Chapter 2

Role of Pyridines in Medicinal Chemistry and Design of BACE1 Inhibitors Possessing a Pyridine Scaffold

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Additional information is available at the end of the chapter

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Abstract

Pyridine is a unique aromatic ring. Although pyridines are used industrially, pyridine moieties are present in many natural products, such as vitamins, coenzymes, and alkaloids, and also in many drugs and pesticides. Pyridine moieties are often used in drugs because of their characteristics such as basicity, water solubility, stability, and hydrogen bond-forming ability, and their small molecular size. Because pyridine rings are able to act as the bioisosteres of amines, amides, heterocyclic rings containing nitrogen atoms, and benzene rings, their replacement by pyridine moieties is important in drug discovery. Recently, we synthesized a series of BACE1 inhibitors by in silico conformational structure-based drug design and found an important role of pyridine moiety as a scaffold. In this chapter, we describe the important role of pyridines in medicinal chemistry and the development of β-secretase inhibitors possessing a pyridine scaffold for the treatment of Alzheimer's disease.

Keywords: Alzheimer's disease, BACE1 inhibitor, bioisostere, drug design, *in silico* conformational structure-based design

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. AD is characterized by progressive intellectual deterioration. In 1906, Alois Alzheimer, a psychiatrist and a neuropathologist, reported on a 51-year-old female at the Frankfurt Asylum. The patient showed strange behavioral symptoms and loss of short-term memory, which was later called "AD." The cause of AD has only been clarified relatively recently, and there have been no therapeutic agents 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-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. We designed a series of peptidomimetic inhibitors possessing a substrate transition-state analog. We followed this with the design of nonpeptidic BACE1 inhibitors possessing a pyridine scaffold, using an approach based on a conformer of the docked ligand in the target biomolecule—the "*in-silico* conformational structure-based design." In this process, we noticed an important and third role of pyridines in medicinal chemistry. Pyridines are contained in many natural products, such as vitamins, coenzymes, and alkaloids. Pyridine moieties are often used in drugs and pesticides because of characteristics that include basicity, water solubility, stability, hydrogen bond-forming ability, and small molecular size. In this chapter, the conventional roles of pyridine in medicinal chemistry are described. We also introduce another role using our example regarding the design of BACE1 inhibitors.

2. Conventional roles of pyridine in medicinal chemistry

Pyridine rings are present in many natural products including vitamins such as niacins and vitamin B_6, coenzymes such as nicotinamide adenine dinucleotide (NAD), and alkaloids such as trigonelline. Trigonelline is an alkaloid that is the product of niacin metabolism. Many drugs and pesticides contain a pyridine moiety. Examples include antimicrobial agents, antiviral agents, antioxidants, antidiabetic agents, anti-malarial agents, anti-inflammatory agents, psychopharmacological antagonists, and antiamoebic agents [1]. These pyridine moieties play critical roles in medicinal chemistry because of their abovementioned characteristics. One role of pyridine in medicinal chemistry is to improve water solubility because of its weak basicity. Although many drugs and pesticides possessing a pyridine ring had been designed for improved water solubility, this improvement is often pH-dependent. For example, the sulfa drug, sulfapyridine **1** (**Figure 1A**) has good antibacterial activity and water solubility under acidic conditions, but there is a risk of crystallization in the bladder or urethra, which leads to pain or blockage of the urethra. The conjugate of sulfapyridine and 5-aminosalicylic acid by an azo bond is the compound called sulfasalazine **2** (**Figure 1A**). It displays good water solubility and is used in clinical practice in the treatment of rheumatoid arthritis, ulcerative colitis, and Crohn's disease [2]. When the parent compound cannot be substituted with a pyridine ring, there is an alternative solution—water-soluble prodrug. Water-soluble prodrug **3b** (isavuconazonium sulfate) of an antifungal agents is shown in **Figure 1B** [3]. Prodrug **3b** hydrolyzed by an esterase to release an intermediate **4b**. Prodrug **4b** can spontaneously release the parent drug, isavuconazole **5**, in physiological conditions. Prodrug **3b**, which possesses a pyridine ring, displays good water solubility (>100 mg/mL) compared to prodrug **3a** (>10 mg/mL) that lacks pyridine ring. Prodrug **3b** was approved as an oral medicine by the United States Food and Drug Administration (FDA) in 2015.

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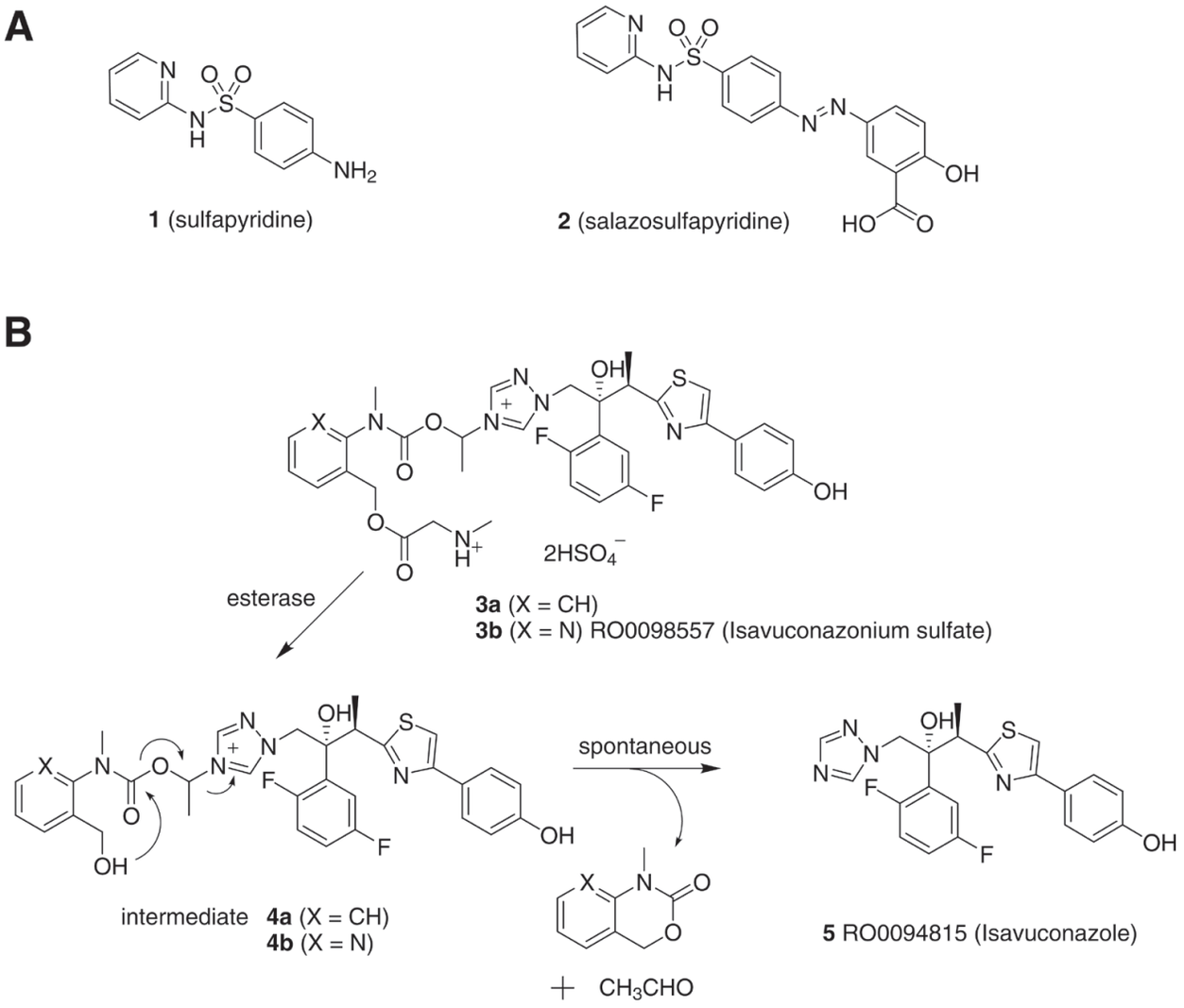

