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Design and synthesis of 3-pyridylacetamide derivatives as dipeptidyl peptidase IV (DPP-4) inhibitors targeting a bidentate interaction with Arg125

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ABSTRACT

We have previously discovered nicotinic acid derivative **1** as a structurally novel dipeptidyl peptidase IV (DPP-4) inhibitor. In this study, we obtained the X-ray co-crystal structure between nicotinic acid derivative **1** and DPP-4. From these X-ray co-crystallography results, to achieve more potent inhibitory activity, we targeted Arg125 as a potential amino acid residue because it was located near the pyridine core, and some known DPP-4 inhibitors were reported to interact with this residue. We hypothesized that the guanidino group of Arg125 could interact with two hydrogen-bond acceptors in a bidentate manner. Therefore, we designed a series of 3-pyridylacetamide derivatives possessing an additional hydrogen-bond acceptor that could have the desired bidentate interaction with Arg125. We discovered the dihydrochloride of 1-{[5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-prolinamide (**13j**) to be a potent and selective DPP-4 inhibitor that could interact with the guanidino group of Arg125 in a unique bidentate manner.

1. Introduction

Type 2 diabetes and obesity are emerging as two major global public health problems of the 21st century. Currently, approximately 194 million people worldwide are affected by these diseases and this number is expected to increase to 366 million by 2030.[1] Among promising drug targets for type 2 diabetes, glucagon-like peptide 1 (GLP-1) has recently come to the forefront.[2] GLP-1 is secreted from the gastrointestinal tract in response to food intake and subsequently stimulates insulin secretion.[3] It has been shown that elevated levels of GLP-1 can lead to improved glycemic control in type 2 diabetic patients.[4] Dipeptidyl peptidase IV (DPP-4) regulates the activity of GLP-1 by cleaving the N-terminus of GLP-1[7-36]-amide to give inactive GLP-1[9-36]-amide. Through this mechanism, the level of GLP-1 in circulation can be increased by inhibition of DPP-4.[5] Thus, extensive effort has been invested to explore DPP-4 inhibitors as novel drugs.[6,7] Among them, MK-0431 (sitagliptin)[8] has been prescribed in the US since 2006 as the first approved DPP-4 inhibitor. SYR-322 (alogliptin),[9] LAF-237 (vildagliptin),[10] and BMS-477118 (saxagliptin)[11] have either been under clinical trials in diabetic patients or launched in some countries (Fig. 1).[12]

Our continued interest in DPP-4 inhibition as a therapy for type 2 diabetes prompted the investigation into other novel small molecule DPP-4 inhibitors. Over the course of this investigation, we discovered structurally novel nicotinic acid derivative **1**[13] as a potent DPP-4 inhibitor.

The X-ray crystal structure of **1** in complex with human DPP-4 was determined (Fig. 3) and, from these data, it was shown that the primary amine group of compound **1** interacts with the Glu motif (Glu205 and Glu206) and Tyr662 of DPP-4. The *p*-tolyl group occupies the well-defined and hydrophobic S1 pocket. In addition, it was shown that the isobutyl group forms a hydrophobic interaction with Phe357 and that the carboxy group interacts with Tyr547. This information prompted us to design more potent pyridine-based DPP-4 inhibitors by adding efficient interactions with the amino acid residues existing near the 3-position of the pyridine ring.

Abbreviations: DPP-4, Dipeptidyl peptidase IV; GLP-1, Glucagon-like peptide 1; SAR, Structure–activity relationship; AcOH, Acetic acid; DIBAL-H, Diisobutylaluminum hydride; Boc, *tert*-Butoxy carbonyl; TMSCN, Trimethylsilyl cyanide; TBAF, Tetrabutylammonium fluoride; *m*CPBA, *m*-Chloroperoxybenzoic acid; ADME, Absorption, distribution, metabolism and elimination; B.A., Bioavailability; MRT, Mean residence time.

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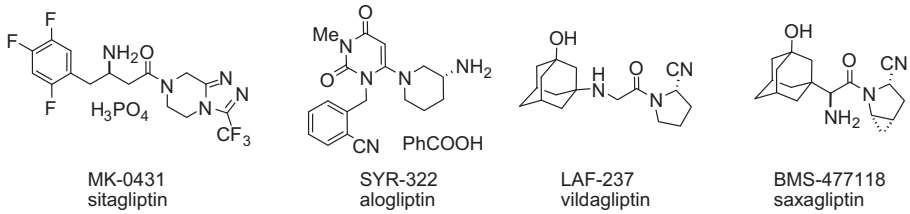


Figure 1. Structures of representative DPP-4 inhibitors.

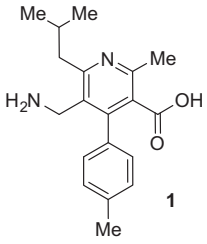


Figure 2. Structure of compound **1**.

We first selected Arg125 as a target residue, indicated by the white circle in Figure 2, and focused on creating a bidentate coordination with this residue to achieve more potent activity. Published studies have reported two distinctive structural classes of DPP-4 inhibitors: peptidomimetic and non-peptidomimetic. MK-0431 (sitagliptin), SYR-322 (alogliptin) and imidazo[1,2-*a*]pyrimidine series[14] are classified in the non-peptidomimetic class. The peptidomimetic class is further subdivided into glycine-based and β-alanine-based inhibitors. The glycine-based inhibitors (α-amino acid series) generate a hydrogen-bond between the carbonyl oxygen and Arg125.[15] Based on this example, Arg125 is a potential target residue for achieving potent DPP-4 inhibitory activities. Moreover, because the two amino groups of guanidine in Arg125 can strongly interact with two hydrogen-bond acceptors, this residue is a desirable target for bidentate coordination. In general, hydrogen-bonds positively contribute to enthalpy. This favorable change in enthalpy could be partially negated by the entropic penalty associated with decreased conformational freedom in the binding region. To reduce the entropic cost, hydrogen-bonds should be targeted towards already structured regions of protein.[16] Formation of a bidentate hydrogen-bond with Arg125 may impart favorable enthalpy change and low conformational entropy loss. Therefore, this bidentate coordination is expected to offer a stronger interaction than that of two separate hydrogen-bonds. To accomplish this goal, we designed a series of 3-pyridylacetamide derivatives with an additional hydrogen-bond acceptor that could make this desired interaction via the amide carbonyl group and the hydrogen-bond acceptor. Although the orientation of both of the two hydrogen-bond acceptors was recognized to be important for attaining a strong bidentate interaction, the carbonyl group of compound **1** is positioned such that it is unable to interact with Arg125 as shown in Figure 3. We hypothesized that the insertion of a methylene linker between the carbonyl group and the pyridine ring would serve to not only position the carbonyl group closer to Arg125 but to also afford the conformational freedom necessary to allow for the proper orientation of this interaction to be realized.

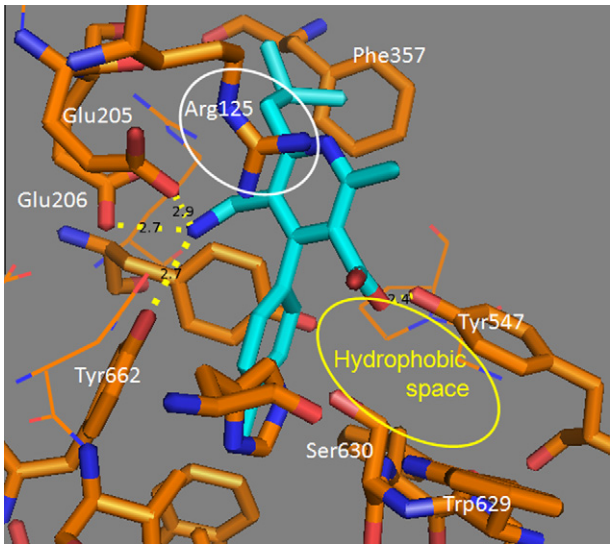


Figure 3. X-ray crystal structure of compound **1** (blue carbons) in complex with human DPP-4. The amino acids (orange carbons, white text) are indicated. Hydrogen bonds are shown as yellow dotted lines. Hydrophobic space is indicated by the yellow circle and the target amino acid residue (Arg125) is indicated by the white circle.

Based on X-ray crystal structure data, we next focused on the hydrophobic space indicated by the yellow circle in Figure 3. We believed that the introduction of a hydrophobic group, for instance, a benzene ring or an aliphatic ring, would help to increase the inhibitory activity via a hydrophobic interaction with residues such as Tyr547 and Trp629.

We hypothesized that compounds exhibiting both the bidentate coordination to Arg125 based on hydrogen-bonding and the hydrophobic interaction with additional residues of DPP-4 would achieve enhanced inhibitory activity (Fig. 4). To validate this hypothesis, we designed and synthesized a series of 3-pyridylacetamide derivatives that combined both of these attributes. In this report, we describe our novel design, structure–activity relationship (SAR) studies, and the identification of the highly potent DPP-4 inhibitor: the dihydrochloride of 1-{[5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-prolinamide (**13j**).

2. Chemistry

3-Pyridylacetic acid **10** was synthesized as described in Scheme 1. The pyridine ring was constructed using modified Hantzsch's pyridine synthesis.[17] Commercially available ester **2** was converted to β-ketonitrile **3**. Benzylidene derivative **4** was prepared using the Knoevenagel condensation reaction from the compound **3**. The benzylidene derivative **4** was converted into dihydropyridine **5** by condensation with an aminoacrylate in acetic acid (AcOH). The dihydropyridine **5** was then oxidized with nitric acid to generate pyridine derivative **6**. Upon reduction of the cyano group with Raney-Ni/H_2, compound **6** was converted to aminomethyl compound **7**. The methoxycarbonyl group at the 3-position of the pyridine ring was converted to a hydoxymethyl group with diisobutylaluminum hydride (DIBAL-H), followed by protection of the amino group with a *tert*-butoxy carbonyl (Boc) group to produce the alcohol **8** in good yield. After mesylation of the hydroxy group, a

