

The Design, Development, and Evaluation of BACE1 Inhibitors for the Treatment of Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is a very serious public health problem. Currently, there is no effective treatment for AD. Among the many biochemical targets for AD drug development, β -secretase (BACE1, memapsin 2) continues to be a promising drug discovery target for AD therapy. This proteolytic enzyme is a membrane-anchored aspartic acid protease that is responsible for the initial step of amyloid precursor protein (APP) cleavage, leading to the production of neurotoxic amyloid- β (A β) peptides in the brain. Since its identification and structural elucidation in 1999, extensive research efforts have led to the development of many promising classes of inhibitors against this protease. Structure-based design strategies led to the evolution of many small-molecule, peptidomimetic, and nonpeptide BACE1 inhibitors that have now overcome the key development challenges including selectivity and brain penetration. To date, 13 BACE1 drug candidates have been brought to clinical trials, and a number of them have advanced to phase II/III human trials. This chapter illustrates structure-based evolution of various classes of BACE inhibitors. Also, it provides a perspective on BACE1 inhibitor drugs for the treatment of AD patients.

Keywords β -Secretase, Alzheimer's disease, BACE1, BACE1 inhibitor, Drug design, Enzyme inhibitors, Memapsin 2, Nonpeptide inhibitor, Peptidomimetic inhibitor, Structure-activity relationship

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Abbreviations

A β	Amyloid- β protein
AD	Alzheimer's Disease
ADAS	Alzheimer's Disease Assessment Scale
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ADCS	Alzheimer's Disease Cooperative Study
ADCS-ADL	Alzheimer's Disease Cooperative Study-Activities of Daily Living
ADCS-PACC	Alzheimer's Disease Cooperative Study-Preclinical Alzheimer's Cognitive Composite
ADME	Absorption distribution, metabolism, and excretion
APP	Amyloid precursor protein
BACE1	β -Site amyloid precursor protein-cleaving enzyme 1
BACE2	β -Site amyloid precursor protein-cleaving enzyme 2
BBB	Blood-brain barrier
CatD	Cathepsin D
CCS-3D	3-Domain Composite Cognition Score
CDR	Clinical Dementia Rating Scale
CDR-SB	Clinical Dementia Rating Sum of Boxes
CFI	Cognitive Function Instrument

CNS	Central nervous system
CSF	Cerebrospinal fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
ECG	Electrocardiogram
FDA	Food and Drug Administration
HIV	Human immunodeficiency virus
HTS	High-throughput screening
IC ₅₀	50% inhibitory concentration
K _i	Inhibitory constant
MMSE	Mini Mental State Examination
MRI	Magnetic resonance imaging
MWM	Morris water maze
NMDA	N-Methyl D-aspartate
NPI	Neuropsychiatric Inventory
NTB	Neuropsychological Test Battery
PET	Positron emission tomography
Pgp	P-glycoprotein
QT	Q-wave to T-wave interval
sAPP α	Soluble amyloid precursor protein α -fragment
sAPP β	Soluble amyloid precursor protein β -fragment
SAR	Structure-activity relationship

1 Introduction

Alzheimer's disease (AD) has emerged as a serious public health issue affecting millions of elderly people around the globe. AD is an irreversible, progressive, degenerative brain disorder that slowly destroys brain function, leading to cognitive decline, behavioral change, and deterioration of bodily function in later stages. Currently, about 44 million people worldwide are believed to have Alzheimer's disease or a related dementia [1, 2]. This number is expected to grow more than 100 million cases by 2050 [3]. The global cost of current AD patient care is estimated to be a staggering \$605 billion, which is equivalent to 1% of the world's gross domestic product [4]. The situation will get worse as there is no cure or disease-modifying treatment for AD at this time. A number of approved drugs are available only to treat symptoms in AD patients [5]. There is an urgent need for mechanism-based, effective AD therapies that will prevent, delay the onset, slow the progression, or improve AD symptoms.

Currently, five drugs are approved for the treatment of AD and related disorders. These include acetylcholinesterase inhibitors, tacrine (approved 1993), donepezil (1996), rivastigmine (1998), and galantamine (2001) [6, 7]. These drugs act by blocking the process that breaks down the neurotransmitter acetylcholine, a key signaling agent responsible for communication between nerve cells. In AD patients, decreased acetylcholine levels and loss of nerve cells are linked to worsening

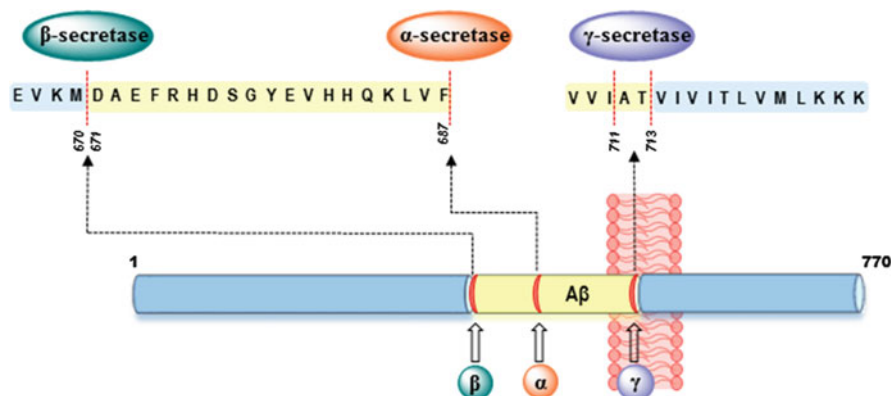


Fig. 1 Processing of APP by α -, β -, and γ -secretase. Cleavage by β -secretase provides the N-terminus of A β , while cleavage by γ -secretase provides the C-terminus of A β . Processing by γ -secretase provides both A β_{40} and A β_{42} by cleaving at residues 711 and 713, respectively. Cleavage by α -secretase occurs in the middle of the A β sequence and precludes the formation of A β

symptoms. Acetylcholinesterase inhibitors prevent breakdown of acetylcholine and help maintain adequate concentration of in the brain. Another Alzheimer's drug, memantine, is an *N*-methyl D-aspartate (NMDA) receptor antagonist, approved in 2004 [8, 9]. The drug regulates the activity of glutamate in the brain. Attachment of the neurotransmitter glutamate to the cell surface of NMDA receptors allows calcium to enter the cell. This is an important event in cell signaling, as well as in learning and memory. However, in AD patients, excess glutamate released from damaged cells leads to overexposure to calcium and accelerates cell damage. Memantine disrupts this chain of events by blocking the NMDA receptors.

AD drug development has been a formidable challenge [5]. A number of current AD drug development efforts are directed towards a breakthrough AD therapy that would treat the underlying disease mechanism and halt or slow down the neuronal cell damage that causes progressive deterioration. One of the major hallmarks of Alzheimer's disease is the accumulation of neuritic plaques containing the 40–42 residue amyloid- β (A β) peptides and neurofibrillary tangles composed of tau protein in the brain [1, 10]. Since a high brain A β level is regarded as an important factor in AD pathogenesis, clinical intervention to reduce its production has become a logical approach for AD therapy development [11, 12]. The origin of neuritic plaques can be attributed to proteolytic processing of amyloid precursor protein (APP) (Fig. 1). More specifically, the sequential cleavage of APP by β -secretase (BACE1, memapsin 2) and γ -secretase yields A β [13, 14]. The two main A β products of APP processing, A β_{40} and A β_{42} , both play a key role in the aggregation of neuritic plaques. While A β_{42} is more prone to aggregation, increasing levels of both peptides are observed early in the pathology of AD, and these rising levels are correlated with the severity of dementia observed in AD patients [15].

The preceding plaques are shown to be neurotoxic and eventually lead to neuronal death [16]. Therapeutic inhibition of β - or γ -secretase has been pursued in AD drug development since these two proteases are the first and second steps in the APP processing leading to $A\beta$ production [11, 17]. Following the cloning and identification of BACE1 in 1999, significant research efforts led to the development of several classes of potent and selective BACE1 inhibitors as a potential treatment of Alzheimer's disease [18, 19]. Herein, we outline structure-based evolution of various classes of BACE1 inhibitors, clinical evaluation of these inhibitors, and their perspective in the treatment of AD.

1.1 The Amyloid Hypothesis of AD

Most AD patients have cases of sporadic AD; however, genetic predispositions to AD can occur within families. While both sporadic and familial AD are pathologically the same, familial AD tends to occur as early-onset AD, with the symptoms appearing as early as age 30 [1]. Familial AD is caused by mutations in either APP or the presenilins responsible for γ -secretase activity. APP mutations result in more efficient cleavage by BACE1, increased propensity for aggregation, or an increase in the more aggregation-prone $A\beta_{42}$ [20]. There are many more presenilin mutations known, which lead to increased γ -secretase processing of APP into pathogenic $A\beta_{42}$ over the more phenotypically normal $A\beta_{40}$. These mutations in early-onset AD and similar pathology of sporadic AD have led to the amyloid hypothesis of AD progression (Fig. 2).

The amyloid hypothesis of AD proposes that the formation of $A\beta$ plaques initiates the flow of outcomes that ultimately leads to dementia in AD patients

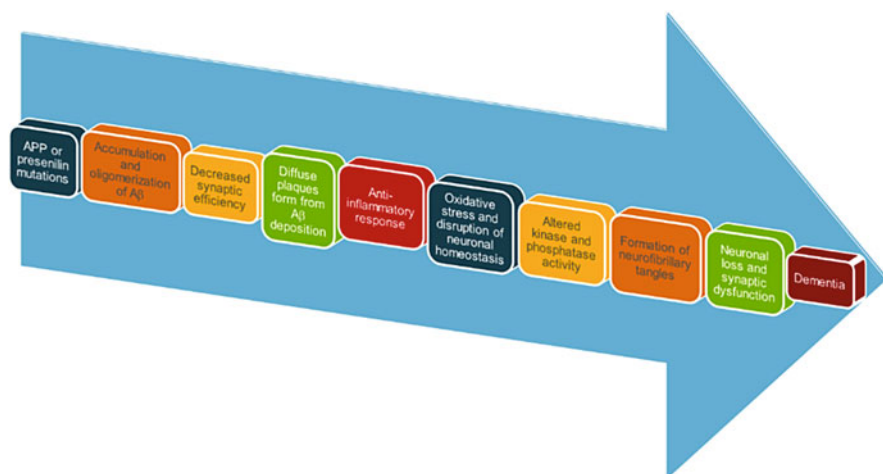


Fig. 2 Progression of events postulated by the amyloid cascade hypothesis

[21]. The accumulation of A β peptides leads to their oligomerization. This event results in decreased activity at the neuronal synapses. These soluble oligomers further associate to form A β plaques. These plaques trigger an anti-inflammatory response causing oxidative stress of the neurons. This can disrupt normal kinase and phosphatase activity, resulting in the hyperphosphorylation of tau protein and subsequent neurofibrillary tangle formation. This adds to abnormal signaling and further synaptic impairment. Ultimately, these events result in neuronal death and dementia in the AD patient [22].

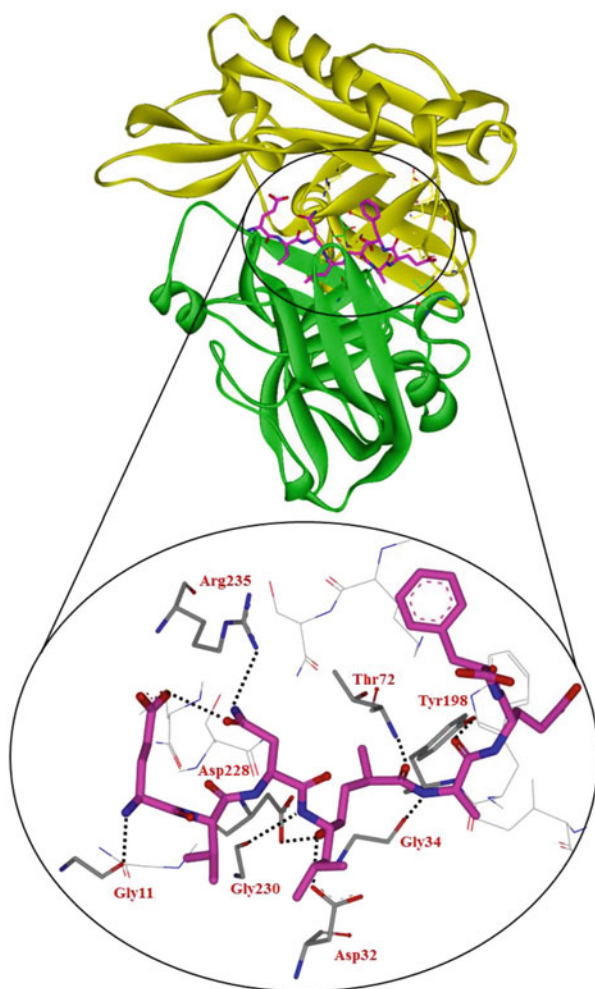
While the occurrence of familial AD and the genetic mutations associated with familial AD are strong arguments for the amyloid hypothesis, some observations remain unexplained [23]. It was once suggested by histological studies that the number of A β plaques in a diseased brain does not directly correlate to the degree of cognitive degeneration in AD patients. However, biochemical and later histological studies have shown that there is a correlation between plaque formation and cognitive decline [15, 23]. While there are further questions regarding the exact neuronal toxicity of A β in vivo, the amyloid hypothesis is still broadly accepted as the general pathological cascade of events in AD [21].

2 BACE1 as a Target for AD

BACE1 is an aspartic acid protease that plays a prominent role in the generation of neurotoxic A β plaques which has been strongly associated with AD pathology (Fig. 3) [24–27]. In light of the initial cloning and further elucidation of the role of BACE1 in AD pathology [26, 28], it has been determined that the inhibition of BACE1 may serve as a promising therapy for AD.

The proteolytic cleavage of APP by BACE1 marks the initial step of amyloidogenesis, which in turn triggers the later events of AD pathogenesis [29–31]. Additionally, the development of successful clinical protease inhibitors (PIs) for HIV protease, another extensively studied aspartic acid protease, has established an invaluable precedent for the development of other aspartic acid protease inhibitors [32]. It was envisioned that knowledge gathered from previous successful aspartic acid protease inhibitors could be utilized to develop effective BACE1 inhibitors. Upon further investigation of the enzyme, it was discovered that BACE1-null mice appeared to be phenotypically normal, with few detectable abnormalities [30, 31, 33]. These mice were viable and fertile and displayed normal tissue histology and blood chemistry. However, upon further analysis of BACE1^{-/-} mice, multiple neuronal phenotypes were discovered, including seizures, memory impairments, reduced myelination, and schizophrenia, among others [34–39]. It is possible that complete cessation of BACE1 activity may lead to unwanted mechanistic side effects. The establishment of a proper therapeutic window would allow for proper inhibition of BACE1 in order to slow or halt the progression of AD while allowing other necessary BACE1 functions to continue.