Figure 1. (A) Drugs with improved water solubility. (B) Isavuconazonium sulfate, a water-soluble prodrug possessing a pyridine ring.

Bioisosteres have an important role in the pyridine ring for medicinal chemistry [4]. Bioisosteres are functional or atomic groups with similar physiochemical properties to the parent functional/atomic groups.

Compounds associated with them exhibit similar biological or physiochemical properties as the parent compound. In medicinal chemistry, a portion of a candidate drug is replaced with other functional/atomic groups with the goal of improving drug efficacy, *in vivo* stability, oral absorption, membrane permeability, and absorption, distribution, metabolism and excretion (ADME). Among the approaches used for drug discovery research, the modification of drug candidates by their corresponding bioisosteres is the first choice in drug design studies. Because pyridine is a unique aromatic ring that features a small molecular size, weak basicity, and good stability, pyridine rings had been used as the bioisostere for other heterocyclic aromatic rings, benzene rings, amides, and amines [4]. Especially, pyridines are often replaced with monocyclic aromatic rings, such as benzenes, imidazoles, pyrrole, and oxazole rings,

because of their same molecular size as the pyridine ring. Some drugs with a pyridine ring as a bioisostere of imidazole and benzene ring are presented in **Figure 2**. Histamine has an amino group and an imidazole ring. Thus, histamine receptor antagonists with the respective bioisosteres of the amino group and an imidazole ring have been designed. Cimetidine **6** (**Figure 2A**) is an H_2 receptor antagonist, which has an imidazole ring and a guanidine derivative as the analog of an amino group [5]. The H_2 receptor belongs to the rhodopsin-like family of G protein-coupled receptors. Because the H_2 receptor stimulates gastric acid secretion, its antagonists such as cimetidine are used in the treatment of heartburn and peptic ulcers. Mepyramine **7** (**Figure 2A**) has a pyridine ring as a bioisostere of the imidazole ring. The compound has histamine H_1 receptor antagonist activity [5]. Histamine receptor H_1 is expressed in smooth muscles, on vascular endothelial cells, in the heart, and in the central nervous system. H_1 receptor antagonists are used as antiallergy drugs. Some histamine antagonists in which imidazole ring of the ligand is replaced with a bulky aromatic ring such as doxepin [6] display H_1 receptor antagonist activity, indicating that this ligand site appears to decide the affinity toward the histamine receptor subtypes.

