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Design and synthesis of BACE1 inhibitors containing a novel norstatine derivative (2*R*,3*R*)-3-amino-2-hydroxy-4-(phenylthio)butyric acid

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Abstract—A novel norstatine derivative, phenylthionorstatine [(2*R*,3*R*)-3-amino-2-hydroxy-4-(phenylthio)butyric acid; Ptns], containing a hydroxymethylcarbonyl (HMC) isostere was designed, synthesized, and stereochemically determined. Then, Ptns was introduced into the structure of BACE1 inhibitors at the P_1 position. Finally, Ptns was found as a suitable P_1 moiety for potent BACE1 inhibitor design.

Alzheimer's disease (AD) is the most common neurodegenerative disease, and the accumulation of amyloid β peptide (Aβ) is a major factor in the pathogenesis of Alzheimer's disease.[1] Aβ is formed by proteolytic processing of amyloid precursor protein (APP). Two enzymes, β-secretase (β-site APP cleaving enzyme, BACE1) and γ-secretase, are responsible for the sequential processing of APP.[2] Since the cleavage of APP by β-secretase is the first step in Aβ formation, BACE1 plays a critical role in the progression of AD. Therefore, the development of BACE1 inhibitors is valuable in the elucidation of AD pathology. BACE1 was identified as a novel membrane-bound aspartic protease and the crystal structure of its catalytical domain was also determined. Based on the common enzymatic mechanism of aspartic proteases, substrate transition-state mimics have been proposed and are currently widely used for the design of highly potent aspartic protease inhibitors.[3]

In our previous study, we applied a hydroxymethylcarbonyl isostere (HMC) at P_1 position of potent inhibitors toward several human disease-related aspartic proteases such as renin, HIV-1protease,[4] plasmepsin II,[5] HTLV-I[6] protease, and BACE1.[7] We also described the importance of the stereochemistry of the transition-state mimetic hydroxyl group for the inhibitory activity.[4b,c,7]

Through the study of the stereochemical preference of the P_1 position, introducing Pns [phenylnorstatine: (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] or its (2*S*,3*S*)-diastereomer, Apns, as a transition-state mimic, we found that (2*R*)-hydroxyl group of HMC, in Pns, was better for efficient inhibitory activity against BACE1.[7]

In order to develop more active compounds, we have focused on the P_1 phenylalanine derivative, since inhibitors containing Pns exhibited higher BACE1 inhibitory activity than those with norstatine [(2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid; Nst], a leucine mimetic from the sequence of Swedish mutant APP ($P_1 - P_1'$: L*D). The phenyl group of Pns enhanced the interaction between inhibitor and protease at the S_1 site.[7] In the sequence of the wild type APP, β-secretase recognizes Met at S_1, what could be a starting point for another mimetic design ($P_1 - P_1'$: M*D). Noteworthy is the SAR study of HIV-1 protease, plasmepsin II, and cathepsin D inhibitors containing HMC, demonstrating the important role of the lipophilic P_1 aromatic ring system, which fits into the S_1 hydrophobic pocket.[8] In Nelfinavir (nelfinavir mesylate, nonpeptidic inhibitor of HIV-1 protease) thiophenyl moiety was introduced at P_1 site and showed

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10-fold greater affinity than a phenyl analogue.[9] These all observations stimulated us to modify the side chain of Pns.

Thus, we designed phenylthionorstatine (Ptns) [(2*R*,3*R*)-3-amino-2-hydroxy-4-(phenylthio)butyric acid] and its (2*S*,3*R*)-diastereomer, and then we have focused our design on Ptns containing peptide inhibitors (**2**, Fig. 1).

We have synthesized Ptns starting from readily available *N*-benzyloxycarbonyl-L-serine (**3**, Scheme 1), and after protecting carboxylic group by Weinreb amide, **4** was transformed into its mesylate and then treated at 0 °C with sodium thiophenolate prepared in situ in DMF to give the phenylsulfide (**5**).[10] Reduction of **5** with $LiAlH_4$ resulted in aldehyde **6**.[11] The key intermediate for the Ptns preparation is the cyanohydrin derivative **7** produced in the next step. Several methods were reported for the synthesis of hydroxymethylcarbonyl units, involving aqueous hydrolysis of cyanohydrin, obtained from protected aldehyde by treatment with potassium cyanide,[12] or a one-pot procedure consisting of reaction of protected aldehyde with (trimethylsilyl)cyanide.[13] In our case, we used acetone cyanohydrin and trimethylaluminum as a reaction accelerator in chloroform at 0 °C.[14] Compound **7** was directly transformed into the methyl esters **8** and **9** by treatment with dry methanolic hydrogen chloride, followed by *in situ* hydrolysis of the intermediate imidate hydrochloride.[13]