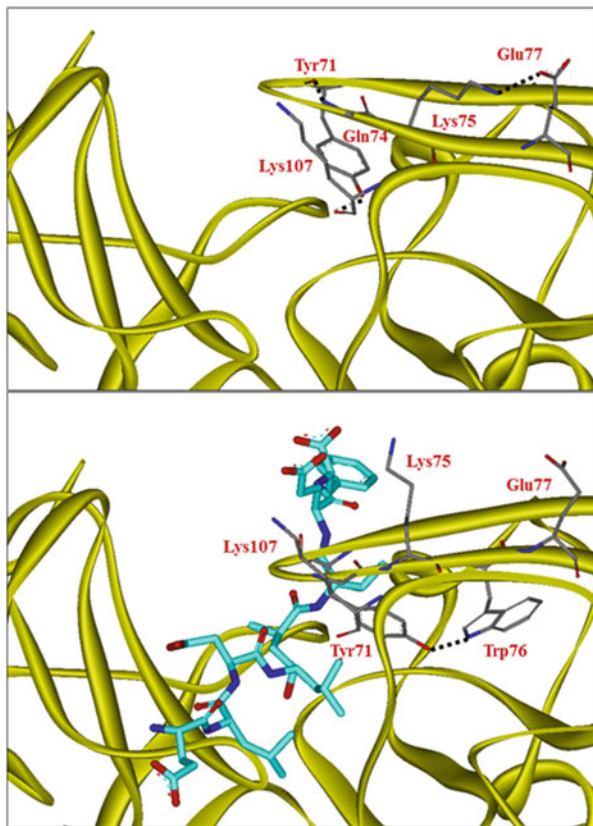
Fig. 3 Overall structure of BACE1 and interactions of inhibitor OM99-2 (**2**) in the BACE1 active site (PDB: 1FKN)



The propensity for membrane penetration of BACE1 inhibitors is vital to clinical success as A β production Leading to AD takes place in the brain [40]. The inability to generate compounds that are permeable through cell membranes and the blood–brain barrier (BBB) would discredit the inhibition of BACE1 as a viable therapy for AD. Validation studies have shown that peptide and sterol linkers have allowed inhibitors to penetrate sites of A β accumulation as well as diminish A β brain levels in AD mice [41, 42]. However, once inhibitors enter into the brain, they face ejection via P-glycoprotein (Pgp). Knockout studies have shown that Pgp-null mice treated with brain-penetrant BACE1 inhibitors resulted in a decline in A β levels, while wild-type mice did not benefit from such treatment [43].

The structural features of BACE1 also provide a challenge when it comes to inhibitor design. The type 1 transmembrane protein is characterized by its

Fig. 4 Flap-open (*top*, apoprotein, PDB: 1SGZ) and flap-closed (*bottom*, bound to OM00-3, PDB: 1M4H) BACE1. Stabilizing hydrogen bonds are shown as *black dashed lines*



expansive catalytic domain which is marked by the centrally located catalytic aspartates Asp32 and Asp228. Free BACE1 features a flap-open conformation that is energetically stable due to the multiple hydrogen bonds in the flap region of the enzyme (Fig. 4, top) [44]. When a substrate is bound, BACE1 assumes a flap-closed conformation, which results in the loss of these optimal hydrogen bonds (Tyr71-Gly74, Lys75-Glu77, Tyr71-Lys107) (Fig. 4, bottom). Interaction with the substrate, along with the formation of a new hydrogen bond between Tyr71 and Trp76, helps to stabilize this conformational shift [44]. In the open conformation, substrates are allowed to enter the active site of the enzyme via a cleft. However, the presence of a bottleneck within the enzyme inherently favors flexible substrates. It is thought that this bottleneck feature may serve as a factor in substrate selectivity [44]. Meanwhile, the somewhat broad specificity in most of the subsites along the active site poses a major hurdle in inhibitor design efforts. X-ray crystal structures of BACE1 validate that the active site is part of a long cleft that serves as a template for potential interactions with designed inhibitors [45, 46].

3 Evolution of BACE1 Inhibitors

BACE1 is an aspartic acid protease which possesses two highly conserved aspartic acid residues in the active site for catalytic cleavage of its peptide substrate, APP. Its catalytic mechanism involves the coordination of a water molecule between the active site aspartates, where one aspartate activates the water molecule for nucleophilic attack on the carbonyl carbon of the substrate scissile site. The resulting tetrahedral intermediate rearranges to provide two peptide fragments. All current aspartic acid protease inhibitor drugs are designed based upon a transition-state mimetic concept which incorporates hydroxyethylene isosteres at the scissile site [19]. Since the catalytic aspartates of BACE1 are similar to other aspartic acid proteases, such as HIV protease and renin, similar principles were utilized for BACE1 inhibitor design at the outset [19]. BACE1 cleaves APP and generates A β mainly in the endosomes of neurons. Therefore, for BACE1 inhibitors to be clinically effective, they must have the necessary properties to cross the BBB and neuronal membranes. Furthermore, clinically relevant inhibitors must exhibit a promising drug-like absorption, distribution, metabolism, and excretion (ADME) profile. Along with good ADME properties, inhibitors must establish selectivity against other aspartic acid proteases. BACE2 is the closest homologue of BACE1 with 64% of the primary structure conserved within both proteases [47]. Cathepsin D (CatD) is a significant off-site target, as it is the most abundant aspartic acid protease found in human cells [48]. Despite many challenges in the design of effective BACE1 inhibitors, there has been enormous progression on this front. Many classes of preclinical inhibitors with impressive potency and selectivity have evolved over the years. As many as 13 drug candidates have entered into clinical development. Herein, we provide a brief overview of the structural evolution of drug-like BACE1 inhibitors.

3.1 Structure-Based Design of Inhibitors

The catalytic properties of BACE1 were thoroughly investigated following its cloning. It has an elongated substrate-binding site that can accommodate up to 11 substrate residues. BACE1 shows a broad specificity. The initial specificity and knowledge of kinetics were obtained from the hydrolysis of several peptides [26, 49]. It is noteworthy that Swedish APP (SEVNL/DAEFR) gets cleaved by BACE1 40-times faster than wild-type APP (SEVKM/DAEFR). Furthermore, specificity studies revealed that alanine is preferred at P1'. This information was used for the design of the first potent substrate-based inhibitors containing seven and eight residues with a nonhydrolyzable Leu-Ala-based hydroxyethylene dipeptide isostere [50]. Inhibitor **1** ($K_i = 36$ nM) is considerably less potent than inhibitor **2** ($K_i = 1.6$ nM), which contains eight residues (Fig. 5). A statin-substituted

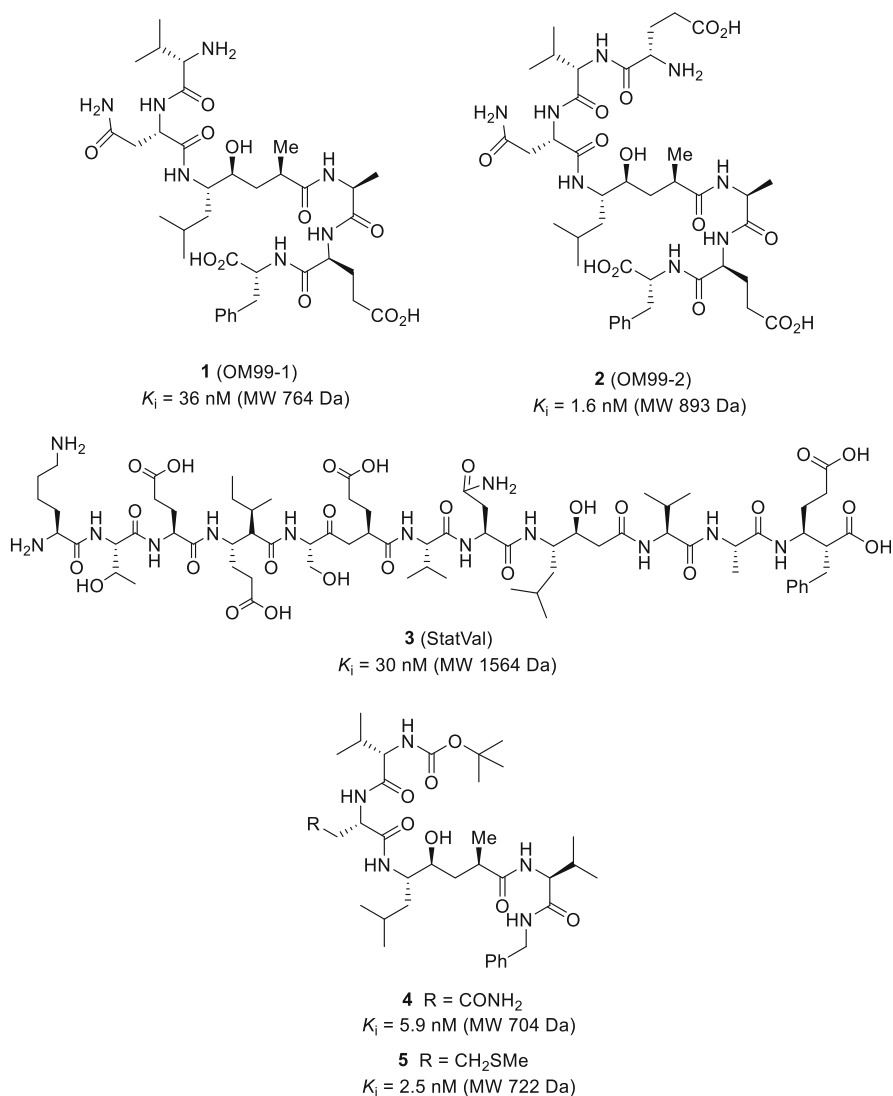


Fig. 5 Structures of pseudopeptidic inhibitors **1–5**

inhibitor was designed by incorporating APP residues. Inhibitor **3** ($IC_{50} = 30$ nM) shows similar inhibitory activity as inhibitor **1** [25].

The X-ray structure of the protease domain of human BACE1 bound to inhibitor **2** was determined at 1.9 Å resolution [45]. This X-ray structure provided critical molecular insight into the ligand-binding site interactions in the active site. The structure revealed that six of the eight residues (P2'–P4) are bound in the active site in an extended conformation. The catalytic aspartates Asp32 and Asp228 form four hydrogen bonds with the hydroxyl group of the transition-state isostere. The

inhibitor also forms ten additional hydrogen bonds throughout the active site including the flap region of BACE1.

The structure shows a rare kink in the S2' subsite where the P2' carbonyl forms a hydrogen bond with the Tyr198 hydroxyl group. Therefore, this interaction may be exploited for selectivity design. Interestingly, both the P3'- and P4'-side-chain residues do not form any specific interactions with BACE1, suggesting that these ligands can be further optimized. Based upon this molecular insight, inhibitor **4**, with an *N*-benzyl amide in place of P3' and P4' residues, showed potent BACE1 activity [51]. The inhibitor makes a number of critical hydrogen bonding interactions in the S2–S4 subsites. These include a hydrogen bond between P2-Asn with Arg235 in the S2 subsite and an intramolecular hydrogen bond with P4-Glu and P2-Asn, which may facilitate hydrogen bonding with Arg235. Structure-based replacement of the P2 ligand resulted in a number of potent inhibitors with reduced molecular weight and peptidic features. Inhibitor **5** is nearly as potent as inhibitor **2** [51].

3.2 Design of Peptidomimetic Inhibitors

The design of substrate-based inhibitors and their X-ray structural studies enabled structure-based design of numerous highly potent peptidomimetic inhibitors with reduced molecular weight and drug-like properties. A variety of dipeptide isosteres, including hydroxyethylene, hydroxyethylamine, and statins, were integrated into the design of these inhibitors. A number of previous reviews cover the evolution of these inhibitors in detail [11, 32]. Herein, we provide a brief highlight of peptidomimetic inhibitors that have addressed specific issues such as reduction of peptidic characteristics, improved cellular and in vivo properties, and design of selective inhibition which are critical to drug development.

3.2.1 Hydroxyethylene-Based Inhibitors

Extensive research efforts in the development of peptidomimetic BACE1 inhibitors led to compounds with molecular weights of 550–650 Da. A number of BACE1 inhibitors have shown good selectivity against other human aspartic acid proteases, particularly against two of the eight aspartic acid proteases deemed necessary, BACE2 and CatD. BACE2 selectivity may be critical, as it has similar specificity as BACE1. The other aspartic acid protease, cathepsin D, is highly abundant in cells, and therefore lack of selectivity will greatly reduce drug concentration of BACE1 inhibitors.

Structure-based design of highly selective inhibitors is shown in Fig. 6. Compound **6** exhibited enhanced selectivity while maintaining the potency of previous inhibitors ($K_i = 0.3$ nM) [52]. It displayed an impressive 436-fold selectivity over CatD and 1,186-fold selectivity over BACE2. The elucidation of a **6**-bound X-ray

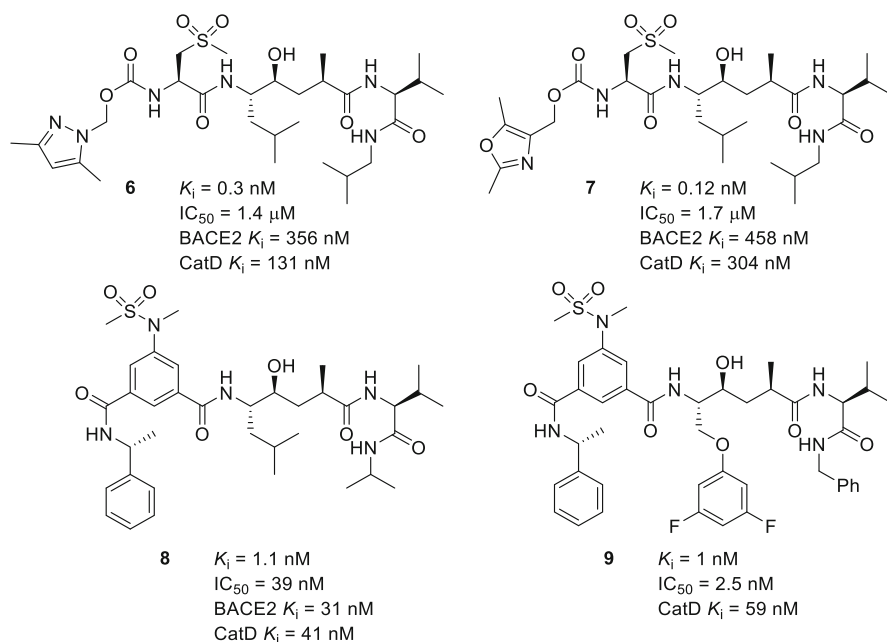
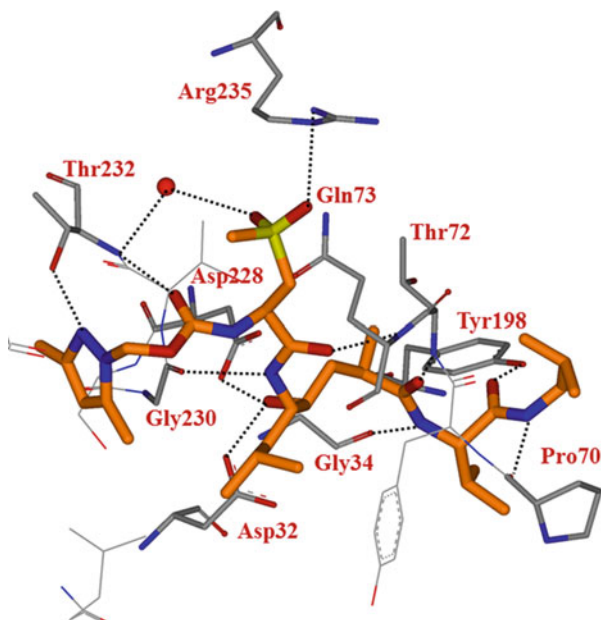


Fig. 6 Structures and activity of hydroxyethylene-based inhibitors **6–9**

crystal structure of BACE1 provided important insight of this observed selectivity. As shown in Fig. 7, the key interactions were observed between Thr232 and a nitrogen within the pyrazole heterocycle, while methyl substituents located on the heterocycle were found to also make interactions within the hydrophobic S3 subsite. The P2 sulfone made other significant hydrogen bonding interactions with Arg235 and a water-mediated hydrogen bond to Thr232 [52]. Substitution of the pyrazolylmethyl ring with the corresponding oxazolylmethyl ligand furnished compound **7**, which was found to have improved potency ($K_i = 0.12 \text{ nM}$) as well as significantly improved selectivity against BACE1 (3,800-fold over BACE2 and 2,500-fold over CatD) [52]. Though inhibitors **6** and **7** were able to display enhanced selectivity, they exhibited IC_{50} values in the micromolar range ($1.4 \text{ }\mu\text{M}$ and $1.7 \text{ }\mu\text{M}$, respectively) in Chinese hamster ovary cells [52]. Since $A\beta$ IC_{50} and BBB penetration are important features for viable AD therapies, further design modifications must be made to improve subcellular membrane permeability.