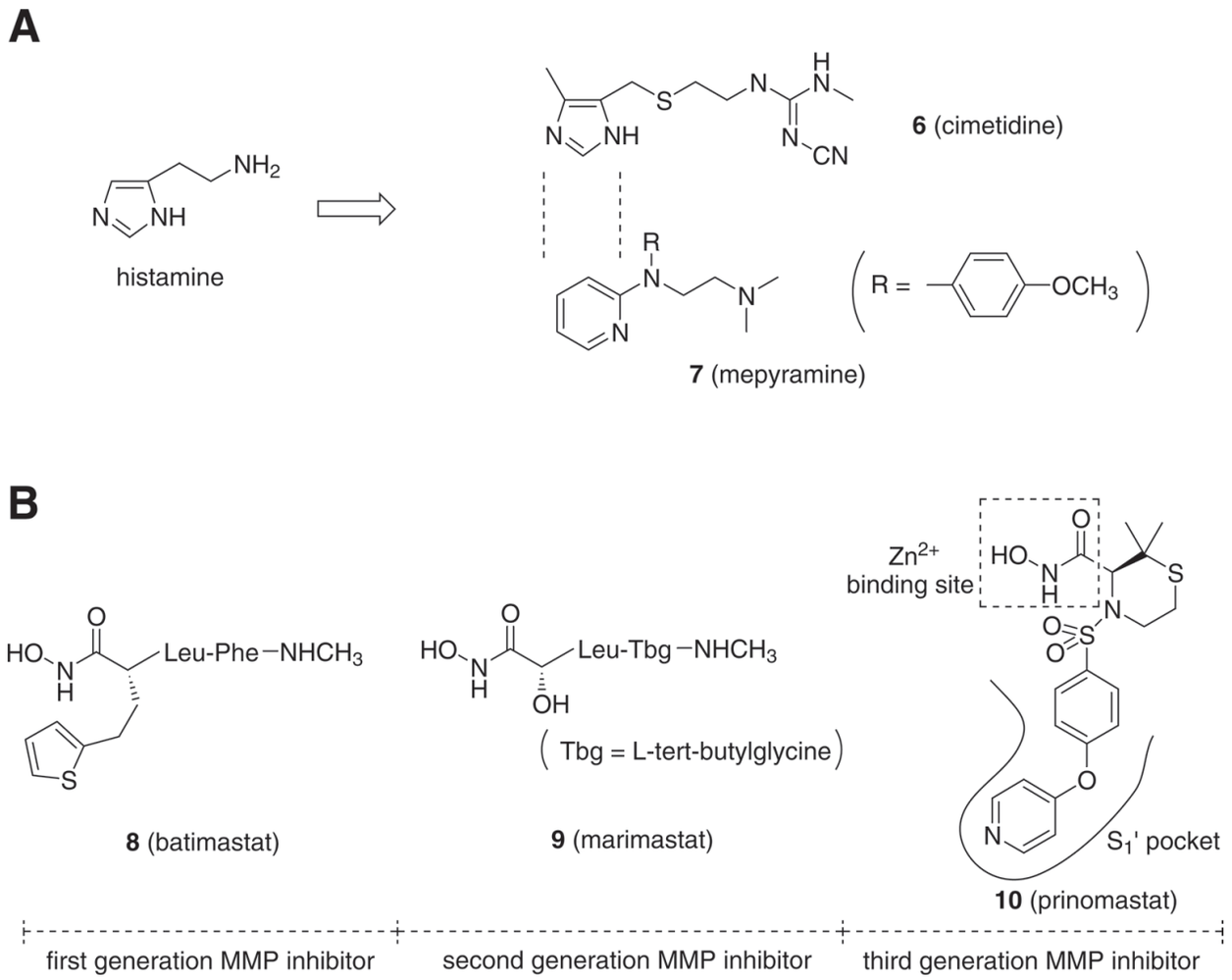

Figure 2. (A) Histamine and histamine receptor antagonists and (B) matrix metalloproteinase (MMP) inhibitors.

Matrix metalloproteinases (MMPs) are calcium- and zinc-containing endopeptidases that have diverse roles in cell behaviors including cell proliferation, migration, and differentiation. Some MMP family subtypes, which include MMP2, MMP3, and MMP9, can degrade the extracellular matrix, resulting in the accelerated infiltration and migration of cancer cells. Inhibitors of some MMP subtypes had been reported for anticancer activity. Most MMP inhibitors have a hydroxamic acid that can bind to the zinc-containing sites of MMPs [7]. The second-generation MMP inhibitors such as batimastat **8** have a more potent inhibitory activity than that of the first-generation MMP inhibitors such as marimastat **9**. (**Figure 2B**), but their selectivity against MMP subtypes is insufficient. The third-generation MMP inhibitor prinomastat **10** has a pyridine ring at the P_1' position, which improves the selectivity to MMP2 and MMP9. A deep hydrophobic pocket corresponding to the S_1' sites is located near the zinc-binding site of MMP, and an alkyl or phenyl groups of amino acids of inhibitors can bind to the S_1' pocket. Researchers at Shionogi & Company Limited reported that the biphenyl group of a third-generation MMP inhibitor, BPHA, can interact tightly with the deep S_1' pocket [8]. The pyridine ring of prinomastat appears to behave as a bioisostere of the benzene ring. As stated earlier, pyridines had been used in medicinal chemistry because of their unique properties, such as weak basicity, water solubility, *in vivo*/chemical stability, hydrogen bond-forming ability, or small molecular size.

3. Design of BACE1 inhibitors

3.1. Pathology of AD and design of peptidomimetic inhibitors

AD is the most common cause of dementia. Its cause has been unclear. A breakthrough was made through the genetic study of some familial AD (FAD) patients with a mutation of the gene encoding amyloid precursor protein (APP) or presenilin gene. As these mutations caused an increase in Aβs that are the main components of senile plaques in the brain of patients with AD, it indicates their involvement in the pathogenesis of AD [9–12]. Aβs are produced from APP by two processing enzymes, β-secretase (BACE1; β-site APP-cleaving enzyme 1) and γ-secretase, which are potential molecular targets for anti-AD drugs [13–16]. The cleavage sites of APP are shown in **Figure 3A**. The full-length APP (APP770) and its isoforms, APP695 and APP751, result from the alternative splicing of its mRNA. BACE1 is a type I transmembrane aspartic protease with 501 amino acids, which triggers Aβ formation in the rate-limiting first step by cleaving at the N-terminus (β-site) of the Aβ domain of APP. Next, the aspartic protease, γ-secretase, cleaves at the C-terminus of the Aβ domain, releasing Aβs that consists mainly of two molecular species, $Aβ_{1–42}$ and $Aβ_{1–40}$. γ-Secretase cleaves two cleavage sites "γ-sites" forming $Aβ_{1–40}$ and $Aβ_{1–42}$. Two processing enzymes, BACE1 and γ-secretase, are categorized as aspartic proteases. They have an acidic optimum pH. Furthermore, BACE1 and γ-secretase, and their substrate, APP, are located in the same intracellular granules, such as endosomes and the trans-Golgi network, which have an acidic environment, suggesting that Aβs are produced in these locations [17]. $Aβ_{1–42}$ displays more potent neurotoxicity and aggregation behavior than $Aβ_{1–40}$ and appears to be critical in the pathogenesis of AD. By contrast,

