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BACE1 inhibitors: Optimization by replacing the P_1' residue with non-acidic moiety

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Abstract—Recently, we reported potent BACE1 inhibitors KMI-429, -684, and -574 possessing a hydroxymethylcarbonyl isostere as a substrate transition-state mimic. These inhibitors showed potent inhibitory activities in enzymatic and cell assays, especially, KMI-429 was confirmed to significantly inhibit Aβ production in vivo. However, acidic moieties at the P_4 and P_1' positions of KMI-compounds were thought to be unfavorable for membrane permeability across the blood–brain barrier. Herein, we replaced acidic moieties at the P_4 position with other hydrogen bond acceptor groups, and these inhibitors exhibited improved BACE1 inhibitory activities in cultured cells. In this study, we replaced the acidic moieties at the P_1' position with non-acidic and low molecular sized moieties.

β-Secretase, also called BACE1 [β-site APP (amyloid precursor protein) cleaving enzyme], is a molecular target for developing drugs against Alzheimer's disease (AD),[1–5] because BACE1 triggers amyloid β (Aβ) peptide formation by cleaving APP at the N-terminus of the Aβ domain.[6–11] Recently, we reported potent BACE1 inhibitors[12–15] KMI-420, -429, and -684 (Fig. 1) possessing phenylnorstatine [Pns: (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] as a substrate transition-state mimic.[16] Specifically, KMI-429 exhibited effective inhibition of BACE1 activity in cultured cells, and significant reduction of Aβ production in vivo (APP transgenic and wild-type mice).[13b] According to structure-activity relationships studies on KMI-compounds, an acidic moiety at the P_4 and P_1' positions is required for improving BACE1 inhibitory activity. However, acidic moieties at the P_4 and P_1' positions

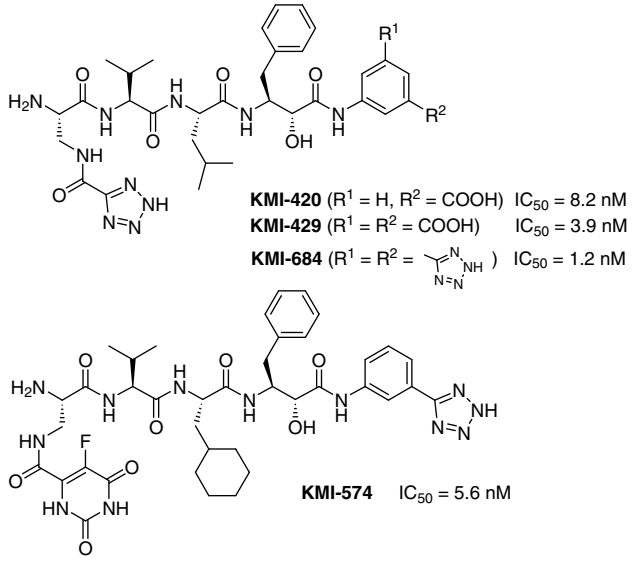

Figure 1. BACE1 inhibitors consisting of penta-peptides.

