Chapter 3

Discovery of BACE1 Inhibitors for the Treatment of Alzheimer's Disease

Yoshio Hamada and Yoshiaki Kiso

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68659

Abstract

Alzheimer's disease is the most common cause of dementia. According to the amyloid hypothesis, β-secretase (BACE1) is a promising molecular target for the development of anti-Alzheimer's disease drugs. BACE1 triggers the formation of the amyloid-β (Aβ) peptides that are the main component of senile plaques in the brain of patients with Alzheimer's disease. As BACE1 cleaves the amyloid precursor protein at the N-terminus of the Aβ domain, BACE1 inhibitors reduce the Aβ level in the brain. Previously, we designed a series of peptidic inhibitors that possessed a substrate transition-state analogue, and the structure-activity relationship of our inhibitors was evaluated, based on docking and scoring, using the docking simulation software Molecular Operating Environment (MOE). However, there was no association between the scoring values and the inhibitory activities at the P_2 position. Hence, we hypothesized that the interaction of the P_2 position of the inhibitor with the S_2 site of BACE1 was critical for the mechanism of inhibition, and we proposed the novel concept of 'electron donor bioisostere' for drug discovery. Using this concept, we designed potent small molecule non-peptidic BACE1 inhibitors.

Keywords: Alzheimer's disease, BACE1 inhibitor, docking simulation, electron donor bioisostere, *in-silico* conformational structure-based design

1. Introduction

Alzheimer's disease (AD), which is the most common cause of dementia, is characterized by progressive intellectual deterioration. In 1901, Alois Alzheimer, a psychiatrist and neuropathologist, observed a 51-year-old female patient at Frankfurt Asylum. The patient showed strange behavioural symptoms and the loss of short-term memory, which was later called 'AD'. Unfortunately, the cause of AD was unclear until recently, and there have been no treatment approaches since that first report by Dr. Alzheimer over 100 years ago. Recently, the development of many drug

candidates based on the amyloid hypothesis has been reported. β-secretase (BACE1; β-site amyloid precursor protein (APP) cleaving enzyme 1) is a promising molecular target for the development of anti-Alzheimer's drugs. BACE1 triggers the formation of the amyloid-β (Aβ) peptide that is the main component of the senile plaques found in the brain of AD patients. Previously, we had designed a series of peptidic inhibitors that possessed a substrate transition-state analogue, and evaluated the structure-activity relationship of our inhibitors, based on docking and scoring, using the docking simulation software Molecular Operating Environment ((MOE), Chemical Computing Group Inc., Canada).

1.1. Pathology of Alzheimer's disease

Although the cause of AD was unclear until recently, a breakthrough was obtained from the genetic study of some patients with familial AD. Certain mutations of the amyloid precursor protein (APP) or presenilin gene increased amyloid-β peptides (Aβs) in the brain, which indicated their involvement in the pathogenesis of AD [1–4]. Aβ is produced from APP by two processing enzymes, β-secretase and γ-secretase, which are potential molecular targets for the development of anti-AD drugs [5–7]. The cleavage sites of APP are shown in **Figure 1A**. BACE1, one of the processing enzymes of APP, triggers Aβ formation in the rate-limiting first step by the cleavage of APP at the Aβ domain N-terminus (β-site). BACE1 is a type-I transmembrane

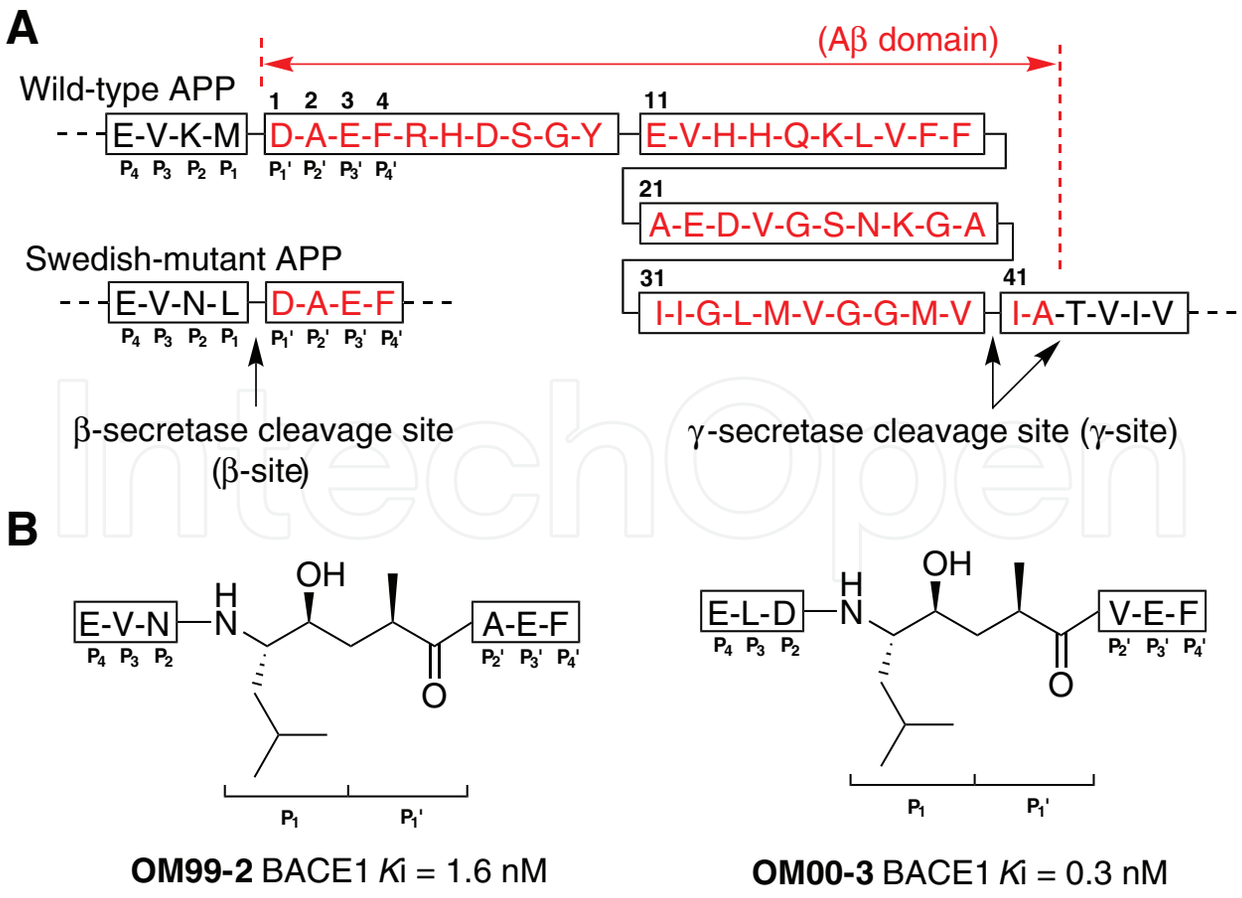


Figure 1. (A) Amyloid precursor protein (APP) and its cleavage site. (B) Early peptidic BACE1 inhibitor by Ghosh et al.

aspartic protease with 501 amino acids. BACE1 and APP are located in the same intracellular granules, the endoplasmic reticulum, Golgi, and trans-Golgi networks, which are acidic environments, which suggested that Aβs are produced in these locations [8]. Next, another aspartic protease, the γ-secretase complex, cleaved at the C-terminus of the Aβ domain and released Aβ peptides. The γ-secretase complex that contains a protein *via* the presenilin gene as a catalytic component cleaved at two cleavage sites, 'γ-sites', which mainly resulted in the formation of two species of Aβs: $Aβ_{1-40}$ and $Aβ_{1-42}$ (**Figure 1A**). $Aβ_{1-42}$ shows greater neurotoxicity and aggregability than $Aβ_{1-40}$ and appears to be a key biomolecular marker of AD pathogenesis. According to the amyloid hypothesis, BACE1 and γ-secretase appear to be molecular targets for the development of anti-AD drugs. However, because γ-secretase can cleave other single-pass transmembrane proteins *in vivo*, such as Notch, which plays a critical role in cell differentiation, γ-secretase inhibitors appeared to lead to serious side effects. As BACE1 knockout transgenic mice demonstrated normal survival, this indicated a promising direction of study, in which BACE1 is a molecular target for the development of AD drugs [9]. At present, many BACE1 inhibitors have been revealed, including those in our study [10–16].

