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Design and synthesis of highly active Alzheimer's β-secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability

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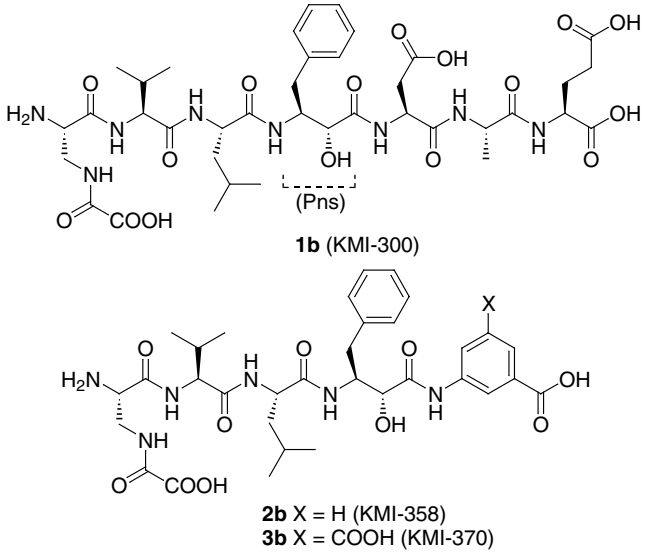
Abstract—Recently, we reported potent and small-sized BACE1 inhibitors KMI-358 and KMI-370 in which the Glu residue is replaced by a β-*N*-oxalyl-DAP (L-α,β-diaminopropionyl) residue at the P_4 position. The β-*N*-oxalyl-DAP group is important for enhancing BACE1 inhibitory activity, but these inhibitors isomerized to α-*N*-oxalyl-DAP derivatives in solvents. Hence, we used a tetrazole moiety as a bioisostere of the free carboxylic acid of the oxalyl group. KMI-420 and KMI-429, containing a tetrazole ring, showed improved stability and potent enzyme inhibitory activity.

1. Introduction

Amyloid β peptide (Aβ), which is the main component of senile plaques found in the brains of Alzheimer's disease (AD) patients,[1] is formed by proteolytic processing of amyloid precursor protein (APP).[2,3] Since BACE1 (β-site APP cleaving enzyme, β-secretase) triggers Aβ formation by cleaving at the *N*-terminus of the Aβ domain,[4–7] it is a molecular target for therapeutic intervention in AD.[8–11] Recently, we reported on the BACE1 inhibitors, KMI-300 (**1b**), -358 (**2b**), and -370 (**3b**)[12] (Fig. 1), which contained phenylnorstatine [Pns: (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] as a substrate transition-state mimic.[13,14] These inhibitors were designed from the octapeptide BACE1 inhibitor KMI-008[11] as the lead compound. However, inhibitors, **1b**–**3b**, have labile β-*N*-oxalyl-DAP residues (DAP: L-α,β-diaminopropionic acid) at the P_4 position. β-*N*-oxalyl-DAP is known as the neurotoxic constituent of the legume *Lathyrus sativus*,[15–17] which thermally isomerizes to an equilibrium mixture with α-*N*-oxalyl-DAP.[18,19] Similarly, the compounds **1b**–**3b** are converted to α-*N*-oxalyl-DAP derivatives (Fig. 2), which show the low enzyme inhibitory activities, in aqueous and organic



1b (KMI-300)

2b X = H (KMI-358)
3b X = COOH (KMI-370)

Figure 1. Structure of BACE1 inhibitors containing β-oxalyl-DAP residue at the P_4 position.

Keywords: Alzheimer's disease; BACE1 inhibitor; β-Secretase; Bioisostere.
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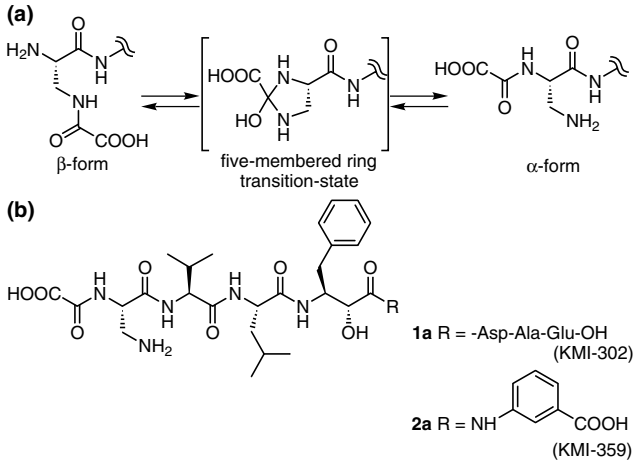


Figure 2. (a) Isomerization of oxalyl-DAP derivatives. (b) Structure of BACE1 inhibitor's isomers containing α-oxalyl-DAP residue at the P_4 position.