In an effort to improve cellular inhibitory activity, inhibitor **8** was designed by incorporating isophthalamide derivatives within the S2 subsite [53]. Inhibitor **8** displayed a K_i of 1.1 nM and a cellular IC_{50} of 39 nM in Chinese hamster ovary cells. Molecular modeling showed that the *N*-methylsulfonamide substituent on the P2 isophthalamide fit nicely in the S2 site. This ligand can make extensive hydrogen bonding interactions with Asn233, Ser325, and Arg235. The model also revealed that the (*R*)- α -methylbenzylamide moiety at the P3 site makes favorable hydrophobic contacts in the S3 pocket [53]. Intraperitoneal injections of 8 mg/kg of

Fig. 7 X-ray crystal structure of **6**-bound BACE1 (PDB: 2G94)



compound **8** reduced plasma A β ₄₀ in Tg2576 transgenic mice by 30% after 8 h. Inhibitor **8** also displayed a 28-fold selectivity over BACE2 and 37-fold selectivity over CatD [53]. Inhibitor **9** featuring a difluorophenyl ether moiety as the P1 ligand proved to be potent with an enzymatic IC₅₀ of 2.5 nM [54].

3.2.2 Hydroxyethylamine-Based Inhibitors

Hydroxyethylamine transition-state mimics have been widely utilized in the design of several FDA-approved HIV-1 protease inhibitors [55, 56]. BACE1 inhibitors with hydroxyethylamine isosteres have been investigated extensively. An early example of a pseudopeptide-based inhibitor containing a hydroxyethylamine isostere is compound **10** in Fig. 8 [57]. This pseudopeptidic inhibitor incorporated a P1 leucine side chain and a transition-state hydroxyl group with an (*S*)-configuration. Further structure-activity relationship studies revealed that a P1-phenylalanine side chain and a (*R*)-hydroxyl configuration are preferred by the BACE1 active site. Inhibitor **11**, with a phenylalanine P1 side chain and a (*R*)-hydroxyl configuration, showed good BACE1 inhibitory activity as well as A β cellular inhibitory activity [58]. It exhibited a 15-fold selectivity over BACE2, 500-fold selectivity over CatD, and greater than 3,000-fold selectivity over renin.

Inhibitor **12** incorporated a lipophilic 3-methoxybenzyl group as the P1 ligand and a phenylalanine side chain as the P1' ligand. This inhibitor exhibited excellent properties, showing an enzymatic *K*_i of 1.8 nM and cellular IC₅₀ of 1.0 nM [59, 60]. An X-ray crystal structure of **12**-bound BACE1 was determined at 2.05 Å resolution.

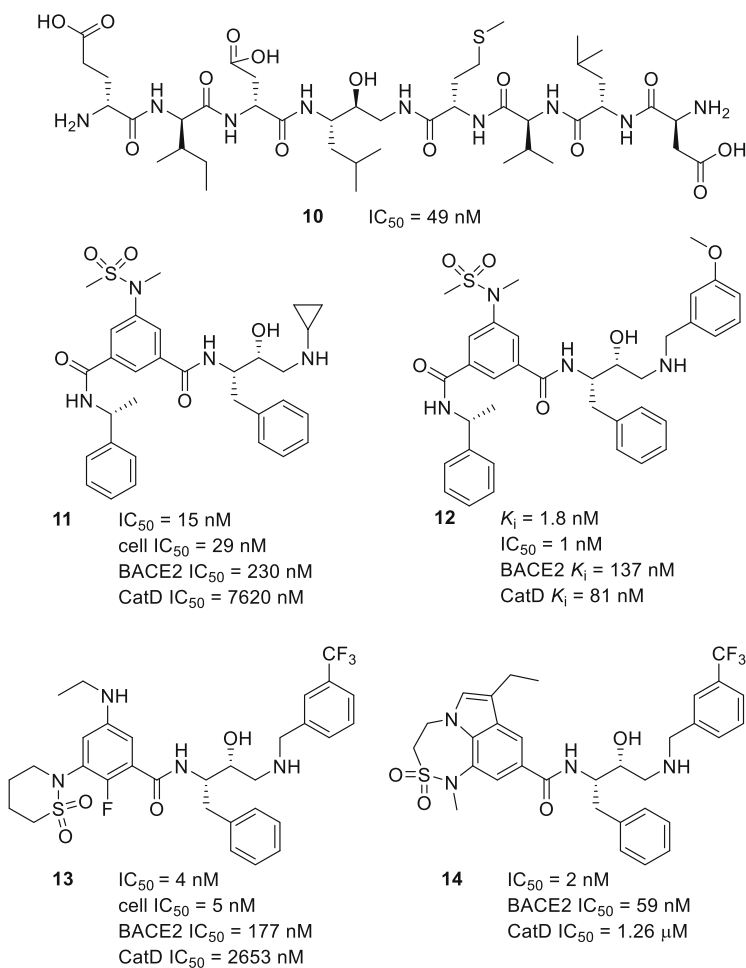
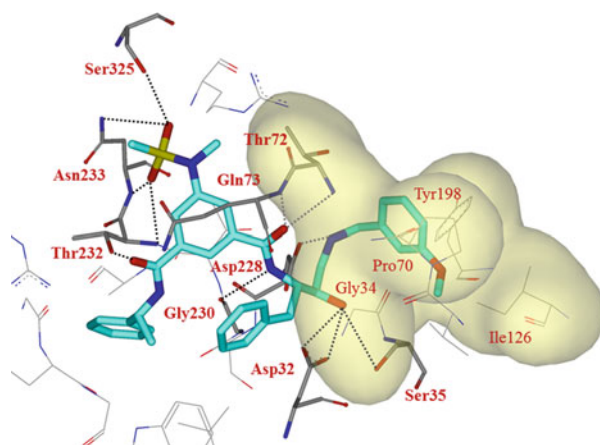


Fig. 8 Structures and activity of hydroxyethylamine-based inhibitors **10–14**

As shown in Fig. 9, incorporation of the P1' methoxybenzylamine resulted in extensive hydrophobic interactions with Gly34, Ile126, Tyr198, Pro70, Tyr71, and Thr72. The transition-state hydroxyl group formed two hydrogen bonds with Asp32; the other catalytic aspartic acid, Asp228, was bound by the hydroxyethylamine nitrogen. Both oxygens of the sulfonamide moiety of the P2 isophthalamide group formed a network of hydrogen bonds with Ser325, Asn233, and Thr232 [60].

Inhibitor **12** showed a 23-fold selectivity over CatD and 39-fold selectivity over BACE2. An intraperitoneal administration of this inhibitor with a dose of 8 mg/kg in Tg2576 mice resulted in a 65% decline of $A\beta_{40}$ levels [60]. An intravenous administration of 4 $\mu\text{g/g}$ ^{14}C -radiolabeled **12** in rats showed the presence of 16% of the inhibitor in the brain over 24 h, demonstrating the BBB

Fig. 9 X-ray crystal structure of **12** in the BACE1 active site (PDB: 2VKM)



penetrability of inhibitor **12** [59]. Further in vivo cognitive studies were carried out by installation of an osmotic pump in transgenic Tg2576 mice. The inhibitor was administered at 33.4 $\mu\text{g/g/day}$. Four experiment groups at varying ages and treatment for varying lengths of time were investigated. The first cohort began treatment with 5.5-months old and sustained treatment for 6.7 months. Cognitive performance, as measured by the Morris water maze (MWM), did not improve at 1.5 or 4.6 months. However, at 6.7 months, the treated group performed significantly better than the control [59]. In older mice (aged 16 months), the administration of inhibitor **12** did not provide any significant cognitive rescue after 4.4 months of treatment, despite the reduction of $\text{A}\beta$ levels similar to that of younger cohorts [59]. These results indicated that even a partial reduction of $\text{A}\beta$ production and neuritic plaque was sufficient to rescue cognitive decline in younger transgenic AD mice. There were no observable signs of toxicity or evidence for the accumulation of unprocessed APP [59].

Inhibitor **13**, with a six-membered sultam, showed good BACE1 IC_{50} and cellular $\text{A}\beta$ activity [61]. The *meta*-ethylamine located in the S3 site provided optimum selectivity and potency with a 44-fold selectivity over BACE2 and a 663-fold selectivity over CatD. A fluorine at the C2 position of the isophthalic aryl ring improved potency in cells expressing both wild-type and Swedish-mutant APP (IC_{50} = 5 nM and 40 nM, respectively) [61]. Inhibitor **13** showed modest oral bioavailability in transgenic mice studies. The administration of oral doses of 250 mg/kg twice daily showed as much as a 23% reduction in $\text{A}\beta_{42}$ levels within the diseased mouse brain. When dosed alongside a Pgp inhibitor, $\text{A}\beta_{42}$ reduction increased to 55% [61]. Tricyclic sultam derivatives, as represented by inhibitor **14**, were developed in order to optimize the metabolic stability of inhibitors. This inhibitor showed a BACE1 IC_{50} value of 2 nM and modest selectivity over BACE2 and CatD [62].

3.2.3 Carbinamine-Based Inhibitors

Further structural modifications of traditional transition-state isosteres were pursued in order to optimize the BBB penetration of inhibitors. As shown in Fig. 10, potent carbinamine-derived inhibitors were designed and evaluated. Inhibitor **15**, containing a primary amine, showed good BACE1 activity and excellent selectivity against renin [63]. As shown in Fig. 11, the primary amine functionality presumably interacts with the catalytic aspartates in the active site. The two oxadiazole nitrogens likely form hydrogen bonds with the backbone of Gln73 and Thr72.

Further optimization of ligand binding led to the evolution of inhibitor **16**, where both the P2 and P3 ligands were modified to improve potency and cellular permeability [64]. It exhibited excellent potency, yet its selectivity against BACE2 was

Fig. 10 Structures and activity of carbinamine-based inhibitors **15–17**

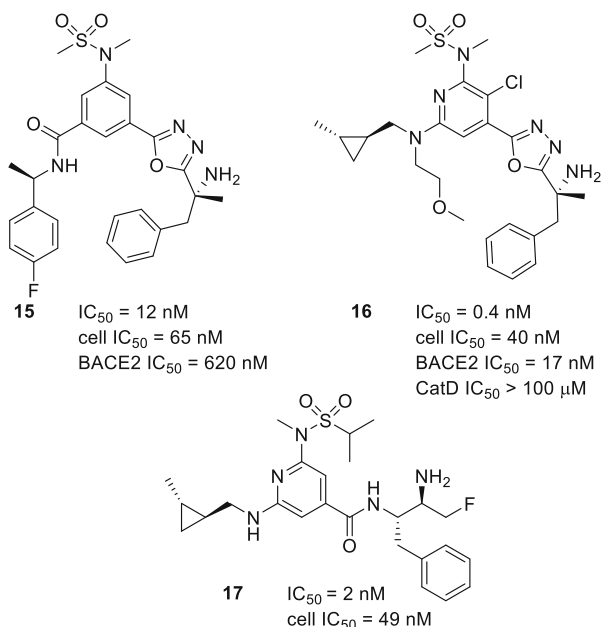
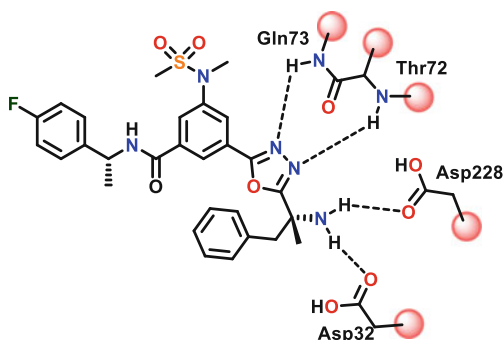


Fig. 11 Binding mode of BACE1 inhibitor **15**



moderate (42-fold). However, it showed enhanced selectivity against CatD. Intra-peritoneal administration of **16** at a 100 mg/kg dose resulted in a 26% decline of postinjection A β_{40} levels. Further, time- and dose-dependent studies in rhesus monkeys showed a 65% reduction of plasma A β_{40} levels after 4 h, a return of levels after 8 h, and a complete recovery of A β_{40} after a 24-hour period. Inhibitor plasma concentration was determined to be 3.8 μ M. The oral bioavailability of the inhibitor was found to be poor. However, when dosed with pharmacokinetic booster ritonavir, the bioavailability of the inhibitor improved to 83% [64, 65].

Inhibitor **17** incorporated the 2,6-diaminoisonicotinamide core of inhibitor **16**. It showed potent BACE1 activity and cellular A β inhibitory activity of 49 nM [66]. In vivo studies in transgenic mice using 50 mg/kg i.v. bolus showed decreased A β_{40} levels in the brain by 34% at 3 h post-dose [66]. Pharmacokinetic evaluation in rats showed a high clearance and volume of distribution (Cl = 45.8 mg/min/kg and Vd = 3.9 l/kg). Unfortunately, the half-life of inhibitor **17** in vivo was very short, and the bioavailability was poor ($t_{1/2}$ = 1.6 h and %F = 13%) [66].

3.2.4 Reduced Amide-Based Inhibitors

X-ray structure-based design of BACE1 inhibitors with a reduced amide isostere provided very potent and selective inhibitors. As shown in Fig. 12, inhibitor **18** showed cellular A β inhibitory activity of 22 nM [67]. Subsequent optimization of ligand binding, particularly in the S1' subsite, led to very potent and highly selective BACE1 inhibitors. As shown in inhibitor **19**, an (*S*)-hydroxyl group was incorporated to form hydrogen bonds with substituents in the S1' subsite [68]. It exhibited a 7,000-fold selectivity against BACE2 and 250,000-fold selectivity against CatD. The corresponding inhibitor with a (*R*)-configuration on the P1' hydroxyl group was significantly less potent and selective. The X-ray crystal structure of **19**-bound BACE1 was determined at 2.2 Å resolution. As shown in Fig. 13, the reduced amide isostere formed two tight hydrogen bonds with the active site aspartates. The allo-threonine hydroxyl group is within proximity to form a hydrogen bond with the Tyr198 side chain hydroxyl group. Since this interaction is absent in inhibitor **18**, the enhanced potency and selectivity of inhibitor **19** may be due to specific interactions with Tyr198. Further structural modification of the P2 ligand with a 7,6,5-tricyclic indole resulted in very potent and selective inhibitors as represented in inhibitor **20**. No in vivo studies were reported for these inhibitors.