1.2. Early peptidic BACE1 inhibitors

An early inhibitor of BACE1, an aspartic protease, was designed on the basis of a substrate transition-state concept, as well as that of other aspartic proteases, such as renin and HIV protease, which have a substrate transition-state analogue at the P_1 position [10–16]. It is well-known that the Swedish mutant APP (K670N and M671L double mutation, **Figure 1A**) is cleaved faster than wild-type APP by BACE1, which results in increased $Aβ_{1-42}$ and $Aβ_{1-40}$ levels. Early BACE1 inhibitors were designed based on the Swedish-mutant APP amino acid sequence. In 1999, Sinha et al. from Elan Pharmaceuticals purified the BACE1 enzyme from the human brain using a transition-state analogue based on the Swedish-mutant sequence, and succeeded in cloning the BACE1 enzyme [17]. Ghosh et al. reported the potent inhibitors **OM99-2** (*K*i = 1.6 nM) and **OM00-3** (*K*i = 0.3 nM) with a hydroxyethylene unit as a substrate transition-state analogue (**Figure 1B**) and the first X-ray crystal structure (PDB ID: 1FKN) of a complex between recombinant BACE1 and OM99-2 [18–21].

We have reported a series of peptidic BACE1 inhibitors that possessed a norstatine-type transition-state analogue [22–30]. Our early inhibitors are shown in **Table 1**. Octapeptide **1** with an Asn residue and (2*R*, 3*S*)-3 amino-2-hydroxy-5-methylhexanoic acid (Nst, Leu-type transition-state analogue) at the P_2 and P_1 positions, respectively, corresponding to the Swedish-mutant APP sequence showed no inhibitory activity. Octapeptide **2** with (2*R*, 3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid (Pns) as a transition-state analogue at the P_1 position, showed weak inhibitory activity. Compound **3** with an Asp residue similar to OM00-3, and compound **4**, with a Met residue at the P_2 position, also showed weak inhibitory activity. Although compound **5** with the P_2-Lys residue that corresponded to the wild-type APP sequence showed no inhibitory activity, octapeptide **6** that possessed a Leu residue at the P_2 position exhibited potent inhibitory activity (>90% at 2 μM). We synthesized some truncated peptides on the N- or C-terminus in order to confirm the essential moiety for the inhibitory effect. N-truncation of peptides eliminated their inhibitory activity (peptides **7**-**8**). Although C-truncated peptides **10**-**13** showed a weaker inhibitory activity than octapeptide **6**, pentapeptide **12** replicated the inhibitory activity

Compound	P_4	P_3	P_2	P_1	P_1'	P_2'	P_3'	P_4'	BACE1 inhibition (%)[1]
1	E	V	N[2]	Nst	D	A	E	F	<20
2	E	V	N[2]	Pns	D	A	E	F	24
3	E	V	D	Pns	D	A	E	F	25
4	E	V	M	Pns	D	A	E	F	42
5	E	V	K[3]	Pns	D	A	E	F	<20
6	E	V	L	Pns	D	A	E	F	>90
7		V	L	Pns	D	A	E	F	<20
8			L	Pns	D	A	E	F	<20
9				Pns	D	A	E	F	<20
10	E	V	L	Pns	D	A	E		60
11	E	V	L	Pns	D	A			46
12	E	V	L	Pns	D				61
13	E	V	L	Pns					34

[1]BACE1 inhibition activities at 2 μM.

[2]P_2 residue corresponding to the Swedish-mutant APP sequence.

[3]P_2 residue corresponding to the wild-type APP sequence.

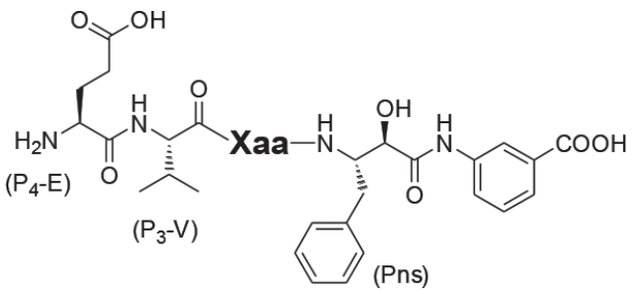
Table 1. BACE1 inhibitory activities of peptidic BACE1 inhibitors.

of heptapeptide **10**. Hence, we designed a series of pentapeptidic BACE1 inhibitors based on compound **12** using a computational approach.

2. Docking simulation and design of pentapeptidic BACE1 inhibitors

Early BACE1 inhibitors were designed using the coordinates of the first reported X-ray crystal structure (1FKN) of a complex between BACE1 and OM99-2. OM99-2 has an Asn residue, which corresponded to the P_2 residue of Swedish mutant sequence. As OM99-2 in 1FKN appeared to interact with the BACE1-Arg235 side chain *via* hydrogen bonding, many researchers have designed BACE1 inhibitors that possessed a hydrogen bond receptor, such as an Asn residue, at the P_2 position, using the 1FKN coordinates. However, our peptidic BACE1 inhibitors with an Asn at the P_2 position showed no inhibitory activity, and, as shown in **Table 1**, the P_2 residue that showed potent BACE1 inhibitory activity was a hydrophobic amino acid residue, Leu. Thus, our design strategy seemed to require a fundamental review. We researched the inhibitory mechanism of our peptides using a computational approach. As we found that pentapeptide **21**, which possessed an aminobenzoic acid residue as a bioisostere of the Asp residue at the P_1' position, showed higher inhibitory activity than pentapeptide **12**, we evaluated the series of pentapeptides **14-24** with an aminobenzoic acid residue by using a docking simulation, and then synthesized the compounds (**Table 2**). The docking

simulation was performed using MOE software under the MMFF94x force field. The calculated active sites of BACE1 were depicted in **Figure 2A** using the Alpha SiteFinder application in MOE software and the coordinate set of X-ray crystal structure, 1FKN. The 3D structure of OM99-2 after a docking simulation is shown in **Figure 2B** as a magenta-coloured stick model. Because the moieties from the P_1 to P_4 positions of OM99-2 almost coincided with that of the X-ray crystal structure (aqua coloured stick model) of OM99-2, we performed the docking simulation study using this calculation model. Although the moieties from the P_2' to P_4' positions of OM99-2 assumed a different pose from that of the X-ray crystal structure of OM99-2, their moieties were placed outside the BACE1 enzyme. It is likely that the difference between the X-ray crystal structure and the docking calculation might occur by a packing at the crystallization of BACE1 complex. The results of the docking simulation are shown in **Table 2**. Peptides **14-24** and OM99-2 were scored using the scoring function in the MOE software.



