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Review Article

Small-sized BACE1 inhibitors

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CONTENTS

Abstract

The determination of the three-dimensional X-ray crystal structure of β-secretase (BACE) complexed with an inhibitor has greatly facilitated the design of BACE inhibitors. Generally, BACE inhibitors can be grouped into two main families: substrate-based inhibitors designed as peptidomimetic inhibitors and nonpeptidomimetic inhibitors. This review focuses on the rational design of inhibitors based on transition-state analogues. The structural nature of peptidomimetic inhibitors usually implies relatively poor catabolic stability and low bioavailability after systemic administration due to low blood-brain barrier permeability. To overcome these drawbacks, several different approaches have been used.

Introduction

The accumulation of β-amyloid peptide (Aβ) in the brain is a major factor in the pathogenesis of Alzheimer's disease (AD) (1). Aβ is formed by the initial cleavage of the β-amyloid precursor protein (β-APP) by β-secretase to form a membrane-bound *C*-terminal fragment, which is then proteolyzed further by γ-secretase to produce two major forms: Aβ(1-40) and Aβ(1-42) (Fig. 1) (2). The detailed characteristics and mechanism of cleavage of γ-secretase have not been elucidated. However, β-secretase has been identified as a novel membrane-bound aspartyl protease known as BACE1, memapsin 2 or Asp2 (3-6), and the crystal structure of its catalytic domain has been determined (7). BACE1 plays a critical role in the progression of AD, since the cleavage of APP by β-secretase is the first step in Aβ formation. Therefore, the development of BACE1 inhibitors is valuable in the elucidation of AD pathology. Several transition-state analogue BACE1 inhibitors have been reported with IC_{50} values in the nanomolar range, but often with a molecular size too large to be viable drug candidates.

This review focuses on small-sized BACE1 inhibitors with potent enzyme-inhibitory activity. An overview of early research is included, with emphasis on a comparison of developments in the design of BACE1 inhibitors as reported by several groups.

Designing BACE1 inhibitors

The initial goal in designing BACE1 inhibitors was to make relatively small peptide inhibitors through substrate transition-state mimetic studies. Since BACE1 is an aspartyl protease, prior experience with related enzymes, especially HIV protease and renin, facilitated the design of inhibitors with a variety of binding motifs. Based on the common enzymatic mechanism of aspartyl proteases, substrate transition-state analogues have been proposed and are currently widely used in the design of highly potent aspartyl protease inhibitors. Previously, potent inhibitors with α-hydroxy-β-amino acids incorporating one of the typical substrate transition-state mimics, hydroxymethylcarbonyl (HMC), were developed against several human disease-related aspartyl proteases, such as renin (8), HIV protease (9-11) and plasmepsin II (12, 13). Along the same lines, we focused on the substrate sequence deduced from the preferred cleavage sequence of Swedish mutant APP, which shows a mutation at the P2-P1 positions (from Lys-Met to Asn-Leu) (4).

Initial BACE1 inhibitors

As reported by Tang and Gosh in an earlier study, replacement of the central P1/P1' residues (Leu/Asp) with the nonpeptidic transition-state isostere Leu*Ala (where the asterisk represents a hydroxyethylene [HE] fragment) (14) led to the discovery of the potent inhibitors OM-99-1 (**1**), OM-99-2 (**2**) and OM-00-3 (**3**),

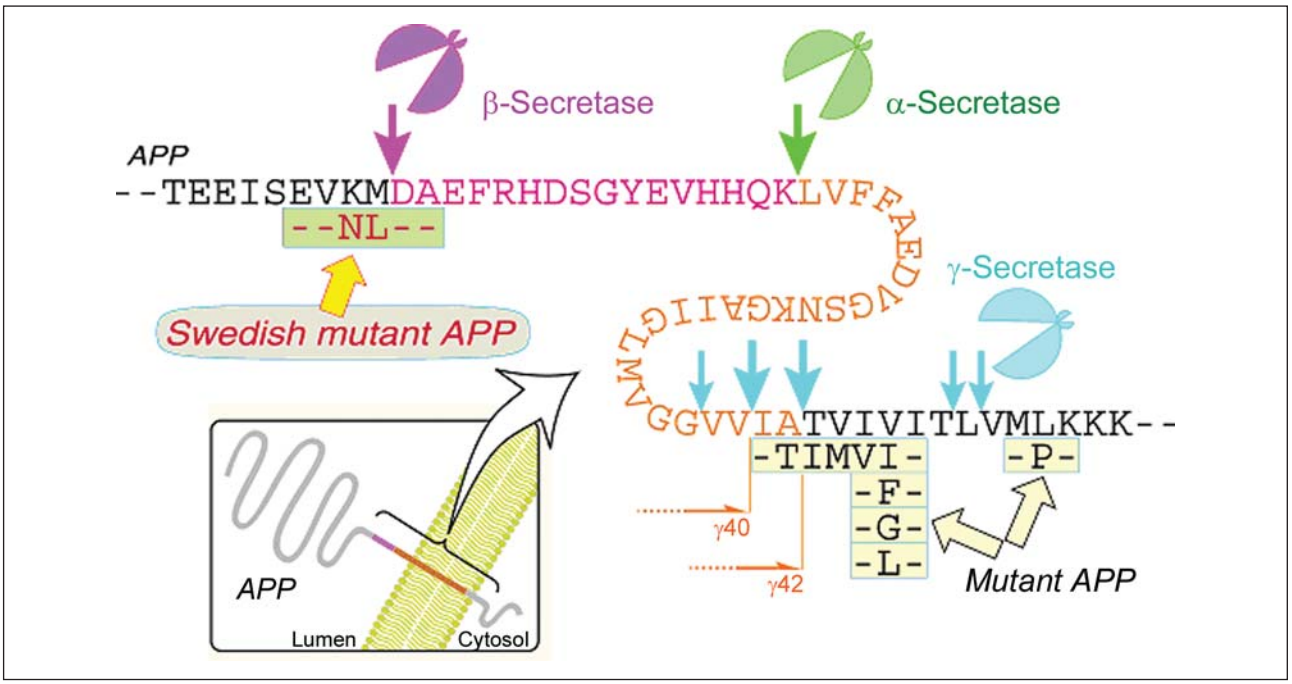


Fig. 1. Secretase cleavage sites with the human β-amyloid (Aβ) peptide sequence. Aβ is formed by an initial cleavage of the β-amyloid precursor protein (APP) by β-secretase to form a membrane-bound *C*-terminal fragment, which is then proteolyzed by γ-secretase to form Aβ(1-40) or Aβ(1-42). The Swedish mutation (KM-NL) in β-APP significantly enhances BACE1-mediated cleavage and production of the γ-secretase substrate C99. α-Secretase mediates nonamyloidogenic cleavage of β-APP in intact cells.

with K_i values of 36, 1.6 and 0.3 nM, respectively (15-18) (Fig. 2). Nonhydrolyzable Leu*Ala is also known as a homostatine, or (2*R*,4*S*,5*S*)-5-amino-4-hydroxy-2,8-dimethyloctanoic acid, and is a leucine mimetic transition-state analogue.