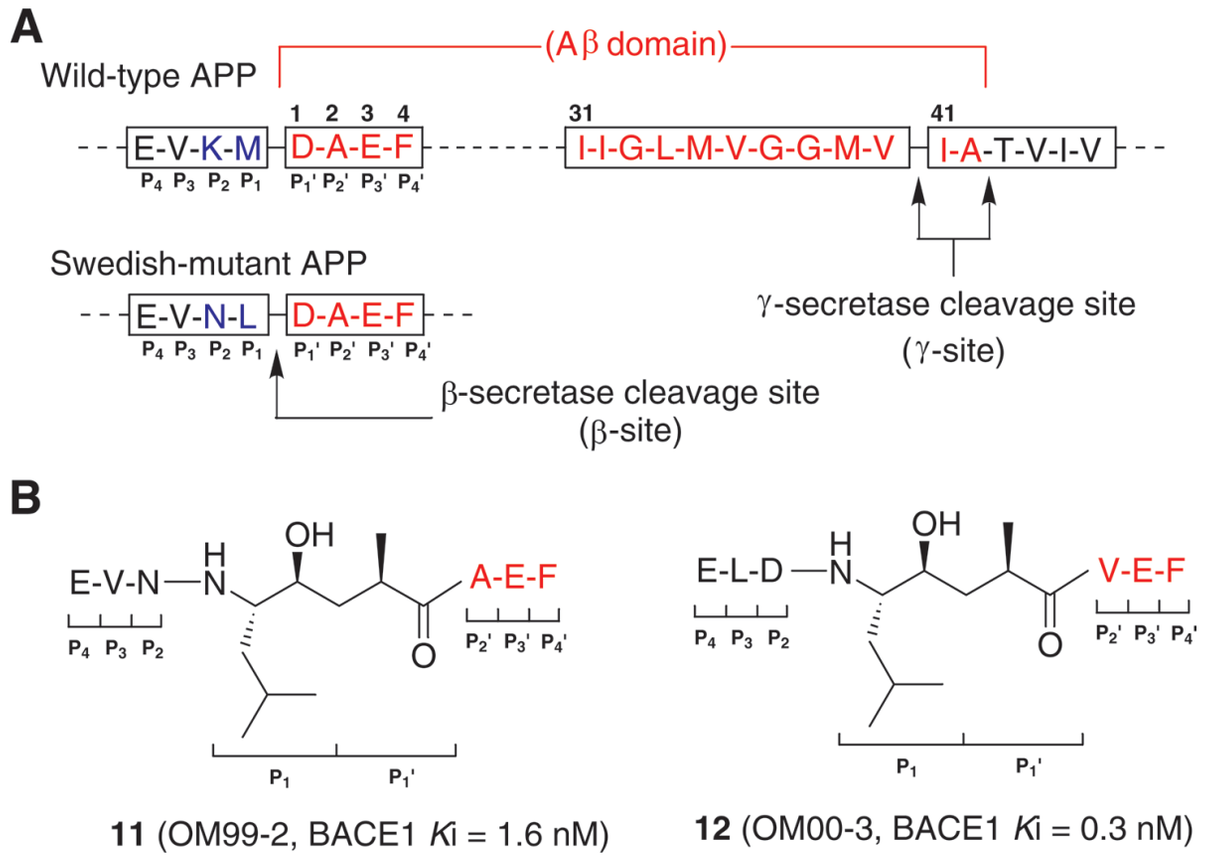

Figure 3. (A) Amyloid precursor protein (APP) and its cleavage site and (B) peptidomimetic BACE1 inhibitors designed on the basis of Swedish-mutant APP sequence by Ghosh et al.

α-secretase is a disintegrin and metalloprotease (ADAM) family metalloprotease, for example, ADAM9, ADAM10, and TNF-α-converting enzyme (TACE, also known as ADAM17), which cleaves APP at the α-site between Lys16 and Leu17 in the Aβ domain [17]. A homolog enzyme of BACE1, BACE2, cleaves at two sites (θ-sites) between Phe19 and Phe20, and between Phe20 and Ala21 in the Aβ domain [18]. Because the α-site and θ-sites are located at the center of the Aβ domain, their cleavage does not lead to Aβ production. According to the amyloid hypothesis, BACE1 and γ-secretase are the molecular targets for 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, its inhibition appears to lead to serious side effects. The fact that BACE1 knockout transgenic mice can survive normally has provided a promising road map, in which BACE1 is a molecular target for the development of AD drugs [19].

As BACE1 is an aspartic protease, early BACE1 inhibitors are peptidomimetic with a substrate transition-state analog. They were designed on the basis of an inhibitor design approach as well as other aspartic proteases such as renin and human immunodeficiency virus protease [20–26]. Many mutations in the APP gene that affect Aβ formation, $Aβ_{1–42}/Aβ_{1–40}$ ratio or Aβ toxicity have been reported. Among them, the Swedish mutation (K670 N, M671 L double mutation, **Figure 3A**) around the β-site induces β-cleavage by BACE1, increasing the $Aβ_{1–42}$ and $Aβ_{1–40}$ levels in the brains of AD patients. Because the Swedish-mutant APP is cleaved faster than the wild-type APP, early BACE1 inhibitors were designed on the basis of the

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Swedish-mutant APP amino acid sequence. In 1999, Sinha et al. at Elan Pharmaceuticals succeeded in purifying BACE1 from the human brain using a transition-state analog based on the Swedish-mutant sequence and cloned the BACE1 enzyme [16]. In 2000 and 2001, Ghosh and Tang described the potent inhibitors, compounds **11** (OM99–2, *K*i = 1.6 nM) and **12** (OM00–3, *K*i = 0.3 nM) with a transition-state analog corresponding to a dipeptide unit at the P_1–P_1' positions of APP (**Figure 3B**) and provided the first X-ray crystal structure (PDB ID: 1FKN) of a complex between recombinant BACE1 and the inhibitor OM99–2 [27–30].

We have also reported a series of peptidomimetic BACE1 inhibitors possessing a norstatine-type transition-state analog, phenyl norstatine (Pns: (*2R,3S*)-3-amino-2-hydroxy-4-phenylbutyric acid), at the P_1 position as shown in **Figure 4** [31–38]. These inhibitors have a Glu bioisostere at the P_4 position and a C-terminus anilide substituted by an acidic group corresponding to the Asp residue at the P_1' position of the APP sequence. Among the compounds, **13** (KMI-429, IC_{50} = 3.9 nM) effectively inhibits BACE1 activity in cultured cells and significantly reduces Aβ production *in vivo* when directly administered into the hippocampi of APP transgenic and wild-type mice [31, 32]. The most potent inhibitor, compound **14** (KMI-684, IC_{50} = 1.2 nM) features two carboxylic acid residues of KMI-429 at the P_1' position that have been replaced with their bioisostere, a tetrazolyl ring [33]. Compound **15** (KMI-574, IC_{50} = 5.6 nM), which possesses a 5-fluoroortyl group in the N-terminus residue, displays improved inhibition in cultured cells because of improved cell membrane permeability [34].

3.2. Design of nonpeptidic BACE1 inhibitors with a pyridine scaffold

We designed and synthesized nonpeptidic BACE1 inhibitors from our peptidic BACE1 inhibitors **13**–**15** as lead compounds [20–25]. Researchers at MSD, Elan, and Pfizer, and Gosh et al. reported a series of BACE1 inhibitors possessing an isophthalic scaffold at the P_2 position [23, 25].