Compound	Xaa	U_ele[1]	U_vdw[2]	U_str[3]	U_dock[4]	BACE1 inhibition (%)[5]
OM99-2 (see **Figure 1**)		−284.7	28.0	14.6	−242.1	—
14	N	−184.4	4.2	10.2	−170.0	28.4
15	M	−196.1	14.8	11.4	−169.9	63.9
16	Y	−195.2	18.5	13.6	−163.1	36.9
17	D	−174.1	2.0	11.2	−160.9	33.2
18	I	−196.3	25.7	11.1	−159.5	65.8
19	F	−176.8	9.0	11.6	−156.2	47.3
20	E	−179.8	11.9	22.3	−145.6	36.3
21	L	−155.1	3.3	10.2	−141.6	83.7
22	W	−187.1	19.6	26.6	−140.9	71.3
23	Q	−148.9	−0.3	9.8	−139.4	14.1
24	Cha[6]	−147.1	−1.2	9.7	−138.6	84.1

[1]Electrostatic energy between BACE1 and inhibitor (kcal/mol).

[2]van der Waals energy between BACE1 and inhibitor (kcal/mol).

[3]Strain energy of inhibitor (kcal/mol).

[4]Docking score (kcal/mol); U_dock = U_ele + U_vdw + U_str.

[5]BACE1 inhibition % at 2 μM.

[6]Cyclohexylalanine (Cha).

Table 2. Docking simulation of pentapeptidic BACE1 inhibitors and their score using the 1FKN X-ray crystal structure.

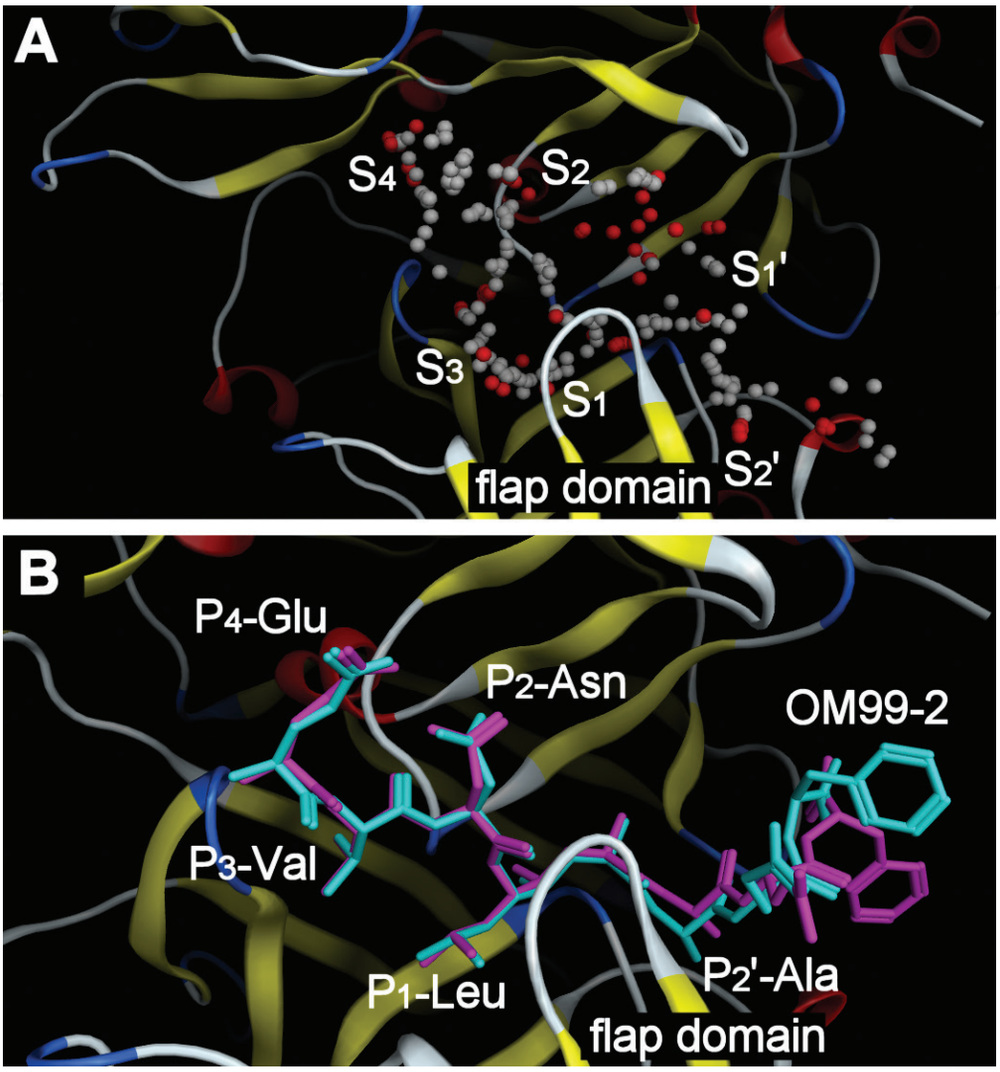


Figure 2. (A) Calculated active sites using the Alpha Site Finder application in MOE software. (B) OM99-2 docked in BACE1. Aqua and magenta colours indicate the X-ray crystal structure 1FKN and the energy-minimized structure under the MMFF94x force field, respectively.

U_ele, U_vdw, and U_str indicated the potential energies of electrostatic interaction, van der Waals interactions between enzyme and ligand, and strain energy of the ligand, respectively, and their sum is a docking score, U_dock. OM99-2 and peptide **14**, which possess an Asn residue at the P_2 position, showed good scores. However, peptides **14** and **17**, that possess a hydrophilic amino acid, such as Asn and Asp, showed a low inhibitory activity. The residues of peptides **21** and **24** that exhibited a high inhibitory activity were hydrophobic amino acids such as Leu and cyclohexylalanine (Cha), as well as the results in **Table 1**. Interestingly, peptides **21** and **24** showed low scores. Thus, there was no association between the scoring values and the inhibitory activity at the P_2 position.

As the docking model using the coordinate set of 1FKN appeared to give an unfavourable score for the BACE1 inhibitor, we compared the publicly available X-ray crystal structures of BACE1-inhibitor complexes. Surprisingly, the guanidine group of BACE1-Arg235 in most

