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Synthesis and SAR of bis-statine based peptides as BACE 1 inhibitors

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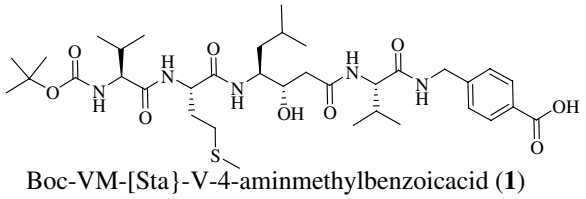
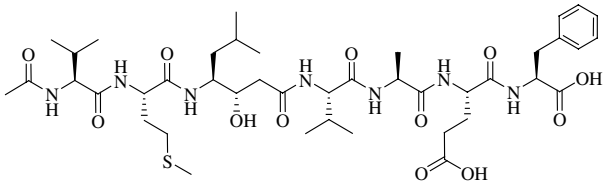
Abstract—A new series of bis-statine based peptidomimetic inhibitors of human β-secretase (BACE 1) was developed by structure-based modification of the three regions to the initial lead **3**: an N-terminus, a central bis-statine core, and a C-terminus. Introduction of a 4-aminomethylbenzoic acid on the C-terminus resulted in a potent BACE 1 inhibitor with an IC_{50} value of 21 nM. The general requirements for the optimal substrate–enzyme interaction are disclosed herein.

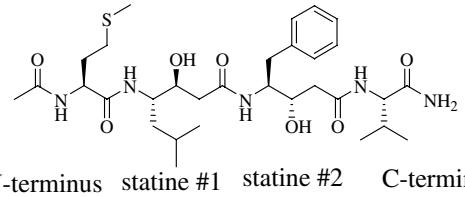
β-Secretases, or β-amyloid converting enzyme (BACE 1), is a membrane-associated aspartic protease that cleaves the β-amyloid precursor protein (APP) and generates the amyloid-β protein Aβ, a major pathological feature of Alzheimer's disease (AD). BACE 1 knockout mice develop normally, show no consistent phenotypic differences from their wild-type littermates, and their Aβ levels in the brains are reduced to negligible levels. Therefore, BACE 1 inhibitors might be beneficial for the treatment of AD.[1] Earlier discovered potent BACE 1 inhibitors, such as Elan's Boc-VM-[Sta]-V-4-aminomethylbenzoic acids (**1**) and Ac-VM-[Sta]-VAEF (**2**), are mono-statine transition state analogues based on the amino acid sequence of APP around the BACE 1 cleavage site.[2,3] Our initial bis-statine lead **3**, with substantially reduced molecular size, was generated by peptide spot synthesis.[4] It is a modestly potent BACE 1 inhibitor with IC_{50} of 0.5 μM. After independent modification of the central core (statine #1 and statine #2), the C-terminus and the N-terminus of **3**, a new lead **9** with IC_{50} of 21 nM was generated, which is described in this communication.[5,6]



Boc-VM-[Sta}-V-4-aminmethylbenzoicacid (**1**)