Keywords: Alzheimer's disease; BACE1; β-Secretase; BACE1 inhibitor.
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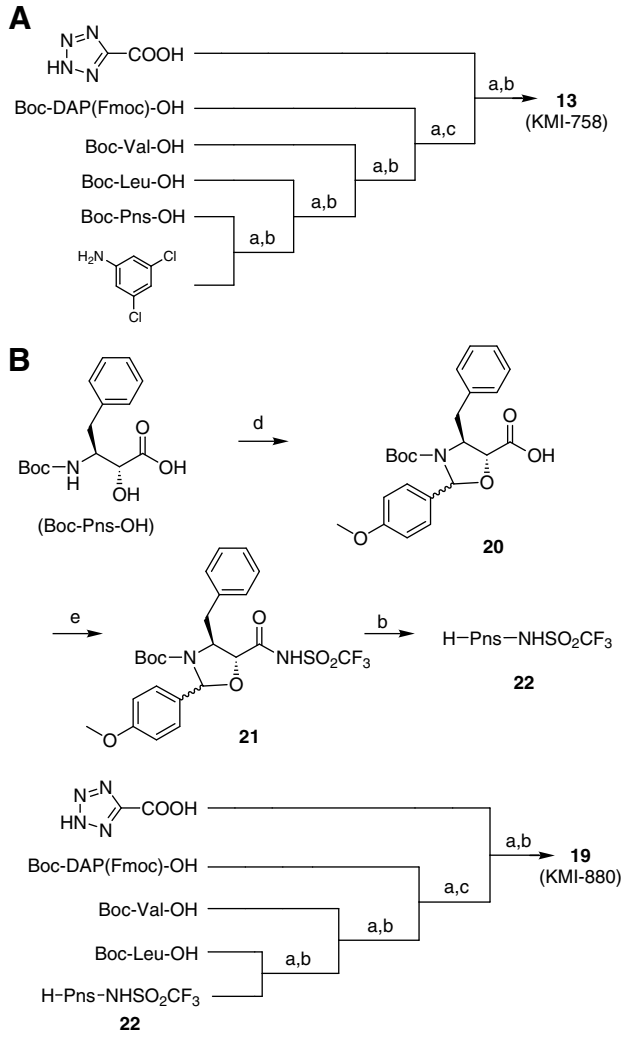
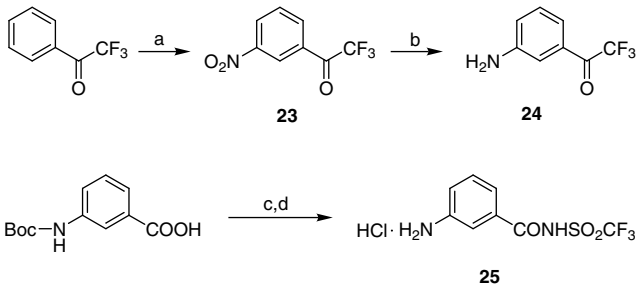
were thought to be unfavorable for membrane permeability across the blood–brain barrier. In order to improve membrane permeability of KMI-compounds, we replaced the acidic moieties at the P_4 position with other hydrogen bond acceptor groups, and these inhibitors (e.g., KMI-574 in Fig. 1) exhibited improved BACE1 inhibitory activities in cultured cells.[15] This paper describes the BACE1 inhibitors, in which the acidic moieties at the P_1' position were replaced with non-acidic and low molecular-sized moieties. In the subsequent paper, we report the small-sized BACE1 inhibitors, in which the P_2 residue was replaced with non-peptidic aromatic moieties.



Scheme 1. Reagents: (a) EDC·HCl, HOBt/DMF; (b) anisole, 4 N HCl/dioxane; (c) 20% diethylamine/DMF; (d) *p*-anisaldehyde dimethyl acetal, pyridinium *p*-toluenesulfonate/toluene, reflux; (e) $NH_2SO_2CF_3$, EDC·HCl, DMAP/DMF.

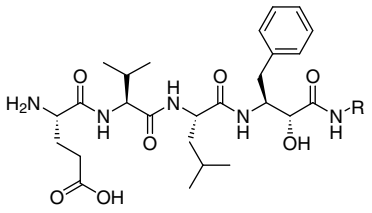


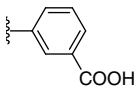
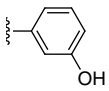
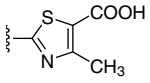
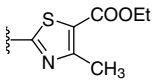
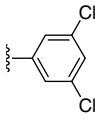
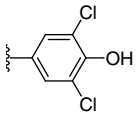
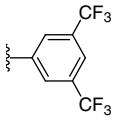
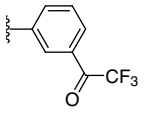
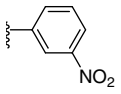
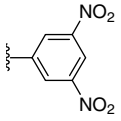
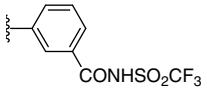
Scheme 2. Reagents: (a) $NaNO_3$/concd. H_2SO_4; (b) H_2, Pd–carbon/MeOH; (c) $NH_2SO_2CF_3$, EDC·HCl, DMAP/DMF; (d) anisole, 4 N HCl/dioxane.