3.2.5 Macrocyclic Peptidomimetic Inhibitors

The large active site of BACE1 provides an opportunity to design macrocyclic BACE1 inhibitors with a prearranged bioactive conformation. As shown in the X-ray structure of substrate-based inhibitor **2**-bound BACE1 (Fig. 1), the S1–S3 subsites are open in nature. Thus, macrocyclic rings formed between the P1 ligand

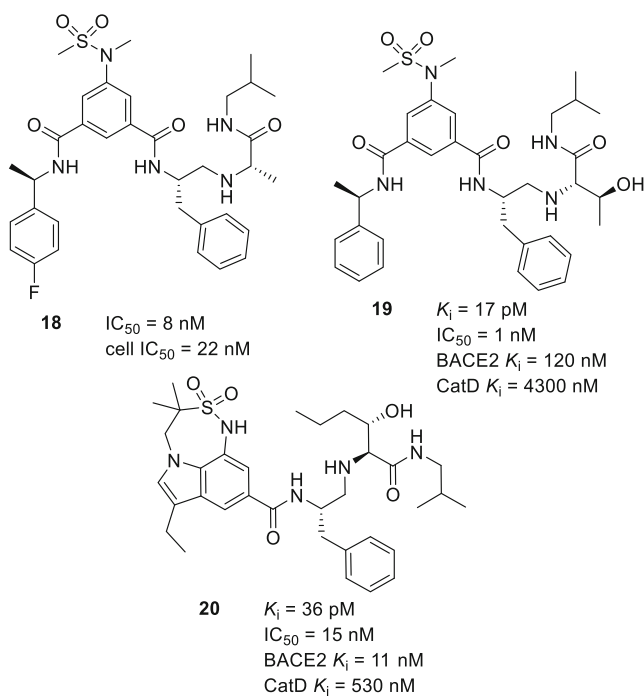


Fig. 12 Structures and activity of reduced amide-based inhibitors **18–20**

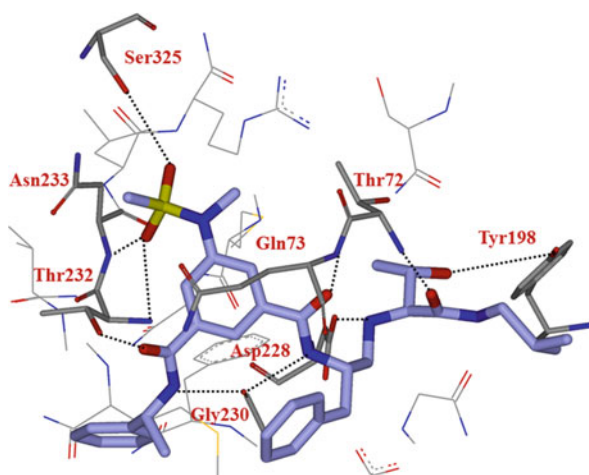


Fig. 13 X-ray crystal structure of inhibitor **19**-bound BACE1 (PDB: 4GID)

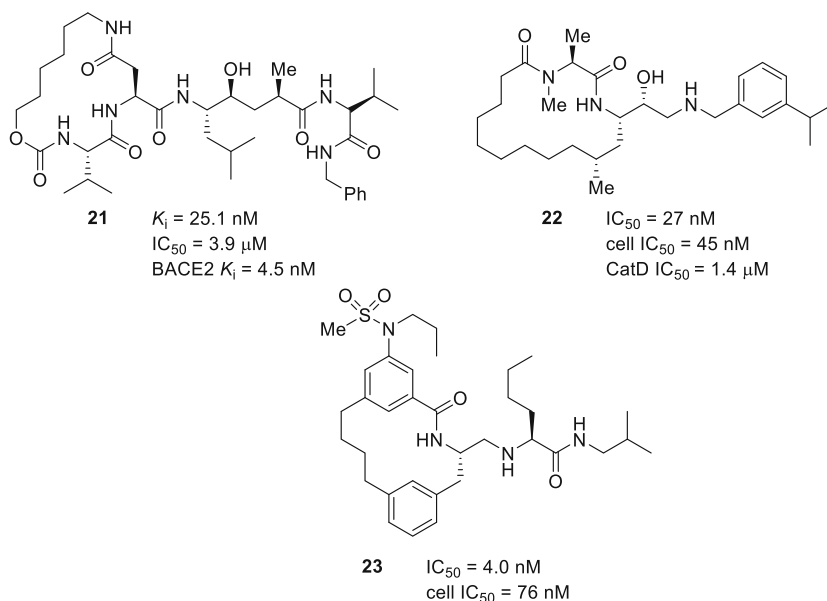


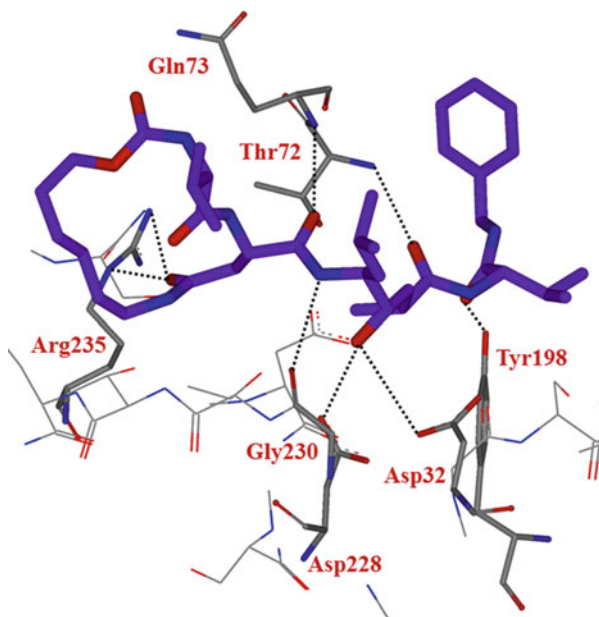
Fig. 14 Structures and activity of peptidomimetic macrocyclic-based inhibitors **21–23**

and the nitrogen of a P2 amide can be accommodated. Macrocyclic inhibitors may lead to more selective inhibitors as the bond rotations are restricted.

Initial examples took advantage of the X-ray structural insight provided by the substrate-based inhibitor **2**-bound BACE1, as shown in Fig. 14. Inhibitor **21**, with a 16-membered macrocycle containing an amide and urethane functionality, showed good BACE1 enzymatic activity [69]. Cyclic inhibitors were more potent than acyclic inhibitors. Also, studies showed that ring sizes 14–16 can be accommodated. An X-ray structure of inhibitor **21**-bound BACE1 showed key ligand-binding site interactions. As shown in Fig. 15, the Leu-Ala dipeptide isostere binds similarly to inhibitor **2**. Furthermore, the P2 asparagine on the macrocycle is able to interact with Arg235 in the S2 subpocket. This class of inhibitors, however, did not show improved cellular activity or selectivity against BACE2.

Inhibitor **22**, with a hydroxyethylamine isostere, showed improved cellular permeability [70]. There was also a modest improvement in selectivity, showing a 63-fold selectivity over CatD. Macrocyclic inhibitors with a reduced amide isostere were designed to bolster the pharmacokinetic profile. The 14-membered macrocyclic inhibitor **23** showed good BACE1 activity and cellular A β inhibitory potency [71]. The inhibitor exhibited a much improved Pgp ratio of 5.5. Further, in vivo studies showed that a 100 mg/kg dose of **23** cleared brain A β_{40} levels by 25% in transgenic mice, while compound concentrations in the brain were found to be approximately 1.1 μM [71].

Fig. 15 X-ray crystal structure of **21**-bound BACE1 (PDB: 1XS7)



3.3 Nonpeptide Inhibitors

In general, the large molecular size and a high degree of peptidic features of peptidomimetic inhibitors led to limitations in the development of effective BACE1 inhibitor drugs. The relatively large active site of BACE1 and the need for efficient brain penetration of BACE1 inhibitors made the design task very challenging. For these reasons, the development of small-molecule and nonpeptide BACE1 inhibitors with different chemotypes has evolved. A number of high-throughput screening approaches have been pursued. These approaches have identified a wide variety of nonpeptidic scaffolds for BACE1 inhibitor design (Fig. 16). Structure-based optimization led to the development of BACE1 inhibitors that are smaller in molecular size and nonpeptidic in nature with improved pharmacokinetic properties and BBB penetration. A number of recent reviews covered this development in detail leading to the discovery of potent and selective BACE1 inhibitors [11, 72]. Herein, we plan to provide a brief outline.

3.3.1 Acyl Guanidine-Based Inhibitors

Acyl guanidine-based BACE1 inhibitors were discerned by high-throughput screening (HTS). The acyl guanidine core shown in Fig. 17 makes interactions with the catalytic aspartic acids (Asp32 and Asp228) of BACE1 [73]. As shown in Fig. 18, inhibitor **24** with an α -substituted 2,4-biphenylpyrrole showed an enzyme

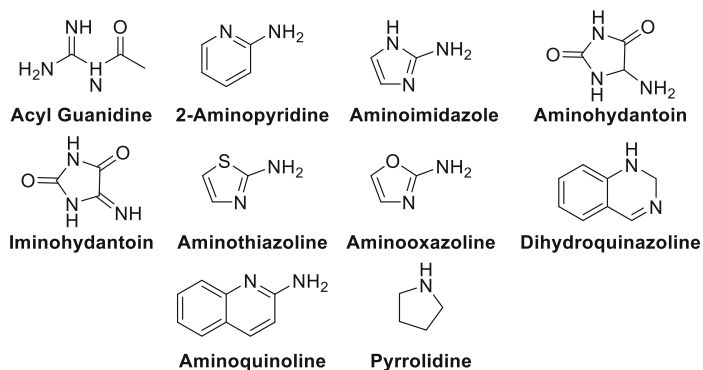


Fig. 16 Common scaffolds for nonpeptide BACE1 inhibitors

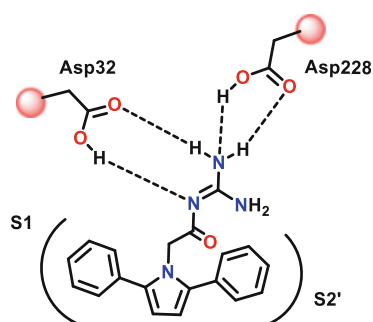


Fig. 17 Binding mode of inhibitor **21**

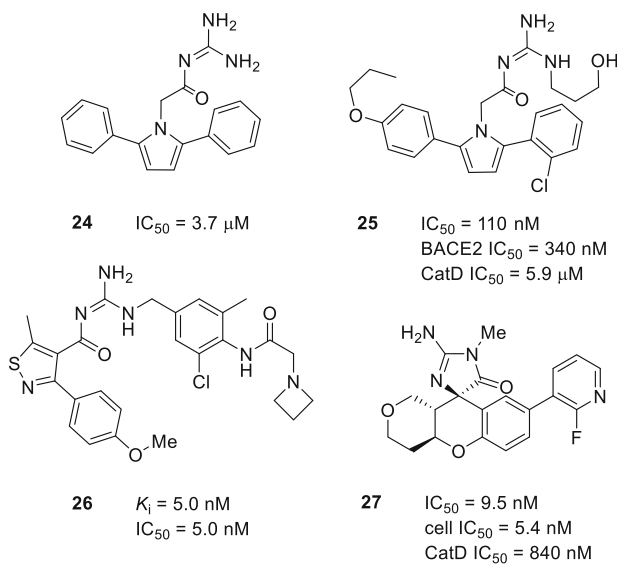


Fig. 18 Structures and activity of acyl guanidine-based inhibitors **24–27**

IC₅₀ of 3.7 μ M [73]. An inhibitor **24**-bound BACE1 X-ray crystal structure revealed that the inhibitor resides in an open conformation in the active site due to stabilizing interactions between Tyr71 and the π -system of the diarylpyrrole ligand (Fig. 17). Furthermore, the biphenyl ligand resides in both the S1 and S2' sites with the P1 phenyl positioned directly toward an unoccupied S3 subsite. To optimize ligand binding in the S1–S3 regions, a *p*-propyloxyphenyl moiety was examined in place of the unsubstituted aryl ring. Similarly, access to potential hydrogen bonding interactions in the S1' pocket was promoted through the functionalization of one of the guanidine nitrogens with a propyl alcohol group. This resulted in a substantial improvement in potency (IC₅₀ 110nM) for inhibitor **25** (Fig. 18). These biaryl pyrrole-based inhibitors show low levels of selectivity over other human aspartyl proteases.

Inhibitor **26**, with an isothiazole derivative, was optimized after the discovery of the scaffold by HTS. This inhibitor exhibited excellent BACE1 affinity (K_i = 5.0 nM, Fig. 18) [74]. In vivo viability was evaluated with dose-dependent studies in mouse models. Plasma A β ₄₀ levels were found to be diminished, while brain and CSF A β ₄₀ levels were maintained during the treatment. This suggests that the inhibitor is a substrate for Pgp. However, further improvement is necessary for better BBB penetration.

A series of chromane-based spirocyclic acyl guanidine-derived BACE1 inhibitors were developed. Inhibitor **27** showed very good BACE1 and A β cell inhibitory activity [75]. The inhibitor displayed a high efflux ratio. However, it was able to reduce CSF A β ₄₀ levels from 53% to as high as 63% over multiple species [75].

3.3.2 2-Aminopyridine-Based Inhibitors

Small-molecule nonpeptide BACE1 inhibitors have been developed based upon the 2-aminopyridine scaffold. BACE1 bound to 2-aminopyridine-based inhibitors adopts a flap-open conformation that results from the displacement of Tyr71 to a position above the pyridine scaffold. In silico screening of varying sites and degree of substitution on the pyridine ring led to the identification of many lead structures. Among the initial hits, 2,6-substituted aminopyridine **28** displayed a modest potency with an IC₅₀ of 25 μ M (Fig. 19) [76]. A 2,3-substituted aminopyridine derivative **29** showed BACE1 IC₅₀ of 690 nM. This lead structure was then optimized for the S1–S3 hydrophobic pockets. The biaryl-substituted pyrrole substituent was incorporated to improve the cellular potency of inhibitors. Inhibitor **30** displayed an improved cellular potency (EC₅₀ = 440 nM) (Fig. 19) [77]. Furthermore, it exhibited >100-fold selectivity against BACE2 and >500-fold selectivity against CatD. The aminopyridine moiety of inhibitor **30** binds to the catalytic aspartic acids, while the pyrimidine moiety forms a hydrogen bond with Ser229 (Fig. 20).

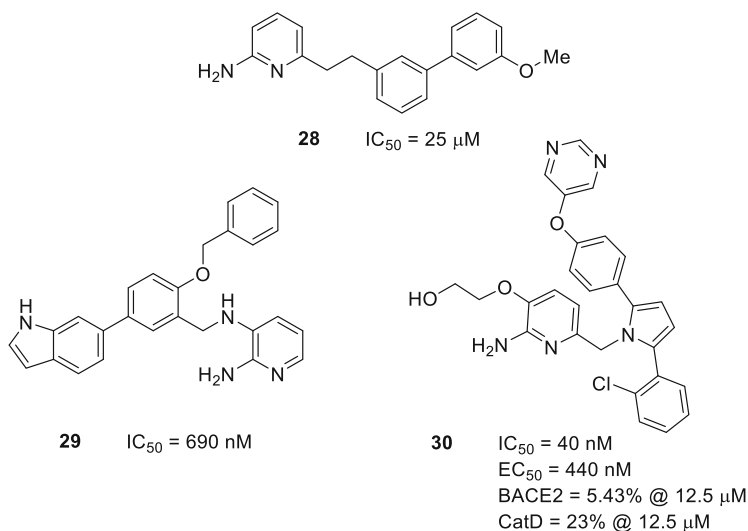


Fig. 19 Structures and activity of 2-aminopyridine-based inhibitors **28–30**

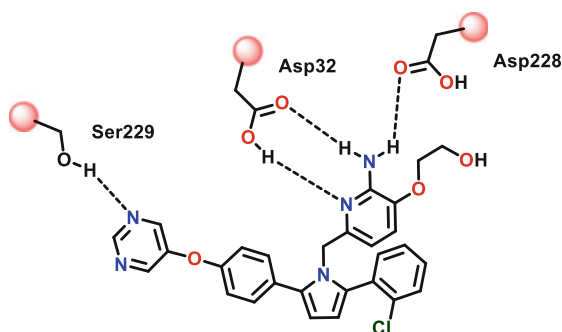


Fig. 20 Binding mode of inhibitor **30**

3.3.3 Aminoimidazole-Based Inhibitors

Potent and selective BACE1 inhibitors emerged based upon the aminoimidazole heterocyclic scaffold. As shown in Fig. 21, HTS initially yielded dibenzyl-substituted imidazole **31** as a weak inhibitor. Molecular modeling studies showed that the amino group of the imidazole heterocycle was responsible for binding with the catalytic aspartic acid residues. Subsequent optimization of the scaffold led to the design of conformationally constrained **32** with an enzymatic IC_{50} of 63 nM [78]. This increase in potency has been attributed to the additional hydrophobic interactions with the flap region of BACE1 as a result of the constrained indane moiety. This inhibitor showed a good Pgp efflux ratio of 3.6, suggesting viable brain penetration.