crystal structures, except 1FKN, showed similar figures flopping over the P_2 region of the inhibitors, and the nearest distances between the guanidino-plane of Arg235 side chain and the P_2 region of the inhibitor showed similar values of approximately 3Å [31]. The P_2 moieties in most of the crystal structures found to interact with the BACE1-Arg235 side chain were a methyl group, carbonyl oxygen atom, or aromatic ring, which were bound to the guanidine-plane of Arg235 side chain by CH-π, O-π, or π-π stacking interactions. This suggested that the π-orbital on the guanidino-plane interacted with the P_2 region by a weak quantum force such as stacking or σ-π interaction. The only exception was the interaction in the first reported X-ray crystal structure, 1FKN. The P_2 moiety of OM99-2 in the crystal structure 1FKN appeared to interact with the BACE1-Arg235 side chain *via* hydrogen bonding (**Figure 3A**). OM00-3, which was reported by the same researchers, was an inhibitor that was structurally similar to OM99-2; surprisingly, the P_2-Asp side chain of OM00-3 docked in BACE1 (PDB ID: 1M4H) interacted with the π-orbital on the guanidine-plane of the BACE1-Arg235 side chain *via* O-π interaction (**Figure 3B**). Many early BACE1 inhibitors that possess a hydrogen bond receptor at the P_2 position were designed using the first reported crystal structure 1FKN. However, the hydrogen bonding interaction between most of the inhibitors and the BACE1-Arg235 side chain was not shown in their crystal structures. For instance, the inhibitor from Merck (MSD), crystal structure (PDB ID: 2B8L), interacted with the BACE1-Arg235 side chain *via* a CH-π interaction (**Figure 3C**). The researchers at MSD most likely based their inhibitor on a structure that possessed an *N*-methyl-*N*-methanesulfonyl group at the P_2 position in anticipation of the hydrogen-bonding interaction between the sulfonyl oxygen atom and the BACE1-Arg235 side chain. However, the *N*-methyl group of the MSD inhibitor interacted with the π-orbital on the guanidine-plane of the BACE1-Arg235 side chain at a distance of 2.8 Å. The inhibitor reported by Pfizer (PDB ID: 2P83) appeared to interact with the BACE1-Arg235 side chain *via* O-π interactions, as shown in **Figure 3D**. As seen above, most of the BACE1 inhibitors, except OM99-2 in the crystal structure 1FKN, interacted with the BACE1-Arg235 side chain by a weak quantum force such as stacking or σ-π interaction. The Arg235 side chain of the BACE1-OM99-2 complex (1FKN) assumed an exceptionally different pose to the other crystal structures because the BACE1 complex appears to be stabilized by intramolecular hydrogen-bonding interaction between the P_4-Glu and P_2-Asn side chains of OM99-2 (**Figure 3A**). Because OM00-3 does not form such intramolecular hydrogen-bonding, the P_2 residue of OM00-3 appears to interact with the BACE1-Arg235 side chain by a quantum chemical interaction. As many researchers have designed BACE1 inhibitors with a hydrogen bond receptor on the basis of the first reported crystal structure 1FKN, docking models using 1FKN will require further review. Furthermore, we found that the side chain of BACE1-Arg235 could move in concert with the inhibitor's size. The superimposed figure of four crystal structures (PDB ID: 2B8L, 1M4H, 1W51, and 2IQG) of the complex between BACE1 and the inhibitors is depicted in **Figure 4**. The guanidino-planes of BACE1-Arg235 in the crystal structures of most BACE1 complexes showed similar distances from the P_2 regions of the inhibitors regardless of their molecular size [31]. This fact suggested a serious issue for a docking simulation for the drug discovery of BACE1 inhibitors. However, the BACE1-Arg235 side chain seems to have a restricted range of motion: the BACE1-Arg235 side chain slides sideways, not up and down, along the wall of the β-sheet structure that consists of four peptide strands behind the flap domain of BACE1; therefore, the location of the BACE1-Arg235 side chain could be predicted by the inhibitor's size. As shown in **Figure 4**, the

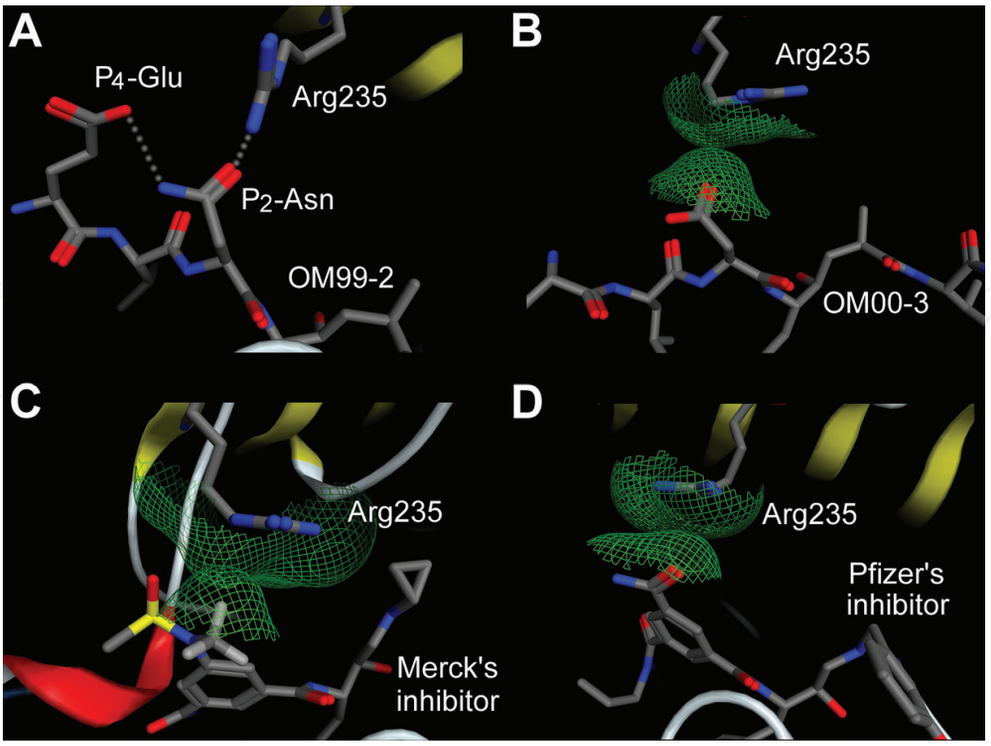


Figure 3. Interaction of BACE1-Arg235 with BACE1 inhibitors in X-ray crystal structures. (A) PDB ID: 1FKN, (B) PDB ID: 1M4H, (C) PDB ID: 2B8L and (D) PDB ID: 2P83.

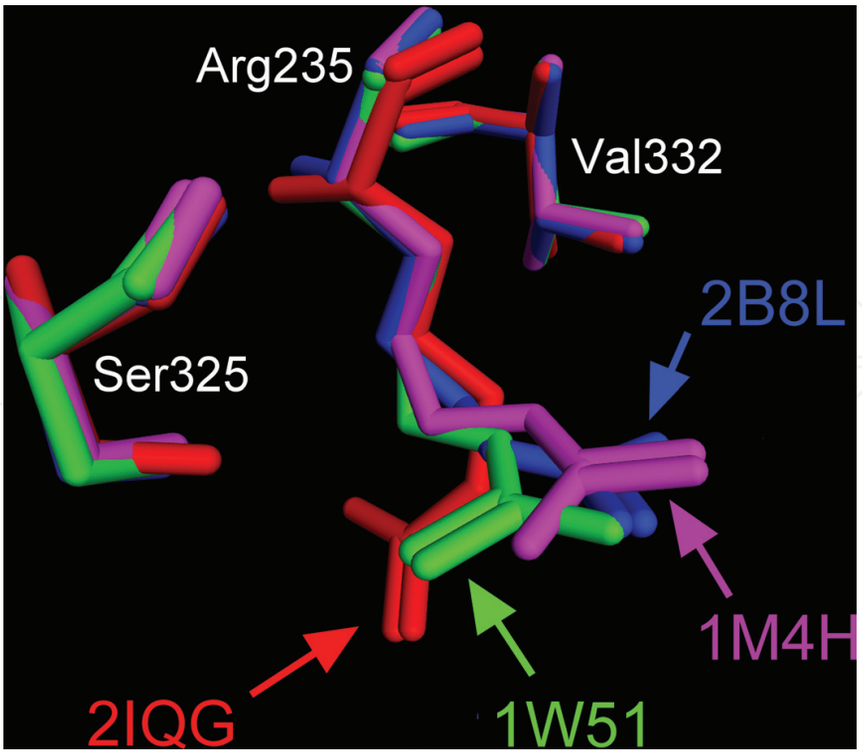
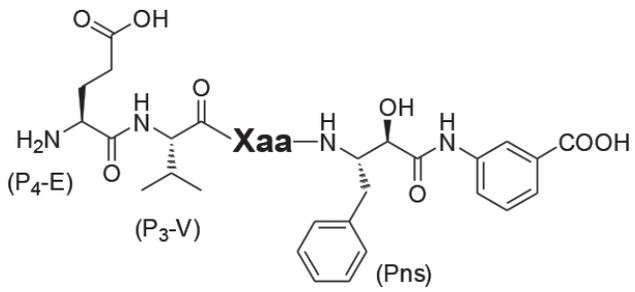


