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Bioorganic & Medicinal Chemistry Letters 13 (2003) 4335–4339

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Statine-Containing BACE Inhibitors

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Received 29 August 2003; revised 9 September 2003; accepted 17 September 2003

Abstract—Utilizing structure-based techniques and solid-phase synthesis, statine-based tetrapeptide BACE inhibitors were designed and synthesized using a heptapeptide BACE transition-state mimetic, **1**, as the starting point. Structure–activity relationship studies at the P_3, P_2, and P_2' positions as well as the N-terminal capping group on scaffold **5** led to the discovery of potent inhibitors **27**, **32**, and **34** (IC_{50} <100 nM). In addition, computational analysis and the X-ray structure of BACE–inhibitor **38** are discussed.

Introduction

Alzheimer's disease (AD), a debilitating disease among aging people, has been extensively studied over the past decade. While the cause of AD is still unknown, increasing evidence implicates the amyloid β-peptide (Aβ, 39–43 residues) in the neurodegenerative pathogenesis.[1–3] Aβ is produced in vivo through proteolytic cleavage of the membrane-bound β-amyloid precursor protein (APP) by β- and γ-secretases sequentially. The polymerization and subsequent aggregation of Aβ cause plaque formation and eventually lead to the loss of neuronal function in AD patients. The amyloid hypothesis suggests that inhibition of secretases responsible for Aβ formation may stop or slow AD progression by preventing its production. This theory prompted extensive structural and functional investigation directed at β- and γ-secretases, and their inhibitors.[4–8] Several groups have recently identified human brain β-APP cleaving enzyme (BACE) as the aspartic protease, β-secretase.[9–13] BACE gene knockout studies provided additional in vivo evidence that BACE is the sole β-secretase in neurons.[14–16] Furthermore, Tang's group disclosed an X-ray crystal structure of an eight-residue transition-state mimic inhibitor, **1** (Fig. 1, OM 99-2, $K_i = 1.6$ nM), bound to BACE.[17] This provided a powerful tool to facilitate structure-based drug design and development of BACE inhibitors as potential therapeutics for AD. Initial BACE–inhibitor studies focused on probing subsite specificity using fairly large peptides.[18,19] To search for small molecule BACE inhibitors, we decided to define a minimal subsite requirement before optimizing the enzymatic activity.

Rational Design

Statine (**2**) has been used as a transition-state dipeptide isostere to develop BACE,[8,19] renin,[20,21] cathepsin D,[22,23] and other aspartic protease inhibitors.[24–26] We here used it as a core to explore BACE structure–activity relationships (SAR). Replacement of the Leu-Ala hydroxyethylene isostere **3** in OM 99-2 with the readily available statine (**2**) afforded scaffold **4** (Fig. 1). In order to quickly probe the SAR of the statine platform, an amino acid library based on the heptapeptide scaffold **4** with various P_2 and P_2' was designed and evaluated.[27] The SAR generated suggested that hydrophobic groups such as the side chains of Ala, Met, Leu, Ile, and Val were tolerated at P_2 and P_2' (inhibitors **6**–**9**, Table 1). To determine the minimal size required for potent BACE inhibition, a systematic truncation of terminal amino acid residues on **10** was subsequently performed. On the N-terminal side, removal of P_4 and P_3 amino acids sequentially resulted in significant loss of activity in each step (**10**→**11**→**12**). On the C-terminal side, removal of P_3'–P_5' amino acids yielded inhibitor **15** with a moderate IC_{50} value (9.4 μM). Further truncation of P_2'-Val resulted in complete loss of activity (compound

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doi:10.1016/j.bmcl.2003.09.037