Ac-VM-[Sta}-VAEF (**2**) IC_{50} 0.3 μM



N-terminus statine #1 statine #2 C-terminus

3

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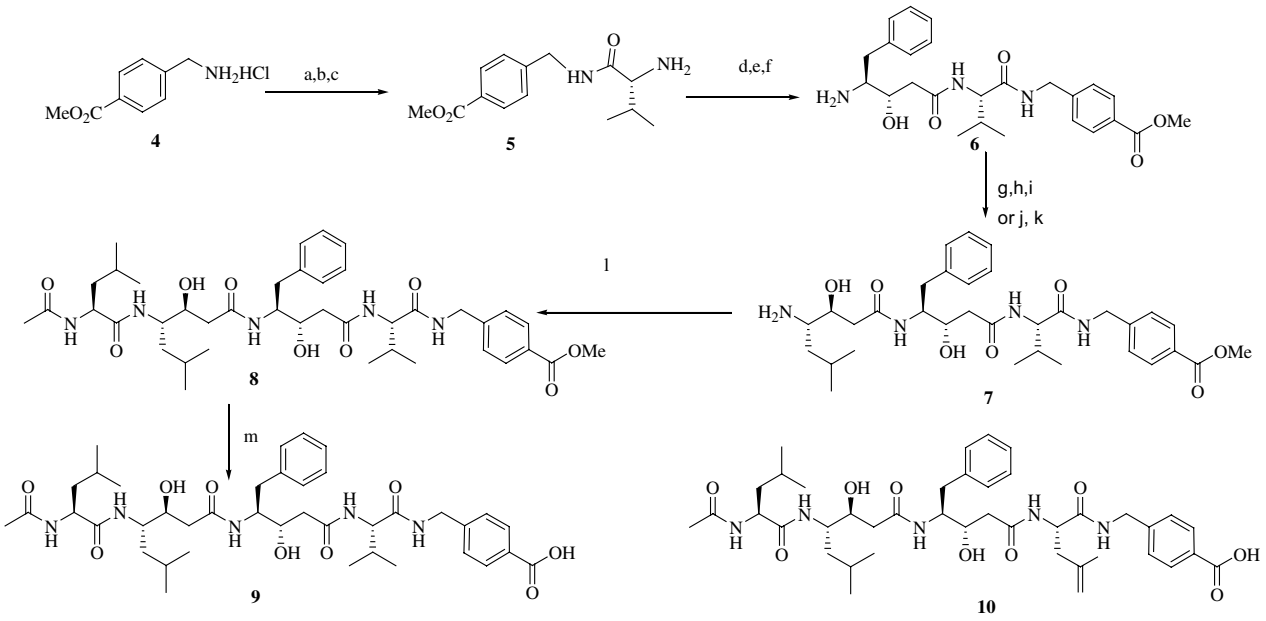
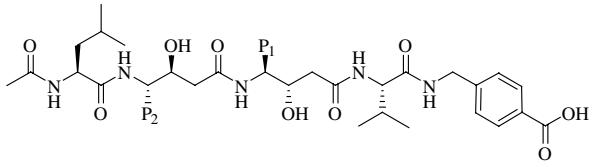


Scheme 1. Initial approach: Reagents and conditions: (a) N-Boc-Val, EDC; (b) TFA; (c) aq $NaHCO_3$, 80% for steps (a)–(c); (d) N-Boc-phenyl statine, EDC; (e) TFA; (f) aq $NaHCO_3$, 37% for steps (d)–(f); (g) N-Boc-statine, EDC; (h) TFA; (i) aq $NaHCO_3$, 22% for steps (g)–(i); (j) N-Fmoc-statine, EDC, HOBt; (k) piperidine, 51% for steps (j)–(k); (l) N-Ac-Leu, EDC, HOBt; (m) LIOH, 37% for steps (l)–(m).

The representative synthesis of various inhibitors containing the bis-statine core is outlined in Scheme 1. Boc-protected valine was coupled to 4-aminomethyl-benzoic acid methyl ester (**4**) using *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the coupling agent and *N*-methyl-morpholine as the base. The Boc-group was removed by treatment with trifluoroacetic acid (TFA) in CH_2Cl_2 and the resulting free amine **5** was allowed to couple subsequently with N-Boc-phenyl statine (4(*S*)-amino-3(*S*)-hydroxy-5-phenyl pentanoic acid) and N-Boc-statine using the same coupling/deprotection sequence to give the amine **7**. However, the yield for the statine coupling/deprotection sequence was only 22% and the amine **7** was isolated with unidentified impurities. This low yield was resulted from the incomplete conversion of **6** and acidic instability of the amine **7**. Modifications were therefore made to use the Fmoc-protected statine instead of Boc-protected statine and 1-hydroxybenzotriazole (HOBt) in additional EDC. The Fmoc-group was then removed by treatment with piperidine to give the pure amine **7** in 55% yield for the coupling/deprotection steps. The N-Ac-terminated leucine inhibitor **9** was successfully prepared by the improved peptide coupling conditions (EDC/HOBt) followed by basic hydrolysis. Other inhibitors (such as 4,5-dehydro-leucine derivative **10**) in Tables 1 and 2 were prepared by analogous sequence as described above.