Figure 4. Location of BACE1-Arg235 in the respective crystal structures. The blue, magenta, green and red stick models represent the X-ray crystal structures of the BACE1-inhibitor complexes, 2B8L, 1M4H, 1W51 and 2IQG, respectively.

orbital follows the same pattern along the wall of the β-sheet structure of BACE1 and might be predicted according to the inhibitor's size. We hypothesized that the role of the BACE1-Arg235 side chain was important for the BACE1 inhibitory mechanism. The guanidine-plane of Arg235 that can move in concert with the inhibitor's size appears to push down on the P_2 region of the inhibitor, which caused them to be affixed to the active site of BACE1 because of this 'flop-over' mechanism by the BACE1-Arg235 side chain. Although a quantum chemical force, such as σ-π interaction, has a weaker binding energy than a hydrogen bonding interaction, this 'flop-over' mechanism permits a strong binding mode with the active site of BACE1.

For the reasons mentioned above, we performed a docking calculation using the X-ray crystal structure 1M4H, in which the P_2 moiety of the inhibitor (OM00-3) had a similar size to that of our inhibitor (**Table 3**). OM00-3 appears to show a high docking score value owing to its large molecular size: OM00-3 has many more amide bonds than our peptapeptidic inhibitors, and



Compound	Xaa	U_ele[1]	U_vdw[2]	U_str[3]	U_dock[4]	BACE1 inhibition (%)[5]
OM00-3 (see Figure 1)		−233.4	0.7	15.7	−217.0	—
21	L	−195.7	11.3	10.3	−174.1	83.7
22	W	−189.3	13.0	10.5	−165.8	71.3
18	I	−194.6	18.5	11.1	−165.0	65.8
19	F	−195.6	23.0	11.7	−160.9	47.3
15	M	−195.6	24.6	10.1	−160.9	63.9
24	Cha[6]	−194.4	26.6	11.6	−156.2	84.1
16	Y	−196.7	26.5	14.1	−156.1	36.9
20	E	−190.7	24.2	10.6	−155.9	36.3
14	N	−190.7	24.2	11.3	−155.2	28.4
23	Q	−196.9	27.6	15.7	−153.6	14.1
17	D	−185.5	28.5	9.7	−147.3	33.2

[1]Electrostatic energy between BACE1 and inhibitor (kcal/mol).

[2]van der Waals energy between BACE1 and inhibitor (kcal/mol).

[3]Strain energy of inhibitor (kcal/mol).

[4]Docking score (kcal/mol); U_dock = U_ele + U_vdw + U_str.

[5]BACE1 inhibition % at 2 μM.

[6]Cyclohexylalanine (Cha).

Table 3. Docking simulation of pentapeptidic BACE1 inhibitors and their scoring using the 1M4H X-ray crystal structure.

can closely interact with the BACE1 active site *via* electrostatic energy. The correlation chart between BACE1 inhibitory activities and the docking score values is shown in **Figure 5**, and it indicated a good correlation coefficient ($r = -0.717$). Peptides that possess a hydrophilic amino acid residue at the P_2 position showed low docking score values, which indicated that these P_2 residues cannot interact with the BACE1-Arg235 side chain *via* electrostatic energy in the X-ray crystal structure 1M4H, and the docking score showed a good correlation with BACE1 inhibitory activity as a result. However, the plot of peptide **24** was outside of the correlation line. Peptide **24**, with the bulky amino acid Cha at the P_2 position, might show van der Waals repulsion against the BACE1-Arg235 side chain.

Furthermore, we designed a series of BACE1 inhibitors that possessed one or more bioisosteres of carboxylic acid from pentapeptide **21** that conferred excellent values to both docking score and BACE1 inhibitory activity, in order to develop practical BACE1 inhibitors as drug candidates (**Figure 6**). A tetrazole ring is known as a carboxylic bioisostere. Because it is well known that 5-aminotetrazole was highly explosive, peptides **25** and **26**, which possessed a carboxylic acid bioisostere at the P_4 position were designed and synthesized using tetrazole-5-carboxylic acid. Peptides **25** and **26** showed potent BACE1 inhibitory activities (IC_{50} = 8.2 nM and 3.9 nM, respectively) [24, 25]. Moreover, peptide **27**, which possessed two tetrazole rings on the P_1' ring, was synthesized. Peptide **27** showed the most potent BACE1 inhibitory activity (IC_{50} = 1.2 nM) [26].

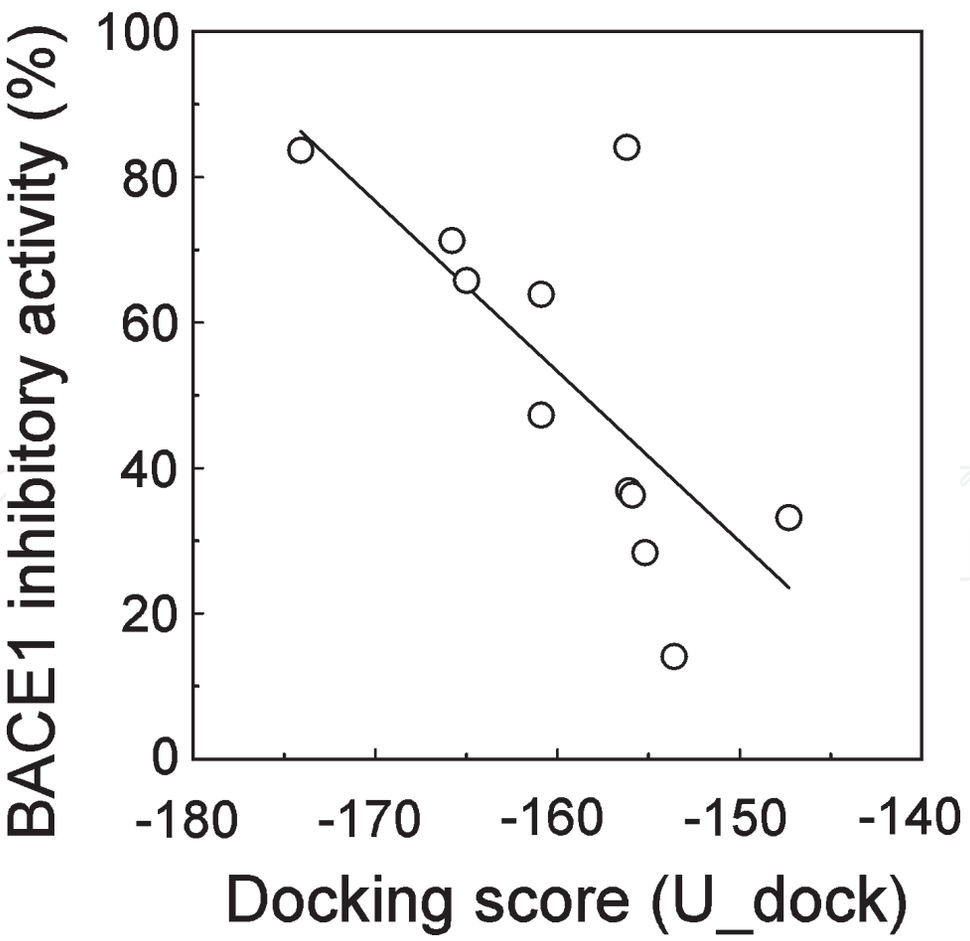
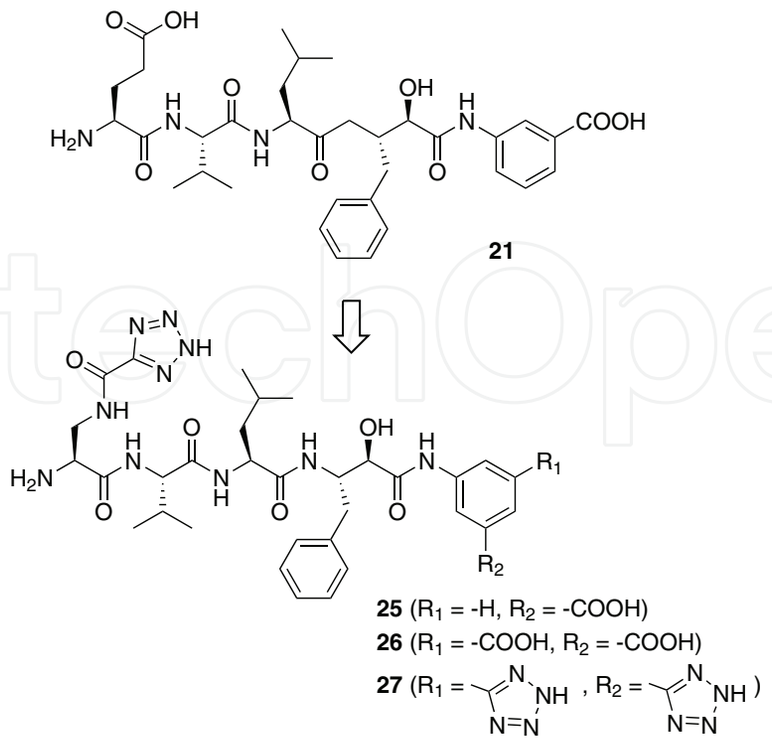