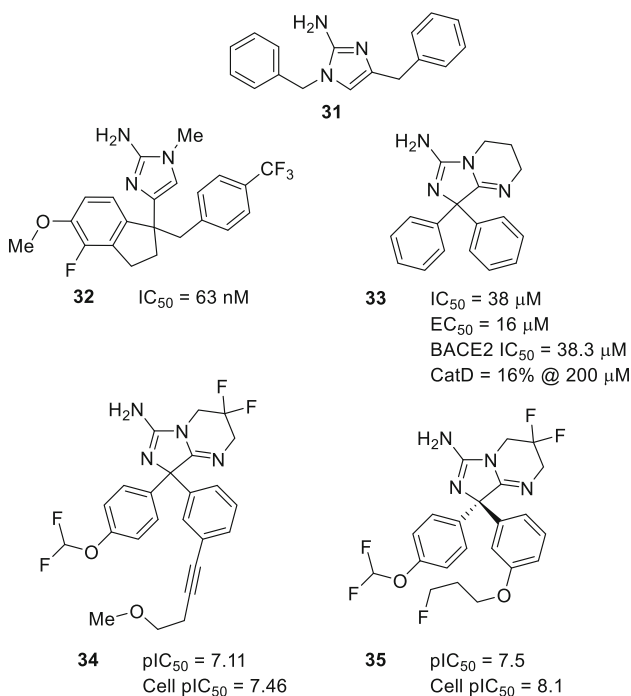
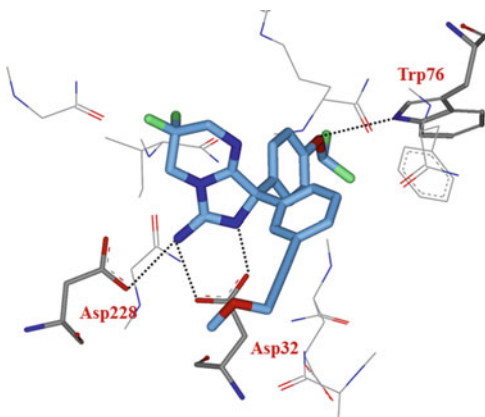


Fig. 21 Structures and activity of aminoimidazole-based inhibitors **31–35**

Further evolution of a bicyclic aminoimidazole scaffold has provided inhibitors with better brain efficacy and overall potency. The initial hit compound **33**, with a bicyclic aminoimidazole core and a biphenyl moiety, exhibited an IC_{50} in the high micromolar range [79]. The removal of the biphenyl motif and functionalization of the aryl rings resulted in potent inhibitors with better cell membrane permeability and Pgp efflux profiles. Inhibitor **34**, with a *p*-difluoromethyl ether substitution on one of the phenyl rings and an *m*-alkynyl substituent on the other ring, displayed good potency in both enzymatic and cellular assays with pIC_{50} values of 7.11 and 7.46, respectively [80]. Cell membrane permeability, as determined by a Caco-2 assay, was $8.4 \times 10^{-6} \text{ cm/s}$, and the efflux ratio was 3.5, indicating potential for BBB penetration. An X-ray crystal structure of **34**-bound BACE1, shown in Fig. 22, provided insight into the ligand-binding interactions of this inhibitor [80]. The structure was determined at 2.0 Å resolution. The aminoimidazole moiety interacted with the catalytic Asp32 and Asp228 residues, binding in a flap-open conformation. This allows Trp76 to be in position for hydrogen bonding to the oxygen of the *p*-difluoromethyl ether. The alkynyl substituent of the second ring extends into the S3 pocket. Replacement of the alkyne chain of inhibitor **34** with a fluorinated propyl ether resulted in inhibitor **35**. This optically active inhibitor showed similar potency as **34** [80]. Interestingly, the efflux ratio of **35** was enhanced to a value of 0.8. Further, in vivo assessment in a mouse model using

Fig. 22 X-ray crystal structure of **34** in the enzyme active site (PDB: 4ACX)



oral coadministration of **35** with a Pgp inhibitor resulted in reduction of brain and plasma A β levels by 17% and 76%, respectively [80].

3.3.4 Aminohydantoin-/Iminohydantoin-Based Inhibitors

Identification of initial hit compound **36** with an aminohydantoin scaffold led to the development of potent and selective BACE1 inhibitors. As shown in Fig. 23, inhibitor **36** displayed an enzymatic IC₅₀ of 3.4 μ M [81]. Subsequent optimization of the hydrophobic core led to inhibitor **37** with an improvement in potency. X-ray structural studies of **37**-bound BACE1 revealed that the catalytic aspartic acid residues were participating in hydrogen bonding interactions with the amino moiety and the N-H nitrogen of the pharmacophore. Furthermore, the phenylpyridine moiety extends into the S3 pocket to make an interaction with Ser229 through a water bridge. Interactions with the Trp76 within the S2' pocket were made possible through the *p*-methoxybenzyl moiety. Inhibitor **37** exhibited good BACE1 activity with an IC₅₀ of 10 nM and a cellular EC₅₀ of 20 nM (Fig. 23) [81]. Furthermore, it displayed greater than 80-fold selectivity against BACE2, CatD, and other aspartic acid proteases. In vivo studies in a transgenic mouse model showed that a 100 mg/kg oral dose of inhibitor **37** cleared 69% of plasma A β ₄₀ [81].

Further exploration of an aminocyclohexyl group as the S1 ligand resulted in inhibitor **38**. This inhibitor displayed a low nanomolar BACE1 activity (IC₅₀ = 22 nM) and a 1136-fold selectivity against CatD [82]. As shown in Fig. 24, the X-ray crystal structure of **38**-bound BACE1 revealed a unique binding mode in the active site. The quinoline group unexpectedly occupies a solvent-exposed region of the active site and makes additional hydrophobic interactions with Ile110 and Lys107. Additionally, it forms a hydrogen bonding interaction with the backbone carbonyl of Phe108. The inhibitor was able to reduce plasma A β ₄₀ by 65% and 55% following oral and subcutaneous administration in rats. Brain A β ₄₀, however, was not reduced when dosed in a mouse model. In whole cell assays, the

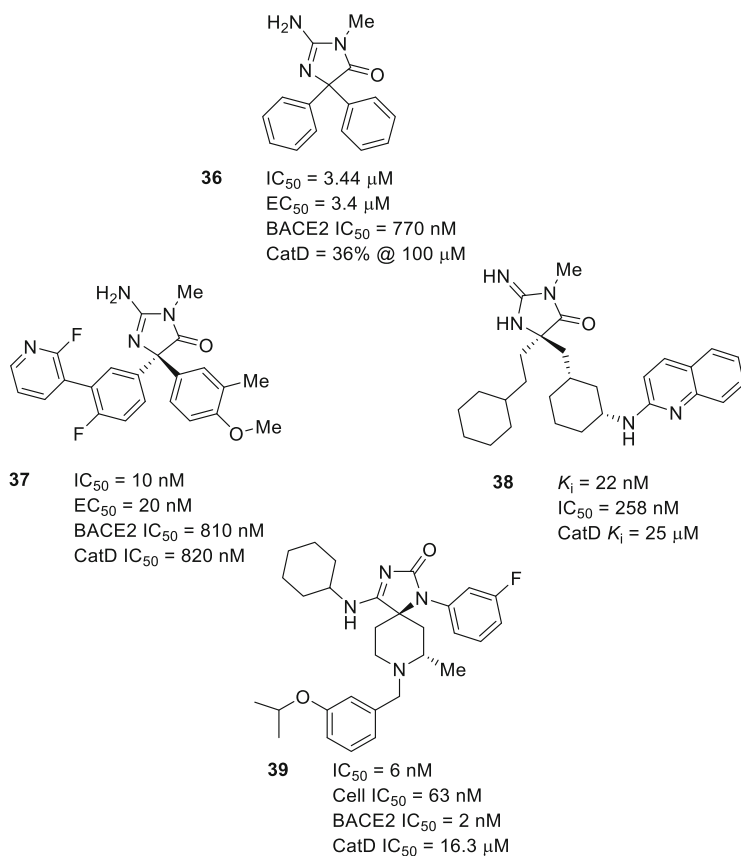


Fig. 23 Structures and activity of aminohydantoin-/iminohydantoin-based inhibitors **36–39**

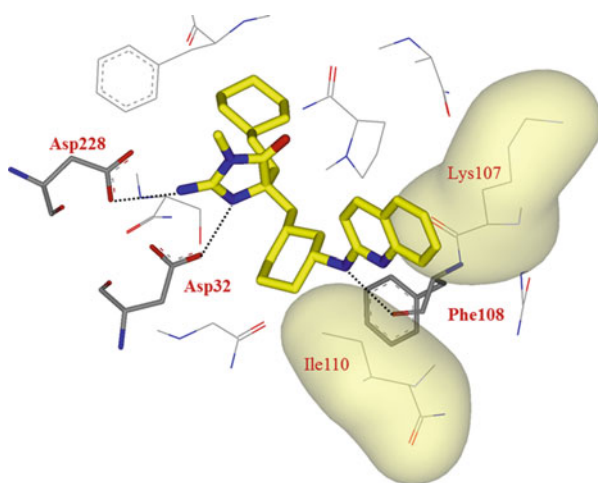


Fig. 24 X-ray crystal structure of **38**-bound BACE1 (PDB: 4R95)

inhibitor showed a cellular EC_{50} of 258 nM. This was further improved to an EC_{50} of 110 nM upon coadministration with a Pgp inhibitor [82].

The development of a closely related spirocyclic piperidine resulted in potent inhibitor **39** [83]. The X-ray crystal structural studies revealed that the binding mode of the inhibitor results in a slightly larger pocket, which can be further optimized. The inhibitor showed no selectivity against BACE2 (IC_{50} = 2 nM) but shows good selectivity against CatD (IC_{50} = 16.3 μ M). It also showed improved cellular potency of 63 nM and a Pgp efflux ratio of 4.4.

3.3.5 Aminothiazoline- and Aminooxazoline-Based Inhibitors

The development of BACE1 inhibitors with an aminothiazoline scaffold was based upon the initial hit compound **40** [84]. As shown in Fig. 25, inhibitor **40** displays a modest enzymatic potency of 41.2 μ M. Initial X-ray studies of **40**-bound BACE1 revealed that meta-substitution of the phenyl moiety could maximize interactions within the S3 subsite. Also, the *meta*-substituted amide group allows the aromatic ring to adopt a nearly planar conformation through a hydrogen bonding interaction with Gly291. Extensive SAR studies led to inhibitor **41**, which shows good BACE1 and cellular A β inhibitory activity [84]. However, inhibitor **41** turned out to be a good Pgp substrate. It shows 10% reduction of A β_{40} levels with an oral dose of 30 mg kg^{-1} .

Inhibitors with related aminooxazoline scaffolds displayed good BACE1 inhibitory and cellular activity. Inhibitor **42** incorporates a trifluoromethyl group on the oxazoline heterocycle. It shows a BACE1 IC_{50} of 12 nM and cellular A β inhibitory activity of 2 nM [85]. The inhibitor showed good selectivity against other common aspartic acid proteases with IC_{50} levels greater than 200 μ M. In vivo studies of

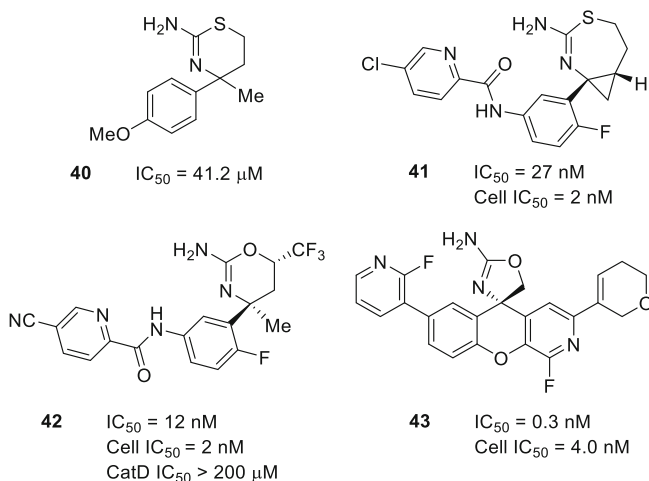
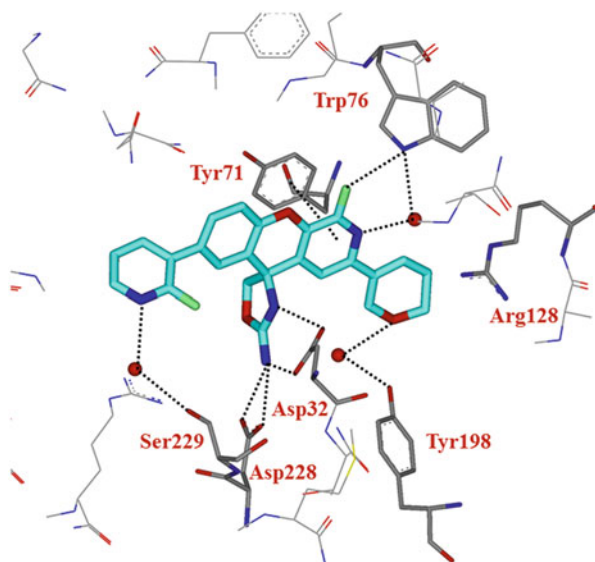


Fig. 25 Structures and activity of aminooxazoline- and aminothiazoline-based inhibitors **40–43**

Fig. 26 X-ray crystal structure of **43** in the BACE1 active site (PDB: 4WTU)



inhibitor **42** with an oral dose as small as 1 mg/kg showed reduction of $A\beta_{40/42}$ levels for up to 24 h. It also displayed a favorable pharmacokinetic profile [85].

Small-molecule BACE1 inhibitors including a xanthene core with an amino-oxazoline head group were explored. Xanthene derivatives generally exhibited good potency but showed high efflux activity. Further modification of the xanthene core and its substituents were pursued in order to develop drug-like inhibitors [86]. Inhibitor **43** with a 3-aza-2-fluoroxanthene scaffold showed excellent BACE1 and cell inhibitory potency [87]. As shown in Fig. 26, an X-ray crystal structure of inhibitor **43**-bound BACE1 revealed that the 3-azaxanthene core formed a crucial hydrogen bonding interaction with Trp76. The oxygen of the dihydropyran also formed a hydrogen bonding interaction with Tyr198. The nitrogen of the pyridine engaged in an additional hydrogen bonding interaction with Ser229. Inhibitor **43** was orally administered to a number of species and showed a robust reduction of central nervous system (CNS) $A\beta_{40}$ levels [87].

3.3.6 Dihydroquinazoline-Based Inhibitors

BACE1 inhibitors containing dihydroquinazoline scaffolds were developed after identification of fragment hit **44** (Fig. 27) [88]. The X-ray structure of **44**-bound BACE1 shows that the inhibitor adopts a hairpin conformation (Fig. 28), enabling the inhibitor to occupy the subsite specific S1 pocket. The X-ray crystal structure reveals a vacant hydrophobic S1' pocket available for optimization of ligand binding. Incorporation of a cyclohexyl group with (*S*)-configuration led to inhibitor **45** [88]. Presumably, the cyclohexyl ligand fills in the S1' hydrophobic pocket. As a result, this inhibitor showed an enzymatic K_{i50} of 11 nM. The inhibitor displayed

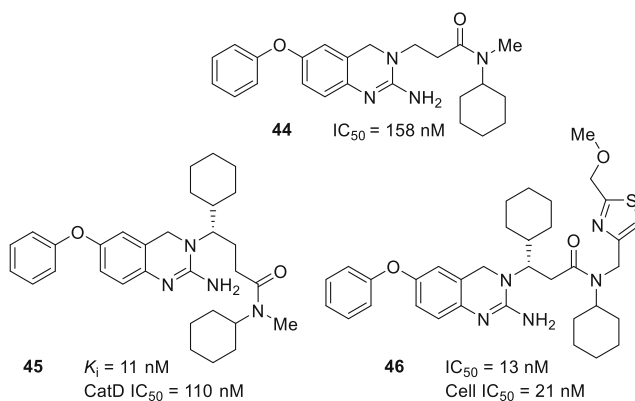


Fig. 27 Structures and activity of dihydroquinazoline-based inhibitors **44–46**

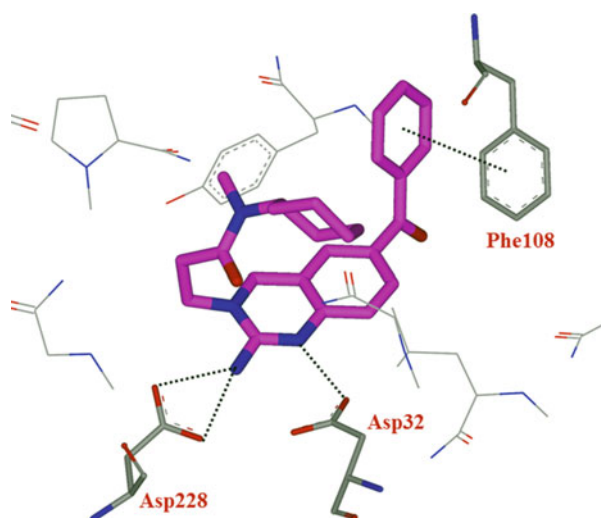


Fig. 28 X-ray crystal structure of **44**-bound BACE1 (PDB: 2Q11)

modest selectivity against common aspartyl proteases such as renin ($IC_{50} = 2.7 \mu\text{M}$) and CatD ($IC_{50} = 0.11 \mu\text{M}$). Oral administration of inhibitor **45** in rats (30 mg/kg) exhibited as much as 70% reduction of $A\beta_{40}$ plasma levels. However, an efflux ratio of 12.2 suggests poor brain permeability for this inhibitor. Further optimization of the ligand binding led to the design of inhibitor **46** [89]. In this inhibitor, a methoxy-methyl thiazole was incorporated in place of the methyl amide. Inhibitor **46** exhibited good BACE1 enzymatic inhibitory activity ($IC_{50} = 13 \text{ nM}$) and cellular $A\beta$ inhibitory activity ($IC_{50} = 21 \text{ nM}$).

