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Tripeptidic BACE1 inhibitors devised by in-silico conformational structure-based design

Yoshio Hamada [a,b,*], Harichandra D. Tagad [b], Yoshinori Nishimura [b], Shoichi Ishiura [c], Yoshiaki Kiso [a,b,d,*]

[a] Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Minatojima, Chuo-ku, Kobe 650-8586, Japan
[b] Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan
[c] Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan
[d] Department of Bio-Science, Nagahama Institute of Bio-Science and Technology, Tamura-cho, Nagahama 526-0829, Japan

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ABSTRACT

Previously reported pentapeptidic BACE1 inhibitors, designed using a substrate-based approach, were used as lead compounds for the further design of non-peptidic BACE1 inhibitors. Although these peptidic and non-peptidic inhibitors, with a hydroxymethylcarbonyl isostere as a substrate transition-state mimic, exhibited potent BACE1 inhibitory activities, their molecular-sizes appeared a little too big (molecular weight of >600 daltons) for developing practical anti-Alzheimer's disease drugs. To develop lower weight BACE1 inhibitors, a series of tripeptidic BACE1 inhibitors were devised using a design approach based on the conformation of a virtual inhibitor bound to the BACE1 active site, also called 'in-silico conformational structure-based design'. Although these tripeptidic BACE1 inhibitors contained some natural amino acid residues, they are expected to be useful as lead compounds for developing the next generation BACE1 inhibitors, due to their low molecular size and unique structural features compared with previously reported inhibitors.

Amyloid β peptide (Aβ), the main component of senile plaques in the brains of Alzheimer's disease (AD) patients,[1] is formed by proteolysis of amyloid precursor protein (APP).[2,3] As β-site APP cleaving enzyme 1 (BACE1, also known as β-secretase) triggers Aβ formation by cleavage at the Aβ domain *N*-terminus,[4–7] it is a molecular target for AD therapeutic intervention.[8–11] In recent reports on pentapeptidic and non-peptidic BACE1 inhibitors,[11–16] peptidic inhibitors KMI-420, -429, -684, and -574 (Fig. 1), which contained phenylnorstatine (Pns: (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) as a substrate transition-state mimic, showed potent BACE1 inhibitory activities.[17,18] Of these, KMI-429 exhibited effective activity in cultured cells and a significant reduction of Aβ production in vivo (by direct administration into APP transgenic and wild-type mice hippocampi).[13] However, some natural amino acid residues in these inhibitors appear necessary for the enzymatic stability in vivo and blood–brain barrier permeability needed to be practical drugs. Accordingly here, non-peptidic BACE1 inhibitors were designed which possessed a heterocyclic scaffold, such as a chelidamic or 2,5-pyridinedicarboxylic moiety, at the P_2 position using 'in-silico conformational structure-based design'.[19] Furthermore, through focusing on interaction with the BACE1-Arg235 side chain, the non-peptidic inhibitor KMI-1303, possessing a halogen atom on the P_2-pyridine scaffold, was designed and found to be a potent inhibitor (Fig. 2). Although these pentapeptidic and non-peptidic BACE1 inhibitors showed potent inhibitory activities, their molecular weights were considered a little high (>600 daltons) for practical anti-AD drugs. With the goal of developing low molecular weight and thus smaller inhibitors, the location of two catalytic Asp residues in BACE1 were examined. As all of the BACE1 active site pockets are created by closure of the flap domain (Fig. 3A), effective, potential inhibitors might involve fixing the 'closed-form' of the flap domain. Additionally, the two catalytic Asp residues are located at some distance from the flap domain (Fig. 3B). Thus, the interactions of the P'_1 region and substrate transition-state analogue with the corresponding pockets of BACE1 were hypothesized to be unnecessary for fixing the flap domain. As most existing BACE1 inhibitors have been designed to optimize interactions with the flap domain and the two catalytic Asp residues, they have naturally had high molecular weights to span the separation. The possible design of an inhibitor lacking the P'_1 position and substrate transition-state analogue was speculated here such that it could stabilize the folding pose around the inhibitor's P_1 phenyl group (Fig. 4B). Previously reported from here, a design tool focused on the conformation of a virtual inhibitor bound in the BACE1 active site was used to allow the synthesis of non-peptidic BACE1 inhibitors.[19] The well known concept that the stability of a specific conformer of a medicine is crucially important for proper drug effect has been employed in computational studies for drug design. In fact, common molecular dynamics calculations

* Corresponding authors. Tel.: +81 749 64 8113; fax: +81 749 64 8140.
E-mail address: y_kiso@nagahama-i-bio.ac.jp (Y. Kiso).

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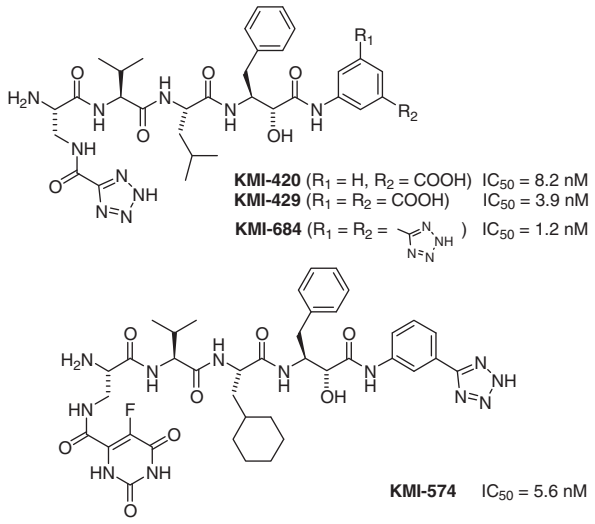

Figure 1. Pentapeptidic BACE1 inhibitor structures.