Figure 5. The correlation between BACE1 inhibitory activities and the docking score values.



Compound	BACE1 inhibition (%) at 2 M	BACE1 inhibition (%) at 0.2 M	IC_{50} (nM)
21	83.7	—	330
25	99.1	87.1	8.2
26	100	98.1	3.9
27	100	100	1.2

Figure 6. Design of practical BACE1 inhibitors using a carboxylic acid bioisostere.

3. Design of small-sized non-peptidic BACE1 inhibitors

At present, many non-peptidic BACE1 inhibitors have been discovered. The research of Elan pharmaceuticals, Merck (MSD), Pfizer, and Schering-Plough, and Ghosh et al. reported the BACE1 inhibitors **28**-**32** (IC_{50} = 15, 15, 5, 3, and 1 nM, respectively) with an isophthalic scaffold at the P_2 position, as shown in **Figure 7** [14, 16]. Because the distance between the flap domain and the cleft domain that forms the S_2 pocket of BACE1 was narrow, a planar aromatic ring, such as an isophthalic scaffold, might dock closely in the S_2 pocket of BACE1. Hence, we designed a series of BACE1 inhibitors from the virtual inhibitor **28** (**Figure 8**), in which the P_2 moiety of our peptidic inhibitors was replaced with an isophthalic scaffold [31–35]. First, we focused on the sterically hindered interaction between the P_3 amide and a proton on the P_2-isophthalic ring of the virtual inhibitor, which restricts the configuration. Using the approach '*in-silico* conformational structure-based design' based on a conformer of the docked inhibitor in BACE1, we adopted a pyridinedicarboxylic scaffold as a P_2 moiety, which lacked the 2-proton from the isophthalic ring, and designed inhibitor **29** [32]. Next, we optimized the P_3-region of inhibitor **29**

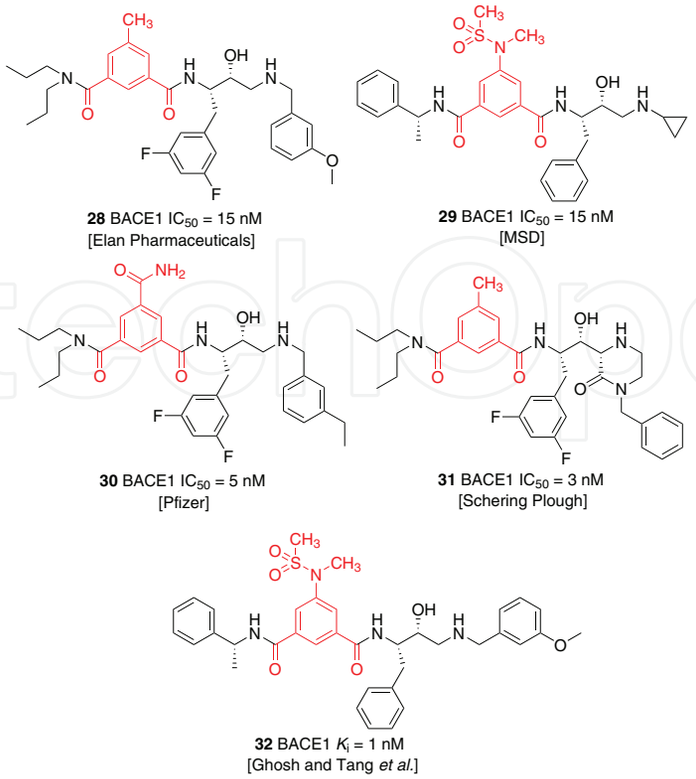


Figure 7. BACE1 inhibitors with an isophthalic scaffold.

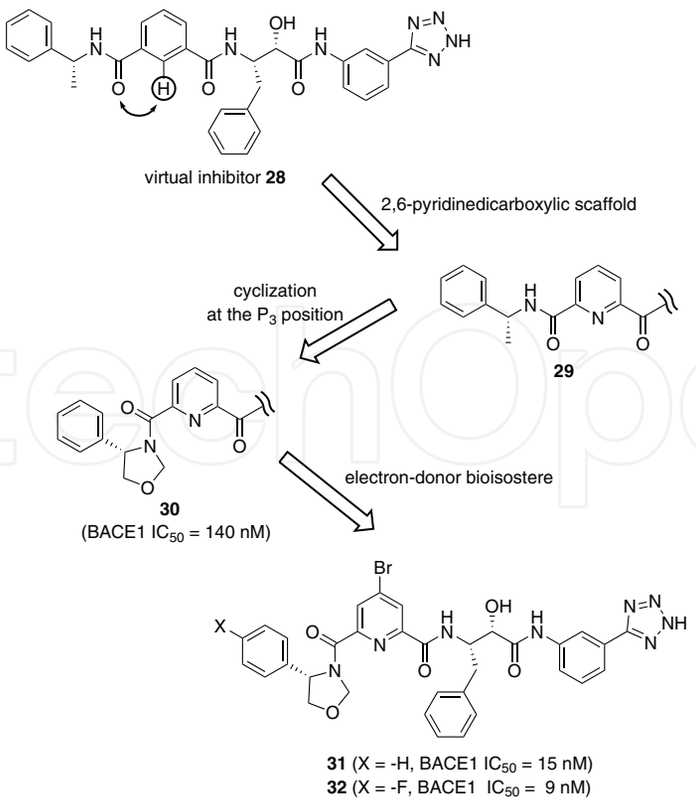


Figure 8. Design of small-sized non-peptidic BACE1 inhibitors with a 2,6-pyridinedicarboxylic scaffold.

with a P_2-pyridinedicarboxylic scaffold. There is a S_3 sub-pocket behind the active site of BACE1, and the P_3 phenyl group of **29** appears to interact with the S_3 sub-pocket. We envisioned that inhibitors with a P_3 benzylamide group assumed a folding pose between the P_2 aromatic scaffold and the P_3 benzylamide, and that the α-methyl group on the P_3 benzylamide of **29** stabilized this folding structure. Hence, we designed inhibitor **30** by the introduction of a five-membered ring, oxazolidine, at the P_3 region to fix the folding structure [32]. The oxazolidine ring fixes the direction of the phenyl ring at the P_3 position, so that the P_3 phenyl ring may be able to bind closely to the S_3 sub-pocket of BACE1. Inhibitor **30** showed moderate BACE1 inhibitory activity (IC_{50} = 140 nM).