The structure[7] of various bis-statine based peptides and their inhibitory potencies against BACE 1 FRET (fluorescence resonance energy transfer) assay[8] are shown in Tables 1 and 2. As illustrated, our initial bis-statine inhibitor **3** was conceptually subdivided into three regions for discussion purposes: an N-terminus, a central core (statine #1, statine #2), and a C-terminus. These regions were individually modified to probe the optimized activity. Based on a SAR study of a previously described mono-statine based BACE 1 inhibitors,[2,3] it was anticipated that the introduction of 4-aminomethylbenzoic acid at the C-terminus would be optimal for BACE 1 inhibition. To our delight, an aminomethylbenzoic acid bis-statine derivative **9** indeed resulted in 24-fold increase in potency compared to **3**. It has an IC_{50} value of 21 nM. Replacement of the isopropyl substituent with isobutene (4,5-dehydroleucine derivative **10**) resulted in a minor increase in BACE 1 inhibition (IC_{50} value of 16 nM for **10** vs 21 nM for **9**). A preliminary study to investigate the amino methyl benzoic acid replacement by peptide spot synthesis had been shown to yield a significant loss of affinity.[4] On the basis of the results of peptide spot synthesis study and the reported mono-statine SARs,[3] we did not manipulate the C-terminus further. Hence, our efforts were directed toward the modification of the center core (P_1/P_2 side chains). As shown in Table 1, a large substituent on P_1 position is preferred for the inhibitory activity.

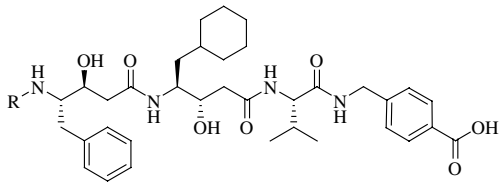
Table 1. SARs on P_1 and P_2 modifications



Compound	P_1	P_2	IC_{50} (μM)[a]
9	Benzyl	2-Methylbutyl	0.021
11	2-Methylbutyl	Benzyl	0.65
12	Methylsulfanyl ethyl	2-Methylbutyl	0.46
13	*c*-Hexyl methyl	Benzyl	0.05
14	Methylsulfinyl ethyl	2-Methylbutyl	34.0

[a] Concentration necessary to inhibit 50% of enzyme activity in BACE 1 FRET assay.

Table 2. SARs on N-terminus modifications



Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)[a]
13	N-Ac-Leu	0.05	**18**	N-Ac-Glu	42
15	N-Ac-Val	0.67	**19**	Pro	1.3
16	N-Ac-Gly	9.0	**20**	H	>100
17	N-Ac-Phe	5.6	**21**	Boc	>100

[a] Concentration necessary to inhibit 50% of enzyme activity in BACE 1 FRET assay.

Replacement of the large benzyl group of phenyl statine at P_1 with a smaller group, such as a 2-methylbutyl group of statine or a methylsulfanyl group of methionine statine (4(*S*)-amino-3(*S*)-hydroxy-6-methylsulfanyl-hexanoic acid), resulted in more than 20-fold decrease in potency (**9** vs **11** and **12**). The potency lost by a small P_1 site of **11** can be recovered back to the level (**13** vs **11**) seen with **9** by a larger size P_1 site of cyclohexyl statine (4(*S*)-amino-5-cyclohexyl-3(*S*)-hydroxy-pentanoic acid). However, the size of substituent on P_2 seems to be not as crucial as P_1 for the activity (**9**, **13** vs **11**, **12**). Interestingly, oxidation of the methyl sulfide of **12** resulted in a significant loss (74-fold) of potency (**12** vs **14**).