Our initial design was based on the sequence of Swedish mutant APP (P4-P4': EVNL/DAEF). First, we designed and synthesized an octapeptide, KMI-061 (**4**), containing an α-hydroxy-β-amino acid, norstatine ([2*R*,3*S*]-3-amino-2-hydroxy-5-methylhexanoic acid [Nst]) (19), at the P1 position (Fig. 3). Because the BACE1-inhibitory activity of compound **4** was very low, we changed the P1 core of the octapeptide to phenylnorstatine ([2*R*,3*S*]-3-amino-2-hydroxy-4-phenylbutyric acid [Pns]) (9, 10), thereby synthesizing the inhibitor KMI-062 (**5**). Since compound **5** exhibited moderate BACE1-inhibitory activity, we chose Pns as a transition-state mimetic. Through structure-activity relationship (SAR) studies of the P3-P3' positions of the octapeptide series, we selected the inhibitor KMI-008 (H-Glu-Val-Leu-Pns-Asp-Ala-Glu-Phe-OH) (**6**), which had the highest activity against recombinant BACE1 enzyme (IC_{50} = 413 nM) among our synthesized octapeptides. Moreover, KMI-008 inhibited the secretion of sAPPβ (a soluble form of APP generated by the action of β-secretase) from COS-7 cells co-transfected with both APP and BACE1 (Fig. 3). Since Pns, a phenylalanine mimetic, had a larger side-chain than Nst, a leucine mimetic, the contribution of the hydrophobic interaction at the S1 subsite increased BACE1 inhibition (20).

Tung *et al.* (Elan Pharmaceuticals) developed a series of substrate-based inhibitors against human brain β-secretase. Based on statine derivatives, these inhibitors mimicked Leu-Gly or Phe-Gly at the P1-P1' positions. Incorporation of statine ([3*S*,4*S*]-4-amino-3-hydroxy-6-methylheptanoic acid) (21) resulted in the potent inhibitor **7** (IC_{50} = 300 nM) (Fig. 4). The 'statine' analogue **8**, with 'AHPPA' ([3*S*,4*S*]-4-amino-3-hydroxy-5-phenylpentanoic acid), was equipotent to **7** (IC_{50} = 500 nM), while the 'ACHPA' ([3*S*,4*S*]-4-amino-3-hydroxy-5-cyclohexylpentanoic acid) analogue **9** was 10-fold less potent, with an IC_{50} in the micromolar range (22).

Development of potent, small-sized inhibitors

Phenylnorstatine-containing analogues

The octapeptide KMI-008 was not suitable as an AD therapeutic agent due to its long peptide chain. It was therefore necessary to minimize the number of natural peptide bonds and the molecular weight. In order to determine the smallest structure having BACE1-inhibitory activity, peptide ladders were designed by gradually removing amino acids from KMI-008. We discovered that the compound lacking the P4-P2 positions displayed no inhibitory activity. This finding suggested that the interactions of *N*-terminal residues were very important, while those of the *C*-terminus were not as significant. Structure-activity relationship (SAR) studies focusing on the P4 and P1' positions led to the optimization of an inhibitor con-

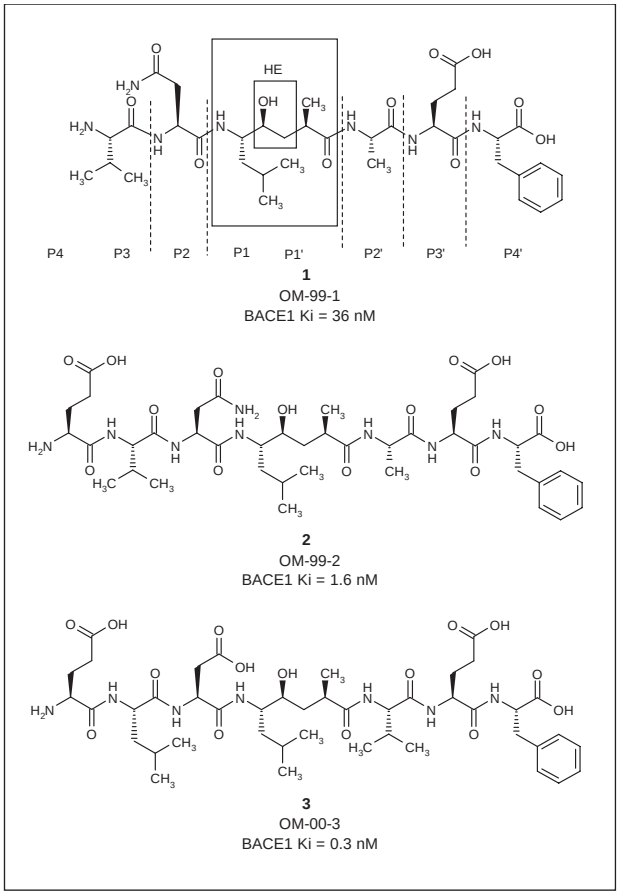


Fig. 2. Structures of selected substrate-based BACE1 inhibitors developed by Tang's group. HE: hydroxyethylene.

sisting of P4-P1'. Based on the SAR results, we designed the pentapeptide BACE1 inhibitors KMI-358 (**10**) and KMI-370 (**11**), with *N*-β-oxalyl-L-2,3-diaminopropionic acid (*N*-β-oxalyl-DAP) at P4 and 3-aminobenzoic acid or 5-aminoisophthalic acid at P1' (Fig. 5). The pentapeptide KMI-370, which has a relatively low molecular weight, was a very potent inhibitor (IC_{50} = 3.4 nM). Finally, we examined the effects of KMI-358 and KMI-370 on β-secretase activity in HEK-293 (human embryonic kidney) cells transfected with BACE1, which can stably express both full-length BACE1 and native APP. The IC_{50} values were 650 and 200 nM for KMI-358 and KMI-370, respectively. The decrease in soluble β-APP secretion from BACE1-transfected HEK-293 cells was concentration-dependent (23). The *N*-β-oxalyl-L-2,3-diaminopropionic group is important for enhancing BACE1 inhibition, but these inhibitors isomerized to *N*-α-oxalyldiaminopropionic derivatives (**12**) in solvents (as it is known that *N*-β-oxalyl-DAP thermally isomerizes to an equilibrium mixture with *N*-α-oxalyl-DAP) (24). Hence, we used a tetrazole moiety as a bioisostere (25) of the free carboxylic acid of the oxalyl group to improve stability. Consequently, we identified the tetrazole-containing BACE1 inhibitors KMI-420 (**13**) and KMI-429 (**14**), which have IC_{50} values of 8.2 and 3.9 nM, respectively, as well as enhanced chemical stability (Fig. 5) (26). We also studied the *in vivo* inhibitory effects of KMI-429, which effectively inhibits β-secretase activity in cultured cells in a concentration-dependent manner. Intrahippocampal injection of KMI-429 in wild-type mice markedly reduced Aβ production in both soluble and insoluble fractions. These results indicate that KMI-429 is a promising candidate for the treatment of AD (27).