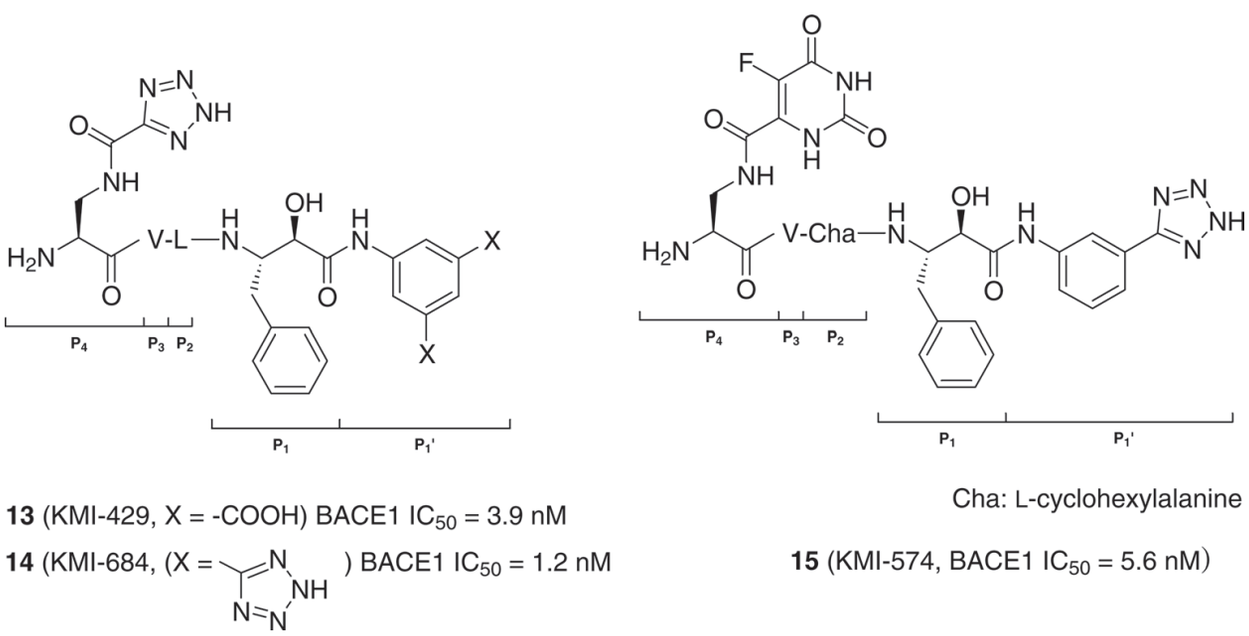
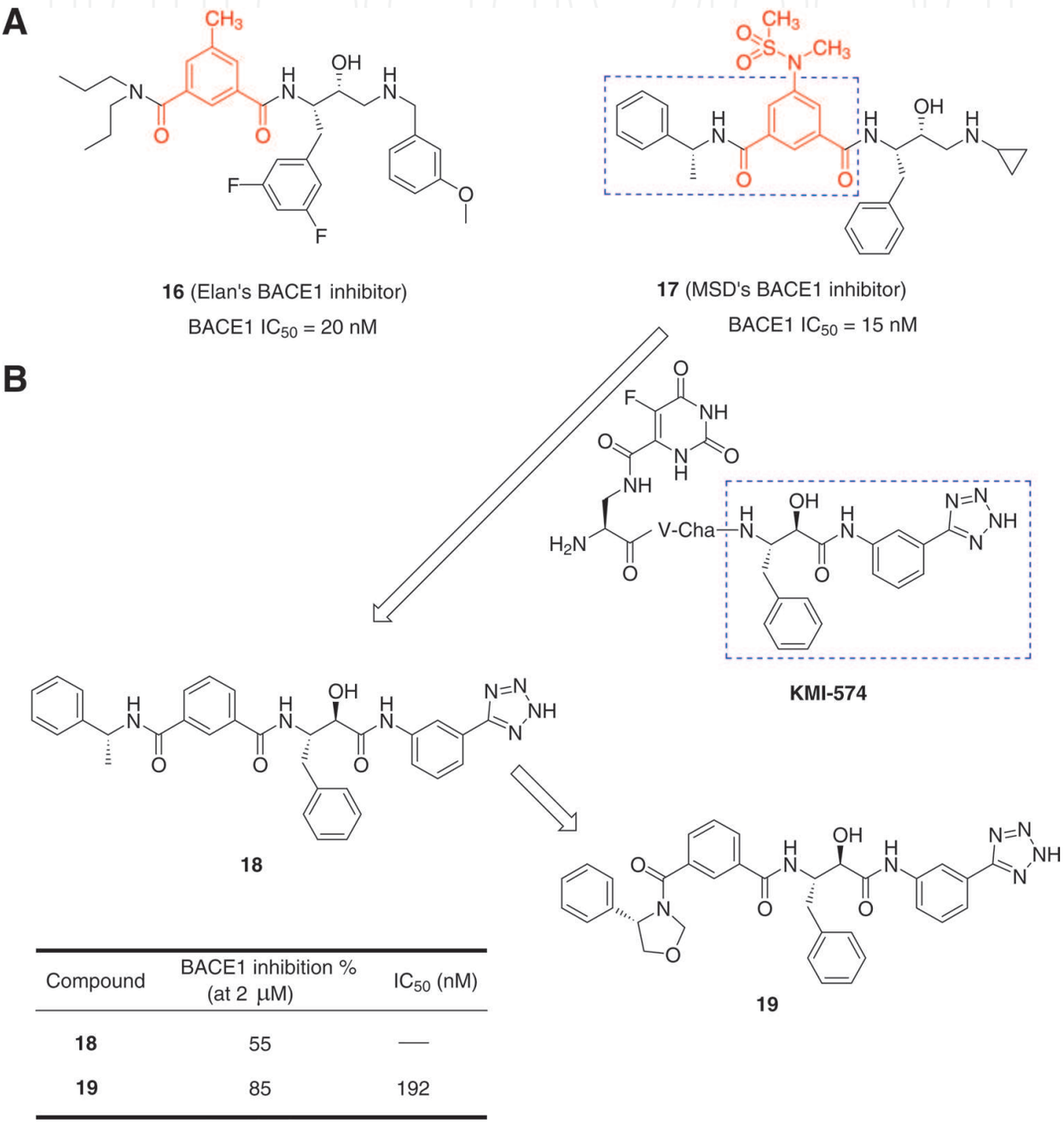


Figure 4. Peptidomimetic BACE1 inhibitor with a norstatine-type substrate transition-state analog.