To determine the stereochemistry of esters **8** and **9**, first, these esters were separated by flash chromatography and then were converted to the corresponding oxazolidinones (**10** and **11**, respectively), by treating the esters with 6 N NaOH in DMF[13] (Scheme 2). The C-2 configuration of these compounds was unequivocally determined by the ^1H NMR spectrum of the oxazolidinones. Thus, **8** gave **10** with an Ha, Hb *trans* disposition, as indicated by their *J* value of 3.9 Hz, while **9** gave **11** with $J_{Ha,Hb}$ = 8.7 Hz, a *cis* configuration.[15]

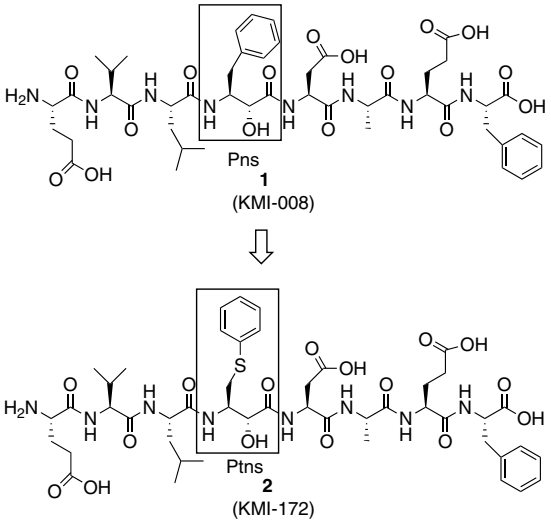
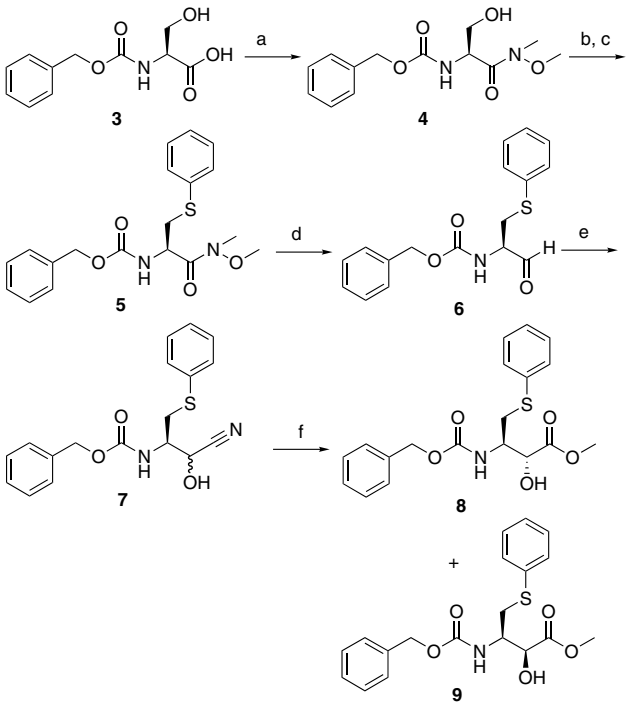
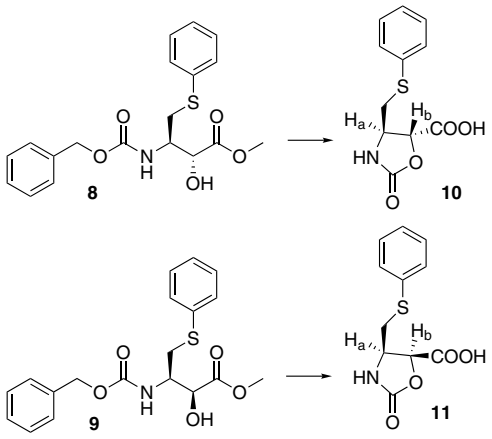


Figure 1. Structure of BACE1 inhibitors containing Pns (**1**, KMI-008) and Ptns (**2**, KMI-172).



