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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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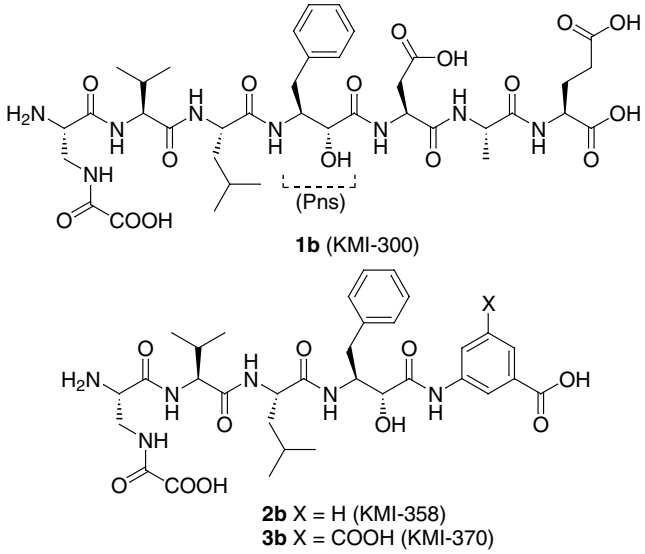
Abstract—Recently, we reported potent and small-sized BACE1 inhibitors KMI-358 and KMI-370 in which the Glu residue is replaced by a β-*N*-oxalyl-DAP (L-α,β-diaminopropionyl) residue at the P_4 position. The β-*N*-oxalyl-DAP group is important for enhancing BACE1 inhibitory activity, but these inhibitors 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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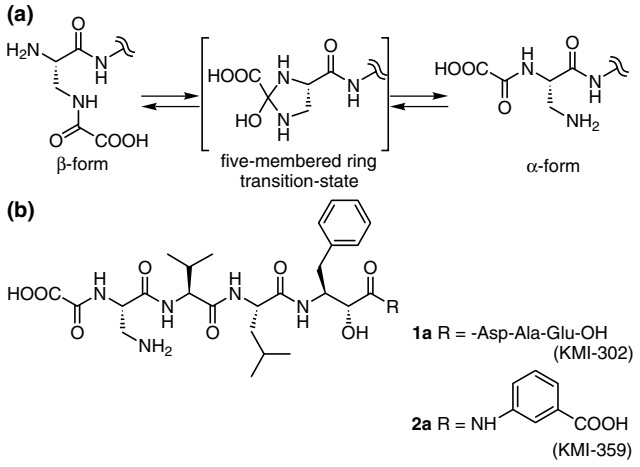


Figure 2. (a) Isomerization of oxalyl-DAP derivatives. (b) Structure of BACE1 inhibitor's isomers containing α-oxalyl-DAP residue at the P_4 position.