Although *in-silico* approaches, such as a docking simulation between an enzyme/receptor and drugs, have contributed greatly to drug discovery research, most docking simulation software programs adopt molecular mechanics/molecular dynamics (MM/MD) calculations based on classical Newtonian mechanics. However, docking simulations using these calculations do not appear to estimate weak quantum chemical interactions, such as stacking or σ-π interaction, between the BACE1-Arg235 side chain and inhibitors. Because the Arg residue is recognized as charged in these software programs, the quantum chemical interactions involving an Arg side chain are unlikely to receive a favourable score. The concept of 'bioisostere' is important for the development of practical drugs. However, in the case of BACE1 inhibitor design, the bioisostere of the P_2 moiety, according to the Swedish-mutant APP, is an Asn or an amide residue based on a classical bioisostere concept that does not assume quantum chemical interactions. Therefore, inhibitors that can interact with the Arg235 side chain on the basis of a quantum chemical interaction could never be designed using such a classical concept. The researchers at Bristol-Myers Squibb (BMS) reported a series of BACE1 inhibitors that can interact with the BACE1-Arg235 side chain by π-π stacking. According to their SAR study, the introduction of an electron-donating methoxy group to the *p*-position of the phenyl ring that interacts with the BACE1-Arg235 side chain enhanced BACE1 inhibitory activity. This indicated that an inhibitor that possessed a P_2 aromatic ring with higher electron density could strongly dock to the active site of BACE1 that has an electron-poor π-orbital on the guanidinoplane of the BACE1-Arg235 side chain. Hence, we proposed a new concept of the 'electron-donor bioisostere', which can interact with an electron-poor π-orbital, such as the guanidine group of Arg235, by quantum chemical interactions [14].

Based on the electron-donor bioisostere concept, we speculated that an electron-rich halogen atom could interact with the electron-poor guanidine π-orbital by Coulomb interaction. Using the *ab initio* molecular orbital approach, Imai et al. indicated that the calculated Cl-π interaction energy was slightly stronger than that of CH-π interaction, and its energy was affected by π-electron density [36]. Inhibitor **31**, which possessed a halogen atom on the P_2 aromatic scaffold, exhibited potent inhibitory activity (IC_{50} = 15 nM). Moreover, inhibitor **32**, which possessed a fluorine atom on the *p*-position of the P_3 phenyl group exhibited the most potent inhibitory activity (IC_{50} = 9 nM), and was available from Wako Pure Chemical Industries (Japan) as a reagent for biological research [31]. The drastic improvement in the inhibition of BACE1 following the introduction of a halogen atom into the P_2 position of our compounds appears to support our hypothesis; namely, the quantum chemical interactions between BACE1 and its inhibitors play a critical role in the mechanism of BACE1 inhibition.

4. Conclusion

Although we calculated the docking scoring values by a docking calculation between BACE1 and its inhibitors using the first reported X-ray crystal structure 1FKN, we found no association between the scoring values and BACE1 inhibition. We found that a specific interaction, a quantum chemical interaction between the Arg235 side chain and the P_2 region of the inhibitor, played a critical role in the inhibitory mechanism of BACE1. Whereas most BACE1 inhibitors, except OM99-2, interacted with BACE1-Arg235 by a quantum chemical interaction, such as stacking and σ-π interaction, many early BACE1 inhibitors were designed using the 1FKN coordinate set. As the crystal structure 1FKN showed a hydrogen bonding between the BACE1-Arg235 side chain and OM99-2, the early studies on BACE1 inhibitor design might have misdirected, as a docking simulation using 1FKN appears to be meaningless. In fact, unlike 1FKN, there is no hydrogen bonding interaction present in most of the X-ray crystal structures. We selected the peptide sequence that showed potent inhibitory activity by a docking simulation using the X-ray crystal structure 1M4H, and designed potent peptidic BACE1 inhibitors with one or more carboxylic acid bioisosteres. Moreover, we focused on a quantum chemical interaction, and designed the potent non-peptidic BACE1 inhibitor **32** using the 'electron-donor bioisostere concept' that we have proposed. Our findings indicated the importance of the X-ray crystal structure in computational drug design.

Acknowledgements

This study was supported in part by the Grants-in-Aid for Scientific Research from MEXT (Ministry of Education, Culture, Sports, Science and Technology), Japan (KAKENHI No. 23590137 and No. 26460163), and a donation from Professor Emeritus Tetsuro Fujita of Kyoto University. At the time of writing, we received word that Prof. Fujita had passed away on January 1, 2017. Prof. Fujita was the teacher of one of the authors, Y. Hamada, and was known as the inventor of a treatment agent for multiple sclerosis. We dedicate this article to Prof. Fujita.

Author details

Yoshio Hamada[1]* and Yoshiaki Kiso[2]

*Address all correspondence to: pynden@gmail.com

1 Faculty of Frontier of Innovative Research in Science and Technology, Konan University, Japan

2 Laboratry of Peptide Science, Nagahama Institute of Bio-Science and Technology, Japan

References

[1] Selkoe DJ. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid β-protein. Annals of the New York Academy of Sciences. 2000;**924**:17-25. DOI: 10.1111/j.1749-6632.2000.tb05554.x

[2] Selkoe DJ. The deposition of amyloid proteins in the aging mammalian brain: Implications for Alzheimer's disease. Annals of Medicine. 1989;**21**:73-76. DOI: 10.3109/07853898909149187

[3] Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature. 1999;**399**:A23-A31. DOI: 10.1038/399a023

[4] Sinha S, Lieberburg I. Cellular mechanisms of β-amyloid production and secretion. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**:11049-11053. DOI: 10.1073/pnas.96.20.11049

[5] Vassar R, Bennett BD, Babu-Khan S, et al. β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999:**286**;735-741. DOI: 10.1523/JNEUROSCI.3657-09.2009

[6] Hussain I, Powell D, Howlett DR, et al. Identification of a novel aspartic protease (Asp 2) as β-secretase. Neuroscience. 1999:**14**;419-427. DOI: 10.1523/JNEUROSCI.3657-09.2009

[7] Yan R, Bienkowski MJ, Shuck ME, et al. Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. Nature. 1999:**402**;533-537. DOI: 10.1038/990107

[8] Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragment in regulating neural activity, plasticity and memory. Progress in Neurobiology. 2003:**70**;1-32. DOI: 10.1016/S0301-0082(03)00089-3

[9] Roberds SL, Anderson J, Basi G, et al. BACE knockout mice are healthy despite lacking the primary β-secretase activity in brain: Implications for Alzheimer's disease therapeutics. Human Molecular Genetics. 2001:**10**;1317-1324. DOI: 10.1093/hmg/10.12.1317

