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Novel non-peptidic and small-sized BACE1 inhibitors

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Abstract—Recently, we reported substrate-based β-secretase (BACE1) inhibitors with a hydroxymethylcarbonyl (HMC) isostere as a substrate transition-state mimic. These inhibitors showed potent BACE1 inhibitory activities (~1.2 nM IC_{50}). In order to improve in vivo enzymatic stability and permeability across the blood–brain barrier, these penta-peptidic inhibitors would need to be further optimized. On the other hand, non-peptidic inhibitors possessing isophthalic residue at the P_2 position were reported from other research groups. We selected isophthalic-type aromatic residues at the P_2 position and an HMC isostere at the P_1 position as lead compounds. On the basis of the design approach focused on the conformer of docked inhibitor in BACE1, we found novel non-peptidic and small-sized BACE1 inhibitors possessing a 2,6-pyridinedicarboxylic, chelidamic or chelidonic residue at the P_2 position.

Amyloid β (Aβ) peptide is a main component of senile plaques in the brains of Alzheimer's disease (AD) patients, that seems to cause ADs pathologies. According to the amyloid hypothesis,[1] β-secretase [BACE1: β-site APP (amyloid precursor protein) cleaving enzyme] appears promising as a molecular target for therapeutic intervention in AD,[2–6] because BACE1 triggers Aβ peptide formation by cleaving APP at the N-terminus of the Aβ domain.[7–12] Recently, we reported potent penta-peptidic BACE1 inhibitors[13–16] containing a hydroxymethylcarbonyl isostere (HMC) as a substrate transition-state mimic[17] as shown in Figure 1. Among them, KMI-429[14] exhibited effective inhibition of BACE1 activity in cultured cells, and significant reduction of Aβ production in vivo (by direct administration into the hippocampuses of APP transgenic and wild-type mice).[14b] However, these inhibitors contained some natural amino acids that seemed to be required to improve enzymatic stability in vivo and permeability across the blood–brain barrier, so as to be practical anti-ADs drugs. Non-peptidic BACE1 inhibitors possessing isophthalic residue such as Elan's and Merck's inhibitors as shown in Figure 2A, were reported from other research groups.[18,19] Herein, we selected isophthalic-type aromatic residues at the P_2 position and an HMC isoste-

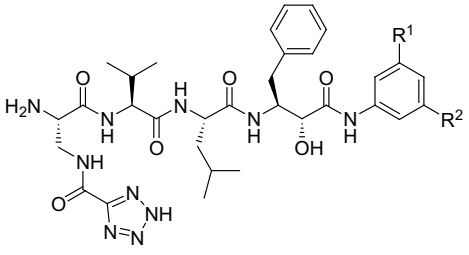

Figure 1. BACE1 inhibitors consisting of penta-peptides.

Keywords: Alzheimer's disease; BACE1; β-Secretase; Non-peptidic B-ACE1 inhibitor.
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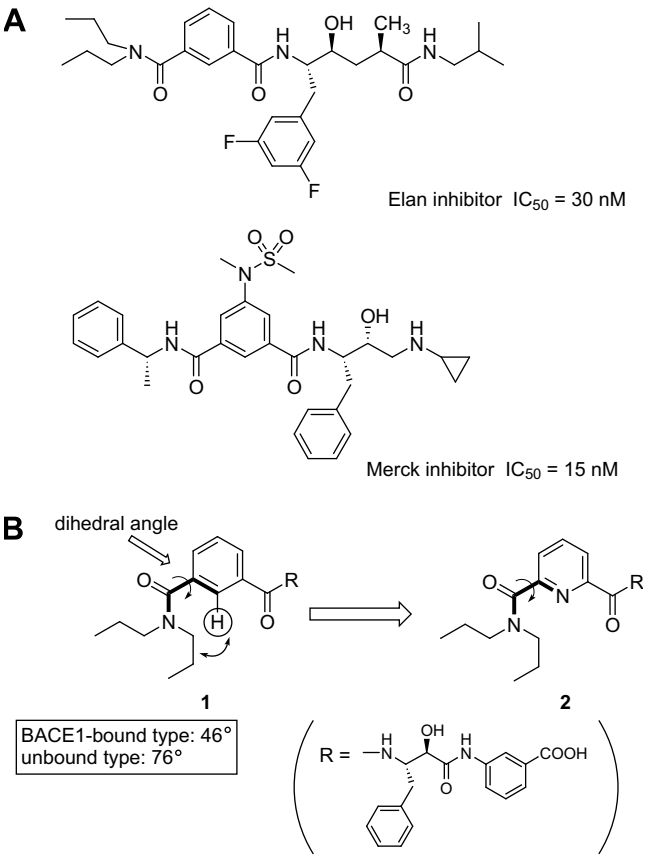
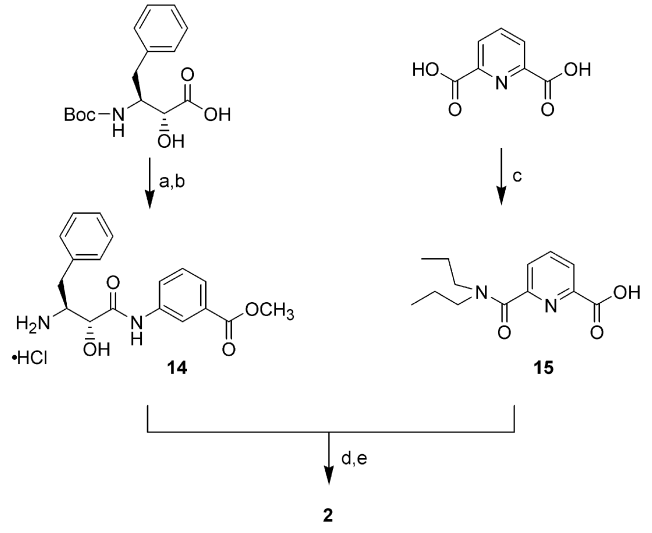