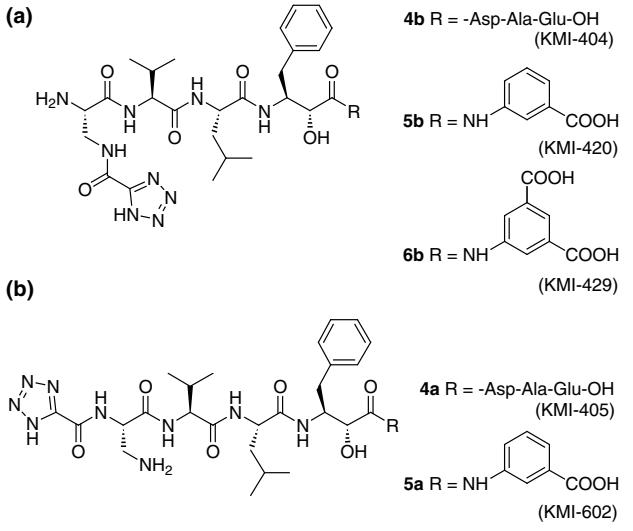
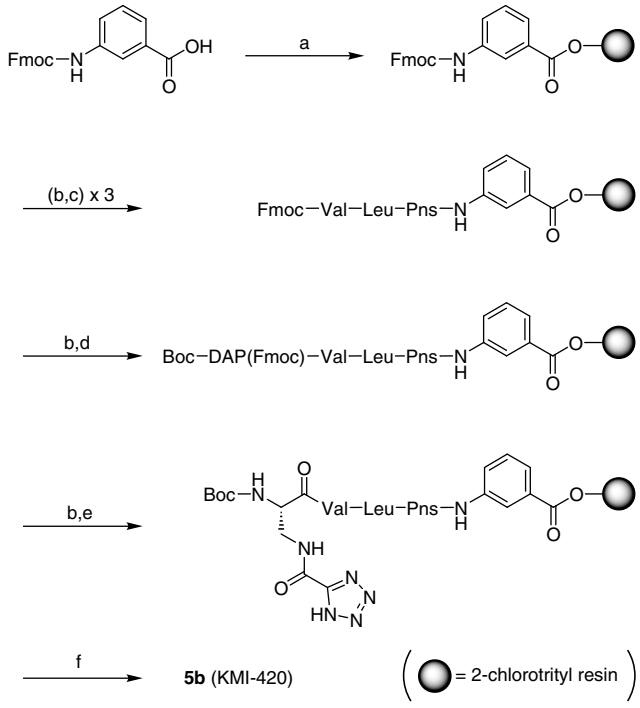


Figure 3. Structure of BACE1 inhibitors containing a tetrazole ring at the P_4 position (a) and their α-isomers (b).

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

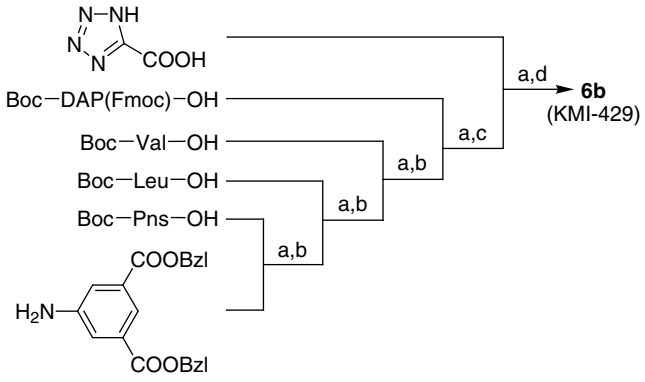
BACE1 inhibitors (**4b** and **5b**) and α-isomers (**1a**, **2a**, **4a**, and **5a**) were synthesized by the 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthesis as previously reported.[12] As an example, Scheme 1 shows the synthesis of **5b** (KMI-420). Namely, the *N*-Fmoc-3-aminobenzoic 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,



Scheme 2. Reagents and conditions: (a) EDC·HCl, HOBt/DMF; (b) 4M HCl/dioxane; (c) 20% Et_2NH/DMF; (d) TMS-Br, thioanisole, *m*-cresol/TFA.

respectively. The final deprotection of the Boc and benzyl groups using trimethylbromosilane (TMS-Br), thioanisole, and *m*-cresol in TFA and subsequent purification by preparative RP-HPLC gave the desired inhibitor **6b**.