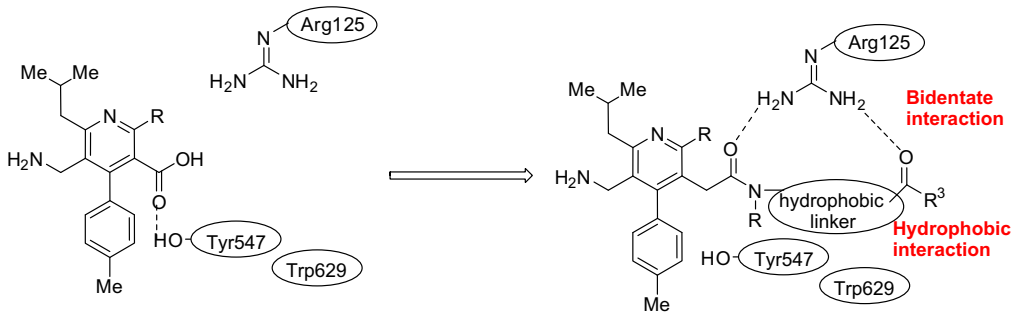
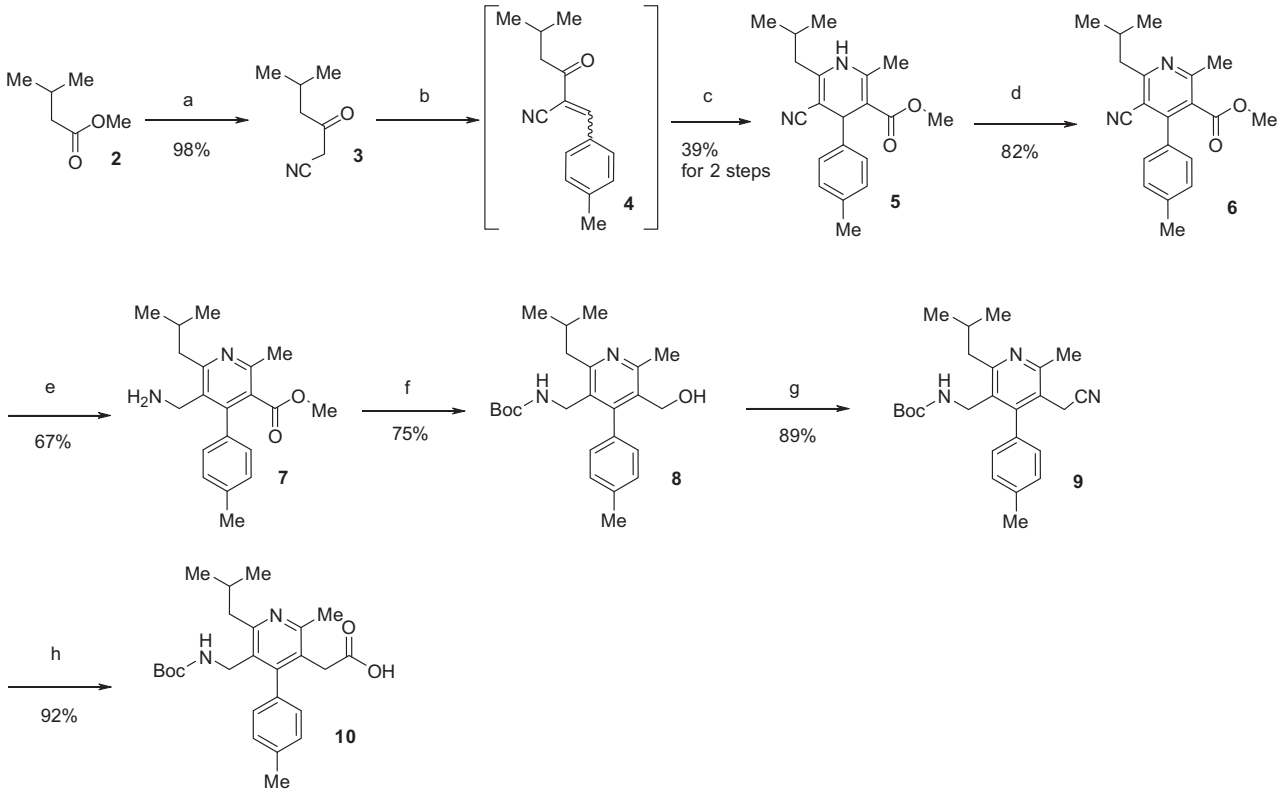


Figure 4. Design of novel 3-pyridylacetamide derivatives.



Scheme 1. Synthesis of compound **10**. Reagents and conditions: (a) NaH, MeCN, THF, reflux, 15 h; (b) *p*-tolualdehyde, AcOH, toluene, reflux, Dean–Stark, 5 h; (c) methyl (2*E*)-3-aminobut-2-enoate, MeOH, 80 °C, 4 h; (d) 2 M HNO_3, 1,4-dioxane, 80 °C, 45 min; (e) Raney-Ni, 25% NH_3, MeOH, 40 °C, 5 h; (f) (1) DIBAL-H, toluene, −78 °C, 68 min; (2) $(Boc)_2O$, AcOEt, 1 M NaOH, room temperature, 30 min; (g) (1) MsCl, Et_3N, THF, 0 °C, 30 min; (2) TMSCN, TBAF, THF, room temperature, 1 h; (h) (1) 6 M HCl, 90 °C, 20 h; (2) $(Boc)_2O$, AcOEt, 8 M NaOH, room temperature, 1 h; (3) neutralization with 1 M HCl.

cyano group was introduced by the treatment with trimethylsilyl cyanide (TMSCN) and tetrabutylammonium fluoride (TBAF). The cyano group was hydrolyzed to a carboxy group under acidic conditions and the subsequent *N*-protection yielded 3-pyridylacetic acid **10**.

The 3-pyridylacetic acid **10** was condensed with several amines (**11a**–**n**) to produce the desired amides **12a**–**n**. The Boc group of **12a**–**n** was cleaved under acidic conditions to give compounds **13a**–**n** (Scheme 2).

The methyl ester of amides **12a**, **12g**, **12h**, **12i**, and **12k** was hydrolyzed with sodium hydroxide then deprotected under acidic conditions to give compounds **15a**, **15g**, **15h**, **15i**, and **15k**. The carboxy group of compound **14k** was converted to the corresponding carbamoyl group to afford **16k**. The Boc group of **16k** was cleaved in acidic conditions to produce compound **17k** (Scheme 3).

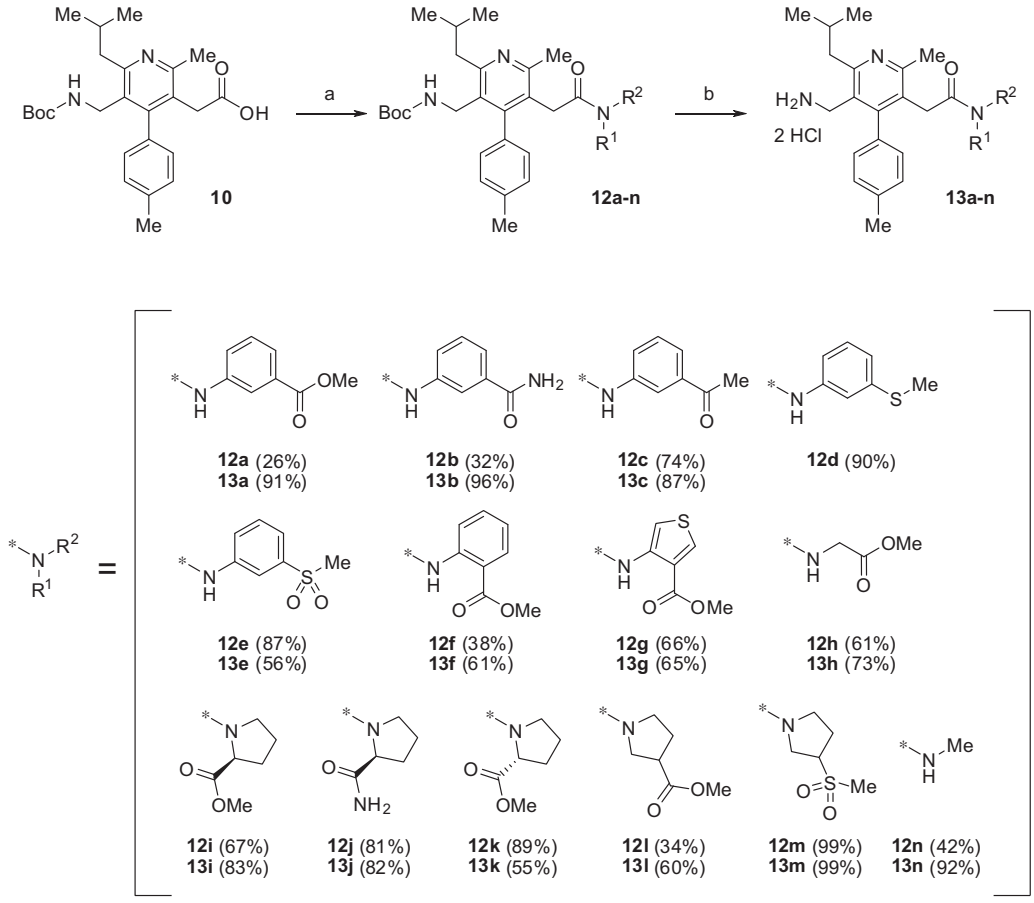
The methylthio group of compound **12d** was oxidized to the methyl sulfoxide using *m*-chloroperoxybenzoic acid (*m*CPBA), followed by deprotection of the Boc group under acidic conditions resulting in compound **19d** (Scheme 4).

3. Results and discussion

The synthesized compounds were evaluated for their in vitro inhibitory activity against human DPP-4 and the results described as IC_{50} values.2

3.1. Comparison of DPP-4 inhibitory activities with compounds 13n, 13h, and 15h

The SAR summary is shown in Table 1. The methyl amide **13n**, which did not interact with Arg125, showed lower potency than compound **1**. The carbonyl group of **13n** is not in a position that allows for interaction with Arg125. Conversely, the compound

