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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

Zyta Ziora, Soko Kasai, Koushi Hidaka, Ayaka Nagamine, Tooru Kimura, Yoshio Hayashi and Yoshiaki Kiso*

Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, 21st Century COE Program, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan

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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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* Corresponding author. Tel.: +81 75 595 4635; fax: +81 75 591 9900; e-mail: kiso@mb.kyoto-phu.ac.jp

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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]

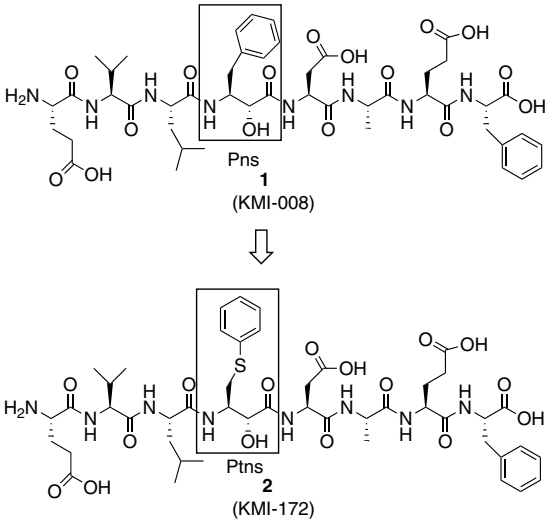
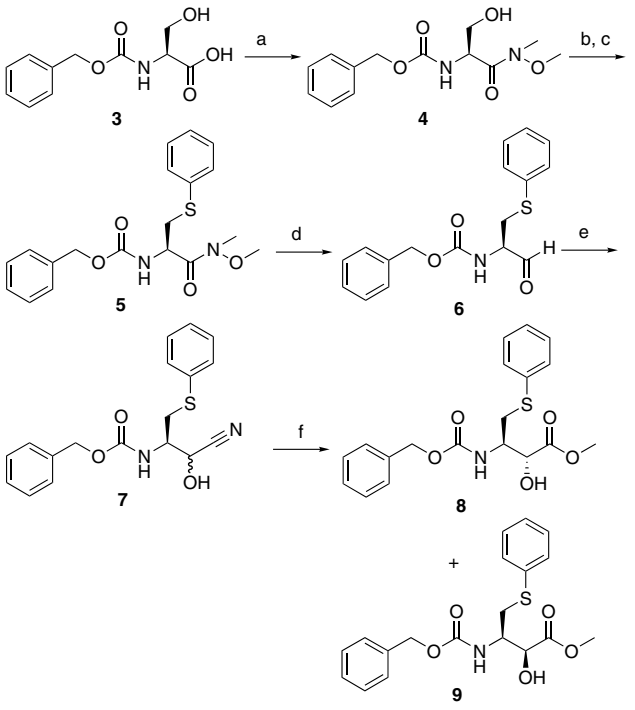
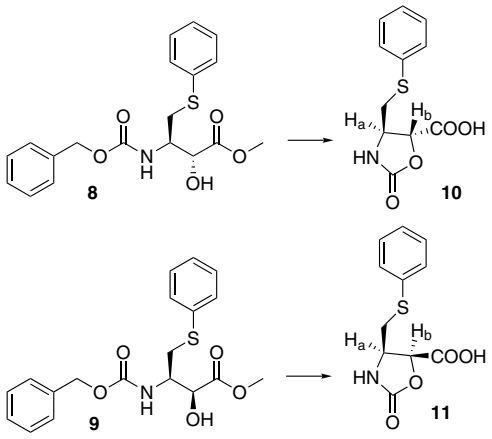


Figure 1. Structure of BACE1 inhibitors containing Pns (**1**, KMI-008) and Ptns (**2**, KMI-172).



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-