Figure 2. (A) Non-peptidic BACE1 inhibitors. (B) Design of small-sized BACE1 inhibitor **2** possessing heterocyclic ring at the P_2 position.

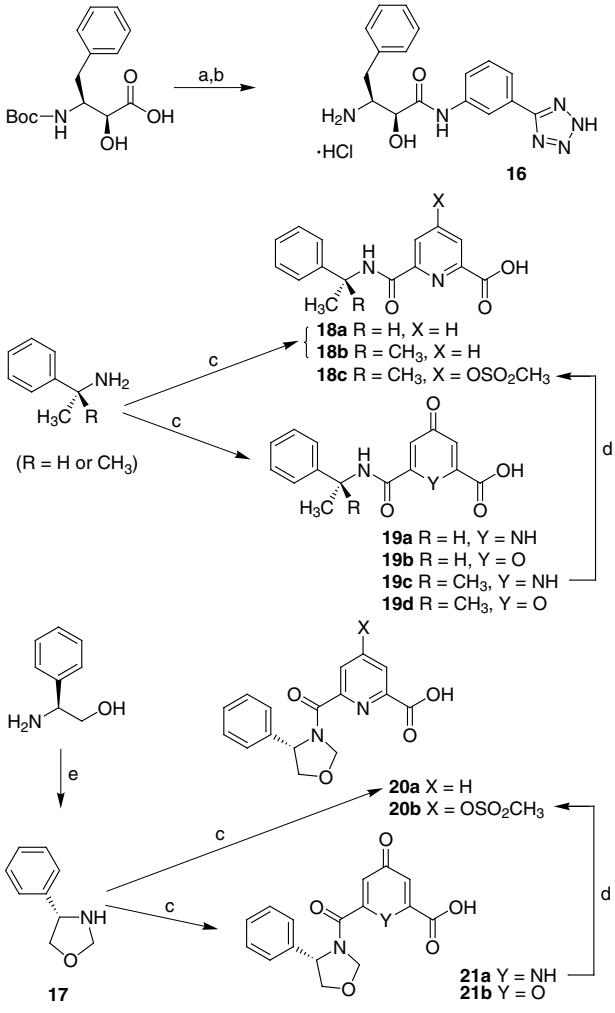
re at the P_1 position as lead compounds. Based on the design approach that a conformer of docked inhibitor in BACE1 was stabilized, we designed novel BACE1 inhibitors.