Statine-containing analogues

The development of statine-based peptidomimetic BACE1 inhibitors was described by researchers at Elan Pharmaceuticals. Their heptapeptide BACE1 inhibitor **7** (Fig. 4) was used as a convenient starting point for an iterative process of screening replacements to peptidic portions of the molecule at both the *N*- and *C*-terminal ends. Hom *et al.* discovered that 4-amino-3-hydroxy-5-phenylpentanoic acid was comparable to statine in terms of activity at the central portion of the molecule, so they began to incorporate fluorine derivatives in their smaller inhibitors (**15-17**). This approach led to the identification of smaller compounds demonstrating BACE1-selective

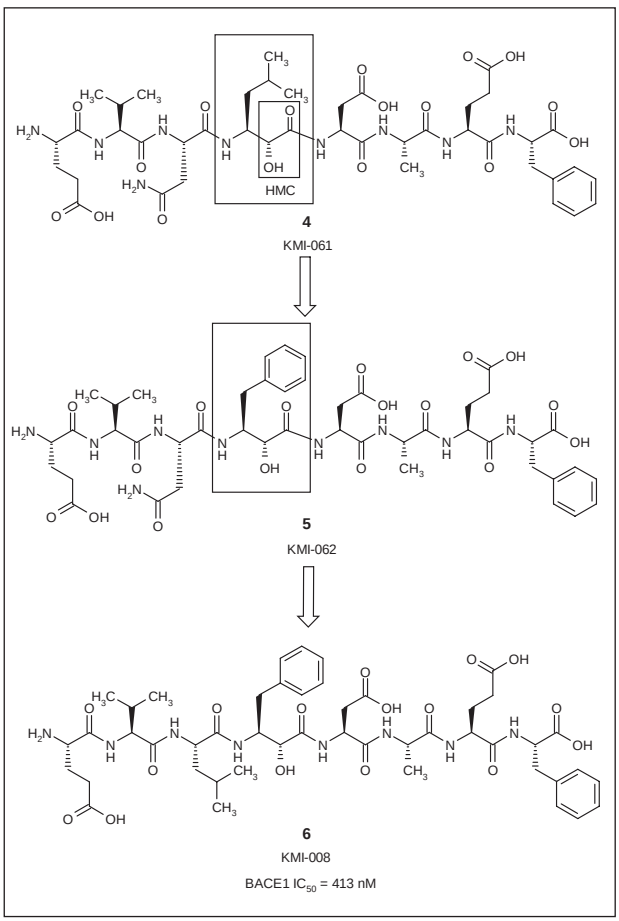


Fig. 3. The design of substrate-based BACE1 inhibitors containing Pns. HMC: hydroxymethylcarbonyl.

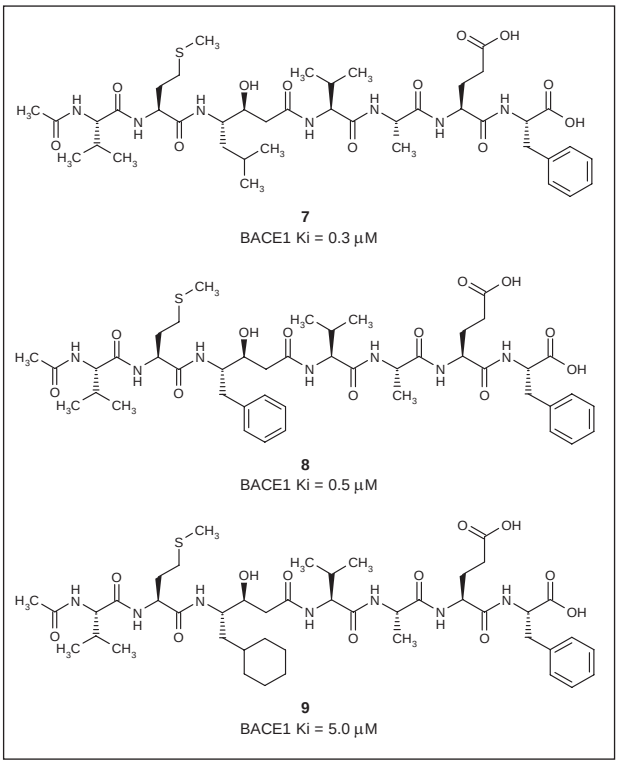


Fig. 4. Structures of statine-containing BACE1 inhibitors.

cellular inhibition, with the most active cell-permeable BACE1 inhibitor (**15**) displaying BACE1 mechanism-selective inhibition of Aβ secretion in HEK-293 cells (IC_{50} = 120 nM for **15a**) (28). They continued their modifications at the *C*- and *N*-termini by synthesizing the dicarboxylate *C*-terminal analogue **16** (BACE1 IC_{50} = 20 nM), and by *N,N*-dipropylisophthalamic moiety replacement of the biphenyl *N*-terminus, which led to the most potent inhibitor (**17**), with an IC_{50} value for inhibition of BACE1 of 1 nM (Fig. 6) (29).

A similar idea was used by Hu *et al.* (Lilly), who started their truncation studies from a heptapeptide mimetic compound. Through systematic modification at the P3, P2 and P2' positions, they synthesized three inhibitors with IC_{50} values for inhibition of BACE1 of < 100 nM, *i.e.*, 69 nM for **18**, 86 nM for **19** and 91 nM for **20** (Fig. 6) (30).

On the basis of SAR study results concerning statine-based BACE1 inhibitors previously described by others, Hu *et al.* (Wyeth Research) anticipated that the introduction of a second statine-like fragment in the central core (P1/P2 side-chain) and 4-aminomethylbenzoic acid at the *C*-terminus would be optimal for BACE1 inhibition. They developed a series of peptide-based BACE1 inhibitors characterized by the presence of a bis-statine central core, and the best example, compound **21**, displayed very high potency (IC_{50} = 21 nM) (Fig. 6) (31).