3.3.7 Aminoquinoline-Based Inhibitors

Initial fragment-based screening efforts identified aminoquinoline **47** with BACE1 K_d of 900 μM [90]. Initial SAR studies showed that incorporation of an aryl ring at the 6 position of the aminoquinoline may lead to improved potency. Further structural modifications with the introduction of a *N*-cyclohexyl propionamide at C3 of the quinoline ring led to inhibitor **48** (Fig. 29) [90]. This inhibitor showed good BACE1 IC_{50} . Further structural studies suggest that a longer and bulkier alkyl chain could potentially extend further into the $\text{S2}'$ subsite. Inhibitor **49** incorporated an (*R*)- α -methyl on the side chain and a chloropyridyl group to improve pharmacokinetic properties [90]. Inhibitor **49** displayed good activity with a BACE1 IC_{50} of 11 nM and cellular IC_{50} value of 80 nM. It also exhibited an efflux ratio of 3.1. In vivo studies with a 60 mg/kg dose administered subcutaneously in rats showed reduction of $\text{A}\beta_{40/42}$ CSF levels by 42% postinjection. However, inhibitor **49** showed rapid clearance from human and rat microsomes. The X-ray structural studies with **49**-BACE1 complex showed that the aminoquinoline formed a tight interaction with flap residue Tyr71, which orients the quinoline and amino nitrogens toward the catalytic Asp32 and Asp228 residues (Fig. 30) [90].

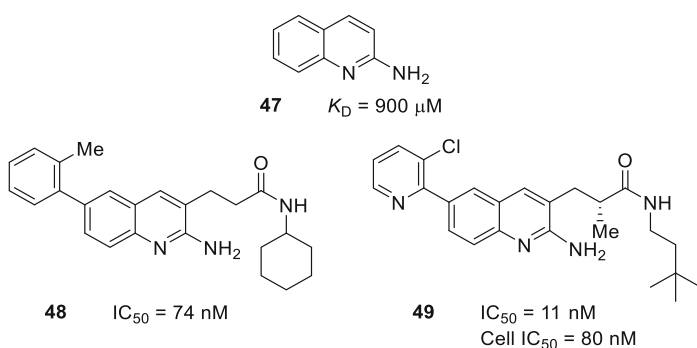


Fig. 29 Structures and activity of aminoquinoline-based inhibitors **47–49**

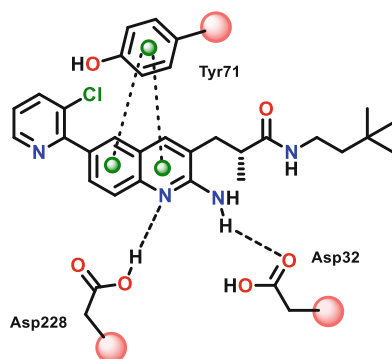


Fig. 30 Binding mode of inhibitor **49**

3.3.8 Pyrrolidine-Based Inhibitors

BACE1 inhibitors containing a pyrrolidine scaffold were identified by HTS. As shown in Fig. 31, initial compound **50** showed a low inhibitory activity of 240 μM [91]. SAR studies and further optimization resulted in inhibitor **51**, which showed BACE1 inhibitory activity of 29 nM. Its cellular activity was 570 nM (IC_{50}), and the inhibitor displayed no selectivity against BACE2 but >200-fold selectivity over CatD [91]. An inhibitor-bound X-ray crystal structure revealed that the pyrrolidine scaffold formed two hydrogen bonds with the catalytic aspartic acid residues. The *cis*-2,4-substituted piperidine ligand nicely occupied a pocket below the flap region (Fig. 32).

3.3.9 Macrocyclic Nonpeptide Inhibitors

The development of macrocyclic nonpeptide inhibitors was carried out in an effort to improve the inhibitors' properties. As mentioned earlier, the dihydroquinazoline-based inhibitors adopt a tight hairpin conformation [88]. A macrocyclic inhibitor may provide stabilization of the bioactive hairpin conformation, leading to an enhancement in potency. As shown in Fig. 33, macrocyclic inhibitor **52** displayed a BACE1 K_i of 5 nM [92]. The cellular A β inhibitory activity was also improved to 7 nM (IC_{50}) compared to the acyclic inhibitor. Further evaluation revealed that

Fig. 31 Structures and activity of pyrrolidine-based inhibitors **50** and **51**

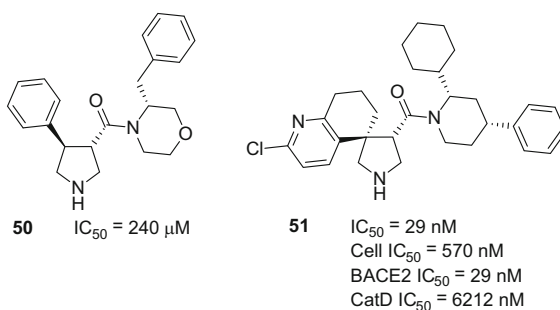


Fig. 32 Binding mode of inhibitor **51**

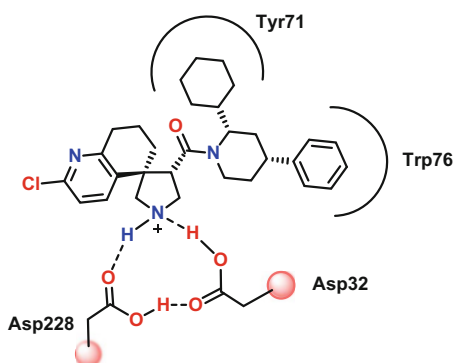


Fig. 33 Structures and activity of nonpeptidic macrocyclic-based inhibitors **52** and **53**

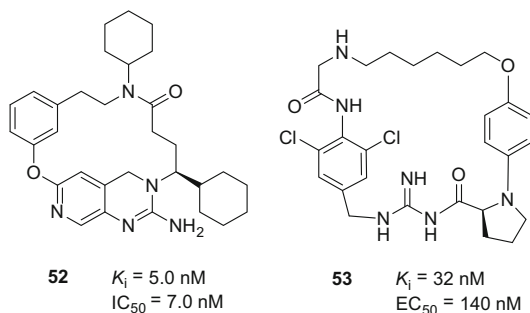
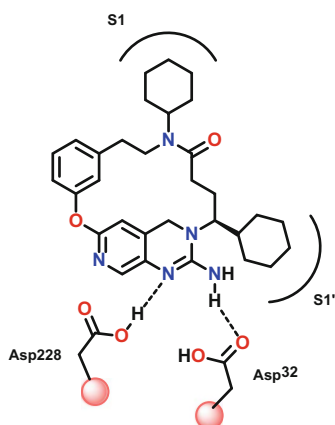


Fig. 34 Binding mode of inhibitor **52**



inhibitor **52** is a Pgp substrate and in vivo studies did not show reduced brain A β in animals. Structural studies suggested that the cyclohexyl moieties occupied the vacant S1 and S1' pockets of the BACE1 active site (Fig. 34).

Macrocyclic inhibitors based upon the acyl guanidine-derived scaffold have been investigated. A representative example is inhibitor **53**, which features a 26-membered macrocycle with an acyl guanidine core, displaying good BACE1 ($IC_{50} = 32 \text{ nM}$) and cellular A β inhibitory activity ($EC_{50} = 140 \text{ nM}$) [93]. Inhibitor **53** also showed good selectivity against multiple aspartic acid proteases, including 700-fold selectivity against CatD. Furthermore, the inhibitor showed a diminished efflux ratio of 2.3. In vivo studies with wild-type mice involving subcutaneous dosing of 40 mg/kg showed a 74% reduction of plasma A β levels and a peripheral drug concentration of 3.8 μM 5 h postinjection. However, A β brain levels were found to be nearly identical to the control levels [93].

4 Clinical Evaluation of BACE1 Inhibitors

The discovery of a clinically translatable BACE1 inhibitor has proven to be a difficult challenge for the medicinal chemistry community. While many highly potent inhibitors have been synthesized and evaluated preclinically, there are many more barriers an inhibitor must cross to be considered clinically viable. Further, a drug intended to treat a brain disorder experiences additional hurdles it must overcome. First, an inhibitor must have the ability to penetrate the BBB. This requires a proper balance of hydrophobicity and hydrophilicity. Also, upon crossing the BBB, Pgp efflux can eject the inhibitor, precluding any therapeutic effect. This efflux can be avoided by carefully designing compounds with minimal recognition by Pgp.

Additionally, due to the chronic nature of AD, any approved therapy would be a maintenance drug, which patients must take for the remainder of their lives. Because of this, accumulation of the drug in body tissues and subsequent toxicity must be monitored and addressed. Further, selectivity over non-pathological aspartic acid proteases is critical, especially in the case of chronic therapy. Selectivities over systemic enzymes such as BACE2 and CatD provide an additional challenge due to the similarity of the proteolytic mechanism and substrate specificity. Unique pockets in each enzyme must be exploited to provide this necessary selectivity.

A final challenge is the balance of inhibitor size. The enzyme has a rather large active site. It has been a difficult challenge to develop compounds large enough to fill the active site and bind efficiently, yet small enough to cross the BBB, cell membrane, and endosomal membrane to inhibit BACE1 at the vesicle. Generally, large peptidomimetic inhibitors tend to have low oral bioavailability, low half-lives, and poor BBB penetration. However, small inhibitors may not adequately fill the enzymatic active site, resulting in poor affinity for the enzyme. Despite these challenges, potential inhibitors have been pursued in clinical trials (Fig. 36). Thirteen drugs have been brought to clinical trials in the United States, six of which have progressed into phase II or III clinical trials and five are currently being tested in the clinic (Figs. 35 and 36)

4.1 Clinical Evaluation of CTS21166

The structure of CTS21166 has not been publically disclosed. This compound, developed by CoMentis, was the first BACE1 inhibitor to be brought to clinical trials. The 8-month phase I trial began in June 2007 and tested CTS21166 in 56 healthy adult males to evaluate the safety profile of single-ascending doses as well as the pharmacokinetics and major plasma metabolites [94]. Intravenous injections of 7.5–225 mg were well tolerated and showed gradual clearance amenable to once-daily dosing across all doses. At 225 mg, plasma A β levels were

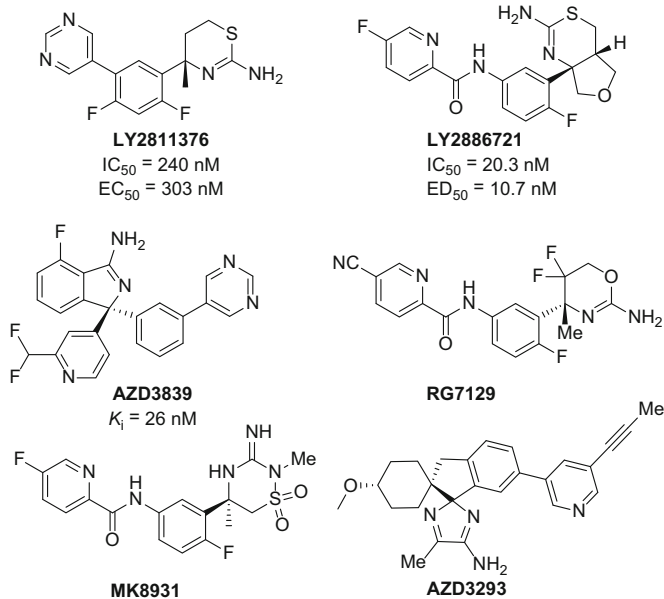


Fig. 35 Known structures of BACE1 clinical drug candidates

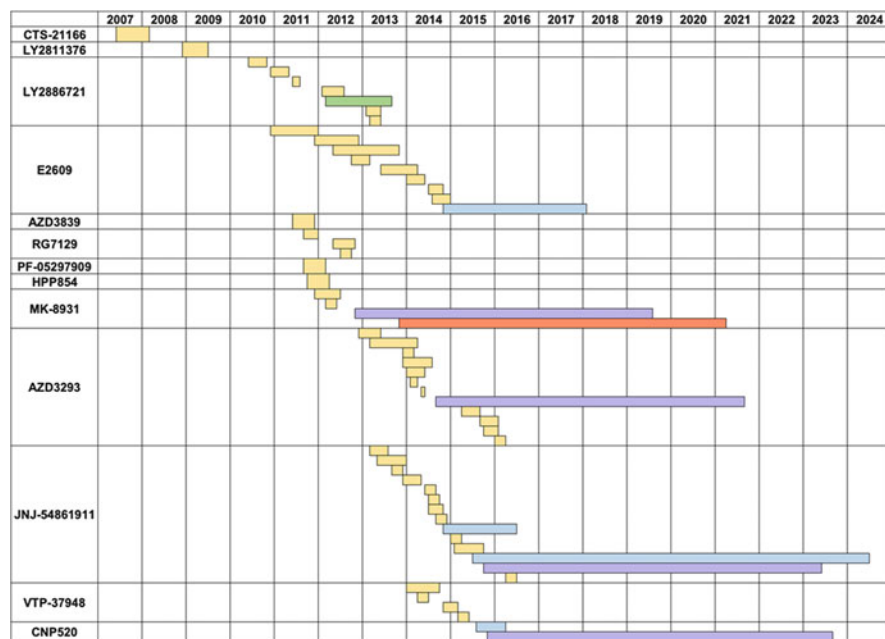
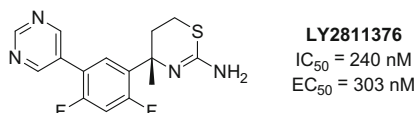


Fig. 36 Timeline of BACE1 inhibitor clinical development. Phase I = yellow; phase I/II = green; phase II = blue; phase II/III = purple; phase III = red

reduced by up to 80% at 3 h postinjection versus placebo [95, 96]. While CTS21166 passed phase I trials, its clinical development was not continued.

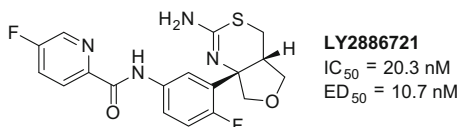
4.2 Clinical Evaluation of LY2811376



LY2811376, Eli Lilly's first clinical BACE1 inhibitor, began phase I clinical trials in 2009. It is a part of the aminothiazoline class of nonpeptidic inhibitors. The inhibitor was given to 61 healthy men and women of non-child-bearing potential to investigate the safety and tolerability at single doses from 5 to 500 mg as oral capsules [97]. LY2811376 was well tolerated with no serious adverse effects reported. Maximum plasma concentrations were reached 2 h post-dose with an average half-life of 40 h. Cerebrospinal fluid (CSF) concentrations reached maximal levels at about 5 h post-dose [98]. Dose-dependent reduction of A β ₄₀ and A β ₄₂ was observed in both the plasma and CSF. At 90 mg, an 80% reduction in A β ₄₀ was observed at 7 h after administration, with no full recovery to pre-dose concentrations within 120 h after dosing. In the CSF, up to a 54% decrease in A β was observed with a statistically significant decrease being observed 7 h after dosing and a maximum reduction at 12–14 h post-dose [98].