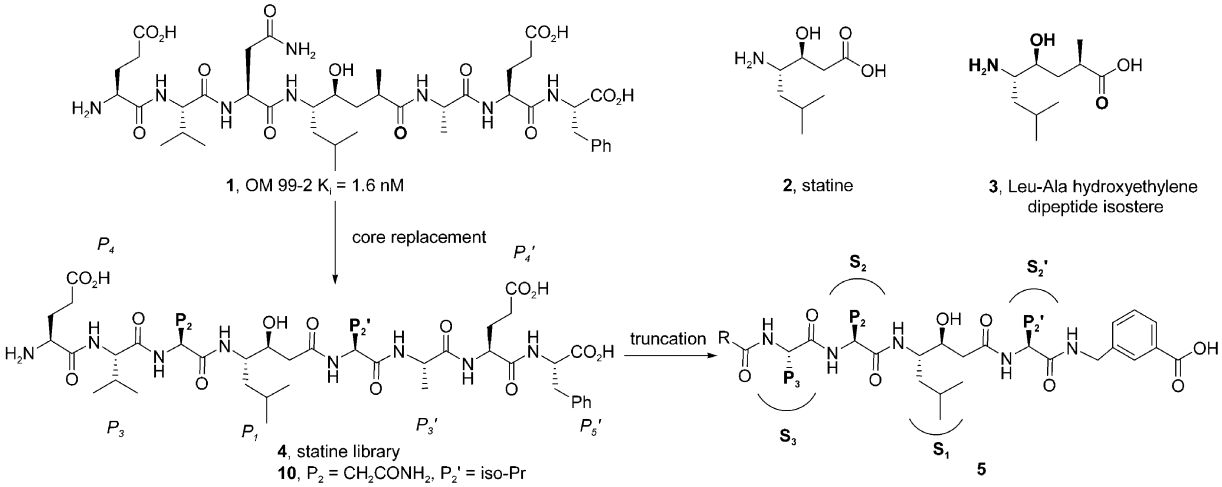


Figure 1. Structure of OM 99-2 and statine.

Table 1. Minimal sequence study of statine analogues

Compd	Sequence P_4-P_3-P_2-statine-P_2'-P_3'-P_4'-P_5'	BACE IC_{50} (μM)
6	Glu-Val-Ala-statine-Val-Ala-Glu-Phe	0.11
7	Glu-Val-Met-statine-Val-Ala-Glu-Phe	0.058
8	Glu-Val-Met-statine-Leu-Ala-Glu-Phe	0.14
9	Glu-Val-Met-statine-Ile-Ala-Glu-Phe	0.22
10	Glu-Val-Asn-statine-Val-Ala-Glu-Phe	0.046
11	Val-Asn-statine-Val-Ala-Glu-Phe	2
12	Asn-statine-Val-Ala-Glu-Phe	>91
13	Glu-Val-Asn-statine-Val-Ala-Glu	0.11
14	Glu-Val-Asn-statine-Val-Ala	0.53
15	Glu-Val-Asn-statine-Val	9.4
16	Glu-Val-Asn-statine	>91

16, IC_{50} > 91 μM). These data suggested that a tetrapeptide mimetic (scaffold **5**) occupying the S_3 to S_2' pockets are essential for moderate binding activity. Molecular docking experiments carried out on **15** and various C-terminal amine analogues of **15** to the published X-ray structure, suggested that a 3-carboxy benzylamine cap could potentially form a salt bridge to Arg 128 or Lys 224.[28] We therefore decided to probe the P_3, P_2, and P_2' positions on the truncated scaffold **5**. A variety of hydrophobic groups derived from the statine library are evaluated. In addition, stereochemistry preferences are discussed.