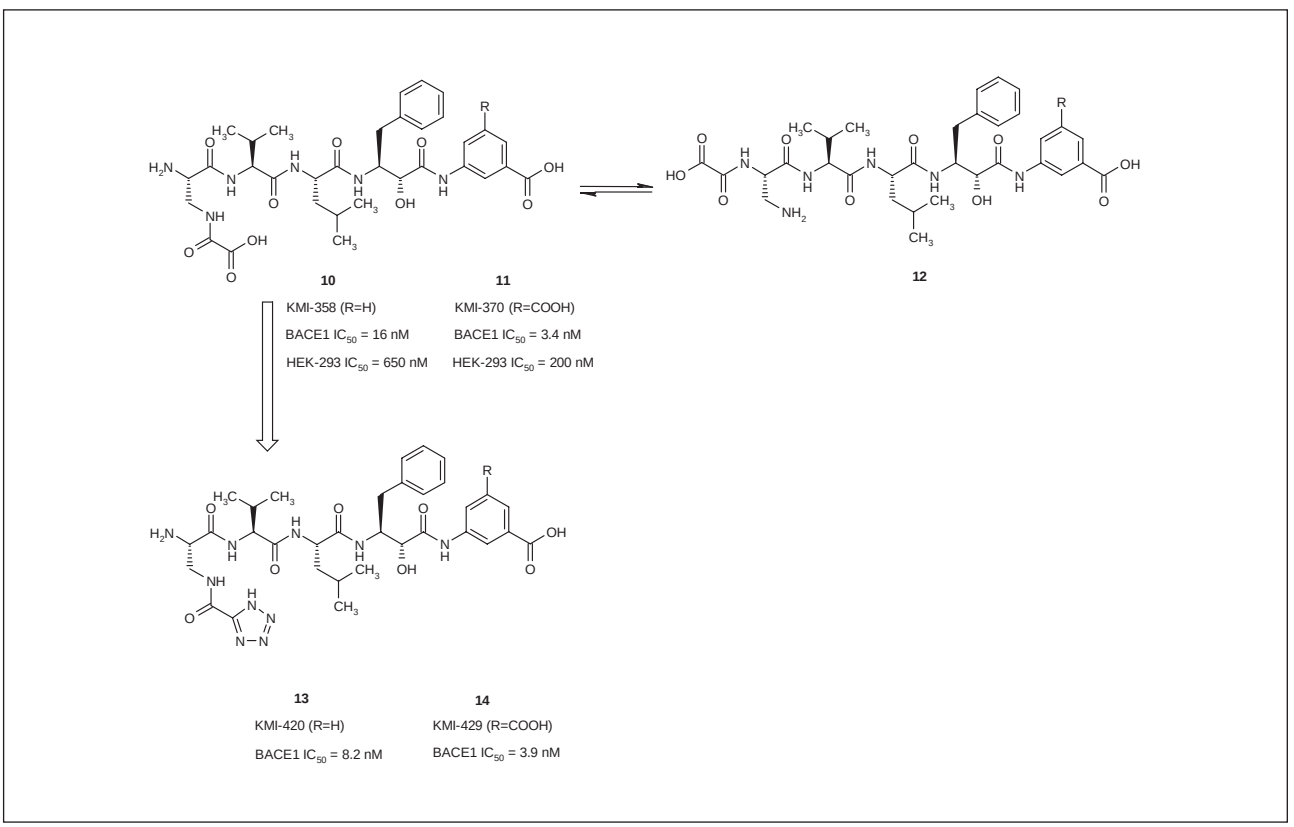


Fig. 5. Structures of Pns peptidomimetics.