In the course of our study on the N-terminus (Table 2), we observed that the N-Ac leucine is the optimal residue in the S3 pocket. A smaller residue, such as N-Ac valine (**15**) or N-Ac glycine (**16**), significantly reduced the potency by 13.5-fold and 180-fold, respectively. On the other hand, a larger residue, such as N-Ac phenylalanine derivative **17**, also resulted in loss more than 100-fold of potency. Furthermore, a polar carboxylic acid analog **18** or a conformationally constrained proline analog **19** was also much less active. Truncation of the N-terminus portion of inhibitor was tried to determine the minimal size requirement for the peptide inhibitor. However, removal of the N-Ac leucine from **9** resulted in complete loss of potency (**20**, $IC_{50} > 100\,\mu M$). Surprisingly, N-terminus Boc protected statine analogs,[3] which have been demonstrated to be potent BACE 1 inhibitors, resulted in an essentially inactive inhibitor **21** in our series. This suggests that peptapeptidic structures are required for decent potency.

Based on the SAR from these peptidic inhibitors and the in-house crystallographic results,[4] a model for inhibitor **9** bound in the active site was constructed using the in-house X-ray structure of BACE 1 in complex with **3**. The program QXP/FLO[9] was used in constructing initial model for inhibitor **9** and then further minimized using MMFF94 force field[10] implemented in SYBYL.[11] The details regarding the interactions with the specific pocket is illustrated in Figure 1. As postulated earlier, the secondary alcohol of the phenyl statine moiety formed hydrogen bonds with the two catalytic aspartic acids (D32 and D228). The S1 is large hydrophobic pocket and has very nice contact with the groups like

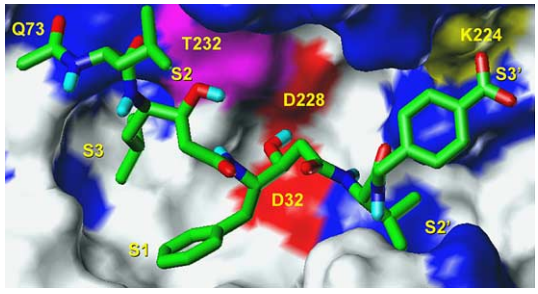


Figure 1. The modeled BACE 1 structure in complex with inhibitor **9**.

benzyl or cyclohexyl methyl, while the S2 is the mixed hydrophobic and polar pocket that seemed to accommodate both large and small residues. The N-Ac leucine on the N-terminus had close contact with S3 pocket and formed three hydrogen bonds with the BACE 1 site including Q73 according to the hypothetical model. The importance of the interaction could be reflected in the substantial loss of the activity by truncation of the N-terminus portion discussed earlier. The C-terminal portion of the inhibitor **9** showed a favorable hydrogen bond interaction between the benzoic acid and lysine K224.

In summary, a new series of peptide-based BACE 1 inhibitors have been developed, characterized by the presence of a bis-statine central core. After independent modifications on the C-terminal, N-terminal, and the central core of the initial lead, the spacer requirements for the preferred substrate–enzyme interaction have been generally defined. This information can serve as a convenient starting point from which small molecular-based BACE 1 inhibitors can be developed.

Acknowledgements

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8. BACE 1 inhibition was measured using recombinant enzyme produced from mammalian expression as described similarly in Ref. 2a. Fluorogenic substrate Abz-SEVNL-DAEFR-DPa was used with 10 nM human BACE 1 in 0.05 M Na-acetate + 0.05% CHAPS and 25% PBS, pH 4.5 at room temperature. The excitation wavelength was 354 nm, and the emission wavelength was 280 nm. Details of this assay will be described shortly.
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