BACE1 inhibitors **1**–**19** were synthesized by traditional solution-phase peptide synthesis methods in a similar manner as previously reported procedures,[13a] as shown in Scheme 1. From amines corresponding to the P_1' residue of the inhibitors as starting materials and N-protected amino acids, peptide bonds were formed sequentially using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC·HCl) in the presence of 1-hydroxybenzotriazole (HOBt) as coupling agents. Aromatic amines **24** and **25** were synthesized from trifluoroacetophenone and 3-(*N*-*tert*-butyloxy)aminobenzoic acid, respectively, as illustrated in Scheme 2. Other amines were commercially available. Previously, we reported that *N*-protected α-hydroxy-β-amino acids, such as Boc–Pns-OH, induced the corresponding homobislactones by dimerization of acids via a condensation reaction with a poor-nucleophilic amine.[17] From this observation, inhibitors **11**, **18**, and **19** were synthesized according to the procedure shown in Scheme 1B. Namely, after the hydroxyl group of Boc–Pns-OH was protected by an introduced 1,3-oxazolidine ring,[18] an amide bond was formed using EDC·HCl in the presence of 4-dimethylaminopyridine (DMAP) or 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling agent. Inhibitor **4** was synthesized from ethyl 2-amino-4-methylthiazole-5-carboxylate corresponding to the P_1' moiety as starting material, followed by a subsequent alkaline hydrolysis. In the case of inhibitors **1**–**12**, Boc–Glu(*O*-*tert*-butyl)-OH were used as an *N*-terminal amino acid moiety. After the elongation of the peptide chain, all inhibitors were synthesized by deprotection using 4 N HCl in dioxane and purified by preparative RP-HPLC.

BACE1 inhibitory activity of the inhibitors was determined by enzymatic assay using a recombinant human BACE1 and FRET (fluorescence resonance energy transfer) substrate as previously reported.[4] Recently, we reported from a computer-assisted docking simulation study of inhibitor KMI-684 docked in BACE1 study[14] that the two P_1' tetrazole rings were found near the hydrophobic amino acids of BACE1, and that the hydrophobic regions above and below these tetrazole rings might interact favorable with the partially hydrophobic inner wall of the S_1' pocket, which consisted of Tyr71, Pro70, Tyr198, Ile126, Ser35 (α and β-carbons), Thr329, Ile226, and Val332. Hence, we considered replacing the P_1' acidic moieties with hydrophobic groups, that could interact with the hydrophobic regions of BACE1. To screen for non-acidic P_1' moiety, we selected previously reported inhibitor KMI-260 (see Table 1) containing a P_4 glutamic acid residue as the reference compound, and replaced its P_1'-containing carboxylic acid group with diverse moieties listed in Table 1. Compounds **1**–**3**, in which the P_1' carboxylic acid groups

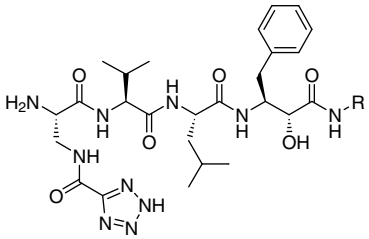
Table 1. Modification at the P_1' position of BACE1 inhibitor KMI-260 possessing P_4 glutamic acid

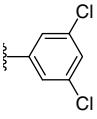
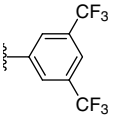
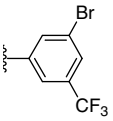
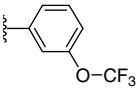
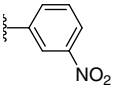
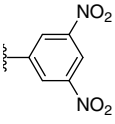
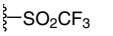