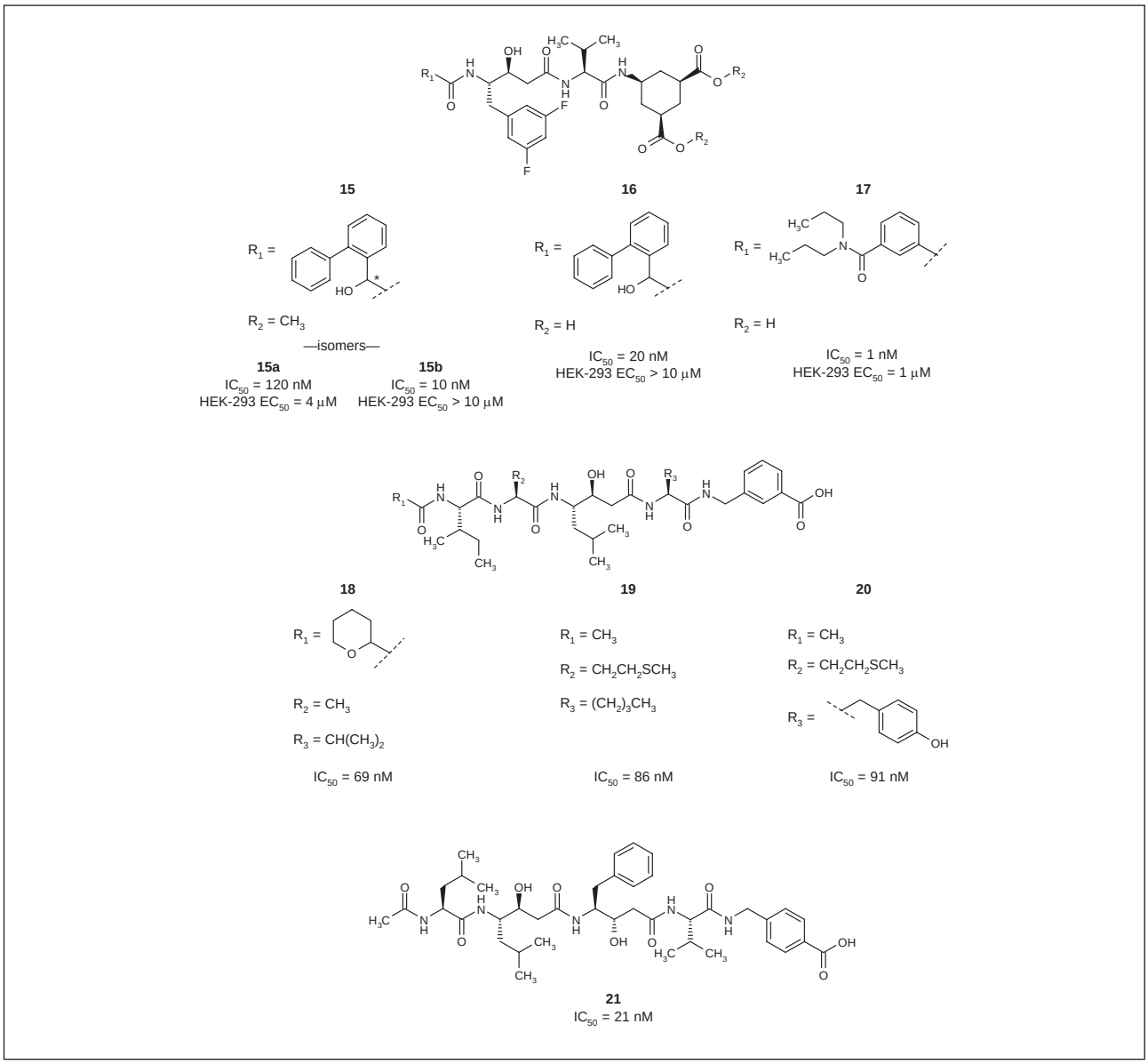


Fig. 6. Structures of statine peptidomimetics.

Homostatine-containing analogues

Elan researchers also investigated the incorporation of homostatine at the P1-Leu position with an HE moiety. They found that the HE derivative **22** (Fig. 7) displayed significantly enhanced potency (IC_{50} = 20 nM), suggesting that HE replacement of the 'Stat-Val' central core (7) (Fig. 4) could result in very potent inhibitors (22). Consequently, they decided to move from statine-like transition-state mimetics towards the HE isostere due to its potential for exhibiting more promising properties, such as increased solubility and enhanced oral bioavailability. The HE transition-state isostere was developed as a scaffold to provide the potent, small-molecule inhibitor **23** with *N*-terminal isophthalamide (IC_{50} = 30 nM) (Fig. 7). Modification at the *C*-terminus by removal of a carboxylic fragment and introduction of a nonpolar isobutyl substituent led to enhanced cell penetration. Unlike in the statine series, nonpolar *C*-termini were tolerated in this series, resulting in inhibitors that were more cell-permeable and contained no amino acid residues (29).

Guided by the findings of Tang's group regarding pentapeptidyl Leu*Ala-based inhibitors, the group from Lilly reported a new series of BACE1 inhibitors. SAR investigations of Phe*Ala-based pentapeptides were focused on both *N*- and *C*-termini, and also on changes at the P2 and P3 positions. As previously mentioned, potent, highly polar inhibitors failed to show any cellular activity because of poor membrane permeability. These researchers therefore carried out rather extensive *C*-ter-

minal modifications and introduced benzylamine at the *C*-terminus to decrease molecular polarity. In cases where whole-cell activity was very low, they hypothesized that it might be attributed to the very low water solubility and consequently replaced the phenyl moiety at the *C*-terminus with a pyridine ring. As a result, they presented inhibitors bearing *C*-terminal Val-pyridine moieties with whole-cell activities at concentrations below the micromolar range, *i.e.*, compound **24**, with a BACE1 IC_{50} value of 42 nM and an IC_{50} value of 410 nM for whole-cell Aβ-lowering activity in HEK-293/APP751$_{Swe}$ cells, and compound **25**, with a BACE1 IC_{50} value of 45 nM and an IC_{50} value of 400 nM for inhibition of Aβ in HEK-293 cells (32). Further modifications at the P3 position gave P3-hydroxylated inhibitors with lower molecular weight and slightly reduced biological activity, *i.e.*, compound **26**, with IC_{50} values in the BACE1 and Aβ HEK-293 assays of 35 nM and 1.23 μM, respectively, and compound **27**, with respective IC_{50} values of 45 nM and 1.08 μM (Fig. 7). Incorporation of fluorine at P3 in **25** and at P2 and P3 in **27** did not increase activity (33).