The inhibitors formulated by Elan and MSD researchers, compounds **16** and **17**, respectively, are shown in **Figure 5**. Because the distance between the flap domain and the cleft domain that form the S_2 pocket of BACE1 is narrow, a planar aromatic ring such as an isophthalic scaffold can closely dock in the S_2 pocket of BACE1. Hence, we designed BACE1 inhibitor **18** with an isophthalic scaffold at the P_2 position [39]. However, compound **18** showed a low inhibitory activity (BACE1 inhibition 55% at 2 μM). Our next innovation involved the S_3 sub-pocket located behind the active site of BACE1. A docking simulation study between



Compound	BACE1 inhibition % (at 2 μM)	IC_{50} (nM)
18	55	—
19	85	192

Figure 5. (A) BACE1 inhibitors with an isophthalic scaffold reported by Elan Pharmaceuticals/Pharmacia and MSD and (B) design of isophthalic-type BACE1 inhibitors using a norstatine-type transition-state analog of KMI-574.

inhibitor **18** and BACE1 revealed that the P_3-phenyl group of inhibitor **18**, which interacts with the S_3 sub-pocket, adopts a folding structure against the P_2-isophthalic scaffold. We envisioned and designed an inhibitor that possessed a folding structure and synthesized inhibitor **19**. This compound featured a five-membered ring, oxazolidine, at the P_3 position in order to fix the folding pose between the P_2-phenyl group and P_3-isophthalic scaffold. Our premise was that the oxazolidine ring fixes the direction of the phenyl ring at the P_3 position, so the P_3-phenyl ring might be able to bind closely to the S_3 sub-pocket of BACE1. Inhibitor **19** showed moderate inhibitory activity (BACE1 inhibition 85% at 2 μM, IC_{50} = 192 nM).

Next, we focused on a proton of the P_2-isophthalic ring of inhibitor **19**. We demonstrated van der Waals repulsion between the proton on the isophthalic ring at the P_2 position and the five-membered ring at the P_3 position in inhibitor **19** docked at the active site of BACE1. We focused on the steric-hindered interaction between the P_3-phenyl group and a proton on the P_2-isophthalic ring of a virtual inhibitor (**Figure 6**), which seemed to restrict its configuration. We calculated the steric energies in the respective conformers around the bond of the P_3 amide and P_2-isophthalic ring of the virtual inhibitors as shown in **Figure 6**. Using an approach based on a conformer of the docked inhibitor in BACE1 (the *in silico* conformational structure-based design) [39, 41], we adopted a pyridinedicarboxylic scaffold as a P_2 moiety, which lacked a proton from the isophthalic ring. Whereas the conformer of the P_2-isophthalic virtual inhibitor with the same dihedral angle to the conformer docked in BACE1 showed a high steric energy, the stable conformer of the virtual inhibitor with a P_2-pyridinedicarboxylic scaffold showed the same dihedral angle to that docked in BACE1 because of the lack of a proton on the pyri-

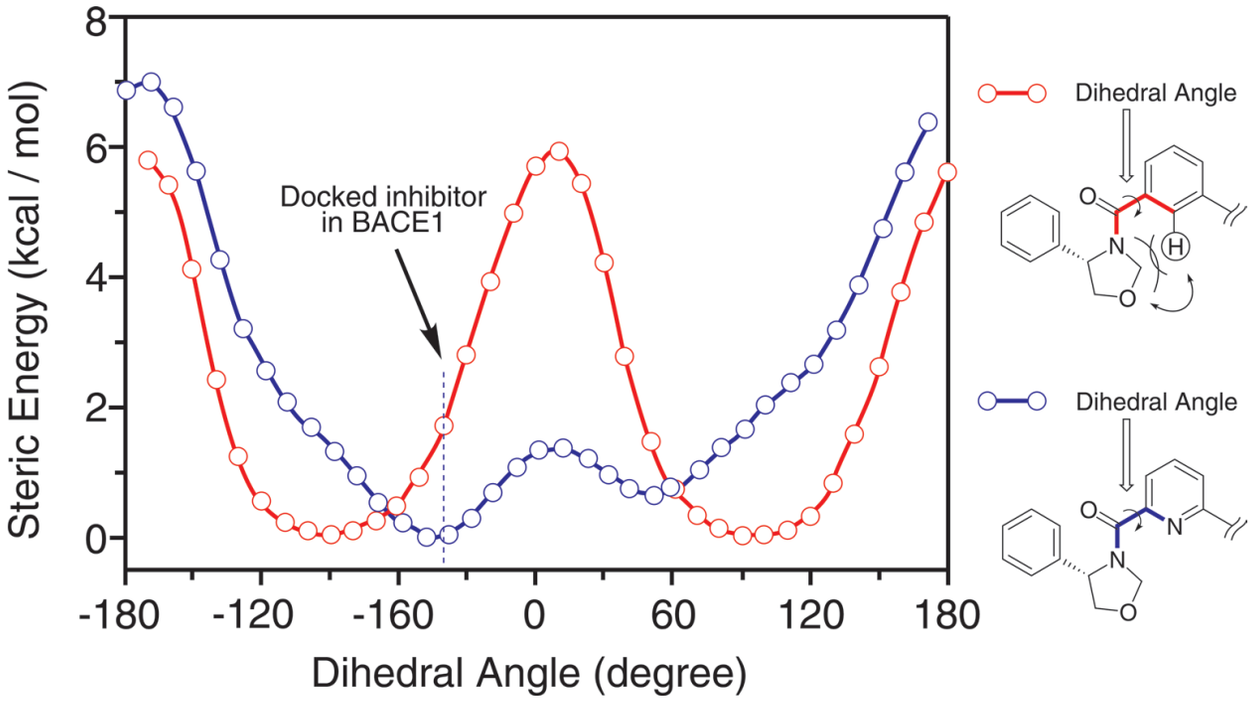
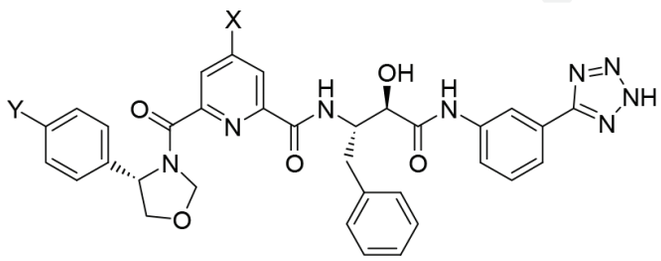


Figure 6. In-silico conformational structure-based design of BACE1 inhibitor possessing a pyridine scaffold at the P_2 position.

dine's amine. Inhibitor **20** with a pyridinedicarboxylic scaffold was designed and synthesized and showed improved inhibitory activity (BACE1 inhibition 93% at 2 μM, IC_{50} = 140 nM) compared to **19** (**Table 1**). A docking simulation demonstrated that inhibitor **20** could adopt a stable folding structure having the same dihedral angle between the P_3-amide and P_2-isophthalic ring to the conformer docked in BACE1. Thus, we could design a potent BACE1 inhibitor (compound **20**) using the computational approach based on the conformer docked in BACE1. However, **20** still showed a lower inhibitory activity than our peptidic inhibitors compared to lead compounds **13–15** (IC_{50} = 1.2–5.6 nM). There is room for further optimization of these inhibitors.