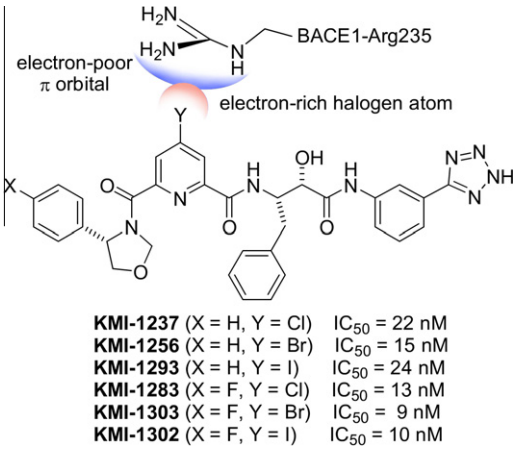

Figure 2. BACE1 inhibitor structures with a halogen atom on the P_2 aromatic ring.

or docking simulation software produce the potential energy values of drug molecules using descriptors regarding steric energy. On the other hand, the design tool used here, 'in-silico conformational structure-based design', is employed as a problem-solving tool for identifying drug candidates. In this study, the proposition of how to design low molecular weight inhibitors lacking a P'_1 moiety and substrate transition-state analogue was solved by seeking to stabilize the folding structure around the P_1-phenyl ring of a virtual inhibitor. For steric energy calculations, low molecular weight model compounds were used to exclude the effect of their intramolecular interactions in solution (Fig. 4A), because an inhibitor docked in the enzyme's active site generally interacts with the pocket of enzyme, not within the inhibitor molecule. Dihedral energy plots were constructed by rotating two covalent bonds, between the *C*-terminus amide bond and its side-by-side carbon atoms, of virtual compounds under an MMFF94x force field, using MOE software (Chemical Computing Group Inc., Canada). The optimal modification around the P_1 region of virtual inhibitors was then screened using a folding conformer by calculating their steric energy. A virtual inhibitor with two methyl groups at the P_1 position was found that showed a folding structure around the P_1-phenyl ring and a most-stable conformer which overlapped the red star symbol in the dihedral energy plot chart corresponding to an inhibitor docked on the enzyme (Fig. 4A); the folding structure appeared likely to be stabilized by a *gem*-dimethyl effect. A series of inhibitors were then designed which had a folding configuration around the P_1-phenyl ring.

BACE1 inhibitors **1**–**38** and **53**–**57** were synthesized by traditional solution-phase peptide synthesis methods, similar to previously reported procedures, from amines corresponding to the P_1 residue 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 a coupling agent.[11–16] For example, the synthesis of inhibitor **6** began with 2-methyl-1-phenylpropane-2-amine as the P_1-amine followed by peptide bond formation with selected amino acids and then protecting group removal (Scheme 1). Other P_1-amines were prepared from 2-methyl-1-phenylpropane-2-amine **39** or phenylacetic acids **44a**–**e** (Scheme 2). In particular, amines **41**–**43** were prepared from amine hydrochloride **40** by bromination, nitration and iodination, respectively, and amine **52** was prepared from amine **39** by catalytic hydrogenation using Adams' catalyst. Inhibitors **11**–**17**, **20**, **23**–**32**, **35**, and **36** were synthesized from P_2 – P_1 blocks **50a**–**b** or **51a**–**e** in a similar manner to above. P_2–P_1 blocks **50a**–**b** or **51a**–**e** were synthesized from alcohols **46a–e**, previously prepared from phenylacetic acids **44a–e** and nitriles **49a–b**. The latter two groups of reactants were prepared from corresponding Fmoc-protected amino-acid amides **48a–b**, using the Ritter reaction and the ensuing Fmoc-deprotec-

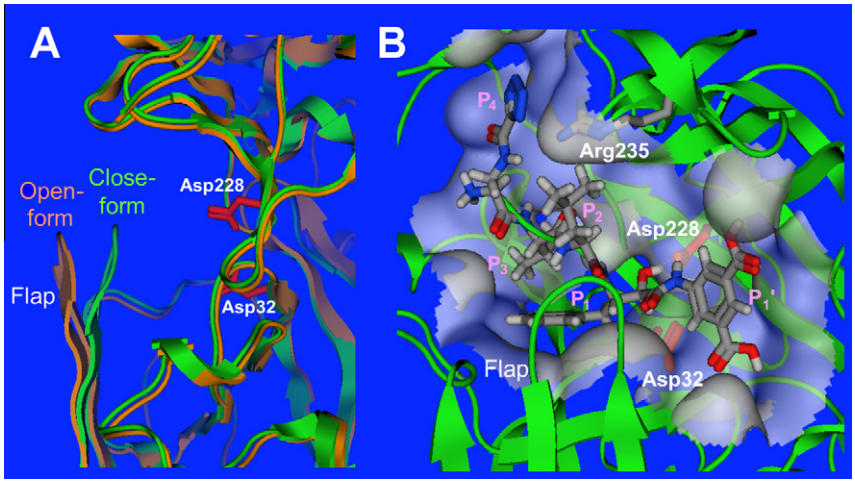

Figure 3. (A) Superimposed flap domain of 'open-form' (PDB ID: 1W50) and 'close-form' (PDB ID: 2B8L) of BACE1. (B) KMI-429 docked in BACE1 enzyme (PDB ID: 2B8L).