Chemistry

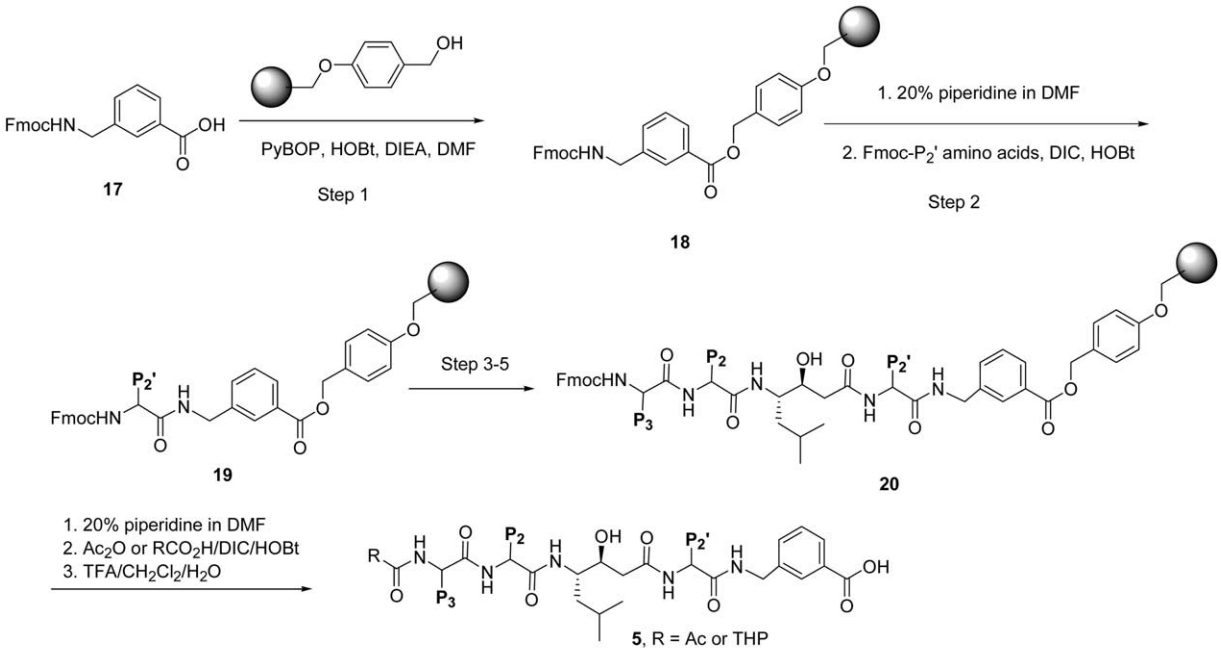
All compounds were prepared using resin-bound peptide synthesis as shown in Scheme 1. The commercially available Fmoc-(3-aminomethyl)-benzoic acid (**17**) was loaded on Wang resin using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) as a coupling reagent to afford resin **18**. Deprotection of Fmoc (20% piperidine in DMF) followed by coupling with an Fmoc-P_2' amino acid by *N,N'*-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) yielded intermediate **19**. Steps 3, 4, and 5 repeated the same sequence as in step 2 with Fmoc-statine, Fmoc-P_2 amino acid, and Fmoc-P_3 amino acid, respectively, in the deprotection/coupling peptide synthesis. The final products **5** were capped and then cleaved from the Wang resin with trifluoroacetic acid (TFA) in methylene chloride.

Results and Discussion

The initial SAR study was designed to explore the S_3 pocket. Seven P_3 modified analogues (Table 2, **21**–**27**) containing branched and linear aliphatic groups were prepared and evaluated in a BACE enzyme assay.[29] Compounds **21**–**25** exhibited potent to moderate inhibitory activity against BACE (IC_{50} = 0.25–3.8 μM). P_3-*n*-Propyl analogue **21** was slightly more potent than the P_3-*n*-butyl analogue **22**. Among P_3-branched inhibitors **23**–**25**, the most intriguing result was the >10-fold increase in potency between the P_3-*s*-butyl **25** and P_3-isopropyl **23** analogues. Replacing P_3-(*S*)-isobutyl group (**24**, IC_{50} = 0.52 μM) with the corresponding (*R*)-isomer resulted in loss of enzyme activity (**26**, IC_{50} > 91 μM). Further elaboration of the N-terminal capping group with tetrahydropyran (THP) resulted in a 3-fold increase in potency (**27**, IC_{50} = 0.069 μM). Overlays of the docked analogues are shown in Figure 2a.

Exploration of the P_2 position SAR focused on the effect of stereochemistry. Two pairs of diastereomers were synthesized and evaluated. Table 3 illustrates that replacing P_2-L-Met or P_2-L-Ala (**28** and **24**) with their corresponding D-isomers (**29**–**30**) resulted in complete loss of enzyme activity. Figure 2b shows the perturbation introduced in the two (*R*)-isomers (yellow and orange structures).

The P_2' SAR was explored using a constant P_3 moiety, namely the (*S*)-*s*-butyl group. Two series of inhibitors featuring two variations at the P_2 position, Met and Ala, were prepared (Table 4). Aliphatic residues at P_2' of both series (**31**–**32**, **35**–**36**) showed potent activity (IC_{50} = 0.086–0.26 μM). P_2' aryl moieties (Phe and Tyr) (**33**–**34** and **37**–**38**) exhibited comparable enzyme potency to their P_2'-aliphatic counterparts. The importance of stereochemistry was shown by the loss of



Scheme 1. Solid-phase synthesis of statine analogues.