[10] Hamada Y, Kiso Y. New directions for protease inhibitors directed drug discovery. Biopolymers. 2016:**106**;563-579. DOI: 10.1002/bip.22780

[11] Hamada Y, Kiso Y. Aspartic protease inhibitors as drug candidates for treating various difficult-to-treat diseases. In: Ryadnov M, Farkas E, editors. Amino Acids, Peptides and Proteins. Vol. 39. London: Royal Society of Chemistry; 2015. pp. 114-147. DOI: 10.1039/9781849739962-00114

[12] Hamada Y. Drug discovery of b-secretase inhibitors based on quantum chemical interactions for the treatment of Alzheimer's disease. SOJ Pharmacy & Pharmaceutical Sciences. 2014:**1**;1-8. DOI: 10.15226/2374-6866/1/3/00118

[13] Hamada Y, Kiso Y. Recent progress in the drug discovery of non-peptidic BACE1 inhibitors. Expert Opinion on Drug Discovery. 2009:**4**;391-416. DOI: 10.1517/17460440902806377

[14] Hamada Y, Kiso Y. The application of bioisosteres in drug design for novel drug discovery: Focusing on acid protease inhibitors. Expert Opinion on Drug Discovery. 2012:**7**;903-922. DOI: 10.1517/17460441.2012.712513

[15] Hamada Y, Kiso Y. Advances in the identification of β-secretase inhibitors. Expert Opinion on Drug Discovery. 2013:**8**;709-731. DOI: 10.1517/17460441.2013.784267

[16] Nguyen J-T, Hamada Y, Kimura T, Kiso Y. Design of potent aspartic protease inhibitors to treat various diseases. Archiv der Pharmazie—Chemistry in Life Sciences. 2008:**341**;523-535. DOI: 10.1002/ardp.200700267

[17] Sinha S, Anderson JP, Barbour R, et al. Purification and cloning of amyloid precursor protein β-secretase from human brain. Nature. 1999:**402**;537-540. DOI: 10.1038/990114

[18] Ghosh AK, Shin D, Downs D, et al. Design of potent inhibitors for human brain memapsin 2 (β-secretase). Journal of the American Chemical Society. 2000:**122**;3522-3523. DOI: 10.1021/ja000300g

[19] Hong L, Koelsch G, Lin X, et al. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. Science. 2000:**290**;150-153. DOI: 10.1126/science.290.5489.150

[20] Ghosh AK, Bilcer G, Harwood, C et al. Structure-based design: Potent inhibitors of human brain memapsin 2 (β-secretase). Journal of Medicinal Chemistry. 2001:**44**;2865-2868. DOI: 10.1021/jm0101803

[21] Ghosh AK, Devasamudram T, Hong L, et al. Structure-based design of cycloamide-urethane-derived novel inhibitors of human brain memapsin 2 (β-secretase). Bioorganic & Medicinal Chemistry Letters. 2005:**15**;15-20. DOI: 10.1016/j.bmcl.2004.10.084

[22] Shuto D, Kasai K, Hidaka K, et al. KMI-008, a novel β-secretase inhibitor containing a hydroxymethylcarbonyl isostere as a transition-state mimic: Design and synthesis of substrate-based octapeptides. Bioorganic & Medicinal Chemistry Letters. 2003:**13**;4273-4276. DOI: 10.1016/j.bmcl.2003.09.053

[23] Kimura T, Shuto D, Kasai K, et al. KMI-358 and KMI-370, highly potent and small-sized BACE1 inhibitors containing phenylnorstatine. Bioorganic & Medicinal Chemistry Letters. 2004:**14**;1527-1531. DOI: 10.1016/j.bmcl.2003.12.088

[24] Kimura T, Shuto D, Hamada Y, et al. Design and synthesis of highly active Alzheimer's β-secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability. Bioorganic & Medicinal Chemistry Letters. 2005:**15**;211-215. DOI: 10.1016/j.bmcl.2004.09.090

[25] Asai M, Hattori C, Iwata N, et al. The novel β-secretase inhibitor KMI-429 reduces amyloid beta peptide production in amyloid precursor protein transgenic and wild-type mice. Journal of Neurochemistry. 2006:**96**;533-540. DOI: 10.1111/j.1471-4159.2005.03576.x

[26] Kimura T, Hamada Y, Stochaj M, et al. Design and synthesis of potent β-secretase (BACE1) inhibitors with P1′ carboxylic acid bioisostere. Bioorganic & Medicinal Chemistry Letters. 2006:**16**;2380-2386. DOI: 10.1016/j.bmcl.2006.01.108

[27] Hamada Y, Igawa N, Ikari H, et al. β-Secretase inhibitors: Modification at the P_4 position and improvement of inhibitory activity in cultured cells. Bioorganic & Medicinal Chemistry Letters. 2006:**16**;4354-4359. DOI: 10.1016/j.bmcl.2006.05.046

[28] Hamada Y, Abdel-Rahman H, Yamani A, et al. BACE1 inhibitors: Optimization by replacing the P_1′ residue with non-acidic moiety. Bioorganic & Medicinal Chemistry Letters. 2008:**18**;1649-1653. DOI: 10.1016/j.bmcl.2008.01.058

[29] Tagad HD, Hamada Y, Nguyen J-T, et al. Design of pentapeptidic BACE1 inhibitors with carboxylic acid bioisosteres at P_1' and P_4 positions. Bioorganic & Medicinal Chemistry. 2010:**18**;3175-3186. DOI: 10.1016/j.bmc.2010.03.032

[30] Tagad HD, Hamada Y, Nguyen J-T, et al. Structure-guided design and synthesis of P_1′ position 1-phenylcycloalkylamine-derived pentapeptidic BACE1 inhibitors. Bioorganic & Medicinal Chemistry. 2011:**19**;5238-5246. DOI: 10.1016/j.bmc.2011.07.002

[31] Hamada Y, Ohta H, Miyamoto N, et al. Significance of interaction of BACE1-Arg235 with its ligands and design of BACE1 inhibitors with P_2 pyridine scaffold. Bioorganic & Medicinal Chemistry Letters. 2009:**19**;2435-2439. DOI: 10.1016/j.bmcl.2009.03.049

[32] Hamada Y, Ohta H, Miyamoto N, et al. Novel non-peptidic and small-sized BACE1 inhibitors. Bioorganic & Medicinal Chemistry Letters. 2008:**18**;1654-1658. DOI: 10.1016/j.bmcl.2008.01.056

[33] Hamada Y, Nakanishi T, Suzuki K, et al. Novel BACE1 inhibitors possessing a 5-nitroisophthalic scaffold at the P_2 position. Bioorganic & Medicinal Chemistry Letters. 2012:**22**;4640-4644. DOI: 10.1016/j.bmcl.2012.05.089

[34] Suzuki K, Hamada Y, Nguyen J-T, et al. Novel BACE1 inhibitors with a non-acidic heterocycle at the P_1′ position. Bioorganic & Medicinal Chemistry. 2013:**21**;6665-6673. DOI: 10.1016/j.bmc.2013.08.016

[35] Hamada Y, Suzuki K, Nakanishi T, et al. Structure-activity relationship study of BACE1 inhibitors possessing a chelidonic or 2,6-pyridinedicarboxylic scaffold at the P2 position. Bioorganic & Medicinal Chemistry Letters. 2014:**24**;618-623. DOI: 10.1016/j.bmcl.2013.12.007

[36] Imai YN, Inoue Y, Nakanishi I, Kitaura K. Cl–π interactions in protein–ligand complexes. Protein Science. 2008:**17**;1129-1137. DOI: 10.1110/ps.033910.107

IntechOpen