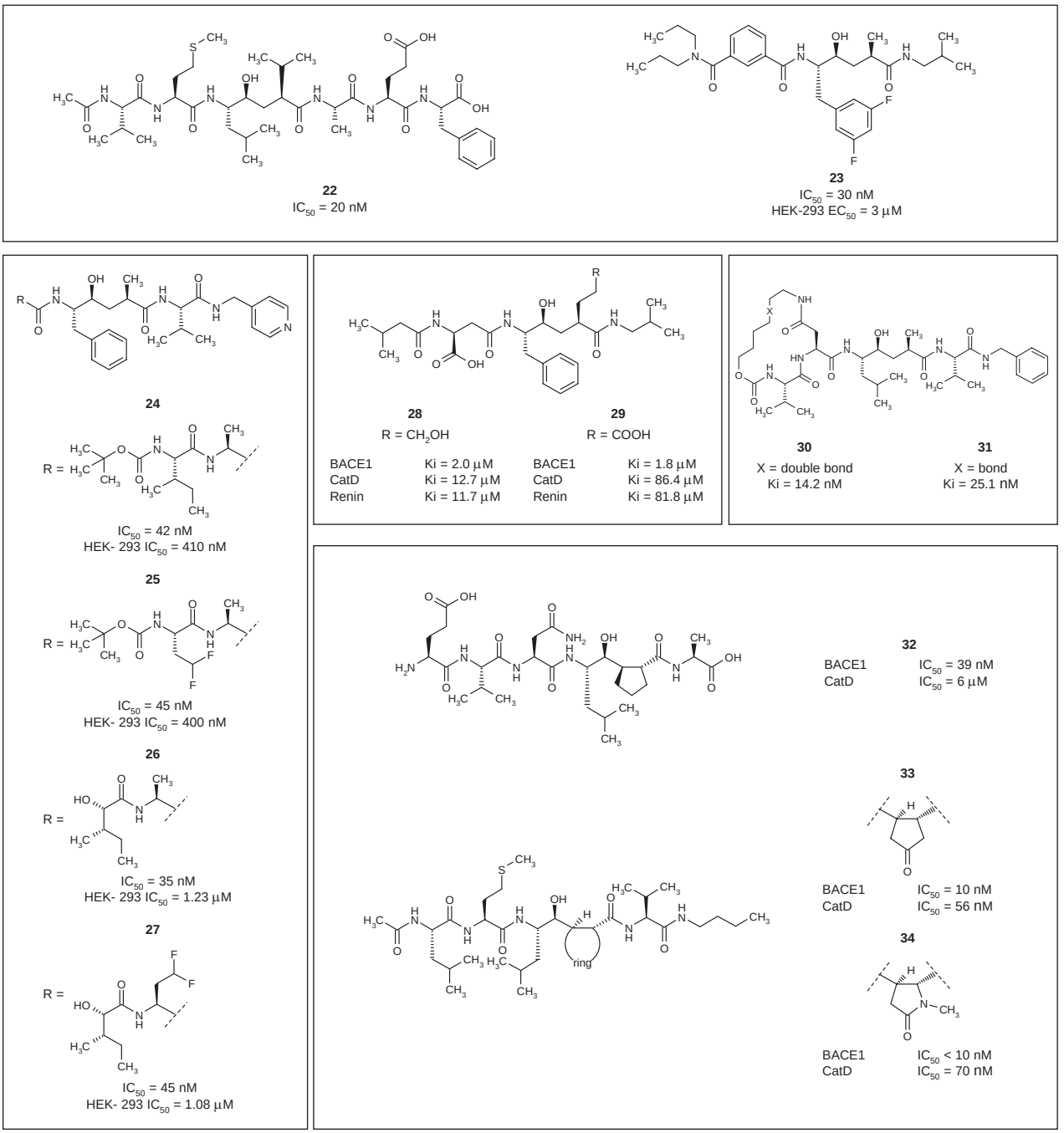


Fig. 7. Structures of homostatine peptidomimetics.

Researchers from Merck and Bachem Bioscience investigated similar homostatine peptidomimetics based on a core disclosed by Elan Pharmaceuticals with 'AHPPA' (**8**) (Fig. 4). They explored modifications of the P1' and *N*-terminal regions to improve BACE1 selectivity over the related aspartyl proteases cathepsin D and renin. When comparing the X-ray structures of these enzymes, they noted significant differences in the length and sequences of the loop. They concluded that the BACE1 loop is truncated and primarily hydrophilic, whereas the renin and cathepsin D loops are extended and primarily hydrophobic. Based on these findings, they deduced that a more polar P1' capping group would be favored. They selected a few candidates for enzyme evaluation via targeted screening of compounds similar to known BACE1 inhibitors but containing side-chains with different polarities at the P1' position. The addition of a hydroxyl group to the P1' side-chain in inhibitor **28** resulted in higher selectivity, *i.e.*, K_i values of 2 μM against BACE1, 12.7 μM against cathepsin D and 11.7 μM against renin. Furthermore, replacement of the hydroxyl moiety with a carboxylic acid functional group in **29** enhanced selectivity approximately 40-fold, with K_i values of 1.8 μM against BACE1, 86.4 μM against cathepsin D and 81.8 μM against renin (Fig. 7). These modifications did not offer any selectivity over BACE2 (also known as Asp1 or memapsin1), the closest homologue of BACE1 (34).

Tang's group also recently reported a series of novel macrocyclic amide-urethanes designed and synthesized on the basis of the X-ray crystal structure of their lead compound (**2**) bound to BACE1 (Fig. 2). Cycloamide-urethane functionalities at P2-P3 containing 14- to 16-membered rings exhibited low nanomolar inhibitory potencies against human brain BACE1 and cellular inhibition of BACE1 in CHO cells. The potent 16-membered monounsaturated compound **30** (K_i = 14.2 nM) and the saturated compound **31** (K_i = 25.1 nM) (Fig. 7) were examined against BACE2, but no significant differences in inhibition were observed. Cellular inhibition of BACE1 in CHO cells was examined for inhibitor **31**, and the average cellular IC_{50} value (5.1 μM) was 10-fold lower than that of compound **2** (IC_{50} = 50 μM) (35).

At almost the same time, Hanessian *et al.* published new potent BACE1 inhibitors. Their design was based on the X-ray crystal structure of the original Tang-Gosh heptapeptide inhibitor (**2**). Molecular modeling led them to a subtle modification at the P1' Ala position with the introduction of a fused cyclopentane ring as a carbocyclic constraint in the HE subunit. Using the same backbone replacement concept, they designed a series of constrained P1' analogues and synthesized cyclopentane (**32**), cyclopentanone (**33**) and 2-pyrrolidinone (**34**) derivatives. These compounds showed low nanomolar potency for BACE1 inhibition, with good selectivity over cathepsin D. Specifically, compound **32** gave IC_{50} values of 39 and 6000 nM for BACE1 and cathepsin D, respectively, compound **33** had respective IC_{50} values of 10 and 56 nM and compound **34** exhibited respective IC_{50} values of < 10 and 70 nM. The X-ray co-crystal structures of **32** and **33** revealed good convergence with the original inhibitor (**2**). Despite their high potency in the enzymatic assay, the inhibitors exhibited low activity in the cellular assay (Aβ production in CHO cells transfected with APP_{wt}) (36).

Other inhibitors

The use of an HE fragment as part of the central core of the inhibitor appears to be quite popular in the design of aspartyl protease inhibitors, especially BACE1 inhibitors. HE is present in statine and homostatine derivatives, as well as in other isosteres that are modified variations of previous designs.

One such modification was disclosed by Fujii's group regarding the design of β-secretase inhibitors containing a hydroxyethylamine (HEA) dipeptide isostere (HDI), another variant of the transition-state isostere. Several β-secretase inhibitors were designed based on the HDI structure. Among these pseudopeptides, effective inhibitors developed through SAR studies include compounds with inhibitory activity at below 100 nM, *i.e.*, compounds **35** (IC_{50} = 47 nM) and **36** (IC_{50} = 87 nM) (Fig. 8) (37).

The HEA isostere is a known motif from early research on HIV protease and renin inhibitors. Studies on HIV protease inhibitors based on this motif have shown that (*S*)-hydroxyl stereochemistry is preferred for a statine or HE type isostere, but the (*R*)-stereochemistry of HEA isosteres is preferred in smaller inhibitors (38). Coburn *et al*. from Merck and NeoGenesis Pharmaceuticals chose to target the HEA motif rather than the related HE fragment because the former was lower in molecular weight and contained one less amide bond. They identified a small molecule with a nonpeptide active site (**37**) as a very selective BACE1 inhibitor (IC_{50} BACE1 = 1.7 μM; IC_{50} BACE2 = 137 μM; IC_{50} cathepsin D and renin > 500 μM) (Fig. 8), via crystallographic determination of the enzyme-inhibitor complex and application of the Automated Ligand Identification System (ALIS) technology developed by scientists at NeoGenesis (39). After examining the enzyme-inhibitor complex, they noted subsite occupation in compound **37**: S4 to S1 subsites of BACE1 and any interaction with 'prime-sides' of the enzyme. They did not observe any direct contact of **37** with catalytic aspartic acids Asp32 and Asp228. Indirect contact with aspartates through a water-mediated hydrogen bond was observed. Inhibitor **37** forms a hydrogen bond from oxyacetamide NH to a water molecule situated between the aspartyl dyad. This nontraditional binding mode is also observed in the apo crystal structures of other aspartyl proteases, such as renin, cathepsin D and endothiapepsin (39). The next goal was to synthesize a hybrid structure involving inhibitor **37** and the HEA isostere. The sulfonate ester was replaced with sulfonamide, yielding the hybrid constructs **38** (IC_{50} = 15 nM for purified BACE1) and **39** (IC_{50} = 14 nM for purified BACE1) (Fig. 8), which show approximately 100-fold greater potency than **37**. Inhibitors **38** and **39** also displayed excellent activity in a cell-based assay optimized for