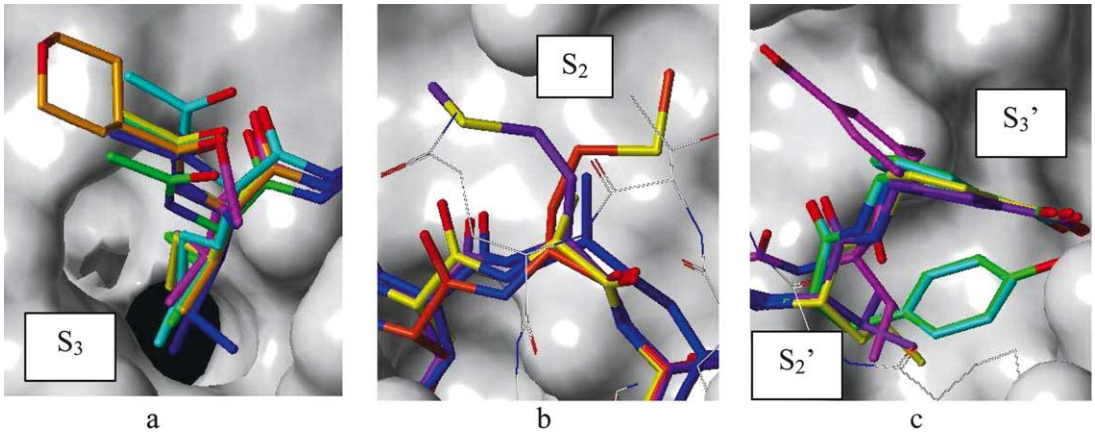
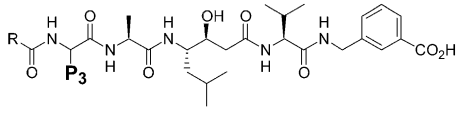


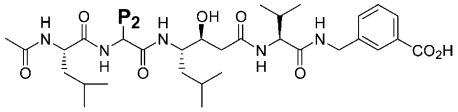
Figure 2. Molecular overlay of analogues docked to the BACE/OM99-2 structure: (a) P_3 SAR (Table 2), (b) P_2 SAR (Table 3), (c) P_2' SAR (Table 4).

Table 2. Observed P_3 SAR trends



Compd	R	P_3	IC_{50} (μM)
21	Me	(*S*)-*n*-Pr	0.83
22	Me	(*S*)-*n*-Bu	1.4
23	Me	(*S*)-*iso*-Pr	3.8
24	Me	(*S*)-*iso*-Bu	0.52
25	Me	(*S*)-*sec*-Bu	0.25
26	Me	(*R*)-*iso*-Bu	>91
27	THP	(*S*)-*sec*-Bu	0.069

Table 3. Evaluation of preferred stereochemistry at P_2



Compd	P_2	IC_{50} (μM)
28	(*S*)-CH_2CH_2SMe	0.89
24	(*S*)-Me	0.52
29	(*R*)-CH_2CH_2SMe	>91
30	(*R*)-Me	>91

activity seen in the replacement of the P_2'-(*S*)-isopropyl group (**28**, $IC_{50} = 0.89$ μM) with the corresponding (*R*)-isomer (**39**, $IC_{50} > 91$ μM). Docking suggests that the (*R*)-isomer (purple structure in Fig. 2c) not only perturbs the backbone from the X-ray conformer, but also forces the terminal benzoic acid group away from a potential interaction with basic side chains in the S_3' pocket.

Based on potency and structural diversity, compounds **24**, **27**, **28**, and **38** were chosen for BACE–inhibitor X-ray

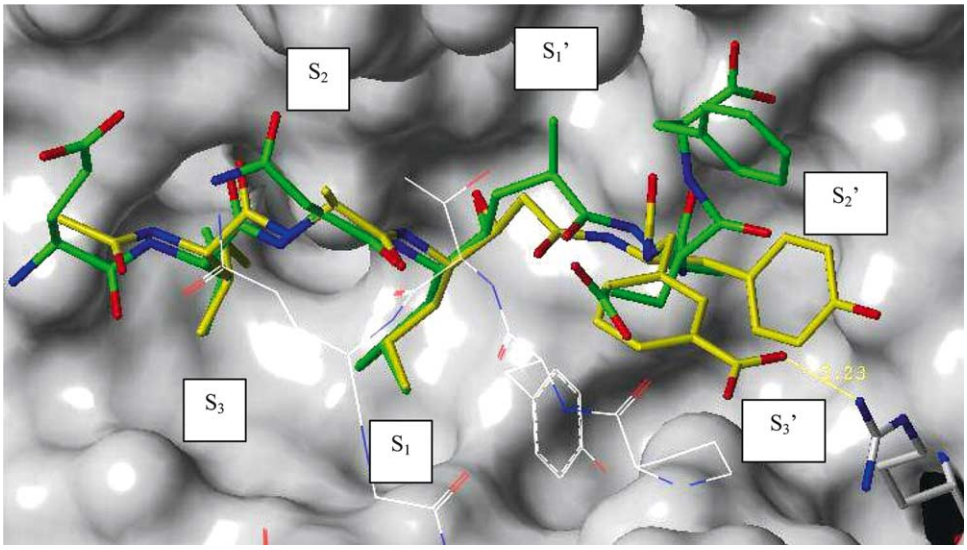
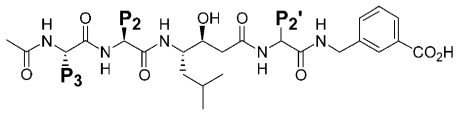