Compound (KMI No)	R	BACE1 inhibition (%) at 2 μM
KMI-260		84
1 (KMI-517)		53
2 (KMI-541)		54
3 (KMI-749)		60
4 (KMI-701)		94
5 (KMI-694)		66
6 (KMI-491)		78
7 (KMI-477)		80
8 (KMI-735)		74
9 (KMI-752)		51
10 (KMI-806)		71
11 (KMI-817)		60
12 (KMI-845)		86

were removed or replaced by a phenolic hydroxyl group, exhibited low BACE1 inhibitory activity. Replacing the P_1' benzene ring with other aromatic ring, **4** possessing a carboxylic acid group exhibited potent BACE1 inhibitory activity, although **5** with no acidic P_1' moiety exhibited low activity. Second, we introduced hydrophobic groups (halogen or nitro group) on the P_1' benzene ring. Compounds **6**–**8** and **10** exhibited higher BACE1 inhibitory activity. However, **9** and **11**, which possessed an elongated side chain or two nitro groups at the P_1' benzene ring, respectively, exhibited moderate BACE1

Table 2. Modification at the P_1' position of BACE1 inhibitors KMI-420 and KMI-429



Compound (KMI No)	R	BACE1 inhibition (%) at	
		2 μM	0.2 μM
KMI-420	See Figure 1	99	87
KMI-429	See Figure 1	100	98
13 (KMI-758)		99	84
14 (KMI-761)		95	68
15 (KMI-811)		92	57
16 (KMI-818)		93	72
17 (KMI-773)		96	70
18 (KMI-819)		96	75
19 (KMI-880)		98	81

inhibitory activity, suggesting that the spatial location of the P_1' moiety interacting with the S_1' hydrophobic regions of BACE1 might be important for improving BACE1 inhibitory activity. On the other hand, compound **12** possessing *N*-sulfonylcarboxamide group at the P_1' position showed higher BACE1 inhibitory activity. The sulfonylamino and carbonyl groups were thought to form hydrogen bond interactions with the S_1' binding sites of BACE1. Moreover, the *N*-sulfonylcarboxamide group has a hydrophilic property, and might function as a bioisostere of carboxylic acid.

From the above results, we replaced the P_1' carboxylic acid groups of inhibitors KMI-420 and -429 with halogen atoms or nitro groups. As shown in Table 2, inhibitors **13**–**19** showed potent BACE1 inhibitory activities. Inhibitor **13** showed especially potent BACE1 inhibitory activities (BACE1 inhibition IC_{50}: 14.0 nM). These results were consistent with our previous findings that hydrophobic regions existed around the hydrophilic S_1' binding sites of BACE1, as shown in Figure 2. Then, we performed docking simulation experiments using a BACE1 crystal structure (PDB ID: 1W51) and previously reported method.[14,15] As shown in Figure 3, the 3D-structure of **13** docked in BACE1 was similar to that of the docked structure of KMI-684, suggesting that these halogen atoms interacted with the hydrophobic regions of S_1' pocket.

We noted that Thr72 was near the P_1' benzene ring, and the P_1' benzene ring could be replaced by other residues (as exemplified by compounds **4** and **5**). Hence, the P_1' phenyl ring could be replaced by a hydrogen bond acceptor that could form hydrogen bonds with Thr72. BACE1 inhibitor **19** (KMI-880) in which we replaced the P_1' benzene ring with a trifluoromethanesulfonyl group exhibited potent BACE1 inhibitory activity as shown in Table 2 (BACE1 inhibition IC_{50}: 29.9 nM).