BACE1 inhibitors **2–13** were synthesized to connect in tandem the blocks corresponding to the P_3–P_2 residues and P_1–P_1' residues, respectively. Amide bonds were formed by traditional solution-phase synthesis methods using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC·HCl) in the presence of 1-hydroxybenzotriazole (HOBt) as coupling agents. As shown in Scheme 1, after amide-bond formation, inhibitor **2** was synthesized by deprotection using alkaline hydrolysis. Synthesis of the blocks for the preparation of inhibitors **3–13** is shown in Scheme 2. 4-Methanesulfonyloxypyridine-2,5-dicarboxlic derivatives **18c** and **20b** were synthesized from chelidamic derivatives **19c** and **21a** by tautomerization and an ensuing methanesulfonation using methanesulfonyl chloride in the presence of triethylamine. Blocks **20–21** possessing a 5-membered ring were synthesized from **17** that was prepared from (*S*)-2-phenylglycinol by cyclization with formaldehyde. As an example, the synthesis of inhibitor **13** is shown in Scheme 3. All of the inhibitors were purified by preparative RP-HPLC.

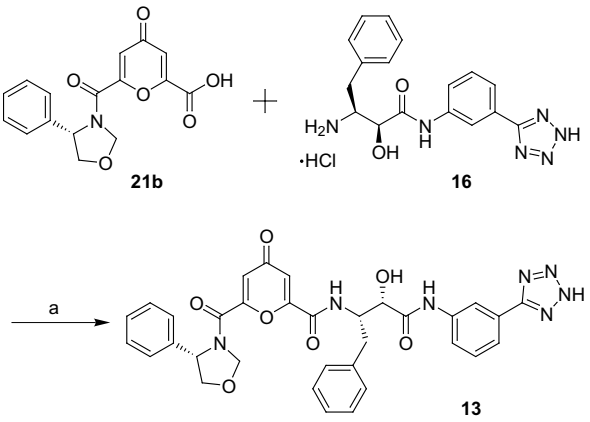


Scheme 1. Reagents and conditions: (a) methyl 3-aminobenzoate, EDC·HCl, HOBt/DMF, 85%; (b) anisole, 4 N HCl/dioxane, 99%; (c) dipropylamine, EDC·HCl, HOBt/DMF, 55%; (d) EDC·HCl, HOBt/DMF, 91%; (e) 1 N NaOH/MeOH, 99% (crude).

BACE1 inhibitory activity of the inhibitors was determined by enzymatic assay using a recombinant human



Scheme 2. Reagents and conditions: (a) 5-(3-aminophenyl)tetrazole, EDC·HCl, HOBt/DMF, 83%; (b) anisole, 4 N HCl/dioxane, 99%; (c) 2,6-pyridinedicarboxylic acid, chelidamic acid or chelidonic acid, EDC·HCl, HOBt/DMF, 55–65%; (d) methanesulfonyl chloride Et_3N/THF, 60–78%; (e) HCHO, water, 86%.



Scheme 3. Reagents: (a) EDC·HCl, HOBt/DMF, 90%.

BACE1 and FRET (fluorescence resonance energy transfer) substrate as previously reported.[5]

We first envisioned virtual inhibitor **1** possessing an isophthalic residue at the P_2 position and an HMC isostere, phenylnorstatine [Pns: (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid], at the P_1 position. A stable conformation docked in BACE1 (PDB ID: 1W51) was calculated using docking simulation software, MOE (Chemical Computing Group Inc., Canada). Second, a stable conformation of unbound inhibitor **1** was searched by calculating the steric energies of conformers around the bond of the P_3 amide and P_2 isophthalic residue in MM2 force field. The ideal dihedral-angles between the P_3 amide and P_2 isophthalic residue of inhibitor **1** in the BACE1-bound and unbound conformers were found to be quite different (BACE1-bound type: 46°, unbound type: 76°). We thought that high-active inhibitors could be found by resolving this variation. Herein, we designed inhibitor **2** possessing a P_2 heterocyclic residue, which lacks a hydrogen atom that would induce steric hindrance between the P_3 moiety and P_2 aromatic ring, as shown in Figure 2B. However, synthesized inhibitor **2** showed no BACE1 inhibitory activity.