Figure 3. The X-ray crystal structure of BACE–inhibitor **38** complex. **38** (yellow) is shown overlaid on OM 99-2 (green). A Connolly surface of the BACE active site is shown but is omitted for the flap residues 70–75 for clarity.

Table 4. Observed P_2' SAR trends



Compd	P_3	P_2	P_2'	IC_{50} (μM)
31	(*S*)-*sec*-Bu	(*S*)-CH_2CH_2SMe	(*S*)-*n*-Pr	0.13
32	(*S*)-*sec*-Bu	(*S*)-CH_2CH_2SMe	(*S*)-*n*-Bu	0.086
33	(*S*)-*sec*-Bu	(*S*)-CH_2CH_2SMe	(*S*)-CH_2Ph	0.14
34	(*S*)-*sec*-Bu	(*S*)-CH_2CH_2SMe	(*S*)-$CH_2C_6H_5$-4-OH	0.091
35	(*S*)-*sec*-Bu	(*S*)-Me	(*S*)-*n*-Bu	0.18
36	(*S*)-*sec*-Bu	(*S*)-Me	(*S*)-*iso*-Bu	0.26
37	(*S*)-*sec*-Bu	(*S*)-Me	(*S*)-CH_2Ph	0.25
38	(*S*)-*sec*-Bu	(*S*)-Me	(*S*)-$CH_2C_6H_5$-4-OH	0.11
28	(*S*)-*iso*-Bu	(*S*)-CH_2CH_2SMe	(*S*)-*iso*-Pr	0.89
39	(*S*)-*iso*-Bu	(*S*)-CH_2CH_2SMe	(*R*)-*iso*-Pr	>91

crystallographic studies.[30] The crystal structure of the inhibitor **38**–BACE complex was shown in Figure 3. As expected, P_3-Ile and P_2-Ala were well positioned in the S_3 and S_2 pockets. The statine core occupied the S_1 pocket with the P_1-isobutyl group but left the S_1' subsite vacant. This suggests that increased potency in Leu-Ala dipeptide isostere analogue **1** ($K_i = 1.6$ nM) is partially due to the additional binding of the P_1'-Me in the S_1' pocket. The interesting geometry of P_2'-Tyr and the C-terminal benzoic acid implies that a modified ring can fit in the S_2' and S_3' pockets very well. This observation led to a further C-terminal truncation effort, which will be reported in due course.

Conclusion

Starting from a heptapeptide mimetic compound **1** and utilizing the results from truncation studies of analogue **10**, a series of statine-based BACE inhibitors, based on scaffold **5**, was designed and evaluated. Systematic modification of P_3, P_2, and P_2' led to the synthesis of three inhibitors with IC_{50}s less than 100 nM (**27**, **32**, **34**). It was shown that the (*S*)-configuration is preferred at the P_3, P_2, and P_2' positions on scaffold **5**. Finally, the X-ray cocrystal structure of BACE–inhibitor **38** provides a well defined template for further structure-based design in the improvement of BACE inhibitors.

Acknowledgements

We would like to thank Richard A. Brier and Debra K. Laigle for performing BACE assay and Amechand Boodhoo for the purification of BACE.

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29. BACE FRET assay uses 20 nM purified recombinant human BACE/Fc. The standard assay format contains 100 mM ELGY-9 (an aminobenzoate based FRET peptide containing Swedish mutation) in 50 mM ammonium acetate, pH 4.6, 1 mg/mL BSA and 1 mM Triton X-100 at room temperature for 4 h. The enzyme activity is determined by an increase in the relative fluorescence of reaction mixture, with umbilliferone excitation/emission filter set.
30. Crystals of the BACE catalytic domain were grown in the presence of a 5-fold molar excess of inhibitor **38** using minor modifications of previously described methods.[17] Diffraction data to 1.8 Å resolution were collected at 100 K using synchrotron beamline 17BM at Argonne National Laboratory and a MAR 165 mm CCD detector. The structure has been refined to a crystallographic *R*-factor of 0.219 ($R_{free}=0.239$) using CNX (Accelyrs, Inc.).