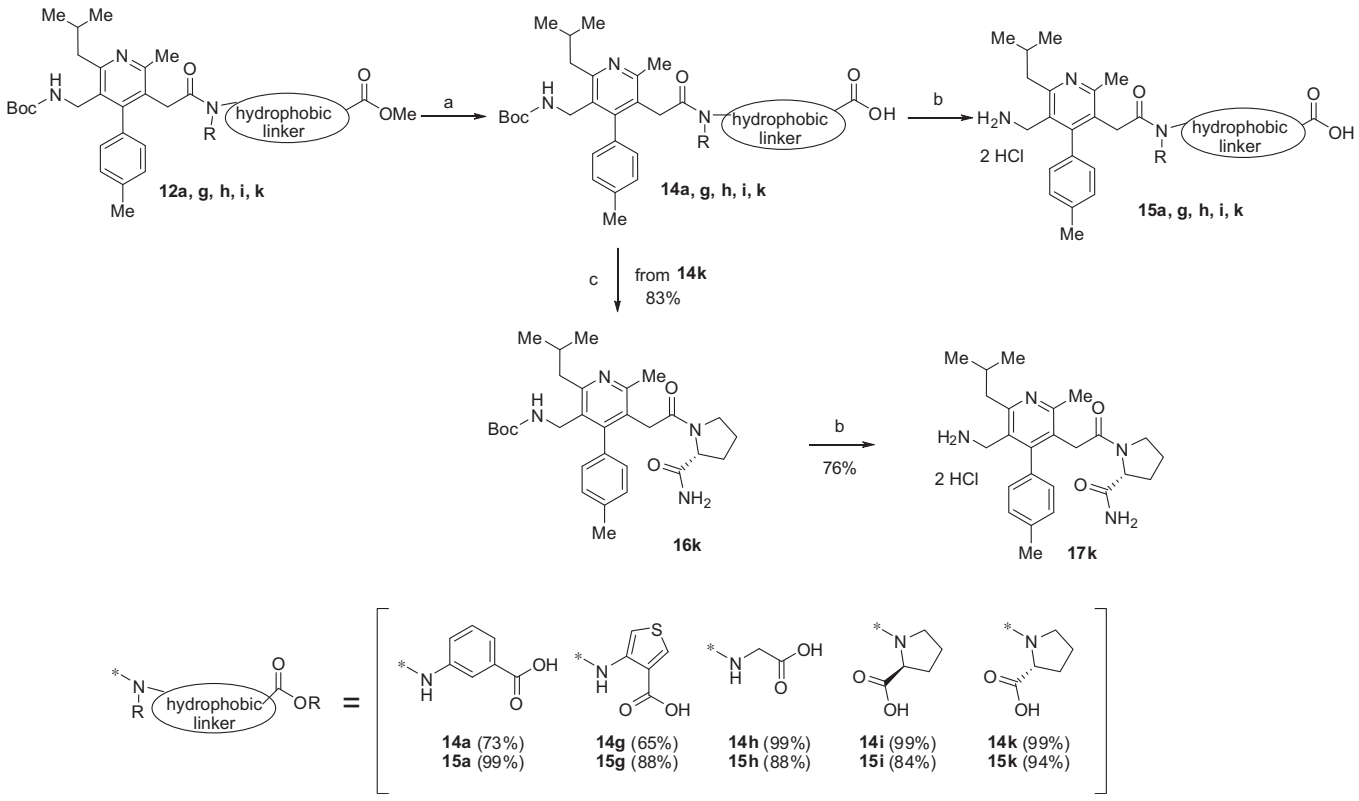


Scheme 2. Synthesis of compounds **13a–n**. Reagents and conditions: (a) amines (**11a–n**), HATU, DMF, room temperature, 14–16 h; (b) 4 M HCl, dioxane, room temperature, 2–16 h.

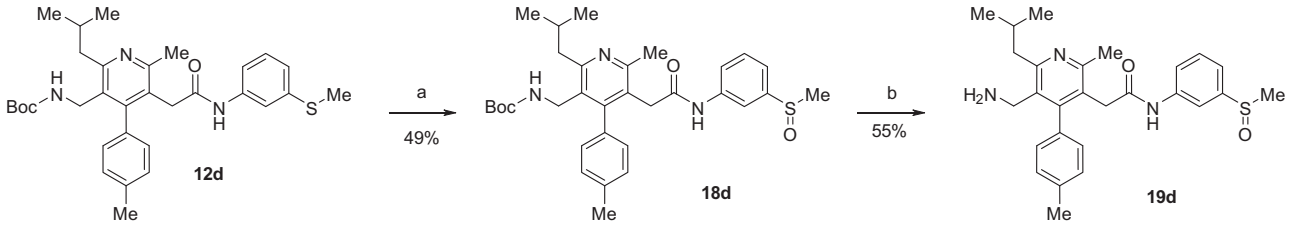
13h shows an activity similar to compound **1**. This indicates that the carbonyl group on the ester contributes to an increase in potency as a result of the hydrogen-bond with some amino acid residues. The compound **15h** displays less potent activity compared with compound **1**. This may be due to the high polarity of the carboxy group, which could potentially induce repulsion against some amino acid residues. The results shown in Table 1 indicate that the two respective carbonyl groups of glycine amide **13h** can form hydrogen-bonds that could serve to increase the inhibitory activity. To make the more effective bidentate coordination, we attempted to immobilize the hydrogen-bond acceptors via the introduction of a cyclic structure. Specifically, in order to allocate a hydrogen-bond acceptor to the appropriate orientation, we explored the use of anilide aliphatic amide derivatives.

3.2. The SAR of anilide-type amides

The SAR summary of the anilide derivatives is shown in Table 2. To achieve the bidentate interaction with the hydrogen-bond acceptor Arg125, a carbonyl group, a sulfinyl group, and a sulfonyl group were introduced individually to the ortho or meta position of the aryl ring. A hydrogen-bond acceptor attached to the para position of the benzene ring may not be able to specifically approach Arg125 and therefore could not interact with Arg125. This is supported by the crystal structure model in Figure 3. As shown in Table 2, the ortho-substituted compounds (**13f**, **13g**, and **15g**) displayed comparable DPP-4 inhibitory activity to compound **1**. We reasoned that the hydrogen-bond acceptor of the ortho-substituted compounds was not oriented in the desired orientation and was incapable of making an additional interaction. Conversely, the meta-substituted compounds exhibited more potent activity than that of compound **1**. Specifically, compounds **13a**, **13b**, and **19d** were found to be extremely potent. Carbonyl and sulfinyl groups at the meta position may form strong interactions with amino acid residues. In order to gain additional insight to these interactions, we performed a docking study of compound **13b** with DPP-4. The docking study revealed that the carbonyl group of 3-pyridylacetamide and the acetyl group of the benzene ring reside near Arg125 and its two respective hydrogen-bond acceptors participate in a bidentate interaction with Arg125 (Fig. 5). Moreover, it was shown that a benzene ring or a thiophene ring can occupy the hydrophobic space to form a hydrophobic interaction with amino acid residues Trp629 and Tyr547. Hydrogen-bond acceptors of the meta-substituted compounds (**13a**, **13c**, **13e**, and **19d**) possessing nanomolar activity could also form a similar bidentate coordination to compound **13b**. Compounds **15a** and **13c** result in less potent activity than compounds **13a**, **13b**, and **19d**, presumably because the high-polarity carboxy and carbamoyl group may interfere with the orientation of the hydrogen-bond acceptor. As predicted, meta-substituted anilide-type compounds exhibit potent inhibitory activities by stabilizing the direction of the hydrogen-bond acceptor. This firm bidentate coordination could help to increase the DPP-4 inhibitory activity. Additionally, a hydrophobic interaction originating from the phenyl group contributes to the potency. Next, we investigated a series of prolinamide-type derivatives whose hydrogen-bond acceptors allowed greater flexibly than those of the anilide-type compounds.



Scheme 3. Synthesis of compounds **15a**, **g**, **h**, **i**, **k**, and **17k**. Reagents and conditions: (a) 1 M NaOH solution, MeOH, room temperature, 1–16 h; (b) 4 M HCl, dioxane, room temperature, 5 min; (c) 25% NH_3 solution, HOBt, WSC, DMF, room temperature, 2 days.



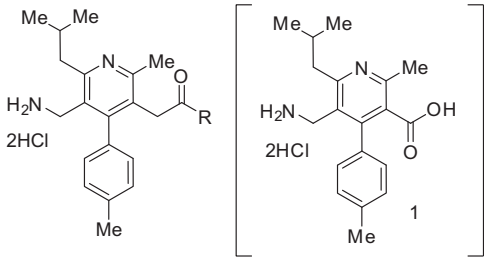
Scheme 4. Synthesis of compounds **19d**. Reagents and conditions: (a) *m*CPBA, $CHCl_3$, room temperature, 1 h; (b) (1) TFA, room temperature, 5 min, (2) neutralization with 1 M NaOH.

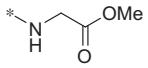
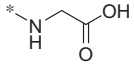
3.3. The SAR of prolinamide derivatives

Since we were able to verify a hydrophobic interaction of the anilide-type compound phenyl group, we surmised that prolinamides would also show promising potencies. Effects of prolinamide-type derivatives were investigated and the SAR summary is shown in Table 3. Compound **13l**, in which the carbonyl of the ester was able to hydrogen-bond with Arg125, displayed potent activity. However, compound **13m** was less potent. Thus, the direction and distance of the sulfonyl group at the 3-position of the pyrrolidine ring is unsuitable to induce the desired interaction. Compounds **13i**, **15i**, and **13j**, derived from L-proline, exhibit high potency and had intensive hydrogen-bond interactions of two respective hydrogen-bond acceptors and a hydrophobic interaction of the pyrrolidine ring. Meanwhile, compounds **13k**, **15k**, and **17k**, derived from D-proline, show less potent activities than the corresponding isomers. The only structural difference between L-proline and D-proline is the direction of carbonyl group on the pyrrolidine ring. The differential activities of these two derivatives can be explained because of the difference in the manner of hydrogen-bond interactions between the carbonyl group and Arg125. Because of the conformation of L-proline ring, the two respective carbonyl groups are positioned in a conformation that facilitates strong coordination. To confirm this coordination, we performed docking studies of compounds **13j** and **17k**.

Results from docking studies of **13j** and its enantiomer **17k** are shown in Figure 6A and B, respectively. The primary amine group of both compounds interacts with the Glu–Glu motif and the *p*-tolyl group occupying the hydrophobic S1 pocket. The isobutyl group creates a hydrophobic interaction with Phe357. There is, however, a significant difference between the two compounds regarding the hydrogen-bond interaction of the two carbonyl groups. The two respective carbonyl groups of compound **13j** make a strong bidentate interaction with the guanidino group of Arg125. Additionally, the pyrrolidine ring forms a hydrophobic interaction with Trp629 and Tyr547. Meanwhile, although the 3-pyridylacetamide carbonyl group of compound **17k** can interact with Arg125, the carbonyl group on the D-proline is directed in an unfavorable direction and cannot bind tightly to Arg125. These results explain the potency discrepancy between the two optical isomers. As was predicted from our design, the two respective hydrogen-bond

Table 1
DPP-4 inhibitory activities of compounds **13n**, **13h**, and **15h**



Compound	R	DPP-4 IC_{50}[a] (nM)
13n		69 (66–73)
13h		17 (15–18)
15h		73 (69–77)
1		18 (16–20)

[a] Inhibitory activity against human DPP-4. IC_{50} values shown are means of duplicate or triplicate measurements. IC_{50} values and 95% confidence limits were calculated from the concentration–response curves generated by GraphPad Prism and shown in parenthesis.

acceptors of **13j** can coordinate bidentately with the guanidino group of Arg125 and these tight interactions contribute to the potent DPP-4 inhibitory activity.