Scheme 1. Reagents and conditions: (a) MeNH(OMe)·HCl, EDC·HCl, NMM, CH_2Cl_2, −10 °C, 2 h, 100%; (b) MsCl, Et_3N, $CHCl_3$, 0 °C, 0.5 h; (c) PhSNa, DMF, 0 °C to rt, 24 h, over 2 steps 95%; (d) $LiAlH_4$, THF, 0 °C, 0.5 h; (e) $Me_2C(OH)CN$, Me_3Al, $CHCl_3$, 0 °C to rt, 10 h, 75% over 2 steps; (f) 4 N HCl/dioxane, MeOH, 4 °C, 24 h, H_2O, 4 °C, 24 h, 52%, ratio **8**:**9** was 1.8:1; separation by flash chromatography (hexane:AcOEt, 2:1).



Scheme 2. Reagents and conditions: 6 N NaOH, DMF, rt, 2 h; ^1H NMR analysis: **10**, $J_{Ha,Hb}$ = 3.9 Hz; **11**, $J_{Ha,Hb}$ = 8.7 Hz.

Esters **8** (2*R*,3*R*) and **9** (2*S*,3*R*) were saponified (1 N NaOH in DMF) to provide corresponding acids. After the removal of benzyloxycarbonyl group by TFA with dimethylsulfide (40 equiv) and anisole (5 equiv),[16] Ptns and its diastereomer, Aptns [(2*S*,3*R*)-3-amino-2-hydroxy-4-(phenylthio)butyric acid; allophenylthionorstatine], were obtained. Aptns, as a (2*S*,3*R*)-diastereomer that is unfavorable for the design of BACE1 pentapeptidic inhibitors, could be used for the design of the other aspartyl protease inhibitors. While Ptns, the (2*R*,3*R*)-di-

astereomer, was finally N-protected by Fmoc masking fragment (Fmoc-OSu in THF/H_2O, standard method) and then applied to SPPS (solid phase peptide synthesis) for the BACE1 inhibitor synthesis.

Compounds **1**, **2**, and **12**–**17** (Figs. 2 and 3) were synthesized by the Fmoc based solid phase method, described previously.[7,17a] These compounds were adopted to enzymatic assay using a recombinant human BACE1. Inhibitory activity was determined based on the decrease% of the cleaved substrate by the enzyme[7] (Table 1).

Our attempts to replace Pns by its thio-derivative in octapeptide type structure led us to the analogue **2** (Table 1, compounds **1** and **2** at 2 μM concentration). Then, we reduced the size of inhibitors from octapeptides to

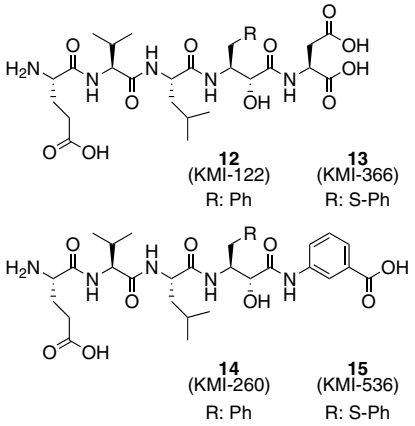


Figure 2. Modification of P_1' position. Structures of pentapeptides containing Pns (**12** and **14**) and Ptns (**13** and **15**).

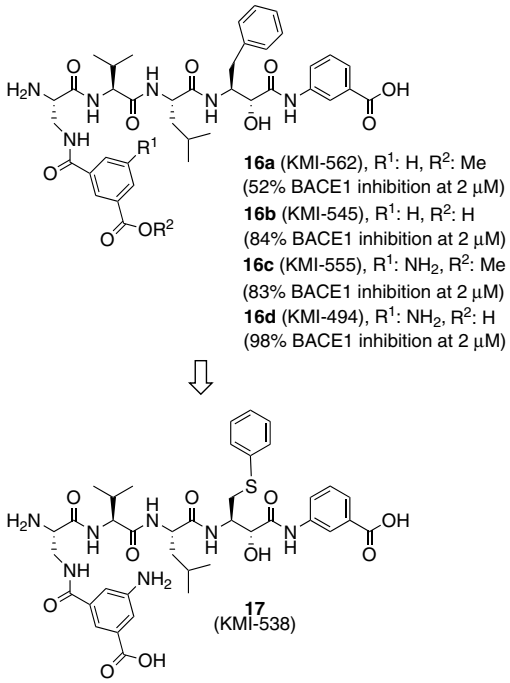


Figure 3. Modification of isophthalic moiety at P_4 position and introduction of Ptns.