In preparation for phase II studies, a rat toxicology study was performed alongside phase I clinical studies. During the 3-month preclinical work, it was observed that autofluorescent granules were accumulating in the retinal epithelium, neurons, and glial cells at doses greater than 30 mg/kg. Based on this data, clinical trials of LY2811376 were immediately terminated. It was hypothesized that the retinal toxicity may be due to off-target effects against other aspartic acid proteases such as BACE2 or CatD. Fortunately, no clinically significant retinal pathology was present in any of the clinical trial participants in follow-up exams [98].

4.3 Clinical Evaluation of LY2886721



It was more than a year before the next clinical candidate was tested in human subjects. LY2886721, developed by Eli Lilly and Company, became the first BACE1 inhibitor to reach phase II trials [99]. Like LY2811376, it is a part of the aminothiazoline class of nonpeptide inhibitors. A total of six phase I studies tested the safety and pharmacology of LY2886721 in 155 subjects. Trials began in June

2010 with a single-ascending dose study in healthy adult males and females, administered as oral capsules in variable doses ranging from 1 to 200 mg or placebo. Concentrations of LY2886721 and $A\beta_{40}$ were measured up to 8 days post-dose in the plasma and up to 36 h post-dose in the CSF [100]. Another study investigated the tolerability of multiple-ascending doses of 5–35 mg or placebo administered as oral capsules in 42 healthy adults. Subjects were monitored for clinically significant effects as well as concentrations of LY2886721 and $A\beta_{40}$ in the plasma and CSF [101].

An open-label study with radioactive ^{14}C -LY2886721 in eight healthy males was used to determine metabolism and excretion pathways [102]. An additional single- and multiple-dose study was performed on healthy volunteers before proceeding to phase I/II studies in Alzheimer's patients [103]. LY2886721 was evaluated in 128 patients with mild cognitive impairment or mild Alzheimer's disease. Doses of 15, 35, or 70 mg or placebo were administered orally once daily for 26 weeks. Concentrations of $A\beta_{40}$ and $A\beta_{42}$ were measured at 12 and 26 weeks in both the CSF and plasma. Other cognitive tests were performed at 26 weeks including a Neuropsychological Test Battery (NTB), Alzheimer's Disease Assessment Scale (ADAS), Clinical Dementia Rating (CDR) Scale, and Mini Mental State Examination (MMSE) [104]. Concurrently, additional phase I studies were performed to evaluate two formulations of LY2886721 and assess safety and side effects in Alzheimer's patients and high-dose tolerability in healthy volunteers [105]. However, in June 2013, Lilly voluntarily halted the phase II trial and all further clinical development of LY2886721 due to abnormal liver biochemistry observed in subjects. It is believed that the abnormalities are not related to the BACE1 mechanism [106]. No clinical trial results of LY2886721 have been published.

4.4 Clinical Evaluation of E2609

Eisai Inc. brought clinical candidate E2609 to trial, starting with an initial phase I safety study beginning in December 2010. The structure of E2609 has not been publically disclosed. Unpublished preclinical work showed that E2609 lowered $A\beta$ levels in the CSF and plasma of rats, guinea pigs, and non-human primates [107]. In the first single-ascending dose study, E2609 was dosed orally in healthy adult volunteers up to 800 mg or placebo and in healthy elderly volunteers at 50 mg or placebo. Subjects were monitored for adverse effects, and pharmacokinetic parameters were assessed in plasma and urine [108]. Adverse effects were reported in 21.8% of subjects treated with E2609 compared to 16.7% dosed with placebo. No serious adverse effects were reported, demonstrating that E2609 was well tolerated in all dosing groups. Plasma concentration of E2609 was dose dependent, with a half-life of 15.9 h at 800 mg. Maximal reduction of plasma $A\beta$ occurred from 6 to

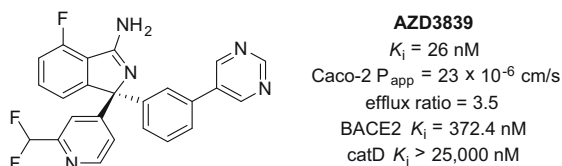
24 h after administration with decreases ranging from 56.3% at 5 mg to 91.0% at 800 mg. At up to 144 h post-dose, an 800 mg dose still afforded a 45% reduction of A β in the plasma [109].

During a multiple-ascending dose study, healthy adult volunteers received 25–400 mg or placebo administered orally once daily for 14 days. Subjects were monitored for adverse effects, as well as A β concentration in the plasma and CSF [110]. E2609 continued to be well tolerated in multiple doses. Plasma levels of E2609 were dose dependent, with a constant concentration achieved at 7 days. Concentration of A β in the plasma was significant, with reduction greater than 85% at the lowest dose. Assessment of CSF showed that at 100 mg, A β_{40} and sAPP β were reduced by 82.1% and 94.2%, respectively, while sAPP α levels increased by 128.6%, indicating enhanced processing by α -secretase due to the inhibition of BACE1 [111].

Other phase I studies included a safety and pharmacodynamic study in 65 mild AD patients given a single oral dose ranging from 5 mg to 400 mg [112] and a bioavailability and absorption assessment in 18 healthy males [113]. Utilizing ^{14}C -radiolabeled compound, the metabolism and excretion pathway of E2609 was investigated in 113 healthy males at a single 100 mg oral dose [114]. To investigate potential drug-drug interactions, 195 healthy adults were dosed with E2609 in the presence or absence of itraconazole, an antifungal and cytochrome P450 3A4 inhibitor; rifampin, an antibiotic and hepatic cytochrome P450 inducer; digoxin, a treatment for heart conditions; and donepezil, an acetylcholinesterase inhibitor used for palliative care in Alzheimer's patients [115]. To assess pharmacokinetic parameters, a single oral dose study in Japanese and white healthy males was performed monitoring plasma concentrations and parameters for up to 10 days [116]. Another drug-drug interaction study was performed on 60 healthy adult volunteers to investigate the effect of moxifloxacin with E2609. Moxifloxacin is an antibiotic which has shown to prolong the QT interval when dosed with some other medications. In this study, particular attention was given to changes in the QTc interval and ECG recordings [117].

In November 2014, Eisai Inc., in collaboration with Biogen, began a phase II study of E2609 in patients with early AD and mild-to-moderate dementia due to AD. An estimated 700 patients will be administered a low, middle, or high dose of E2609 or placebo orally once per day for 18 months. End points will be evaluated via change in derived Alzheimer's Disease Composite Score (ADCS) at 18 months, and change in hippocampal atrophy as determined by MRI, and CSF A β levels [118]. Clinical evaluation of this compound is ongoing, and the current phase II study is estimated to be completed in January 2018.

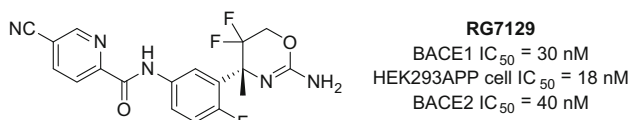
4.5 Clinical Evaluation of AZD3839



In June 2011, AstraZeneca brought clinical candidate AZD3839 into phase I trials. AZD3839 was developed from an aminopyrimidinone NMR hit. Subsequent structural optimization led to the aminoisoindole derivative that was ultimately assessed in the clinic [119]. In preclinical studies, AZD3839 displayed an enzymatic K_i of 26.1 nM/L and a 14-fold and 1,000-fold selectivity over BACE2 and CatD, respectively [119]. In mice, the brain concentration of AZD3839 reached maximum concentration at 30 min and returned to baseline levels at 4.5 h when dosed at 80 $\mu\text{mol/kg}$. When the dose was doubled, the brain exposure elongated to about 8 h. $A\beta_{40}$ levels decreased 30% and 50%, respectively [119]. Guinea pigs were given an oral dose of either 100 or 200 $\mu\text{mol/kg}$. Lasting up to 8 h, guinea pigs which received 200 $\mu\text{mol/kg}$ had 20–60% lower brain $A\beta_{40}$ levels than the control. The lower-dose group showed a brain $A\beta_{40}$ reduction of 20–30% after 1.5–4.5 h post-dose. CSF $A\beta_{40}$ levels in the high-dose group were reduced by 50% at 3 h and 40% at 8 h; however, the reduction was not statistically significant. Plasma $A\beta_{40}$ levels were reduced by 30–80% for both dose classes over the entire test period [119]. In a non-human primate model using cynomolgus monkeys, an intravenous 20 $\mu\text{mol/kg}$ dose of AZD3839 reduced CSF levels of $A\beta_{40}$, $A\beta_{42}$, and sAPP β between 3 and 12 h of exposure. A lower dose of 5.5 $\mu\text{mol/kg}$ did not show any significant effect on biomarker levels. This is proposedly due to low exposure at this concentration, as CSF concentration of AZD3839 peaked at 15 min and decreased tenfold at 3 h [119].

Based on this preclinical data, AZD3839 was brought to phase I clinical studies. A single-dose safety and tolerability study was performed in 72 healthy male volunteers given oral doses ranging from 1 to 300 mg or placebo. Subjects were monitored for adverse effects, as well as for plasma AZD3839, $A\beta_{40}$, and $A\beta_{42}$ levels [120]. AZD3839 was well tolerated at all doses, with 31% of the test group reporting at least one mild adverse event, compared to 39% in the placebo group. A dose-dependent elongation of the QT interval was observed, with an average prolongation of 20 ms at the highest dose of 300 mg. AZD3839 lowered plasma $A\beta_{40}$ and $A\beta_{42}$ in a dose-dependent manner. The maximum reduction of plasma $A\beta_{40}$ and $A\beta_{42}$ was 56% and 39%, respectively [121]. This phase I study was completed in November 2011, and AZD3839 was not brought forward into further clinical studies.

4.6 Clinical Evaluation of RG7129



Hoffmann-La Roche brought inhibitor RG7129 (RO5508887) to clinical trials in September 2011. It is a member of the aminooxazoline class of nonpeptidic inhibitors. Preclinical evaluation showed that RG7129 was potent against BACE1 (IC₅₀ = 30 nM) but was not selective over BACE2 [122]. Single-ascending oral doses were administered to 49 healthy male volunteers. Plasma and urine levels of RG7129 were monitored from 1.5 to 144 h post-dose. Plasma levels of A β ₄₀ and A β ₄₂ were measured in the plasma over the same time frame. The effect of food on the pharmacokinetics of RG7129 and incident of adverse events were also monitored [123]. A secondary study evaluated biomarker levels in the plasma and CSF as well as RG7129 concentrations and adverse effects, in 42 healthy male subjects [124]. A third multiple-ascending dose phase I trial investigated the safety and tolerability of RG7129 over prolonged exposure. Subjects were monitored for the occurrence of adverse effects as well as plasma levels of biomarkers and RG7129 [125]. Results of these studies have not been published or disclosed. In October 2013, Hoffmann-La Roche ended the clinical development of RG7129 but did not provide a clinical explanation for its cessation.

4.7 Clinical Evaluation of PF-05297909

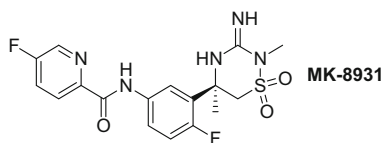
Pfizer brought its first small-molecule BACE1 inhibitor to clinical trials in September 2011; the structure has not been released. PF-05297909 was administered orally as a single dose to 51 healthy volunteers. A thorough pharmacokinetic study was of interest; plasma and CSF levels of biomarkers, in vivo IC₅₀ estimation, half-life, and CSF concentration of PF-05297909 were determined [126]. At all doses, 25–325 mg, PF-05297909 was well tolerated and displayed sufficient plasma concentrations and A β reduction. Unfortunately, this plasma exposure and reduction was not translated to the CSF, and no significant pharmacokinetic or pharmacodynamic effect was observed [127]. Clinical development of PF-05297909 was halted in light of these phase I results.

4.8 Clinical Evaluation of HPP854

High Point Pharmaceuticals developed inhibitor HPP854, which was determined to have significant preclinical activity worthy of translation into a clinical setting.

Phase I trials of HPP854 began in October 2011. HPP854 was administered orally once daily over 28 days in ascending doses to seven patients with mild cognitive impairment or mild AD. Patients were monitored for adverse events, plasma concentration of HPP854, and CSF concentration of A β [128]. Upon the conclusion of the phase I study in March 2012, High Point Pharmaceuticals terminated the clinical development of HPP854. No clinical results or inhibitor structure has been released.

4.9 Clinical Evaluation of MK-8931



MK-8931 (verubecestat) was developed by Merck. It was first brought to clinical trials in the United States in December 2011. Initial clinical studies conducted in healthy human patients consisted of a single-ascending dose and multiple-ascending dose study. Volunteers were given a placebo, a single dose of 20–550 mg, or 10–250 mg once daily for 14 days. Subjects were monitored for adverse effects and CSF biomarker concentrations. MK-8931 was well tolerated across all dosing levels in both single and multiple dosing cohorts. CSF A β_{40} levels were reduced by 25–61% in a dose-dependent manner in the single-dose study; the multiple-dose cohort showed a dose-dependent reduction of A β_{40} ranging from 32 to 94% on day 14 [129]. Exposure of MK-8931 was dose dependent in plasma and CSF with maximum plasma concentrations at 1–4.5 h and maximum CSF concentrations at 4–6 h with a half-life of 14–22 h [130].

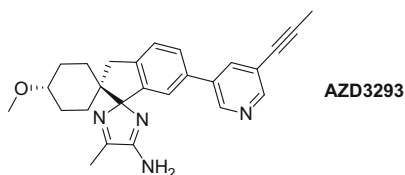
A phase I study of 32 patients with mild-to-moderate AD was performed to evaluate the safety and pharmacokinetics of MK-8931 in diseased patients. Participants received a 12, 40, or 60 mg oral dose of MK-8931 or placebo once daily for 7 days and were monitored for A β_{40} and sAPP β levels in the CSF [131]. An additional study in renal impaired patients evaluated whether renal disease changed the pharmacokinetics of MK-8931. Patients with mild, moderate, or severe renal impairment as well as healthy control groups were given a single oral dose of MK-8931 and monitored for plasma concentration of MK-8931 over 120 h, renal clearance of MK-8931, and excretion in urine [132].

MK-8931 was subsequently brought into a two-part phase II/III trial. The first part consisted of a long-term safety study in which approximately 400 mild-to-moderate Alzheimer's patients were dosed orally, once daily with 12 or 40 mg for 78 weeks or 60 mg for 13 weeks and then 40 mg for the remaining 65 weeks. Efficacy was measured by change in Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) score and Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) score. Secondary outcome

measurements included change in Clinical Dementia Rating Sum of Boxes (CDR-SB) score, Neuropsychiatric Inventory (NPI) score, MMSE score, hippocampal volume, CSF tau concentration, and brain amyloid concentration. Contingent upon the first safety trial, patients would receive the 12 or 40 mg dosages, once daily by mouth up to 260 weeks with the same primary and secondary end point examinations [133].

In December 2013, it was announced that the Data Monitoring Committee recommended continuation into part two of the phase II/III trials based on the data collected during the safety phase of the study [134]. This study is expected to conclude in July 2019. Concurrently, Merck announced the beginning of a phase III study in early AD patients. Patients will receive 12 or 40 mg MK-8931 once daily for 104 weeks. Upon satisfactory completion of part one, a second part will continue the trial for an additional 260 weeks at 12 or 40 mg. Efficacy will be measured via change in 3-domain Composite Cognition Score (CCS-3D), ADCS-ADL score, hippocampal volume, time to progression of probable AD dementia, CSF tau, and composite cortical amyloid standard uptake value ratio as determined by PET imaging [135]. This trial is currently recruiting participants, with an estimated enrolment of 1500. The study is estimated to be completed in March 2021.