3.4. Additional biological evaluation of compound 13j

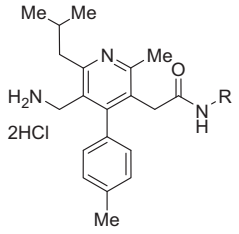
Enzyme selectivity was tested against DPP-2, DPP-8, and DPP-9. These particular isoforms were chosen because it has been shown that inhibition of DPP-2[18] can result in the apoptosis of quiescent T-cells,[19] and safety studies using a DPP-8/9 selective inhibitor suggest that inhibition of both DPP-8 and DPP-9 is associated with profound toxicity in rats and dogs.[20] Remarkably, compound **13j** is significantly more selective relative to related proteases (Table 4).

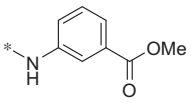
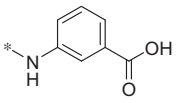
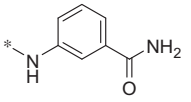
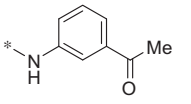
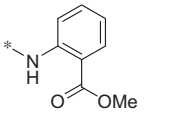
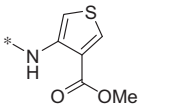
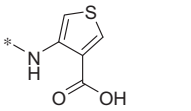
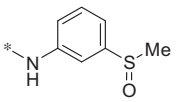
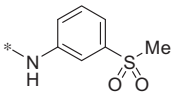
The absorption, distribution, metabolism, and elimination (ADME) properties of **13j** were evaluated as shown in Table 5. Compound **13j** exhibited good metabolic stability and high solubility. Absorption after oral administration was confirmed in dogs. Particularly, the high bioavailability (B.A.) value and the large mean residence time (MRT) in dogs were promising and showed potent and long-lasting effects in in vivo studies. In a dog ex vivo study, compound **13j** rapidly and almost completely inhibited DPP-4 activity in plasma within 1 h after oral administration using a 0.5 mg/kg dose (Fig. 7). Moreover, **13j** was maintained at approximately 90% inhibition of DPP-4 activities for 24 h. These ex vivo results are similar to an actual potent glucose lowering effect in vivo (data not shown) and allow us to expect a reliable efficacy and duration in clinical studies.

4. Conclusion

We optimized lead compound **1** with SBDD to develop a novel and potent DPP-4 inhibitor for the treatment of the type 2 diabetes. Using results from X-ray co-crystallography studies of compound **1**, we focused on Arg125 as a potential target amino acid residue in order to achieve more potent inhibitory activity. We assumed that the guanidino group of Arg125 could interact in a bidentate fashion with two respective hydrogen-bond acceptors. Therefore, we designed a

Table 2
SAR summary for anilide derivatives



Compound		DPP-4 IC_{50}[a] (nM)
13a		3.7 (3.5–4.0)
15a		19 (17–21)
13c		8.1 (6.4–10)
13b		1.8 (1.5–2.0)
13f		19 (18–20)
13g		23 (22–25)
15g		30 (29–31)
19d		2.8 (2.1–3.8)
13e		8.2 (5.9–11)

[a] Refers to Table 1.

series of 3-pyridylacetamide derivatives possessing an additional hydrogen-bond acceptor that could participate in the bidentate interaction with Arg125. To orient the hydrogen-bond acceptor into the appropriate position, we investigated anilide-type and prolinamide-type derivatives. After further investigation, we found that the anilide-type derivatives possessing a hydrogen-bond acceptor at the meta-position of the benzene ring and the L-prolinamide derivatives both showed potent DPP-4 inhibitory activity. Using docking studies, we confirmed that both of these derivatives could achieve the bidentate coordination to Arg125. Additionally, we showed that the phenyl ring of anilides and pyrrolidine ring of prolinamides also contributed to potency via hydrophobic interactions. Finally, we discovered that the dihydrochloride of 1-{[5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methyl-

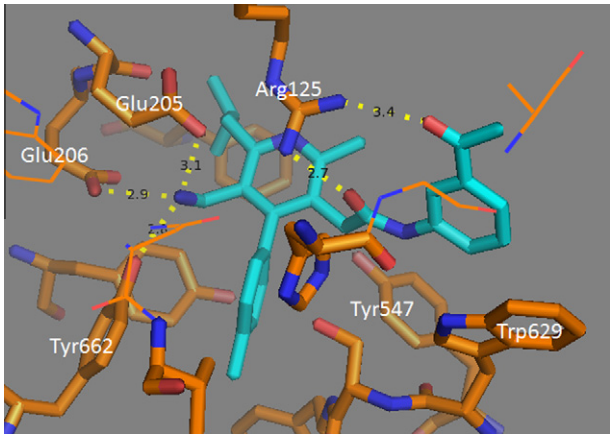


Figure 5. Docking study between DPP-4 protein and compound **13b** (blue carbon). Amino acids (orange carbons, white text) are indicated. Hydrogen bonds are shown as yellow dotted lines.

propyl)pyridin-3-yl]acetyl}-L-prolinamide (**13j**) is a potent, selective and long-lasting DPP-4 inhibitor that can bidentately interact with the guanidino group of Arg125. These results validate our drug design and synthetic strategy; moreover, a new methodology to utilize bidentate coordination to Arg125 was established.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. ^{1}H NMR spectra were obtained at 200 or 300 MHz on a Varian Gemini-200 or a Varian Ultra-300 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; br s, broad singlet; br d, broad doublet; m, multiplet. Elemental analyses and HRMS were carried out by Takeda Analytical Laboratories Ltd. Reactions were followed by TLC on Silica Gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Chromatographic separations were carried out on Silica Gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd) using the indicated eluents. Yields are unoptimized. Chemical intermediates were characterized by ^{1}H NMR.

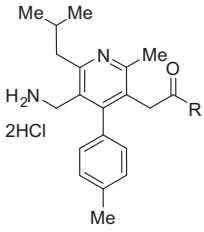
5.1.1. 5-Methyl-3-oxohexanenitrile (3)

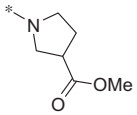
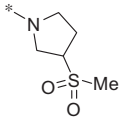
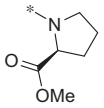
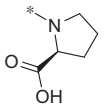
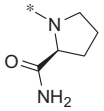
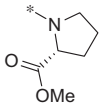
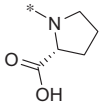
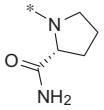
To a suspension of sodium hydride (60% in oil) (30.2 g, 666 mmol) in THF (300 mL) was added a mixture of methyl 3-methylbutanoate (**2**) (50 mL, 333 mmol), and acetonitrile (39 mL, 666 mmol) dropwise at 70 °C. The mixture was stirred at 70 C for 15 h. The reaction mixture was quenched with water and washed with hexane. The aqueous layer was acidified with concentrated HCl and extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **3** (41 g, 98%) as a pale yellow oil. Compound **3** was used for the next reaction without further purifications. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 2.11–2.28 (1H, m), 2.50 (2H, d, J = 6.8 Hz), 3.42 (2H, s).

5.1.2. 5-Methyl-2-[(4-methylphenyl)methylidene]-3-oxohexanenitrile (4)

A mixture of **3** (40 g, 300 mmol), *p*-tolualdehyde (36 g, 300 mmol), piperidine (2.56 g, 30 mmol), AcOH (3.6 g, 60 mmol), and toluene (125 mL) was heated under reflux for 5 h using a Dean–Stark trap. The reaction mixture was allowed to cool to room temperature, washed with brine and dried over anhydrous $MgSO_4$. The solvent was concentrated under reduced pressure to afford **4** (68 g, crude) as a brown oil. The obtained oil was used for the next reaction without further purifications. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89–1.10 (6H, m), 2.18–2.38 (1H, m), 2.44 (3H, s), 2.77 (2H, d, J = 6.8 Hz), 7.31 (2H, d, J = 8.3 Hz), 7.92 (2H, d, J = 8.3 Hz), 8.14 (1H, s).

Table 3
SAR summary for the prolinamide derivatives



Compound	R	DPP-4 IC_{50}[a] (nM)
13l		11 (10–12)
13m		53 (51–56)
13i		1.1 (0.74–1.6)
15i		2.9 (2.7–3.0)
13j		1.1 (0.98–1.1)
13k		23 (19–28)
15k		7.8 (6.5–9.2)
17k		12 (10–14)

[a] Refers to Table 1.

5.1.3. Methyl 5-cyano-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)-1,4-dihydropyridine-3-carboxylate (5)

A mixture of **4** (68 g, 300 mmol), methyl (2*E*)-3-aminobut-2-enoate (34.6 g, 300 mmol) and MeOH (500 mL) was stirred at 80 °C for 4 h. The reaction mixture was ice-cooled and concentrated under reduced pressure. The precipitated crystals were collected by filtration, washed with diisopropyl ether and dried to afford **5** (38.1 g, 39%) as a white powder. ^{1}H NMR (300 MHz,

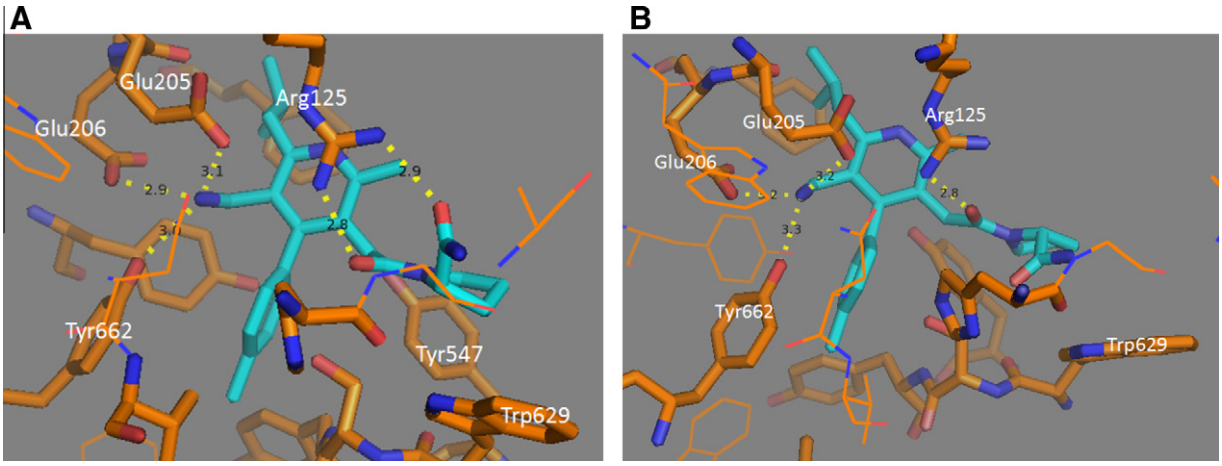


Figure 6. A (left) and B (right). Docking study of DPP-4 protein and compounds **13j** and **17k** (blue carbon). Amino acids (orange carbons, white text) are indicated. Hydrogen bonds are shown as yellow dotted lines.

