drug transport across biological barriers. Moreover, lipid-based nanocarriers can

be engineered to release BACE-1 inhibitors in a controlled manner, optimizing therapeutic efficacy while minimizing systemic toxicity. Polymeric nanoparticles represent another promising approach for BACE-1 inhibition. Utilizing biocompatible polymers, such as poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG), polymeric nanoparticles can encapsulate BACE-1 inhibitors and facilitate their sustained release. Additionally, surface modification with targeting ligands enables specific recognition of BACE-1-overexpressing cells, enhancing therapeutic precision and reducing off-target effects [139–141].

In addition to drug delivery, nanotechnology empowers researchers with advanced imaging techniques for elucidating BACE-1 dynamics in AD. Quantum dots, gold nanoparticles, and superparamagnetic iron oxide nanoparticles offer unparalleled sensitivity and spatial resolution, enabling real-time monitoring of BACE-1 activity in living organisms. By visualizing BACE-1 localization and dynamics, these nanoscale imaging techniques provide invaluable insights into disease progression and therapeutic response [142, 143].

As nanotechnology continues to evolve, the prospects for targeted BACE-1 inhibition in AD appear increasingly promising. By harnessing the precision and versatility of nanoscale platforms, researchers are poised to overcome longstanding challenges in AD therapeutics and pave the way for transformative treatments. With ongoing innovation and collaboration, the intersection of nanotechnology and BACE-1 inhibition holds the potential to revolutionize the landscape of AD management [144, 145].

5. Conclusion

AD is a progressive dementia-type neurological brain disease that causes the destruction of cells in the brain and is common worldwide, especially in people over the age of 60. According to the WHO 2022 data, the number of AD patients worldwide is expected to increase from more than 55 million to 139 million in 2050. One of the notable approaches for the treatment of AD, for which there is no definitive cure, is the development of inhibitors of the enzyme BACE-1, which is involved in the formation of Aβ peptides in the brain by causing proteolytic cleavage of APP. The BACE-1 enzyme consists of five main domains: signal peptide domain, pro-peptide domain, catalytic domain, transmembrane domain and cytosolic domain. The binding pocket of BACE-1 contains three important parts: the catalytic aspartic acid residues, the wing, which is the most flexible part of the binding site and controls substrate access, and the 10-second loop located near the S3 pocket. The fact that the active site of the BACE-1 enzyme is quite large, the difficulty of overcoming the BBB, the requirement for high lipophilicity are the difficulties encountered in the development of inhibitors. BACE-1 enzyme inhibitors, in general, have peptidomimetic or nonpeptidomimetic structure. Peptidomimetic inhibitors are designed to mimic the enzyme's natural substrate, thereby increasing subsite specificity, hydrogen bonding and hydrophobic interactions. The inhibitory effect of these molecules is due to the transition state isoster. These inhibitors were developed by shortening the substrate-based peptide and at the same time replacing the easily cleavable amide bond with a non-cleavable transition state isoster such as hydroxyethylamine, statins, hydroxyethylene, isophthalamide, norstatin, homostatin. In the development of nonpeptidomimetic inhibitors, High Throughput Screening (HTS), substrate-based design, fragment-based approaches, computational screening, modelling methods that can narrow a large compound library down to a few hundred compounds that can be analysed by SAR studies have been used. Thus, it is aimed to develop inhibitors with small molecular size and weight, high permeability to the BBB, low peptidic character and P-gp flow rate, and better metabolic stability. As a result, nonpeptidomimetic enzyme inhibitors carrying Acyl guanidine, 2-aminopyridine, aminoimidazole, amino/iminohydantoin, aminothiazoline, aminooxazoline, aminoquinoline, piperazine fragments were developed. Additionally, natural products such as 5,7,4'-trimethoxyflavone, ursolic acid, lupeol, quercetin and routine have been found to have BACE-1 inhibitory activity. LY2811376, LY2886721, RG7129, BI 1181181, JNJ-54861911, LY3314814, MK-8931, E2609, CNP-520, LY3202626, PF-06751979, CTS21166, and HPP854 are BACE-1 inhibitors entering clinical trials. As a result of the studies, despite the intense efforts spent to develop BACE-1 enzyme inhibitors and some synthesized compounds entering clinical trials, it has not been possible to reach a molecule that can be used as a drug in the treatment of AD. In the relentless pursuit of a cure, nanotechnology emerges as a beacon of hope, illuminating the path towards a future free from the ravages of AD. Through interdisciplinary synergy and unwavering dedication, scientists stand united in their quest to harness the full potential of nanotechnology in the fight against AD.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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