4.10 Clinical Evaluation of AZD3293



AstraZeneca brought this new compound to phase I clinical trials after their termination of AZD3839. AZD3293 is a member of the aminoimidazole class of nonpeptide inhibitors. A single-ascending dose trial of AZD3293 was completed in healthy male and female volunteers. AZD3293 was dosed ranging from 1 to 1000 mg and administered as an oral solution or placebo. Subjects were monitored over 10 days for adverse effects, variation in vital signs, and Columbia-Suicide Severity Rating Scale (C-SSRS). Plasma concentration of AZD3293 and biomarkers were also evaluated [136]. A two-part multiple-ascending dose study was performed on 47 subjects. Part one consisted of healthy elderly volunteers who were dosed with an oral solution of AZD3293 starting at 5 mg upward. Patients were monitored for adverse events, variation in vital signs and C-SSRS scores, as well as plasma and CSF concentrations of biomarkers and AZD3293. Part two consisted of mild-to-moderate AD patients demonstrating AD symptoms at least 6 months prior to the study. Subjects were dosed with an oral solution of AZD3293 and were examined for the same efficacy end points as part one [137].

Potential drug-drug interactions were investigated. AZD3293 was administered to healthy adult volunteers as an oral solution alongside itraconazole, diltiazem, and midazolam. Subjects were monitored for the effects of coadministration on pharmacokinetic parameters of AZD3293 [138]. Pharmacokinetic considerations were assessed in a study of healthy Japanese volunteers who received single doses as an oral solution, 15, 50, and 150 mg for young patients and 1, 15, and 50 mg for elderly patients. A multiple-dose cohort was also established. Subjects were monitored for adverse effects, pharmacokinetic concentrations of AZD3293, and $A\beta_{40}$ and $A\beta_{42}$ concentrations [139]. A study of the effect of AZD3293 on the QT interval was performed in 52 healthy male volunteers. A cohort of each high-dose and low-dose AZD3293 and a moxifloxacin and placebo cohort were given a single dose and monitored for changes in the QT interval as well as inhibitor plasma concentrations and incident of adverse events [140]. Additional phase I studies included a bioavailability assessment of the oral solution versus two different tablet formulations [141]; a study of the effect of food on these tablet formations [142]; an absorption, metabolism, and elimination study with ^{14}C -AZD3293 [143]; and drug-drug interaction studies with simvastatin, midazolam, and donepezil [144], warfarin [145], and dabigatran [146].

In September 2014, AstraZeneca announced it would collaborate with Eli Lilly to develop AZD3293 further and advance into phase II/III studies [147]. This study is currently recruiting participants with an estimated enrolment of 2,202 patients with mild AD. Participants will be given either a 20 or 50 mg dose of AZD3293 (LY3314814) orally, once daily for 104 weeks. Efficacy will be measured primarily by the change in CDR-SB score. Alternative measures include change in ADAS-Cog13 score, ADCS-ADL score, CDR score, NPI score, CSF $A\beta_{42}$ concentration, amyloid PET imaging, whole brain volume, and others [148]. This study is ongoing and results are expected in August 2021.

4.11 Clinical Evaluation of JNJ-54861911

In March 2013, JNJ-54861911 was brought into clinical development by Janssen Research and Development. Structural information for JNJ-54861911 has not been released. Healthy adult volunteers across eight cohorts totaling 56 subjects were given a single dose of JNJ-54861911 ranging from 1 to 160 mg or placebo, administered orally. Patient CSF and blood plasma were assessed for concentration of JNJ-54861911 and $A\beta$ at up to 96 and 36 h post-dose, respectively [149]. A similar study was performed in healthy elderly volunteers. Seventy participants were given a 3–80 mg dose of JNJ-54861911 as an oral suspension, once daily for 14 days. Patients were monitored for adverse effects, while plasma and CSF samples were taken at multiple time points to assess concentration and exposure of JNJ-54861911 as well as $A\beta$ levels [150]. To better understand the pharmacokinetic parameters of JNJ-54861911 and the effect food may have on these parameters, a study was performed in which healthy elderly male volunteers received a single 25 mg dose as

an oral suspension under fasted conditions, a single 25 mg dose as an oral solid formulation under fasted conditions, and a single 25 mg dose as an oral solid formulation under fed conditions with 6 days between each dose and in varying orders of dosing. Bioavailability, absorption, half-life, elimination, and other pharmacokinetic parameters were examined using plasma concentration at discrete time points [151].

A proof-of-mechanism study was performed in early Alzheimer's patients. Forty-five participants were administered a 10 or 50 mg dose as an oral tablet once daily for 28 days. Exposure to JNJ-54861911 and biomarker levels in plasma and CSF were evaluated at discrete time points [152]. Other phase I studies included a study of pharmacokinetic considerations in healthy Japanese males [153] and at-risk asymptomatic Japanese participants [154]; drug-drug interaction studies with clarithromycin and itraconazole [155], caffeine and midazolam and tolbutamide [156], and rosuvastatin and metformin [157]; a study of the effects on QT intervals [158]; and the evaluation of the pharmacokinetic parameters of an alternative oral formulation [159].

Phase II trials for JNJ-54861911 began in November 2014. In a study of 114 participants with early AD, patients received a 10 mg, 50 mg, or placebo oral tablet once daily for 6 months. Safety and tolerability is the primary end point, with adverse events being monitored; however, exposure of JNJ-54861911 in the plasma and CSF as well as concentration of A β , sAPP β , and sAPP α in the CSF and plasma will also be examined [160]. This study is expected to conclude in June 2016. A similar extended study began in July 2015. Patients received a once-daily, oral dose of either 10 mg or 25 mg for 96 weeks or longer until termination of study due to safety issue or registration of JNJ-54861911 [161]. The study is estimated to conclude in June 2024.

A phase II/III study began in October 2015 with an estimated 1,650 asymptomatic at-risk participants. Subjects will be administered 10 mg, 50 mg, or placebo once daily by mouth for 54 months. The primary outcome measurement will be the change in AD Cooperative Study-Preclinical Alzheimer's Cognitive Composite (ADCS-PACC) score, while secondary efficacy end points include change in CFI score and others, incident of adverse events, and concentration of JNJ-54861911 and biomarkers in the plasma and CSF [162]. This study is currently recruiting participants and is expected to be ongoing until May 2023.

4.12 Clinical Evaluation of VTP-37948

Vitae Pharmaceuticals discovered preclinical BACE1 inhibitor VTP-37948 (BI 1181181). This was licensed and developed clinically by Boehringer Ingelheim beginning in January 2014. The structure of VTP-37948 has not yet been disclosed. In phase I development, 65 healthy male volunteers were given a single dose of VTP-37948 in ascending cohorts. The study investigated safety and tolerability as well as pharmacokinetic parameters in three different formulations both with and

without food by assessment of plasma and urine concentrations [163]. Another phase I study assessed pharmacokinetic parameters in healthy males via CSF and plasma analysis after single oral doses [164]. VTP-37948 was determined to be safe across all doses, with a half-life of 16–19 h; CSF A β levels could be lowered by 80% [165]. A multiple-ascending dose study was planned for healthy adult and healthy elderly volunteers. The trial began in September 2014, with participants receiving an oral dose once daily for 10 days [166]. In February 2015, it was announced that Vitae Pharmaceuticals and Boehringer Ingelheim were voluntarily terminating the clinical development of VTP-37948 due to negative skin reactions in some participants [167]. A drug-drug interaction study was withdrawn before participants could be recruited [168]. No further development or data for VTP-37948 has been reported.

4.13 Clinical Evaluation of CNP520

In partnership with Amgen, Novartis has examined CNP520 in phase II clinical trials as a single agent and in phase II/III clinical trials alongside immunotherapy CAD106. The structure of CNP520 has not yet been disclosed. The initial phase II trial was performed in healthy elderly volunteers. Four cohorts were administered a dose of CNP520 orally, once daily for 13 weeks. An additional cohort was given a placebo control. Patients were monitored for adverse effects as well as CNP520, A β_{40} , and A β_{42} levels in the CSF and CNP520 concentrations in the plasma [169]. The study recently ended in March 2016. However, results have not yet been disclosed. A study of at-risk patients is currently recruiting participants to study CNP520 alongside immunotherapy CAD106 when administered separately. Participants will be given only CNP520 or CAD106 or a placebo, but not multiple investigational treatments. Volunteers given CNP520 will be dosed orally, once daily for 60 months. The study is expected to enroll 1340 participants and be completed in August 2023 [170].

5 Conclusions and Outlook

Since the cloning and characterization of BACE1 more than one and a half decades ago, BACE1 continues to be an exciting target for drug development against Alzheimer's disease. To date, several inhibitor drugs have now entered into clinical development for potential new treatment of Alzheimer's disease. This review highlighted design, development, and clinical evaluation of BACE1 inhibitors for the treatment of AD. The early design of BACE1 inhibitors involved replacing the scissile amide bond of the APP β -site with nonhydrolyzable transition-state isosteres. Subsequent determination of the X-ray crystal structure of these substrate-based inhibitors and BACE1 complexes provided necessary drug design

templates and greatly facilitated the structure-based design of drug-like peptidomimetic BACE1 inhibitors. The structural evolution of BACE1 inhibitors has been very impressive. The availability of X-ray structural information of BACE1 with a variety of inhibitors along with past experiences of successful drug design against other aspartic acid proteases, such as renin and HIV protease, expedited the design and discovery of many new classes of BACE1 peptidomimetic inhibitors with therapeutic potential. Several BACE1 inhibitor drugs showed promising results in animal models, as they reduced the amyloid plaque in the brain and rescued cognitive deficits. The first peptidomimetic drug candidate, CTS21166, was evaluated in phase I human clinical trials. The reported results indicated that the drug was well tolerated, and A β reduction was achieved with BACE1 drug treatment.

Despite significant progress with peptidomimetic BACE1 inhibitors, AD drug development brought a different set of medicinal chemistry challenges. BACE1 has a large substrate-binding site which makes it difficult to design a small inhibitor that inhibits the enzyme with desirable drug-like properties. Compounds need to have sufficient lipophilic features to penetrate both plasma and intracellular membranes and gain access to the BACE1 active site in the brain. This requires inhibitor size to be around 550 Da with reduced susceptibility to Pgp-mediated efflux in order to cross the blood–brain barrier efficiently. Furthermore, the high homology among aspartyl proteases makes it particularly important to have high selectivity over cathepsin D, renin, and especially BACE2.

The X-ray structural knowledge of peptidomimetic inhibitors and extensive high-throughput screening efforts ultimately paved the way for the evolution of small-molecule nonpeptide BACE1 inhibitors. Many structural classes have now evolved with novel heterocyclic scaffolds such as acyl guanidine, aminopyridine, and aminoimidazole with unique modes of inhibition. Several optimized inhibitors from these classes have been shown to cross the BBB, reach the brain, and reduce brain A β in transgenic AD mice. As many as 13 such inhibitor drugs with favorable pharmacological properties have entered into clinical development, and two of these drugs have now advanced to phase IIa/b clinical trials with human AD patients. A successful clinical outcome would provide the long-awaited proof for the “amyloid hypothesis” in AD therapy. The accumulation of knowledge of BACE1 and its functions through the years still supports BACE1 as a viable target for Alzheimer’s disease drug development. The progress in the field allows much optimism that β -secretase inhibitor drug treatment of Alzheimer’s disease may soon become a reality.

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γ -Secretase Modulators as A β 42-Lowering Pharmacological Agents to Treat Alzheimer's Disease

Douglas S. Johnson and Martin Pettersson

Abstract γ -Secretase is an intramembrane aspartyl protease comprised of four essential subunits including presenilin, nicastrin (NCT), anterior pharynx defective 1 (Aph-1), and presenilin enhancer 2 (Pen-2). The amyloid precursor protein (APP) is cleaved sequentially by β -secretase and γ -secretase to generate A β peptides including neurotoxic A β 42 monomers and oligomers that are believed to be key pathological species in AD. Familial Alzheimer's disease (FAD) mutations in presenilin and APP increase the relative proportion of A β 42. γ -Secretase modulators (GSMs) have been discovered that bind to presenilin and selectively modulate γ -secretase proteolytic activity. Importantly, GSMs have the opposite effect on the A β cleavage profile as compared to FAD mutations, namely they decrease the relative proportion of A β 42. This review will discuss the initial discovery of GSMs and the recent progress leading to the development of GSMs with improved drug-likeness. These efforts have culminated in GSMs that are currently undergoing proof-of-mechanism studies in the clinic, which is a significant step forward toward testing the amyloid hypothesis.

Keywords A β 42, Alzheimer's disease, Amyloid precursor protein, Medicinal chemistry, Presenilin, γ -Secretase, γ -Secretase modulator

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1 Introduction

Alzheimer’s disease (AD) is a progressive, neurodegenerative disorder and is the most common form of dementia. It results in difficulty remembering newly learned information, memory loss, disorientation, mood and behaviour changes, and eventually difficulty speaking and walking. The pathological hallmarks of AD include deposition of amyloid- β peptides ($A\beta$) in the brain parenchyma [1], formation of paired helical filaments (PHFs) of tau aggregates [2], and ultimately neuronal loss. Genetic linkage studies and positional cloning efforts revealed that rare mutations in amyloid precursor protein (APP), PSEN1, and PSEN2 cause early onset familial AD (EO-FAD) [3]. The vast majority of these mutations (>200) increase the $A\beta_{42}/A\beta_{40}$ ratio, and this relative increase in $A\beta_{42}$ may promote aggregation leading to oligomers and amyloid fibrils. This convergence of disease pathology and genetics led to the amyloid cascade hypothesis, which suggests that a central event leading to AD is the aggregation of amyloid- β peptides ($A\beta$), especially $A\beta_{42}$, which forms toxic soluble oligomers and deposited $A\beta$ in amyloid plaques [4, 5]. Therefore pharmacological approaches to reduce the levels of $A\beta$ peptides have garnered much attention in the field [6–8].

APP is initially cleaved by β -secretase BACE1 to generate $sAPP\beta$ and β -CTF (also referred to as C99), which is cleaved by γ -secretase in the membrane to form APP intracellular domain (AICD) and multiple $A\beta$ peptides (Fig. 1). γ -Secretase initially cleaves APP β -CTF at the ϵ -site at the membrane/cytosolic border to generate $A\beta_{49}$ and/or $A\beta_{48}$, which undergo successive stepwise cleavage every three to four amino acids to generate shorter forms which are released from the

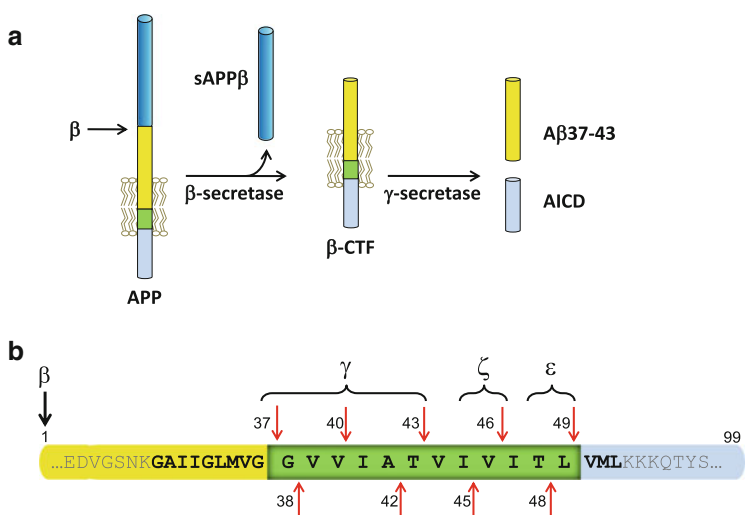


Fig. 1 (a) Sequential cleavage of amyloid precursor protein (APP) by β - and γ -secretases and corresponding products. (b) Sequence of the membrane and nearby regions of the APP β -CTF substrate. Vertical red arrows show locations of γ , ζ , and ϵ cleavages by γ -secretase

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