3. Results and discussion

3.1. Stability of KMI-300 (1b) and KMI-358 (2b)

Since the small-sized BACE1 inhibitors, **1b**–**3b**, showed unstable features, we investigated the mechanism and kinetics for the unstability. It seems that by-products are formed from the isomerization via oxalyl migration. Thus we synthesized the α-oxalyl isomers **1a** and **2a**, corresponding to **1b** and **2b**, respectively, which contained a β-oxalyl DAP group. The HPLC analysis and mass spectra showed that compounds **1a** and **2a** are identical to the by-products of **1b** and **2b**, respectively. Next, we monitored the isomerization of **1b**, **2b**, **1a**, and **2a** in various solvents by HPLC. Compounds **1b**, **2b**, **1a**, and **2a** showed a time-dependent isomerization to an equilibrium mixture (Fig. 4). A slower migration is observed in an aqueous solvent, for example, PBS (phosphate-buffered saline, pH 7.4), than in organic solvents such as MeOH and DMSO. Though the β-isomers (**1b** and **2b**) and α-isomers (**1a** and **2a**), respectively, were isomerized to reach the same equilibrium ratio under the same conditions, the equilibrium ratio and migration rate are dependent on the solvents and the chemical structure of the compounds. Abegaz et al. indicated that the migration rate of oxalyl-DAPs is influenced by the rotamers in the solvents.[19] The difference between α-*N* to β-*N* and β-*N* to α-*N* oxalyl migration rates might determine the equilibrium ratio. Recently, we reported on the water-soluble prodrugs of HIV-1 protease inhibitors based on $O \rightarrow N$ intramolecular acyl migration[21–24] and the kinetic study in different solvents.[23,24] These prodrugs could be converted to the parent drugs via a 'five-membered ring intermediate' under physiological conditions. This mechanism via a five-membered ring intermediate, permitted two equilibrium constants, is consistent with the above findings.

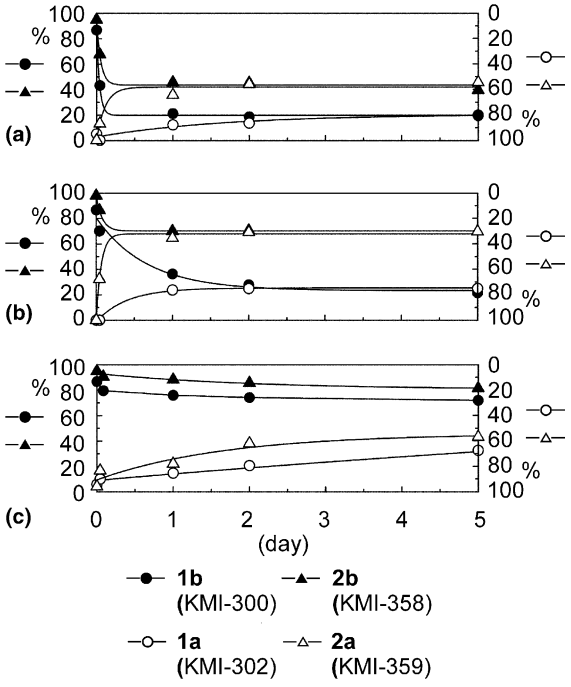


Figure 4. Stability of KMI compounds containing α- or β-oxalyl-DAP residue at the P_4 position in MeOH (a), DMSO (b), or pH 7.4 PBS (c). To unify the β-isomer's ratio to upward direction in the graph, the *y*-axis direction of α-isomers (**1a** and **2a**) was reversed.

3.2. Stability and BACE1 inhibitory activity of KMI-404 (4b), KMI-420 (5b), and KMI-429 (6b)

To improve the stability of BACE1 inhibitors **1b**–**3b**, we replaced the oxalyl group with the 1*H*-tetrazole-5-carbonyl group. The stability of the tetrazole-type BACE1 inhibitors **4b** (KMI-404), **5b** (KMI-420), and **6b** (KMI-429), which correspond to **1b**–**3b**, respectively, was verified by HPLC. **4b**–**6b** showed no isomerization in PBS (pH 7.4), MeOH and DMSO over a period up to 5 days.