Next, we designed inhibitors **3**–**5** possessing (*R*)-α-methylbenzylamino group at the P_3 position and allophenylnorstatine [Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl butyric acid], with syn-stereochemistry similar to Merck's BACE1 inhibitor (Fig. 2A), at the P_1 position. **3**–**5** showed moderate BACE1 inhibitory activity. We noticed the importance of the α-methyl group at the P_3 position for BACE1 inhibitory activity. Inhibitors **6**–**9** possessing an α,α-dimethylbenzylamino group at the P_3 position were designed and showed improved inhibitory activity (Table 1). According to the crystal structure of isophthalic-type Merck's inhibitor (PDB ID: 2B8L, Fig. 3B) and the coordinates of docked inhibitors in BACE1 by our docking simulation study, inhibitors possessing a P_3 benzylamino-type residue displayed

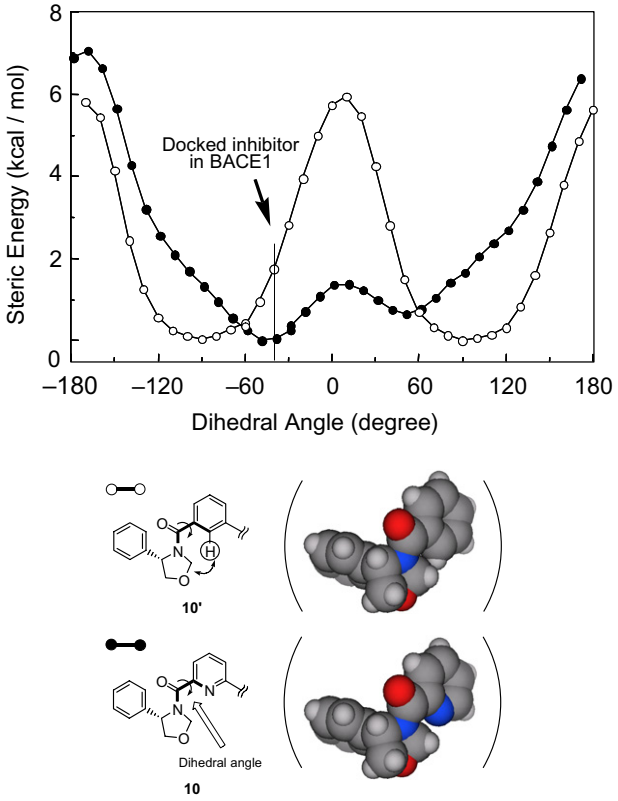


Figure 4. Conformational study of BACE1 inhibitor **10** (KMI-1023). **10′** is virtual isophthalic analogue of inhibitor **10**.

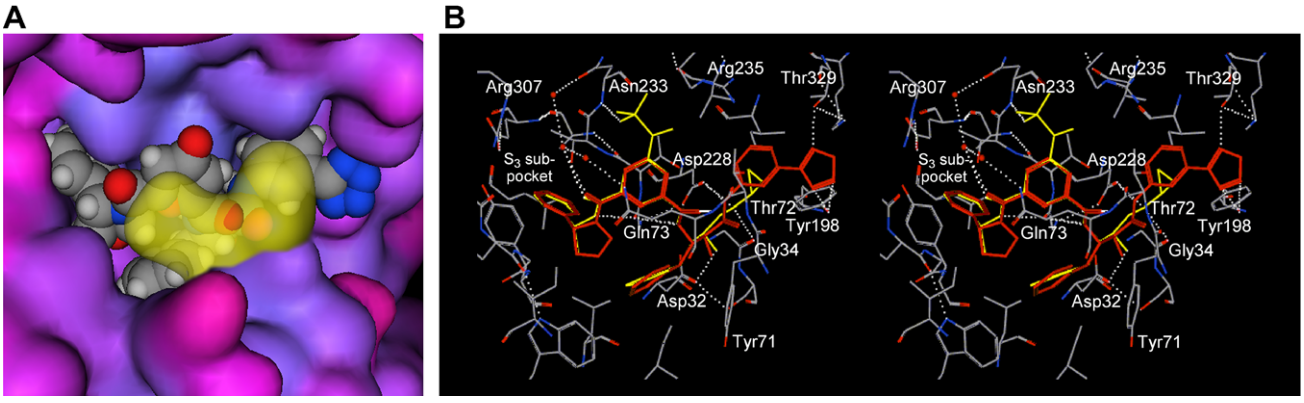
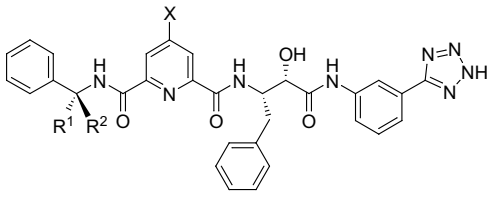