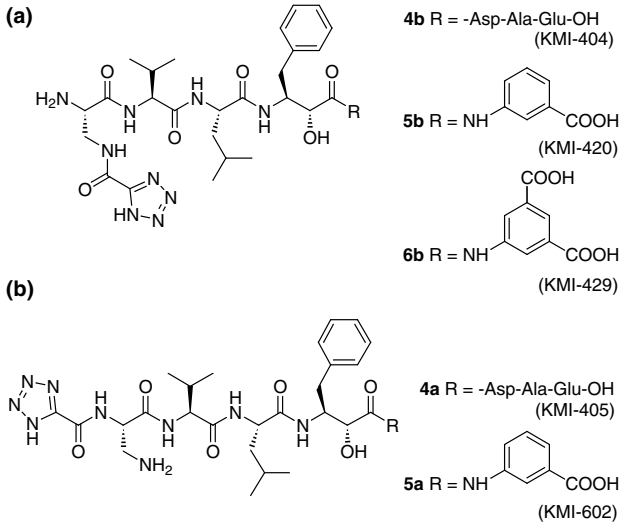
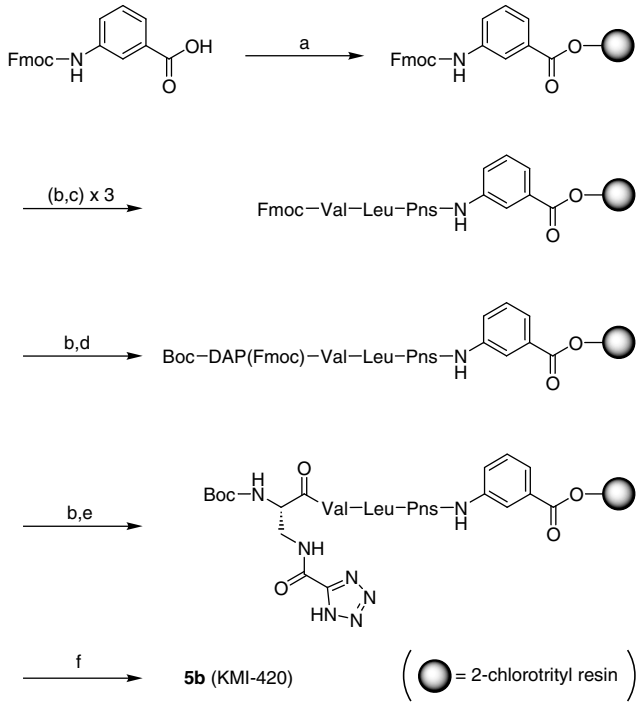


Figure 3. Structure of BACE1 inhibitors containing a tetrazole ring at the P_4 position (a) and their α-isomers (b).

solvents. To improve the stability of compounds **1b**–**3b**, the oxalyl moiety was replaced with tetrazole carbonyl derivatives as a bioisostere[20] of carboxylic acid. Consequently, we found the tetrazole-containing BACE1 inhibitors **4b**–**6b** (Fig. 3), with enhanced chemical stability and enzyme inhibitory activity.

2. Synthesis

BACE1 inhibitors (**4b** and **5b**) and α-isomers (**1a**, **2a**, **4a**, and **5a**) were synthesized by the 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthesis as previously reported.[12] As an example, Scheme 1 shows the synthesis of **5b** (KMI-420). Namely, the *N*-Fmoc-3-aminobenzoic acid was attached to 2-chlorotrityl chloride resin using diisopropylethylamine (DIPEA) in dichloromethane (DCM). The Fmoc group was removed with 20% piperidine in DMF and the peptide



Scheme 1. Reagents and conditions: (a) 2-chlorotrityl chloride resin, DIPEA/DCM; (b) 20% piperidine/DMF; (c) Fmoc-AA-OH, DIPCDI, HOBt/DMF; (d) Boc-DAP(Fmoc)-OH, DIPCDI, HOBt/DMF; (e) 1*H*-tetrazole-5-carboxylic acid, DIPCDI, HOBt/DMF; (f) TFA, *m*-cresol, thioanisole.

bonds were formed using diisopropylcarbodiimide (DIPCDI) in the presence of 1-hydroxybenzotriazole (HOBt). The coupling of Boc-Pns-OH and aminobenzoyl resin was achieved using the same manner reported previously[12] without any problem. The DAP residue at the P_4 position was introduced using N^{α}-Boc-N^{β}-Fmoc-L-2,3-diaminopropionic acid [Boc-DAP-(Fmoc)-OH]. The β-substituted DAP moiety in **4b** (KMI-404) was introduced in a manner similar to that in **5b**. However, for the α-substituted derivatives (**1a**, **2a**, **4a** and **5a**), the DAP residue at the P_4 position was introduced using N^{β}-Boc-N^{α}-Fmoc-L-2,3-diaminopropionic acid [Fmoc-DAP(Boc)-OH]. After peptide chain elongation, the 1*H*-tetrazole-5-carbonyl residue at the β-position of DAP was introduced using 1*H*-tetrazole-5-carboxylic acid. Finally, the peptide was cleaved from the resin by treatment with trifluoroacetic acid (TFA) in the presence of *m*-cresol and thioanisole. The crude peptide was purified by preparative RP-HPLC. On the other hand, the α-oxalyl residue at the P_4 position in compounds **1a** and **2a** was introduced using oxalic acid mono-*t*-butyl ester.

Compound **6b** (KMI-429), which contained 5-aminoisophthalic acid at the C-terminus, was synthesized by a traditional solution method (Scheme 2). Dibenzyl 5-aminoisophthalate was used as a starting compound and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC·HCl) in the presence of HOBt formed the peptide bonds. Boc and Fmoc groups were deprotected using 4 M HCl in dioxane and 20% diethylamine in DMF,