Table 4
Enzyme selectivity of compound **13j** against DPP-2, DPP-8, and DPP-9

DPP-4 IC_{50}[a] (nM)	DPP-2 IC_{50}[b] (nM)	DPP-8 IC_{50}[b] (nM)	DPP-9 IC_{50}[b] (nM)
1.1 (0.98–1.1)	>100,000	>30,000	>30,000

[a] Refers to Table 1.
[b] Inhibitory activity against rat DPP-2, human DPP-8, and human DPP-9. IC_{50} values shown are means of duplicate or triplicate measurements. IC_{50} values and 95% confidence limits are calculated from the concentration–response curves generated by GraphPad Prism and shown in parenthesis.

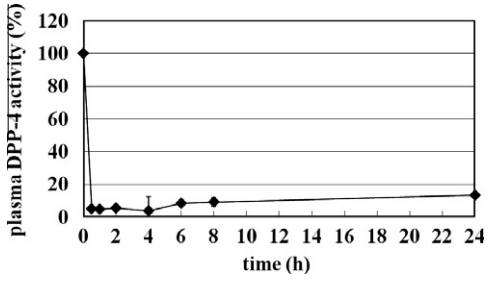


Figure 7. Ex vivo study of compound **13j** in dogs. Compound **13j** was prepared in 0.5% suspension of methylcellulose. Five dogs (age, 28–42 months) were administered orally with the suspension of compound at the dose of 0.5 mg/kg. Blood samples were collected from cephalic vein before (0 h) and 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. Plasma sample was prepared from each blood sample and the residual DPP-4 activity was measured by a method similar to that described in Section 5.

DMSO-d_6) δ: 0.93 (3H, d, J = 6.6 Hz), 0.98 (3H, d, J = 6.6 Hz), 1.80–2.00 (1H, m), 2.10–2.35 (2H, m), 2.30 (3H, s), 2.36 (3H, s), 3.58 (3H, s), 4.57 (1H, s), 5.68 (1H, br s), 7.00–7.20 (4H, m). Mp 171 °C.

5.1.4. Methyl 5-cyano-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylate (6)

To a suspension of **5** (38.1 g, 118 mol) in 1,4-dioxane (100 mL) was added dropwise 2 M HNO_3 solution (480 mL, 960 mmol). The obtained mixture was stirred at 75 °C for 45 min. The residue was neutralized with 8 M NaOH solution and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from hexane and diisopropyl ether to afford **6** (31.2 g, 82%) as a light yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J = 6.6 Hz), 1.32 (3H, t, J = 7.5 Hz), 2.24–2.36 (1H, m), 2.41 (3H, s), 2.85 (2H, q, J = 7.5 Hz), 2.96 (2H, d, J = 6.9 Hz), 3.59 (3H, s), 7.24–7.30 (4H, m). LC/MS m/z 337 (M+H). Mp 87.1–87.4 °C.

5.1.5. Methyl 5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylate (7)

A mixture of **6** (50 g, 155 mmol), Raney-Ni (50 mL), 25% NH_3 solution (50 mL), and MeOH (500 mL) was stirred at 40 °C for 5 h in a sealed tube under a hydrogen atmosphere at 0.5 MPa. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was partitioned between AcOEt and 10% aqueous potassium carbonate solution. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by NH silica gel column chromatography (AcOEt) to afford **7** (33.9 g, 67%) as pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.39 (2H, br s), 2.15–2.30 (1H, m), 2.39 (3H, s), 2.53 (3H, s), 2.80

Table 5
ADME profiles of **13j**

Pharmacokinetic profile[a]				Metabolic stability[d] (μL/min/mg @ 1 μM)			Solubility (μg/mL) (37 °C, 2 h)	
$MRT_{(oral)}$[b] (h)		B.A.[c] (%)						
Rat	Dog	Rat	Dc_{o}	Rat	Dog	Human	JP1[e]	JP2[f]
11.2	4.35	4.9	21.3	<1.0	2.0	4.0	86	71

[a] Rats and dogs were administered intravenously at 0.1 mg/kg and orally at 1.0 mg/kg (n = 3).
[b] MRT: mean residence time.
[c] B.A.: bioavailability.
[d] Metabolic stability in hepatic microsome (rat, dog, and human).
[e] First fluid for the Disintegration test regulated in the Japanese Pharmacopoeia 15th edition (pH 1.2).
[f] Second fluid for the Disintegration test regulated in the Japanese Pharmacopoeia 15th edition (pH 6.8).

(2H, d, J = 7.2 Hz), 3.50 (3H, s), 3.66 (2H, s), 7.11 (2H, d, J = 8.0 Hz), 7.21 (2H, d, J = 8.0 Hz). LC/MS m/z 327 (M+H). Mp 56.7–56.8 °C.

5.1.6. *tert*-Butyl {[5-(hydroxymethyl)-6-methyl-4-(4-methyl phenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (8)

A solution of **7** (9.3 g, 29 mmol) in toluene (150 mL) was cooled to −78 °C, and 1 M DIBAL-H toluene solution (100 mL, 100 mmol) was added dropwise over 30 min. The obtained mixture was allowed to warm, and acetone (10 mL) and sodium sulfate decahydrate (40 g) were added at 0 °C. The reaction mixture was stirred for 15 h at room temperature, and insoluble materials were filtered off and washed with AcOEt. The filtrate and the wash were combined, and 1 M NaOH solution (30 mL, 30 mmol) and $(Boc)_2O$ (6.9 mL, 30 mmol) were added. The mixture was stirred at room temperature for 30 min. The reaction mixture was washed sequentially with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50:50) to afford **8** (8.5 g, 75%) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.6 Hz), 1.32 (9H, s), 2.13–2.25 (1H, m), 2.42 (3H, s), 2.68 (3H, s), 2.75 (2H, d, J = 7.4 Hz), 4.05 (2H, d, J = 4.7 Hz), 4.19 (1H, br s), 4.36 (2H, d, J = 5.7 Hz), 7.05 (2H, d, J = 7.9 Hz), 7.24–7.26 (2H, m). LC/MS m/z 399 (M+H). Mp 140.0–141.0 °C.

5.1.7. *tert*-Butyl {[5-(cyanomethyl)-6-methyl-4-(4-methyl phenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (9)

To an ice-cooled mixture of **8** (17.4 g, 43 mmol), triethylamine (15 mL, 108 mmol), and THF (150 mL) was added dropwise methanesulfonyl chloride (4.0 mL, 52 mmol), and the mixture was stirred at 0 °C for 30 min. Water was added to the reaction mixture, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford [5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]methyl methanesulfonate as a crude product (20 g). The crude product (20 g) was dissolved in acetonitrile (300 mL), and trimethylsilyl cyanide (6.7 mL, 50 mmol) and then 1 M TBAF THF solution (50 mL, 50 mmol) were sequentially added. The obtained mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residual solid was washed with a mixture of hexane and Et_2O to afford **9** (15.6 g, 89%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.8 Hz), 1.38 (9H, s), 2.16–2.25 (1H, m), 2.43 (3H, s), 2.66 (3H, s), 2.77 (2H, d, J = 7.2 Hz), 3.31 (2H, s), 4.07 (2H, d, J = 4.7 Hz), 7.04 (2H, d, J = 8.0 Hz), 7.31 (2H, d, J = 8.0 Hz). LC/MS m/z 408.2 (M+H). Mp 130.3–131.3 °C.

5.1.8. [5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetic acid (10)

Compound **9** (14.5 g, 36 mmol) was suspended in 6 M HCl (150 mL) and the suspension was stirred at 90 °C for 20 h. The reaction mixture was allowed to cool to room temperature and washed with Et_2O. The aqueous layer was alkalified (pH 8) with 8 M NaOH solution, and then AcOEt (200 mL) and $(Boc)_2O$ (10 mL, 44 mmol) were added. The mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with 1 M HCl and partitioned. The aqueous layer was extracted with AcOEt, and the organic layer and the extract were combined. The mixture was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford **10** (14.0 g, 92%) as a white powder. LC/MS m/z 408.2 (M+H).

5.1.9. Methyl 3-({[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)benzoate (12a)

A mixture of compound **10** (500 mg, 1.17 mmol), methyl 3-aminobenzoate (**11a**) (532 mg, 3.52 mmol) and 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (1.34 g, 3.52 mmol) in *N*,*N*-dimethylformamide (DMF) (5 mL) was stirred at room temperature for 16 h. To the reaction mixture was added water, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:70) to afford **12a** (170 mg, 26%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.16–2.31 (1H, m), 2.41 (3H, s), 2.64 (3H, s), 2.77 (2H, d, J = 7.4 Hz), 3.47 (2H, s), 3.91 (3H, s), 4.07 (2H, d, J = 4.5 Hz), 7.02 (2H, d, J = 7.9 Hz), 7.24 (2H, d, J = 7.9 Hz), 7.38 (1H, t, J = 7.9 Hz), 7.72–7.86 (3H, m). LC/MS m/z 560.3 (M+H).

5.1.10. *tert*-Butyl {[5-{2-[(3-carbamoylphenyl)amino]-2-oxo ethyl}-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (12b)

Compound **12b** was prepared in a manner similar to that described for **12a** in 32% yield as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.93 (6H, d, J = 6.6 Hz), 1.35 (9H, s), 2.13–2.28 (1H, m), 2.32 (3H, s), 2.43 (3H, s), 2.59 (2H, d, J = 6.8 Hz), 3.39 (2H, s), 3.80 (2H, d, J = 4.3 Hz), 6.82 (1H, s), 7.09–7.24 (4H, m), 7.28–7.40 (2H, m), 7.51 (1H, d, J = 7.9 Hz), 7.60–7.69 (1H, m), 7.94 (2H, s), 9.93 (1H, s). LC/MS m/z 545.16 (M+H).

5.1.11. *tert*-Butyl {[5-{2-[(3-acetylphenyl)amino]-2-oxoethyl}-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (12c)

Compound **12c** was prepared in a manner similar to that described for **12a** in 74% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.15–2.31 (1H, m), 2.39 (3H, s), 2.58 (3H, s), 2.63 (3H, s), 2.76 (2H, d, J = 7.2 Hz), 3.48 (2H, s), 4.06 (2H, d, J = 4.9 Hz), 4.25 (1H, s), 6.78 (1H, s), 7.02 (2H, d, J = 7.9 Hz), 7.24 (2H, d, J = 7.9 Hz), 7.39 (1H, t, J = 7.9 Hz), 7.67 (1H, d, J = 8.1 Hz), 7.74 (1H, d, J = 8.1 Hz), 7.81 (1H, d, J = 1.7 Hz). LC/MS m/z 544.22 (M+H).