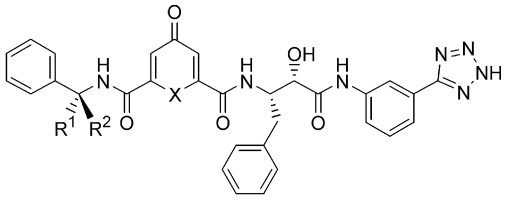
Figure 3. (A) Inhibitor **13** (KMI-1027, space-filling model) docked in BACE1. Molecular surface model indicates BACE1. The yellow transparent part in the center indicates a flap domain. (B) Stereoview of superimposed inhibitor **10** (KMI-1023, red lines) and Merck inhibitor (yellow lines) in BACE1(PDB ID: 2B8L). White dashed lines and red balls indicate hydrogen bond interactions and water molecules, respectively.

folding conformers between the P_2 ring and P_3 benzene rings to bind to the S_3 sub-pocket[19] in BACE1. We assumed that the two methyl groups stabilized this folding conformer by *gem*-dimethyl effect. However, α,α-dimethylbenzylamino group is labile under acidic conditions[20] similar to trityl group. Interestingly, HIV protease inhibitors with α,α-dimethylbenzyl amide showed low oral bioavailability, because of their acid-instability.[21] We designed inhibitors **10**–**13** introducing a 5-membered ring at the P_3 position, in order to improve acid-resistance and fix the folding pose between the P_2 and P_3 rings. Inhibitors **10**–**13** showed potent BACE1 inhibitory activity (Table 2). Modeled structures of docked inhibitors **10** and **13** in BACE1 (PDB ID: 2B8L) by docking simulation using MOE are shown in Figure 3. The P_3 benzene ring was confirmed to take

Table 1. BACE1 inhibitors possessing benzylamino-type groups at the P_3 position and their BACE1 inhibitory activities

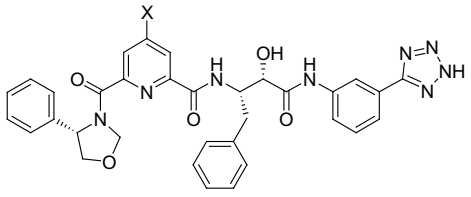


	R^1	R^2	X	BACE1 inhibition % at 2 μM
3	CH_3	H	H	66%
6	CH_3	CH_3	H	69%
7	CH_3	CH_3	OSO_2CH_3	84%

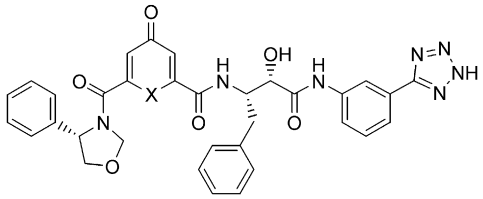


4	CH_3	H	NH	51%
5	CH_3	H	O	70%
8	CH_3	CH_3	NH	65%
9	CH_3	CH_3	O	81%

Table 2. BACE1 inhibitors with a 5-membered ring at the P_3 position and their BACE1 inhibitory activities



Compound (KMI No.)	X	BACE1 inhibition %		IC_{50} (nM)
		at 2 μM	at 0.2 μM	
10 (KMI-1023)	H	93%	63%	140
11 (KMI-1036)	OSO_2CH_3	96%	73%	96



12 (KMI-1030)	NH	83%	36%	360
13 (KMI-1027)	O	96%	78%	50

the form of a folding conformer against the P_2 ring and tightly occupy the S_3 sub-pocket of BACE1 (Fig. 3A). In addition, both ideal conformers around the bond of the P_3 amide and P_2 ring of unbound and BACE1-bound inhibitor **10** were confirmed to coincide. Interestingly, virtual isophthalic analogue **10′** in BACE1 showed high steric energy state caused by the interaction of the P_3 moiety and ring's hydrogen atom (Fig. 4).

In conclusion, on the basis of the design approach focused on the conformer of a docked inhibitor in complex with BACE1, BACE1 inhibitors possessing a heterocyclic ring at the P_2 position and 5-membered ring at the P_3 position were designed. We found novel small-sized and non-peptidic BACE1 inhibitors. These inhibitors are expected improved membrane permeability and bioavailability as practical anti-ADs drugs. This design approach based on a conformer of enzyme-/receptor-bound inhibitor/ligand, which was predicted in silico, was showed the usefulness of the drug discovery research, in contrast to exhaustive combinatorial synthesis.

Acknowledgments

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