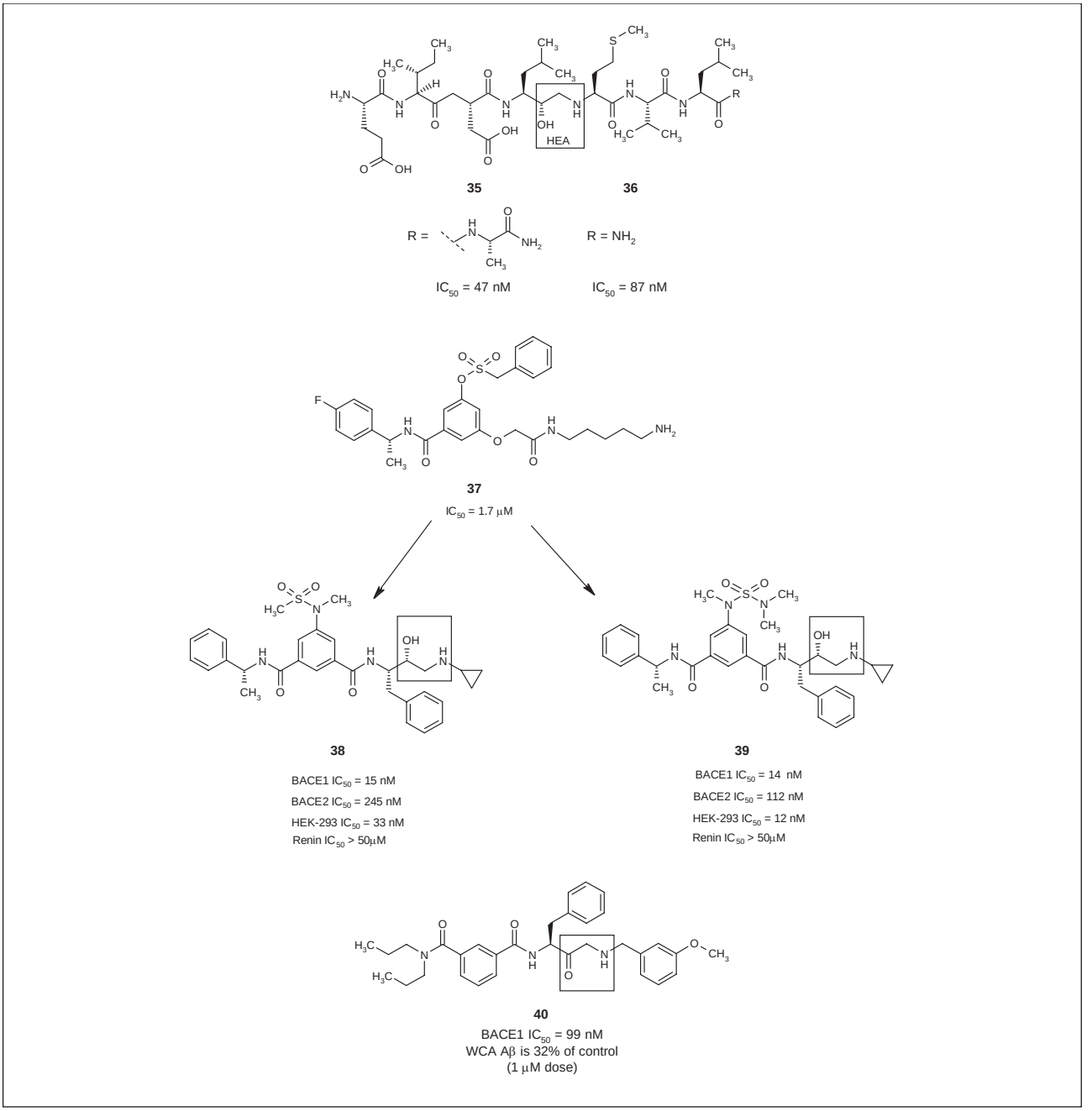
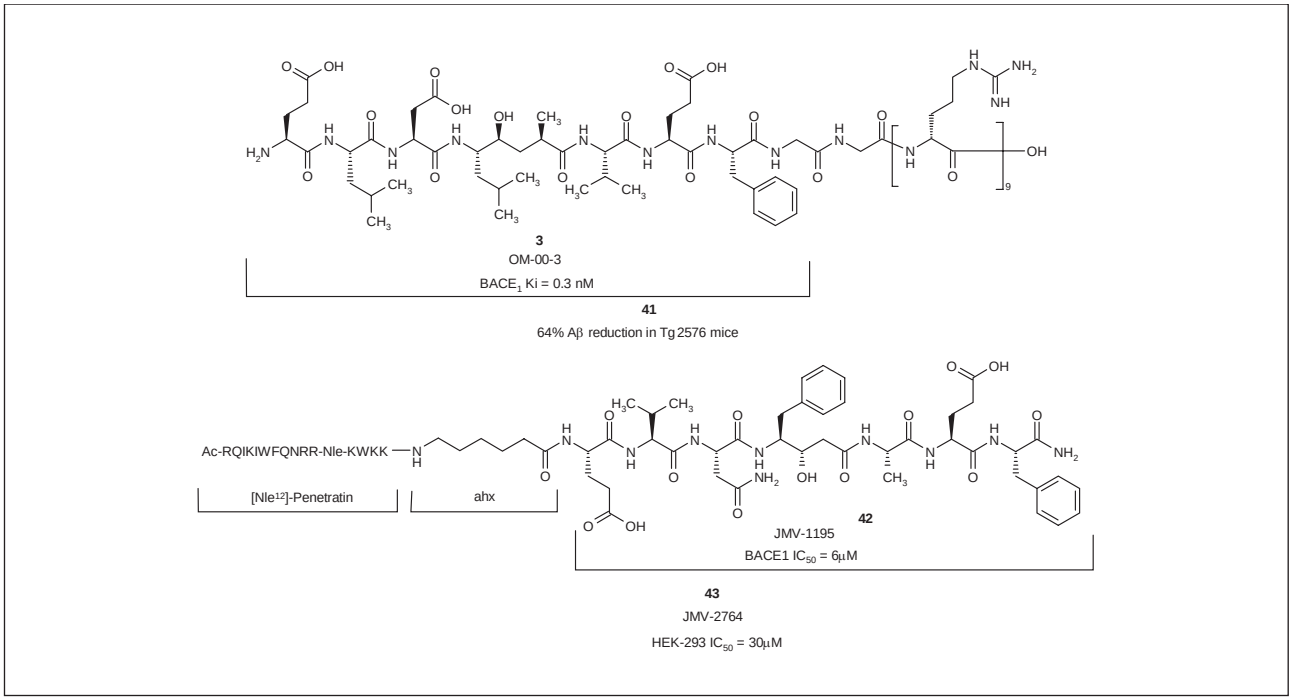