Compound	X	Y	BACE1 inhibition %		IC_{50} (nM)
			at 2 μM	at 0.2 μM	
20	-H	-H	93	63	140
21	$-SO_2CH_3$	-H	96	73	96
22	$-OCH_3$	-H	91	53	151
23	-OEt	-H	79	—	—
24	-OPr	-H	64	—	—
25	$-SCH_3$	-H	95	66	89
26	$-SCH(CH_3)_2$	-H	72	—	—
27	$-N_3$	-H	95	68	79
28	$-NH_2$	-H	78	53	—
29	$-N(CH_3)\ SO_2CH_3$	-H	75	—	—
30	$-CH_3$	-H	95	68	—
31	-Cl	-H	98	87	22
32	-Br	-H	99	88	15
33	-I	-H	99	86	24
34	-Cl	-F	99	91	13
35	-Br	-F	99	93	9
36	-I	-F	99	92	10

Table 1. BACE1 inhibitors with a pyridine scaffold.

3.3. Design based on quantum chemical interaction and electron donor bioisostere

The first reported coordinate set of crystal structure of BACE1-inhibitor (OM99–2) complex is 1FKN by Gosh et al. [27–29]. The P_2 moiety of the inhibitor interacts with the Arg235 side chain of BACE1 by hydrogen bonding in the crystal structure. We compared the publicly available X-ray crystal structures of BACE1-inhibitor complexes and discovered that most inhibitors did not interact with Arg235 by hydrogen bonding [41]. Surprisingly, the guanidino group of BACE1-Arg235 in most crystal structures, except 1FKN, showed the similar "flopping over" feature of 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 3 Å. The P_2 moieties in many crystal structures that interact with the BACE1-Arg235 side chain are a methyl group, carbonyl oxygen atom, or aromatic ring. They appear to interact with the guanidine plane of Arg235 side chain by CH-π, O-π, or π-π stacking interactions. This suggests that the π-orbital on the guanidino plane can interact with the P_2 region of the inhibitors by a weak quantum force. The only exception was the interaction in the first reported X-ray crystal structure, 1FKN. Although the P_2 moiety of OM99–2 in the crystal structure of 1FKN appeared to interact with the BACE1-Arg235 side chain via hydrogen bonding, the P_2-moiety of OM00–3 that was structurally similar to OM99–2 interacted with the π-orbital on the guanidine plane of the BACE1-Arg235 side chain via O-π interaction (PDB ID: 1M4H). Many early BACE1 inhibitors that possess a hydrogen bond receptor at the P_2 position were designed using the 1FKN crystal structure. 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, inhibitor **17** that was synthesized by the MSD researchers interacted with the BACE1-Arg235 side chain via a CH-π interaction (PDB ID: 2B8L) as shown in **Figure 7A** (PDB ID: 2B8L). It is likely that the researchers designed an inhibitor 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 inhibitor interacted with the π-orbital on the guanidine plane of the BACE1-Arg235 side chain at a distance of 2.8 Å. As described earlier, 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. 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 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. This potentially posed 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. We hypothesized that the role of the BACE1-Arg235 side chain is important for the

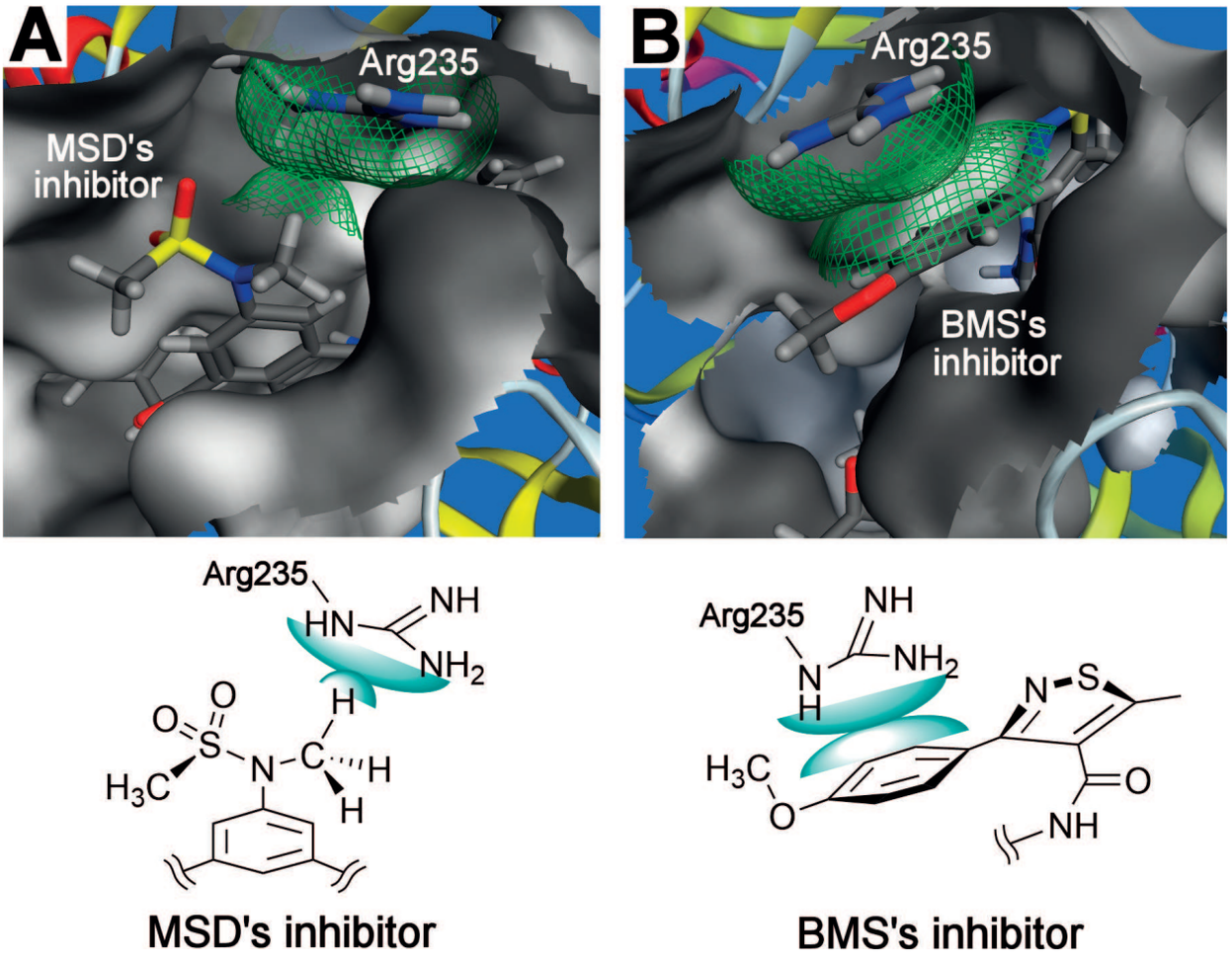


Figure 7. Interaction of BACE1 inhibitors with the Arg235 side chain of BACE1. (A) MSD's inhibitor **17** (PDB ID: 2B8L) and (B) BMS's inhibitor (PDB ID: 4FSL).

inhibitory mechanism of BACE1. 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 causes 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.