Table 1. BACE1 inhibitory activity, compounds **1**–**2** and **12**–**17**

Compound (KMI no.)	HMC type	BACE1 inhibition (%)	
		At 2 μM	At 0.2 μM
1 (KMI-008)	Pns	90[a]	32
2 (KMI-172)	Ptns	82	34
12 (KMI-122)	Pns	61[b]	
13 (KMI-366)	Ptns	69	
14 (KMI-260)	Pns	82[b]	44
15 (KMI-536)	Ptns	96	57
16d (KMI-494)	Pns	98	80
17 (KMI-538)	Ptns	99	86

[a] Data from Ref. 7.
[b] Data from Ref. 16a.

pentapeptides. Compound **12** was a lead compound for pentapeptide series in our previous study.[17a] Its Ptns counterpart **13** possessed potency similar to Pns. After modification at P_1' position, where Asp was replaced by 3-aminobenzoic acid, compound **15** exhibited better inhibitory activity than previously reported inhibitor **14**[17a] (Fig. 2) (Table 1, compounds **12**–**15**).

Then we have focused on the N-terminal optimization of the peptides. We already reported many BACE1 inhibitors with various hydrogen bond acceptor groups at the P_4 position.[17a,d] Through the SAR study we identified also a few potent peptides bearing isophthalic moiety at P_4 (peptides **16a**–**d**, Fig. 3). In that short series of inhibitors with Pns at P_1, compounds containing additional amino group (3-aminoisophthalyl fragment) showed higher activity, and the acidic form (**16d**) was more potent than ester (**16c**). We decided to synthesize an inhibitor with Ptns replacing Pns, and with 3-aminoisophthalic moiety at N-terminal. The Ptns counterpart **17** (KMI-538) exhibited slightly higher activity than **16d** (Table 1.). As we demonstrate here, the modification of P_1 position by introducing Ptns improved the BACE1 inhibitory activity.

The idea to modify the P_1 site by elongation of side chain (thiophenyl fragment instead phenyl) was confirmed by computational simulations. Inhibitor **17** was docked in BACE1 by using a modeling package (MOE 2005.06, Chemical Group, Inc., Montreal, Canada). Additional energy minimization processes with a MMFF94x force field were performed.

Conformation of compound **17**, presented in Figure 4, resulted in almost the same pose as a OM99-2.[18] The exception is the P_1' position. The C-terminal of **17** nicely fixed the pocket, while in OM99-2 the P_1' residue did not reach the S_1' site. The important hydrogen bond interactions between the Ptns anchor and Asp32 and Asp228 are shown in Figure 4B. It is in excellent agreement with our previous observations for inhibitors with Pns.[7,17b,c] In this model, other hydrogen bond interactions with Gly 11, Arg 307, and Arg 235 were also observed, and those coincided with the previously reported modeling study of KMI-429,[17a] KMI-684,[17c] and KMI-574.[17d] The presence of additional amino group at P_4 (3-amino group from aminoisophthalic residue) resulted in new hydrogen bond interaction with Ser 325 (Fig. 4C), and

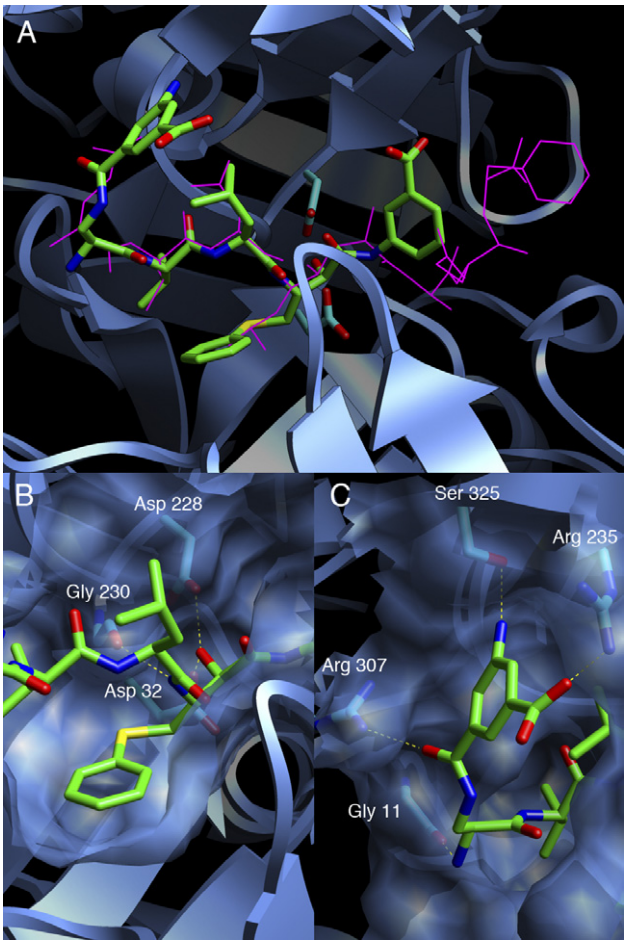


Figure 4. Modeled Structure of **17** (KMI-538, green sticks) in the active site of BACE1 (PDB entry, 1FKN). (A) **17** is superimposed with an inhibitor OM99-2 (magenta line). Catalytic two Asp residues are represented with light blue stick. Close-up views of P_1 position (B) and P_4 position (C) with possible hydrogen bonds (dotted lines) and molecular surface of the enzyme pocket (blue).