5.1.12. *tert*-Butyl {[6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)-5-(2-{[3-(methylsulfanyl)phenyl]amino}-2-oxoethyl)-pyridin-3-yl]methyl}carbamate (12d)

Compound **12d** was prepared in a manner similar to that described for **12a** in 90% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.13–2.24 (1H, m), 2.40 (3H, s), 2.46 (3H, s), 2.62 (3H, s), 2.76 (2H, d, J = 7.5 Hz), 3.44 (2H, s), 4.06 (2H, d, J = 5.1 Hz), 4.22 (1H, s), 6.70 (1H, s), 6.96–7.02 (4H, m), 7.15–7.26 (4H, m), 7.37 (1H, s). LC/MS m/z 548.14 (M+H).

5.1.13. *tert*-Butyl {[6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)-5-(2-{[3-(methylsulfonyl)phenyl]amino}-2-oxoethyl)-pyridin-3-yl]methyl}carbamate (12e)

Compound **12e** was prepared in a manner similar to that described for **12a** in 87% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.18–2.27 (1H, m), 2.41 (3H, s), 2.63 (3H, s), 2.77 (2H, d, J = 7.4 Hz), 3.05 (3H, s), 3.49 (2H, s), 4.06 (2H, d, J = 5.1 Hz), 4.23 (1H, s), 6.85–6.96 (1H, m), 7.01 (2H, d, J = 7.9 Hz), 7.25 (2H, d, J = 5.8 Hz), 7.50 (1H, t, J = 7.9 Hz), 7.64–7.67 (1H, m), 7.76 (1H, d, J = 8.7 Hz), 7.83 (1H, t, J = 1.9 Hz). LC/MS m/z 580.2 (M+H).

5.1.14. Methyl 2-({[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}amino)benzoate (12f)

Compound **12f** was prepared in a manner similar to that described for **12a** in 38% yield as a pale yellow powder. LC/MS *m/z* 560.21 (M+H).

5.1.15. Methyl 4-({[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}amino)thiophene-3-carboxylate (12g)

Compound **12g** was prepared in a manner similar to that described for **12a** in 66% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.4 Hz), 1.40 (9H, s), 2.24–2.33 (1H, m), 2.35 (3H, s), 2.53 (3H, s), 2.77 (2H, d, J = 7.6 Hz), 3.52 (2H, s), 3.79 (3H, s), 4.06 (2H, d, J = 4.1 Hz), 7.02 (2H, d, J = 7.9 Hz), 7.17 (2H, d, J = 7.9 Hz), 7.95–7.98 (1H, m), 7.98–8.02 (1H, m). LC/MS *m/z* 566.17 (M+H).

5.1.16. Methyl *N*-{[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}glycinate (12h)

Compound **12h** was prepared in a manner similar to that described for **12a** in 61% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.8 Hz), 1.38 (9H, s), 2.14–2.29 (1H, m), 2.39 (3H, s), 2.57 (3H, s), 2.76 (2H, d, J = 7.4 Hz), 3.34 (2H, s), 3.74 (3H, s), 3.95 (2H, d, J = 5.1 Hz), 4.05 (2H, d, J = 5.1 Hz), 7.01 (2H, d, J = 7.9 Hz), 7.23 (2H, d, J = 7.9 Hz). LC/MS *m/z* 498.48 (M+H).

5.1.17. Methyl 1-{[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}-L-prolinate (12i)

Compound **12i** was prepared in a manner similar to that described for **12a** in 67% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 1.78–2.23 (5H, m), 2.40 (3H, s), 2.54 (3H, s), 2.72 (2H, d, J = 7.2 Hz), 3.03–3.26 (2H, m), 4.03 (2H, d, J = 4.7 Hz), 4.20 (1H, br s), 4.38 (1H, dd, J = 8.3, 4.0 Hz), 7.17–7.29 (2H, m). LC/MS *m/z* 538.16 (M+H).

5.1.18. *tert*-Butyl {[5-{2-[(2*S*)-2-carbamoylpyrrolidin-1-yl]-2-oxoethyl}-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)-pyridin-3-yl]methyl}carbamate (12j)

Compound **12j** was prepared in a manner similar to that described for **12a** in 81% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 1.39–1.60 (4H, m), 2.13–2.28 (1H, m), 2.39 (3H, s), 2.54 (3H, s), 2.74 (2H, d, J = 7.4 Hz), 3.02–3.12 (1H, m), 3.34 (2H, s), 4.04 (2H, d, J = 4.9 Hz), 4.25 (1H, s), 4.50 (1H, d, J = 7.0 Hz), 5.29 (1H, s), 6.92–7.09 (4H, m), 7.21 (2H, d, J = 8.1 Hz). LC/MS *m/z* 523.25 (M+H).

5.1.19. Methyl 1-{[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}-D-prolinate (12k)

Compound **12k** was prepared in a manner similar to that described for **12a** in 89% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 1.76–2.02 (3H, m), 2.07–2.27 (2H, m), 2.40 (3H, s), 2.54 (3H, s), 2.72 (2H, d, J = 7.2 Hz), 3.01–3.37 (4H, m), 3.71 (3H, s), 4.20 (1H, s), 4.38 (2H, dd, J = 8.3, 4.0 Hz), 7.02 (2H, d, J = 8.3 Hz), 7.14–7.23 (2H, m). LC/MS *m/z* 538.1 (M+H).

5.1.20. Methyl 1-{[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-acetyl}pyrrolidine-3-carboxylate (12l)

Compound **12l** was prepared in a manner similar to that described for **12a** in 34% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 2.07–2.27 (2H, m), 2.40 (3H, s), 2.54 (3H, d, J = 1.7 Hz), 2.74 (2H, d, J = 7.2 Hz), 2.80 (3H, s), 2.90–3.39 (5H, m), 3.51–3.68 (1H, m), 3.71 (2H, d, J = 3.2 Hz), 4.03 (2H, d, J = 4.7 Hz), 4.24 (1H, s), 7.06 (2H, d, J = 8.3 Hz), 7.21 (2H, d, J = 8.3 Hz). LC/MS *m/z* 538.27 (M+H).

5.1.21. *tert*-Butyl {[6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)-5-{2-[3-(methylsulfonyl)pyrrolidin-1-yl]-2-oxoethyl}-pyridin-3-yl]methyl}carbamate (12m)

Compound **12m** was prepared in a manner similar to that described for **12a** in 99% yield as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.92 (6H, d, J = 6.4 Hz), 1.04 (6H, d, J = 6.2 Hz), 1.34 (9H, s), 2.08–2.27 (3H, m), 2.57 (2H, d, J = 7.4 Hz), 3.00 (3H, d, J = 8.7 Hz), 3.21–3.29 (3H, m), 3.60 (2H, d, J = 5.7 Hz), 3.78 (2H, d, J = 4.1 Hz), 3.85–3.95 (1H, m), 6.82 (1H, s), 7.07 (2H, d, J = 7.9 Hz), 7.21 (2H, d, J = 7.9 Hz). LC/MS *m/z* 558.23 (M+H).

5.1.22. *tert*-Butyl ({6-methyl-5-[2-(methylamino)-2-oxoethyl]-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl}methyl)-carbamate (12n)

Compound **12n** was prepared in a manner similar to that described for **12a** in 42% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.10–2.30 (1H, m), 2.40 (3H, s), 2.55 (3H, s), 2.71 (3H, d, J = 4.9 Hz), 2.76 (2H, d, J = 7.4 Hz), 3.28 (2H, s), 4.05 (2H, d, J = 5.1 Hz), 4.21 (1H, s), 5.08 (1H, s), 6.96 (2H, d, J = 8.1 Hz), 7.23 (2H, d, J = 8.1 Hz). LC/MS *m/z* 440.17 (M+H).

5.1.23. Methyl 3-({[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino) benzoate dihydrochloride (13a)

A mixture of **12a** (75 mg, 0.134 mmol) in 4 M HCl in 1,4-dioxane (4 mL, 16 mmol) was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a white powder. The white powder was recrystallized from distilled water and acetonitrile to give **13a** (65 mg, 91%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02 (9H, s), 2.37 (3H, s), 2.59 (3H, s), 3.04 (2H, s), 3.86 (2H, d, J = 5.5 Hz), 7.23 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 8.24 (3H, br s). LC/MS *m/z* 460.2 (M+H). ESI-HRMS calcd for $C_{28}H_{33}N_3O_3$ *m/z* 460.2595 (M+H), found 460.2578 (M+H).

5.1.24. 3-({[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)benzamide dihydrochloride (13b)

Compound **13b** was prepared in a manner similar to that described for **13a** in 96% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 1.00 (6H, d, J = 6.4 Hz), 2.08–2.28 (1H, m), 2.38 (3H, s), 2.88 (3H, s), 3.27 (2H, d, J = 7.0 Hz), 3.65 (2H, s), 3.83 (2H, s), 7.23 (2H, d, J = 7.9 Hz), 7.30–7.44 (4H, m), 7.49–7.73 (3H, m), 7.80–7.94 (1H, m), 7.97 (2H, s), 8.52 (3H, s), 10.29 (1H, s). LC/MS *m/z* 445.14 (M+H). ESI-HRMS calcd for $C_{27}H_{32}N_4O_2$ *m/z* 445.2598 (M+H), found 445.2567 (M+H).

5.1.25. *N*-(3-Acetylphenyl)-2-[5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetamide dihydrochloride (13c)

Compound **13c** was prepared in a manner similar to that described for **13a** in 87% yield as a white powder. ^{1}H NMR

(300 MHz, DMSO-d_6) δ : 1.00 (6H, d, J = 6.6 Hz), 2.11–2.27 (1H, m), 2.37 (3H, s), 2.54 (3H, s), 2.82 (3H, s), 3.18 (2H, s), 3.62 (2H, s), 3.82 (2H, d, J = 4.3 Hz), 7.21 (2H, d, J = 7.9 Hz), 7.34 (2H, d, J = 7.9 Hz), 7.45 (1H, t, J = 7.9 Hz), 7.61–7.78 (2H, m), 8.10 (1H, s), 8.41 (3H, br s), 10.34 (1H, s). LC/MS *m/z* 444.10 (M+H). ESI-HRMS calcd for $C_{28}H_{32}N_3O_2$ *m/z* 444.2646 (M+H), found 444.2620 (M+H).