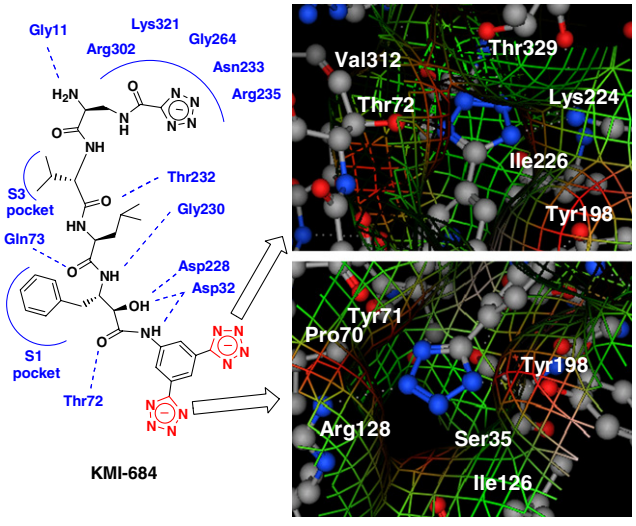


Figure 2. A cartoon depiction of KMI-684 docked in BACE1 and a computer-assisted docking model of the vicinity of the inhibitor's two P_1' tetrazole rings. Meshed surface model indicates BACE1 (green mesh. hydrophobic surface; red mesh, hydrophilic surface).

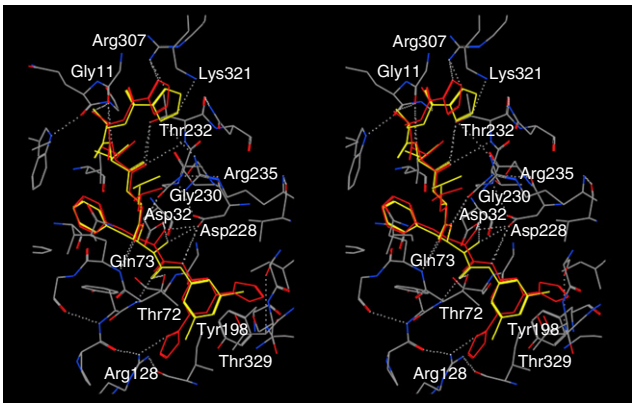


Figure 3. Stereoscopic view of superimposed inhibitors **13** (KMI-758, yellow lines) and KMI-684 (red lines) in BACE1 (PDB ID: 1W51).

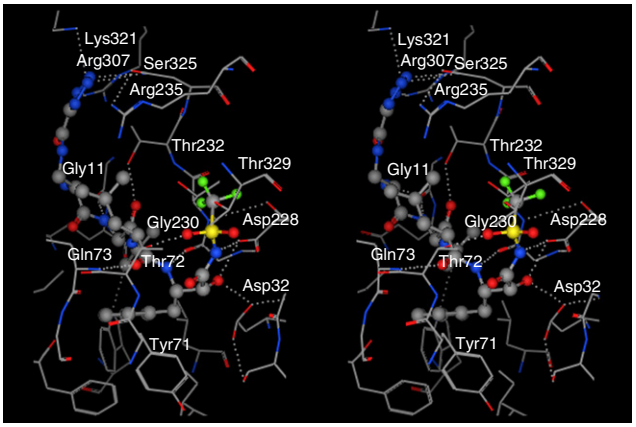


Figure 4. Stereo view of docked inhibitor **19** (KMI-880, colored ball-and-stick model) in BACE1 (PDB ID: 1W51, colored skeleton model).

A modeled structure of the docked inhibitor **19** in BACE1 is shown in Figure 4. Hence, the important role of the P_1' sulfonyl group and P_1 residues as a bridge from Thr72 of the flap domain to the active site (Asp32 and Asp228) on the cleft of BACE1 was confirmed.

In conclusion, BACE1 inhibitors modified at the P_1' position were designed, and we found potent BACE1 inhibitors **13** (KMI-758) having hydrophobic P_1' moieties and **19** (KMI-880) with a P_1' small-sized residue. These inhibitors with non-acidic P_1' moieties are thought to be a further step toward a practical anti-AD's drug.

Acknowledgments

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