that is reasonable explanation of enhanced BACE1 inhibitory activity of **16d** and **17** comparing to **16a–c** (Table 1).

In conclusion, we present for the first time, phenylthionorstatine and its synthesis with full stereochemical determination. The Fmoc-Ptns was applied for the design and synthesis of BACE1 inhibitors. Inhibitor **17** (KMI-538) containing Ptns demonstrated potent inhibitory activity. These results show the possibility of further design of BACE1 inhibitors with Ptns at P_1 position. Our efforts are also directed toward the study of other aspartic protease, and the application of peptides containing Ptns, or its diastereomer Aptns, as inhibitors.

Acknowledgments

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15. Selected physical data: (a) compound **8**: yield = 33%; $[\alpha]_D^{25} = -54.2$ ($c = 0.054$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.44–7.19$ (m, 10H), 5,11 (d, $^3J = 10.3$ Hz, 1H, NH), 5.07 (s, 2H, CH_2), 4.60 (dd, $^3J = 4.0$ and 1.8 Hz, 1H, CH), 4.24–4.20 (m, 1H, CH), 3.73 (s, 3H, CH_3), 3.11 (d, $^3J = 4.0$ Hz, 1H, OH), 3.23, 3.07 (2dd, $^2J = 13.8$ Hz, $^3J = 9.1$ and 5.9 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 173.78, 155.64, 136.14, 135.03, 129.38, 129.06, 128.46, 128.11, 127.92, 126.44, 69.77, 66.89, 52.98, 52.73, 34.92$; HRMS (FAB): calcd for $C_{19}H_{22}O_5NS$ $[M+H]^+$ 376.1219, found 376.1223; purity was higher than 97% (HPLC analysis at 230 nm); (b) compound **9**: Yield = 18%; $[\alpha]_D^{25} = -11.9$ ($c = 0.043$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.37–7.19$ (m, 10H), 5.39 (d, $^3J = 9.2$ Hz, 1H,

NH), 5.09 (s, 2H, CH_2), 4.35 (br s, 1 H, CH), 4.26–4.22 (m, 1H, CH), 3.66 (s, 3H, OCH_3), 3.48–3.45 (br s, 1H, OH), 3.09, 3.04 (2dd, ^2J = 13.9 Hz, ^3J = 5.9 and 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.74, 155.91, 136.14, 135.14, 129.75, 128.99, 128.46, 128.12, 128.06, 126.58, 72.02, 66.96, 52.91, 52.71, 33.91; HRMS (FAB): calcd for $C_{19}H_{22}O_5NS$ $[M+H]^+$ 376.1219, found 376.1224; purity was higher than 95% (HPLC analysis at 230 nm); (c) compound **10**: ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46–7.23 (m, 5H), 4.85 (d, ^3J = 3.9 Hz, 1H), 3.97 (ddd, ^3J = 6.0, 6.0 and 3.9 Hz, 1H), 3.21, 3.12 (2dd, ^2J = 14.1 Hz, ^3J = 6.0 and 6.0 Hz, 2H, CH_2); LRMS (FAB): 254 $[M+H]^+$, 253 $[M^{+\cdot}]$; HRMS (FAB): calcd for $C_{11}H_{11}O_4NS$ $[M^{+\cdot}]$ 253.0409, found 253.0405; (d) compound **11**: ^{1}H NMR (300 MHz, CD_3OD): δ = 7.43–7.22 (m, 5H), 5.14 (d,^3J = 8.7 Hz, 1H), 4.21 (ddd, ^3J = 8.7, 8.7 and 4.2 Hz, 1H), 3.24, 2.90 (2dd, ^2J = 13.7 Hz, ^3J = 8.7 and 4.2 Hz, 2H, CH_2); LRMS (FAB): 254 $[M+H]^+$, 253 $[M^{+\cdot}]$; HRMS (FAB): calcd for $C_{11}H_{11}O_4NS$ $[M^{+\cdot}]$ 253.0409, found 253.0403.

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