5.1.26. 2-[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-*N*-[3-(methylsulfonyl)phenyl]acetamide dihydrochloride (13e)

Compound **13e** was prepared in a manner similar to that described for **13a** in 56% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.99 (6H, d, J = 6.6 Hz), 2.14–2.23 (1H, m), 2.36 (3H, s), 2.80 (3H, s), 3.12–3.18 (2H, m), 3.18 (3H, s), 3.63 (2H, s), 3.79–3.84 (2H, m), 7.21 (2H, d, J = 8.0 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.55–7.64 (2H, m), 7.70–7.74 (1H, m), 8.16–8.18 (1H, m), 8.37 (3H, s), 10.60 (1H, s). Anal. Calcd for $C_{27}H_{33}N_3O_3S{\cdot}2HCl{\cdot}H_2O$: C, 56.84; H, 6.54; N, 7.36. Found: C, 56.92; H, 6.92; N, 7.30. ESI-HRMS calcd for $C_{27}H_{33}N_3O_3S$ *m/z* 480.2315 (M+H), found 480.2294 (M+H).

5.1.27. Methyl 2-({[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)benzo ate dihydrochloride (13f)

Compound **13f** was prepared in a manner similar to that described for **13a** in 61% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 1.00 (6H, d, J = 6.6 Hz), 2.09–2.28 (1H, m), 2.37 (3H, s), 2.84 (3H, s), 3.18 (2H, s), 3.63 (2H, s), 3.78 (3H, s), 3.81 (2H, s), 7.17–7.27 (3H, m), 7.34 (2H, d, J = 7.9 Hz), 7.52–7.65 (1H, m), 7.86 (1H, dd, J = 7.8, 1.4 Hz), 7.98 (1H, d, J = 8.3 Hz), 8.41 (3H, br s), 10.42 (1H, s). LC/MS *m/z* 460.12 (M+H). ESI-HRMS calcd for $C_{28}H_{33}N_3O_3$ *m/z* 460.2595 (M+H), found 460.2599 (M+H).

5.1.28. Methyl 4-({[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)thio phene-3-carboxylate dihydrochloride (13g)

Compound **13g** was prepared in a manner similar to that described for **13a** in 65% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.6 Hz), 2.11–2.27 (1H, m), 2.35 (3H, s), 2.48 (3H, s), 2.80 (2H, s), 3.14 (2H, s), 3.76–3.86 (5H, m), 7.17 (2H, d, J = 7.9 Hz), 7.32 (2H, d, J = 7.9 Hz), 7.80 (1H, d, J = 3.2 Hz), 8.26–8.45 (3H, m), 9.69 (1H, s). LC/MS *m/z* 466.11 (M+H). ESI-HRMS calcd for $C_{26}H_{31}N_3O_3S$ *m/z* 466.2159 (M+H), found 466.2131 (M+H).

5.1.29. Methyl *N*-{[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}glycinate dihydrochloride (13h)

Compound **13h** was prepared in a manner similar to that described for **13a** in 73% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.6 Hz), 2.07–2.30 (1H, m), 2.41 (3H, s), 2.49 (3H, s), 2.74 (2H, s), 3.10 (2H, s), 3.36 (2H, s), 3.63 (3H, s), 3.80 (2H, d, J = 5.7 Hz), 7.21 (2H, d, J = 8.1 Hz), 7.37 (2H, d, J = 8.1 Hz), 8.29 (3H, br s), 8.43 (1H, s). LC/MS *m/z* 398.1 (M+H). ESI-HRMS calcd for $C_{23}H_{31}N_3O_3$ *m/z* 398.2438 (M+H), found 398.2411 (M+H).

5.1.30. Methyl 1-{[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-prolinate dihydrochloride (13i)

Compound **13i** was prepared in a manner similar to that described for **13a** in 83% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.99 (6H, d, J = 6.6 Hz), 1.70–2.28 (5H, m), 2.41 (3H, s), 2.49 (3H, s), 2.82 (3H, s), 3.08–3.57 (6H, m), 3.62 (3H, s), 3.82 (2H, s), 4.20–4.30 (1H, m), 7.20 (2H, d, J = 7.5 Hz), 7.39 (2H, d, J = 7.5 Hz), 8.53 (3H, s). LC/MS *m/z* 438.12 (M+H). ESI-HRMS calcd for $C_{26}H_{35}N_3O_3$ *m/z* 438.2751 (M+H), found 438.2724 (M+H).

5.1.31. 1-{[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-prolinamide dihydro chloride (13j)

Compound **13j** was prepared in a manner similar to that described for **13a** in 82% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.4 Hz), 1.63–2.07 (4H, m), 2.08–2.27 (1H, m), 2.41 (3H, s), 2.74–3.00 (4H, m), 3.11–3.43 (4H, m), 3.82 (2H, d, J = 4.3 Hz), 4.13 (1H, dd, J = 8.2, 2.9 Hz), 6.83–7.54 (6H, m), 8.48 (3H, s). LC/MS *m/z* 424.10 (M+H). ESI-HRMS calcd for $C_{25}H_{34}N_4O_2$ *m/z* 423.2755 (M+H), found 423.2731 (M+H).

5.1.32. Methyl 1-{[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-D-prolinate dihydrochloride (13k)

Compound **13k** was prepared in a manner similar to that described for **13a** in 55% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.4 Hz), 1.73–1.91 (3H, m), 2.05–2.29 (2H, m), 2.41 (3H, s), 2.76 (3H, s), 3.06–3.26 (4H, m), 3.47 (2H, s), 3.62 (3H, s), 3.81 (2H, d, J = 3.8 Hz), 4.19–4.33 (1H, m), 7.18 (2H, d, J = 7.5 Hz), 7.39 (2H, d, J = 7.5 Hz), 8.36 (3H, s). LC/MS *m/z* 438.15 (M+H). Mp 207.6 °C. ESI-HRMS calcd for $C_{26}H_{35}N_3O_3$ *m/z* 438.2751 (M+H), found 437.2736 (M+H).

5.1.33. Methyl 1-{[5-(aminomethyl)-2-methyl-4-(4-methyl phenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}pyrrolidine-3-carboxylate dihydrochloride (13l)

Compound **13l** was prepared in a manner similar to that described for **13a** in 60% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.99 (6H, d, J = 6.4 Hz), 1.76–2.09 (2H, m), 2.11–2.29 (1H, m), 2.40 (3H, s), 2.81 (3H, s), 2.93–3.53 (9H, m), 3.64 (3H, d, J = 7.2 Hz), 3.81 (2H, d, J = 4.5 Hz), 3.98–4.14 (1H, m), 7.08–7.43 (4H, m), 8.43 (3H, s). LC/MS *m/z* 438.16 (M+H). Mp 80.1 °C. ESI-HRMS calcd for $C_{26}H_{35}N_3O_3$ *m/z* 438.2751 (M+H), found 437.2743 (M+H).

5.1.34. 1-[6-Methyl-4-(4-methylphenyl)-2-(2-methylpropyl)-5-{2-[3-(methylsulfonyl)pyrrolidin-1-yl]-2-oxoethyl}pyridin-3-yl]methanamine dihydrochloride (13m)

Compound **13m** was prepared in a manner similar to that described for **13a** in 99% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.96 (6H, d, J = 6.6 Hz), 2.04–2.28 (3H, m), 2.39 (3H, s), 3.02 (3H, d, J = 7.2 Hz), 3.06–4.09 (15H, m), 7.12 (2H, d, J = 7.7 Hz), 7.32 (2H, d, J = 7.7 Hz), 8.29 (3H, s). LC/MS *m/z* 458.13 (M+H). ESI-HRMS calcd for $C_{25}H_{35}N_3O_3S$ *m/z* 458.2472 (M+H), found 458.2442 (M+H).

5.1.35. 2-[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-*N*-methylacetamide dihydrochlo ride (13n)

Compound **13n** was prepared in a manner similar to that described for **13a** in 92% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.6 Hz), 2.06–2.28 (1H, m), 2.40 (3H, s), 2.71 (3H, s), 3.09 (2H, s), 3.28 (2H, s), 3.69 (3H, s), 3.79 (2H, d, J = 4.3 Hz), 7.18 (2H, d, J = 7.9 Hz), 7.37 (2H, d, J = 7.9 Hz), 7.79 (1H, s), 8.28 (3H, br s). LC/MS *m/z* 340.13 (M+H). ESI-HRMS calcd for $C_{21}H_{29}N_3O$ *m/z* 340.2383 (M+H), found 340.2381 (M+H).

5.1.36. 3-({[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl} amino)benzoic acid (14a)

A mixture of **12a** (130 mg, 0.232 mmol) and 1 M NaOH solution (5 mL, 5 mmol) in MeOH (20 mL) was stirred at room temperature for 14 h. The reaction mixture was neutralized with 1 M HCl and extracted with AcOEt. The extract was washed with brine, dried

over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford **14a** (93 mg, 73%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (6H, d, J = 5.7 Hz), 1.38 (9H, s), 2.10–2.27 (1H, m), 2.36 (3H, s), 2.89–3.10 (5H, m), 3.90 (2H, d, J = 5.7 Hz), 4.10 (2H, d, J = 7.2 Hz), 7.13 (2H, d, J = 8.1 Hz), 7.24 (2H, d, J = 8.1 Hz), 7.32 (1H, t, J = 8.0 Hz), 7.65 (2H, d, J = 7.7 Hz), 7.89 (1H, s), 8.17 (1H, s). LC/MS *m/z* 546.3 (M+H).

5.1.37. 4-({[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)thiophene-3-carboxylic acid (14g)

Compound **14g** was prepared in a manner similar to that described for **14a** in 65% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.40 (9H, s), 2.11–2.24 (1H, m), 2.36 (3H, s), 2.52 (3H, s), 2.78 (2H, s), 3.49 (2H, s), 4.03 (2H, s), 6.98–7.25 (4H, m), 7.85–8.05 (2H, m). LC/MS *m/z* 552.17 (M+H).

5.1.38. *N*-{[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}glycine (14h)

Compound **14h** was prepared in a manner similar to that described for **14a** in 99% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (6H, d, J = 6.4 Hz), 1.34 (9H, s), 2.10–2.25 (1H, m), 2.35 (3H, s), 2.41 (3H, s), 2.58 (2H, s), 3.18 (2H, s), 3.69 (2H, d, J = 5.8 Hz), 3.80 (2H, s), 7.13 (2H, d, J = 8.1 Hz), 7.22 (2H, d, J = 8.1 Hz). LC/MS *m/z* 484.1 (M+H).