In silico drug discovery using a docking simulation between a target biomolecule and drugs has provided important information. However, most docking simulation software involves mechanism/molecular dynamics (MM/MD) calculations based on classical Newtonian mechanics. Docking simulations using these calculations do not appear to estimate a weak quantum chemical interaction, such as stacking or σ-π interaction. The quantum chemical interactions that also involve other aromatic amino acids including phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) side chains seem to be approximately optimized using several descriptors based on classical mechanics in the docking simulation software that is based on MM/MD calculations. However, the software programs recognize arginine (Arg) as one of the charged amino acids, and the quantum chemical interactions involving an Arg

side chain are unlikely to yield a reasonable output. Quantum chemical interactions involving a π-orbital of a guanidino group are common in proteins and play an important role in molecular recognition by proteins. Crowley et al. surveyed cation-π interactions in protein interfaces using the Protein Data Bank and the Protein Quaternary Structure server [42]. They evaluated the cation-π interactions using a variant of the optimized potentials for liquid simulations (OPLS) force field and found that approximately half of the protein-protein complexes and one-third of the homodimers contained at least one intermolecular cation-π pair. This finding indicates the significance of these interactions in molecular recognition because the occurrence rate of cation-π pairs in protein-protein interfaces is higher than that in homodimer interfaces, which are similar to the protein interior. Among them, the interactions between an Arg and a Tyr were found to be the most abundant. Moreover, 53% of them involved planar π-π stacking by the quantum chemical interaction between the guanidine group of an Arg residue and the aromatic ring of a Tyr residue. Researchers at Bristol-Myers Squibb Research (BMS) reported a series of BACE1 inhibitors that can interact with the BACE1-Arg235 side chain by a π-π stacking [43] as shown in **Figure 7B** (PDB ID: 4FSL). According to their structure–activity relationship study, the inhibitor possessing an electron-donating methoxy group on the *p*-position of phenyl ring that interacts with BACE1-Arg235 side chain can enhance BACE1-inhibitory activity. This finding indicated that an inhibitor possessing a P_2-aromatic ring with a higher electron density could strongly bind to the electron-poor π-orbital on the guanidino plane of the BACE1-Arg235 side chain. We thought that inhibitors formulated on the basis of such a quantum chemical interaction could never be designed using a classical concept on the basis of Newtonian mechanics, such as MM/MD calculation. Hence, we proposed the new "electron-donor bioisostere," concept, which involves quantum chemical interaction with an electron-poor π-orbital, such as the guanidine group of Arg235 [24].

We hypothesized that the quantum chemical interaction between an inhibitor and the side chain of BACE1-Arg235 plays a critical role in the inhibition mechanism. Therefore, we focused on the optimization around the P_2 region. The finding of a structure–activity relationship study focusing on the inhibitor's P_2 region is shown in **Table 1** [39–41, 44]. Inhibitors **21**, **22**, **25**, **27**, and **30** with hydrophobic and small-sized functional methanesulfonyl, methoxy, methylmercaptan, azide, and methyl groups on the P_2-pyridine ring display a higher inhibitory activity than inhibitors with a bulky or a hydrophilic group such as inhibitors **23**, **24**, **26**, **28**, and **29**. On the basis of the "electron-donor bioisostere" concept, we speculated that an electron-rich halogen atom could interact with the electron-poor guanidine π-orbital by Coulomb's force. Using the *ab initio* molecular orbital approach, Imai et al. described the slightly stronger calculated Cl-π interaction energy than the CH-π interaction and reported that its energy was affected by π-electron density [45]. Hence, we designed inhibitors **31–33** possessing a halogen atom on the P_2-pyridine ring. Inhibitors **31–36** exhibited more potent inhibitory activities (IC_{50} values: 22, 15, and 24 nM, respectively). Next, inhibitors **34–36** possessing a fluorine atom on the *p*-position of P_3-phenyl group exhibited the potent inhibitory activities (IC_{50} values: 13, 9, and 10 nM, respectively) [41]. Among them, inhibitor **35** (KMI-1303) exhibited the most potent inhibitory activity and is available from Wako Pure Chemical Industries (Japan) as a reagent for biological research.

4. Conclusion

Pyridines are important in medicinal chemistry because of their properties, which include weak basicity, water solubility, *in vivo*/chemical stability, hydrogen bond-forming ability, and small molecular size. Pyridine moieties are incorporated in many drugs and pesticides. Water solubility is one role of pyridines. The replacement of a portion of drugs with a pyridine moiety can improve their water solubility for the development of practical drugs that are suitable for an orally administrated or an injectable formulation. This approach is also applicable to the prodrug strategy. The bioisostere is one of the important roles of pyridines in drug design. The abovementioned attributes of pyridines enable the application as a bioisostere of amines, amides, heterocyclic rings containing a/some nitrogen atoms, and the benzene ring. The replacement of a part of lead compounds with pyridines is an important tool for the development of practical drugs. Moreover, the replacement of a portion of a drug molecule with pyridines might control the selectivity against subtypes of a target biomolecule, such as the histamine H_1 receptor antagonist, mepyramine. Recently, we reported a series of BACE1 inhibitors possessing a pyridine scaffold. In this process, we observed an important and third role of pyridines in medicinal chemistry, other than the conventional role of pyridines. This chapter has discussed on this third role. The replacement of the isophthalic scaffold of inhibitors with a pyridinedicarboxylic scaffold enables the control of the conformation of inhibitors. We designed the potent BACE1 inhibitor KMI-1303 using the design approach based on a conformer of the docked inhibitor in the target molecule—the *in silico* conformational structure-based design.

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Conflict of interest

We confirmed independence from the funding source.

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