Fig. 8. Structures of selected hydroxyethylamine (HEA) and ketoamine derivatives.

monitoring BACE1 cleavage, with IC_{50} values of 33 and 12 nM, respectively, for inhibition of sAPP in HEK-293 cells stably transfected with a mutant form of APP containing the synthetic BACE1 cleavage sequence NFEV at the site of proteolysis. Moreover, these two compounds displayed selectivity over BACE2 (about 10-fold) and the structurally related aspartyl protease renin (> 100-fold) and compound **38** also demonstrated selectivity over cathepsin D (> 100-fold) (40).

Other modifications of HEA have been patented by many companies, including the former Bristol-Myers Squibb, Elan Pharmaceuticals and Pfizer, which are thoroughly described in a review by Cumming *et al*. (41). Among the many disclosed structures, Takeda's series of β-ketoamines based on an Elan/Pharmacia core presented an interesting modification of the HEA core. Ketoamine **40** had an IC_{50} value of 99 nM against BACE1 (Fig. 8), and at a concentration of 1 μM it lowered Aβ(1-40) production in a whole-cell assay (41).

Hydroxyethylhydrazide and hydroxyethylhydrazine are other modifications of the HEA motif. A large class of peptidomimetics presented in patent applications



Fig, 9. The design of conjugated BACE1 inhibitors.

deals with amide derivatives, including hydroxyamide, reduced amides, cyclic and substituted forms, and others (42).

The two major categories of nonpeptidic inhibitors disclosed in patent applications involve furylpiperidine and furylpiperazine, as well as their modifications with C3 or C4 substitutions (41).

Summary and future perspectives

Although the design of β-secretase inhibitors was rather difficult at first, it has been made easier by the recent determination of the X-ray crystal structure of an inhibitor complexed with BACE1. Since BACE1 is an aspartyl protease, the design of inhibitors has also been facilitated by applying a variety of binding motifs from early research on HIV protease, renin and closely related enzymes. Some modifications were based on the incorporation of critical SAR data gleaned from previously synthesized BACE1 peptidic inhibitors into subsequent peptidomimetic structures. Peptidomimetic BACE inhibitors are more rationally designed; however, they have displayed relatively poor catabolic stability and bioavailability *in vivo* after systemic administration due to weak blood-brain barrier permeability. Several potent BACE1 inhibitors have been described, but they often lack the ability to inhibit Aβ production in cell systems. The high tertiary homology of aspartyl proteases has offered opportunities for rapidly developing active scaffolds and has also provided challenges for selectivity over other enzymes of the same class (43).

The most promising progress is with peptide isosteres. *N*-Terminal modifications of statine-based compounds have demonstrated high BACE-inhibitory activity. This finding led to the production of HE transition-state isosteres as a scaffold for generating potent small-molecule BACE inhibitors. Most of these inhibitors lack *C*-terminal carboxylic acids and have enhanced cell penetration (44, 45). The HMC isostere was used in the design of highly active inhibitors with enhanced chemical stability and good *in vivo* inhibitory effects (27). Pseudopeptide β-secretase inhibitors containing an HE dipeptide isostere have also been presented.

Conjugated inhibitors

Different approaches have been used to overcome drawbacks related to insufficient lipophilicity and a molecular weight too high to cross the blood-brain barrier. One of the possible strategies employs conjugated inhibitors with “carrier peptides” covalently linked to the inhibitors. This strategy has been tested by two independent research groups, but we have not presented their results in this review. Tang’s group has reported several potent BACE1 inhibitors (**1-3**) with K_i values in the nanomolar range (Fig. 2). However, these compounds were too large to cross the blood-brain barrier and therefore did not possess *in vivo* activity against BACE1. By administering the same compounds linked to a “carrier peptide” (a 12-residue Tat fragment or a 9-residue poly-D-arginine), cellular and blood-brain barrier penetration occurred. After a single i.p. injection (200 mg/25 g body weight) of the

inhibitor (OM-00-3)GG$_D$R$_9$ (**41**) (Fig. 9), the *in vivo* inhibition of Aβ production in the brain of AD mice (Tg2576 mice) was quite efficient (64% reduction) (46).

The same approach was used by Checlerc and Martinez. The BACE1 inhibitor JMV-1195 (**42**) (IC_{50} = 6 μM), one of a series of peptidomimetic statine-derived compounds, was unable to inhibit cellular β-secretase. But after adding a penetration-enhancing sequence as a "carrier peptide" at the *N*-terminus, the analogue JMV-2764 (**43**) penetrated several cell systems (HEK-293 cells) expressing wild-type or Swedish mutated β-APP and reached its cellular target, reducing Aβ production with an IC_{50} value of 30 μM (Fig. 9) (47).

Since "carrier peptides" may be used to deliver AD therapeutics, allowing larger molecules to penetrate the blood-brain barrier, potentially useful drugs can be selected from a wider repertoire of candidate compounds. However, it is not clear whether these compounds will show a good oral distribution. In addition, their pharmacokinetic profiles, toxicities and immunological effects have yet to be studied.

The fact that BACE1 is distributed among various parts of the body outside brain tissues, such as liver and leukocytes, is well known. Only a few BACE1 physiological substrates other than APP have been reported, *e.g.*, sialyltransferase (ST6GL-1), found by Kitazume *et al*. (48, 49), PSGL-1 (the cell adhesion protein P-selectin glycoprotein ligand-1), reported by Lichtenthaler *et al*. (50), and LRP (the low-density lipoprotein receptor-related protein), reported by Von Arnim *et al*. (51). Interestingly ST6GL-1 and PSGL-1 have an important function in the immune system, which suggests that the development of safe and effective BACE1 inhibitors may be problematic.

Hopefully, as more research is published in this field, we will learn to design compounds that will overcome these obstacles.

Acknowledgements

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