5.1.39. 1-{[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-proline (14i)

Compound **14i** was prepared in a manner similar to that described for **14a** in 99% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (6H, d, J = 6.6 Hz), 1.36 (9H, s), 1.75–2.29 (5H, m), 2.42 (3H, s), 2.81 (3H, s), 2.97–3.63 (6H, m), 4.12 (2H, d, J = 7.2 Hz), 4.33–4.45 (1H, m), 4.63 (1H, s), 6.97–7.38 (4H, m). LC/MS *m/z* 524.16 (M+H).

5.1.40. 1-{[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-D-proline (14k)

Compound **14k** was prepared in a manner similar to that described for **14a** in 99% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 1.77–2.32 (7H, m), 2.39 (3H, s), 2.51–2.63 (3H, m), 2.77–2.87 (2H, m), 2.94–3.74 (4H, m), 4.06 (2H, s), 4.41 (1H, s), 6.94–7.32 (4H, m). LC/MS *m/z* 524.16 (M+H).

5.1.41. 3-({[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)benzoic acid dihydrochloride (15a)

Compound **15a** was prepared in a manner similar to that described for **13a** in 99% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 1.00 (6H, d, J = 6.6 Hz), 2.08–2.25 (1H, m), 2.37 (3H, s), 2.51 (3H, s), 2.83 (2H, s), 3.20 (2H, s), 3.82 (2H, s), 7.09–7.51 (5H, m), 7.54–7.79 (2H, m), 8.14 (1H, s), 8.44 (3H, s). LC/MS *m/z* 446.2 (M+H). ESI-HRMS calcd for $C_{27}H_{31}N_3O_3$ *m/z* 446.2438 (M+H), found 446.2421 (M+H).

5.1.42. 4-({[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)thiophene-3-carboxylic acid dihydrochloride (15g)

Compound **15g** was prepared in a manner similar to that described for **13a** in 88% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.99 (6H, d, J = 6.6 Hz), 2.11–2.27 (1H, m), 2.35 (3H, s), 2.50 (3H, s), 2.79 (2H, s), 3.14 (2H, s), 3.81 (2H, s), 7.17 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 7.79 (1H, d, J = 3.6 Hz), 8.29 (2H, d, J = 3.6 Hz), 8.33–8.44 (3H, m), 9.89 (1H, s). LC/MS *m/z* 452.03 (M+H). ESI-HRMS calcd for $C_{25}H_{29}N_3O_3S$ *m/z* 452.2002 (M+H), found 452.1971 (M+H).

5.1.43. *N*-{[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}glycine dihydrochloride (15h)

Compound **15h** was prepared in a manner similar to that described for **13a** in 88% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.6 Hz), 2.07–2.28 (1H, m), 2.40 (3H, s), 2.73 (3H, s), 3.08 (2H, s), 3.34 (2H, s), 3.70 (2H, d, J = 5.7 Hz), 3.81 (2H, s), 7.21 (2H, d, J = 7.9 Hz), 7.36 (2H, d, J = 7.9 Hz), 8.29 (3H, s). LC/MS *m/z* 384.06 (M+H). ESI-HRMS calcd for $C_{22}H_{29}N_3O_3$ *m/z* 384.2282 (M+H), found 384.2258 (M+H).

5.1.44. 1-{[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-L-proline dihydrochloride (15i)

Compound **15i** was prepared in a manner similar to that described for **13a** in 84% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.99 (6H, d, J = 6.6 Hz), 1.74–1.91 (3H, m), 2.02–2.29 (2H, m), 2.41 (3H, s), 2.81 (3H, s), 3.09–3.27 (4H, m), 3.42–3.53 (2H, m), 3.83 (2H, d, J = 5.1 Hz), 4.13–4.30 (1H, m), 7.14–7.28 (2H, m), 7.33–7.46 (2H, m), 8.44 (3H, br s). LC/MS *m/z* 424.05 (M+H). ESI-HRMS calcd for $C_{25}H_{33}N_3O_3$ *m/z* 424.2595 (M+H), found 424.2566 (M+H).

5.1.45. 1-{[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-D-proline dihydrochloride (15k)

Compound **15k** was prepared in a manner similar to that described for **13a** in 94% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.6 Hz), 1.75–1.88 (3H, m), 1.97–2.28 (2H, m), 2.41 (3H, s), 2.80 (3H, s), 3.07–3.27 (4H, m), 3.82 (2H, s), 4.18 (1H, dd, J = 8.4, 3.3 Hz), 7.20 (2H, d, J = 7.9 Hz), 7.37 (2H, d, J = 7.9 Hz), 8.47 (3H, s). LC/MS *m/z* 424.08 (M+H). ESI-HRMS calcd for $C_{25}H_{33}N_3O_3$ *m/z* 424.2595 (M+H), found 424.2567 (M+H).

5.1.46. Methyl 3-({[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}amino)benzoate (16k)

Compound **16k** was prepared in a manner similar to that described for **12a** in 83% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.6 Hz), 1.37 (9H, s), 1.56–2.02 (4H, m), 2.12–2.28 (1H, m), 2.39 (3H, s), 2.54 (3H, s), 2.73 (2H, d, J = 7.2 Hz), 2.85–3.16 (2H, m), 3.34 (2H, s), 4.04 (2H, d, J = 5.1 Hz), 4.27 (1H, s), 4.50 (1H, d, J = 6.8 Hz), 5.34 (1H, s), 6.91–7.07 (3H, m), 7.21 (2H, d, J = 8.1 Hz). LC/MS *m/z* 523.20 (M+H).

5.1.47. 1-{[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetyl}-D-prolinamide dihydro chloride (17k)

Compound **17k** was prepared in a manner similar to that described for **13a** in 76% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ : 0.98 (6H, d, J = 6.4 Hz), 1.63–2.03 (4H, m), 2.08–2.27 (1H, m), 2.40 (3H, s), 2.64–3.50 (11H, m), 3.81 (2H, s), 4.12 (1H, dd, J = 8.2, 2.7 Hz), 6.98–7.56 (4H, m), 8.28 (3H, s). LC/MS *m/z* 424.10 (M+H). ESI-HRMS calcd for $C_{25}H_{34}N_4O_2$ *m/z* 423.2755 (M+H), found 423.2734 (M+H).

5.1.48. *tert*-Butyl {[6-methyl-4-(4-methylphenyl)-2-(2-methyl propyl)-5-(2-{[3-(methylsulfinyl)phenyl]amino}-2-oxoethyl) pyridin-3-yl]methyl}carbamate (18d)

To a solution of **12d** (1.19 g, 2.17 mmol) in $CHCl_3$ (20 mL) was added *m*-chloroperoxybenzoic acid (*m*CPBA) (360 mg, 2.08 mmol)

at 0 °C and stirred for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was extracted with 1 M NaOH solution and AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to afford **18d** (194 mg, 55%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.15–2.29 (1H, m), 2.39 (3H, s), 2.61 (3H, s), 2.70 (3H, s), 2.76 (2H, d, J = 7.2 Hz), 3.48 (2H, s), 4.06 (2H, d, J = 4.8 Hz), 4.26 (1H, s), 7.22–7.28 (3H, m), 7.25 (2H, d, J = 7.8 Hz), 7.35 (1H, s), 7.44 (1H, t, J = 7.8 Hz), 7.68 (2H, d, J = 7.8 Hz). LC/MS m/z 564.14 (M+H).

5.1.49. 2-[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-*N*-[3-(methylsulfinyl)phenyl]acetamide (19d)

A mixture of **18d** (430 mg, 0.763 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 5 min. The reaction mixture was concentrated under reduced pressure. The residue was neutralized with 1 M NaOH solution and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to afford **19d** (194 mg, 55%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (6H, d, J = 6.9 Hz), 2.04–2.29 (1H, m), 2.40 (3H, s), 2.61 (3H, s), 2.71 (3H, s), 2.79 (2H, d, J = 7.2 Hz), 3.48 (2H, s), 3.57 (2H, s), 7.06 (2H, d, J = 8.1 Hz), 7.23–7.29 (4H, m), 7.44 (1H, t, J = 7.8 Hz), 7.64 (1H, br s). LC/MS m/z 464.11 (M+H). ESI-HRMS calcd for $C_{27}H_{33}N_3O_2S$ m/z 464.2366 (M+H), found 464.2340 (M+H).

5.2. Docking study in DPP-4

The X-ray crystal structure of DPP-4 complexed with 5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxyic acid was utilized in the docking calculations. The compounds were docked into DPP-4 using GOLD 3.0.[21] After the automatic docking, the conformations of the substituents in the docked compounds were aligned to the corresponding moiety of 5-(aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxyic acid and then energy-minimized at the MMFF94s force field using MOE 2005.06.[22]

5.3. In vitro DPP-4, DPP-2, DPP-8, and DPP-9 enzyme assay

Human DPP-4 was partially purified from Caco-2 cells (ATCC No. HTB-37). The compounds (1 μL in DMSO) at each concentration were added to 79 μL of assay buffer (0.25 mol/L Tris–HCl pH 7.5, 0.25% bovine serum albumin, 0.125% CHAPS) and mixed with 20 μL of the DPP-IV fraction. After the mixture was incubated at room temperature for 15 min, the reaction was initiated by adding 100 μL of 1 mmol/L of Gly-Pro-pNA·Tos as a substrate and run for 60 min at 37 °C.

Rat DPP-2 was partially purified from rat kidney according to the method previously reported.[23] One microliter of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 mol/L 3,3-dimethylglutamic acid buffer (pH 5.5), and 10 μL of the DPP-2 fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 1 mmol/L of H-Lys-Ala-pNA·2HCl and run at 37 °C for 60 min.

Human DPP-8 and DPP-9 were purified, respectively, by affinity chromatography from the 293-F cells expressing each FLAG-tagged protein. 1 μL of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 mol/L Tris–HCl buffer (pH 7.5), and 10 μL of the enzyme fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 2 mmol/L of Gly-Pro-pNA·Tos for DPP-8 or 4 mmol/L of Gly-Pro-pNA·Tos for DPP-9 and run at 37 °C for 90 min.

Absorbance at 405 nm of each reaction mixture was measured using a microplate reader at the initial time and the end of the reaction. The well containing substrate alone was used as a basal control. The well containing the substrate and the enzyme without the compound was used as a total reaction.

5.4. Pharmacokinetic profile in rats and dogs

Compound **13j** was administered to rats and dogs. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.01 mol/L ammonium acetate and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

5.5. Solubility determination

The compounds were added to each buffer solution. After incubation, precipitates were separated by filtration. The thermodynamic solubility was determined by HPLC analysis of each filtrate.

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