As shown in Table 1, the BACE1 inhibitory activities of β-*N*-tetrazole-5-carbonyl-type inhibitors **4b**–**6b** were enhanced over β-*N*-oxalyl-type inhibitors **1b**–**3b**, respectively. The α-isomers **1a**, **2a**, **4a**, and **5a** showed weaker BACE1 inhibitory activities than the corresponding

Table 1. BACE1 inhibitory activity

Compd (KMI No.)	BACE1 inhibition (%)		IC_{50} (nM)
	At 2 μM	At 0.2 μM	
1b (KMI-300)	96.7	57.6	84
2b (KMI-358)	98.4	85.5	16
3b (KMI-370)	99.8	96.7	4.7
1a (KMI-302)	75.2	—	—
2a (KMI-359)	87.9	58.6	—
4b (KMI-404)	93.0	73.1	—
5b (KMI-420)	99.0	87.1	8.2
6b (KMI-429)	100	98.1	3.9
4a (KMI-405)	23.2	—	—
5a (KMI-602)	~0	—	—

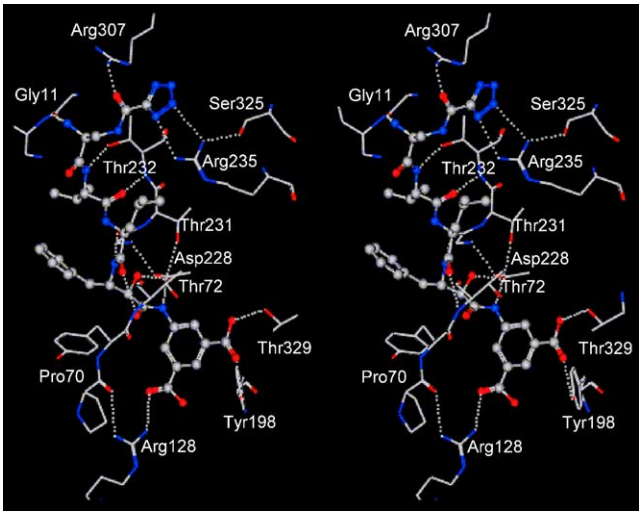


Figure 5. Modeled structure of BACE1 (skeleton model)-**6b** KMI-429 (ball and stick model) complex based on the crystal structure of BACE1 bound to OM99-2.[25] White dashed lines indicate hydrogen bonds to the inhibitor.

β-isomers **1b**, **2b**, **4b**, and **5b**, respectively. As compared with **4a** and **5a** showing no isomerization, relatively high activities of **1a** and **2a** are probably due to the activities of the corresponding β-isomers **1b** and **2b** formed partially by the isomerization of **1a** and **2a** during incubation in this assay. The IC_{50} values of **5b** and **6b** were improved as compared to those of **2b** and **3b**, respectively.

As shown in Figure 5, the tetrazole ring, which is slightly larger than a carboxylic acid, allows the formation of hydrogen bonds to both Arg235 and Arg307 residues of BACE1. Moreover, as previously described,[12] compound **3b**, which contains two carboxylic groups at the P_1' position, exhibited a higher BACE1 inhibitory activity than compound **2b**, which contains a carboxylic group at the P_1' position. Similarly, compound **6b** exhibited higher BACE1 inhibitory activity than compound **5b**. The two carboxylic groups at the P_1' position of compound **6b** allow the formation of rigid hydrogen bonds in BACE1 as shown in Figure 5. As described above, it seems that the acidic functional groups at the P_4 (as an isostere of the carboxylic group) and P_1' sites are important for enhancing BACE1 inhibitory activity.

4. Conclusion

We found that replacing the labile β-*N*-oxalyl-DAP group of **2b** (KMI-358) and **3b** (KMI-370) with a 1*H*-tetrazole-5-carbonyl group resulted in more potent and chemically stable BACE1 inhibitors, **5b** (KMI-420) and **6b** (KMI-429). Replacing carboxylic acid with bioisostere, such as tetrazole ring, is expected to enhance the membrane permeability, which is considered to be a key issue in developing AD's drugs based on the 'amyloid hypothesis'.[26–29]

Acknowledgements

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